Research Article

Evaluation of Comprehensive 2D Gas Chromatography-Time-of-Flight Mass Spectrometry for 209 Chlorinated Biphenyl Congeners in Two Chromatographic Runs

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This research evaluates a recently developed comprehensive two-dimensional gas chromatography 2D GC coupled with a time-of-flight (TOF) mass spectrometer for the potential separation of 209 PCB congeners, using a sequence of 1D and 2D chromatographic modes. In two consecutive chromatographic runs, using a 40 m, Rtx-PCB column, and a 1 m DB-17 column, connected in series, 196 PCB congeners are distinguished, including 43 of the 46 pentachlorobiphenyl isomers. Some of the chlorinated biphenyls that could not be resolved chromatographically are resolved with the use of the “ortho effect,” which distinguishes PCB isomers having 2,2′- and 2,2′,6- chlorine substitution from those isomers without these substitutions. The result of this work falls short of our goal of separating all 209 PCB congeners but still provides investigators with a new tool for a better front-end separation of PCB-specific congeners, and potentially, for use in acquisition of more accurate data.

1. Introduction

Chlorinated biphenyls (CBs), or polychlorinated biphenyls (PCBs), are comprised of 209 distinct chlorine-substituted biphenyl structures (congeners). Ten isomeric groups of congeners exist with varying degrees of chlorination (see Table 1). Approximately 140 to 150 of the 209 PCB congeners listed in Table 2 were found in the complex mixtures (Aroclors) that were used commercially in a variety of applications, including heat transfer and hydraulic fluids, dielectric fluids for capacitors, and as additives in pesticides, sealants, and plastics [1–3]. The dispersion of PCB congeners in the form of the Aroclors by uncontrolled release into the environment, their long-term stability, and possible toxicity, together caused concern for their biological and environmental impact. The World Health Organization (WHO) designated twelve PCBs as “dioxin-like, coplanar PCB congeners” that exhibited high toxicity [4]. Originally, the noncoplanar PCBs were considered the most toxic because they were present in much greater abundances. Later, the “coplanar” PCBs were found to have dioxin-like toxicity and received the most attention. It appears that the toxicity of the noncoplanar PCBs has been re-emphasized because most noncoplanar congeners with ortho chlorine substitution were also found to be toxic in mammalian brains [5–7]. The most toxic coplanar PCB (#126, or 3,3′,4,5,5′-pentachlorobiphenyl) was known to coelute with 2,3,7,8-dibenzo-p-dioxin [8], and with PCB (#159 or 2,3,3′,4,5,5′-hexachlorobiphenyl) in environmental samples using the DB-XLB phase. High resolution mass spectrometry (HRMS) was usually required to resolve PCB 126 and 2,3,7,8-dibenzo-p-dioxin, when both were present in environmental samples in varying concentrations [9]. Tandem mass spectrometry (MS/MS) can also be used for distinguishing dioxins from PCBs. Dioxins can lose -COCl whereas PCBs can only show chlorine losses. To permit congener specific environmental
analysis of the PCBs, scientists invested considerable research effort towards optimizing the gas chromatographic conditions and techniques required for separations on various capillary columns [10–12]. Frame has published work on various and techniques required for separations on various capillary columns [10–12]. Frame has published work on various capillary columns and the fast data acquisition rates (50 spectra/s) of a time-of-flight mass spectrometer (TOFMS) for the identification of PCB congeners in minimal analysis time. Taking advantage of the full mass range data acquisition imparted to TOFMS for a variety of complex sample analyses, such as chlorinated hydrocarbons, and mixtures of environmental analytes [20–25]. A review of comprehensive two-dimensional gas chromatography by Beens and Brinkman in 2005 showed that the separation of chlorinated biphenyls in fish extract, using GC×GC without the necessary summing software for several modulations per analyte, was much improved compared to 1D-GC separation [26]. A goal of this study was to attempt to separate and unambiguously distinguish all of the 209 chlorinated biphenyl congeners in two different chromatographic runs using 1D-GC, with the thermal modulator deactivated in the first run and activated in the second run for 2D-GC.

GC×GC is a relatively new technique [27], yet to be adapted by the CLP program. Since its inception, many researchers have published reviews and experimental results for a variety of complex samples using GC×GC -ECD or GC×GC−µECD, and more recently GC×GC-TOFMS [8, 20, 21, 24, 28, 29], due to the complex nature of a single run in the 2D chromatographic separation method, their studies

Table 1: Polychlorinated biphenyl Congener classes with identification ions.

| PCB Congener Classes | Numbers of PCB isomers per congener class | Molecular Formula | Identification ions M+ | (M-Cl)+ | (M-2Cl)+ |
|----------------------|-------------------------------------------|------------------|------------------------|---------|----------|
| Monochlorobiphenyl   | 3                                         | C_{12}H_{9}Cl     | 188                    | 153     | —        |
| Dichlorobiphenyl     | 12                                        | C_{12}H_{8}Cl_{2} | 222                    | 187     | 152      |
| Trichlorobiphenyl    | 24                                        | C_{12}H_{7}Cl_{3} | 256                    | 221     | 186      |
| Tetrachlorobiphenyl  | 42                                        | C_{12}H_{6}Cl_{4} | 290 (292)*             | 255     | 220      |
| Pentachlorobiphenyl  | 46                                        | C_{12}H_{5}Cl_{5} | 324 (326)              | 289 (291)* | 254 |
| Hexachlorobiphenyl   | 42                                        | C_{12}H_{4}Cl_{6} | 358 (360)              | 323 (325) | 288 (290)* |
| Heptachlorobiphenyl  | 24                                        | C_{12}H_{3}Cl_{7} | 392 (394)              | 357 (359) | 322 (324) |
| Octachlorobiphenyl   | 12                                        | C_{12}H_{2}Cl_{8} | 426 (430)              | 391 (393) | 356 (358) |
| Nonachlorobiphenyl   | 3                                         | C_{12}HCl_{9}     | 460 (464)              | 425 (429) | 390 (392) |
| Decachlorobiphenyl   | 1                                         | C_{12}Cl_{10}     | 494 (498)              | 459 (463) | 424 (428) |

*Most abundant ion of the isotope cluster is in parentheses, if not first member.
Table 2: Polychlorinated biphenyl congener number, IUPAC names, CAS numbers, and retention times in increasing order, using sequential 40 m Rtx-PCB and 1 m DB-17 GC columns (1D Mode).

| Congener no. | IUPAC name                  | CASRN      | Retention time (s) |
|--------------|-----------------------------|------------|--------------------|
| 1            | 2-chlorobiphenyl            | 2051-60-7  | 1395.45            |
| 2            | 3-chlorobiphenyl            | 2051-61-8  | 1705.70            |
| 3            | 4-chlorobiphenyl            | 2051-62-9  | 1777.55            |
| 4            | 2,2′-dichlorobiphenyl       | 13029-08-8 | 1838.05            |
| 10           | 2,5-dichlorobiphenyl        | 34883-39-1 | 2090.25            |
| 7            | 2,4-dichlorobiphenyl        | 33284-50-3 | 2103.35            |
| 6            | 2,3′-dichlorobiphenyl       | 25569-80-6 | 2171.75            |
| 5            | 2,3-dichlorobiphenyl        | 16605-91-7 | 2231.25            |
| 8            | 2,4′-dichlorobiphenyl       | 34883-43-7 | 2261.70            |
| 19           | 2,2′,6-trichlorobiphenyl    | 38444-73-4 | 2381.25            |
| 14           | 3,5-dichlorobiphenyl        | 34883-41-5 | 2413.30            |
| 30           | 2,4,6-trichlorobiphenyl     | 35693-92-6 | 2476.60            |
| 18           | 2,2′,5-trichlorobiphenyl    | 37680-65-2 | 2613.05            |
| 11           | 3,3′-dichlorobiphenyl       | 2050-67-1  | 2628.35            |
| 17           | 2,2′,4-trichlorobiphenyl    | 37680-66-3 | 2640.00            |
| 12           | 3,4-dichlorobiphenyl        | 2974-92-7  | 2683.85            |
| 27           | 2,3′,6-trichlorobiphenyl    | 38444-76-7 | 2699.35            |
| 24           | 2,3,6-trichlorobiphenyl     | 55702-45-9 | 2735.45            |
| 13           | 3,4′-dichlorobiphenyl       | 2974-90-5  | 2737.55            |
| 16           | 2,2′,3-trichlorobiphenyl    | 38444-78-9 | 2789.25            |
| 32           | 2,4′,6-trichlorobiphenyl    | 38444-77-4 | 2826.00            |
| 15           | 4,4′-dichlorobiphenyl       | 2050-68-2  | 2854.10            |
| 34           | 2,3′,5′-trichlorobiphenyl   | 37680-68-5 | 2903.00            |
| 54           | 2,2′,6,6′-tetrachlorobiphenyl | 15968-05-5 | 2910.50            |
| 23           | 2,3,5-trichlorobiphenyl     | 55720-44-0 | 2926.35            |
| 29           | 2,4,5-trichlorobiphenyl     | 15862-07-4 | 2961.45            |
| 50           | 2,2′,4,6-tetrachlorobiphenyl| 62796-65-0 | 3041.90            |
| 26           | 2,3′,5-trichlorobiphenyl    | 38444-81-4 | 3058.20            |
| 25           | 2,3′,4-trichlorobiphenyl    | 55712-37-3 | 3086.65            |
| 53           | 2,2′,5,6′-tetrachlorobiphenyl| 41464-41-9 | 3164.85            |
| 31           | 2,4′,5-trichlorobiphenyl    | 16606-02-3 | 3181.00            |
| 28           | 2,4,4′-trichlorobiphenyl    | 7012-37-5  | 3210.95            |
| 33           | 2,3′,4′-trichlorobiphenyl   | 38444-86-9 | 3217.00            |
| 21           | 2,3,4-trichlorobiphenyl     | 55702-46-0 | 3218.20            |
| 51           | 2,2′,4,6′-tetrachlorobiphenyl| 68194-04-7 | 3226.80            |
| 20           | 2,3′,3′-trichlorobiphenyl   | 38444-84-7 | 3227.75            |
| 45           | 2,2′,3,6-tetrachlorobiphenyl| 70362-45-7 | 3312.35            |
| 22           | 2,3′,4′-trichlorobiphenyl   | 38444-85-8 | 3353.85            |
| 46           | 2,2′,3,6′-tetrachlorobiphenyl| 41464-47-5 | 3372.70            |
| 73           | 2,3′,5′,6-tetrachlorobiphenyl| 74338-23-1 | 3395.50            |
| 69           | 2,3′,4,6-tetrachlorobiphenyl| 60233-24-1 | 3440.00            |
| 43           | 2,2′,3,5-tetrachlorobiphenyl| 70362-46-8 | 3450.95            |
| 36           | 3,3′,5′-trichlorobiphenyl   | 38444-87-0 | 3463.00            |
| 52           | 2,2′,5,5′-tetrachlorobiphenyl| 35693-99-3 | 3485.35            |
| 48           | 2,2′,4,5-tetrachlorobiphenyl| 70362-47-9 | 3498.60            |
| Congener no. | IUPAC name                        | CASRN       | Retention time (s) |
|-------------|-----------------------------------|-------------|--------------------|
| 49          | 2,2′,4,5′-tetrachlorobiphenyl     | 41464-40-8  | 3531.35            |
| 104         | 2,2′,4,6,6′-pentachlorobiphenyl   | 56558-16-8  | 3550.00            |
| 65          | 2,3,5,6-tetrachlorobiphenyl      | 33284-54-7  | 3570.50            |
| 62          | 2,3,4,6-tetrachlorobiphenyl      | 54230-22-7  | 3574.50            |
| 47          | 2,2′,4,4′-tetrachlorobiphenyl    | 2437-79-8   | 3574.95            |
| 75          | 2,4,4′,6-tetrachlorobiphenyl     | 32598-12-2  | 3592.50            |
| 39          | 3,4,5-trichlorobiphenyl         | 38444-88-1  | 3599.50            |
| 38          | 3,4,5-trichlorobiphenyl         | 53555-66-1  | 3602.50            |
| 44          | 2,2′,3,5′-tetrachlorobiphenyl    | 41464-39-5  | 3678.75            |
| 59          | 2,3,3′,6-tetrachlorobiphenyl    | 74472-33-6  | 3714.60            |
| 42          | 2,2′,3,4′-tetrachlorobiphenyl    | 36559-22-5  | 3726.90            |
| 71          | 2,3′,4,6-tetrachlorobiphenyl    | 32690-93-1  | 3791.60            |
| 35          | 2,2′,3,4-tetrachlorobiphenyl    | 52663-59-9  | 3880.25            |
| 64          | 2,3,4′,6-tetrachlorobiphenyl    | 52663-58-8  | 3872.50            |
| 40          | 2,3,3′,5′-tetrachlorobiphenyl   | 73575-52-7  | 3893.50            |
| 68          | 2,3′,4,5′-tetrachlorobiphenyl   | 73575-54-9  | 3923.00            |
| 37          | 2,3′,5,5′-tetrachlorobiphenyl   | 39485-83-1  | 3939.00            |
| 100         | 2,2′,4,4′,6,6′-hexachlorobiphenyl| 33979-03-2  | 3939.00            |
| 94          | 2,2′,3,5,6-pentachlorobiphenyl  | 73575-55-0  | 3952.25            |
| 57          | 2,3,3′,5-tetrachlorobiphenyl     | 70424-67-8  | 3974.00            |
| 67          | 2,3′,4,5-tetrachlorobiphenyl   | 73557-53-8  | 4025.50            |
| 58          | 2,3,3′,5′-pentachlorobiphenyl   | 41464-49-7  | 4026.00            |
| 102         | 2,2′,4,5,6′-pentachlorobiphenyl | 68194-06-9  | 4030.00            |
| 61          | 2,2′,4,5-tetrachlorobiphenyl    | 33284-53-6  | 4061.50            |
| 98          | 2,2′,3,4′,5′-pentachlorobiphenyl| 60233-25-2  | 4077.00            |
| 93          | 2,2′,4,5,5′-pentachlorobiphenyl | 60145-21-3  | 4086.50            |
| 76          | 2,2′,3,4,5′-pentachlorobiphenyl | 70362-48-0  | 4100.00            |
| 63          | 2,2′,3,5,4′-pentachlorobiphenyl | 74472-34-7  | 4124.50            |
| 88          | 2,2′,3,4,6-pentachlorobiphenyl  | 55215-17-3  | 4127.20            |
| 95          | 2,2′,3,5,6′-pentachlorobiphenyl | 38379-99-6  | 4136.40            |
| 121         | 2,2′,3,5,6′-pentachlorobiphenyl | 56558-18-0  | 4155.00            |
| 74          | 2,2′,3,3′-pentachlorobiphenyl   | 32690-93-0  | 4168.50            |
| 155         | 2,2′,4,4′,6,6′-pentachlorobiphenyl| 33979-03-2  | 4194.50            |
| 70          | 2,2′,3,4,5-tetrachlorobiphenyl  | 32598-11-1  | 4199.50            |
| 91          | 2,2′,3,4,6-pentachlorobiphenyl  | 68194-05-8  | 4216.70            |
| 66          | 2,2′,3,4′-pentachlorobiphenyl   | 32598-10-0  | 4240.50            |
| 55          | 2,2′,3,4,5-tetrachlorobiphenyl  | 74338-24-2  | 4304.00            |
| 80          | 2,3′,4,5′-pentachlorobiphenyl   | 33284-52-5  | 4312.50            |
| 92          | 2,2′,3,5,5′-pentachlorobiphenyl | 52663-61-3  | 4342.75            |
| 89          | 2,2′,3,4,6-pentachlorobiphenyl  | 73575-57-2  | 4352.60            |
| 84          | 2,2′,3,3′,6′-pentachlorobiphenyl| 52663-60-2  | 4360.40            |
| 56          | 2,3,3′,4′-pentachlorobiphenyl   | 41464-43-1  | 4378.00            |
| 90          | 2,2′,3,4,5-pentachlorobiphenyl  | 68194-07-0  | 4404.40            |
| 101         | 2,2′,4,5,5′-pentachlorobiphenyl | 37680-73-2  | 4407.40            |
| 113         | 2,2′,3,5,5′-pentachlorobiphenyl | 68194-10-5  | 4421.00            |
| 60          | 2,3,4,4′-pentachlorobiphenyl    | 33025-41-1  | 4446.50            |
| Congener no. | IUPAC name | CASRN | Retention time (s) |
|-------------|------------|-------|-------------------|
| 150         | 2,2′,3,4′,6,6′-hexachlorobiphenyl | 68194-08-1 | 4457.00 |
| 99          | 2,2′,4,4′-pentachlorobiphenyl | 38380-01-7 | 4460.00 |
| 152         | 2,2′,3,5,5,6′-hexachlorobiphenyl | 68194-09-2 | 4507.00 |
| 119         | 2,3′,4,4′-pentachlorobiphenyl | 56558-17-9 | 4543.00 |
| 83          | 2,2′,3,3′,5,5′-pentachlorobiphenyl | 60145-20-2 | 4546.00 |
| 125         | 2,3′,4,5,5′-pentachlorobiphenyl | 74472-39-2 | 4558.00 |
| 86          | 2,2′,3,4,5-pentachlorobiphenyl | 55312-69-1 | 4560.15 |
| 112         | 2,3,5,6-pentachlorobiphenyl | 74472-36-9 | 4562.50 |
| 145         | 2,2′,3,4,6,6′-hexachlorobiphenyl | 74472-40-5 | 4578.00 |
| 108         | 2,3′,4,6-pentachlorobiphenyl | 74472-35-8 | 4584.50 |
| 97          | 2,2′,3,3′,4,4′-pentachlorobiphenyl | 41464-51-1 | 4603.00 |
| 119         | 2,2′,3,3′,5,6-pentachlorobiphenyl | 74472-41-6 | 4644.00 |
| 116         | 2,3,5,6-pentachlorobiphenyl | 18259-05-7 | 4653.00 |
| 79          | 2,3′,4,5′-tetrachlorobiphenyl | 41464-48-6 | 4654.50 |
| 87          | 2,3′,4,5′-pentachlorobiphenyl | 38380-02-8 | 4712.25 |
| 78          | 3,3′,4,5-tetrachlorobiphenyl | 70362-49-1 | 4720.50 |
| 136         | 2,2′,3,3′,6,6′-hexachlorobiphenyl | 38411-22-2 | 4722.90 |
| 154         | 2,2′,4,4′,5,6′-hexachlorobiphenyl | 60145-22-4 | 4731.50 |
| 117         | 2,3,5,6-pentachlorobiphenyl | 68194-11-6 | 4744.50 |
| 115         | 2,3,4,4′-pentachlorobiphenyl | 74472-38-1 | 4752.00 |
| 111         | 2,3′,4,5′-pentachlorobiphenyl | 39635-32-0 | 4757.00 |
| 85          | 2,2′,3,4,4′-pentachlorobiphenyl | 65510-45-4 | 4767.15 |
| 110         | 2,3′,5,5′-pentachlorobiphenyl | 38380-03-9 | 4818.50 |
| 120         | 2,3′,4,5,5′-pentachlorobiphenyl | 68194-12-7 | 4822.50 |
| 81          | 3,4,4′,5-tetrachlorobiphenyl | 70362-50-4 | 4833.50 |
| 151         | 2,2′,3,5,5′,6-hexachlorobiphenyl | 52663-63-5 | 4893.50 |
| 135         | 2,2′,3,3′,5,6′-hexachlorobiphenyl | 52744-13-5 | 4913.95 |
| 82          | 2,2′,3,3′,4-pentachlorobiphenyl | 52663-62-4 | 4915.25 |
| 144         | 2,2′,3,4,5′,6-hexachlorobiphenyl | 68194-14-9 | 4946.00 |
| 147         | 2,2′,3,4,5,6-hexachlorobiphenyl | 68194-13-8 | 4990.60 |
| 77          | 3,3′,4,4′-tetrachlorobiphenyl | 32598-13-3 | 4998.50 |
| 149         | 2,2′,3,4,5′,6-hexachlorobiphenyl | 38380-04-0 | 4999.50 |
| 143         | 2,2′,3,4,5,6′-hexachlorobiphenyl | 68194-15-0 | 5018.00 |
| 139         | 2,2′,3,4,4′,6-hexachlorobiphenyl | 56030-56-9 | 5030.85 |
| 140         | 2,2′,3,4,4′-hexachlorobiphenyl | 59291-64-4 | 5057.35 |
| 124         | 2,3′,4,5′-pentachlorobiphenyl | 70362-41-3 | 5076.30 |
| 107         | 2,3′,4,5′-pentachlorobiphenyl | 65510-44-3 | 5118.00 |
| 123         | 2,3′,4,5′-pentachlorobiphenyl | 74472-48-3 | 5202.50 |
| 188         | 2,2′,3,4,5,6,6′-heptachlorobiphenyl | 74472-46-1 | 5233.50 |
| 122         | 2,3′,4,5′-pentachlorobiphenyl | 76842-07-4 | 5246.50 |
| Congener no. | IUPAC name                        | CASRN        | Retention time (s) |
|-------------|-----------------------------------|--------------|-------------------|
| 146         | $2,2',3,4,5,5'$-hexachlorobiphenyl | 51908-16-8   | 5249.00           |
| 161         | $2,3',4,5',6$-hexachlorobiphenyl  | 74474-43-8   | 5275.50           |
| 114         | $2,3,4,5$-pentachlorobiphenyl     | 74472-37-0   | 5289.50           |
| 168         | $2,3',4,5',6$-hexachlorobiphenyl  | 59291-65-5   | 5310.50           |
| 153         | $2,2',4,5,5'$-hexachlorobiphenyl  | 35065-27-1   | 5324.00           |
| 132         | $2,2',3,3',4,6$-hexachlorobiphenyl| 38380-05-1   | 5333.85           |
| 179         | $2,2',3,3',5,6,6'$-heptachlorobiphenyl | 52663-64-6 | 5383.30           |
| 141         | $2,2',3,4,5,5'$-hexachlorobiphenyl| 52712-04-6   | 5448.05           |
| 176         | $2,2',3,4,6,6'$-heptachlorobiphenyl| 52663-65-7 | 5463.10           |
| 105         | $2,3,3',4,4$-pentachlorobiphenyl  | 32598-14-4   | 5469.00           |
| 186         | $2,2',3,4,5,6,6'$-heptachlorobiphenyl | 74472-49-4 | 5500.00           |
| 137         | $2,2',3,4,5,5'$-hexachlorobiphenyl| 35694-06-5   | 5511.10           |
| 127         | $3,3',4,5,5'$-pentachlorobiphenyl | 39635-33-1   | 5552.00           |
| 130         | $2,2',3,3',4,5$-hexachlorobiphenyl| 52663-66-8   | 5566.30           |
| 164         | $2,3,3',4,5'$-6-hexachlorobiphenyl| 74472-45-0   | 5574.00           |
| 138         | $2,2',3,4,5,5'$-hexachlorobiphenyl| 35065-28-2   | 5627.80           |
| 178         | $2,2',3,3',5,5'$-6-heptachlorobiphenyl | 52663-67-9 | 5632.50           |
| 160         | $2,3,3',4,5,6$-hexachlorobiphenyl | 41411-62-5   | 5637.00           |
| 163         | $2,3,3',4,5$-hexachlorobiphenyl   | 74472-44-9   | 5648.00           |
| 129         | $2,2',3,3',4,5$-hexachlorobiphenyl| 55215-18-4   | 5648.45           |
| 158         | $2,3,3',4,4',6$-hexachlorobiphenyl| 74472-42-7   | 5677.00           |
| 182         | $2,2',3,4,4',5,6'$-heptachlorobiphenyl | 60145-23-5 | 5690.05           |
| 175         | $2,2',3,3',4,5'$-6-heptachlorobiphenyl | 40186-70-7 | 5695.20           |
| 187         | $2,2',3,4,5,5'$-6-heptachlorobiphenyl | 52663-68-0 | 5725.00           |
| 183         | $2,2',3,4,5,5'$-6-heptachlorobiphenyl | 52663-69-1 | 5787.85           |
| 166         | $2,3,4,4',5,6$-hexachlorobiphenyl | 41411-63-6   | 5818.00           |
| 159         | $2,3,3',4,5,5'$-hexachlorobiphenyl| 39635-35-3   | 5893.00           |
| 126         | $3,3',4,4',5$-pentachlorobiphenyl | 57465-28-8   | 5906.50           |
| 185         | $2,2',3,4,5,5',6$-heptachlorobiphenyl | 52712-05-7 | 5915.00           |
| 128         | $2,2',3,3',4,4'$-hexachlorobiphenyl| 38380-07-3   | 5937.80           |
| 162         | $2,3,3',4,5,5'$-hexachlorobiphenyl| 39635-34-2   | 5951.00           |
| 174         | $2,2',3,3',4,5,6'$-heptachlorobiphenyl | 38411-25-5 | 5953.45           |
| 202         | $2,2',3,3',5,5',6,6'$-octachlorobiphenyl | 2136-99-4 | 5998.55           |
| 181         | $2,2',3,4,4',5,6$-heptachlorobiphenyl | 74472-47-2 | 6009.70           |
| 167         | $2,3,4,4',5,5'$-hexachlorobiphenyl| 52663-72-6   | 6023.00           |
| 177         | $2,2',3,3',4,5,6'$-heptachlorobiphenyl | 52663-70-4 | 6069.60           |
| 201         | $2,2',3,3',4,5,6,6'$-octachlorobiphenyl | 52663-73-7 | 6087.50           |
| 204         | $2,2',3,4,4',5,6,6'$-octachlorobiphenyl | 74472-52-9 | 6087.60           |
| 171         | $2,2',3,3',4,4',6$-heptachlorobiphenyl | 52663-71-5 | 6117.90           |
| 173         | $2,2',3,3',4,5,6$-heptachlorobiphenyl | 68194-16-1 | 6139.75           |
| 197         | $2,2',3,3',4,4',6,6'$-octachlorobiphenyl | 33091-17-7 | 6174.95           |
| 172         | $2,2',3,3',4,5,5'$-heptachlorobiphenyl | 52663-74-8 | 6252.10           |
| 156         | $2,3,3',4,4',5$-hexachlorobiphenyl | 38380-08-4   | 6267.00           |
| 192         | $2,3,3',4,5,5'$-6-heptachlorobiphenyl | 74472-51-8 | 6281.00           |
| 157         | $2,3,3',4,4',5'$-hexachlorobiphenyl | 69782-90-7  | 6304.50           |
| 180         | $2,2',3,4,4',5,5'$-heptachlorobiphenyl | 35065-29-3 | 6330.60           |
| 200         | $2,2',3,5',4,5,6,6'$-octachlorobiphenyl | 52663-75-9 | 6341.45           |
| 193         | $2,3,3',4,5,5'$-6-heptachlorobiphenyl | 69782-91-8  | 6348.50           |
have not sufficiently resulted in the transfer of the 2D-GC technology to the commercial industry.

2. Experimental

The GC × GC-TOFMS instrument used for this experiment was the liquid nitrogen quad-jet modulator, with two nozzles for heating and two for cooling, Pegasus 4D (Leco Corp., St Joseph, MI, USA). The system is equipped with a secondary oven, and a 6890 series auto-injector. Liquid nitrogen and breathable air are the sources of the cold and hot jets, respectively. The detector is a time-of-flight mass analyzer with a setup, which can be described as postacceleration, where all ions leave the drift region with approximately 900 eV kinetic energy, and then accelerate to 2000 eV or higher before striking the microchannel plate surface. The first column used in this study was a nonpolar phase Rtx-1R (proprietary phase that separated compounds based upon their volatility, that is, with retention times increasing with compound boiling points [30]. A nonpolar phase GC column was connected with a press-fit connector (Varian universal quick seal, Varian-Chrompack, Palo Alto, CA, USA), of 1 meter length, 0.10 mm ID, and 0.10 µm film thickness.

3. Materials and Method

Two hundred and nine certified standard solutions of individual chlorinated biphenyls (International Union of Pure and Applied Chemistry (IUPAC) # 1 through 209) in isooctane were used for this study (see Table 2) and were purchased from AccuStandard, Inc., (New Haven, CT, USA). Each isooctane solution contained 100 ng/µL of an individual chlorinated biphenyl congener and was diluted 5-fold with 99.9% n-hexane (B&J GC2 grade, Burdick and Jackson, Muskegon, MI, USA) to produce 20 ng/µL. To compensate for the observed decreasing signals of the more highly chlorinated congeners, the concentrations of the injected congener solutions were adjusted to between 0.20 and 4.25 ng/µL. The concentration adjustments yielded approximately equal signal intensities upon analysis by GC × GC-TOFMS.

In GC × GC-TOFMS, the injected sample was transferred from the first column into the second column, aided by means of a thermal-based modulator. The GC × GC column configurations as used here consisted of a long first dimension (1D) column, at 40 m in length and containing a proprietary phase that separated compounds based upon their volatility, that is, with retention times increasing with compound boiling points [30]. A nonpolar phase GC column was connected with a press-fit connector (Varian universal quick seal, Varian-Chrompack, Palo Alto, CA, USA) serially to a second, but shorter (about 1 m), polar phase GC column. After several temperature program cycles, a DB-17 GC column was determined to be optimal for the PCB separations due to its thermal stability (280 °C) at the GC × GC-TOFMS interface.

The modulator period was 4 s with the hot-pulse duration set at 1200 ms and the cool time between modulation stages set at 800 ms. When a 2D run was begun, the modulator operated throughout the entire analysis and performed uninterrupted trapping and releasing of the effluent from the primary column. The GC × GC-TOFMS system may also be operated in the 1D mode by deactivating the modulator operated throughout the entire analysis and performed uninterrupted trapping and releasing of the effluent from the primary column. The GC × GC-TOFMS system may also be operated in the 1D mode by deactivating the modulator operated throughout the entire analysis and performed uninterrupted trapping and releasing of the effluent from the primary column. The GC × GC-TOFMS system may also be operated in the 1D mode by deactivating the modulator operated throughout the entire analysis and performed uninterrupted trapping and releasing of the effluent from the primary column. The GC × GC-TOFMS system may also be operated in the 1D mode by deactivating the modulator operated throughout the entire analysis and performed uninterrupted trapping and releasing of the effluent from the primary column.  

| Congener no. | IUPAC name | CASRN | Retention time (s) |
|-------------|------------|-------|--------------------|
| 191         | 2,3,3′,4,4′,5,5′-heptachlorobiphenyl | 74472-50-7 | 6399.00 |
| 198         | 2,2′,3,3′,4,5,5′-hexachlorobiphenyl | 68194-17-2 | 6610.75 |
| 199         | 2,2′,3,3′,4,4′,5,6′-heptachlorobiphenyl | 40186-71-8 | 6624.80 |
| 170         | 2,2′,3,3′,4,4′,5,6′-heptachlorobiphenyl | 35065-30-6 | 6626.15 |
| 190         | 2,3,3′,4,4′,5,6′-heptachlorobiphenyl | 41411-64-7 | 6670.50 |
| 196         | 2,2′,3,3′,4,4′,5,6′-octachlorobiphenyl | 42740-50-1 | 6682.60 |
| 203         | 2,2′,3,4,4′,5,5′-octachlorobiphenyl | 52663-76-0 | 6691.25 |
| 169         | 3,3′,4,4′,5,5′-hexachlorobiphenyl | 32774-16-6 | 6724.00 |
| 208         | 2,2′,3,3′,4,5,5′,6′-nonachlorobiphenyl | 52663-77-1 | 6835.70 |
| 207         | 2,2′,3,3′,4,4′,5,6′-nonachlorobiphenyl | 52663-79-3 | 6893.05 |
| 205         | 2,2′,3,3′,4,4′,5,5′-octachlorobiphenyl | 52663-78-2 | 6915.85 |
| 189         | 2,3,3′,4,4′,5,5′-heptachlorobiphenyl | 39635-31-9 | 6917.20 |
| 194         | 2,2′,3,3′,4,4′,5,5′-heptachlorobiphenyl | 35694-08-7 | 7071.45 |
| 205         | 2,3,3′,4,4′,5,5′-octachlorobiphenyl | 74472-53-0 | 7121.15 |
| 206         | 2,2′,3,3′,4,4′,5,5′-octachlorobiphenyl | 40186-72-9 | 7288.20 |
| 209         | decachlorobiphenyl | 2051-24-3 | 7471.40 |
and deconvoluted by commercially available software (ChromaTOF), that came with the instrument, which generated contour plots, as well as three-dimensional chromatograms, based upon the user defined modulation period. The TOFMS detector was utilized because the fast chromatographic technique that generated multiple narrow peaks from the short secondary column required a fast scanning detector, capable of producing sufficient data points to accurately define a chromatographic peak, and to deconvolute nearly overlapping peaks. The first GC run used both columns in the GC configured in a one-dimensional mode of separation (with the modulator turned off) to define where more separation using the 2D mode of separation (with the modulator turned on) was needed. The second GC run was configured in a two-dimensional mode of separation, to distinguish some of the most difficult to separate coeluting PCB congeners. Successful separation and measurement of specific PCB congeners held potential for significant improvements in estimating environmental exposure to PCB congeners and toxicological evaluations of PCB congeners in biological tissues.

4. GC × GC-TOFMS Analysis of Chlorinated Biphenyl Congeners

The GC × GC-TOFMS chromatographic conditions were optimized as follows: initial primary oven temperature containing the first column was set at 70°C held for 0.5 min, ramped at 10°C/min to 150°C, then at 1°C/min to 250°C, followed by a 4°C/min ramp to 275°C and held for 15 min. The second-dimension column in the secondary oven ran with a 15°C offset to the primary oven temperature. Using ultra pure helium as carrier gas, the target flow rate was set at a corrected constant flow of 1.20 mL/min. The inlet temperature was set at 280°C for splitless injection of 1 µL. The conditions above allowed for the early elution of volatile compounds, which would eliminate them as potential background interferences. Instrument control (data acquisition) and data processing (postacquisition) were conducted with commercially available software—the integrated Leco ChromaTOF. Mass spectrometric conditions were set as follows: the filament was turned on at 1100s into the run, until the end. The mass range was 45 to 550 amu, mass acquisition rate at 3 spectra/s (1D mode), and 100 spectra/s (2D mode), detector voltage at 1.65 KV, electron energy at 70 V, transfer line temperature at 280°C, ion source temperature set at 200°C.

5. Relative Retention Time and Mass Spectral Library for all 209 PCB Congeners

In this research, one PCB congener was taken from each homologue group to create a solution. Exactly 46 solutions were created to bracket all 46 pentachlorobiphenyl isomers. The first solution contained 10 congeners, the second and third contained 9 each, the fourth through twelfth contained 7 each, and so forth. For the purpose of general matching between congener classes, a mass spectral library of 209 PCB individual congeners was created using 46 different solutions, containing unique groups of the PCB congeners. The design was such that no two isomeric PCBs were included in any of the 46 solutions. All 46 solutions were individually analyzed (46 chromatographic runs) using the 1D mode (thermal modulation deactivated) because most of the congeners (188) were separated by the 1D mode. The instrument conditions for the 1D mode of separation were the same as previously described above, except that the thermal modulator was not activated. The PCB standard used in this research was constructed as follows: (Congener number range, chlorine number, concentration in ng/µL; PCBs 1–3 (1Cl), 0.20; PCBs 4–15 (2Cl), 0.40; PCBs 16–39 (3Cl), 0.85; PCB 40 (4Cl), 1.25; PCBs 41–81 (4Cl), 1.50; PCB 82 (5Cl), 1.50; PCBs 83–127 (5Cl), 2.00; PCBs 128–169 (6Cl), 3.00; PCB 170 (7Cl), 3.40; PCBs 171–193 (7Cl), 4.00; PCB 194 (8Cl), 3.25; PCBs 195–196 (8Cl), 4.05; PCBs 197–205 (8Cl), 4.25; PCB 206 (9Cl), 3.25; PCBs 207–208 (9Cl), 4.00; and PCB 209 (10Cl), 3.00). The first solution contained the following ten congeners of IUPAC numbers: 1, 4, 16, 40, 82, 128, 170, 194, 206, and 209, using individual concentration as shown above. The second solution contained nine congeners of IUPAC numbers: 2, 5, 17, 41, 83, 129, 171, 195, and 207. One congener was taken from each of the ten chlorination levels or homologue groups each time until 46 different solutions of congeners were achieved. Each congener was chromatographically separated in each of the mixtures, and this enabled the identification of every member of the group by molecular weight and chlorine isotope cluster. As each run was completed, the file was manually transferred to a dedicated workstation computer, for postacquisition data processing, while the instrument-dedicated computer system continued to acquire raw data. In this way, using the same chromatographic conditions, several runs were made each day, and retention times and mass spectra were archived for all of the congeners. Some of the data acquired with 50 scans/s in the 1D chromatographic mode were resampled using the default data resampling function of the instrument to smooth the data.

During a preliminary chromatographic run of the first solution containing mono through decachlorobiphenyl congeners, in the 1D mode, it was noticed that there was a sharp decrease in peak signal or intensity from the early eluting PCB congeners to the later eluting ones, with decachlorobiphenyl's ion chromatogram being extremely small. Due to the fact that this TOFMS is equipped with a postacceleration system, we therefore attributed this phenomenon to the decreasing molar concentration for a given weight of sample, as the molecular weight increased, and the larger number of fragments of the more highly chlorinated PCB congeners, resulting in more ions with low intensity. It thus became necessary to adjust the concentration of each component within the ten chlorination levels or first solution of ten PCB congeners. This achieved approximately equivalent signals for each component by increasing the concentrations of the later, higher molecular weight eluters. Also, to raise the sensitivity level for the purpose of detecting higher masses, auto-tuning was performed using m/z 414 from perfluorotributylamine (PFTBA) instead of the default m/z 69.
6. Analysis of the 209 Polychlorinated Biphenyl Mixture in the 2D Mode

A combined solution of 9 ampoules containing a mixture of all 209 PCB congeners at adjusted concentration of 6 ng/µL (AccuStandard, New Haven, CT, USA), was analyzed using two different separation modes—1D and 2D chromatography.

Experimental conditions were the same as previously described above. The versatility of the 2D chromatographic mode in this research was of major importance because it was used to separate additional pentachlorobiphenyls to the 188 congeners that were distinguished using the 1D chromatographic mode. It may be optimized and used for the separation of those congeners previously or partially separated in the 1D chromatographic mode for isomers with great concentration differentials.

7. Results and Discussion

The chemical identity of all PCB congeners rested primarily on the accuracy of the individual labeled ampoules supplied by AccuStandard Inc. (New Haven, CT, USA), for the creation of our mass spectral library. However, all individual PCB congeners were also verified by mass spectrometry as providing ions consistent with Table 1 and as having the correct chlorine isotope clusters. Only 140 of the PCB congeners were available in the National Institute of Standards and Technology (NIST ’05) mass spectral library [34]. The information recorded from the acquired full-scan data was used to create a new mass spectral library for all 209 PCB congeners. The intensities of key ions from this newly created mass spectral library were compared with those from the mass spectra available in the NIST ’05 library. This 209 congener library provided an opportunity to include and test another parameter relating chemical structures and the mass spectra, with the potential to help distinguish isomers. This was the mass spectrometric “ortho effect” observed for chlorinated biphenyls having 2,2′-; and 2,2′,6,6′-chlorine substitution, and to a much lesser extent, 2,2′,6,4′-substitution [35–38]. This effect showed itself as an increase, for the above ortho substituted isomers and congeners, of the chlorine cluster resulting from the loss of the first chlorine (M-Cl)+ relative to the chlorine cluster containing the molecular ion, M*. PCBs having 2,6-di-ortho substitution on the same ring, or no ortho substitution at all, showed quite different spectra, with no or very small losses of the first chlorine. All standards were therefore also checked for consistency of the structure with the presence or absence of the expected “ortho-effect,” and with the spectra present in the NIST ’05 Library. As a result of this cross-checking, it was also observed that congener IUPAC number 99 (2,2′,4,4′-pentachlorobiphenyl) yielded an “ortho effect” consistent with 2,2′-chlorine substitution for our standard (83 +119), whereas the value in the NIST library (110 + 120), was inconsistent with this assignment. The remaining two penta PCBs with IUPAC numbers 90 and 101 could not be separated with the 2D mode. Because both of these penta isomers had 2,2′-ortho substitution, their respective “ortho effects” were also too similar to allow them to be distinguished. However, the 2D separation mode distinguished the tetra isomers 55 and 80. Difficulty was encountered in the separation of a pair of dichlorobiphenyls.

Figure 1: Surface view showing the use of 2D in the separation of four pairs of polychlorinated biphenyl isomers with IUPAC numbers beginning from the right of (110 + 120), (115 + 111), (86 + 112), and (119 + 83). The PCB isomers (left pair) numbers (90 + 101) were inseparable under the chromatographic conditions used. Both axes are in seconds.
| Elution Order | IUPAC # | Structure Ring1 : Ring 2 | RT sec<sup>a</sup> | “Ortho Effect” %<sup>b</sup> |
|---------------|---------|--------------------------|-----------------|------------------|
| 1             | 104     | 246 : 26                 | 3550            | 10               |
| 2             | 96      | 236 : 26                 | 3814            | 13 h             |
| 3             | 103     | 246 : 25                 | 3864            | 38 h             |
| 4             | 100     | 246 : 24                 | 3939            | 31               |
| 5             | 94      | 235 : 26                 | 3952            | 50               |
| 6             | 102     | 245 : 26                 | 4030            | 36               |
| 7             | 98      | 246 : 23                 | 4077            | 51               |
| 8             | 93      | 2356 : 2                 | 4093            | 59               |
| 9             | 88      | 2346 : 2                 | 4127            | 48               |
| 10            | 95      | 236 : 25                 | 4136            | 46 h             |
| 11            | 121     | 246 : 35                 | 4155            | 6 h              |
| 12            | 91      | 236 : 24                 | 4217            | 42 h             |
| 13            | 92      | 235 : 25                 | 4343            | 49               |
| 14            | 89      | 234 : 26                 | 4353            | 51               |
| 15            | 84      | 236 : 23                 | 4360            | 63               |
| 16            | 90      | 235 : 24                 | 4404 c          | 42               |
| 17            | 101     | 245 : 25                 | 4407 c          | 29               |
| 18            | 113     | 236 : 35                 | 4421            | 7 h              |
| 19            | 99      | 245 : 24                 | 4460            | 20 h             |
| 20            | 119     | 246 : 34                 | 4543 d          | 3 h              |
| 21            | 83      | 235 : 23                 | 4546 d          | 69 h             |
| 22            | 125     | 345 : 26                 | 4558 e          | 7 h              |
| 23            | 86      | 2345 : 2                 | 4560 e          | 44 h             |
| 24            | 112     | 2356 : 3                 | 4562 e          | 6 h              |
| 25            | 108     | 2346 : 3                 | 4584            | 4                |
| 26            | 97      | 245 : 25                 | 4603            | 38 h             |
| 27            | 116     | 23456                   | 4653            | 6 h              |
| 28            | 87      | 234 : 25                 | 4712            | 41 h             |
| 29            | 117     | 2356 : 4                 | 4744            | 4 h              |
| 30            | 115     | 2346 : 4                 | 4752 f          | 3                |
| 31            | 111     | 235 : 35                 | 4757 f          | 3                |
| 32            | 85      | 234 : 24                 | 4767            | 33 h             |
| 33            | 110     | 236 : 34                 | 4818 g          | 3 h              |
| 34            | 120     | 245 : 35                 | 4822 g          | 3                |
| 35            | 82      | 234 : 23                 | 4915            | 54 h             |
| 36            | 124     | 345 : 25                 | 5068            | 5 h              |
| 37            | 107     | 234 : 35                 | 5016            | 3                |
| 38            | 123     | 345 : 24                 | 5118            | 3                |
| 39            | 109     | 235 : 34                 | 5128            | 3                |
| 40            | 106     | 2345 : 3                 | 5134            | 3                |
| 41            | 118     | 245 : 34                 | 5186            | 1                |
| 42            | 122     | 345 : 23                 | 5246            | 4                |
| 43            | 114     | 2345 : 4                 | 5290            | 3                |
| 44            | 105     | 234 : 34                 | 5469            | 1                |
| 45            | 127     | 345 : 35                 | 5552            | 2                |
| 46            | 126     | 345 : 34                 | 5906            | 3                |

<sup>a</sup>: Values rounded off from Table 2.

<sup>b</sup>: Most abundant ions of isotope clusters of ([M-Cl]/[M]) ×100%.

c, d, e, f, g: Groups of isomers with separations of 5 sec or less.

<sup>h</sup>: “Ortho effect” used with the elution order can distinguish these nearest neighbors.

<sup>H</sup>: From groups c, d, e, f, g, these coeluters can be distinguished using the “ortho effect.”
Table 4: Congeners and Some PCB Coeluting Isomers (1D Mode) with their Percent “Ortho Effects”.

| IUPAC Numbers of Coeluting Isomers (“Ortho Effects”) |  |  |
|------------------------------------------------------|-----------------------------------------------------------|
| Tri                                                  | 28(5%) + 33(5%) + 21(6%)                                  |
| Tri                                                  | 39(5%) + 38(7%)                                          |
| Tetra                                                | 64(4%) + 40(82%)*                                        |
| Tetra                                                | 65(7%) + 62(5%) + 47(26%)                                |
| Tetra                                                | 67(3%) + 58(4%)                                          |
| Hexa                                                 | 163(4%) + 129(56%)*                                     |
| Hepta                                                | 182(32%) + 175(44%)                                     |
| Octa                                                 | 201(4%) + 204(2%)                                       |

*This isomer can be distinguished from other coeluting isomers by the “ortho effect.” The penta isomers were given previously in the text and figures.

However, as illustrated in Figure 2, PCB congeners numbers 4 and 10 (2,2′- and 2,6-dichlorobiphenyl, resp.) were ultimately distinguished by taking advantage of their “ortho-effect” (38). The 2,2′-dichlorobiphenyl had a higher abundance of the m/z 187 (M-Cl)⁺ ion relative to the molecular ion at m/z 222. This fragment was not nearly as significant in 2,6-dichlorobiphenyl, where the two ortho chlorines were on the same ring. Note, there was partial resolution in this case. For complete coelution, if the magnitude of the “ortho effect” were known for each isomer, and were sufficiently different, it would be possible to estimate the relative concentrations of each isomer present. For example, if equal concentrations of each isomer were present, the observed “ortho effect” would be expected to be half way between the two values.

It was observed that the PCB congener retention times in the 1D chromatographic mode were different by a few seconds from those of the 2D retention times due to the modulation effect. Based on our analytical conditions, as stated previously, the isomeric peak separations, achieved using the 2D chromatographic mode, occurred within approximately 2-3 sec on the 2D plane. When the instrument was optimized (setting the trapping time to 1.2 sec) for pentachlorobiphenyl isomer separations (Figure 1) in a different 2D run, the isomeric peak separations occurred at about 1-2 sec on the 2D plane. The significance of this adjustment was that most of the potential interferences would be separated twice from the target analytes: once, mostly volatile interferences, in the first 1000 sec of the 40 m column of the 1D plane, and second, interfering hydrocarbons, on the first 1 sec of the 1 m column of the 2D plane (Figure 1).

The early elution of these potential interferences would leave the analytes of interest in a purer form, resulting in more accurate full-scan library identification. This tool may prove very useful for the identification of individual PCB congeners in extracts of complex mixtures such as sewage treatment plant sludge or river sediments.

Table 4 shows 18 PCBs that were not distinguishable using the 1D chromatographic mode and three of these 18 PCB congeners were additionally distinguished from their isomers, using the “ortho effect.” This further raised the numbers of distinguishable PCB congeners by three. As indicated earlier in this paper (see Figure 2), PCB isomers 4 and 10 (2,2′- and 2,6-dichlorobiphenyl, resp.) were distinguished by their “ortho-effect,” and this also increased the total number of distinguishable congeners by one. In spite of the use of the GC×GC-TOFMS, not all of the congeners were distinguishable. The 2D chromatographic conditions permitted the separation of most of the pentachlorobiphenyls, but this was found to be at the expense of resolving the tetrachlorobiphenyls (e.g., IUPAC #62 and #65). It was necessary to optimize the system for those separations of interest. Based on these experiments, GC×GC-TOFMS provided more complete separation of isomeric PCBs that were unresolved after the 1D mode was used. Even though not all 209 PCB congeners (33) were chromatographically resolved, the use of the 2D chromatographic mode provided superior data for assessing the risk posed by a complex mixture of PCBs. We believe that complete separation of all 209 PCB congeners may eventually be achieved by two consecutive GC runs and with the advent of enhanced columns and different chromatographic conditions, optimized to resolve the most difficult to separate PCB isomers or congeners.

More chromatographic work and superior deconvolution software may be needed to take full advantage of this promising technology for isomeric compound separations. The authors used the default data re-sample feature of the software to re-sample large data files to reduce the file size and better define chromatographic peaks, which resulted in enhanced peak separations.

8. Conclusion

In light of recent scientific findings that noncoplanar PCB congeners were also culpable in human health deterioration, as well as planar PCBs, we felt it was important to develop a method that would potentially separate all possible PCB congeners, which would be ready for use on demand. It would be highly unlikely for all congeners to be simultaneously present in any given environmental sample, for example, heavily chlorinated biphenyls with all chlorines on one ring and none on the other ring would be inconsistent with the known electrophilic method of preparation of the Aroclors. However, these or other non-Aroclor PCB congeners, could result from different processes. Because we have examined all PCB congeners, this study has included all those that were found to be ubiquitous, toxic, or potentially toxic. This study considered all congeners that would result from all possible routes: intentional, or incidental, chlorinated biphenyl syntheses, for example, coupling reactions, and as byproducts, and environmental alteration products by selective vaporization or absorption, and reductive dechlorination during metabolism, photochemical reaction, combustion, and chemical remediation [44–47]. Rather than relying on the common method of total or combined PCB quantification, the electron capture detector (for high sensitivity), this study employed technology that provided excellent separation of 196 PCB congeners that were either present in or absent from Aroclor formulations. Indeed, the
need to overcome the challenges of individual PCB congener separation, in not more than two chromatographic runs, remained an approachable, but not yet achieved goal. The quest for a congener-specific analysis of all PCB congeners in complex environmental mixtures, without sacrificing separation quality, is on-going and this paper presents a step towards this goal.

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