Full Congener CBs Analysis by GC/HRMS: QA/QC Considerations

Ikonomou, M.G.; Sather, P.; He, T.; Crewe, N.; Fraser, T.

Department of Fisheries and Oceans, Institute of Ocean Sciences, Contaminants Science Section, 9860 West Saanich Road, Sidney, B.C., V8L-4B2, CANADA

Introduction: In recent years, there has been a growing call for congener specific CBs quantitative analysis (1,2), for reasons that include providing measures of total CB load in environmental samples, and for congener specific risk assessments without the inaccuracies due to sample weathering that occur when these are estimated using non-specific Aroclor determinations by GC-ECD. In response to this we have been interested in developing an analytical method towards the quantitative determination of all 209 CB congeners by GC/HRMS. The approach was based on the method our lab has used for some years for the quantitative determination of non-ortho (NO-) and mono-ortho (MO-) CBs in environmental samples (3,4). In addition to the NO- and MO-CBs all of the di, tri and tetra ortho congeners (which will be referred to as Di-Ortho-CBs, DO-CBs, in this paper) were also determined by GC/HRMS.

In an attempt to identify areas that could compromise the quality of the analytical data every part of the analytical procedure was scrutinized. The objectives were: a) to assess the efficiency of the carbon fiber fractionation process especially for samples that had a wide range of CBs concentrations, i.e. certain congeners at the ppm level and others at ppt; b) to have absolute separation for all the CB congeners with recognized TEF values (5); c) obtain maximum separation for all CB congeners on a single column and with the shortest possible GC program; d) to assess the accuracy of the analytical standards by evaluating their relative response factors (RRFs) in terms of structural and analytical parameters and by data comparisons against standard reference materials (SRMs).

Materials and Methods: The overall analytical method was tested for the following matrices: tissue, blubber microsamples (100mg), sediment, sludge, SPMD and municipal waste incinerator extracts. Samples were spiked with a suite of representative ^{13}C internal standards, see Table 1, and processed and analyzed by GC/HRMS through the procedures reported in references 3 and 4. Carbon fiber (CF) fractionation was optimized using samples spiked with all three groups of CB standards -- both natives and surrogates, see Table 1. The CBs chosen for these solutions were those with: a) recognized TEF values; b) analyzed for in most environmental investigations and c) were components of NIST CB SRMs. Fractions were eluted from a 7.5cm x 5mm i.d. stainless steel column hand packed with a 12:1 homogenate of glass filter paper (nucleopore, 124 mm P100 prefilter sn. 211707) and carbon (Amoco PX-21) using 20 mL of 3% DCM in hexane at 2 mL/min to elute DO- congeners (fraction I); 44 mL of 1:1 DCM:cyclohexane at 2 mL/min to

elute MO- congeners (fraction-II); and 50 mL of 1:1 benzene:ethyl acetate at 2 mL/min to elute the NO-CB congeners (fraction-III). All fractions were analyzed separately by GC/HRMS.

The GC columns tested were:

DB5:	60m x .25 mm i.d. x .1 μ film	CP-Sil-19CB:	60m x .25 mm i.d. x .15 μ film;
BPX-5:	50m x .22 mm i.d. x .25 μ film	DB-225:	30m x .25 mm i.d. x .15 μ film;
HP-5:	60m x .25 mm i.d. x .1 μ film	CP-Sil-5/C18:	50m x .25 mm i.d. x .1μ film (6)

Conditions were as follows:

- Cond. A: 80 C (2 min) - 150 C, 8 C/min; 150 C - 295 C, 4 C/min, (15 min) 62 min total
- Cond. B: 80 C (2 min) - 160 C, 4 C/min; 160 C - 220 C, 1.5 C/min, (8 min) 62 min total
- Cond. C: 75 C (2 min) - 150 C, 15 C/min; 150 C - 300 C, 1.5 C/min, 107 min total

Native CBs were quantitated against the surrogate with the same chlorination level except for the following: a) all dichloro MOs against ^{13}C-CB28; b) all tetrachloro MOs and 118 against ^{13}C-CB118 (other pentaclorinated MOs against ^{13}C-CB105); c) heptachloro MO CB189 against ^{13}C-CB156; and di- and trichlorinated DOs against ^{13}C-CB52.

Table 1: Composition of Calibration Standard Solutions

Cl no.	Non-Ortho	Mono-Ortho	Di-Ortho
di	11, 13, 15[a]	8	4
tri	39, 35, 37, 38[b]	31, 28[a]	18, 27, 24
tetra	80, 79, 78, 80, 77[a]	58, 61, 74, 70, 66, 60	45, 46, 52[a], 49, 47, 44, 42, 41
penta	127, 126[a]	118[a], 124, 108, 123, 114, 122, 105[a]	95, 91, 84, 101[a], 90, 99, 119, 97, 125, 87, 85, 110, 82
hexa	169[a]	159, 162, 167 156, 157	151, 144, 149, 131, 146, 153, 168, 141, 137, 130, 138, 158, 128[a]
hepta		189	179, 178,175, 187, 182, 183, 185, 174, 177, 171, 172, 180[a], 193, 170
octa			200, 197, 199, 203, 196, 194[a], 205
nona			208[a], 206

a) native and ^{13}C surrogate
b) ^{5}D surrogate

Results and Discussion: The separation efficiency of the six different GC columns was evaluated using the calibration standard solutions tabulated above. The conditions used and the coeluters detected for each column are listed in Table 2. The DB5 column provided optimum separation with the lowest number of coeluters in the shortest run time. When real samples containing all 209 congeners were analyzed additional coeluters were identified. These were: MOs 7/9, 8/5, 33/20, 61/74, 70/76, 56/60, 108/107; and DOs 4/10, 27/24, 73/52, 47/75/48, 59/42, 71/41/64, 102/93, 92/84, 101/90, 113/99, 109/83, 97/86, 116/125/117, 115/87, 134/144, 139/140, 143/134, 142/131/133, 165/146/161, 132/153, 160/163/164/138, 187/182, 174/181, 192/172, **170/190**, 203/196. The DB5 provided complete separation of all the CB congeners with TEF values except CB170. CB170 has a TEF of 0.0001 while its coeluter 190 has no TEF reported. Both have a relative retention times of 0.874 on the DB5 column (7), but are separable on the C18 column with RRTs: 0.6987(170) and 0.7088 (190) (8). Thus, the toxicity calculated for CB170 analyzed on the DB5 column might be overestimated without confirmation on another column.

Since the fractionation of CBs into NO-, MO- and DO- using CF columns is not absolute the quantitation interferences caused by this condition had to be identified and solutions to improve the data quality had to be found. One example is the partitioning of di- and tri-chlorinated MO-CBs into both the MO- and the DO fractions. The extent of cross fractionation

was affected by the composition of the eluting solvent and the condition of the CF column(s). In an attempt to optimize separation of the two fractions a number of solvent systems were examined, the 3% DCM in hexane combination was the best compromise. However even under these conditions there was a large variability on the degree of fractionation from one experiment to the next. As a result for every sample the actual concentrations of di- and tri-chlorinated MO-CBs had to be calculated using both the DO and the MO analytical reports. The di- and tri-chloronative CBs and ^{13}C-CB28 were monitored in both DO and MO GC/MS analyses. Quantitations were performed individually and were determined by the recovery of ^{13}C28 in each fraction. The following system of calculations was found to give the most accurate results:

$$\text{If } \frac{\text{13C28 recovery in DO report}}{\text{13C recovery in MO report}} < 0.54 \text{ then:}$$

-dichlorinated mono-ortho congeners cannot be accurately reported.
-tri-chlorinated congeners are taken from the MO report

$$\text{If } \frac{\text{13C28 recovery in DO report}}{\text{13C28 recovery in MO report}} > 0.54 \text{ then:}$$

-dichlorinated congeners taken from the DO report
-congeners 23, 34, 29, 26 are taken as the mean between the DO and MO reports
-congeners 25, 31, 28, 21, 20, and 22 are taken from the MO report
-^{13}C28 recovery is taken as the mean between the DO and MO reports

Prior to allpying these corrections the criterion that had to be met was that the total % recovery of ^{13}C28 had to be higher than 30%.

Table 2: CB coeluters in Six Different GC Columns

Cl no.	DB5 Cond. A		CP Sil 19cb Cond. C		BPX5 Cond A		DB225 Cond. B		HP5 Cond. A		CP-Sil C18 Cond. A	
	mo	do	mo	do	mo	do	mo	do	mo	do	mo	do
di												
tri		27/ 24	31/ 28		31/28	27/24	31/28		31/28	27/24	31/28	
tetra	61/ 74		58/ 61				58/61	52/45			70/74	44/47
penta		101/ 90			114/122		124/108/123	101/90 125/87/85	124/108	97/125	124/108	101/90 119/97 125/87
hexa				153/ 168		130/138/158 131/146 153/168		137/130		138/158 153/168	156/157	153/168 158/178[a] 128/187[a]
hepta		187/ 182		178/ 175		187/182				187/182		185/174 193/180
octa		203/ 196		203/ 196		203/196		203/196		203/196		

[a] hepta congener

Another CF fractionation problem was the tailing of high concentration congeners from one fraction into the next, resulting in small percentages of fraction I congeners (DOs) to elute in fraction II (MOs) and fraction III (NOs) and also fraction II congeners to elute into fraction III. Because environmental samples contain CB congeners at widely varying concentrations (ppt to ppm) even low levels of CF tailing can result in inaccurate quantitations especially when the tailing congener and/or their decomposition products happen to have identical GC retention times with a low concentration target congener. When all of the CB congeners were examined considering their relative concentrations in typical environmental samples and their elution times

on the DB5, 8 congener pairs were found to share these characteristics and those are listed in Table 3. In such cases two approaches, one direct and one indirect, were used to estimate the levels of carry-over, and an example of each is described in the paragraphs following.

Table 3: Cross-Fractionated and GC Coeluters Needing Correction

Description	Action Required
MO105 coelutes with NO127	direct estimate using 105 surrogate
MO66 coelutes with NO80	estimate based on MO56/60
(M-2Cl) of DO178 coelutes with NO126	estimate based on DO187/182
(M-Cl) of DO49 coelutes with NO38	estimate based on DO47/75/48
(M-Cl) of DO109/83 coelutes with NO78	estimate based on DO97/86
DO85 coelutes with MO120	estimate based on DO110
(M-Cl) of DO187/182 coelutes with MO159	estimate based on DO174/181
(M-Cl) of DO183 coelutes with MO162	estimate based DO174/181

The use of ^{13}C-CB105 as a surrogate enabled a direct calculation of native CB105 carry-over into the NO fraction using the data obtained from both (MO- and NO-CB) analyses. The peak area response of the ^{13}C-CB105 in the NO fraction was normalized against the MO response using the area responses of ^{13}C-CB101 which was used as the method recovery standard and thus was present in both fractions (NO and MO) at the equal concentrations. Using this approach the percentage of ^{13}C-CB105 fractionated into the NO fraction was calculated, and assuming that the native CB105 partitioned similarly to its surrogate, the response of the native CB105 in the NO fraction was calculated. When the contribution of CB105 was significant the concentration of CB127 peak was adjusted by subtracting from it the CB105 component. In the samples so far examined it has been found that carry-over varied from 1% - 3% of the MO105 response.

The indirect method was used to estimate the level of carry-over between DO178 and NO126. Since CB126 has a very high TEF (0.1) it is important to determine precisely the amount contributed by CB178. This can be achieved in two ways: a) by monitoring the heptachloro DO natives and surrogates in the NO GC/MS analysis in which case the amount of CB178 into the NO fraction could be calculated in a similar way as was discussed above for the 105/126 coeluters; or b) by monitoring degradation products of closely related congeners. Heptachloro DO187/182 is a strong component in many environmental samples and elutes close to CB178. It is possible to see the degradation products (M-2Cl) of this congener pair in the pentachloro NO chromatograms at approximately 0.03% of the intensity of its parent. Assuming the same level of CF cross-over elution and similar degradation for DO178 allows the estimation of contamination should levels be so high as to warrant the correction, and otherwise provides the assurance that the reported NO126 concentration is correct.

Finally, in order to determine possible sources of analytical inaccuracies due to inpure standards, an evaluation of all RRFs was undertaken. This evaluation took two forms. The first was a detailed examination of inter and intra surrogate RRF comparisons and intra-group surrogate-native RRF comparisons with emphasis on the mass balance of the isotopes used for analysis, the structure of the congeners (particularly with respect to chlorination level), and external factors such as the appearance of impurities recognized during preparation. The second was the analysis ofNIST CB SRMs, both solutions and matrices, for direct confirmation of problems that may have arisen. The results from this investigation are being evaluated.

References:

1. Martin, M., Tjeerdema, R.S.. Selection of Analytical Chemical Protocols for Polychlorinated Biphenyl Measurement in Ecological Risk Assessment. SETAC'97 Abstarcts, PCB workshop, San Francisco CA, .Nov. 16-20, 1997.
2. Davis, J.A., Hoenicke, R., Risebrough, R.W., Jarman, W.M., Bacon, C.A., Vedder, J., Sericano, J.L.. PCB Congener Data Are Advantageous, But Proceed with Caution. SETAC'97 Abstarcts, PCB workshop, San Francisco CA, .Nov. 16-20, 1997.
3. MacDonald, D.D., Ikonomou, M.G., Rantalainen, A-L., Rogers, I.H., Sutherland, D., Van Oostdam, J.. Environm.Tox. and Chem. 1997. Vol. 16, No. 3, 479-490.
4. Rantalainen, A-L., Ikonomou, M.G., Rogers, I.H.. Chemosphere, in press.
5. Ahlborg, U.G., Becking, G.C., Birnbaum, L.S., Brouwer, A., Derks, H.J.G.M., Feeley, M., Golor, G., Hanberg, A., Larsen J.C., Liem A.K.D., Safe, S.H., Schlatter, C., Wærn, F., Younes, M. Yrjänheikki, E.. Chemosphere. 1994. Vol. 28, No. 6, 1049-1067.
6. Ballschmiter, K., Schäfer, W., Buchert, H.. Fresenius Z. Anal Chem. 1987. 326, 253-257.
7. Mullin, M.D., Pochini, C.M, McCridle, S., Romkes, M., Safe, S.H., Safe, L.M. Environ. Sci. Technol. 1984, 18, 468-476.
8. de Zeeuw, J., Zwiep, D., Marinissen, J.W., Investigation of Retention Characteristics of Several Bonded Phases for the Separation of all 209 PCB Isomers. Chrompack Technical Report, Chrompack International, Middelburg, Netherlands