GC/MS Analysis for PCB Congeners in Lake Ontario Salmon

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Introduction

Eighteenmile Creek is designated as an Area of Concern (AOC) by the Environmental Protection Agency (EPA)[1]. Contamination of sediments and wildlife with Polychlorinated Biphenyl congeners (PCB) has resulted in a "DON'T EAT" advisory from the New York State Department of Health (NYSDOH) for any fish caught in this AOC[2]. Tissue samples from multiple fish of two species of salmon were gathered out of Olcott harbor and subjected to Gas Chromatography/Mass Spectrometry (GC/MS) analysis to estimate PCB concentrations to investigate if they remain cause for concern, particularly in fish that only temporarily reside in Eighteenmile Creek to spawn. PCB concentrations in both edible and inedible portions of samples were compared.

Procedure

Full Analysis

The procedure for this analysis was adapted from EPA Method 1668B[3]. Following section numbers refer to sections of this method. A sample of fish tissue is collected per 8.4, and homogenized per 11.8.1. The sample is then extracted per 12.4. To prepare for cleanup, the sample is macro-concentrated per 12.6.1, and then micro-concentrated by blowing down with nitrogen gas, by attaching a tube with a pipette at the end to the gas cylinder regulator, and applying just enough pressure to slightly disturb the surface of the solvent. The residue is then

exchanged into hexane and eluted through an anthropogenic isolation column which is prepared per 7.5.3 and operated per 13.6. The sample is again concentrated per 12.6.1 and the nitrogen blowdown, and then exchanged into dichloromethane for gel permeation chromatography (GPC). A manual method of GPC was created, whereby a glass column was packed with 70 g of SX-8 bio-beads (Bio-Rad Laboratories, Richmond, CA) that were soaked for 24 hours in dichloromethane, with an approximately 2 cm layer of glass wool on top to prevent the beads from floating, and a liquid level of dichloromethane is consistently maintained at least a centimeter above the glass wool. Additionally, a 250 mL dropping funnel is placed above the column to provide dichloromethane for the elution. The sample is loaded on to the top of the column and the stopcock of the column is fully opened which results in a dropping rate of approximately 4-5 mL/min, and the dropping funnel's stopcock is opened enough to have an equal dropping rate to the column. A total of 400 mL of dichloromethane is eluted, and the eluant between 90-140 mL is collected for further analysis. The sample is again concentrated per 12.6.1 and the nitrogen blowdown, and then exchanged into hexane to be subjected to Florisil cleanup. The Florisil column is prepared per 7.5.4 and operated per 13.7. The sample is again concentrated per 12.6.1 and the nitrogen blowdown, and then exchanged into 1 mL of nonane in a 2 mL septum vial, and then loaded into an autosampler for GC/MS analysis.

Column Material Preparation

Multiple of the solid materials required for the chromatographic columns must be specially prepared before use. Sodium sulfate was prepared per 7.2.1 and the rinsing was done via suction filtration. The activated silica gel was prepared per 7.5.1.1 and the rinsing was done via suction filtration, and acid silica gel was prepared per 7.5.1.2. Potassium silicate was prepared per 7.5.1.4, but the activation caused it to harden, resulting in difficulty of pouring. To

loosen chunks of the potassium silicate, a power drill with an auger drill bit cleaned with soap and rinsed with hexane was used to drill holes in the hardened mass to create a free flowing powder ready for use. Florisil was prepared per 7.5.4.1.1. The bio-beads for GPC were prepared per 13.2.1.1 and 13.2.1.2, however SX-8 beads were used.

Sample Collection

The fish tested in this experiment were provided the Reel Fishing Adventures charter which runs out of Olcott harbor. Details on the fish from each sample are shown in Table 1.

Table 1: Details of each sample and their respective fish.

Sample	Sample Mass	Fish Part	Species	Catch Site	Fish Weight
bellyA2	10.060	Belly fat	Coho Salmon	N 43 22.226 W 78 47.955	7 lb, 15 oz
bellyA3	10.262	Belly fat			
filetA1	10.198	Edible filet			
filetA2	10.290	Edible filet			
filetD1	10.136	Edible filet	Coho Salmon	N 43 22.464 W 78 49.557	8 lb, 10 oz
filetD3	11.000	Edible filet			
stomachE1	10.523	Stomach contents	Coho Salmon	N 43 22.451 W 78 49.282	4 lb, 7 oz
filetE1	10.484	Edible filet			
filetF1	10.457	Edible filet	King Salmon	N 43 20.223 W 78 39.453	15 lb, 14 oz
filetF2	10.413	Edible filet			
filetG1	10.461	Edible filet	King Salmon	N 43 29.297 W 78 39.987	11 lb, 3 oz
filetG2	10.743	Edible filet			
filetH1	10.001	Edible filet	King Salmon	N 43 28.347 W 78 40.848	17 lb, 10 oz
filetH2	10.829	Edible filet			

Calibration

A standard solution for calibration was made using a mixture of the following 19 congeners with the following concentrations:

Table 2: Congeners and respective concentrations in Agilent RPCM-8082-1 congeners mixture[4].

Congener Name	Congener #	CAS #	Concentration (µg/mL)
2-Chlorobiphenyl	1	2051-60-7	100
2,3-Dichlorobiphenyl	5	16605-91-7	100
2,2',5-Trichlorobiphenyl	18	37680-65-2	100
2,4',5-Trichlorobiphenyl	31	16606-02-3	100
2,2',3,5'-Tetrachlorobiphenyl	44	41464-39-5	100
2,2',5,5'-Tetrachlorobiphenyl	52	35693-99-3	100
2,3',4,4'-Tetrachlorobiphenyl	66	32598-10-0	100
2,2',3,4,5'-Pentachlorobiphenyl	87	38380-02-8	100
2,2',4,5,5'-Pentachlorobiphenyl	101	37680-73-2	100
2,3,3',4',6-Pentachlorobiphenyl	110	38380-03-9	100
2,2',3,4,4',5'-Hexachlorobiphenyl	138	35065-28-2	100
2,2',3,4,5,5'-Hexachlorobiphenyl	141	52712-04-6	100
2,2',3,5,5',6-Hexachlorobiphenyl	151	52663-63-5	100
2,2',4,4',5,5'-Hexachlorobiphenyl	153	35065-27-1	100
2,2',3,3',4,4',5-Heptachlorobiphenyl	170	35065-30-6	100
2,2',3,4,4',5,5'-Heptachlorobiphenyl	180	35065-29-3	100
2,2',3,4,4',5',6-Heptachlorobiphenyl	183	52663-69-1	100
2,2',3,4',5,5',6-Heptachlorobiphenyl	187	52663-68-0	100
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	206	40186-72-9	100

The 1 mL of this solution solution was diluted into 10 mL of nonane, resulting in a concentration of 10 µg/mL for each congener, 1 mL of which was again diluted in 10 mL of nonane resulting in a concentration of 1000 ng/mL for each congener. This was used as the calibration standard solution.

To determine the elution volumes of the PCBs in the GPC column, 200 µL of the standard spiking solution was diluted in 10-15 mL of dichloromethane and eluted through it, and every 10 mL, a 2 mL septum vial was filled halfway and loaded into the GC/MS autosampler. Analyzing all of these fractions indicated that the PCBs are eluted at 90-130 mL, shown in

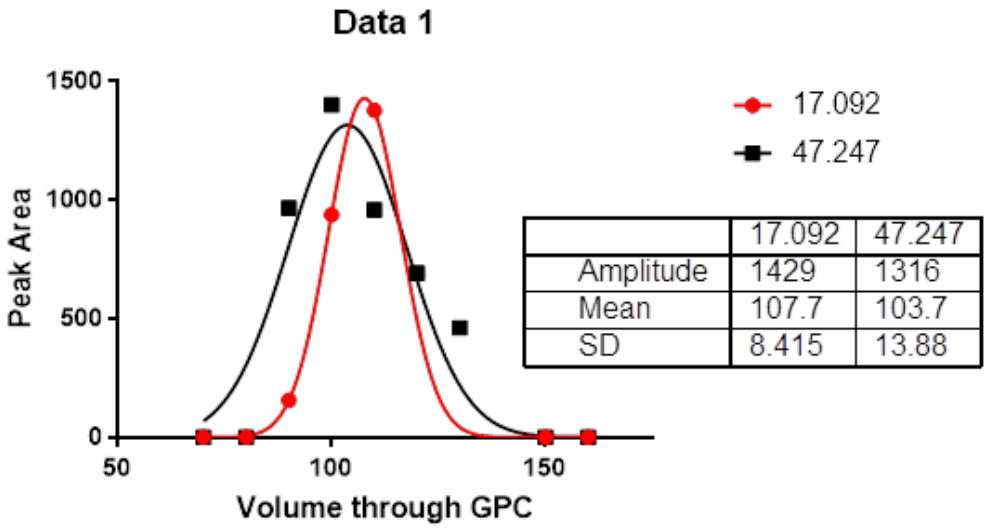


Figure 1.

Figure 1: GPC fraction volume against PCB peak area on GC/MS chromatogram

The GC operating conditions used for this analysis are those suggested in section 10.1.1 of EPA Method 1668B. To establish retention times and major ion peaks of congeners of interest, 200 µL of the standard spiking solution was diluted to 1 mL in nonane, and injected into the GC/MS. The gathered information is in Table 3.

Congener	Retention Time (min)	Major Ion Peak	Minor Ion Peak
2-Chlorobiphenyl	10.6	188	153

Congener	Retention Time (min)	Major Ion Peak	Minor Ion Peak
2,3-Dichlorobiphenyl	14.9	222	152
2,2',5-Trichlorobiphenyl*	16.8	186	256
2,4',5-Trichlorobiphenyl	19.5	256	186
2,2',3,5'-Tetrachlorobiphenyl	22.7	292	220
2,2',5,5'-Tetrachlorobiphenyl	21.4	292	220
2,3',4,4'-Tetrachlorobiphenyl	25.5	292	220
2,2',3,4,5'-Pentachlorobiphenyl	28.6	326	254
2,2',4,5,5'-Pentachlorobiphenyl	26.8	326	254
2,3,3',4',6-Pentachlorobiphenyl	29.3	326	254
2,2',3,4,4',5'-Hexachlorobiphenyl	34.3	360	290
2,2',3,4,5,5'-Hexachlorobiphenyl	33.3	360	290
2,2',3,5,5',6-Hexachlorobiphenyl	30.0	360	290
2,2',4,4',5,5'-Hexachlorobiphenyl	32.4	360	290
2,2',3,3',4,4',5-Heptachlorobiphenyl	40.7	394	324
2,2',3,4,4',5,5'-Heptachlorobiphenyl	38.7	394	324
2,2',3,4,4',5',6-Heptachlorobiphenyl	35.6	394	324
2,2',3,4',5,5',6-Heptachlorobiphenyl	35.3	394	324
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	47.2	464	392

Table 3: Retention times and major ion peaks of congeners of interest.

*2,2',5-Trichlorobiphenyl was the only congener with a parent ion peak that was not the largest peak.

This information was used to create a set of MS parameters to operate in single ion mode (SIM) to more narrowly search for the congener retention times and ion abundance, shown in Table 4. Dwell time for each ion is 100 ms.

Table 4: MS SIM method parameters.

Zone	Start Time (min)	Plotted Ion 1	Plotted Ion 2
1 — MonoCBP	8.00	188	153
2 — DiCBP	12.75	222	152
3 — TriCBP	15.85	256	186
4 — TetraCBP	20.45	292	220
5 — PentaCBP	25.50	326	254
6 — HexaCPB	29.65	360	290
7 — HeptaCBP	34.80	394	324
8 — NonaCBP	43.95	464	392

The final GC/MS parameters used for sample analysis were those in section 10.1.1 but with narrowed scan parameters to avoid cluttering peaks from non congener material retained in the extract. The scan parameters for the total ion operation are shown in Table 5.

Table 5: MS TIC method parameters

Scan Group	Start Time (min)	Start at (amu)	End at (amu)
1	8.00	145	263
2	20.50	215	370
3	35.10	315	475

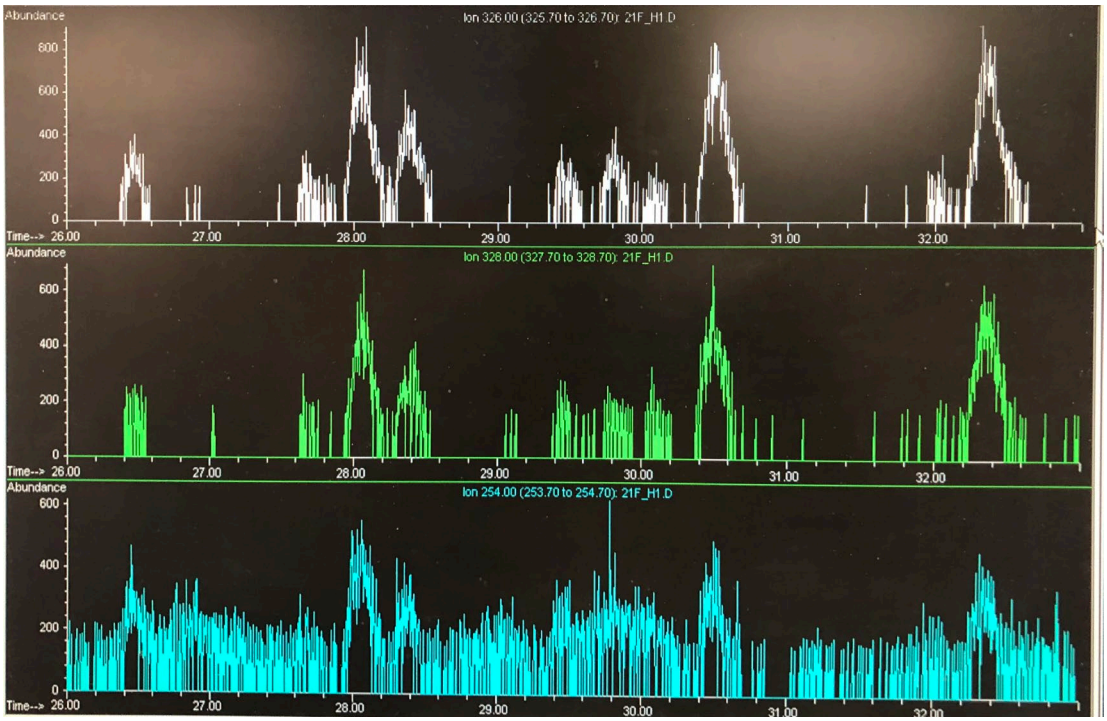
To provide a rugged estimate of the concentration of each congener, the abundance of the major ion of each congener was compared to that found in the standard with known concentration.

Table 6: Each congener's major ion abundance (averaged across peak at half height) at 200 ng/mL

Congener	Ion	Abundance
1	188	1100
5	222	450
18	186	850
31	256	260
44	292	600
52	292	850
66	292	60
87	326	550
101	326	720
110	326	650
138	360	550
141	360	600
151	360	900
153	360	700
170	394	550
180	394	600
183	394	850
187	394	850
206	464	800

Results

The peaks were found by extracting an ion chromatogram of each congener's major ion, minor ion, and the ions of the isotope 2 amu higher than both. Then, the presence of a congener was apparent by all four chromatograms sharing a peak at a retention time near those of congeners in the calibration standard, with ratios in abundances between these similar to those in



the calibration standard. An example of these aligned chromatograms is shown in Figure 2.

Figure 2: Aligned extracted chromatograms of filetH1 in the pentachlorobiphenyl range (major ion, major ion Cl-37 isotope, minor ion)

Table 7 shows the abundance of the major ions of the 19 congeners in each injected unspiked sample averaged across half of the peak's height.

Congener	bellyA2	bellyA3	filetA1	filetA2	filetD1	stomE1	filetE1	filetF1	filetF2	filetG1	filetG2	filetH1	filetH2
1													
5		600	220										
18													
31		350	300									150	160
44										250	280		110
52										180	250	110	150
66													
87		220				220	100	250		220	300	370	400
101		200	120	140	100	220	120	100	120	500	500	550	700
110		270	150	140	130	320	150	200	90	400	400	550	650
138	300	290	120	120	150	350	220	220	130	300	600	750	850
141		400	110	140	110	420	160	400	160	600	650	1100	1100
151	475										200		
153	120	150				220	80			380	150	500	500
170						50		50		120	200	220	220
180												70	70
183												40	60
187						50		35		170	150	300	250
206													

Table 7: Abundance of major ions of congeners in each tested sample (blank spaces indicate no or negligible presence).

Other isomers of these congeners were visible, but were not considered due to not being present in the calibration standard. Their abundances were not exceedingly higher than those considered.

The concentration of each congener was found by

$$c_i = \frac{200\frac{ng}{ml}}{a_0}(a_i) \quad (1)$$

where a_0 is the abundance of the respective ion in Table 6, and a_i is the abundance of the major ion of congener *i* in the sample shown in Table 7. Total concentration in a sample was found by

$$c_T = \frac{\sum_{i=1}^{206} a_i}{m} \quad (2)$$

where *m* is the mass of the sample. The estimated concentrations found in each sample are shown in Table 8.

Table 8: Determined individual and total concentrations of congeners in each tested sample (blank spaces indicate no or negligible presence).

Congener	bellyA2	bellyA3	filetA1	filetA2	filetD1	stomE1	filetE1	filetF1	filetF2	filetG1	filetG2	filetH1	filetH2
1 (ng/mL)													
5 (ng/mL)		267	98										
18 (ng/mL)													
31 (ng/mL)		269	231									115	123
44 (ng/mL)										83	93		37
52 (ng/mL)										42	59	26	35
66 (ng/mL)													
87 (ng/mL)		80				80	36	91		80	109	135	145
101 (ng/mL)		56	33	39	28	61	33	28	33	139	139	153	194
110 (ng/mL)		83	46	43	40	98	46	62	28	123	123	169	200
138 (ng/mL)		105	44	44	55	127	80	80	47	109	218	273	309
141 (ng/mL)		133	37	47	37	140	53	133	53	200	217	367	367
151 (ng/mL)	106										44		
153 (ng/mL)	34	43				63	23			109	43	143	143
170 (ng/mL)						18		18		44	73	80	80
180 (ng/mL)												23	23
183 (ng/mL)												9	14
187 (ng/mL)						12		8		40	35	71	59

Congener	bellyA2	bellyA3	filetA1	filetA2	filetD1	stomE1	filetE1	filetF1	filetF2	filetG1	filetG2	filetH1	filetH2
206 (ng/mL)													
Total (ppb)	25	101	48	17	16	57	26	40	16	93	107	156	160

Conclusions

The NYSDOH guidelines for men over 15 and women over 50 state that fish containing <1ppm total PCBs can be eaten up to 4 meals/month, ≥1 ppm to <2 ppm up to 1 meal/month, and ≥2 ppm not be eaten at all[5]. The findings of this experiment suggest that fish caught off of Olcott harbor represented by these samples are not cause for concern regarding PCB concentrations if consumed, as the estimates fall significantly below limited meals/month advisories, and fall below DON'T EAT advisories by more than an order of magnitude. Additionally, these findings suggest that PCB concentrations are higher in inedible portions of the fish, as demonstrated by the difference between fish E's stomach contents and edible flesh, in addition to the difference between fish A's belly fat and edible flesh. More fish and their different body portions would need to be analyzed in parallel to confirm this suspicion, as this experiment only showed such with two samples, but it is a conjecture consistent with information provided by the NYSDOH[6].

To provide a more robust analysis capable of handling a greater number of samples with more precise results, some portions of the procedure should be modified. Firstly, an automated gel permeation chromatograph would save significant time due to the attention required to monitor the manual setup performed in this experiment. The same is true for the nitrogen blowdown. To specifically improve the GC/MS analysis, 0.3 mL conical vials instead of the 2 mL vials used would greatly improve resolution, as the extracted sample would be concentrated

further. Additionally, more of the calibration standard would allow for more precise concentration values via a more rigorous method of analysis such as a detailed calibration curve or the standard addition method, as only 1 mL of the original standard was available.

References

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