AN INVESTIGATION OF WILD-CAUGHT AND FARM-RAISED SHRIMP SAMPLES WITH HIGH CONCENTRATIONS OF POLYCHLORINATED BIPHENYLS

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Abstract
As part of a study to characterize levels of different persistent organic pollutants in a variety of aquatic food products, 168 PCB congeners/congener pairs were measured in samples of uncooked shrimp and crab and cooked shrimp. Ten uncooked samples of shrimp were determined to be outliers, 60% of which were wild-caught and 40% of which were farm-raised. All but one of the outliers were from North American countries, with the majority of samples originating in the United States. As expected, the average number of congeners detected in the outliers was greater than the number detected in all other uncooked shrimp samples. While the percent contribution of PCBs 28, 52/69, 90/101, 106/118, 138/163/164, 153, and 180 were generally similar between the outliers and all other uncooked shrimp samples, homologue fractions of mono-, di-, octa-, nona- and deca-PCBs varied considerably. Results from this evaluation suggest that for a few outliers, high concentrations of PCBs may be due to contaminated feed or local point sources. For other samples, excess loading of PCBs could be due to broader regional contamination.

Introduction
As part of a study to describe levels of different persistent organic compounds in a variety of seafood products, we collected 84 raw samples of warm-water shrimp from various geographic regions around the world.[1] A more general, objective of the study was to determine whether levels of PCBs in shrimp were a result of regional contamination or possibly due to other factors such as contaminated commercial feeds used in shrimp farms. Findings from our initial analyses indicated summed concentrations of seven PCB congeners (PCBs 28, 52, 101, 106/118, 138, 153, and 180) did not vary significantly between wild-caught and farm-raised shrimp; however, a significant difference in PCB concentrations among shrimp samples from different countries was observed.[1] As expected, the distribution of PCB concentrations in uncooked shrimp was fairly skewed, with several samples having very high concentrations of PCBs. Given that food and food products are a primary pathway of exposure to PCBs, with seafood being the major contributor to total dietary intake,[2] we further sought to characterize and understand samples with unusually high levels of these chemicals. Accordingly, in this study, we examined corresponding sampling information, the number of detected congeners, and congener and homologue patterns to help identify potential sources of variation in the observed concentrations for samples with outlying values.

Materials and Methods
Detailed methodology regarding sample collection and analysis has been described previously[1]. Briefly, 84 raw shrimp samples, three cooked shrimp samples, and three raw crab samples were randomly acquired from local fish markets, supermarkets, and grocery stores in the San Francisco and Sacramento areas in Northern California between February and April 2009. Samples were analyzed by Vista Analytical Laboratory (El Dorado Hills, CA) using high resolution gas chromatography-mass spectrometry according to the EPA Method 1668. Samples with non-detected concentrations were assumed to have a value equal to the LOD divided by the square root of two.

Total PCB concentrations were estimated using all 168 congeners/congener pairs (∑tPCB), all congeners in which more than 50% of the samples had concentrations greater than the congener specific LOD (∑51 PCB), the seven indicator congeners (∑7 PCB: PCBs 28, 52, 101, 106/118, 138/163/164, 153, and 180), and the 12 congeners with dioxin-like activity (∑DLC PCB: PCBs 77, 81, 126, 169, 105, 114, 106/118, 123, 156, 157, 167, and 189). For this investigation, the average percent contribution of selected congeners was calculated by averaging the percent contribution of the specified congener to the ∑51 PCB.

Only data for uncooked shrimp were evaluated in these analyses. A probable outlier was defined as a sample with ∑51 PCB concentrations more than three times the interquartile range and a possible outlier was defined as a sample with ∑51 PCB concentrations between 1.5 and 3.0 times the interquartile range. For the purposes of this assessment, we focused only on outliers at the upper end of the distribution as these are most relevant to human exposure and risk. All data analyses were completed using SAS software (Cary, NC).

Results and Discussion

Our evaluation of ∑51 PCB concentrations in uncooked shrimp samples identified ten possible and probable outliers (hereafter referred to as "outliers"). Table 1 presents the ∑tPCB, ∑51 PCB, and ∑7 PCB concentrations for each outlier as well as the sample type (i.e. wild vs. farmed) and country of origin. Of these ten samples, 60% were wild-caught and 40% were farm-raised. Only one sample (outlier #10) did not originate in a North American country. Fifty percent of the samples were from the United States (U.S.), followed by Belize (20%), Panama (10%), Mexico (10%), and Ecuador (10%). Of the samples from the U.S., 80% were wild-caught.

∑51 PCB concentrations ranged from 349 to 2024 pg/g wet weight with an average concentration of 1040 pg/g wet weight. Although ∑51 PCB levels for these ten samples are considered outliers in this study, they are relatively similar to concentrations measured in wild-caught shrimp from Thailand (range ∑49 PCBs: 50-2700 pg/g wet weight) and wild-caught and farm-raised shrimp collected in Canada (both domestic and imported samples) (range ∑36 PCBs: 42-1980 pg/g wet weight).[3,4] In addition, average ∑7 and ∑51 PCB concentrations for these ten outliers were consistent with the ∑11 PCB level measured in pooled shrimp samples from Catalonia, Spain and average ∑PCB concentrations measured in two shrimp samples from Zhoushan, China, respectively.[5,6]

Interestingly, the two samples from Belize, which had the highest PCB concentrations of all uncooked shrimp samples, were obtained from two separate, independent markets in the San Francisco area. Given that both of these outliers were farm-raised samples, the high levels observed may be due to contaminated feed or even a local point source if the samples came from the same farm. All other outliers with ∑51 PCB concentrations >1000 pg/g wet weight were wild-caught. Three of these were from the U.S. and one was from Mexico. Given that shrimping for warm-water shrimp in these countries predominantly occurs in the Gulf of Mexico,[7] it is not unreasonable to suggest a regional source of contamination may have impacted the PCB loading of these samples.

Table 1: PCB concentrations and other relevant information for ten outliers

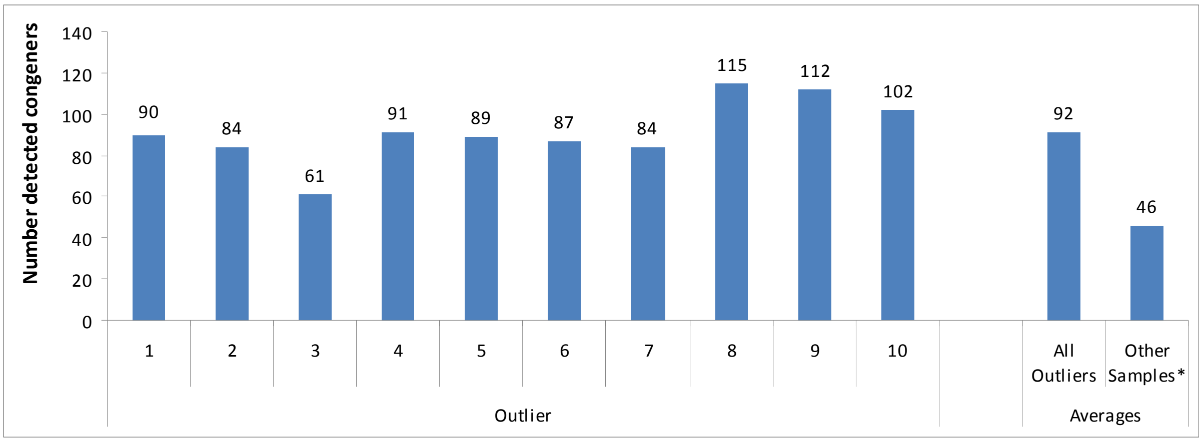
Outlier	Sample Type	Country	∑tPCB	∑51 PCB	∑7 PCB
1	Wild	Panama	474.0	378.7	163.1
2	Wild	USA	1648.3	1030.4	166.3
3	Farm	USA	607.5	501.6	218.3
4	Wild	USA	661.7	547.8	233.4
5	Wild	Mexico	1227.1	1075.2	474.8
6	Wild	USA	1476.8	1313.7	600.3
7	Wild	USA	1472.3	1296.2	595.8
8	Farm	Belize	2230.9	1885.4	908.3
9	Farm	Belize	2403.3	2024.3	962.4
10	Farm	Ecuador	470.3	348.7	128.3

Table 2 presents the ∑51 congeners detected for each of the ten outliers. Interestingly, all of these congeners were detected in the majority of outliers, except for PCB 3. Though this congener was detected in more than 50% of all uncooked shrimp samples (a prerequisite of the ∑51 profile), it was detected only in outliers #8 and #10. Thirty-nine congeners in Table 2 were detected in all the samples with outlying values. Other congeners with measurable levels for all ten of these samples included PCBs 42, 154, 174, 195, 202, 206, and 209. Not surprisingly, the outliers all had a greater number of detected congeners than the average number of detected congeners for all other uncooked shrimp samples (Figure 1). In fact, the average number of detected congeners for the outliers was 92, double the average number of detected congeners for all other uncooked shrimp samples.

Table 2: Detected congeners (for selected congeners) by outlier

	Outlier									
PCB Congener	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
PCB 3								X		X
PCB 5/8		X		X	X	X	X	X	X	X
PCB 11	X	X	X	X	X	X	X	X	X	X
PCB 16/32	X			X	X	X	X	X	X	X
PCB 18	X	X			X	X	X	X		X
PCB 28	X	X	X	X	X	X	X	X	X	X
PCB 31	X		X	X	X	X	X	X	X	X
PCB 41/64/71/72	X	X	X	X	X	X	X	X	X	X
PCB 43/49	X	X	X	X	X	X	X	X	X	X
PCB 44		X	X	X	X		X	X	X	X
PCB 47	X	X	X	X	X	X	X	X	X	X
PCB 52/69	X	X	X	X	X	X	X	X	X	X
PCB 56/60	X	X	X	X	X	X	X	X	X	X
PCB 61/70	X	X	X	X	X	X	X	X	X	X
PCB 74	X		X	X	X	X	X	X	X	X
PCB 76/66	X	X	X	X	X	X	X	X	X	X
PCB 77	X	X	X	X	X			X	X	X
PCB 85/116	X	X	X	X	X	X	X	X	X	X
PCB 87/117/125	X	X	X	X	X	X	X	X	X	X
PCB 90/101	X	X	X	X	X	X	X	X	X	X
PCB 97	X	X	X	X	X	X	X	X	X	X
PCB 99	X	X	X	X	X	X	X	X	X	X
PCB 105	X	X	X	X	X	X	X	X	X	X
PCB 106/118	X	X	X	X	X	X	X	X	X	X
PCB 107/109	X	X	X	X	X	X	X	X	X	X
PCB 110	X	X	X	X	X	X	X	X	X	X
PCB 128/162	X	X	X	X	X	X	X	X	X	X
PCB 130	X	X	X	X	X	X	X	X	X	X
PCB 133/142	X	X	X	X	X	X	X	X	X	X
PCB 138/163/164	X	X	X	X	X	X	X	X	X	X
PCB 139/149	X	X	X	X	X	X	X	X	X	X
PCB 141	X	X	X	X	X	X	X	X	X	X
PCB 146/165	X	X	X	X	X	X	X	X	X	X
PCB 153	X	X	X	X	X	X	X	X	X	X
PCB 156	X	X		X	X	X	X	X	X	X
PCB 157	X		X	X	X	X	X	X	X	X
PCB 158/160	X	X	X	X	X	X	X	X	X	X
PCB 167	X		X	X	X	X	X	X	X	X
PCB 170	X	X	X	X	X	X	X	X	X	X
PCB 171	X	X	X	X	X	X	X	X	X	X
PCB 172	X	X	X	X	X	X	X	X	X	X
PCB 177	X	X	X	X	X	X	X	X	X	X
PCB 178	X	X	X	X	X	X	X	X	X	X
PCB 180	X	X	X	X	X	X	X	X	X	X
PCB 182/187	X	X	X	X	X	X	X	X	X	X
PCB 183	X	X	X	X	X	X	X	X	X	X
PCB 190	X	X	X	X	X	X	X	X	X	X
PCB 193	X	X	X	X	X	X		X	X	X
PCB 194	X	X	X	X	X	X	X	X	X	X
PCB 196/203	X	X	X	X	X	X	X	X	X	X
PCB 199	X	X	X	X	X	X	X	X	X	X

Figure 1: Number of detected congeners for each outlier and average number of detected congeners for all outliers and all other uncooked shrimp samples.



*Uncooked shrimp excluding the nine outliers (*N*=74)

The percent contribution of PCBs 28, 52/69, 90/101, 106/118, 138/163/164, 153 and 180 to ∑51 PCB concentration are presented for each outlier in Table 3. As expected, PCBs 153 and 138/163/164 contributed, overall, the most to ∑51 PCB concentrations. The congener pattern of all seven indicator congeners was reasonably consistent with the profile for all other uncooked shrimp samples, as demonstrated by the similar average percent contributions for each of the two groups. Both outlier #2 (wild, U.S.) and #10 (farmed, Ecuador) had very different profiles with respect to other outliers as well as the general pattern observed for all other shrimp samples. In particular, PCB 28 was the dominant congener for outlier #10 and contributed between 17 and 100 times more to PCB concentrations than the other outliers. For outlier #2, the percent contribution for each of the seven indicator PCBs was considerably lower than that observed for the other outliers.

Table 3: Percent contribution of select PCBs to ∑51 PCB concentrations

PCB Congener	Outlier										Average	
	1	2	3	4	5	6	7	8	9	10	Outliers (*N*=10)	All others (*N*=74)
28	0.7	0.2	1.1	1.2	0.7	1.1	0.6	0.6	0.5	20.2	2.7	2.3
52/69	1.0	0.3	0.5	1.7	2.0	1.4	2.0	0.1	0.2	1.7	1.1	1.1
90/101	5.5	1.9	7.7	5.7	6.8	5.1	7.1	7.5	6.4	3.0	5.7	5.2
106/118	6.0	2.6	6.1	5.7	5.7	6.1	6.1	6.8	6.4	3.6	5.5	6.4
138/163/164	11.4	3.1	10.7	10.6	11.7	12.1	11.1	11.3	11.4	3.3	9.7	8.6
153	15.4	5.3	13.3	14.1	13.2	14.8	14.1	16.4	16.9	3.9	12.7	10.9
180	3.0	2.6	4.1	3.7	4.0	5.1	4.9	5.4	5.8	1.2	4.0	3.9

More notably, although the congener profiles were similar between the two groups, homologue fractions varied considerably, particularly for the mono-, di-, octa-, nona- and deca-PCBs (Table 4). Indeed the average di-PCB homologue fraction for outliers was approximately 1/10th of that for the other shrimp samples and the average deca-PCB homologue fraction for the outliers was nine times that for the other uncooked shrimp. For most of the outliers, hexa-PCBs were the dominant homologue, followed by penta-, hepta-, and tetra-PCB homologues. As with congener pattern, however, the two exceptions were outliers #2 and #10, which had dominant homologues of octa- and nona-PCBs and tri- and tetra-PCBs, respectively.

Table 4: Homologue fraction (%)

PCB Homologue	Outlier										Average	
	1	2	3	4	5	6	7	8	9	10	Outliers (*N*=10)	All others (*N*=74)
Mono-PCBs	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	1.7
Di-PCBs	0.5	0.4	0.5	0.9	0.9	0.5	0.4	0.2	0.1	5.4	1.0	9.5
Tri-PCBs	1.8	0.2	1.6	2.3	1.8	2.3	1.3	1.1	0.9	37.9	5.1	5.9
Tetra-PCBs	8.9	2.1	13.1	12.4	12.7	10.5	12.2	5.8	4.9	30.8	11.3	11.2
Penta-PCBs	24.5	5.5	27.4	25.1	26.6	22.2	27.3	23.4	20.9	11.8	21.5	23.6
Hexa-PCBs	39.4	9.8	36.0	35.7	37.1	38.3	37.3	35.5	36.5	9.6	31.5	32.8
Hepta-PCBs	17.5	17.5	16.8	17.2	16.6	19.8	16.5	19.5	21.6	3.5	16.7	13.0
Octa-PCBs	5.4	44.0	3.7	4.8	3.8	5.5	4.1	8.4	8.9	0.7	8.9	2.1
Nona-PCBs	1.0	18.8	0.7	0.8	0.4	0.7	0.5	4.4	3.9	0.1	3.1	0.1
Deca-PCBs	0.9	1.7	0.3	0.7	0.2	0.3	0.2	1.9	2.3	0.1	0.9	0.1

For outliers #1, 4, 5, 6, and 7 (all wild samples from the U.S., Mexico and Panama), both the congener profiles and homologue fractions were very similar among these samples, indicating that PCB levels in shrimp from the Gulf region may be a result of broader regional contamination. In contrast, while the congener patterns and homologue fractions for outliers #8 and #9 (both farmed samples from Belize) are highly comparable with each other, they vary just enough from the profiles and homologue patterns of other Gulf Coast samples to suggest that excess PCB levels in these outliers may be due to contaminated feed or a more local point source.

Taken together, the results of this follow-up investigation provide useful information with regard to potential sources of contamination for samples with high levels of PCBs and may likely explain much of the variation in these concentrations. Additional analyses using other statistical methods, such as principal component analysis, and other definitions for outliers may also be useful in further understanding potential sources of contamination that are contributing to PCB loadings for these samples.

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