BACKGROUND CONTAMINATION BY COPLANAR POLYCHLORINATED BIPHENYLS (PCBs) IN TRACE LEVEL HIGH RESOLUTION GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMETRY (HRGC/HRMS) ANALYTICAL PROCEDURES

Joseph Ferrario, Christian Byrne, Aubry E. Dupuy, Jr.

jferrario@nrlssc.navy.mil

U. S. Environmental Protection Agency,

Office of Prevention, Pesticides, and Toxic Substances, OPP/BEAD/ACB,

Environmental Chemistry Section, John C. Stennis Space Center, Mississippi 39529 USA

Abstract

The addition of the "dioxin-like" polychlorinated biphenyl (PCB) congeners to the assessment of risk associated with the 2,3,7,8-chlorine substituted dioxins and furans has dramatically increased the number of laboratories worldwide that are developing analytical procedures for their detection and quantitation. Most of these procedures are based on established sample preparation and analytical techniques employing high resolution gas chromatography/ high resolution mass spectrometry (HRGC/HRMS), which are used for the analyses of dioxin/furans at low parts-per-trillion (ppt) levels. A significant and widespread problem that arises when using these sample preparation procedures for the analysis of coplanar PCBs is the presence of background levels of these congeners. Industrial processes, urban incineration, leaking electrical transformers, hazardous waste accidents, and improper waste disposal practices have released appreciable quantities of PCBs into the environment. This contamination has resulted in the global distribution of these compounds via the atmosphere and their ubiquitous presence in ambient air. The

background presence of these compounds in method blanks must be addressed when determining the exact concentrations of these and other congeners in environmental samples.

In this study reliable procedures were developed to accurately define these background levels and assess their variability over the course of the study. The background subtraction procedures developed and employed increase the probability that the values reported accurately represent the concentrations found in the samples and were not biased due to this background contamination. **Published by Elsevier Science Ltd**

Introduction

The USEPA Dioxin Exposure Initiative has recently required that the dioxin-like PCB congeners be added to the list of 2,3,7,8-chlorine substituted dioxins and furans when considering the total toxic equivalent (TEQ) resulting from the various congeners. As part of the program to assess these dioxin-like congeners in various food items, beef fat samples ,which were originally collected as part of a statistically designed national survey to determine the concentrations of dioxins and furans, were re-analyzed for the following coplanar PCBs: the non-ortho 77, 126, 169, and the mono-ortho 105, 118, 156, and 157.

During the method development phase of the study, background contaminations from these PCBs were found in the method blanks at concentrations that compromised the analysis of samples at the desired low parts-per-trillion (ppt) levels. The various steps in the analytical procedure were isolated and checked in hopes of identifying and, thus, eliminating the source of the contamination. The solvents were checked by combining the appropriate amounts of all the solvents used in the procedure, fortifying the mixture with ^{13}C recovery surrogates, and analyzing the 20 μl concentrate. Besides a small amount of background that we concluded had originated from exposure to the air during the testing procedures, the reagents, glassware, equipment, and solvents were found not to be the source of the background. Upon further investigation, it was learned that the transformers for the fluorescent lighting in our laboratory facility contained PCBs as the dielectric fluid. Air samples taken in the laboratory matched the PCB pattern identified in the method blanks and the pattern of the Aroclor mixture removed from the transformer.

Although transformers were replaced with the PCB-free type, the background contamination was not reduced to an acceptable level. In addition, repeated efforts at modifying the analytical procedure to minimize exposure to the air failed to adequately reduce the contamination.

The sample preparation activities were then moved into a mobile laboratory trailer isolated from the main building. The results of air samples taken in the trailer showed a substantial reduction in PCB concentrations as compared to the main building; however, the concentrations still precluded the preparation of samples without unacceptable PCB contamination of the blanks. The trailer was subsequently equipped with a five-ton air conditioner and the air was passed through an air filtration system comprised of several layers of activated charcoal. The air flow was sustained at a level that resulted in the trailer maintaining positive pressure, thereby substantially reducing any inflow of unfiltered air. These efforts resulted in a reduction in the actual concentration and variability of these congeners in our method blanks. The results are presented in this paper and the background contamination compared with the findings of other investigators from around the world. The method blanks were then evaluated to assess the background and the variability of the blanks over the course of the study and to develop an approach to determine the minimum concentration of each congener that can be reliably distinguished from the background. Detection/quantitation limits with a known amount of associated risk of false positives are proposed based on the mean and variability of the background as determined from the method blanks.

Methods and Materials

During the course of this ten-week study to determine the concentration of the PCB congeners in beef fat, eighteen method blanks were generated. A sample set in this study consisted of 12 possible sample positions including one or two method blanks, one control matrix, one control matrix fortified with the native congeners, and eight or nine field samples. Glassware was washed with Chem-Solv® and warm water and then thoroughly rinsed with tap water. It was then rinsed with acetone, methylene chloride, and hexane and placed on aluminum trays. When the solvent had evaporated, the trays were loosely covered with aluminum foil and placed in an oven and baked for at least ten hours at 450°C. The oven was allowed to cool to 100°C and, while still

hot, the foil was sealed around the edge of the trays and the trays placed in the trailer to cool. The glassware was again immediately rinsed before use with the solvent to be used with or contained in that particular piece of glassware. In addition, the tissue homogenizer, evaporator-needle tips etc. were thoroughly rinsed immediately before use. The analytical and QA/QC methods employed were similar to those described in USEPA Method 1613[1] with modifications[2].

Briefly, a 10 g sub-sample taken from a 100 g homogenate of beef fat was fortified at 10 ppt with ^{13}C labeled surrogates for the seven coplanar PCBs mentioned earlier and extracted with methylene chloride using a tissue homogenizer. The lipid was removed by stirring the crude extract with acid-impregnated silica gel and passing the extract through an acid/base silica gel column. The coplanar PCBs were separated from the other non-target PCBs and from the dioxins and furans using a graphitized carbon column consisting of a 95/5 mixture of Biosil A (100/200 mesh) and AMOCO PX-21 carbon. The separations were achieved using the following elution procedure: 5 ml of 25/75 methylene chloride/hexane [this fraction contains the non-planar PCBs and was discarded]; 5 ml methylene chloride [this fraction contains the mono-ortho PCBs 105, 118, 156, and 157]; 14 ml 75/25 benzene/methylene chloride [this fraction contains non-ortho PCBs 77, 126 and 169]. The column was then reversed and the dioxins/furans eluted with 14 ml of toluene.

The two fractions containing the coplanar PCBs were combined, reduced in volume, fortified with ^{13}C internal standards and further reduced to a 20 μl final volume. One μl was analyzed using a 60 meter DB5-MS® column by HRGC/HRMS. The KRATOS Concept® mass spectrometer was operated in the mass drift correction mode and the native analyte concentrations were determined by isotope dilution.

All standards were purchased from Cambridge Isotope Laboratory Inc. as solutions and prepared in nonane. The concentrations of the target analytes in the calibration standards were selected to reflect the relative distribution of the various congeners found in the background air and method blanks and were prepared to bracket the expected sample concentrations. The concentrations of the various PCB congeners in picograms per microliter (pg/μl) in Calibration Standard #3 were as follows: 77 = 2.0, 118 = 50.0, 105 = 25.0, 126 = 0.5, 156 = 5.0, 157 = 1.0

and $169 = 0.5$. ^{13}C labeled surrogate recovery standards were present in all calibration standards at 5.0 pg/µl, and the two ^{13}C internal standards, PCB 111 and 128, were present at 10.0 pg/µl. All the native compounds in the remaining calibration standards were multiples of Calibration Standard #3 as follows: 0.25 x, 0.50 x, 2.5 x, 5.0 x and 10 x.

All solvents used in the analysis were obtained from Fisher Scientific and were OPTIMA® grade. All inorganic reagents were also obtained from Fisher Scientific and were ACS grade or better. The sulfuric acid used in the preparation of the acid silica gel column was Baker Ultrex® grade. The silica gel used in the lipid extraction and acid silica gel cleanup was a combination of Fisher Chromatographic Silica Gel, Davisil Grade 646, Types 60Å and 150Å. The HRGC/HRMS analytical column was a J&W Scientific DB-5MS® 60 meter, 0.32 mm widebore, 0.25 µm film thickness.

The heating/ventilation/air conditioning (HVAC) system used for the trailer was a Carrier Weathermaster® Series, 5-ton, 208 volt unit with an American Air Filtration (AAF) air handling system, using high efficiency pleated pre- and post-filters and a bank of eight G 210 activated carbon filters (84 cm x 84 cm x 2 cm, # 4 mesh). Initial environmental engineering calculations suggested that further carbon banks and other air filtration modifications would be required to reach the extremely low level reduction wanted; however, the cost was prohibitive.

Results and Discussions

The results of the analysis of method blanks generated before and after the installation of the HVAC/filtration system are presented in Table 1. As is evident from the table, these measures failed to completely eliminate the PCB background contamination from the air and detectable concentrations of several congeners remained as chronic contaminants in the method blanks. However, these efforts resulted in not only a reduction in the actual concentration of these congeners, but also a decrease in the variability associated with the various PCB congeners in the method blanks. The RSDs remain relatively high and emphasize the importance of systematically evaluating the background over the course of the study period.

As is evident from Table 1, the relative distribution of the PCBs observed in the method blanks remained constant during the course of the study. PCB 118 is always the congener found

at the highest concentration followed by PCBs 105, 156, 157, 77, 126, and 169. PCB 126 was not detected in all the method blanks and was only detected as the concentrations of PCBs 118 and 105 increase substantially. PCB 126 was approximately 0.2% of PCB 118. PCB 169 was never detected in the blanks [instrumental LOD = 0.025-0.050 pg/µl]. The relative distribution of these congeners in the blanks, contaminated from exposure to ambient air, is consistent with that found in various PCB commercial mixtures.

Table 1. **Concentrations of PCBs (pg/µl) in Method Blanks Before and After the Introduction of a HVAC/Filtration System, Final Volume = 20 µl**

PCB Congener	PCB 77	PCB 118	PCB 105	PCB 126	PCB 156	PCB 157	PCB 169
BEFORE (n = 14)							
Average	3.7	79	54	0.33	26	5.6	ND*
Std Dev	1.8	53	37	0.18	28	6.0	-
RSD	50	68	68	53	106	107	-
AFTER (n = 18)							
Average	1.4	35	15	0.07	4.0	0.8	ND*
Std Dev	0.4	14	6.0	0.02	2	0.4	-
RSD	31	41	41	27	49	51	-

* Not detected in method blanks: instrumental detection limit = 0.05 pg/µl

Commercial PCB mixtures contain PCBs 118, 105, and 156 at between 1-10% depending on the specific formulation, PCBs 77 and 157 at approximately 0.5-1.0%, and PCBs 126 and 169 at <0.05%[3]. J.C. Duinker et al [4] analyzed several Aroclors and Clophens as well as seal blubber using multidimensional GC/electron capture and observed this relative distribution in all tested materials. The relative proportions of the coplanar PCBs in water and in various aquatic biota can be appreciably altered from that found in the commercial mixtures but generally the relative distribution is constant[5].

The relative distribution for the most toxic non-ortho PCBs 77,126,169 in the method blanks of this study is consistent with the amounts and relative distribution found by other

investigators. J.de Boer *et al* [6] from the Netherlands found that background contamination from PCBs 77 and 126 in method blanks generated while preparing fish and marine mammal samples prevented the lowering of detection limits below 1.0 and 0.1 ppt, respectively. It is noteworthy that our detection limits were also limited by background concentrations of the same two congeners to virtually the same values of 1.0 and 0.3 ppt. J.A. van Rhijn *et al* [7] reported background contamination, especially from PCB 77, during the processing of Dutch milk samples for PCBs 77, 126, and 169. Similarly, D.G. Patterson *et al* [8] in the USA and H. Beck *et al* [9] in Germany both reported problems arising from PCB 77 background during the processing of milk and human adipose/milk samples for these three congeners. As more investigators analyze samples for the presence of additional planar PCB congeners (PCBs 105, 118, 156, 157), it is expected that they will detect background levels of these congeners in their method blanks at concentrations proportional to that found in ambient air.

Additional documentation of the problems caused from PCB contaminated laboratory air is provided by R.E. Alcock *et al* [10] in the United Kingdom. These investigators reported measurable increases in the concentrations of PCBs in freshly sampled ancient peat samples after only a few hours of exposure to laboratory air. Several days of exposure to laboratory air resulted in PCB contamination at levels similar to those found in contemporary U.K. field soils. They also noted, as have others, that the PCB concentration in ambient air is often greater indoors. This relationship was also observed at our facility presumably due to the leaking of PCBs from ballasts used in the fluorescent lighting system. Other laboratories in the U.S. conducting analyses for the USEPA Dioxin Exposure Initiative also have reported problems associated with background PCB contamination of method blanks with the same congeners reported here at remarkably similar concentrations and always displaying the same relative distribution.

It is interesting that laboratories from around the world are experiencing the same background problems arising from these ubiquitous contaminants. It appears that the widespread use and release of PCBs have resulted in their unwanted and persistent presence in the environment. It is ironic that the advances in technology that have allowed the progressive lowering of detection limits have reached a limit imposed by the very contaminants the technology was designed to measure. Nevertheless, this background contamination poses a

serious problem in terms of defining the limit of detection and quantitation above background that can be reliably detected for various sample matrices.

Background Subtraction and Detection Limits

Background contamination is an important consideration when analyzing samples for compounds at the ppt level that are also globally distributed in the environment. In fact, background contamination will define the lower limit of detection if it cannot be eliminated. In cases where background contamination is routinely present, the central issue to be resolved is the level above background that can be reliably determined to be "real" (i.e., contributed from the sample matrix). To define the level of background contamination and its variability over the course of a study, one must retrospectively examine the method blanks.

Upon completion of the analyses of all samples and method blanks associated with the project, the blanks were evaluated and the means and standard deviations were determined for all target analytes found in the blanks. The results from the analysis of the method blanks of this study are shown in Table 2 with the mean and standard deviation for each congener. Included in the table is the mean + 1 standard deviation (s) and the mean + 2 standard deviations (s) for each congener. For the majority of the method blanks, there was a positive response (area counts above baseline) at the retention time for PCB 126; however, isotope and S/N ratios were insufficient for a positive identification. Nevertheless, a background value was generated since it would contribute to the amount reported in samples, if not properly subtracted. PCB 169 was not detected in the method blanks at the instrumental detection limit of 0.05 pg/μl.

Table 2. **Concentrations of the PCBs (pg/μl) in Method Blanks (n=18), Final Volume = 20 μl**

PCB Congener	Mean	Std Dev (s)	RSD	Mean + 1s	Mean + 2s
77	1.39	0.44	31.4	1.83	2.26
118	33.7	13.8	40.9	47.5	61.3
105	15.6	6.49	41.6	22.1	28.6
126	0.07	0.02	30.7	0.09	0.11

156	3.86	1.91	49.5	5.77	7.68
157	0.78	0.41	51.6	1.18	1.59
169	-	-	-	-	-

After examining the blanks, it was decided to subtract from each sample analyzed over the course of the study the mean value + 2s for each congener. These resulting values subtracted for the congeners were greater than the background concentration found in 95% (17/18) of the method blanks and, frequently, were greater than all the method blanks. If one were to subtract the mean + 1s for each congener, the resulting values subtracted would be greater than the background concentration in only 67% of the method blanks. Subtraction of just the means would have resulted in an even greater probability of false positives arising from background. These relationships are graphically depicted in Figure 1, which shows the results of the concentrations of PCB 118 in the method blanks. By subtracting the mean +2s rather than just the mean or the mean + 1s, the likelihood of reporting positive results for samples when the compounds were actually derived from the background is substantially reduced to less than five percent.
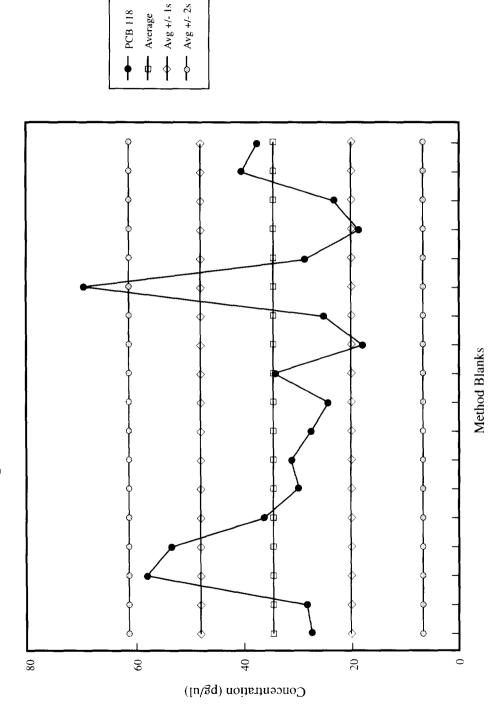
Average of PCB 118 in Method Blanks

Method Blanks

Figure 1. **Average of PCB 118 in Method Blanks**

Our observations regarding the analyses of multiple method blanks processed on the same day and multiple blanks processed on different days over the course of several months revealed that considerable variation can exist in the amount of the background under both conditions. The approach described above addresses this variation over the course of the study in determining the amount of background to subtract, takes this random variation into consideration, and adjusts the amount subtracted accordingly.

This method of background subtraction is quite conservative and increases the possibility of false negatives for values close to the detection limits. However, it increases the confidence associated with concentration values near the LOD and minimizes the likelihood of false positives. In the presence of consistent background the probability of false positives becomes greater than that for false negatives. Therefore, the risk of reporting false positives should be proportionally reduced (by subtracting a certain amount of background) based on the mean and the variability of the measurement of interest, which, in this case, is the standard deviation of the mean of the blanks.

After subtracting the background, another issue that must be addressed is defining the minimum amount of each congener that can be reliably distinguished from the average amount of background present. This minimum amount functionally defines the method LOD. No value for an analyte was reported until the amount remaining after background subtraction exceeded $1s$ of the mean concentration of the blank values for that particular congener. The value (s) then becomes a critical factor in defining the method detection limit, since it is an objective determination of the minimum amount that can be reliably distinguished from that present in the blanks. This procedure for the determination of the method detection limits, like that used for background subtraction, considers not only the amount of the background for each congener, but also the variability of each for the duration of the study. The values for $1s$ are then used to calculate the above-background detection limit based on a 10 g sample. For example, the standard deviation for PCB 118 found in Table 2 is 13.8 pg/μl (approximately 15.0 pg/μl). This corresponds to a LOD of 30.0 ppt.

To examine the validity of this approach, four replicate subsamples were fortified with the PCB congeners at concentrations approaching the detection limits as determined using the

procedure described above. Subsamples from a beef fat sample homogenate were fortified rather than method blanks to demonstrate the detection and quantitation of the "spike" using real samples. In this way the effects of the matrix on analyte recovery and/or any problems associated with coextracted interferences would be addressed. As would be expected, the concentrations of the various PCB congeners measured in the control matrix were higher than the average method blank since the total amount present was a combination of contamination from background and the amount present in the beef tissue. Therefore, the fortification level was increased proportionally to compensate for this increased background. Again using PCB 118 as an example; the unspiked control contained 75.2 pg/µl , approximately twice the mean value of 33.7 pg/µl in the method blanks. Therefore, the fortification level was approximately doubled from 13.8, the standard deviation for PCB 118, to 25 pg/µl. These fortification levels in pg/µl are listed in Table 3 and correspond approximately to two times the value of s.

Table 3.   **Beef Fat Samples Fortified at the LOD (in pg/µl), Final Volume = 20 µl**

PCB Congener	Unspiked Control: Mean Conc	Spiking Level	Conc Expected in Spiked Sample	Conc Found in Spiked Sample	% Difference	Standard Deviation	RSD
77	1.7	2.5	4.2	4.1	2.4	0.26	6.3
118	75.2	25.0	100.2	114.1	12.2	11.9	10.4
105	26.7	12.5	39.2	44.1	11.1	5.4	12.2
126	0.5	0.25	0.75	0.79	5.1	0.08	10.1
156	9.8	2.5	12.3	14.3	14.0	1.5	10.5
157	2.1	0.5	2.6	3.2	18.8	0.3	9.4
169	0.08	0.25	0.33	0.33	0.0	0.02	6.1

In Table 3 are listed the mean values measured for each congener in the control and the fortified tissue along with the (s) and the RSD. As is evident from Table 3 there is close agreement between the expected and observed results and low RSDs. This illustrates that the precision and accuracy of the procedure even at these low fortification levels above background

are within the acceptable 20% target range. Moreover, these results prove that the value of $1s$ of the mean of the blanks is a reliable indicator of the minimum amount that can be consistently distinguished from background and thereby can be used to objectively determine a method LOD when background is present.

These procedures resulted in the following LODs/LOQs above background (in ppt): PCB 77, 1.0/1.0; PCB 118, 30/30; PCB 105, 15/15; PCB 126, 0.3/0.3; PCB 156, 4/4; PCB 157, 1.0/1.0; PCB 169, 0.2/0.3. As is evident above the LODs and LOQs are equal for all the congeners except PCB 169. As proposed herein, these values were used to establish our LODs. Since these minimum amounts are well above our instrumental detection limits and are in fact above our lowest calibration standard, they also define the LOQs. For PCB 169, the LOD is less than the LOQ and illustrates the situation that exists in the absence of background; where the LOD is defined as that amount below which a signal- to-noise ratio of three can not be consistently obtained and the ion ratios of characteristic ions from the molecular ion clusters are not reproducible.

When analyzing samples for compounds that are globally distributed via the atmosphere, the importance of evaluating the method blanks in a systematic fashion cannot be overemphasized. By calculating the means and standard deviations for each target compound in the blanks generated over the course of the entire study period, one can determine the amount and the variability of the background. This information can then be used to objectively decide the acceptable level of risk from false positives arising from the background and choose the background subtraction procedure and reporting limits accordingly[11].

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