PRINCIPAL COMPONENTS ANALYSIS (PCA) OF PBDE CONCENTRATIONS IN FISH TISSUE FROM SOUTHERN MISSISSIPPI

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Introduction

Polybrominated diphenyl ethers (PBDEs) are commonly measured in the environment, wildlife and human tissues; however, the primary sources of release to these media not yet well understood. The objective of this study was to apply Principal Components Analysis (PCA) to a robust dataset which evaluated 43 PBDE congeners in 61 individual catfish samples from Southern Mississippi (both farmed and wild)[1], in an effort to further characterize trends among fish type and collection locations relative to commercial mixtures. PCA is a multivariate statistical technique that is used to reduce the dimensions of a data set so that trends in the data can be visualized more easily. This approach was used to conduct two analyses. The first PCA included only the fish tissue data collected across southern Mississippi. The purpose of this PCA was to determine if the samples could be distinguished by their sampling location or sample type (e.g. Leaf River vs. Mississippi River or farm raised vs. wild caught). All 43 PBDE congeners measured in the catfish samples were used in this analysis. The second PCA included all fish tissue data as well as data from 6 widely used technical flame-retardant mixtures of PBDEs[2]: penta-PBDEs DE-71 and Bromkal 70-5DE, octa-PBDEs DE-79 and Bromkal 79-8DE, and deca-PBDEs Saytex 02E and Bromkal 82-ODE. The purpose of this PCA was to determine whether any of these commercial mixtures show similar PBDE profile characteristics to the fish tissue samples. In this PCA, there were 18 congeners that were present in both commercial mixture characterizations and analyzed in our fish tissue samples; only these 18 congeners were used in the second PCA.

Materials and methods

PBDEs in Catfish

Measurements of 43 PBDE congeners in 61 Southern Mississippi catfish were previously reported by Staskal et al (2008)[1]. Briefly, 28 farm-raised catfish samples were purchased from local grocery/seafood markets (SM) or catfish farms (CF) throughout southern Mississippi and 33 wild catfish were collected from two locations along the Leaf River (LR), one location along the Mississippi River (MR), and two locations along the Pearl River (PR); one of which is referred to as the Ross Barnett Spillway (RBS). Fish tissue samples were analyzed for 43 PBDEs (mono-throuth deca- congeners) using high resolution gas chromatography-mass spectrometry (HGC-MS). BDEs 8 and 11, 12 and 13, and 28 and 33 co-eluted; each co-eluting pair was reported as a single concentration (i.e., reported as BDE 8/11, 12/13 and 28/33). Non-detect concentrations were assumed to have a concentration equal to the limit of detection (LOD) divided by the square root of two. Lipid-adjusted concentrations were used for PCA.

General Approach

In PCA, a set of correlated variables is transformed into a subset of factors, or principal components, that are linear functions of the original variables and are not correlated with each other. Each factor explains a percentage of the variance in the original data; with the first factor explaining the largest proportion and each subsequent factor explaining successively less of the proportion of variance. A factor describes a concentration profile for the chemicals included in the PCA. In some cases, a factor profile may mimic a known source, indicating the contribution from that source. PCA analysis yields two main components: factor loadings and factor scores. Factor loadings are coefficients that indicate the relative contribution (positive or negative) of each chemical in the PCA to a particular factor. Factor scores indicate the plotting position of each sample in a new coordinate system described by the factors. Together, these provide data to graphically evaluate the congener signatures in the fish tissue and commercial mixtures.

Data Analysis

To decrease the impact of differences in measurement scale among the congeners for the PCA, PBDE concentrations were range transformed. First, the percent contribution to total concentration for each chemical was

calculated for each sample (both fish tissue and commercial mixtures) by dividing each PBDE congener by the sum of all PBDE concentrations for that sample, as appropriate. The effect of this calculation was to scale all concentrations for an individual between zero and one. The data were then range transformed for each chemical across all samples included in a PCA, using the transformation presented below:

$$r`_{ij} = \frac{(r_{ij} - r_{\min j})}{(r_{\max j} - r_{\min j})}$$

where

r_{ij} = Relative concentration of the *j*th congener in the *i*th sample
$r_{min\,j}$ = Minimum relative concentration of the *j*th congener
$r_{max\,j}$ = Maximum relative concentration of the *j*th congener

The purpose of the range transformation was to minimize the effects of large concentrations on the percent contribution calculation. After range transforming the data, PCA was performed using the factor analysis tool in SYSTAT for Windows, version 11.00.01 (2004). Because the data set was range transformed, the covariance matrix option was used. The minimum eigenvalue criterion was set at 0.0001. The focus of this study is on factors explaining more than 5% of variance. Rotation and resampling options were not used.

Results and discussion

PCA including fish tissue concentrations

Factors 1, 2, and 3 describe 42%, 15% and 10% percent of the variance associated with this data, respectively (a total of 67% of variance described). From the factor score plots in figure 1, several interesting trends are notable. First, the samples collected from the catfish farms (CF) and seafood markets (SM) tended to plot together for all factors, and are separate from the wild-caught catfish samples. Of the wild-caught samples, catfish from the Leaf River (LR) were generally segregated from catfish collected from other rivers in all plots based on negative factor 1 and positive factor 2 and 3 scores. Pearl River (PR), Ross Barnett Spillway (RBS) and Mississippi River (MR) samples tend to plot together for all scores, although some MR samples share common plotting position with the CF and SM samples.

Figure 3 illustrates the fingerprints associated with each factor. Factor 1 tends to show negative scores for BDE 47, 99, 100, 153, and 154 and positive for all others. These congeners are associated with strong negative factor 1 scores such as samples from PR, RBS, and MR (Figure 1). The relative absence of these congeners would characterize CF/SM samples. Factor 2 shows positive loading for BDE 85, 99, 138, 53, 207, and 209. These congeners are associated with strong positive factor scores such as samples from LR, and a few CF/SM samples. Factor 3 shows negative loading for BDE 17, 49, 207, and 209; characteristics which appear to be unique to the farmed samples (i.e., CF and SM).

PCA including fish tissue and commercial mixture concentrations

Factors 1, 2, 3, and 4 describe 26%, 23%, 15% and 8% percent of the variance associated with this data, respectively (a total of 72% of variance described). Figure 2 illustrates the factor score plots for the PCA, and Figure 4 illustrates the loading associated with each factor. An association was observed between catfish samples and penta-BDE mixtures, based on factor 2 and a distinct contrast with octa- and deca- mixtures (Figure 2). Factor 2 loading, depicted in Figure 4, shows strong positive loading associated with BDE 17, 28/33, 47, 66, 100, and 154; and negative loading for BDE 183-209. Positive loaded congeners are associated with catfish and penta-BDE mixtures, while negative loading was associated with octa- and deca-BDE mixtures.

Other factors did not show a similar remarkable trend. Generally, positive factor 4 scores characterized octa-BDE mixtures; associated with BDE 66, 183, 197, and 207 and negative factor 3 scores characterized deca-BDE mixtures; associated with BDE 49. Positive factor 3 scores tend to characterize penta-BDE mixtures; associated with BDE 99, 126, and 138. None of these associations demonstrated a particular relevance to catfish samples.

Conclusion

PCA can be an effective tool for comparing PBDE congener profile in catfish collected from various locations.. In the first PCA analysis presented, samples purchased at a seafood market or catfish farm plotted together, but separately from the wild-caught catfish. Catfish from the Leaf River were even further distinguished from the other

wild-caught fish. These findings demonstrate the potential variability of PBDE exposure, even in a study which evaluated catfish from a small region of the United States. The differences observed between farmed and wild fish may likely be a function of the diet. However, the differences observed between the collection locations of the wild-caught catfish is less clear, but may indicate proximity to a release source in some of the fish.

Results of the second PCA analysis indicated that only penta-BDE mixtures demonstrated similarity with fish tissue samples. This finding is generally consistent with other studies which have evaluated congener profiles found in fish, other food products, as well as in human tissue. However, evaluation of profiles relative to potential sources of release is incredibly limited for PBDEs given that release sources have not been well characterized. For example, congener profiles associated with releases of dioxins are generally well characterized (e.g., incineration processes); but for PBDEs, evaluation was limited to comparison of commercial mixtures rather than direct sources of release into the environment. Further understanding of the fate and transport of these compounds will greatly enhance the ability to evaluate PBDE profiles in humans, wildlife and the environment.

References:

1. Staskal, D. F.; Scott, L.L.F; Williams, E. S.; Haws, L. C.; Nguyen, L. M.; Luksemburg, W. J.; Birnbaum, L. S.; Paustenbach, D. J.; Harris, M. A., *Chemosphere* **Submitted**.
2. La Guardia, M, Hale, RC, and Harvey, E. (2006)..*Environmental Science and Technology*. Oct 15;40(20):6247-54

Figure 1: Factor score plots for PCA including fish tissue data

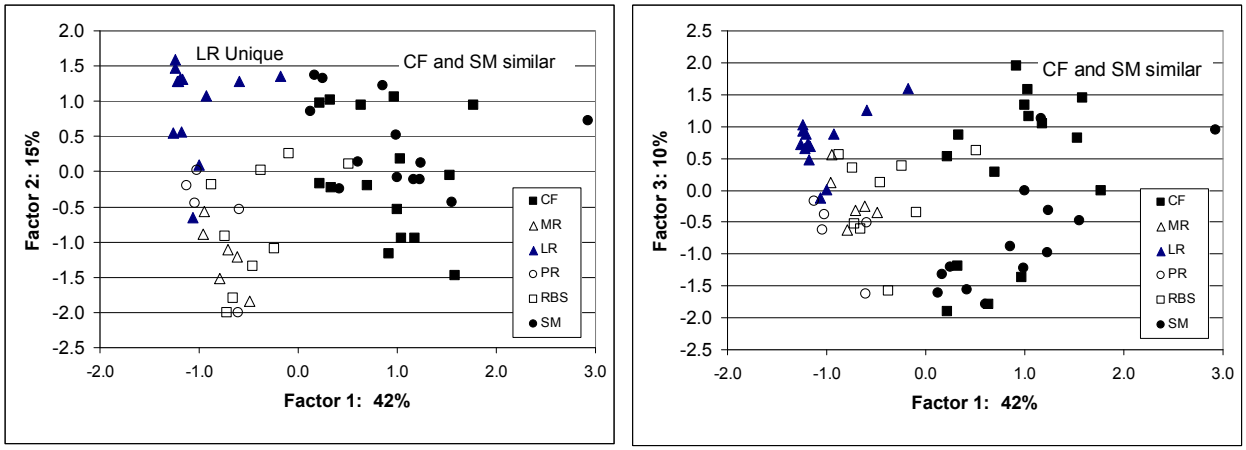

Figure 2: Factor score plots for PCA including fish tissue and industrial source data

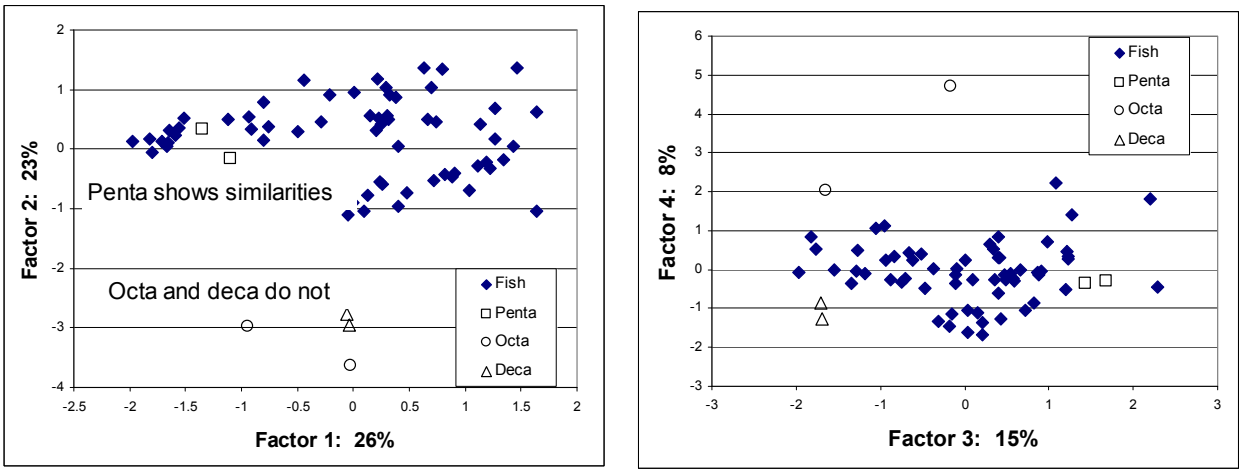

Figure 3: Factor loading plots for PCA including fish tissue data

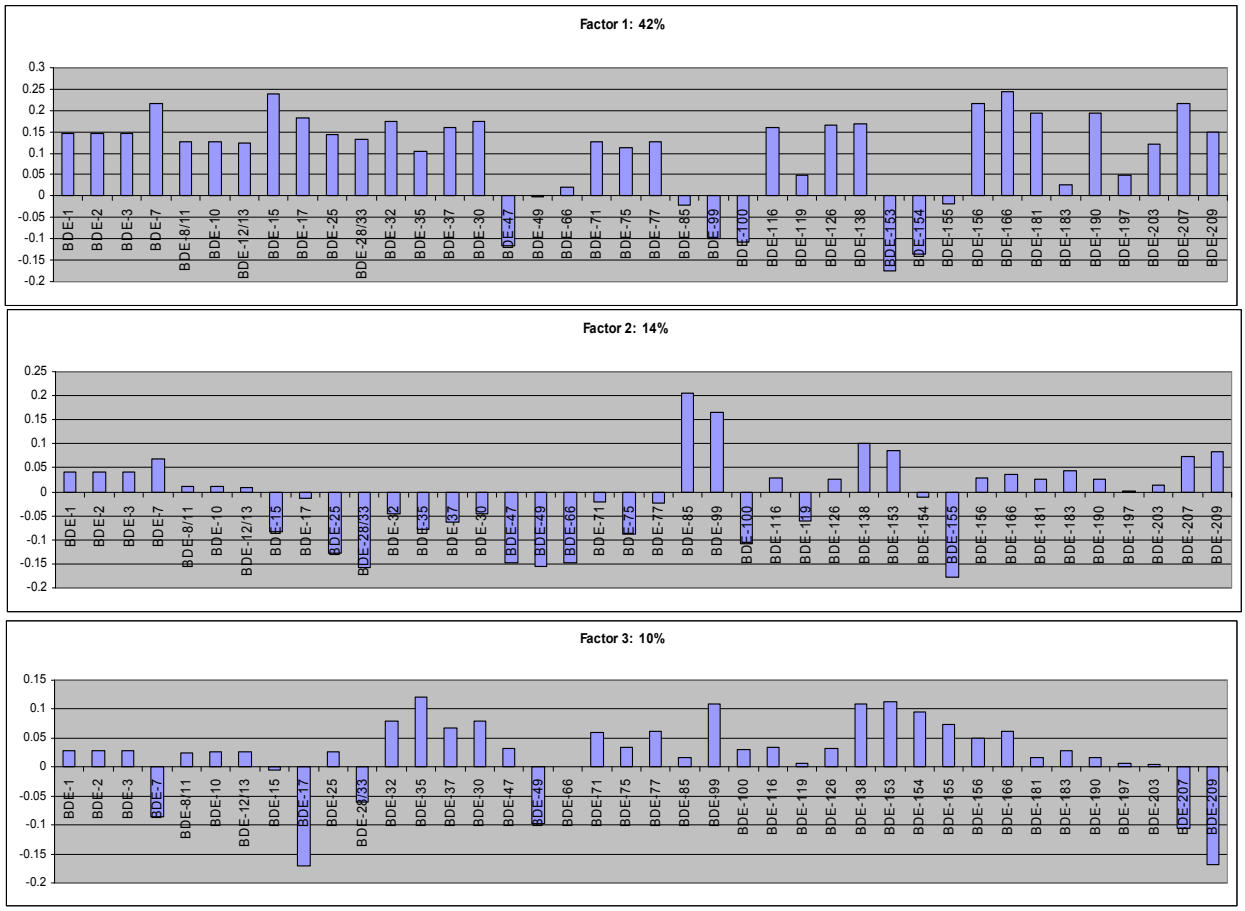

Figure 4: Factor loading plots for PCA including fish tissue and industrial source data