AGE ADJUSTMENT APPROACHES FOR MULTIVARIATE ANALYSIS OF SERUM DIOXINS AND FURANS

[1]Towey, Timothy; [1]Barabás, Noémi; [2]Demond, Avery; [3]Hedgeman, Elizabeth; [3]Garabrant, David; [2]Adriaens, Peter

[1]LimnoTech, Inc., 501 Avis Dr. Ann Arbor MI 48108 USA; [2]Department of Civil and Environmental Engineering, 1351 Beal Ave, The University of Michigan, Ann Arbor, MI 48109 USA; [3]School for Public Health, 1420 Washington Heights, The University of Michigan, Ann Arbor, MI 48109 USA

Introduction

Polytopic vector analysis (PVA) is a multivariate statistical technique used to evaluate source contributions to complex chemical mixtures. PVA was applied to the University of Michigan Dioxin Exposure Study (UMDES) serum dataset to evaluate possible exposure sources of dioxin-like compounds and to compare exposure sources between the UMDES populations. Analysis of the unadjusted serum data suggested that the congener patterns were strongly correlated with age, and that the patterns may be more representative of differential congener elimination rather than differential exposure. In order to obtain patterns that may be more representative of exposure sources, three methods for adjusting for age were applied. Results from all three methods suggest that some regional differences are evident among the study populations in terms of serum congener patterns.

Materials and Methods

Serum Collection

As part of the UMDES, serum samples were collected from 946 individuals from five study populations: 1) Individuals whose property is in the floodplain of the Tittabawassee River (Floodplain - FP); 2) Individuals who live in census blocks that intersect the Tittabawassee River floodplain, but whose property is outside of the floodplain (Near Floodplain - NFP); 3) Residents of Midland and Saginaw counties living outside of the Tittabawassee River floodplain census blocks and incinerator plume (Other Midland/Saginaw - MS); 4) Residents of the City of Midland living downwind of a former Dow incinerator (Plume - PL); 5) Residents of Jackson and Calhoun counties, which served as the comparison population (Jackson/Calhoun - JC). Details of respondent selection and collection procedures are presented elsewhere[1].

Samples were analyzed for the 29 WHO designated dioxin, furan, and PCB congeners. However, preliminary analysis using all 29 congeners indicated that the variability of the PCB congeners was greater than the variability in the dioxin and furan congeners, and findings related to dioxins and furans were obscured. Therefore, the dioxin and furan congeners were analyzed separately. This paper presents results from the analysis of the dioxins and furans.

Data Treatment

Values below detection limits can affect correlations between congeners and, therefore, impact the results of PVA. In order to minimize the effect of values below the limit of detection (LOD), congeners with greater than 50% of the values below LOD were excluded from the analysis. Samples with greater than 50% of the remaining congeners below LOD were also excluded. These steps resulted in the exclusion of 6 of the 10 furan congeners (no dioxin congeners were excluded) and 21 of the 946 samples.

Polytopic Vector Analysis

PVA is a type of factor analysis that has been demonstrated to be useful in determining source contributions in environmental systems[2,3,4]. The factors, or stable congener patterns, that are outputs from PVA represent potential exposure sources. They are referred to as end-members (EMs). The contribution of each EM to a sample is referred to as a loading.

PVA was performed using Matlab[5]. The steps in the PVA algorithm can be summarized as: constant row sum and range transformations of the dataset; principal components analysis; varimax (orthogonal) rotation of the principal components axes; oblique rotation towards extreme values; and iterative rotations until end-members and loadings satisfy a positivity constraint.

The PVA algorithm is run using a range of different numbers of end-members. Determining the appropriate number of EMs to retain is based on a number of criteria, including: parsimony, stability of EMs, interpretability of EMs, percent of variance explained, coefficient of determination (reproducibility of each congener), and communality (reproducibility of each sample). Further details regarding the PVA process can be found elsewhere[3].

Age Adjustment Methods

Two reasons why age may be important in terms of serum congener patterns are differential congener elimination and differential exposure in time. Differential elimination is important because slowly eliminated congeners tend to increase in importance with age. Differential exposure may be important because congeners that were historically larger contributors to the total fraction of PCDD/Fs would tend to increase in importance with age. Both of these factors could influence the correlations among congeners and therefore impact PVA results.

Three methods of adjusting for age were applied. The first method was age stratification. The dataset was stratified into four age groups (18-29, 30-44, 45-59, ≥60 yrs) and PVA was applied to each group. This method allowed for the evaluation of differences in congener patterns between the different age groups, including how exposures have changed over time. A similar age-stratification method was applied in a study using PVA on the serum of the Akwesasne Indians[6]. A potential disadvantage to this method is that even over a relatively short age range (15 years), differential elimination may impact the correlations between congeners, which would affect the formation of end-members. Additionally, this method does not account for other factors (e.g., BMI, sex, breastfeeding, smoking) that affect dioxin elimination rates.

The second method was to control for age and other demographic factors that impact elimination rates based on the parameter estimates from the UMDES linear regression models[7]. A predicted concentration for each congener for each respondent was calculated. PVA was applied to the residuals or the differences between the actual and predicted concentrations for each congener. This method adjusts for both differential elimination and differences in exposures over time.

The third method was to control for age, sex, BMI, breastfeeding, and smoking only as they affect congener elimination rates. The methods presented in Milbrath et al[8] were used to predict elimination rates for each study respondent. A predicted concentration was calculated for each congener for each respondent assuming a constant intake rate over time. As with the second method, PVA was applied to the residuals. This method adjusts only for differential elimination. Evaluating the end-member loading as a function of age allowed for an assessment of how exposures have changed over time.

Results and Discussion

Three EM models were selected for the 18-29 and 30-45 year age groups. Four EM models were selected for the 45-59 and 60 and over age groups based primarily on the coefficient of determination criteria. Specifically, the reproducibility of TCDD values was improved for those age groups by expanding the model to four end-members.

Figure 1 shows the end-members for the four age groups. Three similar EMs occur in each of the age groups: a pattern dominated by OCDD; and a pattern that includes all of the congeners, with a large fraction of 1,2,3,6,7,8-HxCDD. The additional end-member included in the 45-59 and 60 and over age groups is similar to the end-member that includes all of the congeners, with a larger contribution of 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD. It should be noted that expanding the models for the 18-29 and 30-44 age groups to four EMs does not yield an EM similar to the additional EM included in the 45-59 and 60 and over age groups.

Figure 2 shows the distribution of loadings for each EM in the 60 and over age group by study population. The whiskers in the box-and-whisker plots represent the 5^{th} and 95^{th} percentiles, the edges of the box represent the 25^{th} and 75^{th} percentiles, the line in the box between colors represents the median, and the hollow dot indicates the mean. Two trends associated with study population are noticeable: the Plume population has noticeably higher loadings from the OCDD EM and all of the Midland/Saginaw populations (FP, NFP, MS, PL) have higher loadings for the EM with a larger fraction of 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD. These two trends are noticeable in the 45-60 age group also, although the difference in the Plume population is less pronounced in that stratum.

Preliminary analysis using the other age adjustment methods yields similar results in terms of regional differences. The various methods of incorporating age all show some differentiation by region in terms of EM loading. The end-members that vary between the study populations are:

- an elevated OCDD signature in the Plume, and
- a signature that is elevated in all Midland/Saginaw populations that varies in composition across methods but always includes 2,3,7,8-TCDD; 1,2,3,7,8-PeCDD; 2,3,4,7,8-PeCDF; and 1,2,3,4,7,8-HxCDF.

The analysis also shows that the observed regional differences are only present in older age groups or that the differences increase with age, suggesting a historic exposure source.

Acknowledgements
Financial support for this study comes from the Dow Chemical Company through an unrestricted grant to the University of Michigan. The authors acknowledge Ms. Sharyn Vantine for her continued assistance and Drs. Linda Birnbaum, Ron Hites, Paolo Boffetta and Marie Haring Sweeney for their guidance as members of our Scientific Advisory Board.

References

1. Garabrant, D., Hong, B., Chen, Q., Franzblau, A., Lepkowski, J., Adriaens, P., Demond, A., Adriaens, P., Hedgeman, E, Knutson, K., Zwica, L., Chang, C-W., Towey, T., Luksemburg, W., Maier, M., Gillespie, B.W. 2008. In Review.
2. Johnson, G., Ehrlich, R., Full,W. *Introduction to Environmental Forensics*; Academic Press: New York, 2002; pp 461-515.
3. Barabas, N., Goovaerts, P., Adriaens, P. 2004. *Environ. Sci. Technol*., 38: 1813-1820.
4. Barabas, N., Goovaerts, P., Adriaens, P. 2004. *Environ. Sci. Technol*., 38: 1821-1827.
5. The Mathworks. Matlab R2008a. Natick, Massachusetts.
6. DeCaprioa, A.P., Johnson, G.W., Tarbellc, A.M, Carpentera, D.O., Chiarenzellid, J.R., Morsee, G.S.,. Santiago-Riverae, A.L, Schymuraf, M.J. Akwesasne Task Force on the Environment. 2005. *Environ. Res*. 98: 284–302.
7. Garabrant, D., Franzblau, A., Lepkowski, J., Gillespie, B.W., Adriaens, P., Demond, A., Hedgeman, E., Knutson, K., Zwica, L., Towey, T., Chen, Q., Hong, B., Chang, C-W., Lee, S-Y., Ward, B., LaDronka, K., Luksemburg, W., Maier, M. 2008. In Review.
8. Milbrath, M.O., Chang, C-W., Emond, C., Garabrant, D., Gillespie, B.W., Wenger, Y., Jolliet, O. 2008. In review.

Figure 1. Comparison of the PVA end-members from four age groups.

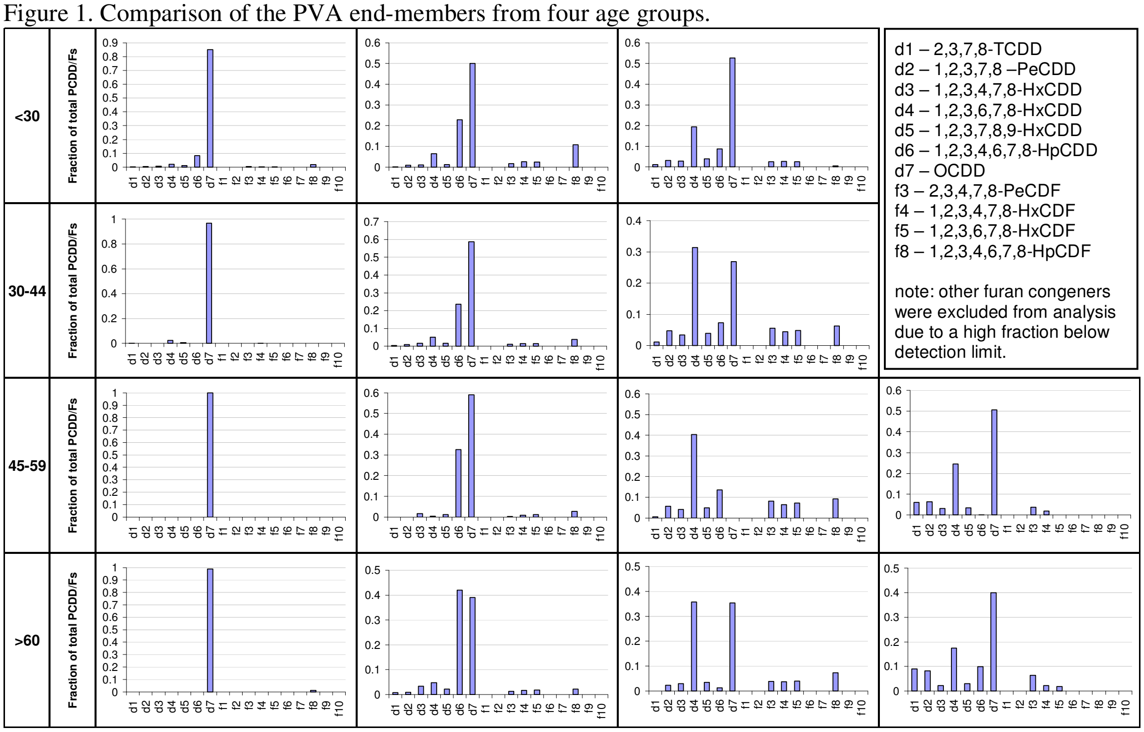
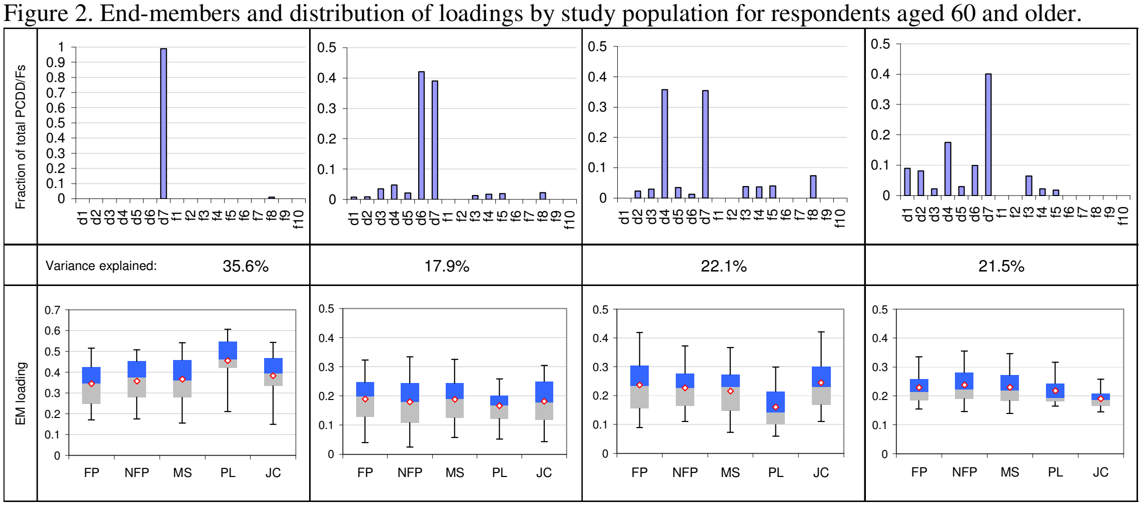


Figure 2. End-members and distribution of loadings by study population for respondents aged 60 and older.



FP- Floodplain, NFP – Near Floodplain, MS – Other Midland/Saginaw, PL – Plume, JC – Jackson/Calhoun