Multivariate Data Evaluation of PCB Profiles in Human Background Samples from Sweden and Spain.

Håkan Wingfors[1], Bert van Bavel[1], Gunilla Lindström[1], Patrik Fredriksson[1], Marta Schuhmacher[2] and Lennart Hardell[3]

[1] Institute of Environmental Chemistry, Umeå University, SE-901 87 Umeå, Sweden
[2] Laboratory of Toxicology and Environmental Health "Rovira i Virgili" University, San Lorenzo 21, 43201 Reus, Spain
[3] Department of Oncology, Örebro Medical Center, SE-701 85 Örebro, Sweden

Introduction

The analysis of organochlorines in human samples yields large data sets, especially when polychlorinated biphenyls (PCBs), dibenzodioxins (PCDDs) and dibenzofurans (PCDFs) are measured congener specific. In human exposure studies the interpretations of large data sets can be succesfully performed by multivariate data evaluation. Data sets of 50 samples x 70 measured congeners are not exceptional in such studies. Multivariate data interpretation of complex data matrixes provides an overview of the data and can also be used for classification of groups. Both can be done by *soft independent modeling of class analogy* (Simca)(1,2). Simca uses principal components (PCs) to get an overview of dominant and/or latent patterns. Local PC models can be used for classification, and overlapping of the data can be investigated between models in Cooman plots. Relationships between samples and between variables can be evaluated in score and loading plots respectively. The objective of this study was to reveal differences between the patterns of 30 PCBs in human background data from Spain and Sweden.

Material and methods

Blood (n=20) and tissue (n=15) samples from Spain were obtained from persons living in the area around Tarragona for at least 10 years. Further tissue (n=28) samples were collected from persons with no known PCB exposure living in the sorroundings of Örebro, in the center of Sweden. All samples were kept at -20°C before extraction and clean up. Organic solvent extraction methods (3,4) were applied for samples from Spain. Supercritical fluid extraction coupled to on-line liquid chromatography (SFE-LC)(5) was applied for the samples from Sweden. High-resolution gas chromatography (HRGC) coupled to a quadropole mass spectrometer (LRMS) was used for determination of the PCBs.

Statistical evaluation

Results from the analysis of PCBs are usually reported in ng/g extracted lipids (ppb). The unit nanogram for tri-PCB #28, 256 g/mole, compared to deca-PCB #209, 498 ng/mole, actually implies a factor two regarding the amount of molecules. Further normalisation of data to the most abundant PCB isomer (#153) keeps the PCB pattern in the data but removes the concentration differences. The PCB congeners analysed, variables (i), often show a positive skewness for the samples while plotted in a histogram. The log-transformation forces the data to follow a normal distribution. The multivariate data analysis was performed on a PC with the SIMCA 6.01 package.

Results

The results from the congener specific analysis are summerized in Table 1 where the sum PCB of each sample group is given. In total, thirty PCB congeners were detected in all 63 samples. Basic statistical analysis has limitations in describing the different groups especially when data are unbalanced ($n_1 \neq n_2$) or when age and sex distribution differ i.e. data is not designed.

Table 1. Mean values of the sum PCB (ng/g and nmole/g extracted lipids) and standard deviation for nmole/g in Sweden and Spain.

Sum PCB	n	Mean ng/g	Mean nmole/g	stdev
Sweden adipose	28	1260	3.42	1.18
Spain all	35	1420	3.86	2.27
Spain adipose	15	1710	4.63	2.69
Spain plasma	20	1210	3.28	1.75

Information can be disguised or hidden within the data by the correlated variables. Therefor after the normalisation to PCB#153 a score plot is calculated by PCA. The scoreplot, Figure 2 is a low dimensional sample oriented projection describing maximum variance of the X-space.

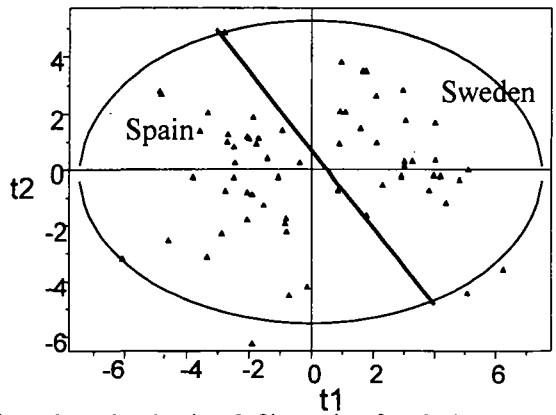

Figure. Scoreplot, t1against t2. Observations from Spain are separated from observations from Sweden based on the PCB profiles.

The Swedish group is almost perfectly seperated from the Spanish group in the first two principal components explaining 42 % of the variance in the X-space. In the score plot from PC1 against PC3 (not shown) the two groups are also separated (54% explained). From the corresponding loading plots, which are variable oriented, the dependence from each variable can be interpreted.

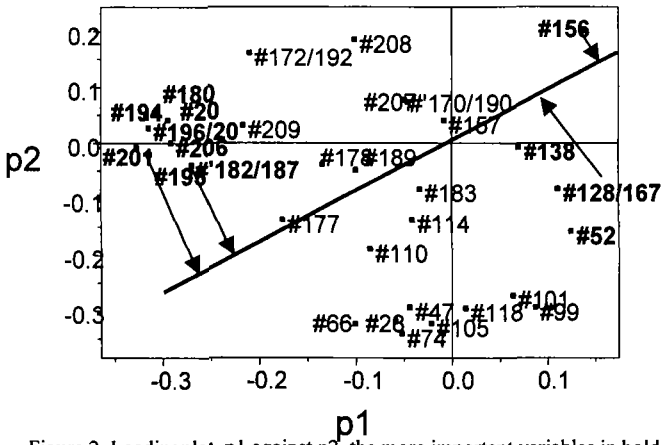

Figure 2. Loadingplot, p1 against p2, the more important variables in bold.

Some of the higher chlorinated PCBs like PCB #180, #201, #194 are separating the Spanish group from the Swedish group. In the same manner the Swedish group is separated from the Spanish group by PCB #156, #52. From p1 to p3 (not shown) also PCB #138 and the coeluting PCBs #128/#167 are important for separating the two groups. Using this information two local PCA models were calculated for each country. The overlapping of the models was tested in a Cooman plot where all objects' distances to each model are plotted. No overlapping of the models could be seen.

Cooman's Plot

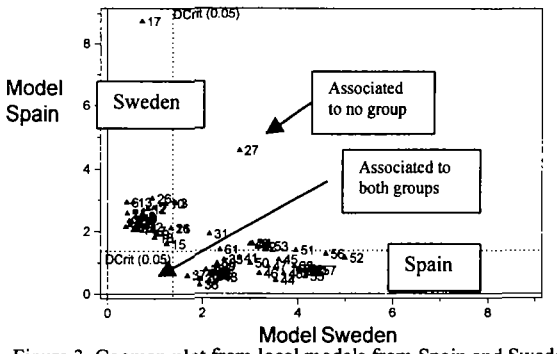

Figure 3. Cooman plot from local models from Spain and Sweden.

Conclusion and Discussion

No significant differences are seen between the mean concentrations of the PCBs analysed in human background samples from Spain and Sweden.

By multivariate projection analysis it is revealed that the PCB pattern in human background data from Spain and Sweden differs. The differences depend on the PCB profiles present in the samples. Since most of the exposure for the general population of PCBs is via food the differences origin from dietary habits or different food compositions in the two countries. Also varying primary sources and meteorological circumstances may contribute.

In this paper we used a transformation of PCB data from ng/g (fat) to nmole/g (fat). This pretreatment of data should in our opinion be used when regressions to biological responses like age, lactation or toxicity are studied.

Acknowledgements

The author thanks "Cancer- och Allergifonden" and "IngaBritt och Arne Lundbergs forskningsstiftelse".

References

1. Wold S, Albano C, Dunn WJ, Edlund U, Esbensen K, Geladi P, Hellberg S, Johansson E, Lindberg W, Sjöström M. **1984**, 1. D. Reidel publishing company, Dordrecht Holland

2. Stalling DL, Dunn WJI, Schwartz TR, Hogan JW, Petty JD, Johansson E, Wold S, *ACS Symp. Ser.* **1985**, 284, 195.

3. Päpke O, Ball M, Lis Z.A and Scheunert K. *Chemosphere*, **1989**, 19, 941

4. Hansson M, Holma S and Rappe C, *Organohalogen Compd.* **1993**, 11, 177

5. van Bavel B, Järemo M, Karlsson L and Lindström G, *Anal. Chem.* **1996**, 68 (7), 1279