Multivariate Data Analysis (MVDA) Applied in Toxicokinetic Studies on the Retention of PCDFs in the Liver and Fat Tissue of Test Animals.

B. v. Bavel*, G. Lindström**, M. Sjöström***, H. Håkanson****

*Dept. of Environmental and Toxicological Chemistry, University of Amsterdam, 1018 WV Amsterdam, The Netherlands. **Dept. of Environmental Chemistry, University of Umeå. ***Dept. of Organic Chemistry University of Umeå, Sweden. ****Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden.

Abstract

Multivariate Data Analysis (MVDA) was performed on part of a matrix containing data of a toxicokinetic study. Test animals were fed with a low concentration mixture of PCDF's. Levels of PCDF's in liver and fat together with biological variables were measured at different days after the administration of the PCDF's. By using MVDA trends and anomalies were discovered.

Keywords

Multivariate Data Analyses(MVDA),Polychlorinated Dibenzofurans(PCDF), Toxicokinetic, Male Sprague-Dawley Rats, Male C57Bl/6 Mice.

Introduction

This study is part of a project in which more than 180 PCDF-analyses (each containing 19 congeners) have been made and over 1000 biological values were measured. The main purpose of the study was the determination of the half lives of PCDF's in liver and fat of the different test animals (mice, rat, guinea pig and hamster). For the interpretation of the data MVDA was used. The here presented results for mice and rat show some preliminary results and possibilities of MVDA. The whole data set will be analyzed by MVDA in the near future. The purpose of this study is to monitor the dominating trends in PCDF-patterns in fat and liver by time as well as in the biological measurements. We also want to compare the trends between the fat, liver and biological responses with each other.

Materials and Methods

The test animals(Male Sprague-Dawley rats and male C57Bl/6 mice) were fed a single low oral dose (0.1 µg of each congener/kg body weight) of a mixture of polychlorinated dibenzofurans(PCDF's). The mixture contained the following congeners: 1,3,6,8-TCDF; 1,3,7,8-TCDF; 1,2,7,8-TCDF; 1,4,6,9-TCDF; 2,3,4,7-TCDF; 2,3,7,8-TCDF; 3,4,6,7-TCDF; 1,2,4,7,8-PCDF; 1,2,3,7,8-PCDF; 2,3,4,6,8-PCDF; 2,3,4,7,8-PCDF; 2,3,4,6,7-PCDF; 1,2,3,4,7,8-H_xCDF; 1,2,3,6,7,8-H_xCDF; 1,2,3,4,6,9-H_xCDF; 1,2,3,7,8,9-H_xCDF; 2,3,4,6,7,8-H_xCDF; 1,2,3,4,6,7,8-H_pCDF and OCDF. The mixture was taken up in corn oil and given as a single oral doses, the control animals only received the corn oil. Two control and four PCDF fed animals were killed 1, 5, 10, 30 and 90 days after the administration of the corn oil(1).
Biochemical assays such as tissue extraction and vitamin A status were carried out as earlier described(2).

PCDF analyses and clean up were performed using activated KOH-silica, H_2SO_4, acidic alumina oxide and Carbopack C on Celite. The method used was developed specially for the clean up and enrichment of a large number of pharmacokinetic samples(3). The resolution during the measurements was 10,000 and the detection limit was 0.1-1.0 ppt depending on the congener analyzed. The data analysis was performed using the Simca 3-B program (SEPONOVA AB, Sweden) on an IBM PC microcomputer(4).

Analytical data sets. Principal component analyses(PCA) were performed on fat and liver PCDF-levels and a series of biological values such as weight gain, EROD-activity of the liver, liver-, kidney-, thymus- and bitestical-weight, vitamin A in liver, kidney and serum. The animals were, as already mentioned, divided in several groups. For the MVDA the rats of day 1,5,10 and the mice of day 1,5, 10,30,90 were used. Data of PCDF-levels in liver for the mice were only available from day 1,5 and 10.
The objects in the PCA were either mice(11 obj.) or rats(19 obj.) killed on different days. Three types of variables were used: PCDF-level in liver(17 var.), PCDF's in fat(17 var.) and the biological measurements (17 var.)
Although PCA was only performed on the not yet complete data set, already anomalies and trends were easily discovered. Especially the graphical displays of the data by the principal component analysis were informative and surveyable.

Results and Discussion

PCA of mice data. From the projection of the first two principal compounds (PC) of fat and liver several anomalies were directly discovered. Three mice (no.2,5 and 8) were far outside their group of terminating day for one or more types of the variables: PCDF in fat, PCDF in liver or the biological data.
Mice no.2 showed a too high level of PCDF in fat and liver (compared to its group day 1)(fig.1a,b) but no significant difference in biological data. A possible explanation is that mice 2 has been administered a too high dose of PCDF's but not sufficiently high enough to affect the biological response.
Mice no.5 had a higher level of PCDF's in fat but not in liver (compared to its group, day 5)(fig.1a,b). This mice also showed a difference in biological data. Here bitestical- and thymus weight were rather low. The weight gain was negative and vitamin A in liver was high.
Mice no.8 also showed a different behavior to its group (day 5) (fig 1a,b).It showed a low level of PCDF in fat, a normal level in the liver and from the biological data a low thymus weight and a low EROD-activity in the liver but a very high weight gain compared to its group.

PCA of rat data. Also the PCA showed some anomalies of the rat data. As an example Rat no.24 is used. This rat had somewhat higher levels of PCDF's in fat but no significant difference in liver (compared with his group day 5). From the PC score plot of the biological data (fig.2a) it is seen that also rat 24 is an outlier. The corresponding variable loading plot (fig.2b) it can be seen which biological variables that pull rat 24 from his group (0 is the center in fig.2a and MM is the center in fig.2b). The variables responsible are 43 (vitamin A in liver) which is low, 44 (erod activity in liver) which is high and 42 (vitamin a in kidney) which is also high.

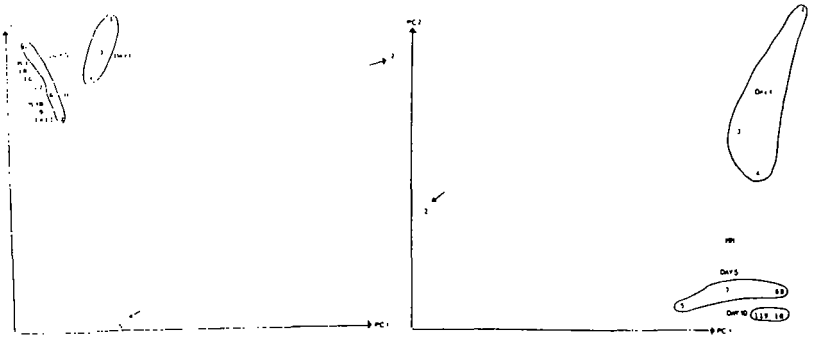

Figure 1a PC score plot mice fat PCDF-levels
(PC 1 explaining 87% and PC 2 explaining 10% of the variance in the data)
Figure 1b PC score plot mice liver PCDF-levels
(PC 1 explaining 64% and PC 2 explaining 18% of the variance in the data)

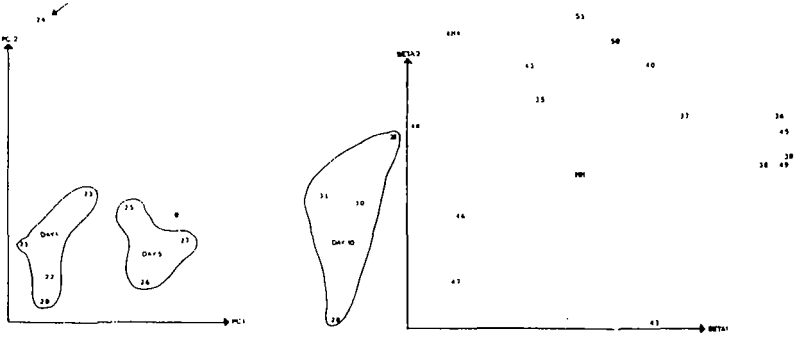

Figure 2 PC score(a) and loading(b) plots of biological data of rats
(PC 1 explaining 28% and PC 2 explaining 21% of the variance in the data)

Trends. From PCA where the anomalies had been excluded trends could be visualized by PC score plots.
Plots of the first PC's of PCDF-levels of fat from mice and rat showed that day 1 and the days 5,10,30 and 90 are situated in two groups(fig 3a,b). Also for the liver samples the same distinction can be made for day 1 and days 5 and 10. This indicates that more sampling between, for example, day 1 and 5 gives much more information than sampling at later days.

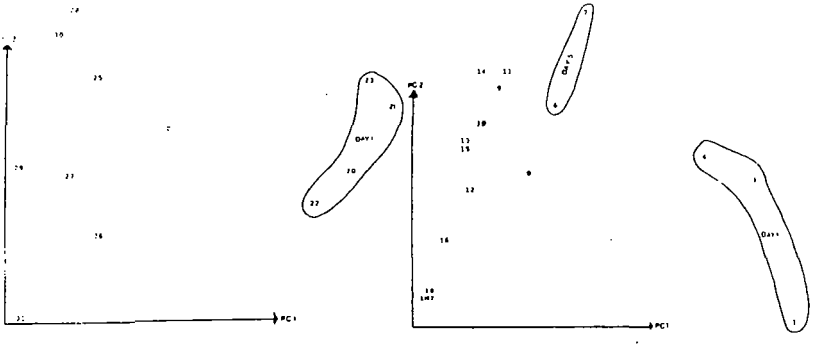

Figure 3a PC score plot PCDF-levels of mice fat (without mice no.2,5 and 8)
(PC 1 explaining 73% and PC 2 explaining 16% of the variance in the data)

Figure 3b PC score plot PCDF-levels of rat fat (without rat no.24)
(PC 1 explaining 64% and PC 2 explaining 14% of the variance in the data)

Again PCA proved to be an excellent tool for getting information out of a large data set as presented here. In this project MVDA will further be used to interpret available measurements (over 1500) and for the construction of a regression models which can predict the distribution of administered low concentration PCDF-mixtures over liver and fat.

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