Novel multivariate methods for evaluation of PCB levels, and of CYP1A, vitamin E, and haematological parameters as PCB-exposure biomarkers, in seal

Magnus Bergknut[1], Lennart Eriksson[2], Madeleine Nyman[3], Patrik Andersson[1], Peter Haglund[1], Mats Tysklind[1]

[1]Environmental Chemistry, Department of Chemistry, Umeå University
[2]Umetrics AB
[3]Finnish Environment Institute, P.O.Box 140, FIN-00251 Helsinki, Finland

Introduction

Biomarkers can be used to assess the exposure and effect of the combined contaminant load of an individual. Attempts have been made to use constituents of blood samples[1] as well as induction of CYP1A[2], as biomarker for PCB exposure in seals. Vitamin E has also been proposed as a biomarker of contaminant exposure[3], but has rarely been used. In the work by Nyman *et al* (2003)[4], CYP1A and vitamin E levels were proposed as the most useful biomarkers from a set of potential biomarkers (haematological and clinical chemistry parameters, cholesterol, cytochrome P4501A, vitamin A, and vitamin E). The current study was based on the data set reported Nyman et al (2003), and was performed to further assess the potential of haematological and clinical chemistry parameters as biomarkers for exposure to PCBs, using novel multivariate techniques. In order to facilitate the interpretation of correlations observed between biomarkers and PCB levels, external factors of potential significance were also included in the study. These included QSAR descriptors, UV data, environmental fate variables, PCB levels in a food web, and PCB levels in food sources which were correlated with the contaminant load and the biological response. The correlation was successfully studied using the two novel multivariate techniques, Hi-PLS and 3BIF-PLS, which proved to be useful tools for simultaneously exploring the relationships between biological effects, contaminant load, and physicochemical properties of the included contaminants.

Materials and methods

Baltic ringed seals and grey seals were sampled on the ice in the Bothnian Bay in April and May, 1996-1998 (for details, see Nyman *et al* (2003)[4]). Reference samples, with lower levels of PCBs compared to the Baltic Sea samples, were collected from Svalbard, in the Arctic (ringed seals) and Sable Island, Canada (grey seals) in May and June 1996-1998.The preparation, analytical methods and results of the chemical and biochemical analyses, including haematological and clinical chemistry parameters, have been reported in detail previously[4,5,6]. Of the 34 PCB congeners reported, 24 (IUPAC nos. 52, 74, 77, 99, 101, 105, 110, 114, 118, 126, 128, 138, 153, 156, 157, 169, 170, 180, 183, 187, 189, 194, 206, 209) were included in this study. The remaining ten PCBs were ignored because they were present at levels below the detection limit in a majority of the investigated seals.

Before the multivariate evaluation, the studied data set, consisting of data on the levels of 24 PCBs in 81 seals, was organized into three matrices. Biomarker variables (35 in total: haematological and clinical chemistry parameters, CYP1A induction, and vitamin E) were compiled in a matrix named X, while PCB concentrations (24 in total) were compiled in a matrix named Y and variables describing physicochemical properties and descriptors of the environmental fate of the PCBs (73 in total) in a matrix named Z. The strategy was to find correlations between biomarkers (X) and levels of PCBs (Y), and then to investigate Y using PCB descriptors (Z) and the 3BIF-PLS method. Z included five blocks of data that could in some way aid the interpretation of the X/Y correlation; (i) 32 molecular descriptors and (ii) UV spectra (200-300 nm) for all PCBs[7], (iii) eight environmental variables including ease of metabolizing the PCBs[8,9], the bioconcentration factors (BCF), persistence, and PCB distribution between air, water, soil and sediment[10], (iv) PCB levels of two different benthic food webs in the Baltic (sediment, amphipod, isopod, and fourhorned sculpin; and zooplankton, *Mysis*, and herring)[11,12], and (v) PCB, levels in lesser and greater sand eel, cod, perch, and herring[13].

Principal component analysis (PCA)[14] was used for classifying the data and identifying relationships within the data

while partial least squares projections to latent structures (PLS) analysis[15,16] was used to study correlations between an independent and a dependent matrix (X and Y or Z' and Y', respectively). Three-block bi-focal PLS (3BIF-PLS, Fig 1A)[17] was used to increase the understanding of correlations observed between the biochemical data (X) and the measured PCB levels (Y), and thus the grouping of the PCBs in the multivariate space. In 3BIF-PLS, the X/Y model is modified by the Z'/Y' model and correlations between Y and X that are governed by Z will then be distinguishable by observing changes in the X/Y-model's score (relationships among samples) and loading (relationships among variables) plots. As an alternative to PLS, and in preparing data for 3BIF-PLS analysis, hierarchical-PLS (Hi-PLS)[18] was used (Fig 1B). With Hi-PLS, the blocks of variables were first evaluated by PLS to reveal correlations to the measured PCBs. The score vectors from these models (base level) were then transferred to a new matrix, which was used in a final model (top level) correlating scores and PCB concentrations. All calculations were carried out using the software packages SIMCA-P 10 (Umetrics, Umeå, Sweden) and MATLAB (The MathWorks, Natick, MA, US).

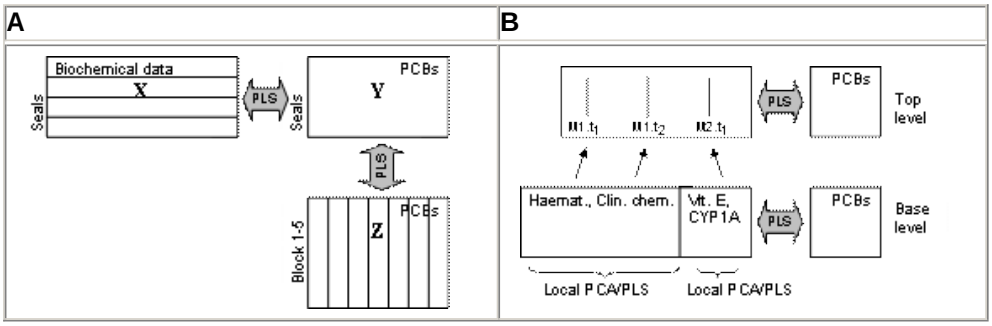

Fig. 1. Schematic overview of 3BIF-PLS (A) and Hi-PLS (B). (A) adapted from Eriksson *et al* (2004)[17], (B) adapted from Eriksson et *al* (2002)[18].

Results and discussion

A PCA of all biomarker variables (matrix X) revealed that the seals were grouped according to species and degree of PCB exposure. Generally, seals with high levels of PCBs had high levels of both CYP1A induction and vitamin E. The first two principal components reflected exposure, as well as species-dependent variations, in the biomarker data. A PCA on the levels of PCBs (matrix Y) detected one major component, describing the degree of exposure, with a second minor component describing differences in individual PCB concentrations. Closer examination showed that PCBs resistant to metabolic transformations[8] (PCBs 99, 128, 138, 153, 170, 180, 183, 187, 189, 194, 206, 209) were more abundant in grey seals, whereas easily metabolized PCBs (PCBs 52, 74, 77, 101, 105, 110, 114, 118, 126, 156, 157, 169) were more abundant in ringed seals. This indicates that grey seals have a higher metabolic capacity, i.e. a metabolic system that is more easily induced, or has different specificity, compared to ringed seals. Two principal components for the Y matrix picked up both exposure- and species- dependent variations within the data.

PLS between the haematological and clinical chemistry parameters and levels of PCBs gave a explained variance of only 29 %. However, when the Hi-PLS method was used, i.e. when the information in the haematological and clinical chemistry parameters was summarized in base model PLS and then correlated to the PCB levels in a top level model, the explained variance increased to 42 %. Linear regression models obtained using either CYP1A, vitamin E or the first score from the Hi-PLS and the log10-transformed sum of all PCBs showed a stronger correlation for CYP1A than for the others (R^2 values of 61.4 %, 53.2 %, and 45.1 %, respectively). None of the evaluated biomarkers showed any species-dependences. Overall, and taking the confounding effects of biological variation into consideration, the performance was acceptable.

3BIF-PLS was utilized in order to further investigate (i) if the observed correlation between biomarkers and exposure to PCBs was due to specific PCBs , e.g. PCBs 77, 126, and 169 that are known to be strong inducers of CYP1A [3], and if these PCBs had particular structural characteristics, and (ii) if the species-dependent PCB grouping in Y was attributable to PCBs with certain properties. In general, there was a weak correlation between X/Y and Z'/Y', with the strongest link between the two models being due to the second component of Z'/Y'. The weak correlation in the first

component strongly indicates that the observed biological effects were due to the total load of the PCBs and not to PCBs with particular properties. This is consistent with the hypothesis that PCBs have concentration-additive effects[19]. When interpreting the differences between seal species and levels of individual PCBs through the use of the second component of Z'/Y', Block 3 (combined effect of BCF, ease of metabolization, and persistence), Block 1 (mainly absolute hardness, QSAR data), and Block 2 (mainly 220-240 nm, UV data) were the main contributors. Hence it seems like absolute hardness and parts of the UV spectra reflects the ease with which seals metabolize individual PCBs.

In conclusion, the main advantages of using Hi-PLS were that it provided better models and that a large number of variables could be incorporated while still producing easy to interpret graphical plots. 3BIF-PLS, on the other hand, appears to facilitate interpretation of the data through inclusion of additional (interconnected) data, in this case biological effects, and levels of PCBs and properties of PCBs, into a single model.

References

1. Jenssen, B. M.; Skaare, J. U.; Ekker, M.; Vongraven, D.; Silverstone, M. *Chemosphere* **1994,** *28*, 3-10.

2. Troisi, G. M.; Mason, C. F. *Chemosphere* **1997,** *35*, 1933-46.

3. Stegeman, J. J.; Brouwer, M.; Di Giulio, R. T.; Förlin, L.; Fowler, B. A.; Sanders, B. M.; Van Veld, P. A. *In: Biomarkers, Biochemical, Physiological, and Histological Markers of Anthropogenic Stress.Editors, Hugget, R.J., Kimerle, R.A., Mehrle, P.M.and Bergman, H.L.(Lewis Publishers, USA* **1992,** 235-335.

4. Nyman, M.; Bergknut, M.; Fant, M.L.; Raunio, H.; Jestoi, M.; Bengs, C.; Murk, A.; Koistinen, J.; Backman, C.; Pelkonen, O. *Marine Environ. Research* **2003,** *55*, 73-99.

5. Mattson, M.; Raunio, H.; Pelkonen, O.; Helle, E. Elevated levels of cytochrome P4501A (CYP1A) in ringed seals from the Baltic Sea. *Aquat. Toxicol.* **1998**, *43* (1), 41-50.

6. Nyman, M.; Raunio, H.; Taavitsainen, P.; Pelkonen, O. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **2001,** *128*, 99-112.

7. Andersson, P.; Haglund, P.; Rappe, C.; Tysklind, M. *J. of Chemometrics* **1996,** *10*, 171-85.

8. Boon, J. P.; vanderMeer, J.; Allchin, C. R.; Law, R. J.; Klunsoyr, J.; Leonards, P. E.; Spliid, H.; Storrhansen, E.; Mckenzie, C.; Wells, D. E. *Arch. Environ Contam. Toxicol.* **1997,** *33*, 298-311.

9. Thomas, G. O.; Sweetman, A. J.; Jones, K. C. *Chemosphere* **1999,** *39*, 1533-44.

10. U.S.EPA *Epi Suite:http://www.epa.gov/opptintr/exposure/docs/episuitedl.htm (v.3.10)* **2001**.

11. Strandberg, B.; Bandh, C.; van Bavel, B.; Bergqvist, P.-A.; Broman, D.; Näf, C.; Pettersen, H.; Rappe, C. *Science Tot. Environ.* **1998,** *217*, 143-54.

12. van Bavel, B.; Näf, C.; Bergqvist, P.A.; Broman, D.; Lundgren, K.; Papakosta, O.; Rolff, C.; Strandberg, B.; Zebuhr, Y.; Zook, D.; Rappe, C. *Mar.Pollut.Bull.* **1996,** *32*, 210-18.

13. Falandysz, J.; Wyrzykowska, B.; Puzyn, T.; Strandberg, L.; Rappe, C. *Food Additives and Contaminants* **2002,** *19*, 779-95.

14. Wold, S.; Esbensen, K.; Geladi, P. *Chemometrics Intell. Lab. Syst.* **1987,** *2*, 37-52.

15. Dunn III, W. J.; Wold, S.; Edlund, U.; Hellberg, S.; Gasteiger, J. *Quant. Struc -Act Relat.* **1984,** *3*, 131-37.

16. Geladi, P.; Kowalski, B. R. *Anal. Chimica Act.* **1986,** *185*, 1-17.

17. Eriksson, L.; Damborksy, J.; Earll, M.; Johansson, E.; Trygg, J.; Wold, S. *SAR and QSAR in Environ. Research* **2004,** *15*, 481-99.

18. Eriksson, L.; Johansson, E.; Lindgren, F.; Sjöström, M.; Wold, S. *J. of Computer-Aided Molecular Design* **2002,** *16* , 711-26.

19. Escher, B. I.; Hermens, J. L. M. *Environ.Sci.Technol.* **2002,** *36*, 4201-17.