Organohalogen Compounds, Volume 61, Pages 96-99 (2003)

MULTIVARIATE (QSAR) MODELLING OF POLYBROMINATED DIPHENYL ETHERS ACTIVITY IN DIFFERENT SPECIES CELL LINES AND THEIR CAPACITY TO INDUCE CYP1A BY THE AH RECEPTOR MEDIATED PATHWAY

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Introduction

The widely used flame retardants polybrominated diphenyl ethers (PBDEs) are persistent and ubiquitous organic pollutants (POPs) that biomagnify and may have endocrine disrupting effects similar to the effects observed for other halogenated compounds such as polychlorinated and polybrominated biphenyls (PCBs and PBBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Concern with the risks to human health, particularly infants, is increasing due to observations of increasing PBDE concentrations in human breast milk, although available data suggest that current levels of PBDEs are an order of magnitude lower than those of PCBs.[1,5] While PBDEs are in extensive production and use, existing data on the receptor mediated toxicology of PBDEs is very limited, but there are indications of toxicity via the estrogen, thyroid and Ah receptors (AhR).

Multivariate techniques are useful for data analyses of selected compounds, tested in a broad battery of test systems where there is a large variation within some of the variables. There is a renewed interest in Quantitative Structure Activity Relationships (QSARs) due to social and political pressures, particularly with respect to reducing animal testing and the proposed changes in EU chemical management regulations. QSARs are simplified mathematical representations of complex chemical-biological interactions, and consequently QSAR predictions are potentially more uncertain than the underlying test data. However QSARs are suitable for activity estimation, and they can provide warnings/alerts about possible toxic properties of the test compounds. QSARs can be used for decision support in early phases of product development to regulatory decision-making such as in risk assessment and risk classification.

We have generated a principal component analysis (PCA) and quantitative structural activity relationship (QSAR) for the AhR, using multivariate techniques, specific descriptors, and biological data sets. This QSAR utilized experimentally generated PBDE data sets[6], together with an unpublished data set from the same laboratory, for 12 PBDEs capacities to bind and activate the AhR in rat and human hepatocytes, human intestinal cells and to induce CYP 1A1, which here was assayed as 7-ethoxyresorufin-*O*-deethylase (EROD) activity in cells from rainbow trout, chick, rat and human.

VolSurf and physico-chemical descriptors were generated for each of the compound structures for which biological data was available and subsequently used to develop a partial least squares regression based model. 2,3,7,8 TCDD was an outlier compared to the PBDEs, and major differences were apparent between the different cell line EC50s (n=7) reflecting PBDE ligand potencies in AhR, and cell line EROD % values (n=7) reflecting P450 CYP1A induction.
We have supported these multivariate analyses by utilising a homology model of AhR (based on the ERα crystal structure)[7], to examine the mode of binding of representative compounds in the ligand-binding domain (LBD) of the receptor model. Key commonalities and differences between the ligands have been observed.

Methods

1. Generation of PBDE biological data using *in vitro* assays. The following PBDEs IUPAC No's 28, 47, 66, 77, 85, 99, 100,119, 126, 153, 154, 183 were tested relative to 2,3,7,8 TCDD in the following cell lines: RTL-W1: rainbow trout hepatoma; CEH: primary chick embryo hepatocytes; PRH: primary rat hepatocytes; H4IIE: rat hepatoma; Caco-2: human adenocarcinoma; Hep G2: human hepatoma, from which EC50's and EROD%, representing the highest EROD activities from individual congeners compared with the activity of the positive control 1nM TCDD. Methodologies are described in Chen et al 2001.[6]
2. To enable the evaluation and interpretation of this multivariate data the following multivariate techniques (Umetrics, SIMCA-P v.10.0) and descriptors were used:
2. (i) Principal components analysis (PCA): a projection method that helps turn data into information by providing a graphical overview (Similarity/Dissimilarity/Outliers) and classification (Characterisation of groups).
2. (ii) Generation of VolSurf descriptors: VolSurf is a computational procedure to produce & explore the physicochemical property space of a molecule starting from 3D interaction energy grid maps. The information present in 3D grid maps is compressed into a few quantitative 2D numerical descriptors using image analysis software. Each 3D map is considered as 3D image, but the image compression process is made adding chemical knowledge. The pharmacokinetic properties of a compound often depend on a variety of physicochemical parameters and therefore, require a multivariate description. VolSurf descriptors quantitatively characterize size, shape, polarity, hydrophobicity and the balance between them. VolSurf descriptors are fast to calculate and are independent of alignment of molecules.[8]
2. (iii) Statistical tools: Partial Least Squares Regression (PLS) PLS assumes that there are a few 'principal properties' of the molecule which are directly related to the Y response, which in this model are the EROD and EC50 values in different cell lines. Different linear combinations of all the descriptors called 'components' are made, in turn, to describe each principal property. By evaluating the regression coefficients of the PLS model in conjunction with the variable influence on the prediction, the relative importance of each descriptor can be identified.
3. Utilisation of a homology model of AhR based on the ERα crystal structure for docking studies of PBDE, TCDD and PCB ligands in the hAhR model, using SYBYL biopolymer software and visualised on a Silicon graphics Indigo 2 IMPACT 10000 Unix work station (Tripos Assocs. St Louis, MO).[7]

Results and Discussion

The data sets were analyzed using PCA and PLS techniques. Initial analysis suggested the presence of an outlier, 2,3,7,8-TCDD, which was subsequently excluded in the 3-component model shown. In this way the PLS model R2X value improved from 0.4 to 0.77 (n=12, RMSEE=15.71), with a good fit (R2Y=0.71) but poor predictability (Q2=0.16). The primary variables summary plot of the model fit, (fig 1) show the success of the model fit for each of the Y responses. Examination of the loadings plot (fig 2) highlight specific descriptors which were positively correlated with the biological responses, in the top left corner, and negatively correlated, in the bottom right corner.

Figure 1. Primary variables plot

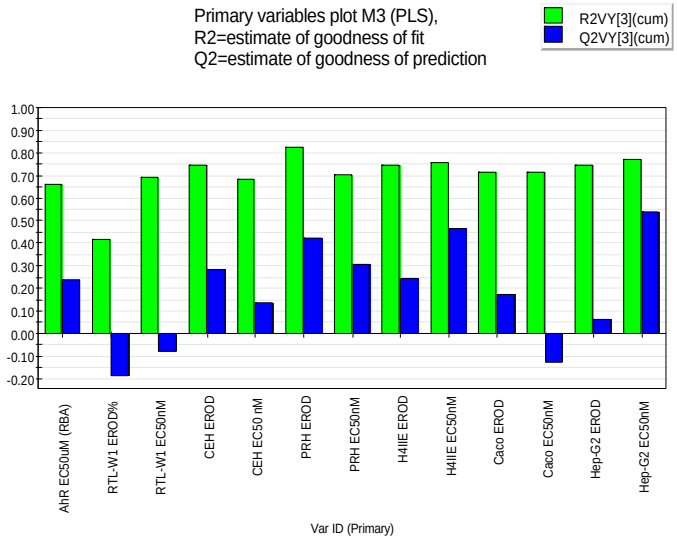
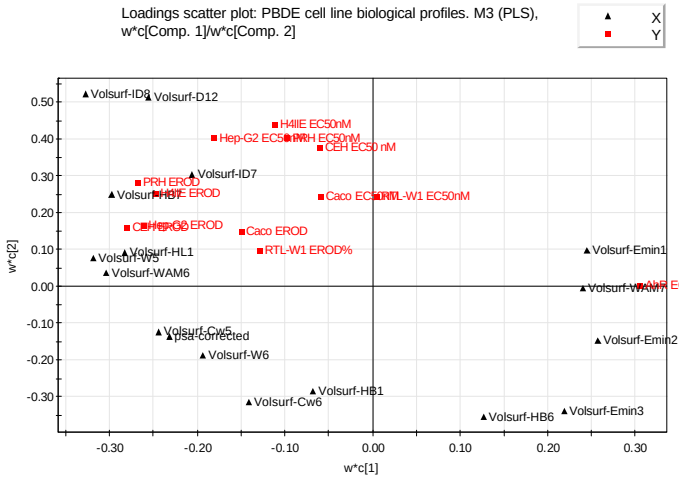


Figure 2. Loadings scatter plot



Taking the best-predicted biological parameter, the human hepatocyte cell line Hep G2 EC50, the following Volsurf variable importance (fig 3) and coefficients (fig 4) plots were derived.

Figure 3. Variable Importance plot

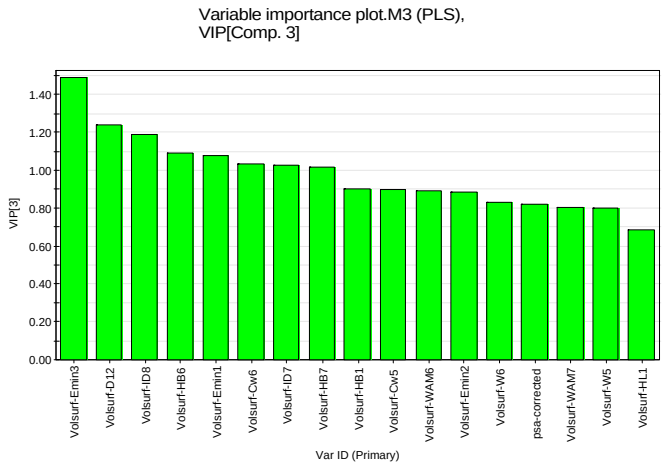
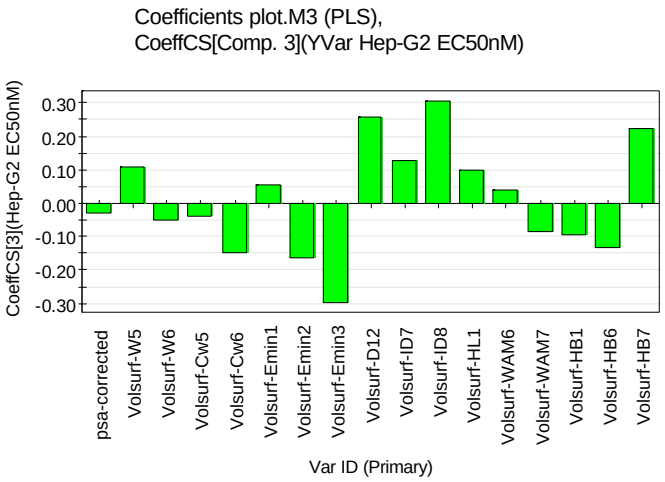


Figure 4. Coefficients plot: Hep G2 EC50



The key variables were: The local interaction energy minima (Emin3) had a large negative correlation; this represents the energy of interaction in kcal/mol of the best three local minima of interaction energies between a water probe and the target molecule. Positive correlations were observed for: Local interaction energy minima distances (D12), these are shape descriptors for the distances between the best three local minima of interaction energies when a water probe interacts with a target molecule; Hydrophobic integy moments (ID7 and ID8) which measure the imbalance between the centre of mass of a molecule and the position of the hydrophobic regions around them, and hydrogen bonding (HB7) capabilities of the ligands.

Less important negatively correlated variables were: capacity factors (Cw5 and Cw6), which represent the amount of hydrophilic regions per surface unit, and amide responsive regions (WAM7), suggesting that there is some indication that good acceptor abilities may not increase the potency of a high affinity AhR ligands. Both *in vitro*[6] and *in silico*[7] docking studies suggest different modes of binding in the AhR for TCDD compared to other ligands, and this is reflected in the key Volsurf variables observed from the QSAR exercise. 2,3,7,8-TCDD appears to bind in the AhR more potently by π-π stacking, while other less potent ligands such as PCBs and PBDEs hydrogen bond with specific key amino acid residues triggering differing conformational shapes of the receptor-ligand binding sites and energies to that of 2,3,7,8-TCDD.[7]

Conclusions

There are many considerations for cell lines of choice when conducting *in vitro* assay screening. In this study the human Hep G2 cell line was the best for predicting receptor binding and CYP1A1 induction for PBDEs. Analyses of the Volsurf variable contributions together with observed *in silico* modes of ligand binding in the AhR model allow additional insights into receptor activation on a compound and chemical family specific basis as well as ligand promiscuity and ligand-receptor cross talk when compared to other receptor studies. This is of relevance to consideration of AhR and CYP 1A mediated xenobiotic species-specific interactions, and a better understanding of the molecular mechanisms of PBDE pollutant toxicity and endocrine disruption.

Acknowledgments

MNJ gratefully acknowledges PhD funding from the BBSRC GSK CASE studentship scheme.

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