Modelling of species differences and interspecies responses after PCB exposure *in vitro*

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Introduction

The induction of the cytochrome P450 1A (CYP1A) activity is thought to occur via ligand binding to the Ah-receptor and can be measured as, e.g. ethoxyresorufin-*O*-deethylase (EROD) and methoxyresorufin-*O*-deethylase (MROD) catalytic activity. Structural characteristics for high affinity to the Ah-receptor of the ligand are planar conformation and chlorine atoms in the lateral positions. The toxic equivalence factors (TEFs) have been developed for use in risk assessment of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs). In the recent re-evaluation of the TEF-scale, quantitative structure-activity relationships (QSARs) were used as a supporting tool (1). A QSAR model relates the X-variables, which describe the chemical characteristics of the compounds, to the response Y, which in this case are biological responses. The concluding goal of the developed model is to achieve as good predictions as possible for untested compounds. In the present study we have applied a large set of physico-chemical parameters of the PCBs (X-matrix) and previously measured CYP1A induced activities of 20 congeners (Y-matrix) in order to model species differences and estimate an interspecies activity measure.

Material and Methods

A set of 52 physico-chemical descriptors is used to describe the features of the PCBs. In Table 1, these parameters, which is presented in detail elsewhere (2), are grouped in blocks. Briefly the blocks include following descriptors; 1) GC retention times on different columns and octanol/water partitions, 2) HOMO and LUMO energies etc., 3) sub-molecular energies derived from AM1 calculations, 4) dipole moments in the x-, y-, and z-directions, and 5) digitised UV-spectra from 200 to 300 nm. In the present study following PCBs were used, which are selected by using statistical design in combination with principal component analysis (PCA) (3, 4); #41, #51, #58, #60, #68, #78, #91, #99, #104, #112, #115, #126, #143, #153, *#169, #173, #184, #188, #190,* and #193.

Table 1. The physico-chemical parameters (X-matrix) of the PCBs divided in blocks.

Block	Type of parameter	No. of Parameters
1	Partition and retention characteristics	11
2	Reactivity measures	7
3	Molecular energies	5
4	Dipole moments	8
5	UV-spectra	21

The CYP1A induced activity was measured in intact hepatocyte monolayers as EROD and MROD catalytic activities (5). Hepatocytes from three different species were used, viz. cynomolgus monkeys (*Macaca fascicularis*), male castrated pigs (Great Yorkshire x Dutch Landrace), and chicken embryos (White Leghorn). The induced activity of the PCBs was measured in triplicates in 12 doses from concentrations of pM to μM. The responses determined from the dose-response curves were EC10, EC50, EC90, and Ymax relative to PCB#169. In the chicken system only MROD was measured whereas both catalytic activities were measured in the pig and monkey hepatocytes. As the X-matrix in Table 1, the responses are grouped in blocks; 1) monkey EROD, 2) monkey MROD, 3) pig EROD, 4) pig MROD, and 5) chicken MROD.

The X-matrix, i.e. the 52 physico-chemical parameters and the Y-matrix, i.e. the 20 measures of the PCB induced CYP1A activities, are of a multivariate nature. Partial least-squares projections to latent structures (PLS) is a suitable tool for multivariate calibration and to establish QSARs (6). Autoscaling and mean centring were used to pre-process the data and crossvalidation to validate the dimensionality of the models (7). In addition, PCA (4) and hierarchical multiblock PLS (Hi-PLS) (8) were used for characterisation of the structural diversity of the PCBs and to relate different parts of the X- and Y-matrix, respectively. Briefly, Hi-PLS works with the X-and Y-matrices divided in blocks. Further, the variation within and between them are modeled. The PCA and PLS calculations were performed using the SIMCA-S 6.0 (Umetri AB, S-907 19 Umeå, Sweden).

Results and Discussion

A two-step QSAR procedure was applied where 1) the active and non-active inducers were separated and 2) the CYP1A induced activity for untested PCBs was predicted. Valid QSARs were found for all systems and are presented elsewhere (4,9). In order to analyse species differences, predicted responses were compared as only five congeners were active in all assays. In Figure 1, the predicted logEC50 EROD values are plotted of the pig versus the monkey system. The responses of the two species are correlated, but a change in sensitivity is found at approximately 2 μM. The monkey hepatocytes are more sensitive than the pig to the non-*ortho* substituted PCBs and show only minor CYP1A activity for multi-*ortho* PCBs. The lines in Figure 1 indicate the breaking point in sensitivity, e.g. the congeners found in the lower left area are more active in the monkey than the pig system. The corresponding analysis of the predicted responses of pig versus chicken, shows an even earlier shift in sensitivity (0.2 μM) and a higher correlation (r^2=0.9). In Figure 2, the physico-chemical characteristics of the PCBs are represented by the three first score vectors (t1-t3) from a PCA describing 75% of

the variation. PCBs predicted as the most active inducers were marked in the PCA by encircling "high priority" area of each species. As can be seen in Figure 2, the pig and chicken assays correlate whereas the area of the monkey system is shifted towards lower chlorinated biphenyls.

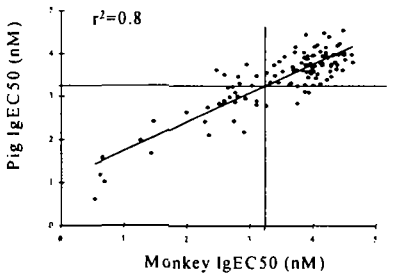

Figure 1. The predicted lgEC50 values for pig versus monkey. The lines indicate the change in sensitivity of the two species.

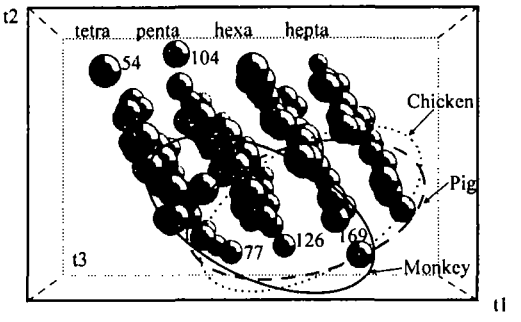

Figure 2. Tetra to hepta CBs in a PCA and the areas of highest CYP1A induction for monkey, pig and chicken.

The variation in responses from the three different species as well as the two catalytic activities measured have been studied by using Hi-PLS. In Hi-PLS the original X- and Y-matrices are blocked and the variation within and between the blocks are modelled in a sub- and super-level, respectively. The calculated model including 5 blocks of both the X- and Y-matrix described 69% and 72% of the variation in X and Y, respectively. Each Y-block included EC10, EC50 and EC90. The EROD and MROD blocks (1, 2) of the monkey are clustered and separated from the pig blocks (3, 4). This indicates species differences and may also indicate a variation in the EROD/MROD responses for the pig assays. The important X-blocks for the species separation are block 1 and 3, which both are related to the size of the compounds. Notably, this observation is well correlated with the findings in Figure 2.

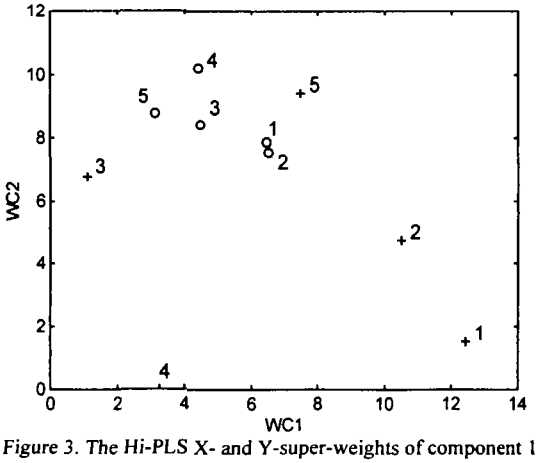

Figure 3. The Hi-PLS X- and Y-super-weights of component 1 versus 2. The Y-blocks are marked with (o) and the X-blocks with (+).The blocks used in the model are described in the material and methods part.

A general response measure of the PCBs for all different species and endpoints measured is calculated as the principal induction potencies (PIPs). This interspecies estimate of the CYP1A induced activity is based on the principal properties calculated of the complete Y-matrix by using PCA. One component of the PCA explained 85% of the variation and the generated score values for the 20 tested PCBs was in a second step used as the dependent variable in a PLS-model. By using PLS, the "principal toxicity" of untested congeners was predicted. The principal induction potency of all tetra to hepta chlorinated biphenyls was calculated relative to PCB#126, which was given PIP=1. The PCBs included in the TEF scale (1) and the PCBs of PIPs between 1 to 0.1 are summarised in Table 2. The PIP is the sum over all activities in the three species and can be used to estimate Ah-receptor mediated toxicity of untested congeners and to identify high priority congeners. By using this interspecies toxicity measure a number of congeners is identified which are suggested to be considered in environmental monitoring and risk assessments of the PCBs.

Table 2. The PCBs ranked to PIPs from 1 to 0.1. Marked in bold are congeners included in the TEF concept (1).

126, **169**, **77**, **81**, 127, 79, 78, **167**, 74, **189**, **157**, **118**, **114**, **156**, 80, 124, **105**, 159, 60, 66, 63, **123**, 162, 120, 122, 191, 107, 61

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