STRUCTURE-ACTIVITY RELATIONSHIPS OF PCBS POTENCY AS NEUROTOXICANTS

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Introduction

Polychlorinated biphenyls (PCBs) are abundant environmental contaminants with a complex pattern of toxicological responses. It is well known that the so-called coplanar or dioxin-like PCBs (DL-PCBs) exert their toxicity via the aryl hydrocarbon (Ah) receptor. These PCBs include 12 congeners, which have been assigned toxic equivalency factors, and these share a common molecular structure including chlorine atoms in the *para-para* positions and at least one *meta* position plus at most one chlorine in the *ortho* position. Reported toxicological effects in addition to those related to the Ah-receptor include cancer, disturbances of the immune- and endocrine systems, and neurotoxic effects. These effects with specific focus on non dioxin-like PCBs (NDL-PCBs) will be studied in detail the coming years in the EU funded research project ATHON. The central nervous system (CNS) is sensitive even for low levels of PCBs and various effects have been observed. Recently, neurotoxic effects related to PCB exposure were reviewed by Mariussen and Fonnum.[2] These effects include disturbances of calcium homeostasis, altered levels of neurotransmitters (e.g. dopamine), and induction of oxidative stress. The neurotoxic effects as determined *in vitro* have been shown by several research groups to be closely related to the molecular structures of the PCBs.[3-6] Here quantitative structure-activity relationships (QSARs) models are discussed and reported related to PCBs potency as neurotoxicants. In addition, QSARs based on a wider range of effects are compared with focus on both DL-PCBs and NDL-PCBs.

Materials and Methods

A set of 20 PCBs was selected using statistical experimental design combined with principal component analysis (PCA).[7] In summary, this strategy is initiated by a chemical characterisation using a multitude of chemical descriptors. Here 52 descriptors were used including those calculated in the semiempirical method AM1, UV absorption spectra, relative retention times on various gas chromatographic columns, and the octanol-water partition coefficient.[8] The inherent information in the multitude of descriptors were summarised using PCA. PCA can handle correlated variables and extract the largest variation in a few latent variables, so-called principal components. These can be plotted in a score plot where the most dominant pattern among the objects can be visualised. The corresponding loading plot shows the importance of the studied variables. The second step in the selection strategy was to use the formed latent variables as descriptors of the PCBs structural features. The design is next used as an aid to select congeners that together represents the entire chemical domain. Here factorial design was used including four variables varied on two levels plus four centre points representing the interior region of the domain. Furthermore, partial least squares projections to latent structures (PLS) was used to establish relationships between the chemical descriptors and the biological responses. In PLS, latent variables are formed from each matrix plus a relationship in-between the dependent and independent variables. The PCA and PLS calculations were performed in Simca-P+ 11.0 (Umetrics Inc, Umeå, Sweden). Most biological response data that were used in the modelling were produced in the laboratory of Fonnum and co-workers.

Results and Discussion

The training set of 20 PCBs was selected to cover the chemical domain of the tetra to hepta chlorinated congeners. The set includes 6 tetra-, 6 penta-, 3 hexa-, and 5 hepta-chlorinated biphenyls, and 3 non-, 3 mono-, 7 di-, 4 tri-, and 3 tetra-ortho substituted. In Figure 1, biological responses from nine various tests of the 20 PCBs of the training set are summarised using PCA. These tests cover competitive binding to the estrogen receptor (cER), inhibition of gap junction intercellular communication (GJIC), induction of porphyrin accumulation in chicken embryo hepatocytes (PA), induction of CYP1A in primary monkey hepatocytes (CYP1A), biomagnification in various fish species (BMF), inhibition of dopamine uptake in rat brain synaptic vesicles (DU

ves), inhibition of dopamine uptake in rat brain synaptosomes (DU syn), induction of formation of reactive oxygen species (ROS), and activation of respiratory burst (RB).[3,9-11] The responses show a wide variation in structure-activity relationships (SARs) for the tested PCBs, however, forming roughly four clusters. In the upper left corner of the loading plot in Figure 1b cER, GJIC, and PA are found. By simultaneously reading the corresponding score (Figure 1a) and loading plots (Figure 1b) it can be seen that these responses are most significant for highly chlorinated, tetra-*ortho* substituted compounds (e.g. PCBs 104, 184, and 188). The CYP1A response and BMF show unique SARs where CYP1A is clearly associated with non-*ortho* PCBs, here represented by PCBs 126 and 169. Highest BMF values were in general found for highly chlorinated congeners with two or less *ortho* chlorines (e.g. PCBs 99, 190, and 193). The responses related to CNS, viz. DU (ves), DU (syn), and ROS form together with RB, which is an effect related to the immune system, a distinct cluster. These responses are in general correlated with lower chlorinated, *ortho* substituted PCBs, such as PCBs 91 and 112. In summary, Figure 1 illustrates that the PCBs constitute a group of compounds with a number of distinct SARs.

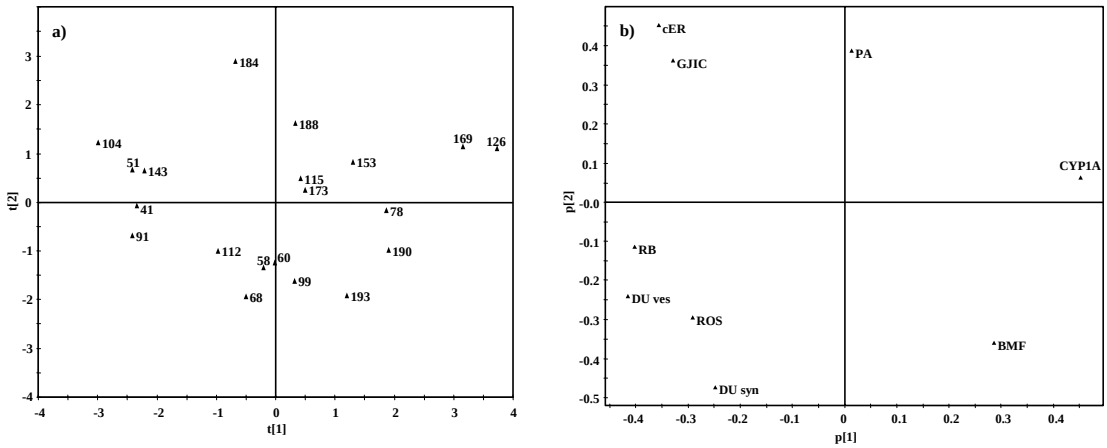

Figure 1: Score plot (a) and loading plot (b) from a PCA including 20 PCBs described by their relative potencies ranked from 0 to 3 from nine biological test systems (the abbreviations of the test systems are given in the text above).

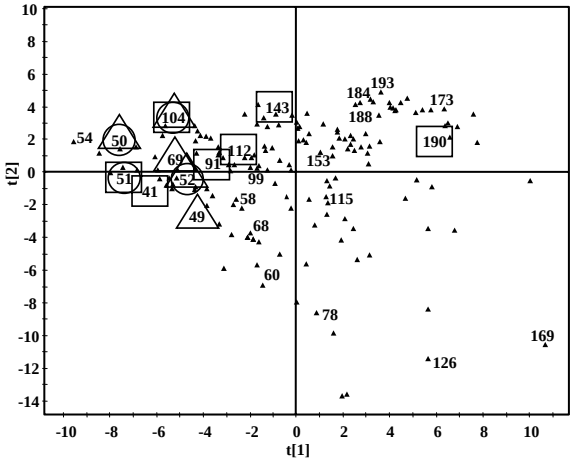

Figure 2: Score plot from a PCA including the 154 tetra to hepta chlorinated biphenyls described using 52 chemical descriptors. The PCBs of the training set plus PCBs with measured CNS related effects are indicated and those marked with rectangles, circles, and triangles were the most potent congeners in the assays employed by Mariussen[3], Shain[5], and Kodavanti[6], respectively.

An illustration of the chemical variation of the 154 tetra to hepta chlorinated biphenyls is shown in Figure 2. In brief, the first principal component (t1) is related to the molecular size whereas the second principal component t(2) reflects the number of *ortho* chlorines. In the score plot the training set of 20 PCBs are marked plus the most potent congeners as reported in the studies by Shain *et al*[5], and Kodavanti *et al*[6]. Marked are also the most potent inhibitors of dopamine uptake in synaptic vesicles.[3] The most active congeners are clearly found in a specific region of the chemical domain, i.e. the domain of lower chlorinated biphenyls with two or more *ortho* chlorines. One hexa (PCB 143) and one hepta (PCB 190) chlorinated biphenyl were among the most potent congeners, remaining potent PCBs were substituted with only four or five chlorines.

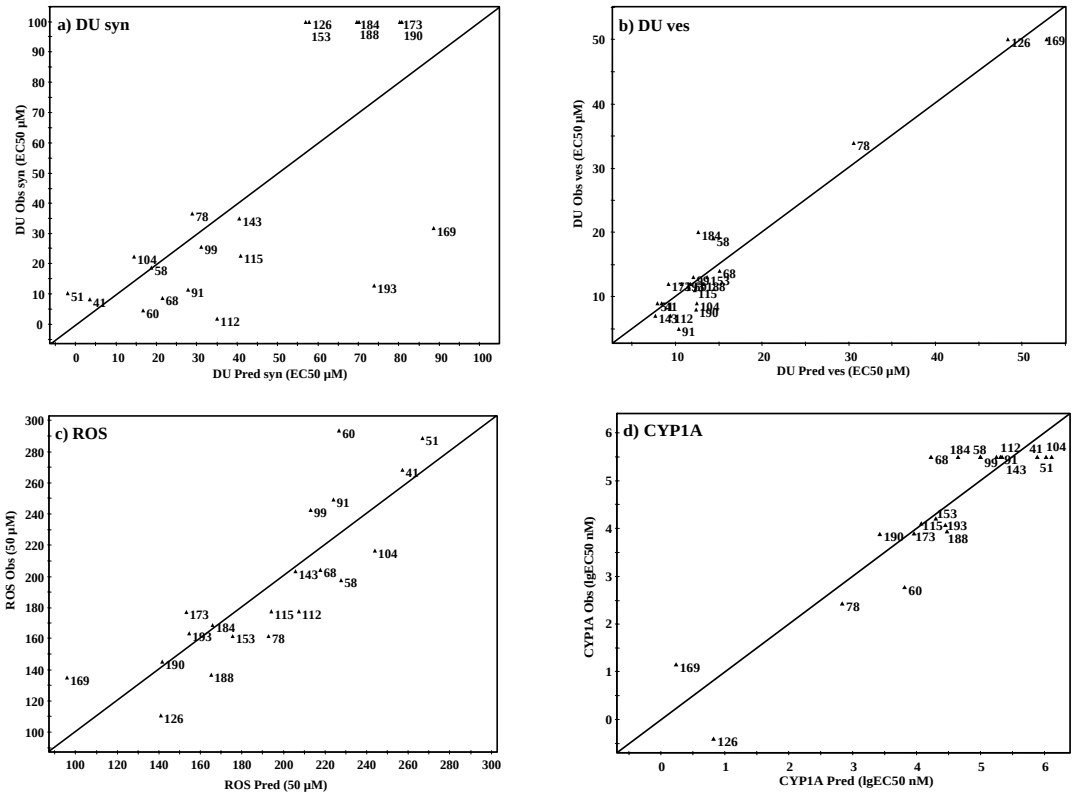

Figure 3: Plots showing observed versus predicted responses from QSAR models including 20 PCBs based on *in vitro* data from four assays; a) inhibition of dopamine uptake into synaptosomes (DU syn), b) inhibition of dopamine uptake into synaptic vesicles (DU ves), c) induction of formation of reactive oxygen species (ROS), d) EROD induction in primary monkey hepatocytes (CYP1A).

A number of QSAR models have been developed using data produced for the 20 selected PCBs.[9] Four models are illustrated in Figure 3 with plots of observed versus predicted responses. Clearly, the response window of the four responses differs where DU ves (Figure 3b) has a very narrow response range whereas ROS (Figure 3c) and CYP1A (Figure 3d) show wider variation in responses among the assessed PCBs. Potent congeners of the DU syn and DU ves responses are PCBs 41, 51, and 112, which are di- and tri-*ortho* substituted congeners. No activity in these systems is found for the co-planar PCBs 126 and 169 plus for DU syn PCBs 153, 173, 184, 188, and 190. The QSARs indicated that the model of DU syn was heavily influenced by measures of size whereas the DU ves model was dependent on reactivity descriptors and certain bands of the UV-spectra typical of co-planar biphenyls. The ROS response, measured at 50µM as percentage of control, shows highest response for PCBs 41, 51, and 60 and lowest for the co-planar PCBs 126 and 169. In this model a mixture of descriptors related to size, hydrophobicity and reactivity compose the most significant variables. Finally, the CYP1A model

shows as expected a correlation with descriptors related to the compounds ability to be co-planar. In comparison with the other responses CYP1A showed activity for less number of congeners, viz. for only 10 out of the 20 studied PCBs.

In summary, the SARs of PCBs are complex showing certain well defined and distinct relationships. The neurotoxic responses of PCBs, as determined *in vitro*, indicate an in-common SAR where a high activity is caused by lower chlorinated biphenyls with two or more *ortho* chlorines. QSARs were here presented for both effects related to DL- and NDL-PCBs and validated QSARs can be used to predict effects of untested PCBs and for ranking and classification. In the EU funded research project ATHON various effects of NDL-PCBs will be explored. In ATHON, QSARs will be applied to provide data for ultimate use to generate a new classification system of PCBs. For this project the models presented here will form the basis for the development of more precise QSARs.

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