Multivariate QSAR modelling of the endocrine effects of polybrominated diphenyl ethers

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Introduction

Polybrominated diphenyl ethers (PBDEs) have been extensively used as flame-retardants in different polymers, in e.g. electronic equipment such as computers and television sets[1]. Mainly two technical mixtures have been used, viz. the Deca-BDE and Bromkal70-5DE.
PBDEs were first discovered in 1981 in samples of pike, eel, and sea trout[2] and has from this point been found in a variety of environmental samples but most disturbing is their increasing levels found in mother's milk for the past 25 years[3].
As a part of a hazard identification approach for brominated flame retardants (BFRs), within the framework of FIRE[4], a selection of test compound was made with 17 BFRs based on their environmental relevance and availability for testing. An additional set of substances of 12 BFRs was selected to complement the originally selected BFRs by making a statistical molecular design with the use of a D-optimal approach, which maximized the coverage of the physicochemical space. The total test-set of 27 BFRs consisted of tetrabromobisphenol A (TBBPA), 6OH-BDE47, 2,4,6-tribromophenol (246TBP), hexabromocyclododecane (HBCDD), tetrabromobisphenol-dibromopropyl ether (TBBPA-DBPE) and polybrominated diphenyl ethers (PBDE). The nineteen selected BFRs are a choice of brominated diphenyl ethers (BDEs) ranging from tri to deca brominated diphenyl ethers. Seven of these BDEs were pre-selected due to their occurrence in used technical products and will be considered environmentally significant i.e. BDEs 28, 47, 99, 100, 153, 183 and 209 while 12 congeners were selected by statistical molecular design. This study focuses on the 19 PBDEs only, with the QSAR models established on the antagonistic potential of these BDEs towards the androgen (AR) and progesterone receptors (PR) determined by the AR and PR CALUX® bioassays, respectively[5].

Material and methods

Physicochemical and additional properties often used in QSAR studies were generated by the molecular modeling software HyperChem®. Molecular geometry optimization was made for all the 209 PBDEs with the semi-empirical AM1[6]. The properties generated were e.g. a diverse set of molecular energies, heat of formation, (x, y, z) moments of inertia, frontier orbital energies, dipole moments, volumes and surface areas. A few charge descriptors were calculated within the DRAGON software (v.3.0, TALETE srl©, Milano, Italy), see **Table 1**. The dataset was complemented with experimental data by scanning the

UV spectra in the 200-320 nm range (GBC Scientific Equipment, Pty, Ltd.). The test-set of 19 PBDEs i.e. BDEs 19, 28, 38, 39, 47, 49, 79, 99, 100, 127, 153, 154, 169, 181, 183, 185, 190, 206 and 209 was prepared individually in cyclopentane (UV cut-off = 198nm) at a concentration of 20µM. QSAR models were calculated using partial least squares projections to latent structures (PLS) using Simca-P+® (Umetrics Inc., Umeå, Sweden). QSAR modelling was based on the antagonistic activities of PBDEs determined by the *in vitro* AR and PR CALUX® bioassays on androgen and progestin mediated gene transcription to determine their endocrine disrupting potential. Stock solutions of BFRs were made in THF/toluene and DMSO for analytical purposes and *in-vitro* pre-screening, respectively.

Table 1. Calculated semi-empirical parameters and UV spectra used in the PLS models.

Electronic, spectral and molecular properties	Abbreviation	Unit
UV absorbance spectra	200-320	nm
Heat of formation	H_f	kcal/mol
Stretch-bend energy	SBE	kcal/mol
Charge descriptors[a]	qpmax,qnmax,Qpos,Qneg,Qtot,Q2, RPCG,RNCG,SPP,TE1-2, PCWTe	
Moments of Inertia	Mom(x, y, z)	
Frontier Molecular Orbitals (FMOs)	HOMO, LUMO, Gap	eV
Total Dipole Moment & vectors	µ, µ (x,y,z)	Debye
Local dipole index[a]	LDip	
Van der Waals Area & Volume	VdWA, VdWV	$Å^2$, $Å^3$
Solvent Accessible Surface Area & Volume	SAS, SAV	$Å^2$, $Å^3$

a) Charge descriptors are defined in DRAGON (v3.0), also see Todechini et al.[7].

Results and discussion

In this study 30 physicochemical parameters were either calculated or experimentally determined. Whereas none of the tested BDEs had any AR or PR agonistic potency in the CALUX® bioassays, only AR and PR antagonistic potencies are considered in this paper. The calculated PLS models for the antagonistic activities on AR and PR mediated gene induction gave two significant principal components (PCs) for each response Y-data, which explained 85 and 71 % (R^2Y=0.85 and 0.71), respectively, of the variation in the response Y-data (log $1/IC_{50}$), see **Figure 1**. Both correlation coefficients show an acceptable explained variation in the Y-data, as is evident from the observed vs. predicted plots, see **Figure 2**. Model diagnostics was used to verify the validity of the models using cross-validation (Q^2) and response permutation testing. The cross-validated predicted variance was adequate with a Q^2 of 69% and 45%, respectively, for antagonistic AR and PR activity while the permutation testing revealed some over fitting of the model[8].

We found that both the antagonistic AR and PR mediated gene transcription was primarily influenced by two groups of physicochemical descriptors. Descriptors influencing the PLS model are mainly the strongly interrelated descriptors for the molecular electronic properties such as the frontier molecular orbitals (FMOs). The UV absorbance spectrum has a high degree of correlation with the FMOs but also describes the size, shape and the substitution pattern of the BDEs. Generally, an increase in FMO energy, negative charge and polarity has a positive influence on the antagonistic AR and PR activity while an

increasing size has a negative influence. The substitution pattern influences the antagonistic activity as bromines in *ortho* position increases the antagonistic effects. This is evident from the UV spectrum as BDEs with many *ortho* bromines has the absorption maximum at shorter wavelengths as is the case for the tri-*ortho* BDE 19, while higher brominated congeners as BDE 209 absorbs UV light at longer wavelengths.
Comparing the progestin and androgen mediated gene transcription, see **Figure 1**, the responses are moderately correlated with a slight difference in responses. Relating the response data with the physicochemical variables the differences in position of AR and PR suggests that the PR responses might be more dependent on more polar or polarisable BDEs while size/lipophilicity are important for the AR antagonistic activity.

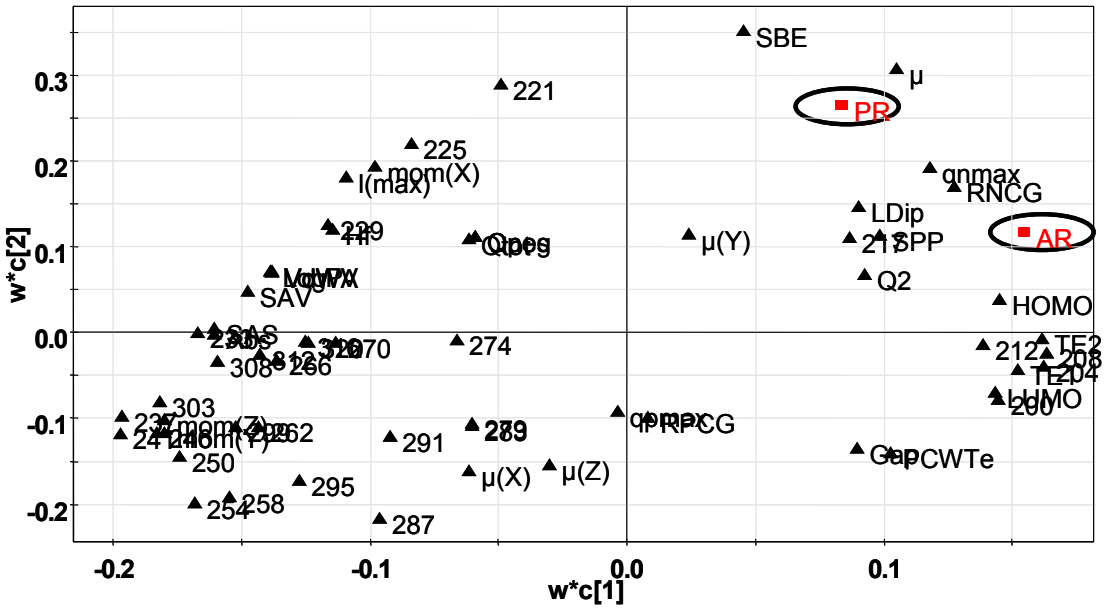

Figure 1. Loading plot for the latent variables relating antagonistic effects from the AR and PR CALUX® bioassay to the physicochemical variables.

Based on the QSAR model, five BDEs that were not included in the test set were predicted to have an equal or stronger antagonistic PR activity than BDE-19 (IC_{50} at 0.8 μM). These five BDEs are the congeners 53, 54, 96, 104 and 152 all being tri or tetra-*ortho* brominated congeners. The PBDEs with the highest predicted activity was BDE 54 (2,2',6,6') with a predicted IC_{50} at 0.5 μM. For antagonistic AR activity on the other hand we found no congeners to have a predicted activity higher than BDE 19 (IC_{50} at 0.06 μM). The BDEs with the highest predicted antagonistic activity were low brominated BDEs with substitutions in *ortho* or *ortho-para* positions, i.e. BDEs 4, 10, 17, 32, 51 and 54. Only BDE 17 (2,2',4) as it is found in Bromkal 70-5DE could be considered to be of more environmental significance. BDE 17 was predicted to have a similar antagonistic AR activity as BDE 100 with an IC_{50} of 0.10 μM. Similarly, the predicted antagonistic PR activity also found BDE 17 as the only environmentally significant PBDE with a predicted IC_{50} of 1.9 μM which is significantly lower than the IC_{50} for BDE 19.

Conclusions

Comparing the molecular structures of progesterone and testosterone, the natural ligands for AR and PR respectively, one of the differences in structure is that there is a hydroxyl-group on testosterone while progesterone has a ketone-group. BDEs predicted to have an increased PR antagonistic effect are congeners that are tetra to hexa brominated which have a significantly higher log P (6.6 to 8.2) compared to those predicted for the AR antagonism which might relate to the differences in log P for progesterone and testosterone, as progesterone is slightly more lipophilic. If these differences have any real correlation with the lipophilicity or electronic properties of BDEs is not clearly evident from this QSAR model. Finally, as this work is a continuous project within the FIRE project[4] more QSARs will be generated in the near future for other endocrine endpoints.

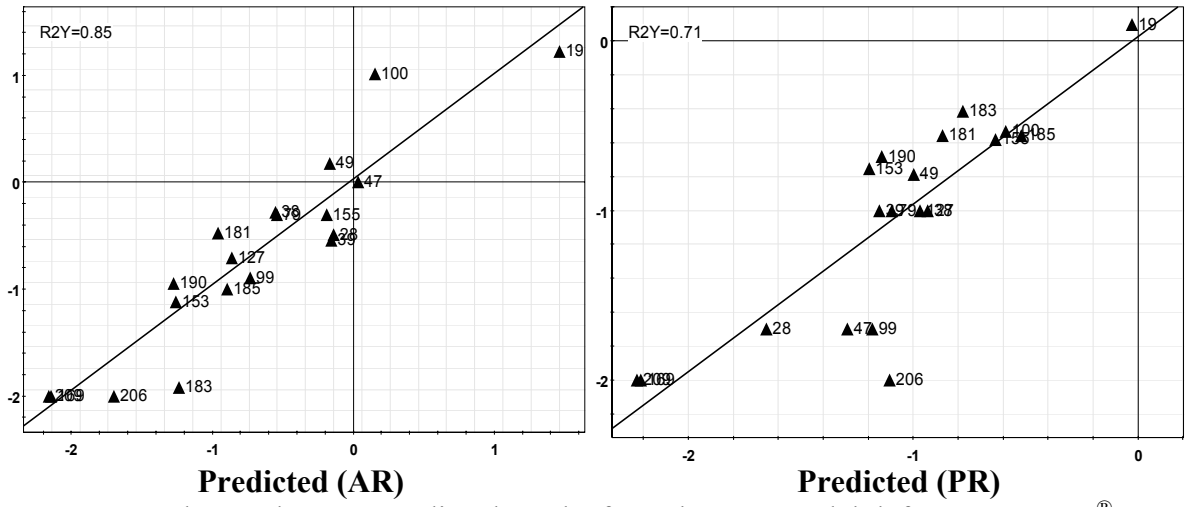

Figure 2. Observed versus predicted results from the PLS model, left, AR CALUX® bioassay, right, PR CALUX® bioassay.

References

1. WHO. Environmental health criteria 162: brominated diphenyl ethers. **1994,** 31-34.
2. Andersson, O.; Blomkvist, G. *Chemosphere* **1981**, *10*, 1051-1060.
3. Meironyte, D.; Noren, K.; Bergman, A. *J. Toxicol. Environ. Health,* **1999**, *58*, 329-341.
4. www.rivm.nl/fire, site update 2003-05-14, accessed 2004-03-30.
5. Hamers, T., Kamstra, J.H., Sonneveld, E., Murk, A.J., Zegers, B. N., Boon, J. P., Andersson, P.L., and Brouwer, A. Abstract BFR2004 Toronto, **2004**.
6. Dewar, M.J.S.; Zoebisch, E.G.; Healy, E.F.; Stewart, J.J.P. *J. Am. Chem. Soc.* **1985**, *107*, 3902-3909.
7. Todeschini, R.; Consonni, V.; Mannhold, R.; Kubinyi, H.; Timmermann, H. *Handbook of Molecular Descriptors*, 1ed. Vch Verlagsgesellschaft Mbh: **2002**.
8. Eriksson, L.; Johansson, N.; Wold, K.; Wold, S. *Introduction to multi- and megavariate data analysis using projection methods (PCA & PLS).*, Umetrics Inc., Umeå, Sweden, **1999**.