# QSAR prediction of endocrine disruption potencies of brominated flame retardants

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**ABSTRACT**

**In the last decade, brominated flame retardants (BFRs) became an emerging class of POPs. Because of their chemical similarity with other classes of organohalogenated compounds, such as PCBs and dioxins, these compounds can act as endocrine disruptors. In this study, QSAR models were developed on different responses related to endocrine disruption potency of some BFRs, in particular polybrominated diphenyl ethers (PBDEs). The multiple linear regression (MLR) approach was applied, in combination with the Genetic Algorithm variable selection procedure, to a variety of theoretical molecular descriptors representing the molecular structures. The best models were internally validated for their performance using the leave-one-out (Q2loo=72-91%) procedure and scrambling of the responses. External validation was provided, when possible, by splitting the data sets in training and test sets (range of Q2ext=80-94%), which confirmed the predictive ability of the models.**

**This topic is included in the FP7- EU Project CADASTER under negotiation.**

## MATERIALS and METHODS

**DATA SET** The experimental data related to endocrine disruption potencies of BFRs were taken from the literature [1, 2, 3]. The selected responses included Ah Receptor Binding Affinities (RBA), EROD induction potencies ($EC_{50}$ERODind), Ah Receptor, Estrogen Receptor and Progesterone Receptor interaction potencies as agonist or antagonist ($EC_{50}$DRag, $EC_{50}$ERag, $IC_{50}$PRant), T4-TTR competing potencies ($IC_{50}$T4-TTR) and estradiol sulfotransferase inhibiting potencies ($IC_{50}$E2SULT). All the responces, reported in μM, have been transformed to logarithmic units and, if necessary, multiplied by -1 to obtain positive values. The experimental data set, very restricted in most of the cases, was formed by some PBDE congeners and other BFRs (i.e. BPA, TBBPA, HBCD).

**MOLECULAR DESCRIPTORS**

- 615 molecular descriptors (0D; 1D; 2D; 3D) were calculated by the software *DRAGON* [4].
- 4 quantum-chemical descriptors (Highest Occupied Molecular Orbital (HOMO), Lowest Unoccupied Molecular Orbital (LUMO), HOMO-LUMO gap (DHL) and the ionisation potential (P ion)) were calculated by the semi empirical PM3 Hamiltonian for the geometry optimisation method available in the HYPERCHEM package [5].

**MULTIPLE LINEAR REGRESSION MODELS** and Variable Selection were performed by *Ordinary Least Squares* regression (OLS) method [6].

**EXTERNAL VALIDATION**

Prediction set selection based on the molecular structure (by Kohonen Maps - Artificial Neural Networks (K-ANN) [7]) or using the Random by response approach.

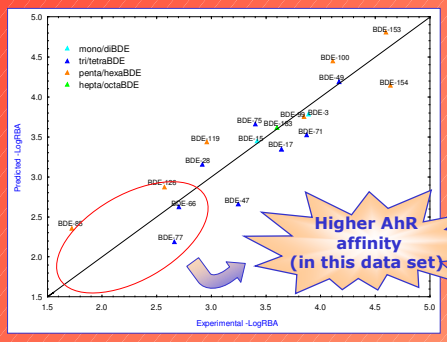
**TOOLS OF VALIDATION AND DIAGNOSTICS**

Models were developed taking into account the recently proposed OECD principles for QSAR validation [8].

- Internal (by $Q^2_{LOO}$ and $Q^2_{LMO}$, Y-scrambling) and external validation (verified by $Q^2$ext).
- Check of the quality of the best models by Residuals and Williams plot
- Applicability Domain (AD% for 223 BFRs) verified by leverage approach.

## RESULTS

**-LogRBA = 0.80 + 0.45 RDF035v + 1.01 RDF080v**



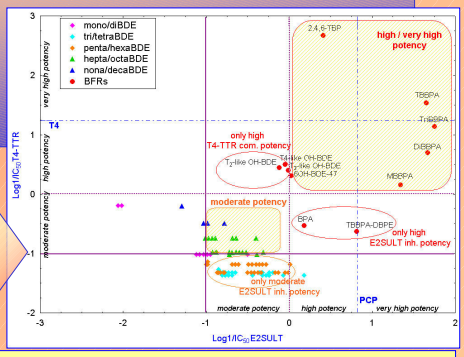

| Endpoint | Models | Training obj. | Pred obj. | Variables | $R^2$ | $Q^2_{LOO}$ | $Q^2_{BOOT}$ | $Q^2_{EXT}$ | RMSE | AD% (223) |
|---|---|---|---|---|---|---|---|---|---|---|
| Log1/RBA | Full Model | 18 | - | RDF035v RDF080v | 86.13 | 79.34 | 79.75 | - | 0.34 | 89.69 |
| | Random | 15 | 3 | | 86.51 | 78.28 | 77.93 | 74.65 | - | - |
| | K-ANN | 15 | 3 | | 84.71 | 75.30 | 73.81 | 93.50 | - | - |
| Log1/$EC_{50}$ERODind | Full Model | 8 | - | HATS5e | 86.97 | 79.92 | 81.82 | - | 0.33 | 94.17 |
| Log1/$EC_{50}$DRag | Full Model | 8 | - | Mor29u | 83.19 | 71.89 | 72.93 | - | 0.38 | 76.68 |
| Log$EC_{50}$ERag-1 | Full Model | 8 | - | Mor19u | 90.91 | 83.03 | 83.53 | - | 0.10 | 85.20 |
| Log$EC_{50}$ERag-2 | Full Model | 7 | - | RDF075u | 95.37 | 90.88 | 91.64 | - | 0.04 | 78.48 |

**Classification of BFRs based on predicted toxicity results (according to Hamers *et al.*, 2006 [2]):**

| Criteria | Potency |
|---|---|
| Log1/E(I)C50 < -1 | no/low potency |
| -1 < Log1/E(I)C50 < 0 | moderate potency |
| 0 < Log1/E(I)C50 < +1 | high potency |
| Log1/E(I)C50 > +1 | very high potency |

| Endpoint | Models | Training obj. | Pred obj. | Variables | $R^2$ | $Q^2_{LOO}$ | $Q^2_{BOOT}$ | $Q^2_{EXT}$ | RMSE | AD% (223) |
|---|---|---|---|---|---|---|---|---|---|---|
| Log1/$IC_{50}$PRant | Full Model | 19 | - | GATS8e EEig09x | 83.45 | 76.85 | 73.39 | - | 0.18 | 95.52 |
| | Random | 16 | 3 | | 85.18 | 78.19 | 74.39 | 61.19 | - | - |
| | K-ANN | 16 | 3 | | 84.24 | 80.18 | 67.68 | 79.9 | - | - |
| Log1/$IC_{50}$T4-TTR | Full Model | 12 | - | MATS1v C-024 | 97.44 | 98.69 | 97.03 | - | 0.20 | 96.86 |
| Log1/$IC_{50}$E2SULT | Full Model | 16 | - | X5Av GATS2p | 87.96 | 83.69 | 84.12 | - | 0.33 | 98.65 |

**E2SULT Inhibition Pot. vs T4-TTR Competing Pot.**




## CONCLUSIONS

- Different QSAR models for prediction of endocrine disrupting potencies of BFRs, particularly PBDE, are proposed, and AD was verified for 223 BFRs.
- Despite the limited amount of experimental data available, the developed models have good predictive power, and were verified by internal and, when possible, external validations.
- According to RBA experimental data [1], all predicted RBA values show weaker AhR affinity than the reference toxicant TCDD (< 2-5 orders of magnitude).
- Predicted EROD induction potencies result higher for planar compounds than for those non planar (with two or more *ortho*-bromines).
- T4-TTR competing potency seems greater for highly brominated diphenyl ethers (hepta-nonaBDEs), as well as for diBDEs, and for all the other BFRs, specially 2,4,6-TBP and TBBPA, whose TTR-binding potency exceeds that of the natural ligand T4.
- E2SULT inhibition potency appears moderate for almost all PBDEs (except mono-diBDEs) and high to very high for the other BFRs, particularly M/Di/Tri/TBBPA. These BFRs are more potent than the well-known inhibitor pentachlorophenol (PCP).
- According to the literature [2], a correlation was found between T4-TTR competing potency and E2SULT inhibition. In agreement with this, our models predicted a moderate and high toxicity respectively for highly brominated BDE congeners and BFRs with hydroxylated aromatic group.
- Not enough experimental data are available to identify a quantitative relationship between bromination degree, or the bromine position, and BFRs' interaction with Ahryl, Estrogen and Progesterone receptors (DR agonism, ER agonism, PR antagonism).
- The variability of interactions of the studied chemicals with different hormone receptors and hormonal systems prevented us from defining a general ranking based on their ED potency.

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