QSAR modelling of the endocrine disrupting activity of Brominated Flame Retardants (BFRs)

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INTRODUCTION

In the last decade, brominated flame retardants (BFRs), and in particular polybrominated diphenyl ethers (PBDEs), have been recognised as an emerging class of persistent organic pollutants. Endocrine disrupting (ED) effects, especially on thyroid and sex-related hormones, have been observed for some BFR congeners. In the REACH legislation the crucial step of Authorisation is mandatory for chemicals with PBT and ED behaviour: the identification of safer alternatives to these chemicals is required.

Unfortunately, the available amount of experimental data is very small and is mainly related to already banned BFRs. According to REACH there is urgent need to maximize the value of existing data, also by using them to predict unknown activities for existing or even not yet synthesized chemicals. The development of QSAR models is among the successful strategies which can meet these needs.

The aim of this study was to develop QSAR models for the prediction of T4-TTR competing potency and E2SULT inhibition potency of BFRs, which are linked to endocrine disruption activity. Two approaches are here proposed: multiple linear regression, by Ordinary Least Squares (OLS), and classification, by K-NN method.

MATERIALS and METHODS

DATA SET The experimental data related to endocrine disruption potencies of BFRs were available for several PBDE and OH-BDE congeners, TBBPA (tetrabromobisphenol-A), TBBPA-DBPE (tetrabromobisphenol-A-bis(2,3)dibromopropyl ether), 246-TBP (2,4,6-tribromophenol) and HBCD (hexabromocyclododecane) [1-2].

Regression endpoints: T4-TTR relative competing potencies (**T4-REP** = IC_{50}T4-TTR$_{T4}$/IC_{50}T4-TTR$_{BFR}$) and estradiol sulfotransferase relative inhibiting potencies (**E2SULT-REP** = IC_{50}E2SULT$_{PCP}$/IC_{50}E2SULT$_{BFR}$). All the responses, reported in μM, have been converted into logarithmic units.

Classification: 3 classes (C1=no potency; C2=low/moderate potency; C3=(very) high potency) selected according to Hamers *et al.* [1].

MOLECULAR DESCRIPTORS The input files for descriptor calculation, containing information relative to the minimum energy conformation of the molecule, were obtained by the Semi-empirical method AM1 in *HYPERCHEM* [3]. 483 molecular descriptors (0D; 1D; 2D; 3D) were then calculated by the software *DRAGON* [4].

REGRESSION MODELS Multiple linear regression was performed by *Ordinary Least Squares* regression (OLS) method and *All Subset Selection* method was applied to select the best variables and models [5].

CLASSIFICATION MODELS K-NN method was applied to model the three classes of ED potency [6]. The selection of the best subset of variables has been realised by the *All Subset Selection* method.

SPLITTING TECHNIQUE Prediction set selection was carried out by Random through activity sampling.

TOOLS OF VALIDATION AND DIAGNOSTICS

Models were developed taking into account the recently proposed OECD principles for QSAR validation [7].

- Internal (by Q^2_{LOO} and Q^2_{BOOT}, Y-scrambling) and external validation (verified by Q^2_{EXT}) [8].
- Check of the quality of the best models by Residuals and Williams plot.
- Applicability Domain (AD% for 238 BFRs) verified by leverage approach (regression models) or by descriptor's range (classification models).

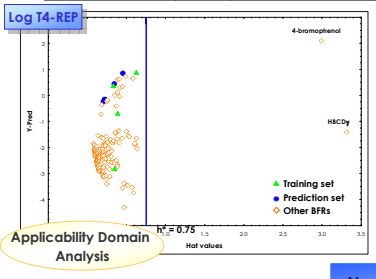
REGRESSION MODELS

External validation of previously developed models [9] with new data available for OH-BDEs [2]

Endpoint	N_{TR}	N_P[2]	Variables	R^2%	Q^2_{LOO}%	Q^2_{BOOT}%	R^2_{YS}%	Q^2_{EXT}%
LogT4-REP	12	5	PW4 qpmax	96.12	92.77	86.96	17.45	89.1
LogE2SULT-REP	16	5	GATS1v B08[C-O]	82.71	78.46	67.85	13.39	95.12

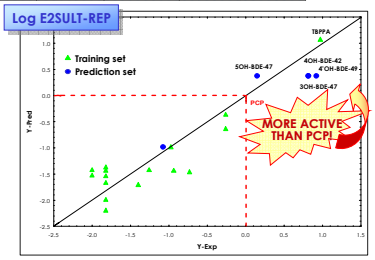
THE OLD MODELS WORK!!

Log T4-REP



Applicability Domain Analysis

Log E2SULT-REP



New models developed with all available data [1,2]

Endpoint	Model	N_{TR}	N_P	Variables	R^2	Q^2_{LOO}	Q^2_{BOOT}	R^2_{YS}%	Q^2_{EXT}	AD% (238)
LogT4-REP	Full Model	17	-	MATS6v qpmax	95.20	92.96	92.86	13.42	-	98.74
	Split 30%	12	5		94.74	90.18	89.72	17.24	96.21	
LogE$_2$SULT-REP	Full Model	21	-	GGI7 B08[C-O]	87.57	83.61	81.96	9.12	-	100
	Split 30%	15	6		88.94	83.61	83.80	14.65	82.41	

CLASSIFICATION MODELS

Classification criteria according to Hamers *et al.*, 2006 [1]

CRITERIA	POTENCY
Response < 20% of control	no potency
IC_{50} > 10 μM & resp > 20% of control	low potency
1.0 μM < IC_{50} < 10 μM	moderate potency
0.1 μM < IC_{50} < 1.0 μM	high potency
0.01 μM < IC_{50} < 0.1 μM	very high potency

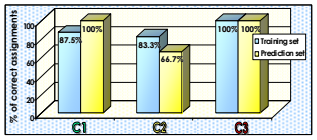
CLASSES
1 NO POTENCY
2 LOW/MODERATE POTENCY
3 (VERY) HIGH POTENCY

T4-TTR competition

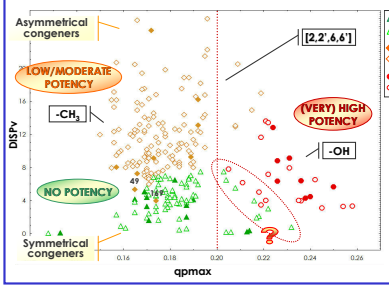
Split Model (35%)

Variables	K	n_{TR}	n_P	NER%	NER_{EXT}%
DISPv qpmax	3	19	10	89.5	90

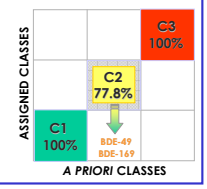
NER (non-error rate) = correct classifications/tot. obj.



Full Model	Variables	K	n	NER%
	DISPv qpmax	3	29	93.1



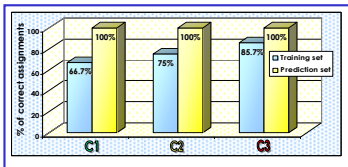
DISPv qpmax
Geometrical descriptor
Charge descriptor



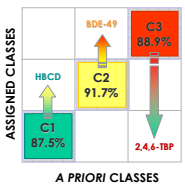
E2SULT inhibition

Split Model (30%)

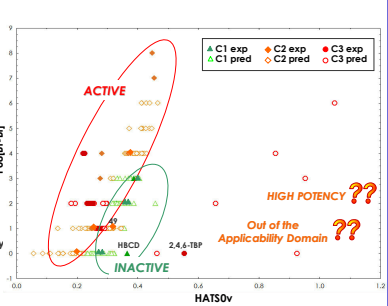
Variables	K	n_{TR}	n_P	NER%	NER_{EXT}%
HATS0v F08[Br-Br]	1	21	8	76.2	100



Full Model	Variables	K	n	NER%
	HATS0v F08[Br-Br]	1	29	89.6



HATS0v F08[Br-Br]
GETAWAY descriptor
2D Frequency Fingerprint



CONCLUSIONS

- ✓ Endocrine disrupting potency of BFRs has been modelled by two different QSAR approaches.
- ✓ Models have been developed according to OECD principles [6].

REGRESSION MODELS

- ✓ The availability of new toxicity data for some hydroxilated PBDEs [2] allowed for the external validation of the previously developed models, which confirmed their robustness and real predictive power.
- ✓ New models have been developed using all the available data [1,2]: the models show high performances (both in fitting and prediction) and are expected to give reliable predictions for almost all the 238 BFRs considered in this study.

CLASSIFICATION MODELS

- ✓ The developed models are characterized by good performance both in fitting (NER ≈ 90%) and in prediction (NER_{EXT} > 90%).
- ✓ According to literature [1,10], ED activity of BFRs is strongly increased by the presence of –OH group on the aromatic ring.
- ✓ The presence of Br substituents in [2,2',6,6'] seems to increase T4-TTR competition. The same behaviour was not observed for E2SULT inhibition.
- ✓ In REACH context, classification models here proposed represent an important and simple tool to predict the level of endocrine disruption potency of BFRs.

Financial support, given by European Union through the project CADASTER (FP7-ENV-2007-1-212668), is gratefully acknowledged.

REFERENCES
(1) Hamers T. *et al.*, 2006. *Tox.Sci.*, 92, 157-173;
(2) Hamers T. *et al.*, 2008. *Mol. Nutr. Food. Res.* 52, 284-298;
(3) HYPERCHEM. Rel. 7.03 for Windows, 2002. Hypercube, Inc. Florida, USA;
(4) Talete srl., 2007. *DRAGON – Software for Molecular Descriptors Calculations*, ver. 5.5 for Windows; http://www.talete.mi.it/
(5) Todeschini R., 2001. *MOBY DIGS. Rel.* 2.3 for Windows, Talete srl, Milan (Italy);
(6) SCAN Software for Chemometric Analysis, 1995, ver. 1.1 for Windows, Minitab (USA);
(7) Available online at: http://www.oecd.org/document/23/ (accessed April 2009);
(8) Gramatica P., 2007. *QSAR Comb. Sci.*, 26, 694-701;
(9) Papa E., Kovarich S., Gramatica P. Poster presented at SETAC Europe 18th Annual Meeting, 25-29 May 2008, Warsaw, Poland;
(10) Liu H. *et al.*, 2007. *J. Mol. Graph. Model.*, 26, 135-144.