Classification QSAR Models for the prediction of endocrine disruption potencies of brominated flame retardants

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INTRODUCTION

Increasing concern is shown by the scientific community, regulators, and the public, about **endocrine-disrupting** chemicals (EDCs) that are adversely affecting human and wildlife health through a variety of mechanisms of toxicity. The potential activity as endocrine disruptors (EDs) of Brominated Flame Retardants (BFRs), has already been experimentally demonstrated and deserves particular attention since the production and use of potential EDs will be strictly regulated through the authorization process of the **REACH** regulation. To overcome the problem of insufficient experimental data necessary to complete the toxicological profile of these chemicals, the QSAR/QSPR approach can be applied to predict the missing information [1]. In this study **QSAR classification** models were developed, according to the **OECD principles**, to predict endocrine disrupting potencies of BFRs.

ENDPOINTS and CLASSES

Classification criteria according to Hamers *et al.*, 2006 [2].

Endpoints	inactive	active
Dioxin (Ah) Receptor (DR) agonism	1 NO ED POTENCY	2 ED POTENCY
Dioxin (Ah) Receptor (DR) antagonism		
Estrogen Receptor (ER) agonism		
Estrogen Receptor (ER) antagonism		
Progesterone Receptor (PR) antagonism		
Androgen Receptor (AR) antagonism		

	1	2	3
T4-TTR competing potency	NO ED POTENCY	LOW/MODERATE ED POTENCY	(VERY) HIGH ED POTENCY
E2SULT inhibiting potency			

EXPERIMENTAL DATA SET [2-3]

PBDEs (tri-deca)	Data available for 29 BFRs
OH-PBDEs	
TBBPA	
TBBPA-DBDE	
HBCD	
246-TBP	

K-NN Classification Models [4]

The *All Subset Selection* method was applied for the selection of the best subset of variables.

"SCREENING" DATA SET

Screening of 243 BFRs

209 PBDEs	HBB
18 PBDE metabolites (OH- and CH_3O-)	HBCD
	DBDE
7 TBBPA analogs	EBTPI
4 Bromo Phenols	TBE

alternatives to deca-BDE

MOLECULAR DESCRIPTORS

The chemical structures of BFRs were drawn using the Semi-empirical method AM1 in the HYPERCHEM program (ver. 7.03 for Windows, 2002) and used as input files for descriptors calculation. 701 molecular descriptors (0D; 1D; 2D; 3D) were computed by the software *DRAGON* (ver. 5.5 for Windows, 2007).

VALIDATION and Applicability Domain (AD)

Data were split into training set (development of the models) and prediction set (validation of the models) by random selection (30%). Models were developed taking into account the OECD principles for QSAR validation for regulatory purposes [5].

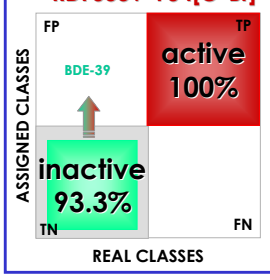
- Internal and external validation: Sn (sensitivity), Sp (specificity), NER (non-error rate), NER_{EXT}.
- Applicability Domain (AD% for 243 BFRs) verified by descriptor's range.

PARAMETERS

$$Sn = \frac{TP}{TP+FN} \quad Sp = \frac{TN}{TN+FP} \quad NER\% = \frac{TP+TN}{Tot} \times 100$$

DR agonism

RDF055v F04[O-Br]



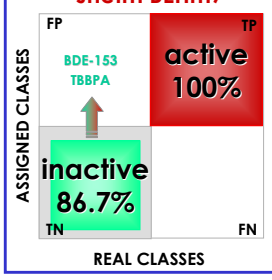
Model		Desc.	K	n	Sn	Sp	NER%	NER_{EXT}%	AD%
Full Model		RDF055v F04[O-Br]	4	24	1	0.94	95.8	-	98
Split	Training set		4	16	0.83	0.8	81.3	-	
Split	Prediction set		4	8	1	1	-	100	

Predicted as ACTIVE: **mono-hexa BDEs** (1-2-3 Br in *meta*); **PBDE metabolites** (Br in *meta*); **Pentabromophenol**

Out of AD: BDE-5; 4-BP; PBP; TBBPA-DE

DR antagonism

Jhetm BEHm7



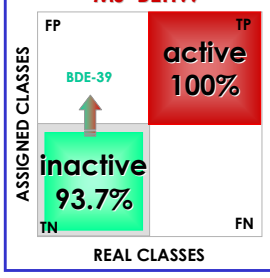
Model		Desc.	K	n	Sn	Sp	NER%	NER_{EXT}%	AD%
Full Model		Jhetm BEHm7	1	24	1	0.87	91.7	-	93
Split	Training set		1	16	0.83	0.80	81.3	-	
Split	Prediction set		1	8	1	0.80	-	87.5	

Predicted as ACTIVE: **mono-penta BDEs** (Br in *meta/para*); **OH-BDEs** (-OH in *meta/para*); **small bisphenols**; **HBCD**; **TBE**

Out of AD: mono-BDEs: 4-BB, PBP; di-BDEs (Br in *para*): 4-PP, HBB; BPA, MBBPA: EBTPI, TBE

ER agonism

Ms BEHv7



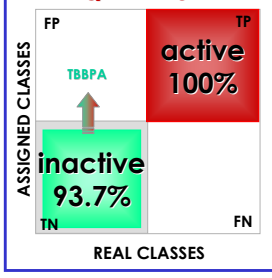
Model		Desc.	K	n	Sn	Sp	NER%	NER_{EXT}%	AD%
Full Model		Ms BEHv7	1	24	1	0.94	95.8	-	99
Split	Training set		1	16	1	0.91	93.7	-	
Split	Prediction set		1	8	1	1	-	100	

Predicted as ACTIVE: **tri- BDEs**; **di-/tetra- BDEs** (most symmetrical or Br in [2,4,6])

Out of AD: 4-BP; EBTPI

ER antagonism

QW nArOH



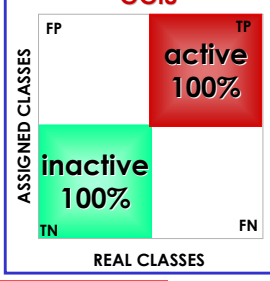
Model		Desc.	K	n	Sn	Sp	NER%	NER_{EXT}%	AD%
Full Model		QW nArOH	1	24	1	0.94	85.8	-	100
Split	Training set		1	16	0.80	0.91	87.5	-	
Split	Prediction set		1	8	1	0.80	-	87.5	

Predicted as ACTIVE: **hepta-/octa- BDEs**; **OH-BDEs**; **OH- Bromo Phenols**; **HBCD**

All BFRs in AD!

AR/PR antagonism

GGI8



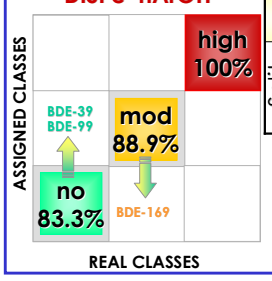
Model		Desc.	K	n	Sn	Sp	NER%	NER_{EXT}%	AD%
Full Model		GGI8	1	24	1	1	100	-	99
Split	Training set		1	16	1	1	100	-	
Split	Prediction set		1	8	1	1	-	100	

Predicted as ACTIVE: **all PBDEs** (except those with Br in [3,3',4,4',5,5'])); **PBDEs metabolites**; **Bromo Phenols + small bisphenols**; **HBB + HBCD**

Out of AD: EBTPI

T4-TTR competition

DISPe nArOH



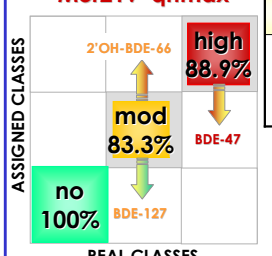
Model		Desc.	K	n	C1%	C2%	C3%	NER%	NER_{EXT}%	AD%
Full Model		DISPe nArOH	3	29	83.3	88.9	100	93.1	-	92
Split	Training set		3	20	87.5	100	100	95	-	
Split	Prediction set		3	9	100	66.7	100	-	88.8	

Predicted as ACTIVE: **all BFRs with OH- group**; **75% PBDEs** (moderate) — most asymmetric?

Out of AD: **16 PBDEs** (tri- to hepta-BDEs): TriBBPA; HBB; DBDE

E2SULT inhibition

Mor21v qnmax



Model		Desc.	K	n	C1%	C2%	C3%	NER%	NER_{EXT}%	AD%
Full Model		Mor21v qnmax	1	29	100	83.3	88.9	89.6	-	88
Split	Training set		1	20	83.3	62.5	83.3	75	-	
Split	Predict. set		1	9	100	100	100	-	100	

Predicted as ACTIVE: **PBDE metabolites**; **BromoPhenols + bisphenols**; **50% PBDEs** (mod); **HBB** (mod)); **EBTPI + TBE** (mod)

Out of AD: **22 PBDEs** (bi- to octa-BDEs) → [3,3'] [5,5']: 4-PP, HBB; BPA, MBBPA; TBBPA-DE; **2'OH-BDE-68**: DBDE, EBTPI

CONCLUSIONS

- ✓ New CLASSIFICATION models were developed for different endpoints related to the endocrine potency of BFRs.
- ✓ The proposed models were selected by balancing:
  - number of false negative FN (highest Sn)
  - external predictivity (NER_{EXT})
  - simplicity and interpretability of descriptors
- ✓ The most dangerous compounds and/or important structural alerts were identified for each ED activity (i.e., nArOH, F04[O-Br]).
- ✓ According to literature [2, 6], ED activity of BFRs (DR/ER/AR/PR_{ant}, T4-TTR_{comp}, $E2SULT_{inh}$) is strongly increased by the presence of -OH group on the aromatic ring.
- ✓ The variability of interactions of the studied chemicals with different receptors prevented us from defining a general ranking based on their ED potency.
- ✓ The here proposed classification models are simple tools, with defined Applicability Domains, which can be applied to screen BFRs in relation to their ED activity and, for identification of safer alternatives. This is in agreement with requirements of REACH regulation (Title VII, Chapter 1, Article 57-f).

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