CADASTER MODELS FOR BROMINATED FLAME RETARDANTS

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The EU-REACH regulation encourages the use of alternative *in vitro* and *in silico* methods in order to minimize animal testing, costs and time. In this context the use of Quantitative Structure Activity Relationships (QSAR) becomes particularly useful to predict unknown activities/properties for existing or even not yet synthesized chemicals. The development and validation of QSAR models for four classes of emerging pollutants (brominated flame retardants, fragrances, perfluorinated compounds and (benzo)triazoles) is the central topic of Work Package 3 (WP3) within the FP7 European project CADASTER (CAse studies on the Development and Application of in-Silico Techniques for Environmental hazard and Risk assessment). The final goal of the project is to exemplify the integration of information, models and strategies for carrying out hazard and risk assessments for large numbers of substances, organized in the four representative chemical classes.
In this study are presented the QSAR models that were developed for Brominated Flame Retardants (BFRs) during the first year of the project. Briefly, QSPR models were developed for some SIDS physico-chemical properties2 (i.e. Henry's low constant, vapour pressure, water solubility, LogKOW, LogKOA, photodegradation rate) and then compared with publicly available EPI Suite models.
In addition, endocrine disrupting activities of BFRs3, 4 (i.e. interaction with Aryl hydrocarbon receptor, Estrogen receptor, Progesterone receptor, Androgen receptor, T4-TTR competition and E2SULT inhibition) were modelled by both regression (MLR)5 and classification (K-NN) methods. All the QSAR models were developed taking into account the OECD principles for validation, for regulatory purposes, of QSARs6. This implied internal and external validations, the analysis of the applicability domain and, when possible, a mechanistic interpretation of the models.

FINAL GOAL of CADASTER (1)

to exemplify the integration of information, models and strategies for carrying out hazard and risk assessments for four classes of emerging pollutants:

- **Brominated Flame Retardants**
- **Perfluorinated Compounds**
- **Fragrances**
- **Triazoles/ benzotriazoles**

WP2 Collection of data and models

WP3 Development and validation of QSARs

WP4 Integration of QSARs within hazard and risk assessment

WP5 Development of website and stand-alone tools for dissemination and project results

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).

DESCRIPTOR SELECTION

The *ALL Subset Selection* method was applied to select the best subset of variables.

QSAR DEVELOPMENT

REGRESSION MODELS → Multiple linear regression (OLS)
CLASSIFICATION MODELS → K-NN classification method

VALIDATION AND APPLICABILITY DOMAIN (AD)

Models were developed taking into account the OECD principles for QSAR validation for regulatory purposes (2):

- Internal and, when possible, external validation
- Applicability Domain (AD% for more than 200 BFRs) verified by leverage approach (regression models) or by descriptor's range and similarity (classification models)

QSAR MODELS FOR PHYSICO-CHEMICAL PROPERTIES

Property	Endpoint	N_{obj}	Descriptors	R^2	Q^2_{LOO}	AD (% of 220 BFRs)
Melting Point	MP*	25	X2A	0.84	0.82	98.6
Vapour Pressure	Log VP*	34	T(O..Br)	0.99	0.98	91.4
K_{OA}	Log K_{OA}*	30	T(O..Br)	0.97	0.97	90.4
K_{OW}	Log K_{OW}*	20	T(O..Br)	0.96	0.96	93.6
Photolysis rate	Log K_P	15	MW	0.95	0.94	91.0
Photolysis half-life	Log HL_P	15	T(O..Br)	0.94	0.93	83.9

* Externally validated models ($0.92 < Q^2_{ext} < 0.99$)

Papa et al. (2009) *QSAR Comb. Sci.*, 28, 790-796. (3)

T(O..Br) → descriptor related to both the number and position of Br substituents

PCA on predictions for 209 PBDEs

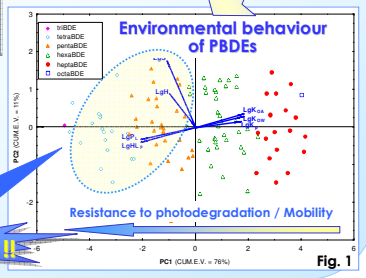

Fig. 1

Tri-Penta BDE

- ✓ Higher volatility
- ✓ Higher persistence
- ✓ High lipophilicity $5.3 < LogK_{OW} < 7.2$

Potential for LRT !!

COMPARISON WITH SOME EXISTING MODELS

Endpoint	RMSE Papa et al. (2009)	RMSE EPI Suite (4)
MP	18.92	57.47
Log K_{OA}	0.23	0.81
Log K_{OW}	0.19	0.91

Proceedings Euro-QSAR 2010 ?

Comparison of Exp. Data vs Predictions by *AD HOC* models & genral model (K_{OA}WIN)

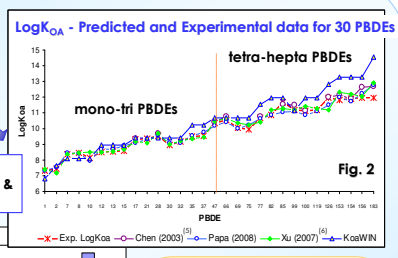

Fig. 2

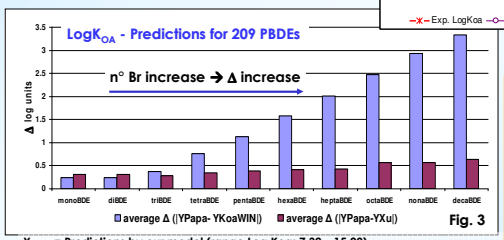

Fig. 3

Y_{Papa} = Predictions by our model (range Log Koa: 7.32 – 15.09)
Y_{KoaWIN} = Predictions by KoaWIN (Δmax = 3.33 log units; range Log Koa: 6.81-18.23)
Y_{Xu} = Predictions by Xu *et al.* (2007) (Δmax =1.06 log units; range Log Koa: 7.4-15.73)

Fig. 2 shows that *AD HOC* models have higher accuracy in prediction than the genral K_{OA}WIN model , independently of the bromination degree.

Fig. 3. The increasing Δ among predicted values of *AD HOC* models vs K_{OA}WIN highlights the risk of overstimation of the predicted property by general models, in particular for highly brominated PBDEs.

QSAR MODELS FOR ENDOCRINE DISRUPTION POTENCY

REGRESSION MODELS

Activity	Endpoint	N_{obj}	Descriptors	R^2	Q^2_{LOO}	AD % of 243 BFRs
AhR rel. binding affinity	Log RBA *	18	L1v, Mor22u	0.82	0.73	75
EROD induction	Log $1/EC_{50}EROD_{ind}$	8	pilD	0.85	0.75	93
AhR agonism	Log $1/EC_{50}DR_{ag}$	8	Mor08e	0.91	0.85	81
ER agonism	Log $1/EC_{50}ER_{ag}$	8	RGyr	0.95	0.88	99
PR antagonism	Log $1/IC_{50}PR_{ant}$*	19	RDF045m, GATS4m	0.87	0.82	93
T4-TTR rel. competition	Log $T4_{REP}$ *	17	qpmax, MATS6v	0.94	0.91	98
E2SULT rel. inhibition	Log $E2SULT_{REP}$ *	21	B08[C-O], GGI7	0.88	0.84	100

* Externally validated models ($0.95 < Q^2_{ext} < 0.99$)

Papa et al. (2010) *Chem. Res. Toxicol.* 23, 946-954. (7)

Importance of variables related to electronic properties, such as electronegativity or the charge distribution along the molecules (Mor08e, qpmax, GGI7), and to molecular size and complexity (L1v, RGyr, pilD)

CLASSIFICATION MODELS

External validation (87 < NERext% < 100)

% of correct classifications

Endpoint	Descriptors	k	Real class	N_{obj}	Assigned class 1	2	3	NER_{class}%	NER%
DR_{ag}	F04[O-Br], RDF055v	4	1	15	14	1		93.3	95.8
			2	9	0	9		100	
DR_{ant}	Jhetm, BEHm7	1	1	15	13	2		86.7	91.7
			2	9	0	9		100	
ER_{ag}	Ms, BEHv7	1	1	16	15	1		93.7	95.8
			2	8	0	8		100	
ER_{ant}	QW, nArOH	1	1	16	15	1		93.7	95.8
			2	8	0	8		100	
AR/PR_{ant}	GGI8	1	1	5	5	0		100	100
			2	19	0	19		100	
$T4\text{-}TTR_{comp}$	DISPe, nArOH	3	1	12	10	2	0	83.3	89.6
			2	9	1	8	0	88.9	
			3	8	0	0	8	100	
$E2SULT_{inh}$	Mor21v, qnmax	1	1	8	8	0	0	100	89.6
			2	12	1	10	1	83.3	
			3	9	0	1	8	88.9	

1 = INACTIVE (no ED potency)
2 = ACTIVE (low-high ED potency)

CLASSES (8,9)

1 = INACTIVE
2 = MODERATELY ACTIVE
3 = VERY ACTIVE

- ✓ PBDE congeners without (or few) ortho-substituents show higher affinity with the Ah Receptor
- ✓ ED activity of BFRs ($DR_{ag/ant}$, ER_{ant}, AR/PR_{ant}, $T4\text{-}TTR_{comp}$, $E2SULT_{inh}$) is strongly increased by aromatic –OH group.
- ✓ Screening of 243 BFRs (including three deca-BDE alternatives) according to their ED potency

Submitted to *ES&T*

Identification of most dangerous compounds for each ED activity

REFERENCES
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