**Introduction**

In the last decade, brominated flame retardants (BFRs), and in particular polybrominated diphenyl ethers (PBDEs), have been recognized 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 a T-TR compete 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.

**Pre-requisite Models**

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

| Endpoint | N_p | N_f | Variables | R² | Q_100 | Q_1000 | R² | Q_100 | AD% (20%)
|----------|-----|-----|-----------|----|-------|--------|----|-------|-------
| LogT4-REP | 12  | 5   | PWA apmax | 96.12 | 92.77 | 86.96 | 17.45 | 89.1 |
| LogE2SULT-REP | 16  | 5   | GAT/S1 BOEC C-Cl | 82.71 | 78.46 | 67.85 | 13.39 | 95.12 |

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

| Endpoint | Model | N_p | N_f | Variables | R² | Q_100 | Q_1000 | R² | Q_100 | AD% (20%)
|----------|-------|-----|-----|-----------|----|-------|--------|----|-------|-------
| LogT4-REP | Full Model | 17  | 5   | MAT500 apmax | 95.20 | 92.96 | 92.86 | 15.42 | 99.74 |
| LogT4-REP | Split 30% | 12  | 5   | MAT500 apmax | 94.74 | 90.18 | 89.72 | 17.24 | 96.21 |
| LogE2SULT-REP | Full Model | 21  | 6   | GSP7 [B0C-C] | 87.57 | 83.61 | 81.96 | 9.12 | 100 |
| LogE2SULT-REP | Split 30% | 18  | 6   | GSP7 [B0C-C] | 88.94 | 83.61 | 83.68 | 14.65 | 82.41 |

**Classification Models**

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

<table>
<thead>
<tr>
<th>Potency</th>
<th>Criteria</th>
<th>RIC</th>
<th>Desired</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO POTENCY</td>
<td>Response &lt; 20% of control</td>
<td>no potency</td>
<td></td>
</tr>
<tr>
<td>LOW/MODERATE POTENCY</td>
<td>1.0 µM &lt; IRIC &lt; 1.0 µM</td>
<td>moderate potency</td>
<td></td>
</tr>
<tr>
<td>(VERY) HIGH POTENCY</td>
<td>IRIC &gt; 1.0 µM</td>
<td>high potency</td>
<td></td>
</tr>
</tbody>
</table>

**Results**

- 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 hydroxylated 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 (NERf = 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 [2,7,6,4] seems to increase T-TR competition. The same behavior 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 disrupting potency of BFRs.

**Conclusion**

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

**Tools of Validation and Diagnostics**

- Models were developed taking into account the recently proposed OECD principles for QSAR validation [7]:
  - Internal (by Q_100 and Q_1000 Y-scrambling) and external validation (verified by Q_100) [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 descriptor's range (classification models).

**APPENDIX**

(3) HYPERCHEM. Rev. 7.02 for Windows, 2002. Hypercube, Inc. Florida, USA.

**References**

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

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**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-dibromopropyle) ether), 248-TPB (2,4,6-tetrabromophenol) and HBCD (hexabromocyclododecane) [1-2].

- Regression endpoints: T-TR relative competing potencies (T-REP = IC_{50,T-TR}/IC_{50,T-TR,obs}) and estradiol sulfotransferase relative inhibiting potencies (E2SULT-REP = IC_{50,E2SULT}/IC_{50,E2SULT,obs}). 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 (EE; 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.

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