	QMRF identifier (JRC Inventory):To be entered by JRC	
QMRF	QMRF Title: Insubria QSAR PaDEL-Descriptor model for prediction of Pharmaceuticals toxicity in Daphnia magna. Keywords: QSARINS; PaDEL-Descriptor; GA-OLS; Daphnia magna toxicity EC50; Pharmaceuticals; INSUBRIA	QMRF
	Printing Date:Jan 25, 2017	

1.QSAR identifier

1.1.QSAR identifier (title):

Insubria QSAR PaDEL-Descriptor model for prediction of Pharmaceuticals toxicity in *Daphnia magna*.

Keywords: QSARINS; PaDEL-Descriptor; GA-OLS; *Daphnia magna* toxicity EC50; Pharmaceuticals; INSUBRIA

1.2.Other related models:

A. Sangion, P. Gramatica, Hazard of pharmaceuticals for aquatic environment: Prioritization by structural approaches and prediction of ecotoxicity, Environ. Int. 95 (2016) 131–143. doi:10.1016/j.envint.2016.08.008 [1]

1.3.Software coding the model:

[1]PaDEL-Descriptor A software to calculate molecular descriptors and fingerprints, version 2.21 [2] Yap Chun Wei, phayapc@nus.edu.sg http://padel.nus.edu.sg/software/padeldescriptor/index.html

[2]QSARINS Software for the development, analysis and validation of QSAR MLR models, version 2.2.1 [3,4] Prof. Paola Gramatica, paola.gramatica@uninsubria.it http://www.qsar.it/

2.General information

2.1.Date of QMRF:

13/01/2017

2.2.QMRF author(s) and contact details:

[1]Alessandro Sangion Insubria University, Department of Theoretical and Applied Sciences (DiSTA), via J.H. Dunant 3, 21100 Varese (Italy) +390332421439 alessandro.sangion@uninsubria.it http://www.qsar.it/
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2.3.Date of QMRF update(s):

2.4.QMRF update(s):

2.5.Model developer(s) and contact details:

[1]Paola Gramatica Insubria University, Department of Theoretical and Applied Sciences (DiSTA), via J.H. Dunant 3, 21100 Varese (Italy) +390332421573 paola.gramatica@uninsubria.it http://www.qsar.it/
[2]Alessandro Sangion Insubria University, Department of Theoretical and Applied Sciences (DiSTA), via J.H. Dunant 3, 21100 Varese (Italy) +390332421439 alessandro.sangion@uninsubria.it http://www.qsar.it/

2.6.Date of model development and/or publication:

Developed and Published in 2016.

2.7.Reference(s) to main scientific papers and/or software package:

[1]A. Sangion, P. Gramatica, Hazard of pharmaceuticals for aquatic environment: Prioritization by structural approaches and prediction of ecotoxicity, Environ. Int. 95 (2016) 131–143 doi:10.1016/j.envint.2016.08.008

[2]C.W. Yap, PaDEL-Descriptor: An open source software to calculate molecular descriptors and fingerprints., JComput Chem. 32 (2011) 1466–1474. doi:10.1002/jcc.21707

[3]P. Gramatica, N. Chirico, E. Papa, S. Cassani, S. Kovarich, QSARINS: A new software for the development, analysis and validation of QSAR MLR models, J Comput Chem. 34 (2013) 2121–2132 doi:10.1002/jcc.23361.

[4]P. Gramatica, S. Cassani, N. Chirico, QSARINS-Chem: Insubria Datasets and New QSAR/QSPR Models for Environmental Pollutants in QSARINS, J.Comput.Chem. 35 (2014) 1036–1044. doi:10.1002/jcc.23576.

2.8.Availability of information about the model:

Non-proprietary. Defined algorithm, available in QSARINS [3,4]. Training and prediction sets are available in the Supporting Information of the related paper [1], in the attached sdf files of this QMRF (section 9) and in the QSARINS-Chem database [4].

2.9.Availability of another QMRF for exactly the same model:

No other information available

3.Defining the endpoint - OECD Principle 1

3.1.Species:

Daphnia magna

3.2.Endpoint:

3.Ecotoxic effects 3.1.Short-term toxicity to Daphnia (immobilisation)

3.3.Comment on endpoint:

A selected set of experimental EC50 (48h) data was taken from ECOTOX online database [5].

3.4.Endpoint units:

The median lethal concentrations are reported as the minus logarithm of the millimolar concentration: -log(mmol/l)

3.5.Dependent variable:

pEC50

3.6.Experimental protocol:

OECD test 202

3.7.Endpoint data quality and variability:

The experimental data were specifically filtered for the species, defined time of exposure, endpoints and measured effects, trying to ensure the highest degree of homogeneity in experimental measures. If different and multiple values were found for a specific chemical, the minimum LC/EC50 value was taken and modelled, considering the "worst case scenario"(i.e.the most toxic value). Once these experimental values

were selected and filtered, the data were additionally carefully checked, removing the
duplicates and measures reported as "nominal concentration".

4.Defining the algorithm - OECD Principle 2

4.1.Type of model:
QSAR - Multiple linear regression model (OLS - Ordinary Least Square)

4.2.Explicit algorithm:
pEC50 D.magna split
MLR-OLS method. Model developed on a training set of 88 compounds

pEC50 D.magna Ordered Response split
MLR-OLS method. Model developed on a training set of 84 compounds

pEC50 D.magna Structural Similarity split
MLR-OLS method. Model developed on a training set of 84 compounds.

pEC50 D.magna Full Model
MLR-OLS method. Model developed on 125 compounds.

Random split equation: $\text{pEC50 (48h)}_{D.magna} = 0.61 + 0.33\text{CrippenLogP} - 0.06 \text{ SpMAD\_Dzs} - 0.08 \text{ minHBint2} + 0.14\text{C3SP2} - 0.62\text{AATSC4i}$

Ordered Response split equation: $\text{pEC50 (48h)}_{D.magna} = 0.77 + 0.32\text{CrippenLogP} - 0.07\text{SpMAD\_Dzs} - 0.08\text{minHBint2} - 0.76\text{AATSC4i} + 0.13\text{C3SP2}$

Structural Similarity split equation: $\text{pEC50 (48h)}_{D.magna} = 0.64 + 0.30\text{CrippenLogP} - 0.09\text{minHBint2} - 0.06\text{SpMAD\_Dzs} + 0.16\text{C3SP2} - 0.69\text{AATSC4i}$

Full model equation: $\text{pEC50 (48h)}_{D.manga} = 0.65 + 0.32 \text{ CrippenLogKow} - 0.08\text{minHBint2} - 0.06 \text{ SpMAD Dzs} - 0.75 \text{ AATSC4i} + 0.13 \text{ C2SP3}$

4.3.Descriptors in the model:
[1]CrippenLogP Crippen's LogP
[2]SpMAD_Dzs Spectral mean absolute deviation from Barysz matrix / weighted by I-state
[3]minHBint2 Minimum E-State descriptors of strength for potential Hydrogen Bonds of path length 2
[4]C3SP2 Doubly bound carbon bound to three other carbons
[5]AATSC4i Average centered Broto-Moreau autocorrelation - lag 4 / weighted by first ionization potential

4.4.Descriptor selection:

A total of 1444 molecular descriptors of differing types (0D, 1D, 2D) were calculated by PaDEL-Descriptor 2.21 [2]. Constant and semi-constant values and descriptors found to be correlated pairwise were excluded in a pre-reduction step (one of any two descriptors with a correlation greater than 0.98 was removed to reduce redundant information), and a final set of 699 molecular descriptors were used as input variables for variable subset selection. All the possible combinations of two descriptors were investigated by the all-subset procedure, then, the Genetic Algorithm (GAVSS) was applied to explore new combinations with additional descriptors using Q^2_{LOO} (leave one out) as fitness function to be optimized during the variable selection procedure.

4.5. Algorithm and descriptor generation:

Multiple linear regression (Ordinary Least Square method) was applied to generate the model. Molecular descriptors were generated by PaDEL-Descriptor software 2.21. The input files for descriptor calculation contain information on atom and bond types, connectivity, partial charges and atomic spatial coordinates, relative to the minimum energy conformation of the molecule, and were firstly obtained by the semi empirical AM1 method using the package HYPERCHEM 7.03 [6]. Then, these files were converted by OpenBabel 2.3.2 [7] into MDL-MOL format and used as input for the calculation of descriptors in PaDEL-Descriptor. Any user can re-derives the model calculating the molecular descriptors by PaDEL-Descriptor 2.21 software (included in QSARINS 2.2.1) and applying the given equation (automatically done by QSARINS 2.2.1).

4.6. Software name and version for descriptor generation:

PaDEL-Descriptor
A software to calculate molecular descriptors and fingerprints, version 2.21
Yap Chun Wei, Department of Pharmacy, National University of Singapore
http://padel.nus.edu.sg/software/padeldescriptor/index.html

HYPERCHEM
Software for molecular drawing and conformational energy optimization, ver. 7.03, 2002
Hypercube, Inc., 1115 NW 4th Street, Gainesville, Florida 32601, USA, 2002
http://www.hyper.com/

OpenBabel
Open Babel: The Open Source Chemistry Toolbox. Used for conversion between HYPERCHEM files (hin) and MDL-MOL files, version 2.3.2, 2012.
http://openbabel.org/wiki/Main_Page

4.7. Chemicals/Descriptors ratio:

Random split equation: 88/5=17.4
Ordered Response split equation: 84/5=16.8
Structural Similarity split equation: 84/5=16.8
Full model equation: 125/5=25

5.Defining the applicability domain - OECD Principle 3

5.1.Description of the applicability domain of the model:

The applicability domain of the model was verified by the leverage approach and fixed thresholds has been used to define both structural and response outliers (see section 5.4). The plot of standardized residuals versus leverages (hat diagonals), i.e. the Williams plot, verified the presence of response outliers (i.e.compounds with cross-validated standardized residuals greater than 2.5 standard deviation units) and chemicals very structurally influential in determining model parameters (i.e. compounds with a leverage value (h) greater than 3p'/n (h*), where p' is the number of model variables plus one, and n is the number of the objects used to calculate the model). For new compounds without experimental data, leverage can be used as a quantitative measure for evaluating the degree of extrapolation: for compounds with a high leverage value (h > h*), that are structural outliers, predictions should be considered less reliable. In QSARINS the Insubria graph allows to identify for which new chemicals the predictions are inter- or extrapolated by the model.

Response and descriptor space:
Range of experimental pEC50 *D.magna* values: -0.64 / 2.55 [-log(mmol/L)]
Range of descriptor values: CrippenLogP: -1.5 / 7.51; SpMAD_Dzs: 1.21/ 17.95; minHBint2: -0.54 / 8.47; C3SP2: 0/ 5 AATSC4i: -0.88 / 0.8

5.2.Method used to assess the applicability domain:

As it has been stated in section 5.1, the response applicability domain can be verified by the standardized residuals in cross-validation; chemicals with a standardized residual greater than 2.5 deviation units were considerate outliers. The structural applicability domain of the full model was assessed by the leverage approach, providing a cut-off hat value (h*=0.144 for the full model). HAT values are calculated as the diagonal elements of the HAT matrix: $H = X(X^TX)^{-1}X^T$.

5.3.Software name and version for applicability domain assessment:

QSARINS
Software for the development, analysis and validation of QSAR MLR models, version 2.2.1
Prof. Paola Gramatica; paola.gramatica@uninsubria.it
http://www.qsar.it/

5.4.Limits of applicability:

Random split model domain: Outliers for structure (hat>0.205): 52-24-4,   79-57-2. Ordered response split model domain: Outliers for structure (hat>0.214):   52-24-4, 54-85-3, 79-57-2. Structural Similarity split model domain: Outliers for structure (hat>0.214):   52-24-4, 54-85-3, 79-57-2. Full model domain: Outlier for structure (hat>0.144): 52-24-4, 79-57-2

6.Internal validation - OECD Principle 4

6.1.Availability of the training set:
Yes

6.2.Available information for the training set:
CAS RN:Yes
Chemical Name:No
Smiles:Yes
Formula:No
INChI:No
MOL file:Yes

6.3.Data for each descriptor variable for the training set:
All

6.4.Data for the dependent variable for the training set:
All

6.5.Other information about the training set:
To verify the predictive capability of the proposed models, the whole   dataset (n=125) was split, before model development, into training sets   used for model development and prediction sets used later for external   validation. Three different splitting techniques were applied: Random (n   training = 88), by ordered response (n training =84) and by structural   similarity (n training= 84).

In the Random splitting chemicals are randomly assigned to the training set.

In the Ordered response splitting chemicals have been ordered according   to their increasing toxicity and one out of every three chemicals has   been assigned to the prediction set (always including the most and the   least toxic compound in the training set, i.e. the lowest and the   highest pEC50). This splitting guarantees that the training set covers   the entire range of the modeled response.

In the structural similarity splitting, training and prediction set are structurally balanced, being the splitting based on the structural similarity analysis (performed with Principal Component Analysis of molecular descriptors).

6.6.Pre-processing of data before modelling:
Transformation of EC50 (mg/L) into EC50 (mmol/L) and than in the   logarithmical form Log(1/EC50)

6.7.Statistics for goodness-of-fit:
Random split:
R^2= 0.75; RMSE= 0.39

Ordered Response split:
R^2= 0.75; RMSE= 0.40
Structural Similarity split:
R^2= 0.75; RMSE= 0.41

Full model:
R^2= 0.75; RMSE= 0.40

6.8. Robustness - Statistics obtained by leave-one-out cross-validation:

Random split:
Q^2_{loo}=0.71
Ordered Response split:
Q^2_{loo}=0.72
Structural Similarity split:
Q^2_{loo}=0.71

Full model: Q^2_{loo}=0.72

6.9. Robustness - Statistics obtained by leave-many-out cross-validation:

Random split:
$Q^2_{LMO30\%}$=0.70
Ordered Response split:
$Q^2_{LMO30\%}$=0.71
Structural Similarity split:
$Q^2_{LMO30\%}$=0.70

Full model: $Q^2_{LMO30\%}$=0.72

6.10. Robustness - Statistics obtained by Y-scrambling:

Random split:
R^2_{Yscr}=0.06
Ordered Response split:
R^2_{Yscr}=0.06
Structural Similarity split:
R^2_{Yscr}=0.06
Full model:
R^2_{Yscr}=0.04

6.11. Robustness - Statistics obtained by bootstrap:

No information available (since only Q^2_{LMO} was   calculated)

6.12. Robustness - Statistics obtained by other methods:

Random split:
$RMSE_{CV}$= 0.42; CCC_{CV}=0.83
Ordered Response split:
$RMSE_{CV}$= 0.42; CCC_{CV}=0.84
Structural Similarity split:
$RMSE_{CV}$= 0.44; CCC_{CV}=0.84
Full model: $RMSE_{CV}$=0.41; CCC_{CV}=0.84

7.External validation - OECD Principle 4

7.1.Availability of the external validation set:
Yes

7.2.Available information for the external validation set:
CAS RN:Yes

Chemical Name:No

Smiles:Yes

Formula:No

INChI:No

MOL file:Yes

7.3.Data for each descriptor variable for the external validation set:
All

7.4.Data for the dependent variable for the external validation set:
All

7.5.Other information about the external validation set:
To verify the predictive capability of the proposed models, the dataset (n=125) was split, before model development, into a training set used for model development and a prediction set used later for external validation.

7.6.Experimental design of test set:
In the Random splitting chemicals are randomly assigned to the training set.

In the case of split by Ordered response model, chemicals were ordered according to their increasing activity, and one out of every three chemicals was put in the prediction set (always including the most and the least active compounds in the training set).

The splitting based on structural similarity, allowed the selection of a structurally meaningful training set and an equally representative prediction set. The selection is based on Principal Component Analysis that is able to project chemicals in the multivariate descriptors space. This method ensures that both sets are homogeneously distributed within the entire area of the descriptors space.

7.7.Predictivity - Statistics obtained by external validation:
Random split model:

Q^2_{extF1} [8]= 0.74; Q^2_{extF2} [9]= 0.74; Q^2_{extF3}[10]= 0.7; CCC_{ext} [11]=0.86; RMSE= 0.42

Ordered response split model:

Q^2_{extF1}= 0.72; Q^2_{extF2}= 0.72; Q^2_{extF3}=0.74; CCC_{ext}=0.84; RMSE= 0.41   Structural Similarity split model:

Q^2_{extF1}= 0.74; Q^2_{extF2}= 0.73; Q^2_{extF3}=0.79; CCC_{ext}=0.84; RMSE= 0.37

The high values of external Q^2 and concordance correlation coefficient-CCC (threshold for accepting the external $Q^2_{F1-F2-F3}$ is

0.70, threshold for CCC is 0.85,[11,12]), show that the proposed model is predictive, when applied to chemicals never seen during the model development (prediction sets)[13].

7.8.Predictivity - Assessment of the external validation set:

The splitting methodology based on similarity analysis, by ordered response and random allowed for the selection of meaningful training sets and representative prediction sets.

Training and prediction sets were balanced according to both structure and response and the predictivity was assessed even on random training and prediction set. In particular:

Random split model:
-response range of pEC50 values: training [-0.5 / 2.54] prediction [-0.64 / 2.55]
-descriptor range:
CrippenLogP: training [-1.1 / 7.51] prediction [-1.5 / 6.41]
SpMAD_Dzs: training [1.21 / 17.95] prediction [2.34 / 15.11]
minHBint2: training [-0.54 / 8.47] prediction [0 / 7.68]
C3SP2: training [0 / 4] prediction [0 / 5]
AATSC4i: training [-0.88/0.8] prediction [-0.63/ 0.32]

Ordered response split model:
-response range of pEC50 values: training [-0.64 / 2.55] prediction [-0.5 / 2.37]   -descriptor range: CrippenLogP: training [-1.5 / 7.51] prediction [-1.41 / 6.41]
SpMAD_Dzs: training [1.21 / 16.5] prediction [2.79 / 17.95]
minHBint2: training [0 / 8.36] prediction [-0.54 / 8.47]
C3SP2: training [0 / 5] prediction [0 / 4]
AATSC4i: training [-0.63/0.8] prediction [-0.89/ 0.31]
            Structural Similarity split model: -response range of pEC50 values: training [-0.64 / 2.55] prediction   [-0.5 / 2.17]   -descriptor range:
CrippenLogP: training [-1.5 / 6.41] prediction [-0.82 / 7.51]
SpMAD_Dzs: training [1.21 / 17.95] prediction [1.93 / 16.8]
minHBint2: training [0 / 8.47] prediction [-0.54 / 8.13]
C3SP2: training [0 / 5] prediction [0 / 4]
AATSC4i: training [-0.88/0.8] prediction [-0.63/ 0.34]
The applicability domain of the model on the prediction set has been verified by the Williams plot.

7.9.Comments on the external validation of the model:
no other information available

8.Providing a mechanistic interpretation - OECD Principle 5

8.1.Mechanistic basis of the model:
The model was developed by statistical approach. No mechanistic basis   for this biological activity was set a priori, but a mechanistic

interpretation of molecular descriptors was provided a posteriori (see 8.2).

8.2. A priori or a posteriori mechanistic interpretation:

The most important descriptor in modelling the acute toxicity in D.magna was the CrippenLogKow (std coefficient 0.74), the logarithm of the octanol-water coefficient calculated by the Wildman-Crippen model. This descriptor is related to the lipophilicity of the compounds and has a positive contribution in the model equation. The presence of this descriptor in an acute toxicity model is not surprising since the relationship between LogKow and acute toxicity by narcosis was well established since the Könemann and Veithworks.Most lipophilic compounds are the most bioaccumulative and are potentially toxic compounds, since they can interact with the biological membranes in the organisms. However CrippenLogKow as single descriptor is not sufficient to model adequately acute toxicity in a general model, so the GA selected also four additional theoretical descriptors, in order to obtain a model with higher predictive performances. MinHBint2 (std coefficient −032) belongs to the E-State atomtype descriptors that account for the intrinsic electronic state of the atom as perturbed by the electronic influence of all other atoms in the molecule. In particular minHBint2 encodes for the minimum E-State of strength for potential hydrogen bonds and increases with the hydrophilicity of the substance.CrippenLogKow and MinHBint2 are the two most important descriptors with obviously opposite sign in the full model equation. These two descriptors together give information about the lipophilic and hydrophilic behaviour respectively and about the interactions that a chemical can have with the external environment. The other three descriptors selected by the GA-VSS are less important in defining the regression model and their role is simply of support of the two main descriptors integrating their information. [1]

8.3. Other information about the mechanistic interpretation:

no other information available

9. Miscellaneous information

9.1. Comments:

To predict pEC50 for new Pharmaceuticals without experimental data, it is suggested to apply the equation of the Full Model, developed on all the available chemicals (N Training=125).

Full model equation: pEC50 (48h) *D.magna* =0.65 + 0.32 CrippenLogKow −0.08minHBint2 −0.06 SpMAD Dzs −0.75 AATSC4i + 0.13 C2SP3

N Training set= 125; R^2= 0.75; Q^2_{LOO} = 0.72; $Q^2_{LMO30\%}$= 0.72; CCC_{CV} = 0.84; RMSE= 0.40; $RMSE_{CV}$ = 0.41.

End-point, descriptors and splitting status for each chemical

are   reported in the supporting information.

9.2.Bibliography:

[1]A. Sangion, P. Gramatica, Hazard of pharmaceuticals for aquatic environment: Prioritization by structural approaches and prediction of ecotoxicity, Environ. Int. 95 (2016) 131–143 doi:10.1016/j.envint.2016.08.008

[2]C.W. Yap, PaDEL-Descriptor: An open source software to calculate molecular descriptors and fingerprints., JComput Chem. 32 (2011) 1466–1474 doi:10.1002/jcc.21707.

[3]P. Gramatica, N. Chirico, E. Papa, S. Cassani, S. Kovarich, QSARINS: A new software for the development, analysis and validation of QSAR MLR models, J Comput Chem. 34 (2013) 2121–2132. doi:10.1002/jcc.23361.

[4]P. Gramatica, S. Cassani, N. Chirico, QSARINS-Chem: Insubria Datasets and New QSAR/QSPR Models for Environmental Pollutants in QSARINS, J.Comput.Chem. 35 (2014) 1036–1044. doi:10.1002/jcc.23576.

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[10]V. Consonni, D. Ballabio, R. Todeschini, Comments on the Definition of the Q(2) Parameter for QSAR Validation, J. Chem. Inf. Model. 49 (2009) 1669–1678. doi:10.1021/ci900115y.

[11]Real External Predictivity of QSAR Models: How To Evaluate It? Comparison of Different Validation Criteria and Proposal of Using the Concordance Correlation Coefficient, 51 (2011) 2320-2335 10.1021/ci200211n

[12]N. Chirico, P. Gramatica, Real External Predictivity of QSAR Models. Part 2. New Intercomparable Thresholds for Different Validation Criteria and the Need for Scatter Plot Inspection, J. Chem. Inf. Model. 52 (2012) 2044–2058 doi:10.1021/ci300084j.

[13]P. Gramatica, A. Sangion, A Historical Excursus on the Statistical Validation Parameters for QSAR Models: A Clarification Concerning Metrics and Terminology, J. Chem. Inf. Model. 56 (2016) 1127–1131. doi:10.1021/acs.jcim.6b00088.

9.3.Supporting information:

Training set(s)Test set(s)Supporting information

10.Summary (JRC Inventory)

10.1.QMRF number:

To be entered by JRC

10.2.Publication date:

To be entered by JRC

10.3.Keywords:

To be entered by JRC

10.4.Comments:

To be entered by JRC