	QMRF identifier (JRC Inventory): **To be entered by JRC**
	QMRF Title: **MOA toxicity classification (EPA T.E.S.T.) Model version 1.0.2**
	Printing Date: **27-10-2022**

1.QSAR identifier

1.1. QSAR identifier (title):

MOA toxicity classification (EPA T.E.S.T.) Model version 1.0.2

1.2. Other related models:

NA

1.3. Software coding the model:

VEGA (https://www.vegahub.eu/)

The VEGA software provides QSAR models to predict tox, ecotox, environ, phys-chem and toxicokinetic properties of chemical substances.

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2.General information

2.1. Date of QMRF:

October 2022

2.2. QMRF author(s) and contact details:

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2.3. Date of QMRF update(s):

NA

2.4. QMRF update(s):

NA

2.5. Model developer(s) and contact details:

[1] Alberto Manganaro Istituto di Ricerche Farmacologiche Mario Negri - IRCSS Via Mario Negri 2,20156 Milano, Italy alberto.manganaro@marionegri.it https://www.marionegri.it/

[2] Martin T.M National Risk Management Research Laboratory, U.S. Environmental Protection Agencymartin.todd@epa.gov

2.6. Date of model development and/or publication:

The model was developed in 2013.

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

[1] Martin, T. M.; Grulke, C. M.; Young, D. M.; Russom, C. L.; Wang, N. Y.; Jackson, C. R.; Barron,M. G. Prediction of Aquatic Toxicity Mode of Action Using Linear Discriminant and Random Forest Models. J. Chem. Inf. Model. 2013, 53, 2229-2239 https://pubs.acs.org/doi/10.1021/ci400267h

[2] Martin, T. M.; Young, D. M.; Lilavois, C. R.; Barron, M. G. Comparison of global and mode of action-based models for aquatic toxicity. SAR QSAR Environ. Res. 2015, 26, 245-262. DOI:10.1080/1062936X.2015.1018939

[3] Benfenati E, Roncaglioni A, Lombardo A, Manganaro A. Integrating QSAR, Read-Across, and Screening Tools: The VEGAHUB Platform as an Example. Advances in Computational Toxicology;Springer; 2019. p. 365-81.

2.8. Availability of information about the model:

The model is non-proprietary and the training set is available.

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

Another QMRF is not available.

3.Defining the endpoint - OECD Principle 1

3.1. Species:

Fathead Minnow (Pimephales promelas)

3.2. Endpoint:

[1] Eco-Tox AChE Inhibition: acetylcholinesterase (AChE) inhibition

[2] Eco-Tox Anticoagulation

[3] Eco-Tox ACHr Antagonism: acetylcholine receptor (AChR) antagonism

[4] Eco-Tox Narcosis

[5] Eco-Tox Neurotoxicity

[6] Eco-Tox Reactivity

[7] Eco-Tox Uncouple

3.3. Comment on endpoint:

The model provides a qualitative estimation for the Mode of action of Chemicals in Fathead Minnow (Pimephales promelas) 96 h. It is based on a series of Linear Discriminant Analysis (LDA) models and it is an implementation of US EPA T.E.S.T mode of action profiler. Seven endpoints namely, AChE Inhibition, Anticoagulation, ACHr Antagonism, Narcosis, Neurotoxicity, Reactivity, and Uncoupler were modelled for mode of action (MOA) of the chemicals

3.4. Endpoint units:

Adimensional

3.5. Dependent variable:

MOA

3.6. Experimental protocol:

NA

3.7. Endpoint data quality and variability:

The dataset was developed using a combination of high confidence assignments, including biological responses in fish acute toxicity assays [3], international consensus classifications such as the Insecticide Resistance Action Committee's (IRAC) MOA classification tool [4], ASTER [5] predictions, and weight of evidence professional judgment based on assessment of structure and literature information [2].

The final dataset is composed of 957 mono constituent organic compounds.The dataset was split in training (757 mono constituent organic compounds) and test (200 mono constituent organic compounds)

4.Defining the algorithm - OECD Principle 2

4.1. Type of model:

The model is based on 957 chemicals collected from T.E.S.T. training set

Classification model: LDA

4.2. Explicit algorithm:

LDA generates a series of multilinear regression models which clearly show which descriptors are associated with each MOA

4.3. Descriptors in the model:

US EPA T.E.S.T

4.4. Descriptor selection:

Genetic Algorithms

4.5. Algorithm and descriptor generation:

NA

4.6. Software name and version for descriptor generation:

US EPA T.E.S.T

4.7. Chemicals/Descriptors ratio:

80:1

5.Defining the applicability domain - OECD Principle 3

5.1. Description of the applicability domain of the model:

The Applicability Domain (AD) is assessed using the original algorithm implemented within VEGA. An overall AD index is calculated, based on a number of parameters, which relate to the results obtained on similar chemicals within the training and test sets.

ADI is defined in this way for this QSAR model´s predictions:

If 1 ≥ AD index > 0.8, the predicted substance is regarded in the Applicability Domain of the model. It corresponds to “good reliability” of prediction.

If 0.8 ≥ AD index ≥ 0.6, the predicted substance could be out of the Applicability Domain of the model. It corresponds to “moderate reliability” of prediction.

If AD index < 0.6, the predicted substance is regarded out of the Applicability Domain of the model. It corresponds to “low reliability” of prediction.
Indices are calculated on the first *k* = 2 most similar molecules, each having S_k similarity value with the target molecule.

Similarity index (*IdxSimilarity*) is calculated as:

$$\frac{\sum_k S_k}{k} \times (1 - Diam^2)$$

where *Diam* is the difference in similarity values between the most similar molecule and the *k*-th molecule.

Accuracy index (*IdxAccuracy*) is calculated as:

$$\frac{\sum_c log\ (1 + S_c)}{\sum_k log\ (1 + S_k)}$$

where the molecules with *c* index are the subset of the *k* molecules where the prediction of the model matches with the experimental value of the molecule.

Concordance index (*IdxConcordance*) is calculated as:

$$\frac{\sum_c log\ (1 + S_c)}{\sum_k log\ (1 + S_k)}$$

where the molecules with *c* index are the subset of the *k* molecules where the experimental value of the molecule matches with the prediction made for the target molecule.

ACF contribution (*IdxACF*) index is calculated as

$$ACF = rare \times missing$$

where: *rare* is calculated on the number of fragments found in the molecule and found in the training set in less than 3 occurences as following: if the number is 0, *rare* is set to 1.0; if the number is 1, *rare* is set to 0.6; otherwise *rare* is set to 0.4

missing is calculated on the number of fragments found in the molecule and never found in the training set as following: if the number is 0, *missing* is set to 1.0; if the number is 1, *missing* is set to 0.6; otherwise *missing* is set to 0.4

Descriptors Range (*IdxDescRange*) index is calculated as 1.0 if all molecular descriptors used in the prediction fall within the range of descriptors used in the whole training set, 0.0 otherwise.

AD final index is calculated as following:

$$ADI = (IdxSimilarity^{0.5} \times IdxAccuracy^{0.25} \times IdxConcordance^{0.25}) \times IdxACF \times IdxDescRange$$

5.2. Method used to assess the applicability domain:

The Applicability domain and chemical similarity are measured with the algorithm developed for VEGA. Full details are in the VEGA website (www.vegahub.eu), including the open access paper describing it[6]. The VEGA AD also evaluates the correctness of the prediction on similar compounds (accuracy), the consistency between the predicted value for the target compound and the experimental values of the similar compounds, the range of the descriptors, and the presence of unusual fragments, using atom centred fragments.
These indices are defined in this way for this QSAR model:

Similar molecules with known experimental value:
This index takes into account how similar are the first two most similar compounds found. Values near 1 mean that the predicted compound is well represented in the dataset used to build the model, otherwise the prediction could be an extrapolation. Defined intervals are:

If 1 ≥ index > 0.8, strongly similar compounds with known experimental value in the training set have been found

If 0.85 ≥ index > 0.6, only moderately similar compounds with known experimental value in the training set have been found

If index ≤ 0.6, no similar compounds with known experimental value in the training set have been found

Accuracy (average error) of prediction for similar molecules:
This index takes into account the classification accuracy in prediction for the two most similar compounds found. Values near 1 mean that the predicted compounds fall in an area of the model's space where the model gives reliable predictions (no misclassifications), otherwise the lower is the value, the worse the model behaves. Defined intervals are:

If 1 ≥ index < 0.8, accuracy of prediction for similar molecules found in the training set is good

If 0.8 ≥ index > 0.6, accuracy of prediction for similar molecules found in the training set is not optimal

If index ≤ 0.6, accuracy of prediction for similar molecules found in the training set is not adequate

Concordance for similar molecules:
This index takes into account the difference between the predicted value and the experimental values of the two most similar compounds. Values near 0 mean that the prediction made disagrees with the values found in the model's space, thus the prediction could be unreliable. Defined intervals are:

If 1 ≥ index < 0.8, molecules found in the training set have experimental values that agree with the target compound predicted value

If 0.8 ≥ index > 0.6, similar molecules found in the training set have experimental values that slightly disagree with the target compound predicted value

If index ≤ 0.6, similar molecules found in the training set have experimental values that completely disagree with the target compound predicted value

Model descriptors range check:
This index checks if the descriptors calculated for the predicted compound are inside the range of descriptors of the training and test set. The index has value 1 if all descriptors are inside the range, 0 if at least one descriptor is out of the range. Defined intervals are:

If index = True, descriptors for this compound have values inside the descriptor range of the compounds of the training set

If index= False, the maximum error in prediction of similar molecules found in the training set has a moderate value, considering the experimental variability

Atom Centered Fragments similarity check:
This index takes into account the presence of one or more fragments that aren't found in the training set, or that are rare fragments. First order atom centered fragments from all molecules in the training set are calculated, then compared with the first order atom centered fragments from the predicted compound; then the index is calculated as following: a first index RARE takes into account rare fragments (those who occur less than three times in the training set), having value of 1 if no such fragments are found, 0.85 if up to 2 fragments are found, 0.7 if more than 2 fragments are found; a second index NOTFOUND takes into account not found fragments, having value of 1 if no such fragments are found, 0.6 if a fragments is found, 0.4 if more than 1 fragment is found. Then, the final index is given as the product RARE * NOTFOUND. Defined intervals are:
If index = 1, all atom centered fragment of the compound have been found in the compounds of the training set

If 1 > index ≥ 0.7, some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments

If index < 0.7, a prominent number of atoms centered fragments of the compound have not been found in the compounds of the training set or are rare fragments

5.3. Software name and version for applicability domain assessment:

VEGA

The VEGA software provides QSAR models to predict tox, ecotox, environ, and phys-chemproperties of chemical substances. emilio.benfenati@marionegri.it https://www.vegahub.eu/

5.4. Limits of applicability:

The model is not applicable on inorganic chemicals and those including unusual elements (i.e., different from C, O, N, S, Cl, Br, F, I). Salts can be predicted only if stripped of the counter ion and converted to the neutralized form

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: No

NanoMaterial: No

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

No

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

All

6.5. Other information about the training set:

Training set = 757 compounds

6.6. Pre-processing of data before modelling:

See [1]

6.7. Statistics for goodness-of-fit:

After the implementation in VEGA the accuracy is of 0.90 with 22 not predicted compounds

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

NA

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

NA

6.10. Robustness - Statistics obtained by Y-scrambling:

NA

6.11. Robustness - Statistics obtained by bootstrap:

NA

6.12. Robustness - Statistics obtained by other methods:

NA

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: No

NanoMaterial: No

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

No

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

All

7.5. Other information about the external validation set:

Implementation test set = 200 compounds

7.6. Experimental design of test set:

Random Splitting of data set

7.7. Predictivity - Statistics obtained by external validation:

The statistics reported in [1] for the original T.E.S.T fish acute toxicity MOA model are: Balanced Accuracy = 0.796, MCC= 0.792, Not Predicted= 22

After the implementation in VEGA the statistics are the following:

Test set in AD: n=125, Accuracy 0.97

Test set Could be out AD: n=28, Accuracy 0.64

Test set out AD: n=35, Accuracy 0.74

22 compounds are not predicted

7.8. Predictivity - Assessment of the external validation set:

NA

7.9. Comments on the external validation of the model:

NA

8.Providing a mechanistic interpretation - OECD Principle 5

8.1. Mechanistic basis of the model:

The model gives information about the mechanism of action (MOA) towards fish acute toxicity. These MOA were retrieved from literature

8.2. A priori or a posteriori mechanistic interpretation:

Both a priori and a posteriori

8.3. Other information about the mechanistic interpretation:

NA

9.Miscellaneous information

9.1. Comments:

NA

9.2. Bibliography:

[1]Martin, T. M.; Grulke, C. M.; Young, D. M.; Russom, C. L.; Wang, N. Y.; Jackson, C. R.; Barron,M. G. Prediction of Aquatic Toxicity Mode of Action Using Linear Discriminant and Random ForestModels. J. Chem. Inf. Model. 2013, 53, 2229-2239. https://pubs.acs.org/doi/10.1021/ci400267h

[2]Martin, T. M.; Young, D. M.; Lilavois, C. R.; Barron, M. G. Comparison of global and mode ofaction-based models for aquatic toxicity. SAR QSAR Environ. Res. 2015, 26, 245-262. DOI:7.External validation - OECD Principle 48.Providing a mechanistic interpretation - OECD Principle 59.Miscellaneous information

10.1080/1062936X.2015.1018939

[3] C.L. Russom, S.P. Bradbury, S.J. Broderius, D.E. Hammermeister, and R.A. Drummond, Predicting modes of toxic action from chemical structure: Acute toxicity in the fathead minnow (Pimephales Promelas), Environ. Toxicol. Chem. 16 (1997), pp. 948–967

[4] Insecticide Resistance Action Committee (IRAC), IRAC MoA Classification Scheme, IRAC International MoA Working Group, 2012; software available at http://www.irac-online.org/eClassification/

[5] Un Environmental Protection Agency (US EPA), ASTER (Assessment Tools for the Evaluation of Risk), US EPA, Washington DC, 2012; software and information available at https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHEERL&count=10000&dirEntryId=136286&searchall=&showcriteria=2&simplesearch=0&timstype
search for “ASTER: AN INTEGRATION OF THE AQUIRE DATABASE AND THE QSAR SYSTEM FOR USE IN ECOLOGICAL RISK ASSESSMENTS Christine L. Russom"(Eric B. Anderson , Brad E. Greenwood, and Anne Pilli 'U.S. EPA, Environmental Research Laboratory-Duluth 6201 Congdon Boulevard, Duluth, MN 55804 U.S.A Computer Sciences Corporation, EPA/600/D-90/206 by use of this web site: https://ntrl.ntis.gov/NTRL/

[6] Floris, M., Manganaro, A., Nicolotti, O. et al. A generalizable definition of chemical similarity for read-across. J Cheminform 6, 39 (2014). https://doi.org/10.1186/s13321-014-0039-1

9.3. Supporting information:

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

All available dataset are present in the model inside the VEGA software.

10.Summary (JRC QSAR Model Database)

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