Leadscope Enterprise model for binding to the human Estrogen Receptor alpha (hERalpha) *in vitro* (Japanese METI data, balanced training set)

1. QSAR identifier

1.1 QSAR identifier (title)

Leadscope Enterprise model for binding to the human Estrogen Receptor alpha (hERalpha) *in vitro* (Japanese METI data, balanced training set), Danish QSAR Group at DTU Food.

1.2 Other related models

MultiCASE CASE Ultra model for binding to the human Estrogen Receptor alpha (hERalpha) *in vitro* (Japanese METI data, balanced training set), Danish QSAR Group at DTU Food.

SciMatics SciQSAR model for binding to the human Estrogen Receptor alpha (hERalpha) *in vitro* (Japanese METI data, balanced training set), Danish QSAR Group at DTU Food.

1.3. Software coding the model

Leadscope Predictive Data Miner, a component of Leadscope Enterprise version 3.1.1-10.

2. General information

2.1 Date of QMRF

January 2015.

2.2 QMRF author(s) and contact details

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2.3 Date of QMRF update(s)

2.4 QMRF update(s)

2.5 Model developer(s) and contact details

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2.6 Date of model development and/or publication

January 2014.

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

Roberts, G., Myatt, G. J., Johnson, W. P., Cross, K. P., and Blower, P. E. J. (2000) LeadScope: Software for Exploring Large Sets of Screening Data. *Chem. Inf. Comput. Sci.*, 40, 1302-1314.

Cross, K.P., Myatt, G., Yang, C., Fligner, M.A., Verducci, J.S., and Blower, P.E. Jr. (2003) Finding Discriminating Structural Features by Reassembling Common Building Blocks. *J. Med. Chem.*, *46,* 4770-4775.

Valerio, L. G., Yang, C., Arvidson, K. B., and Kruhlak, N. L. (2010) A structural feature-based computational approach for toxicology predictions. *Expert Opin. Drug Metab. Toxicol.*, 6:4, 505-518.

2.8 Availability of information about the model

The training set is non-proprietary and was compiled from the Japanese Ministry of Economy, Trade and Industry (METI) report (see 9.2, METI 2002). The model algorithm is proprietary from commercial software.

2.9 Availability of another QMRF for exactly the same model

3. Defining the endpoint

3.1 Species

Human (a cell-free assay containing the human Estrogen Receptor alpha, hERalpha).

3.2 Endpoint

QMRF 4. Human Health Effects

QMRF 4.18.a. Endocrine Activity. Receptor-binding (human Estrogen Receptor alpha)

3.3 Comment on endpoint

There is increasing evidence that a variety of environmental chemicals have the potential to disrupt the endocrine system by mimicking or inhibiting endogenous hormones such as estrogens and androgens. These endocrine disrupting chemicals (EDCs) may adversely affect development and/or reproductive function.

Natural estrogens are involved in the development and adult function of organs of the female genital tract, neuroendocrine tissues and the mammary glands; their role in reproduction spans from maintenance of the menstrual cycle to pregnancy and lactation. These effects are mediated through the estrogen receptors (ERs), members of the nuclear receptor superfamily. When estrogen binds to the ER in the cytoplasm a receptor-hormone complex dimer is formed. This dimer translocates to the nucleus, where it recruits co-factors to form the active transcription factor (TF) complex. The active TF complex binds to the estrogen response element (RE) upstream to the target gene. This binding activates transcription of mRNA and subsequent translation to the proteins that exert the hormone effects. Two isoforms of the ER exists in humans, alpha and beta, and both are widely expressed in different tissue types although there are some differences in their expression pattern.

Exogenous compounds able to bind to and activate the ERs (i.e. ER agonists) have the ability mimic natural estrogens and cause adverse effects to the reproductive system. Likewise, exogenous compounds that bind to the ERs without subsequent activation (i.e. ER antagonists) can potentially disturb the effect of the natural estrogens by blocking the receptors.

In this model data from a cell-free *in vitro* assay measuring the binding affinity of a chemical to the human Estrogen Receptor alpha (hERalpha) were used. The assay does not say anything about if the binding induces transcription of the target genes (i.e. if the chemical is an ER agonist or antagonist).

3.4 Endpoint units

No units, 1 for positives and 0 for negatives.

3.5 Dependent variable

Binding to the human Estrogen Receptor alpha (hERalpha) *in vitro*, positive or negative.

3.6 Experimental protocol

The experimental protocol is described in METI (2002). Briefly, an assay to detect binding of chemicals to the estrogen receptor was established and performed by the Japanese Ministry of Economy, Trade and Industry (METI). The assay measures the binding affinity of chemicals to the human Estrogen Receptor alpha (hERalpha) by measuring the extent of the competition reaction with a reference hormone labelled with a radioisotope (RI). The hERalpha was produced from *Escherichia coli* by a genetic engineering method and was used for the receptor binding assay with RI-labelled estradiol as the reference ligand.
The chemical concentration that inhibits 50% of the binding of the reference hormone to the receptor is measured and defined as IC_{50}. The relative binding affinity (RBA) is the ratio between the IC_{50} value of the chemical tested and the IC_{50} for the natural hormone, which is set to 100.

3.7 Endpoint data quality and variability

The training set data originate from a single source (METI 2000) and therefore no or only very limited variability in the experimental protocol is expected.

4. Defining the algorithm

4.1 Type of model

A categorical (Q)SAR model based on structural features and numeric molecular descriptors.

4.2 Explicit algorithm

This is a categorical (Q)SAR model made by use of partial logistic regression (PLR). The specific implementation is proprietary within the Leadscope software.

4.3 Descriptors in the model

structural features,

aLogP,

polar surface area,

number of hydrogen bond donors,

Lipinski score,

number of rotational bonds,

parent atom count,

parent molecular weight,

number of hydrogen bond acceptors

4.4 Descriptor selection

Leadscope Predictive Data Miner is a software program for systematic sub-structural analysis of a chemical using predefined structural features stored in a template library, training set-dependent generated structural features (scaffolds) and calculated molecular descriptors. The feature library contains approximately 27,000 pre-defined structural features and the structural features chosen for the library are motivated by those typically found in small molecules: aromatics, heterocycles, spacer groups, simple substituents. Leadscope allows for the generation of training set-dependent structural features (scaffold generation), and these features can be added to the pre-defined structural features from the library and be included in the descriptor selection process. It is possible in Leadscope to remove redundant structural features before the descriptor selection process and only use the remaining features in the descriptor selection process. Besides the structural features Leadscope also calculates eight molecular descriptors for

each training set structure: the octanol/water partition coefficient (alogP), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), Lipinski score, atom count, parent compound molecular weight, polar surface area (PSA) and rotatable bonds. These eight molecular descriptors are also included in the descriptor selection process.

Leadscope has a default automatic descriptor selection procedure. This procedure selects the top 30% of the descriptors (structural features and molecular descriptors) according to X^2-test for a binary variable, or the top and bottom 15% descriptors according to t-test for a continuous variable. Leadscope treats numeric property data as ordinal categorical data. If the input data is continuous such as IC_{50} or cLogP data, the user can determine how values are assigned to categories: the number of categories and the cut-off values between categories. (Roberts *et al.*2000).

When developing this model, intermediate models with application of different modelling approaches in Leadscope were tried:

1. 'Single model' using only the Leadscope pre-defined structural features, i.e. no scaffolds, and calculated molecular descriptors for descriptor selection.
2. 'Single model' using both the Leadscope pre-defined structural features and the training set dependent features (scaffolds generation) as well as the calculated molecular descriptors in the descriptor selection.
3. 'Single model' using both Leadscope pre-defined structural features and the training set dependent features (scaffolds generation), with subsequent removal of redundant structural features, and calculated molecular descriptors for descriptor selection.
4. 'Composite model' using only the Leadscope pre-defined structural features, i.e. no scaffolds, and calculated molecular descriptors in the descriptor selection.
5. 'Composite model' using both Leadscope pre-defined structural features and the training set dependent features (scaffolds generation) as well as the calculated molecular descriptors in the descriptor selection.

Based on model performance as measured by a preliminary cross-validation the model developed using approach number 2. was chosen.

For this model scaffolds were generated by Leadscope for the training set structures and added to the Leadscope library of structural features. Descriptors were then automatically selected among the structural features and the eight molecular descriptors.

4.5 Algorithm and descriptor generation

For descriptor generation see 4.4.

After selection of descriptors the Leadscope Predictive Data Miner program performs partial least squares (PLS) regression for a continuous response variable, or partial logistic regression (PLR) for a binary response variable, to build a predictive model. By default the Predictive Data Miner performs leave-one-out or leave-groups-out (in the latter case, the user can specify any number of repetitions and percentage of structures left out) cross-validation on the training set depending on the size of the training set. In the cross-validation made by Leadscope the descriptors selected for the 'mother model' are used when building the validation submodels and they therefore have a tendency to be overfittet and give overoptimistic validation results.

In this model, because of the categorical outcome in the response variable, PLR was used to build the predictive model. For this model 168 descriptors were selected to build the model. These include 6 Leadscope calculated molecular descriptors, 74 hierarchy features, and 88 scaffolds. The 168 descriptors were distributed on 8 PLS factors.

4.6 Software name and version for descriptor generation

Leadscope Predictive Data Miner, a component of Leadscope Enterprise version 3.1.1-10.

4.7 Descriptors/chemicals ratio

In this model 168 descriptors were used and distributed on 8 PLS factors. The training set consists of 595 compounds. The descriptor/chemical ratio is 1:3.5 (168:595).

5. Defining Applicability Domain

5.1 Description of the applicability domain of the model

The definition of the applicability domain consists of two components; the definition of a structural domain in Leadscope and the in-house further probability refinement algorithm on the output from Leadscope to reach the final applicability domain call.

1. Leadscope

For assessing if a test compound is within the structural applicability domain of a given model Leadscope examines whether the test compound bears enough structural resemblance to the training set compounds used for building the model (i.e. a structural domain analysis). This is done by calculating the distance between the test compound and all compounds in the training set (distance = 1 - similarity). The similarity score is based on the Tanimoto method. The number of neighbours is defined as the number of compounds in the training set that have a distance equal to or smaller than 0.7 with respect to the test compound. The higher the number of neighbours, the more reliable the prediction for the test compound. Statistics of the distances are also calculated. Effectively no predictions are made for test compounds which are not within the structural domain of the model or for which the molecular descriptors could not be calculated in Leadscope.

2. The Danish QSAR group

In addition to the general Leadscope structural applicability domain definition the Danish QSAR group has applied a further requirement to the applicability domain of the model. That is only positive predictions with a probability equal to or greater than 0.7 and negative predictions with probability equal to or less than 0.3 are accepted. Predictions within the structural applicability domain but with probability between 0.5 to 0.7 or 0.3 to 0.5 are defined as positives out of applicability domain and negatives out of applicability domain, respectively. When these predictions are wed out the performance of the model in general increases at the expense of reduced model coverage.

5.2 Method used to assess the applicability domain

Leadscope does not generate predictions for test compounds which are not within the structural domain of the model or for which the molecular descriptors could not be calculated.

Only positive predictions with probability equal to or greater than 0.7 and negative predictions with probability equal to or less than 0.3 are accepted.

5.3 Software name and version for applicability domain assessment

Leadscope Predictive Data Miner, a component of Leadscope Enterprise version 3.1.1-10.

5.4 Limits of applicability

The Danish QSAR group applies an overall definition of structures acceptable for QSAR processing which is applicable for all the in-house QSAR software, i.e. not only Leadscope. According to this definition accepted

structures are organic substances with an unambiguous structure, i.e. so-called discrete organics defined as: organic compounds with a defined two dimensional (2D) structure containing at least two carbon atoms, only certain atoms (H, Li, B, C, N, O, F, Na, Mg, Si, P, S, Cl, K, Ca, Br, and I), and not mixtures with two or more 'big components' when analyzed for ionic bonds (for a number of small known organic ions assumed not to affect toxicity the 'parent molecule' is accepted). Calculation 2D structures (SMILES and/or SDF) are generated by stripping off ions (of the accepted list given above). Thus, all the training set and prediction set chemicals are used in their non-ionized form. See 5.1 for further applicability domain definition.

6. Internal validation

6.1 Availability of the training set

Yes

6.2 Available information for the training set

CAS

SMILES

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

595 compounds are in the training set: 284 positives and 311 negatives.

Of the 948 entries in Appendix I (METI 2002), 843 contained test information for ERalpha binding. Among these 802 were discrete organic substances with available SMILES (see 5.4 for this step). Data was categorised to either active or inactive for binding to ERalpha based on the given RBA values in Appendix 1 (METI 2001); all chemicals, that were assigned a numerical RBA value, were defined as actives and all chemicals assigned with 'N.B' (Not Bound) or 'N.D.' (Not Determined) were defined as negatives (i.e. for these, whether [N.B] or [N.D], the RBA value was less than 0.001). This gave 284 compounds positive and 518 compounds negative for ERalpha binding. To balance the training set in order to prevent bias on the predictive performance of the model 311 negatives were randomly chosen among the 518 negatives.

6.6 Pre-processing of data before modelling

Only structures acceptable for Leadscope were used in the final training set. That is only discrete organic chemicals as described in 5.4 were used. In case of replicate structures, one of the replicates was kept if all the compounds had the same activity and all were removed if they had different activity. No further structures accepted by the software were eliminated (i.e. outliers).

6.7 Statistics for goodness-of-fit

Not performed.

6.8 Robustness – Statistics obtained by leave-one-out cross-validation

Not performed. (It is not a preferred measurement for evaluating large models).

6.9 Robustness – Statistics obtained by leave-many-out cross-validation

A five times two-fold 50 % cross-validation was performed. This was done by randomly removing 50% of the full training set used to make the "mother model", where the 50% contains the same ratio of positive and negatives as the full training set. A new model (validation submodel) was created on the remaining 50% using the same settings in Leadscope but with no information from the "mother model" regarding descriptor selection etc. The validation submodel was applied to predict the removed 50% (within the defined applicability domain for the submodel). Likewise, a validation submodel was made on the removed 50% of the training set and this model was used to predict the other 50% (within the defined applicability domain for this submodel). This procedure was repeated five times.

Predictions within the defined applicability domain for the ten validation submodels were pooled and Cooper's statistics calculated. This gave the following results for the 76.0% (2262/(5*595)) of the predictions which were within the applicability domain:

- – Sensitivity (true positives / (true positives + false negatives)): 83.7%
- – Specificity (true negatives / (true negatives + false positives)): 89.0%
- – Concordance ((true positives + true negatives) / (true positives + true negatives + false positives + false negatives)): 86.3%

6.10 Robustness - Statistics obtained by Y-scrambling

Not performed.

6.11 Robustness - Statistics obtained by bootstrap

Not performed.

6.12 Robustness - Statistics obtained by other methods

Not performed.

7. External validation

7.1 Availability of the external validation set

7.2 Available information for the external validation set

7.3 Data for each descriptor variable for the external validation set

7.4 Data for the dependent variable for the external validation set

7.5 Other information about the training set

7.6 Experimental design of test set

7.7 Predictivity – Statistics obtained by external validation

7.8 Predictivity – Assessment of the external validation set

7.9 Comments on the external validation of the model

External validation not performed for this model.

8. Mechanistic interpretation

8.1 Mechanistic basis of the model

The global model identifies structural features and molecular descriptors which in the model development was found to be statistically significant associated with effect. Many predictions may indicate modes of action that are obvious for persons with expert knowledge for the endpoint.

8.2 A priori or posteriori mechanistic interpretation

A posteriori mechanistic interpretation. The identified structural features and molecular descriptors may provide basis for mechanistic interpretation.

8.3 Other information about the mechanistic interpretation

9. Miscellaneous information

9.1 Comments

The model can be used to predict if a chemical can bind to the human Estrogen Receptor alpha (hERalpha) *in vitro*.

9.2 Bibliography

METI (2002) Current status of testing methods development for endocrine disrupters. In 6th Meeting of the Task Force on Endocrine Disrupters Testing and Assessment (EDTA) 24-25 June 2002, Tokyo. Ministry of Economy, Trade and Industry, Japan. Data can be found in Appendix I. Available online on: http://www.meti.go.jp/english/report/data/g020205ae.html

Jensen, G.E., Niemelä, J.R., Wedebye, E.B., and Nikolov, N.G. (2008) QSAR models for reproductive toxicity and endocrine disruption in regulatory use – a preliminary investigation. *SAR and QSAR in Environmental Research*, 19:7–8, 631–641.

9.3 Supporting information