Leadscope Enterprise model for Androgen Receptor (AR) antagonism (human vector) *in vitro*

1. QSAR identifier

1.1 QSAR identifier (title)

Leadscope Enterprise model for Androgen Receptor (AR) antagonism (human vector) *in vitro*, Danish QSAR Group at DTU Food.

1.2 Other related models

MultiCASE CASE Ultra model for Androgen Receptor (AR) antagonism (human vector) *in vitro*, Danish QSAR Group at DTU Food.

SciMatics SciQSAR model for Androgen Receptor (AR) antagonism (human vector) *in vitro*, 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

QSAR Group at DTU Food;

Danish National Food Institute at the Technical University of Denmark;

http://qsar.food.dtu.dk/;

qsar@food.dtu.dk

Eva Bay Wedebye;

National Food Institute at the Technical University of Denmark;

Nikolai Georgiev Nikolov;

National Food Institute at the Technical University of Denmark;

Marianne Dybdahl;

National Food Institute at the Technical University of Denmark;

Sine Abildgaard Rosenberg;

National Food Institute at the Technical University of Denmark;

2.3 Date of QMRF update(s)

2.4 QMRF update(s)

2.5 Model developer(s) and contact details

Jay Russel Niemelä;

National Food Institute at the Technical University of Denmark;

Gunde Egeskov Jensen;

National Food Institute at the Technical University of Denmark;

Eva Bay Wedebye;

National Food Institute at the Technical University of Denmark;

Nikolai Georgiev Nikolov;

National Food Institute at the Technical University of Denmark;

Danish QSAR Group at DTU Food;

National Food Institute at the Technical University of Denmark;

http://qsar.food.dtu.dk/;

qsar@food.dtu.dk

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 is composed of experimental data from our own laboratory and additional data from the literature (see references under 9.2). 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 (human androgen receptor in Chinese Hamster Ovary (CHO) cells).

3.2 Endpoint

QMRF 4. Human Health Effects

QMRF 4.18.c. Endocrine Activity. Other (human Androgen Receptor antagonism in a reporter gene assay)

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.

Among the many biological mechanisms that can result in endocrine disruption, one important is the expression of an antiandrogenic response. Chemicals with antiandrogenic activity counteract the effect of the male sex steroid hormones either by affecting their synthesis or metabolism or by blocking the effects of androgens. Androgens such as testosterone and dihydrotestosterone play a crucial role at several stages of male development and in the maintenance of the male phenotype. The development of the male phenotype during gestation is totally dependent on the action of androgens, and interference with the androgen receptor (AR) at this point of development is hypothesized as being linked to the increased frequency of male reproductive disorders such as testicular dysgenesis syndrome. Blocking of the androgen action may be exerted by antagonism of the AR, that is, by direct interaction of a chemical with AR.

The AR is a member of the nuclear receptor superfamily. Upon ligand binding to the AR in the cytoplasm the receptor undergoes a conformational change and the receptor-ligand dimer is transported to the nucleus where it binds to an androgen response element (RE) on the DNA. This binding modulates the transcription of target genes downstream the RE. The structural diversity of chemicals, which can bind to and affect the activity of AR is very broad. *In vivo* assays for the detection of antiandrogenic action are time-consuming, costly, and labour intensive, which makes them impractical for routine screening and testing of a large number of chemicals. Although *in vitro* data for AR antagonism alone are not sufficient to characterize a compound as an EDC, information on the ability of a chemical to antagonize AR *in vitro* provides an important piece of information for priority setting of chemicals for the more elaborate *in vivo* assays.
For this model training set data originates from reporter gene assays using hAR plasmid transfected Chinese Hamster Ovary (CHO) cells. The training set consists of data from our own laboratory (Vinggaard *et al.* 2008) and data compiled from the literature.

3.4 Endpoint units

No units, 1 for positives and 0 for negatives.

3.5 Dependent variable

Human Androgen Receptor (hAR) antagonism *in vitro*, positive or negative.

3.6 Experimental protocol

The experimental protocol for the data obtained in our own laboratory can be found in Vinggaard *et al.* (2008). Briefly, Chinese Hamster Ovary (CHO) cells were transfected with a plasmid containing a gene coding for the human androgen receptor (AR) and a plasmid containing a gene coding for the reporter enzyme Luciferase. The synthetic androgen, R1881, responsible for AR activity, was added, and the response of 0.1 nM R1881 was set to 100%. Chemicals were tested at various concentrations and data was related to the response of 0.1 nM R1881. Cytotoxicity was determined in parallel using CHO cells transfected with a plasmid containing a gene coding for a constitutively active AR lacking the ligand binding domain. The IC_{25}, defined as the concentration of the test compound that caused a 25% inhibition of the luciferase activity induced by R1881, was calculated for each compound.

For the data obtained from the literature different experimental protocols have been employed. We therefore refer to the references in 9.2 for a specific description of the different protocols.

All the AR antagonism data was separated in to two groups: chemicals reaching an IC_{25} at non-cytotoxic concentration ≤10 μM were defined as positives, and chemicals with IC25 > 10 μM or showing no activity were defined as negatives.

3.7 Endpoint data quality and variability

The dataset from our own laboratory is expected to have low data variability. Because multiple different experimental protocols were used for the data obtained from the literature a certain degree of interlaboratory variability in the data is expected. Jensen *et al.* (2012) compared data where different laboratories had tested the same substances and found an agreement of 83% (29/35) in one case and 91% (40/44) in another. Some chemicals were excluded from the training set due to significant discrepancies between data from different sources without other supporting data.

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 1. was chosen.

In this model the descriptors were automatically selected among the pre-defined 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 177 descriptors were selected to build the model. These include 9 Leadscope calculated molecular descriptors, 164 hierarchy features, and 4 dynamic features. The 177 descriptors were distributed on 3 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 177 descriptors were used and distributed on 3 PLS factors. The training set consists of 874compounds. The descriptor/chemical ratio is 1:4.9 (177:874).

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

874 compounds are in the training set: 231 positives and 643 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 64.8% (2831/(5*874)) of the predictions which were within the applicability domain:

- Sensitivity (true positives / (true positives + false negatives)): 51.7%
- Specificity (true negatives / (true negatives + false positives)):91.2 %
- Concordance ((true positives + true negatives) / (true positives + true negatives + false positives + false negatives)): 80.4%

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 has an antagonistic effect on the human androgen receptor *in vitro*.

9.2 Bibliography

Andersen, H.R., Vinggaard, A.M., Rasmussen, T.H., Gjermandsen, I.M., and Bonefeld-Jørgensen, E.C. (2002) Effects of currently used pesticides in assays for estrogenicity, androgenicity, and aromatase activity *in vitro. Toxicology and Applied Pharmacology*, 179, 1-12.

Araki, N., Ohno, K., Takeyoshi, M., and Iida, M. (2005a) Evaluation of a rapid in vitro androgen receptor transcriptional activation assay using AR-EcoScreen™ cells. *Toxicology In Vitro*, 19, 335-352.

Araki, N., Ohno, K., Nakai, M., Takeyoshi, M., and Iida, M. (2005b) Screening for androgen receptor activities in 253 industrial chemicals by in vitro reporter gene assays using AR-EcoScreen™ cells. *Toxicology In Vitro*, 19, 831-842.

Jensen, G.E., Nikolov, N.G., Wedebye, E.B., Ringsted, T., and Niemela, J.R. (2011) QSAR models for anti-androgenic effect--a preliminary study. *SAR and QSAR in Environmental Research*, 22:1-2, 35-49.

Jensen, G.E., Nikolov, N.G., Dreisig, K., Vinggaard, A.M., and Niemelä, J.R. (2012) QSAR Model for Androgen Receptor Antagonism - Data from CHO Cell Reporter Gene Assays. *J Steroids and Hormonal Science*, S2:006, doi:10.4172/2157-7536.S2-006.

Kawamura, Y., Mutsuga, M., Kato, T., Iida, M., and Tanamoto, K. (2005) Estrogenic and Anti-Androgenic Activities of Benzophenones in Human Estroge and Androgen Receptor Mediated Mammalian Reporter Gene Assays. *J Health Sci,* 51:1, 48-54.

Kojima, H., Iida, M., Katsura, E., Kanetoshi, A., Hori, Y., and Kobayashi, K. (2003) Effects of a diphenyl ether-type herbicide, chlornitrofen and its amino derivative on androgen and estrogen receptor activities. *Environ. Health Perspect*, 11:4, 497–502.

Kojima, H., Takeuchi, S., Uramaru, N., Sugihara, K., Yoshida, T., and Kitamura, S. (2009) Nuclear hormone receptor activity of polybrominated diphenyl ethers and their hydroxylated and methoxylated metabolites in transactivation assays using Chinese hamster ovary cells. *Environ Health Perspect*, 117:8, 1210-1218.

Körner, W., Vinggaard, A.M., Térouanne, B., Ma, R., Wieloch, C., Schlumpf, M., Sultan, C., and Soto, A.M. (2004) Interlaboratory comparison of four in vitro assays for assessing androgenic and antiandrogenic activity of environmental chemicals. *Environ Health Perspect*, 112:6, 695-702.

Satoh, K., Ohyama, K., Aoki, N., Iida, M., and Nagai, F. (2004) Study on anti-androgenic effects of bisphenol a diglycidyl ether (BADGE), bisphenol F diglycidyl ether (BFDGE) and their derivatives using cells stably transfected with human androgen receptor, AR-EcoScreen. *Food Chem Toxicol,* 42, 983-993.

Satoh, K., Nonaka, R., Ohyama, K., Nagai, F., Ogata, A., and Iida, M. (2008) Endocrine disruptive effects of chemicals eluted from nitrile-butadiene rubber gloves using reporter gene assay systems. *Biol Pharm Bull,* 31:3, 375-379.

Takeuchi, S., Iida, M., Kobayashi, S., Jin, K., Matsuda, T., and Kojima, H. (2005) Differential effects of phthalate esters on transcriptional activities via human estrogen receptors alpha and beta, and androgen receptor. *Toxicology*, 210, 223-233.

Takeuchi, S., Takahashi, T., Sawada, Y., Iida, M., Matsuda, T., and Kojima, H. (2009) Comparative study on the nuclear hormone receptor activity of various phytochemicals and their metabolites by reporter gene assays using Chinese hamster ovary cells. *Biol Pharm Bull*, 32:2, 195-202.

Vinggaard, A.M., Bonefeld-Joergensen, E.C., and Larsen, J.C. (1999) Rapid and sensitive reporter gene assays for detection of antiandrogenic and estrogenic effects of environmental chemicals. *Toxicol Appl Pharmacol*, 155, 150-160.

Vinggaard, A.M., Nellemann, C., Dalgaard, M., Jørgensen, E.B., and Andersen, H.R. (2002) Antiandrogenic effects in vitro and in vivo of the fungicide prochloraz. *Toxicol Sci*, 69, 344-353.

Vinggaard, A.M., Niemelä, J.R., Wedebye, E.B., and Jensen, G.E. (2008) Screening of 397 Chemicals and Development of a Quantitative Structure - Activity Relationship Model for Androgen Receptor Antagonism. *Chem. Res. Toxicol*., 21, 813-823.

9.3 Supporting information