Leadscope Enterprise model for Syrian Hamster Embryo (SHE) Cell Transformation *in vitro*

1. QSAR identifier

1.1 QSAR identifier (title)

Leadscope Enterprise model for Syrian Hamster Embryo (SHE) Cell Transformation *in vitro*, Danish QSAR Group at DTU Food.

1.2 Other related models

MultiCASE CASE Ultra model for Syrian Hamster Embryo (SHE) Cell Transformation *in vitro*, Danish QSAR Group at DTU Food.

SciMatics SciQSAR model for Syrian Hamster Embryo (SHE) Cell Transformation *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

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2.3 Date of QMRF update(s)

2.4 QMRF update(s)

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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 data were compiled from Isfort *et al.* (1996), Kerckaert *et al.* (1996), Gibson *et al.* (1997), Kerckaert *et al.* (1998) and Park *et al.* (2002) (see 9.2). In addition, 39 physiological chemicals from Grant *et al.* (2000), which are assumed to have a low probability of activity in this assay, were added as negatives to balance the training set against overrepresentation of positive test results. 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

Syrian hamster (embryo cells).

3.2 Endpoint

QMRF 4. Human Health Effects

QMRF 4.10. Mutagenicity

3.3 Comment on endpoint

Syrian hamster embryo (SHE) cells are genetically stable, diploid, metabolically and p53-competent primary cells, that have the ability to biotransform a wide range of xenobiotics. SHE cells have been used since the mid-1960ies to study the transforming ability of a variety of chemicals and physical agents. Exposure of the SHE cells to mutagenic chemicals results in an increase of morphologically transformed (MT) colonies, which are characterised by disorganised growth patterns and mimicking an early stage in carcinogenesis. It has been shown that SHE cells can be morphologically transformed by treatment with both genotoxic and non-genotoxic carcinogens. The exact molecular mechanisms involved in cell transformations are only partially understood. The transformation of these primary, diploid SHE cells is considered a model of the multistep process of carcinogenesis, as it appears to follow a staged process. The transformants are thought to be stem cells with blockages in their differentiation pathways. The transformed phenotype is characterized as a neoplastic progression-predisposing state that permits further steps toward acquisition of immortality, tumourigenicity and, finally, full malignancy. Upon further passages *in vitro*, transformed colonies clonally isolated from treated cultures, frequently generate cells with an infinite cellular lifespan or an ability to form tumours in syngenic (i.e. genetically identical) hosts. Untransformed clones on the other hand become senescent. The cell transformation results from structural alterations and changes in the expression of genes involved in cell cycle control, genomic stability, proliferation and differentiation. Genetic changes affecting these processes may result from direct genotoxic mechanisms or from non-genotoxic disturbance of gene expression and genomic stability through hyper- or hypomethylation of DNA, histone modifications and nucleosomal remodelling. In morphologically transformed SHE cell lines, cell cycle checkpoint control (G2) is often compromised. (OECD Guideline Draft 2013)

The SHE cell transformation assay is a short-term *in vitro* assay that predicts rodent carcinogenicity of chemicals by detecting the earliest identifiable stage in carcinogenesis; morphological transformation (MT) of cell colonies induced by chemicals. In contrast to most other short-term *in vitro* assays, both genotoxic and non-genotoxic carcinogens are identified.
A high correlation between results from the SHE cell transformation assay and rodent carcinogenicity data has been shown (Isfort *et al.* 1996). It was also shown that this assay was better at predicting rodent carcinogens compared to the Salmonella mutagenicity test (i.e. the Ames test). This is probably due to the fact the Salmonella mutation test only identifies genotoxic carcinogens.

3.4 Endpoint units

No units, 1 for positives and 0 for negatives.

3.5 Dependent variable

Syrian Hamster Embryo (SHE) cell transformation *in vitro*, positive or negative.

3.6 Experimental protocol

The experimental protocol for the SHE cell transformation *in vitro* assay is described in an OECD Guideline Draft (2013). Briefly, SHE cells are seeded at clonal density onto a feeder layer of x-ray-irradiated SHE cells in culture conditions allowing for the development of colonies. After plating, the cells are exposed to the test substance for 7 days. Then cells are washed, fixed and stained, and the colonies are scored for their morphological phenotype by stereomicroscopy. Cytotoxicity is evaluated by inhibition of cloning efficiency and reduction in size or density of the colonies. The number of morphologically transformed (MT) colonies relative to the total number of scorable colonies is calculated for each concentration tested. The frequency of MT colonies relative to total number of colonies in the substance-treated test groups is compared to the frequency of MT colonies in the solvent-treated control group.

3.7 Endpoint data quality and variability

Data for the training set originates from multiple sources and therefore some degree of variability in data is expected. Difference in the use of physiological pH (approx. 7.4) or reduced pH (6.7) in the experimental protocol has been shown not to affect the final results significantly (OECD Guideline Draft 2013) and therefore data from assays using either of the pH values are useful. The assay has been thoroughly validated since it was first introduced in the 1960ies and data are in general of high quality (Gibson *et al.* 1997, Kerckaert *et al.* 1998).

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 model was made using the composite model building option in Leadscope (see 4.4). The composite model consisted of only 1 submodel which uses all the positives and negatives. 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 4. 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 the single submodel in this composite model 172 descriptors were selected to build the submodel. These include 9 Leadscope calculated molecular descriptors, 159 hierarchy features, and 4 dynamic features. The 172 descriptors were distributed on 2 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 the 172 descriptors were used in the single submodel and they were distributed on 2 PLS factors. The training set consists of 352 compounds. The descriptor/chemical ratio is 1:2.0 (172:352).

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 CASE Ultra. 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

352 compounds are in the training set: 176 positives and 176 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 of the ten validation submodels were pooled and Cooper's statistics calculated. This gave the following results for the 48.3% (850/(5*352)) of the predictions which were within the applicability domains of the respective submodels:

- Sensitivity (true positives / (true positives + false negatives)): 71.6%
- Specificity (true negatives / (true negatives + false positives)): 76.5%
- Concordance ((true positives + true negatives) / (true positives + true negatives + false positives + false negatives)): 74.5%

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.

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 applied to predict a result for the Syrian Hamster Embryo (SHE) cell transformation *in vitro* assay.

9.2 Bibliography

Gibson, D.P., Brauninger, R., Shaffi, H.S., Kerckaert, G.A., LeBoeuf, R.A., Isfort, R.J., and Aardema, M.J. (1997) Induction of micronuclei in Syrian hamster embryo cells: comparison to results in the SHE cell transformation assay for national toxicology program test chemicals. *Mutation Research*, 392, 61-90.

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Isfort, R.J., Kerckaert, G.A., and LeBoeuf, R.A. (1996) Comparison of the standard and reduced pH Syrian Hamster Embryo (SHE) cell in vitro transformation assays in predicting the carcinogenic potential of chemical. *Mutation Research*, 356, 11-63.

Kerckaert, G.A., Isfort, R.J., Carr, G.J., Aardema, M.J., and LeBoeuf, R.A. (1996) A comprehensive protocol for conducting the Syrian hamster cell transformation assay at pH 6.70. *Mutation Research*, 356, 65-84.

Kerckaert, G.A., LeBoeuf, R.A., and Isfort, R.J. (1998) Assessing the Predictiveness of the Syrian Hamster Embryo Cell Transformation Assay for Determining the Rodent Carcinogenic Potential of Single Ring Aromatic/Nitroaromatic Amine Compounds. *Toxicological Sciences*, 189-197.

Park, J., Kamendulis, L.M., and Klaunig, J.E. (2002) Mechanisms of 2-Butoxyethanol Carcinogenicity: Studies on Syrian Hamster Embryo (SHE) Cell Transformation. *Toxicological Sciences*, 68, 43-50.

OECD Guideline Draft (2013) *In Vitro* Carcinogenicity: Syrian Hamster Embryo (SHE) Cell Transformation Assay. Available online at: http://www.oecd.org/env/ehs/testing/CTA%20TG_Feb2013.pdf

9.3 Supporting information