Leadscope Enterprise model for carcinogenicity exclusively in rodent liver *in vivo*

1. QSAR identifier

1.1 QSAR identifier (title)

Leadscope Enterprise model for carcinogenicity exclusively in rodent liver *in vivo*, Danish QSAR Group at DTU Food.

1.2 Other related models

MultiCASE CASE Ultra model for carcinogenicity exclusively in rodent liver *in vivo*, Danish QSAR Group at DTU Food.

SciMatics SciQSAR model for carcinogenicity exclusively in rodent liver *in vivo*, 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 in 2003 from the Cancer Potency Database (CPDB 1999). 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

Rodent (rat and mouse).

3.2 Endpoint

QMRF 4. Human Health Effects

QMRF 4.12. Carcinogenicity

3.3 Comment on endpoint

Data compiled from the Cancer Potency Database (CPDB 1999) were used to train this model. The CPDB is a unique and widely used international resource currently holding the results of 6540 chronic, long-term animal cancer tests on 1547 chemicals. The CPDB provides easy access to the bioassay literature, with qualitative and quantitative analyses of both positive and negative experiments that have been published over the past 50 years in the general literature through 2001 and by the National Cancer Institute/National Toxicology Program (NCI/NTP) through 2004. The CPDB standardizes the diverse literature of cancer bioassays that vary widely in protocol, histopathological examination and nomenclature, and in the publishing author's choices of what information to provide in their papers. Results are reported in the CPDB for tests in rats, mice, hamsters, dogs, and nonhuman primates (CPDB 1999).

From the CPDB data for substances with organ specific tumour information from rodent (rat and/or mouse) *in vivo* experiments were compiled. Chemicals causing tumours exclusively in the liver of rodents were defined as positives. Chemicals that caused cancer not only in the liver but also in others of the investigated organs were defined as negatives. Due to differences in liver metabolism, rat and mice are more sensitive to certain mechanisms associated with cell proliferation compared to humans. This model is intended to identify substances which are acting by these mechanisms and therefore may possibly not give the same effects in humans.

3.4 Endpoint units

No units, 1 for positives and 0 for negatives.

3.5 Dependent variable

Carcinogenicity in rodent liver (exclusively), positive or negative.

3.6 Experimental protocol

For data to be included in the CPDB, experiments should meet a set of standard inclusion criteria. These inclusion rules can be seen online at: http://toxnet.nlm.nih.gov/cpdb/methods.html#sources. These inclusion criteria for the CPDB were designed to identify reasonably thorough, chronic, long-term tests of single chemical agents (whether positive or negative). The two sources of data are the bioassays of the NCI/NTP and the general published literature. For NCI/NTP bioassay data the standard protocol from the 1970s is described in Sontag *et al.*(1976) and recommends that tests be conducted in two species of rodents (rats and mice) with both sexes tested individually at the maximally tolerated dose (MTD) and half that dose, using a control group and a vehicle control where appropriate. In the early 1990s the standard number of dose groups was increased to 3, and the standard range of doses tested was 4-10 folds.

In order for experiments from the general literature to be included in the database a set of standard inclusion criteria should be met.

For the data in CPDB the following should be noted: For any single chemical, the number of experiments in the database may vary. Some chemicals have only one test in one sex of one species, while others have multiple tests including both sexes of a few strains of rats and mice, possibly using quite different protocols.

3.7 Endpoint data quality and variability

Data for the training set originated from multiple sources and therefore some degree of variability is expected. The inclusion rules (see 3.6) for CPDB reduces some of this variability in 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 3. was chosen.

For this model scaffolds were generated by Leadscope for the training set structures and added to the Leadscope library of structural features. The number of structural features was then reduced further using the built-in filter to remove similar (reduntant) features (the "less similar" features removed). Descriptors were then automatically selected among the remaining 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 172 descriptors were selected to build the model. These include 8 Leadscope calculated molecular descriptors, 105 hierarchy features, and 59 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 172 descriptors were used and distributed on 2 PLS factors. The training set consists of 320 compounds. The descriptor/chemical ratio is 1:1.9 (172:320).

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 weeded 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

320 compounds are in the training set: 109 positives and 211 negatives.

6.6 Pre-processing of data before modelling

From (CPDB 1999) substances with organ specific tumour information from rodent in vivo experiments were compiled. For 626 structure information in the form of SMILES were available. Of these, 109 chemicals exclusively caused tumours in the liver and these were defined as positives. Chemicals causing tumours in the liver as well as in other organs were defined as 'negative'. To balance the model so that the ratio of negatives to positives was not too high a random selection was made among them giving a total of 211 negatives. The total number of substances in the training set was therefore 320. The remaining 306 negatives were originally used in an external validation which has not yet been repeated for this new version of the model (originally in MC4PC: 182 of the negatives were within AD giving a specificity of 86.3%).

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 49.8% (796/(5*320)) of the predictions which were within the applicability domain:

- Sensitivity (true positives / (true positives + false negatives)): 35.6%
- Specificity (true negatives / (true negatives + false positives)): 88.6%
- Concordance ((true positives + true negatives) / (true positives + true negatives + false positives + false negatives)): 69.3%

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 validation 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 applied to predict if a chemical has the potential to cause liver tumours exclusively in rodents (rat and/or mouse). A negative result does not mean that the predicted chemical is not a carcinogen but that the chemical is not exclusively causing tumours in the rodent liver.

9.2 Bibliography

CPDB (1999) The Carcinogenic Potency Database (CPDB) [online]. By Lois Swirsky Gold. Last updated September 2011. Available at http://toxnet.nlm.nih.gov/cpdb/

Sontag, J.M., Page, N.P. and Saffiotti, U. (1976) Guidelines for carcinogen bioassay in small rodents. DHHS Publication (National Institutes of Health) 76-801, National Cancer Institute, Bethesda, Maryland.

9.3 Supporting information