Leadscope Enterprise version of commercial MultiCASE model A2E for Structural Alerts for DNA Reactivity (NTP data)

1. QSAR identifier

1.1 QSAR identifier (title)

Leadscope Enterprise version of commercial MultiCASE model A2E for Structural Alerts for DNA Reactivity (NTP data), Danish QSAR Group at DTU Food.

1.2 Other related models

MultiCASE CASE Ultra version of commercial MultiCASE model A2E for Structural Alerts for DNA Reactivity (NTP data), Danish QSAR Group at DTU Food.

Leadscope Enterprise version of commercial MultiCASE model A2E for Structural Alerts for DNA Reactivity (NTP data), Danish QSAR Group at DTU Food.

SciMatics SciQSAR version of commercial MultiCASE model A2E for Structural Alerts for DNA Reactivity (NTP data), 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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MultiCASE Inc. has kindly given their permission that remodelling of their training set for the commercial A61 model in Leadscope was performed by:

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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 proprietary and commercially available from MultiCASE Inc. It was originally compiled by MultiCASE Inc. and used to train the commercial MultiCASE A2E model. The Danish QSAR Group bought this model from MultiCASE Inc. in 1999. Permission to remodel the training set in Leadscope was kindly granted by MultiCASE Inc. 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

Rodents (rats and mice, both sexes, multiple organs).

3.2 Endpoint

QMRF 4. Human Health Effects

QMRF 4.10. Mutagenicity

3.3 Comment on endpoint

Structural alerts (SAs) were introduced by Ashby and co-workers back in the late 80'ies and are chemical moieties that are either electrophilic or can be metabolized to electrophiles and thereby have the potential to cause electrophilic attack on DNA (Ashby & Tennant 1988). Chemicals containing SAs may in this way be genotoxic and therefore potentially carcinogenic. The SAs reflect specific rules, all of which presumably have been enumerated *a priori*.
Ashby and co-workers identified SAs in chemicals with related US National Toxicology Program (NTP) cancer bioassay and *Salmonella typhimurium* mutagenicity assay data (Ashby & Tennant 1988, Ashby *et al.*1989). They then made a comparison of SA, mutagenicity in Salmonella and ability to induce cancer in rats and mice at several sites and in both sexes (this is a typical pattern seen for genotoxic carcinogens as opposed to non-genotoxic carcinogens that are generally restricted in their range of site, sex and species specificity). A strong correlation between the SA and mutagenicity was found and this was not surprising as the Salmonella mutagenicity assay identifies mutagenic chemicals that exert their effect through electrophilic attack on DNA, just like SA is expected to do. Also a strong correlation was found between chemicals containing a SA and causing cancer in rats and mice at several sites and in both sexes (i.e. genotoxic carcinogens). In fact, SA appeared to perform as well as the Salmonella mutagenicity assay in predicting the genotoxic carcinogens (Ashby & Tennant 1988, Ashby *et al.*1989). SA is therefore useful to identify genotoxic carcinogens and mutagens but it is important to be aware of the fact that the SAs are not necessarily an exhaustive list of possible alerts for genotoxic carcinogens, and moreover that chemicals that do not contain a SA may be non-genotoxic carcinogens or non-carcinogens. It should be noted that genotoxic chemicals are not necessarily also mutagens (i.e. lead to mutations after the DNA damage) so the presence of a SA in a chemical does not that mean it thereby is also mutagenic.

In the fact sheet for the A2E model (personal communication with MultiCASE in 2001), MultiCASE Inc. refer to three publications of previous versions of the model made with smaller training set (Rosenkranz & Klopman 1990a,b,c). The description of the endpoint for this model is based on the assumption that it is similar to the endpoint described in the paper by Rosenkranz & Klopman (1990b):
Chemicals with cancer bioassay results from US NTP and other databases, as well as results from the *Salmonella typhimurium* mutagenicity assay constitute the data in the training set. The training set chemicals are categorised as positive if they contain a SA, as defined by Ashby and co-workers, and as negative if no SA is found in the chemical. Rather than programming the software to recognize the specific SAs, the chemical structures and the final decisions by Ashby and co-workers as to whether or not the chemicals were classified as possessing or lacking a SA were submitted to the program for modelling. In the modelling process the program identified the structural moieties which were found to be related to activity (biophores) or lack of activity (biophobes).

3.4 Endpoint units

No units, 1 for positives and 0 for negatives.

3.5 Dependent variable

Structural alerts for DNA reactivity, positive or negative.

3.6 Experimental protocol

As the training set is proprietary from MultiCASE Inc. and the data sources are unknown an experimental protocol cannot be described, but as mentioned under 3.3 the training set probably consist of rodent carcinogenicity data from US NTP and other data bases categorized by experts using the rules for SA as described by Ashby and co-workers.

3.7 Endpoint data quality and variability

As the training set is commercial by MultiCASE Inc. the quality and variability of the data used is unknown.

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 is a composite model consisting of 2 submodels, using all the negatives (278 chemicals) in each of these and different sub-sets of the positives (see 4.5). 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 5. 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. Because of the unbalanced training set (i.e. 500 positives vs. 278 negatives) 2 submodels for smaller individual training sets consisting of the 278 negatives and an equal number of positives selected by random among the 500 positives were made. The descriptors for each of the 2 submodels were automatically selected from the Leadscope feature library based solely on the training set compounds used to build the individual submodel and was not affected by the training set chemicals in the composite model. Therefore, a different number of descriptors (structural features and molecular descriptors) were selected and distributed on varying number of PLS factors for each submodel.

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

As this model is a composite model consisting of 2 submodels with varying training set size and using different descriptors and number of PLS factors (see 4.5), an overall descriptor/chemical ratio for this model cannot be calculated.

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

No

6.2 Available information for the training set

SMILES

6.3 Data for each descriptor variable for the training set

No

6.4 Data for the dependent variable for the training set

No

6.5 Other information about the training set

778 compounds are in the training set: 500 positives and 278 negatives.

6.6 Pre-processing of data before modelling

Only structures acceptable for SciQSAR 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 66.5% (2587/(5*778)) of the predictions which were within the applicability domains of the respective submodels:

- Sensitivity (true positives / (true positives + false negatives)): 87.5%
- Specificity (true negatives / (true negatives + false positives)): 90.7%
- Concordance ((true positives + true negatives) / (true positives + true negatives + false positives + false negatives)): 88.5%

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 predict if a chemical contain a structural alert, i.e. a moiety that can cause electrophilic attack on DNA, and thereby have the potential to be a genotoxic carcinogens. A negative prediction means that the chemical does not contain a structural alert and the chemical can be either a non-genotoxic carcinogen or a non-carcinogen.

9.2 Bibliography

Ashby, J., and Tennant, R.W. (1988) Chemical structure, Salmonella mutagenicity and extent of carcinogenicity as indicators of genotoxic carcinogenesis among 222 chemicals tested in rodents by the U.S. NCI/NTP. *Mutat Res*., 204, 17-115.

Ashby, J., Tennant, R.W., Zeiger, E., and Stasiewicz, S. (1989) Classification according to chemical structure, mutagenicity to Salmonella and level of carcinogenicity of a further 42 chemicals tested for carcinogenicity by the U.S. National Toxicology Program. *Mutat Res*., 223, 73-103.

Rosenkranz, H.S., and Klopman, G. (1990a) Structural basis of carcinogenicity in rodents of genotoxicants and non-genotoxicants. *Mutat Res.*, 228:2, 105-124.

Rosenkranz, H.S., and Klopman, G. (1990b) Structural alerts to genotoxicity: the interaction of human and artificial intelligence. *Mutagenesis*, 5:4, 333-361.

Rosenkranz, H.S., and Klopman, G. (1990c) Evaluating the ability of CASE, an artificial intelligence structure-activity relational system, to predict structural alerts for genotoxicity. *Mutagenesis*, 5:6, 525-527.

9.3 Supporting information