MultiCASE CASE Ultra version of commercial MultiCASE model A2E for Structural Alerts for DNA Reactivity (NTP data)

1. QSAR identifier

1.1 QSAR identifier (title)

MultiCASE CASE Ultra 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

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

MultiCASE CASE Ultra 1.4.6.6 64-bit.

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

MultiCASE Inc.;

23811 Chagrin Blvd Ste 305, Beachwood, OH, 44122, USA;

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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

Klopman, G. (1992) MULTICASE 1. A Hierarchical Computer Automated Structure Evaluation Program. *Quant. Struct.-Act. Relat.,* 11*,* 176 - 184.

Chakravarti, S.K., Saiakhov, R.D., and Klopman, G. (2012) Optimizing Predictive Performance of CASE Ultra Expert System Models Using the Applicability Domains of Individual Toxicity Alerts. *J. Chem. Inf. Model*., 52, 2609 −2618.

Saiakhov, R.D., Chakravarti, S.K., and Klopman, G. (2013) Effectiveness of CASE Ultra Expert System in Evaluating Adverse Effects of Drugs. *Mol. Inf*., 32, 87 – 97.

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. It has been remodeled by MultiCASE Inc. in MC4PC and CASE Ultra. 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

CASE unit, 39 for positives and 10 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 fragments and calculated molecular descriptors.

4.2 Explicit algorithm

This is a categorical (Q)SAR model composed of multiple local (Q)SARs made by use of stepwise regression. The specific implementation is proprietary within the MultiCASE CASE Ultra software.

4.3 Descriptors in the model

Fragment descriptors,

Distance descriptors,

Physical descriptors,

Electronic descriptors,

Quantum mechanical descriptors

4.4 Descriptor selection

Automated hierarchical selection (see 4.5).

4.5 Algorithm and descriptor generation

MultiCASE CASE Ultra is an artificial intelligence (AI) based computer program with the ability to learn from existing data and is the successor to the program MultiCASE MC4PC. The system can handle large and diverse sets of chemical structures to produce so-called global (Q)SAR models, which are in reality series of local (Q)SAR models. Upon prediction of a query structure by a given model one or more of these local models, as well as global relationships if these are identified, can be involved if relevant for the query structure. The CASE Ultra algorithm is mainly built on the MCASE methodology (Klopman 1992) and was released in a first version in 2011 (Chakravarti *et al.* 2012, Saiakhov *et al.* 2013).

CASE Ultra is a fragment-based statistical model system. The methodology involves breaking down the structures of the training set into all possible fragments from 2 to 10 heavy (non-hydrogen) atoms in length. The fragment generation procedure produces simple linear chains of varying lengths and branched fragments as well as complex substructures generated by combining the simple fragments.

A structural fragment is considered as a positive alert if it has a statistical significant association with chemicals in the active category. It is considered a deactivating alert if it has a statistically significant relation with the inactive category.

Once final lists of positive and deactivating alerts are identified, CASE Ultra attempts to build local (Q)SARs for each alert in order to explain the variation in activity within the training set chemicals covered by that alert. The program calculates multiple molecular descriptors from the chemical structure such as molecular orbital energies and two-dimensional distance descriptors. A stepwise regression method is used to build the local (Q)SARs based on these molecular descriptors. For each step a new descriptor (modulator) is added if the addition is statistically significant and increases the cross-validated R2 (the internal performance) of the model. The number of descriptors in each local model is never allowed to exceed one fifth of the number of training set chemicals covered by that alert. If the final regression model for the alert does not satisfy certain criteria ($R2 \geq 0.6$ and $Q2 \geq 0.5$) it is rejected. Therefore, not all alerts will necessarily have a local (Q)SAR.

The collection of positive and deactivating alerts with or without a local (Q)SAR constitutes a global (Q)SAR model for a particular endpoint and can be used for predicting the activity of a test chemical.

More detailed information about the algorithm can be found in Chakravarti *et al.* (2012), Saiakhov *et al.* (2013).

4.6 Software name and version for descriptor generation

MultiCASE CASE Ultra 1.4.6.6 64-bit.

4.7 Descriptors/chemicals ratio

The program primarily uses fragment descriptors specific to a group of structurally related chemicals from the training set. Therefore estimation of the number of descriptors used in a specific model, which is a collection of local models as explained under 4.5, may be difficult. In general, we estimate that the model uses an order of magnitude less descriptors than there are observations. The number of descriptors in each local (Q)SAR model is never allowed to exceed one fifth of the number of training set chemicals covered by that alert (Saiakhov *et al.* 2013).

It should be noted that due to CASE Ultra's complex decision making scheme overfitting is rare compared to simpler linear models. Warnings are issued in case of statistically insufficient overall number of observations to produce a model, which is not the case in the present model.

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 in CASE Ultra and the in-house further refinement algorithm on the output from CASE Ultra to reach the final applicability domain call.

1. CASE Ultra

CASE Ultra recognizes unknown structural fragments in test chemicals that are not found in the training data and lists these in the output for a prediction. Fragments this way impose a type of global applicability domain for the overall model. The presence of more than three unknown structural fragments in the test chemical results in an 'out of domain' call in the program. (Chakravarti *et al.*2012, Saiakhov *et al.*2013).

For each structural alert, CASE Ultra uses the concept of so-called domain adherences and statistical significance.

The domain adherence for an alert in a query chemical depends on the similarity of the chemical space around the alert in the query chemical compared to the chemical space (in terms of frequencies of occurrences of statistically relevant fragments) of the training set chemicals used to derive the alert. The domain adherence value (between zero and one) is the ratio of the sum of the squared frequency of occurrence values of the subset of the fragments that are present in the test chemical and sum of the squared frequency of occurrence of all the fragments that constitute the domain of the alert in question. The more fragments of the domain of the alert in the test chemical the closer the domain adherence value is to 1. The value will never be zero as the alert itself is part of the alerts domain.

Furthermore, all alerts come with a measure of its statistical significance, and this depends on the number of chemicals in the training set which contained the alert and the prevalence within these of actives and inactives. (Chakravarti *et al.*2012).

2. In-house refinement algorithm to reach the final applicability domain call

The Danish QSAR group has applied a stricter definition of applicability domain for its MultiCASE CASE Ultra models.

An optimization procedure based on preliminary cross-validation is applied to further restrict the applicability domain for the whole model based on non-linear requirements for domain adherence and statistical significance, giving the following primary thresholds:

Domain adherence = 0.77 and significance = 70%

Any positive prediction is required to contain at least one valid positive alert, namely an alert with statistical significance and domain adherence exceeding thresholds defined for the specific model.

The positive predictions for chemicals which only contain invalid positive alerts are considered 'out of domain' (in CASE Ultra these chemicals are predicted to be inactive).

Furthermore, only query chemicals with no unknown structural fragments are considered within the applicability domain, except for chemicals predicted 'positive', where one unknown fragment is accepted. Also no significant positive alerts are accepted for an inactive prediction.

5.2 Method used to assess the applicability domain

The applicability domain is assessed in terms of the output from CASE Ultra with the Danish QSAR group's further refinement algorithm on top as described in 5.1.

Because of the complexity of the system (see 5.1), the assessment of whether a test chemical is within the applicability domain of the model requires predicting the chemical with the specific model, and the application of the Danish QSAR group model-specific thresholds for domain adherence and significance.

This applicability domain was also applied when determining the results from the cross-validations (6.9).

5.3 Software name and version for applicability domain assessment

MultiCASE CASE Ultra 1.4.6.6 64-bit.

5.4 Limits of applicability

All structures are run through the DataKurator feature within CASE Ultra to check for compatibility with the program. Furthermore, 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). Structures with less than two carbon atoms or containing atoms not in the list above (e.g. heavy metals) are rendered out as not acceptable for further QSAR processing. Calculation 2D structures (SMILES and/or SDF) are generated by stripping off accepted organic and inorganic ions. 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

781 compounds are in the training set: 503 positives and 278 negatives.

6.6 Pre-processing of data before modelling

As the training set is commercial by MultiCASE Inc. the pre-processing of data is unknown.

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”, thereby splitting the full training set into two subsets A and B, each containing the same ratio of positives to negatives as the full training set. A new

model (validation sub-model) was created on subset A without using any information from the “mother model” (regarding e.g. descriptor selection etc.). The validation sub-model was applied to predict subset B (within the CASE Ultra applicability domain for the validation sub-model and the in-house further refinement algorithm for the full model). Likewise, a validation sub-model was made on subset B and this model was used to predict subset A (within the CASE Ultra applicability domain for the validation sub-model and the in-house further refinement algorithm for the full model). This procedure was repeated five times.

Predictions within the defined applicability domain for the ten validation sub-models were pooled and Cooper’s statistics calculated. This gave the following results for the 49.3% (1926/(5*781)) of the predictions which were within the applicability domain:

- Sensitivity (true positives / (true positives + false negatives)): 92.0%
- Specificity (true negatives / (true negatives + false positives)): 91.8%
- Concordance ((true positives + true negatives) / (true positives + true negatives + false positives + false negatives)): 91.9%

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 has not been performed for this model.

8. Mechanistic interpretation

8.1 Mechanistic basis of the model

The model identifies statistically relevant substructures (i.e. alerts) and for each set of molecules containing a specific alert it further identifies additional parameters found to modulate the alert (e.g. logP and molecular orbital energies, etc.). Many predictions may indicate modes of action that are obvious for persons with expert knowledge about 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