Leadscope Enterprise model for Chromosome Aberrations (CA) in Chinese Hamster Lung (CHL) cells *in vitro*

1. QSAR identifier

1.1 QSAR identifier (title)

Leadscope Enterprise model for Chromosome Aberrations (CA) in Chinese Hamster Lung (CHL) cells *in vitro*, Danish QSAR Group at DTU Food.

1.2 Other related models

MultiCASE CASE Ultra model for Chromosome Aberrations (CA) in Chinese Hamster Lung (CHL) cells *in vitro*, Danish QSAR Group at DTU Food.

Leadscope Enterprise model for Chromosome Aberrations (CA) in Chinese Hamster Lung (CHL) cells *in vitro*, Danish QSAR Group at DTU Food.

SciMatics SciQSAR model for for Chromosome Aberrations (CA) in Chinese Hamster Lung (CHL) cells *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)

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 data set is non-proprietary and was compiled from 'Data Book of Chromosomal Aberration Test In Vitro' (revised by Ishidate in 1988, and later by Sofuni in 1998) and the publication of Kusakabe *et al.* (2002). 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

Chinese hamster (all tests were performed using a Chinese Hamster Lung (CHL) fibroblast cell line, which has been kept as a single cell sub-clone since 1973 (Ishidate 1988, Sofuni 1998, Kusakabe *et al.* 2002)).

3.2 Endpoint

QMRF 4.10. Mutagenicity

OECD 473 In Vitro Mammalian Chromosome Aberration Test

3.3 Comment on endpoint

The chromosome aberration test using cultured mammalian cells is one of the sensitive methods to predict environmental mutagens and/or carcinogens, and is a complementary test to the *Salmonella* assay (also known as the Ames test). The purpose of the *in vitro* chromosome aberration test is to identify agents that cause structural chromosome aberrations in cultured mammalian cells arrested in metaphase. The structural aberrations detected may be of two types, chromosome (i.e. breakage, or breakage and reunion, of both chromatids at an identical site) or chromatid (i.e. breakage of single chromatids or breakage and reunion between chromatids). With the majority of chemical mutagens, induced aberrations are of the chromatid type, but chromosome-type aberrations also occur. Chromosome mutations and related events are the cause of many human genetic diseases and there is substantial evidence that chromosome mutations and related events causing alterations in oncogenes and tumor suppressor genes of somatic cells are involved in cancer induction in humans and experimental animals. The *in vitro* assay systems for clastogenicity (i.e. any process resulting in the breakage of chromosomes or the loss or rearrangement of pieces of chromosomes) testing have certain advantages over *in vivo* systems such as, cells of human origin can be used if desired, a chemical can be tested for both direct effect and in the presence of metabolic activating systems, active but short-lived metabolites can be more easily detected, tests can be repeated with the same or different cell types under the same experimental conditions, and numerical aberrations – such as aneuploidy (i.e. abnormal number of chromosomes) and polyploidy (i.e. more than two paired (homologous) sets of chromosomes) - are more easily detected.

The mammalian chromosome aberration *in vitro* test is used to screen for possible mammalian mutagens and carcinogens. Many compounds that are positive in this test are mammalian carcinogens; however, there is not a perfect correlation between this test and carcinogenicity. Correlation is dependent on chemical class. In addition, it is important to be aware that positive results may arise from changes in pH, osmolality or high levels of cytotoxicity and do not reflect intrinsic mutagenicity (OECD guideline 473, 1997).

3.4 Endpoint units

No units, 1 for positives and 0 for negatives.

3.5 Dependent variable

Chromosome aberrations (CA) in Chinese Hamster Lung (CHL) cells *in vitro*, positive or negative.

3.6 Experimental protocol

Data has been generated using similar experimental protocols to that described in OECD guideline 473 (1997). In 'Data Book of Chromosomal Aberration Test in Vitro' (revised by Ishidate 1988, and later Sofuni 1998) and the publication of Kusakabe *et al.* (2002) further descriptions of experimental protocols can be found in addition to the OECD guideline 473 (1997). Briefly, the cell cultures (CHL cells) are exposed to the test substance both with and without metabolic activation. In the data set compiled from 'Data Book of Chromosomal Aberration Test in Vitro' (revised by Ishidate 1988, and later Sofuni 1998) activation was only used in 170 of the 513 chemicals selected for the training set. At predetermined intervals after the exposure, the cell cultures are treated with a metaphase-arresting substance, harvested, stained and metaphase cells are analysed microscopically for the presence of chromosome aberrations (OECD guideline 473, 1997). Results from the experimental studies were indicated as positive (active) or negative (inactive).

3.7 Endpoint data quality and variability

The vast majority of the training set data were taken from a single source, the "Data Book of Chromosomal Aberration Test In Vitro" (revised by Ishidate 1988, and later Sofuni 1998), and supplemented with a smaller data set (Kusakabe *et al.* 2002) continuing the work from the first source. Therefore a relatively low degree of variability is expected. The data source is very detailed and well-documented, and judged by the performance of the (Q)SAR model the quality of the data is high.

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 positives (293 chemicals) in each of these and different sub-sets of the negatives (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

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. Because of the unbalanced training set (i.e. 293 positives vs. 305 negatives) 2 submodels for smaller individual training sets consisting of the 293 positives and an equal number of negatives selected by random among the 305 negatives/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

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

598 compounds are in the training set: 293 positives and 305 negatives.

6.6 Pre-processing of data before modelling

The data in the model consist of data from a preliminary model with a training set of 513 chemicals (Niemelä & Wedebye 2004) and data for 87 chemicals used for external validation (Kusakabe *et al.* 2002) of the preliminary model. Out of 911 substances from the Data Book (Sofuni 1998), 513 were used to establish the preliminary model. The exclusion criteria used include inorganic status, inadequate smile code, etc. A decision was made to include chemicals as being positive if they were active in inducing either aberrations or polyploidy. Polyploidy is not included in the current test guideline (OECD guideline 473 1997). 87 chemical from Kusakabe *et al.* (2002) completed the training set for the model.

From the original training set (513 compounds from "Data Book of Chromosomal Aberration Test In Vitro" (revised by Ishidate 1988, and later Sofuni 1998) and 87 compounds from Kusakabe *et al.* (2002)) only compounds for which SMILES codes could be found and that had a structure acceptable for the commercial software 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 59.2% (1770/(5*598)) of the predictions which were within the applicability domains of the respective submodels:

- Sensitivity (true positives / (true positives + false negatives)): 74.6%
- Specificity (true negatives / (true negatives + false positives)): 75.2%
- Concordance ((true positives + true negatives) / (true positives + true negatives + false positives + false negatives)): 74.9%

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 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 used to predict a result for chromosome aberrations in cultured Chinese Hamster Lung (CHL) cells *in vitro*.

9.2 Bibliography

Ishidate, M. Jr., Ed. (1988) Data Book of Chromosomal Aberration Test In Vitro, Revised Edition. Elsevier; Amsterdam, New York, Oxford.

Kusakabe, H., Ymakage, K., Wakuri, S., Sasaki, K., Nakagawa, Y., Watanabe, M., Hayashi, M., Sufuni, T., Ono, H., and Tnanka, N. (2002) Relevance of chemical structure and cytotoxicity to the induction of chromosome aberrations based on testing of 98 high production volume industrial chemicals. *Mutation Research,* 517, 187-198.

Niemelä, J., and Wedeby, E. (2004) Evaluation of the setubal principles for establishing the status of development and validation of (Q)SARs, Annex 4, chapter 2, pp. 115-120, A "global" MULTI-CASE model for in vitro chromosomal aberrations in mammalian cells. In OECD Environment Health and Safety Publications, Series on Testing and Assessment, no. 49, Report from the expert group on (Quantitative) Structure-Activity Relationships ((Q)SARs) on the principles for the validation of (Q)SARs.

OECD guideline 473(1997) OECD Guidelines for the Testing of Chemicals No. 473: *In Vitro* Mammalian Chromosome Aberration Test. Organisation for Economic Cooperation and Development; Paris, France. Available online at: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788.

Sofuni, T., Ed. (1998) Data Book of Chromosomal Aberration Test In Vitro, Revised Edition. Life-Science Information Center; Tokyo, Japan.

9.3 Supporting information