SciMatics SciQSAR version of commercial CASE Ultra A61 for Chromosome Aberrations in Chinese Hamster Ovary cells *in vitro* (NTP data)

1. QSAR identifier

1.1 QSAR identifier (title)

SciMatics SciQSAR version of commercial CASE Ultra A61 for Chromosome Aberrations in Chinese Hamster Ovary cells *in vitro* (NTP data), Danish QSAR Group at DTU Food.

1.2 Other related models

MultiCASE CASE Ultra commercial model A61 for Chromosome Aberrations in Chinese Hamster Ovary cells *in vitro* (NTP data), Danish QSAR Group at DTU Food.

Leadscope Enterprise version of commercial CASE Ultra model A61 for Chromosome Aberrations in Chinese Hamster Ovary cells *in vitro* (NTP data), Danish QSAR Group at DTU Food.

1.3. Software coding the model

SciQSAR version 3.1.00.

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 commercial A33 model in SciQSAR 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

Contrera, J.F., Matthews, E.J., Kruhlak, N.L., and Benz, R.D. (2004) Estimating the safe starting dose in phase I clinical trials and no observed effect level based on QSAR modelling of the human maximum recommended daily dose. *Regulatory Toxicology and Pharmacology*, 40, 185 – 206.

SciQSAR (2009) Reference guide: *Statistical Analysis and Molecular Descriptors*. Included within the SciMatics SciQSAR software.

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 A61 model. The Danish QSAR Group bought this model from MultiCASE Inc. in 1999. Permission to remodel the training set in SciQSAR 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

Chinese Hamster (Chinese Hamster Ovary (CHO) cells).

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 typhimurium* mutagenicity assay.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 aberrations and related events are the cause of many human genetic diseases and there is substantial evidence that chromosome aberrations and related events causing alterations in oncogenes and tumor suppressor genes of somatic cells are involved in cancer induction in humans and experimental animals. Chromosome aberration *in vitro* tests have been used as an effective screen for chemicals which may have mutagenic, teratogenic, or tumorigenic potential. 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 and there is increasing evidence that there are carcinogens that are not detected by this test because they appear to act through mechanisms other than direct DNA damage (i.e., non-genotoxic carcinogens). I 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).
In the fact sheet for the A61 model (personal communication with MultiCASE in 2001), MultiCASE Inc. refer to two publications of the MultiCASE A61 model (Rosenkranz *et al.* 1990, Liu *et al.*1997). The compilation of the training set has been described by Rosenkranz *et al.*(1990) and the training set consists of chromosome aberration results from US National Toxicology Program (NTP) (Galloway *et al.*1985, 1987, Gulati *et al.*1989, Loveday *et al.*1989).

3.4 Endpoint units

No units, 1 for positives and 0 for negatives.

3.5 Dependent variable

Chromosome aberrations in Chinese Hamster Ovary cells, positive or negative.

3.6 Experimental protocol

The training set data were compiled by MultiCASE Inc. from US NTP and consist of results from the chromosome aberration test in Chinese Ovary Cells. The experimental protocol for this test is described in OECD guideline 473 (1997). Briefly, the cell cultures (a variety of cell lines can be used, e.g. CHO cells) are exposed to the test substance both with and without metabolic activation. At predetermined intervals after exposure of cell cultures to the test substance, they are treated with a metaphase-arresting substance, harvested, stained and metaphase cells are analysed microscopically for the presence of chromosome aberrations.

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. NTP).

4. Defining the algorithm

4.1 Type of model

This is a categorical (Q)SAR model based on calculated molecular descriptors, and if available the modeller's own or third-party descriptors or measured endpoints can be imported and used as descriptors.

4.2 Explicit algorithm

This is a categorical (Q)SAR model made by use of parametric discriminant analysis to create a linear discriminant function (see 4.5). The specific implementation is proprietary within the SciQSAR software.

4.3 Descriptors in the model

Molecular connectivity indices

Molecular shape indices

Topological indices

Electrotopological (Atom E and HE-States) indices

Electrotopological bond types indices

SciQSAR software provides over 400 built-in molecular descriptors. Additionally, SciQSAR makes it possible to import the modeller's own or third-party descriptors or use measured endpoints as custom descriptors.

4.4 Descriptor selection

The initial descriptor set is manually chosen by the model developer from the total set of built-in descriptors. Furthermore, the set of descriptors applied in the modelling by the program is on top of this selection determined by thresholds for descriptor variance and number of nonzero values likewise defined by the model developer.

57 descriptors were selected from the initial pool of descriptors by the system and used to build the model.

4.5 Algorithm and descriptor generation

For a binary classification problem SciQSAR uses discriminant analysis (DA) to make a (Q)SAR model. SciQSAR implements a broad range of discriminant analysis (DA) methods including parametric and non-parametric approaches. The classic parametric method of DA is applicable in the case of approximately

normal within-class distributions. The method generates either a linear discriminant function (the within-class covariance matrices are assumed to be equal) or a quadratic discriminant function (the within-class covariance matrices are assumed to be unequal). When the distribution is assumed to not follow a particular law or is assumed to be other than the multivariate normal distribution, non-parametric DA methods can be used to derive classification criteria. The non-parametric DA methods available within SciQSAR include the kernel and *k*-nearest-neighbor (kNN) methods. The main types of kernels implemented in SciQSAR include uniform, normal, Epanechnikov, bi-weight, or tri-weight kernels, which are used to estimate the group specific density at each observation. Either Mahalanobis or Euclidean distances can be used to determine proximity between compound-vectors in multidimensional descriptor space. When the kNN method is used, the Mahalanobis distances are based on the pooled covariance matrix. When the kernel method is used, the Mahalanobis distances are based on either the individual within-group covariance matrices or the pooled covariance matrix. (Contrera *et al*. 2004)

If the data outcome is continuous, regression analysis is used to build the predictive model. Within SciQSAR several regression methods are available: ordinary multiple regression (OMR), stepwise regression (SWR), all possible subsets regression (PSR), regression on principal components (PCR) and partial least squares regression (PLS). The choice of regression method depends on the number of independent variables and whether correlation or multicollinearity among the independent variables exists: OMR is acceptable with a small number of independent variables, which are not strongly correlated. SWR is used under the same circumstances as OMR but with greater number of variables. PSR is used for problems with a great number of independent variables. PCR and PLS are useful when a high correlation or multicollinearity exist among the independent variables. (SciQSAR 2009)

To test how stable the developed models are, SciQSAR have built-in cross-validation procedures (see 6.).

For this model, the quadratic method was used.

4.6 Software name and version for descriptor generation

SciQSAR version 3.1.00.

4.7 Descriptors/chemicals ratio

In this model 57 descriptors were used. The training set consists of 229 compounds. The descriptor/chemical ratio is 1:4 (57:229).

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

1. SciQSAR

The first criterion for a prediction to be within the models applicability domain is that all of the descriptor values for the test compound can be calculated by SciQSAR. If SciQSAR cannot calculate each descriptor value for the test chemical no prediction value is given by SciQSAR and it is considered outside the model's applicability domain.

2. The Danish QSAR group

The Danish QSAR group has applied a stricter definition of applicability domain for its SciQSAR models. In addition to the applicability domain definition made by SciQSAR a second criterion has been applied for predictions generated from (Q)SAR models with a binary endpoint. For each prediction SciQSAR calculates the probability (p) for the test compound's membership in one of the two outcome classes (positive or negative). The probability of membership in a class is a measure of how well training set knowledge is able to discriminate a positive prediction from a negative prediction within the nearest space of the subject compound-vector. The probability of membership value is also a measure of the degree of confidence of a prediction. The Danish QSAR group uses this probability for a prediction to further define the model's applicability domain. Only positive predictions with a probability equal to or greater than 0.7 and negative predictions with a probability equal to or less than 0.3 are accepted. Positive predictions with a probability between 0.5 and 0.7 as well as negative predictions with a probability between 0.3 and 0.5 are considered outside the model's applicability domain. When these predictions are wed out the accuracy of the model in general increases at the expense of reduced model coverage. Furthermore, as SciQSAR does not define a structural domain, only predictions which were within either Leadscope structural domain (defined as at least one training set chemical within a Tanimoto distance of 0.7) or CASE Ultra structural domain (no unknown fragments for negatives and maximum 1 unknown fragment for positives) were defined as being inside the SciQSAR applicability domain.

5.2 Method used to assess the applicability domain

The system does not generate predictions if it cannot calculate each descriptor value for the test compound.

Only positive predictions with probability equal to or greater than 0.7 and negative predictions with probability equal to or less than 0.3 were accepted.

5.3 Software name and version for applicability domain assessment

SciQSAR version 3.1.00.

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 SciQSAR. 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

229 compounds are in the training set: 95 positives and 134 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

SciQSARs own internal performance test of the model gave the following Cooper's statistics for predictions within the applicability domain as defined by SciQSAR (i.e. the first criterion described in 5.1):

- Sensitivity (true positives / (true positives + false negatives)): 100%
- Specificity (true negatives / (true negatives + false positives)): 85.1%
- Concordance ((true positives + true negatives) / (true positives + true negatives + false positives + false negatives)): 91.3%

6.8 Robustness – Statistics obtained by leave-one-out cross-validation

Not performed.

6.9 Robustness – Statistics obtained by leave-many-out cross-validation

SciQSAR's own internal 10-fold cross-validation (10*10% out) procedure was used for predictions within the applicability domain as defined by SciQSAR (i.e. the first criterion described in 5.1). As the probability domain was not applied (i.e. the second criterion described in 5.2) the accuracy of the predictions when applying this domain can be expected to be higher than reflected in these cross-validation results. This gave the following Cooper's statistics:

- Sensitivity (true positives / (true positives + false negatives)): 50.5%
- Specificity (true negatives / (true negatives + false positives)): 84.3%
- Concordance ((true positives + true negatives) / (true positives + true negatives + false positives + false negatives)): 70.3%

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 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 SciQSAR software provides over 400 calculated physico–chemical, electrotopological E-state, connectivity and other molecular descriptors. The descriptors selected for the model 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 descriptors selected for the model may provide a 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 results for the chromosome aberration test in Chinese Hamster Ovary (CHO) cells *in vitro*.

9.2 Bibliography

Ishidate, M. Jr., Miura, K.F., and Sofuni, T. (1998) Chromosome aberration assays in genetic toxicology testing in vitro. *Mutation Research*, 404, 167–172.

Galloway, S.M., Bloom, A.D., Resnick, M., Margolin, B.H., Nakamura, F., Archer, P. and Zeiger, E. (1985) Development of a standard protocol for *in vitro* cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. *Environ. Mutagenesis,* 7, 1-51.

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C, Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B. and Zeiger, E. (1987) Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells. Evaluation of 108 chemicals. *Environ. Mol. Mutagenesis,* 10 (Suppl. 10), 1-175.

Gulati, D.K., Witt, K., Anderson, B., Zeiger, E. and Shelby, M.D. (1989) Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells *in vitro.* III: Results with 27 chemicals. *Environ. Mol. Mutagenesis*, 13, 133-193.

Liu, M., Grant, S.G., Macina, O.T., Klopman, G., and Rosenkranz, H.S. (1997) Structural and mechanistic bases for the induction of mitotic chromosomal loss and duplication ('malsegregation') in the yeast Saccharomyces cerevisiae: relevance to human carcinogenesis and developmental toxicology. *Mutation Research,* 374, 209-231.

Loveday, K.S., Lugo, M.H., Resnick, M.A., Anderson, B.E. and Zeiger, E. (1989) Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells in vitro: II. Results with 20 chemicals. *Environ. Mol. Mutagenesis,* 13, 60-94.

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.

Rosenkranz, H.S., Ennever, F.K., and Klopman, G. (1990) Relationship between carcinogenicity in rodents and the induction of sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells. *Mutagenesis,* 5, 559-571.

9.3 Supporting information