PHARM_BMUT

	Element	Explanation
1.	**QSAR identifier**	
1.1.	QSAR identifier (title)	PHARM_BMUT Statistical model for bacterial mutagenicity with extended pharma coverage version 1.9.2.0.20514.450
1.2	Other related models	GT1_BMUT Statistical model for bacterial mutagenicity as per OECD 471 test guidance version 1.9.2.0.15546.500
1.3.	Software coding the model	**Name** CASE Ultra Version 1.9.2.0 **URL** http://www.multicase.com/case-ultra **Description** QSAR based bioactivity and toxicity prediction software. **Contact** sales@multicase.com, MultiCASE Inc, 5885 Landerbrook Dr. #210 Mayfield Heights, OH 44124 USA www.multicase.com
2.	**General information**	
2.0	Abstract	PHARM_BMUT is a statistical model for predicting bacterial mutagenicity (Ames test). This model was trained using proprietary chemicals to increase coverage for pharmaceutical compounds. Once the model was built, the proprietary training chemical structures and related information were removed from the model's database. However, the extracted knowledge, including any identified alerts, remains in the model.
2.1.	Date of QMRF	June 13, 2022
2.2.	QMRF author(s) and contact details	**Name** Dr Roustem D Saiakhov **Affiliation** MultiCASE Inc., **Contact** +1-440-565-7221 **URL** www.multicase.com **Email** saiakhov@multicase.com **Name** Mounika Girireddy **Affiliation** MultiCASE Inc., **Contact** +1-440-565-7221 **URL** www.multicase.com **Email** girireddy@multicase.com
2.3.	Date of QMRF update(s)	October 10, 2024

2.4.	QMRF update(s)	Mounika Girireddy, entire document
2.5.	Model developer(s) and contact details	**Name** Model was constructed using the data, provided by the US Food and Drug Administration's Center for Drug Evaluation and Research and MultiCASE Inc Datasharing Consortium, as well as MultiCASE Ames mutagenicity database. **Affiliation** MultiCASE Inc., **Contact** +1-440-565-7221 **URL** www.multicase.com **Email** sales@multicase.com
2.6.	Date of model development and/or publication	The model was developed in 2019, updated in 2020 and 2024.
2.7.	Reference(s) to main scientific papers and/or software package	1. Chakravarti SK, Saiakhov RD, Klopman G. Optimizing predictive performance of CASE Ultra expert system models using the applicability domains of individual toxicity alerts. J Chem Inf Model. 2012 Oct 22;52(10):2609-18. 2. Chakravarti SK, Saiakhov RD. Computing similarity between structural environments of mutagenicity alerts. Mutagenesis. 2019 Mar 6;34(1):55-65. 3. Honma M, Kitazawa A, Cayley A, Williams RV, Barber C, Hanser T, Saiakhov R, Chakravarti S, Myatt GJ, Cross KP, Benfenati E, Raitano G, Mekenyan O, Petkov P, Bossa C, Benigni R, Battistelli CL, Giuliani A, Tcheremenskaia O, DeMeo C, Norinder U, Koga H, Jose C, Jeliazkova N, Kochev N, Paskaleva V, Yang C, Daga PR, Clark RD, Rathman J. Improvement of quantitative structure-activity relationship (QSAR) tools for predicting Ames mutagenicity: outcomes of the Ames/QSAR International Challenge Project. Mutagenesis. 2019 Mar 6;34(1):3-16. 4. Chakravarti SK, Saiakhov RD. MultiCASE Platform for In Silico Toxicology. https://pubmed.ncbi.nlm.nih.gov/35188644/
2.8.	Availability of information about the model	Model is commercial. Although the training set is not publicly available, Information about the non-proprietary training set chemicals, assay conditions and details, information about the alerts are available through CASE Ultra interface. For any other specific details contact: sales@multicase.com, MultiCASE Inc. 5885 Landerbrook Dr. #210 Mayfield Heights, OH 44124 USA. Phone: +1-440-565-7221.
2.9.	Availability of another QMRF for exactly the same model	None

3	**Defining the endpoint - OECD Principle 1: "A DEFINED ENDPOINT"**	**PRINCIPLE 1: "A DEFINED ENDPOINT". ENDPOINT refers to any physicochemical, biological, or environmental property/activity/effect that can be measured and therefore modelled. The intent of PRINCIPLE 1 (a (Q)SAR should be associated with a defined endpoint) is to ensure clarity in the endpoint being predicted by a given model, since a given endpoint could be determined by different experimental protocols and under different experimental conditions. It is therefore important to identify the experimental system and test conditions that is being modelled by the Q)SAR.**
3.1.	Species	Salmonella composite: Combination of results from the S. typhimurium histidine reversion gene mutation test using tester strains TA97, TA98, TA100, TA1535, TA1536, and TA1537 and E.Coli WP2 family of bacteria. See also OECD 471 guidance.
3.2.	Endpoint	**Group** Bacterial Mutagenicity **Name** Salmonella Mutagenicity; E Coli mutagenicity. **Protocol** As described in OECD 471.
3.3	Comment on endpoint	As per OECD 471 guideline: "The bacterial reverse mutation test uses amino-acid requiring at least five strains of Salmonella typhimurium and Escherichia coli to detect point mutations by base substitutions or frameshifts. The principle of this bacterial reverse mutation test is that it detects mutations which revert mutations present in the test strains and restore the functional capability of the bacteria to synthesize an essential amino acid. Suspensions of bacterial cells are exposed to the test substance (liquid or solid) in the presence and in the absence of an exogenous metabolic activation system. At least five different analysable concentrations of the test substance should be used. The recommended maximum test concentration for soluble non-cytotoxic substances is 5 mg/plate or 5 ml/plate. There are two methods: the plate incorporation method and the preincubation method. For both techniques, after two or three days of incubation at 37°C, revertant colonies are counted and compared to the number of spontaneous revertant colonies on solvent control plates." - Source OECD 471 guideline (https://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en).
3.4.	Endpoint units	Binary score
3.5.	Dependent variable	Overall Ames Positive (1) or Negative (0). The final calls were determined as a summary of all the strains used in the Ames test.
3.6.	Experimental protocol	As described in OECD 471.
3.7.	Endpoint data quality and variability	High quality curated data for Ames bacterial mutagenicity. Data were acquired from public domain sources and provided by the proprietary contributors. Negative data were accepted only if tested against all 5 types of strains as per OECD 471.

4	**Defining the algorithm - OECD Principle 2 : "AN UNAMBIGUOUS ALGORITHM"**	**PRINCIPLE 2: "AN UNAMBIGUOUS ALGORITHM". The (Q)SAR estimate of an endpoint is the result of applying an ALGORITHM to a set of structural parameters which describe the chemical structure. The intent of PRINCIPLE 2 (a (Q)SAR should be associated with an unambiguous algorithm) is to ensure transparency in the model algorithm that generates predictions of an endpoint from information on chemical structure and/or physicochemical properties. In this context, algorithm refers to any mathematical equation, decision rule or output approach.**
4.1.	Type of model	Model built using Statistical Machine Learning techniques.
4.2.	Explicit algorithm	**Definition** Logistic regression QSAR **Description** Training: Multiple Logistic Regression model with occurrence of Alerts and Deactivating Features as independent and overall Ames outcome as dependent variable. Prediction: Application of the logistic regression model using the identification of alerts and deactivating features in the query compounds.
4.3.	Descriptors in the model	**Name** Fragments **Units** Count **Description** Occurrence of molecular fragment-based Alerts and Deactivating Features as independent and overall Ames outcome as dependent variable.
4.4.	Descriptor selection	An initial pool of approximately 20000 Molecular fragment-based descriptors were subjected to a descriptor selection process which picks up the fragments with positive and negative contributions so as to give the best predictive ability to the whole model. The final model contains 1276 fragments with positive contribution and 1532 fragments with negative contribution.
4.5.	Algorithm and descriptor generation	Descriptors for this model are molecular fragments which are generated from splitting the training set compounds systematically and creating a dictionary of unique fragments. After selecting a few most relevant fragments, a statistical logistic regression data fitting was applied between the X and Y variables to give the final model.
4.6.	Software name and version for descriptor generation	**Name** CASE Ultra Version 1.9.2.0 **URL** http://www.multicase.com/case-ultra **Description** QSAR based bioactivity and toxicity prediction software **Contact** sales@multicase.com, MultiCASE Inc, 5885 Landerbrook Dr. #210 Mayfield Heights, OH 44124 USA www.multicase.com
4.7.	Chemicals/Descriptors ratio	Number of Chemicals = 20514 (7498 positives/13016 negatives) Number of Descriptors = 2808

5	**Defining the applicability domain - OECD Principle 3: "A DEFINED DOMAIN OF APPLICABILITY"**	**PRINCIPLE 3: "A DEFINED DOMAIN OF APPLICABILITY". APPLICABILITY DOMAIN refers to the response and chemical structure space in which the model makes predictions with a given reliability. Ideally the applicability domain should express the structural, physicochemical and response space of the model. The CHEMICAL STRUCTURE (x variable) space can be expressed by information on physicochemical properties and/or structural fragments. The RESPONSE (y variable) can be any physicochemical, biological or environmental effect that is being predicted. According to PRINCIPLE 3 a (Q)SAR should be associated with a defined domain of applicability. Section 5 can be repeated (e.g., 5.a, 5.b, 5.c, etc) as many times as necessary if more than one method has been used to assess the applicability domain.**
5.1.	Description of the applicability domain of the model	The applicability domain of the model is defined by fragment based chemical space defined by the training set chemicals and range in the computed prediction probabilities where the model has weakest differentiability.
5.2.	Method used to assess the applicability domain	The CASE Ultra program evaluates automatically whether a tested molecule is within the domain of applicability of the model it is tested with. A combination of two criteria were used: 1. Checking for 3-atom fragments that are not present in the training chemicals, and 2. Calculated prediction probabilities that fall between 0.40 - 0.50 where the model has weakest differentiability.
5.3.	Software name and version for applicability domain assessment	**Name** CASE Ultra Version 1.9.2.0 **URL** http://www.multicase.com/case-ultra **Description** QSAR expert system for in-silico prediction of toxicity and bioactivity of chemicals. **Contact** sales@multicase.com, MultiCASE Inc, 5885 Landerbrook Dr. #210 Mayfield Heights, OH 44124 USA www.multicase.com
5.4.	Limits of applicability	Inorganic compounds, mixtures and large biomolecules are not covered.
6	**Defining goodness-of-fit and robustness (internal validation) – OECD Principle 4: "APPROPRIATE MEASURES OF GOODNESS-OF-FIT, ROBUSTENESS AND PREDICTIVITY"**	**PRINCIPLE 4: "APPROPRIATE MEASURES OF GOODNESS-OF-FIT, ROBUSTENESS AND PREDICTIVITY". PRINCIPLE 4 expresses the need to perform validation to establish the performance of the model. GOODNESS-OF-FIT and ROBUSTNESS refer to the internal model performance.**
6.1.	Availability of the training set	Training set data and associated references can be seen in CASE Ultra interface under "Display Training Set Chemicals".
6.2.	Available information for the training set	a) Chemical names (common names and/or IUPAC names) b) CAS c) SMILES d) Mol
6.3.	Data for each descriptor variable for the training set	Some data available.

6.4.	Data for the dependent variable for the training set	Some data available.
6.5.	Other information about the training set	Within CASE Ultra interface, all the alerts are supported by the training chemicals that are not proprietary. Every alert is supported by statistical details. Majority of the training set chemicals' Ames outcome, particularly the non-proprietary ones, are explained with assay conditions, strain information, scientific publications etc.
6.6.	Pre-processing of data before modelling	As published in Chakravarti SK, Saiakhov RD. Computing similarity between structural environments of mutagenicity alerts. Mutagenesis. 2019 Mar 6;34(1):55-65.
6.7.	Statistics for goodness-of-fit	Sensitivity 92.7% Specificity 97.9% Positive predictivity 96.1% Negative predictivity 96.0%, Coverage 94.0% AUC 0.994, Self-validation, Classification cut-off 0.45
6.8.	Robustness - Statistics obtained by leave-one-out cross-validation	Not performed.
6.9.	Robustness - Statistics obtained by leave-many-out cross-validation	Sensitivity 89.0% Specificity 95.8% Positive predictivity 92.3% Negative predictivity 93.9%, Coverage 91.3% AUC 0.983, 10 iterations, 10% off Classification cut-off 0.45
6.10.	Robustness - Statistics obtained by Y-scrambling	Sensitivity 13.8% Specificity 84.1% Positive predictivity 33.4% Negative predictivity 62.8%, Coverage 51.9% AUC 0.492, 10 iterations, 10% off Classification cut-off 0.45
6.11.	Robustness - Statistics obtained by bootstrap	Sensitivity 88.5% Specificity 96.0% Positive predictivity 92.6% Negative predictivity 93.6%, Coverage 90.8% AUC 0.983, 10 iterations, 10% off Classification cut-off 0.45
6.12.	Robustness - Statistics obtained by other methods	Not performed.

7	**Defining predictivity (external validation) – OECD Principle 4: "APPROPRIATE MEASURES OF GOODNESS-OF-FIT, ROBUSTENESS AND PREDICTIVITY"**	**PRINCIPLE 4: "APPROPRIATE MEASURES OF GOODNESS-OF-FIT, ROBUSTENESS AND PREDICTIVITY". PRINCIPLE 4 expresses the need to perform validation to establish the performance of the model. PREDICTIVITY refers to the external model validation. Section 7 can be repeated (e.g., 7.a, 7.b, 7.c, etc) as many times as necessary if more validation studies need to be reported in the QMRF.**
7.1.	Availability of the external validation set	Not available.
7.2.	Available information for the external validation set	Not available.
7.3.	Data for each descriptor variable for the external validation set	Some data available.
7.4.	Data for the dependent variable for the external validation set	Some data available.
7.5.	Other information about the external validation set	Not available.
7.6.	Experimental design of test set	The test set contains 1323 chemicals (308 Positives, 1015 Negatives) randomly taken out from the internal mutagenicity database.
7.7.	Predictivity - Statistics obtained by external validation	Sensitivity 83.0% Specificity 83.0% Positive predictivity 91.0% Negative predictivity 86.0%, Coverage 92.0% AUC 0.9333, Classification cut-off 0.45
7.8.	Predictivity - Assessment of the external validation set	This external validation set is well balanced and sufficiently represents the structural domain.
7.9.	Comments on the external validation of the model	Not available.
8	**Providing a mechanistic interpretation - OECD Principle 5: "A MECHANISTIC INTERPRETATION, IF POSSIBLE"**	**PRINCIPLE 5: "A MECHANISTIC INTERPRETATION, IF POSSIBLE". According to PRINCIPLE 5, a (Q)SAR should be associated with a mechanistic interpretation, if possible.**
8.1.	Mechanistic basis of the model	CASE Ultra models do not have any predefined knowledge of molecular mechanism that explains the activity of a molecule. However, the way the modules were built, splitting the entire learning set into clusters of molecules with a dedicated QSAR in every cluster, suggests very close links with a mechanistic explanation of activity. Indeed, many of the resulting biophores have modes of action that are obvious to persons with expert knowledge for the endpoint in question. For example, the presence of an alert containing N-nitroso fragment in bacterial mutagenicity model will undoubtedly suggest potential mutagenicity activity. Other fragments, which do not have such a clear mechanism of action assigned to them, can support an intelligent guess about possible sets of events causing activity. Either way, it is certain that the results of a MultiCASE analysis can serve as a mechanistic research tool as well as a QSAR builder. When Pharm_BMUT models is complimented by a rule-based GT_EXPERT

		system, the mechanistical interpretation of every discovered alert in comparison with a corresponding expert rule can be greatly simplified.
8.2.	A priori or a posteriori mechanistic interpretation	Posteriory mechanistic interpretation is simplified when a matching expert rule from GT_EXPERT system is reviewed.
8.3.	Other information about the mechanistic interpretation	None.
9	**Miscellaneous information**	
9.1.	Comments	Model is compatible with all 1.9.x.x versions of CASE Ultra.
9.2.	Bibliography	1. Optimizing predictive performance of CASE Ultra expert system models using the applicability domains of individual toxicity alerts; Chakravarti, S.K., Saiakhov, R.D. and Klopman, G., Journal of Chemical Information and Modeling, 2012, 52, 2609-2618. DOI: 10.1021/ci300111r. 2. Effectiveness of CASE Ultra Expert System in Evaluating Adverse Effects of Drugs; Saiakhov, R.D., Chakravarti, S.K. and Klopman, G.; Molecular Informatics, 2012, 32, 87-97. DOI : 10.1002/minf.201200081. 3. Computing similarity between structural environments of mutagenicity alerts, Chakravarti, S.K.,Saiakhov, R. D.; Mutagenesis,October20,2018,DOI: https://doi.org/10.1093/mutage/gey032
9.3	Supporting information	Not available.