(Q)SAR Model Reporting Format (QMRF)

(The present QMRF v.2.1 is prepared in accordance with (Q)SAR Assessment Framework (QAF) document developed by OECD)

(https://one.oecd.org/document/ENV/CBC/MONO(2023)32/ANN1/en/pdf**)**

Welcome

Model version: Ames mutagenicity v.20.20
Platform version: OASIS TIMES 2.33.1
Name: Ames mutagenicity
Author: LMC, University "Prof. As. Zlatarov", Bourgas, Bulgaria
Date: 31 March 2024
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www: http://www.oasis-lmc.org/

Section 1. QSAR identifier

1.1. QSAR identifier (title)

In vitro Ames Mutagenicity with S9 metabolic activation v.20.20

1.2. Other related models

Not applicable

1.3. Software coding the model

Model version: Ames mutagenicity v.20.20
Platform version: OASIS TIMES 2.33.1
Name: *In vitro* Ames Mutagenicity with S9 metabolic activation
Developer: LMC, University "Prof. As. Zlatarov", Bourgas, Bulgaria
Coding language: Delphi 10.2

Section 2. General information

2.0. Abstract

The in vitro Ames model identifies chemicals which are able to elicit mutagenicity as a result of interactions with DNA. Detected are point mutations including substitution, addition or deletion of one or a few DNA base pairs in Salmonella typhimurium and E. coli [1].

The TIMES system integrates in a same modelling platform metabolic simulation of chemicals and their interaction with target macromolecules [2]. The Ames mutagenicity model combines the alerting group approach with a pattern recognition type of model to delineate reactivity of chemicals toward DNA within a given interaction mechanism. The mechanistic interrelation between alerts and related parametric ranges generalizing the

effect of the rest of the molecules on the alert is also considered. The explicit generation of metabolites allows the DNA reactivity model to address simultaneously mutagenicity of parents and their activated metabolites. The in vitro S9 mix metabolic simulator which is associated with the model is trained to reproduce 441 documented metabolic maps for mammalian liver metabolism. Parent chemicals and each of the generated metabolites are submitted to a battery of models to screen for a general effect and mutagenicity mechanisms. Thus, chemicals are predicted to be mutagenic as parents only, parents and metabolites, and metabolites only.

The training set consists of 4268 chemicals with experimental Ames data separated in two groups: 2092 positive as parents and after S9 metabolic activation (302 are proprietary), and 2176 negative as parents and after S9 metabolic activation (1428 are proprietary). Chemicals with proprietary data are used for deriving alert boundaries and estimating performance of the model (and its domain) but are not disclosed for public.

2.1. Date of QMRF

31 March 2024

2.2.QMRF author(s) and contact details

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2.3. Date of QMRF update(s)

20 November 2014; 12 June 2015, 11 May 2016; 12 July 2016; 31 August 2016; 26 May 2017; 20 July 2018; 22 Jan 2020; 23 Nov 2021; 20 March 2023; 31 March 2024

2.4. QMRF update(s)

Information which has been modified:
Sections 1.1 QSAR identifier (title)**; Sections 1.3** Software coding the model; **Section 2.** General information; **Sections 2.0** Abstract; **Sections 2.1** Date of QMRF; **Sections 2.3** Date of QMRF update(s); **Sections 2.6** Date of model development and/or publications; **Sections 2.7** Reference(s) to the main scientific and/or software package; **Sections 2.8.** Availability of information about the model; **Sections 3.3** Comment on endpoint; **Section 3.6** Experimental protocol; **Section 3.7.** Endpoint data quality and variability; **Section 4.2.** Explicit algorithm; **Section 4.4.** Descriptor section**; Section 4.6.** Software name and version for descriptor generation; **Section 5.3.** Software name and version for the applicability domain assessment; **Section 5.4.** Limits of applicability; **Section 6.1** Availability of the training set; **Section 6.4** Data for the dependent variable for the training set; **Section 6.7** Statistics for goodness-of-fit; **Section 6.9** Robustness - Statistics obtained

by leave-many-out cross-validation; **Section 6.11** Robustness - Statistics obtained by bootstrap; Section **6.13** Comment on the internal validation of the model;

2.5. Model developer(s) and contact details

Name: P. Petkov, A. Chapkanov, C. Kuseva, H. Ivanova, E. Kaloyanova, G. Dimitrova, D. Yordanova, R. Serafimova, M. Todorov, T. Pavlov, S. Kotov, E. Jacob, A. Aptula, O. Mekenyan
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2.6. Date of model development and/or publication

Date of the model development: 2006/2012

2.7. Reference(s) to the main scientific and/or software package

1. B. Ames, J., McCann, E., Yamasaki, Methods for Detacting Carcinogens and Mutagens with the Salmonella/Mammalian-Microsome Mutagenicity Test. 1975. *Mutation Res*., Vol. 31, 347-364.
2. R. Serafimova, M. Todorov, T. Pavlov, S. Kotov, E. Jacob, A. Aptula, O. Mekenyan. 2007. Identification of the structural requirement for mutagenicity by incorporating molecular flexibility and metabolic activation of chemicals. II. General Ames mutagenicity model. *Chem. Res. Toxicol*., 662-676.
3. O. Mekenyan, S. Dimitrov, T. Pavlov, G. Dimitrova, M. Todorov, P. Petkov & S. Kotov. 2012. Simulation of chemical metabolism for fate and hazard assessment. V. Mammalian hazard assessment, *SAR and QSAR in Environmental Research*, Vol. 23, 553-606.
4. S. Dimitrov, G. Dimitrova, T. Pavlov, N. Dimitrova, G. Patlewicz, J. Niemela and O. Mekenyan, 2005. *J. Chem. Inf. Model*. Vol. 45, 839-849.

2.8. Availability of information about the model

In vitro Ames Mutagenicity with S9 metabolic activation model is proprietary and its use is subject of licence agreement.
Information that cannot be disclosed:

- External validation sets,
- Proprietary chemicals,
- Source code.

For more details, please contact Professor Ovanes Mekenyan: omekenya@btu.bg
Details of the model is provided in the sections bellow as well as in the following link:
http://oasis-lmc.org/products/models/human-health-endpoints/mutagenicity-(ames).aspx

2.9. Availability of another QMRF for exactly the same model

Not available.

Section 3. Defining the endpoint – OECD Principle 1

3.1. Species

Chemicals included in the training set of the TIMES Ames model are collected according to the recommendation in the OECD technical guideline 471 addressing the number of *Salmonella typhimurium* strains (TA100, TA98, TA1535, TA1537 (TA97, TA97a), TA102) and/or *E. coli*, associated with each data:

- For negative effect, all five *Salmonella* strains must show simultaneously negative data as described in the corresponding OECD guideline for testing of chemicals: https://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en.
- For positive effect, positive data in a single *Salmonella* strain/ E. coli would be enough.

3.2. Endpoint

Bacterial Reverse Mutation Test

According to JRC pre-classification list of endpoints:
No. 207 QMRF Human Health Effects, QMRF 4.10 Mutagenicity.

3.3. Comment on endpoint

Mutagenic toxicity is the capacity of a substance to cause genetic mutations. This property is of high public concern because it has a close relationship with carcinogenicity and eventually reproductive toxicity: most of the mutagenic substances are suspected carcinogenic substance in case a genotoxic mechanism is considered. The Ames test - simple and inexpensive *in vitro* assay is the basic in vitro assay to detect mutagens. The Ames test detects single nucleotide base change, base insertion or deletion in different *Salmonella* strains. All *Salmonella* strains carry some type of defective (mutant) gene that prevents them from synthesizing the amino acid histidine. In the presence of mutagenic chemicals, the defective gene may be mutated back to the functional state allowing the bacterium to grow. The relevant test guideline covering this endpoint is OECD TG 471. The endpoint covers the DNA base-pair substitution and frameshift mutagenic mechanisms that are covered by the Ames tester strains: TA 1535, TA100, TA 98, and TA 1537 or TA97 or TA 97a. A part of the training set data additionally covers cross-linking mutagenic events measured by the inclusion of the E. coli WP2 or E. coli WP2 (pKM101) or TA 102 test strains. The endpoint is measured on the parent compound and the metabolites generated in vitro by the employed S9 mix of enzyme-induced rodent liver homogenates.

3.4. Endpoint units

Qualitative – positive/ negative

3.5. Dependent variable

Observed Mutagenicity with S9

3.6. Experimental protocol

OECD technical guideline 471: Bacterial Reverse Mutation Assay (e.g. Ames test). https://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en

3.7. Endpoint data quality and variability

References associated with each documented mutagenicity data (except for proprietary data) included in the training set of the model are provided in *Appendix 1*.

Section 4. Defining the algorithm – OECD Principle 2

4.1. Type of model

Structural alerts based model

4.2. Explicit algorithm

Prediction of Bacterial (Ames) mutagenicity is based on modelling of the two events deemed to be crucial for the effect – interaction of the chemicals with DNA and their activation as a result of liver S9 metabolism.
Ames mutagenicity predictions are obtained using an alerting group approach. Only alerts having clear interpretation of mechanisms leading to DNA mutagenicity are included in the model. To obtain predictions, a set of alerts (125) is applied on parents and each of the generated *in vitro* rat liver S9 metabolites. The *in vitro* S9 metabolic simulator is trained to reproduce documented maps for mammalian liver metabolism for 441 parents. Match of alerts either on parents or metabolites is sufficient for obtaining positive prediction. Chemicals are predicted to be mutagenic as parents only, parents and metabolites, or as metabolites only.
Details about the alerts included in the model are provided in the next sections.

4.3. Descriptors in the model

Descriptors in the model are structural alerts related to interactions with DNA. Alerts in the TIMES Ames model (+S9) constitute expertly-derived sets of structural fragments incorporating knowledge for the interactions of chemicals (parents and metabolites) with DNA. Application of the alerts on the training set of the model forms fractions of representative chemicals for the alerts, i.e. so-called ‘local’ training sets. All chemicals captured by the alerts are considered as validation sets of the introduced expert knowledge addressing reactivity of chemicals with DNA. The procedure for obtaining local training

sets includes applying the structural boundaries of the alert searching among all chemicals from the training set of the model after application of S9 metabolic simulator. According to this, local training sets contain parent chemicals in which general fragments are:

- found in their structures;
- not found in the parent structures but found in their metabolite(s).

Description of these alerts is provided in the next sections.

4.4. Descriptor selection

Table 1 summarizes the main characteristics of each DNA alert in TIMES Ames (+S9):

- Alert name (corresponding to the name of the chemical class which is addressed);
- Performance of alert (correct/incorrect predictions) which is estimated based on proportion of observed positive chemicals from all chemicals captured by the alert. Performance of each alert is provided with its confidence range. As smaller is the size of local training sets as wider are the confidence ranges and vice versa.
- P-values addressing the reliability of alert performance estimation and taking into account possible bias of positive/negative chemicals in the training set of the model. Low p-values could be obtained only if both are satisfied:
  - The number of chemicals in local training set is high enough;
  - The alert performance is significantly higher than the proportion of positive/negative chemicals in the model training set, i.e. so-called naïve alert.

Analogically, high p-values could be obtained in case of:

- Small number of local training set chemicals (1-2 chemicals); or
- Performance comparable to the performance of the naïve alert.

High performance associated with low *p-values* indicate for High Reliability of alerts. The above statistical measures along with the underlying mathematical formalisms are discussed in details in **Section 6** (Internal validation).

Table 1. Main characteristics of the DNA alerts in the TIMES Ames model (+S9).

No.	Alert name	Correct	Incorrect	Performance	p-value
1	Fused-Ring Nitroaromatics	61	0	0.984 (0.953 ÷ 1.000)	< 1.0E-10
2	Polynitroarenes	48	0	0.980 (0.941 ÷ 1.000)	< 1.0E-10
3	Nitrogen and Sulfur Mustards	41	0	0.977 (0.931 ÷ 1.000)	< 1.0E-10
4	Diazenes	40	0	0.976 (0.930 ÷ 1.000)	< 1.0E-10
5	Nitroarenes with Other Active Groups	36	0	0.974 (0.922 ÷ 1.000)	< 1.0E-10
6	Sulfonates and Sulfates	72	1	0.973 (0.937 ÷ 0.999)	< 1.0E-10

7	N-Nitroso Compounds	35	0	0.973 (0.920 ÷ 1.000)	< 1.0E-10
8	Heterocyclic nitro compounds	32	0	0.971 (0.913 ÷ 1.000)	1.4E-10
9	Nitrobiphenyls and Bridged Nitrobiphenyls	32	0	0.971 (0.913 ÷ 1.000)	1.4E-10
10	N-Acyloxy(Alkoxy) Arenamides	30	0	0.969 (0.908 ÷ 1.000)	5.7E-10
11	Aminoacridine DNA Intercalators	28	0	0.967 (0.902 ÷ 1.000)	2.3E-9
12	N-Nitrosamines	27	0	0.966 (0.899 ÷ 1.000)	4.7E-9
13	N-Acetoxyamines	26	0	0.964 (0.895 ÷ 1.000)	9.6E-9
14	Acyl Halides	25	0	0.963 (0.891 ÷ 1.000)	2.0E-8
15	Haloalkane Derivatives Containing Chain Heteroatom	100	3	0.962 (0.925 ÷ 0.993)	< 1.0E-10
16	DNA Intercalators with Carboxamide and Aminoalkylamine Side Chain	22	0	0.958 (0.878 ÷ 1.000)	1.6E-7
17	Halofuranones	19	0	0.952 (0.861 ÷ 1.000)	1.4E-6
18	p-Substituted Mononitrobenzenes	38	1	0.951 (0.886 ÷ 0.999)	< 1.0E-10
19	Hydrazine Derivatives	93	4	0.949 (0.906 ÷ 0.987)	< 1.0E-10
20	Conjugated Nitroalkenes and Five-Membered Nitro- and Amino Heterocycles	55	2	0.949 (0.893 ÷ 0.994)	< 1.0E-10
21	Acridone, Thioxanthone, Xanthone, Phenazine and Other Fused-Ring Heterocyclic DNA Intercalators	35	1	0.947 (0.877 ÷ 0.999)	3.1E-10
22	Quinolone Derivatives	17	0	0.947 (0.847 ÷ 1.000)	5.6E-6
23	Organic Peroxy Compounds	33	1	0.944 (0.871 ÷ 0.999)	1.2E-9
24	Alkylphosphates, Alkylthiophosphates and Alkylphosphonates	16	0	0.944 (0.838 ÷ 1.000)	1.1E-5
25	Haloalkane Derivatives with Labile Halogen	180	10	0.943 (0.909 ÷ 0.973)	< 1.0E-10

26	Haloalkene Derivatives with Electron-Withdrawing Groups	30	1	0.939 (0.859 ÷ 0.998)	9.2E-9
27	Nitroazoarenes and p-Monosubstituted Azobenzene Derivatives	59	3	0.938 (0.878 ÷ 0.988)	< 1.0E-10
28	Acyclic Triazenes	14	0	0.938 (0.819 ÷ 1.000)	4.7E-5
29	C-Nitroso Compounds	299	20	0.935 (0.907 ÷ 0.960)	< 1.0E-10
30	N-Hydroxylamines	521	36	0.934 (0.913 ÷ 0.954)	< 1.0E-10
31	Specific Imine and Thione Derivatives	26	1	0.931 (0.841 ÷ 0.998)	1.4E-7
32	Nitrophenols, Nitrophenyl Ethers and Nitrobenzoic Acids	79	5	0.930 (0.876 ÷ 0.978)	< 1.0E-10
33	Geminal Polyhaloalkane Derivatives	52	3	0.930 (0.864 ÷ 0.986)	< 1.0E-10
34	Benzanthrone Derivatives	11	0	0.923 (0.779 ÷ 1.000)	0.0004
35	Diazoalkanes	11	0	0.923 (0.779 ÷ 1.000)	0.0004
36	Fused-Ring Primary Aromatic Amines	69	5	0.921 (0.860 ÷ 0.975)	< 1.0E-10
37	Polarized Haloalkene Derivatives	45	3	0.920 (0.845 ÷ 0.984)	< 1.0E-10
38	p-Aminobiphenyl Analogs	21	1	0.917 (0.809 ÷ 0.998)	3.9E-6
39	Alkylnitrites	10	0	0.917 (0.762 ÷ 1.000)	0.0008
40	Hydroxamic acid	10	0	0.917 (0.762 ÷ 1.000)	0.0008
41	Epoxides, Aziridines, Thiiranes and Oxetanes	185	16	0.916 (0.878 ÷ 0.953)	< 1.0E-10
42	Quinoneimine, Thionine and Phenoxazinium Derivatives	30	2	0.912 (0.818 ÷ 0.990)	7.7E-8
43	Polycyclic Aromatic Hydrocarbon, Naphthaleneimide and Carbazole Derivatives	29	2	0.909 (0.812 ÷ 0.989)	1.5E-7
44	Four-and Five-Membered Lactones	9	0	0.909 (0.741 ÷ 1.000)	0.0016
45	Heterocyclic N-Hydroxylamines	37	3	0.905 (0.816 ÷ 0.981)	5.7E-9

46	Coumarins and Thiocoumarins	8	0	0.900 (0.717 ÷ 1.000)	0.0034
47	N,N-Dialkyldithiocarbamate Derivatives and Azaarene Dithiocarbamates	8	0	0.900 (0.717 ÷ 1.000)	0.0034
48	Organic Azides	8	0	0.900 (0.717 ÷ 1.000)	0.0034
49	Quinones and Trihydroxybenzenes	69	7	0.897 (0.830 ÷ 0.959)	< 1.0E-10
50	Alpha-Haloethers	16	1	0.895 (0.761 ÷ 0.997)	0.0001
51	Amino Anthraquinones	23	2	0.889 (0.772 ÷ 0.986)	6.7E-6
52	Quinone Methides	7	0	0.889 (0.688 ÷ 1.000)	0.0068
53	Thiols	7	0	0.889 (0.688 ÷ 1.000)	0.0068
54	Haloalcohols	55	7	0.875 (0.794 ÷ 0.950)	< 1.0E-10
55	Flavonoids	6	0	0.875 (0.652 ÷ 1.000)	0.013
56	Haloalkene Cysteine S-Conjugates	6	0	0.875 (0.652 ÷ 1.000)	0.013
57	N-Aryl-N-Acetoxy(Benzoyloxy) Acetamides	6	0	0.875 (0.652 ÷ 1.000)	0.013
58	Substituted Benzoindoline and Indole Derivatives	6	0	0.875 (0.652 ÷ 1.000)	0.013
59	Substituted Chlorophenylalkylurea Derivatives	6	0	0.875 (0.652 ÷ 1.000)	0.013
60	Single-ring Substituted Primary Aromatic Amines	288	41	0.873 (0.837 ÷ 0.908)	< 1.0E-10
61	Formaldehyde Releasers	12	1	0.867 (0.701 ÷ 0.996)	0.0014
62	Nitroaniline Derivatives	123	19	0.861 (0.804 ÷ 0.915)	< 1.0E-10
63	N-methylol derivatives	11	1	0.857 (0.681 ÷ 0.995)	0.0026
64	4,4’-Bipyridinium Salts and N-Oxides	5	0	0.857 (0.607 ÷ 1.000)	0.028
65	Chlorinated Diphenylmethane and Benzophenone Derivatives	5	0	0.857 (0.607 ÷ 1.000)	0.028
66	Pyrrolizidine derivatives	5	0	0.857 (0.607 ÷ 1.000)	0.028

67	Substituted Nitropyridines, Aminopyridines and N-Oxides	5	0	0.857 (0.607 ÷ 1.000)	0.028
68	Vicinal Dihaloalkanes	15	2	0.842 (0.682 ÷ 0.979)	0.0009
69	Arenediazonium and Diazonium Salts	9	1	0.833 (0.632 ÷ 0.994)	0.0092
70	Specific Acetate Esters	9	1	0.833 (0.632 ÷ 0.994)	0.0092
71	Alpha-Beta Conjugated Alkene Derivatives with Geminal Electron-Withdrawing Groups	4	0	0.833 (0.549 ÷ 1.000)	0.057
72	Sultones	4	0	0.833 (0.549 ÷ 1.000)	0.057
73	Tri-Methylindole derivatives	4	0	0.833 (0.549 ÷ 1.000)	0.057
74	Alpha,Beta-Unsaturated Aldehydes	31	6	0.821 (0.700 ÷ 0.931)	1.3E-5
75	Triarylimidazole and Structurally Related DNA Intercalators	8	1	0.818 (0.602 ÷ 0.993)	0.017
76	Haloazaarene and Fused-Ring Haloquinoline Derivatives	12	2	0.813 (0.627 ÷ 0.973)	0.0053
77	Quinoline Derivatives	15	3	0.800 (0.628 ÷ 0.954)	0.0030
78	Monohaloalkanes	7	1	0.800 (0.567 ÷ 0.991)	0.031
79	Anthrones	3	0	0.800 (0.473 ÷ 1.000)	0.11
80	Benzofuranyl carbamate derivatives	3	0	0.800 (0.473 ÷ 1.000)	0.11
81	Non-Aromatic Hydroxylamine Derivatives	3	0	0.800 (0.473 ÷ 1.000)	0.11
82	Quinoxaline-Type 1,4-Dioxides	3	0	0.800 (0.473 ÷ 1.000)	0.11
83	Specific 5-Substituted Uracil Derivatives	3	0	0.800 (0.473 ÷ 1.000)	0.11
84	Sulfonyl Halides	3	0	0.800 (0.473 ÷ 1.000)	0.11
85	Dicarbonyl Compounds	46	11	0.797 (0.693 ÷ 0.894)	9.4E-7
86	Heterocyclic Nitroso compounds	13	3	0.778 (0.590 ÷ 0.947)	0.0087
87	Aminophenoxazinone derivative	2	0	0.750 (0.368 ÷ 1.000)	0.24

88	Benzoyl cyclohexanedione derivatives	2	0	0.750 (0.368 ÷ 1.000)	0.24
89	Bleomycin and Structurally Related Chemicals	2	0	0.750 (0.368 ÷ 1.000)	0.24
90	Dichlorophosphine and Dichlorophosphonium Derivatives	2	0	0.750 (0.368 ÷ 1.000)	0.24
91	Fused-Ring Conjugated Lactones	2	0	0.750 (0.368 ÷ 1.000)	0.24
92	Halogenated Oxetanes and Haloepoxides	2	0	0.750 (0.368 ÷ 1.000)	0.24
93	Hypoxanthine Derivatives	2	0	0.750 (0.368 ÷ 1.000)	0.24
94	N-Alkylindolinium and N-Alkylbenzothiazolium Salts	2	0	0.750 (0.368 ÷ 1.000)	0.24
95	Polyethylene Polyamines	2	0	0.750 (0.368 ÷ 1.000)	0.24
96	Hydroxybenzophenone Derivatives	7	2	0.727 (0.478 ÷ 0.954)	0.080
97	Nitroalkanes	4	1	0.714 (0.409 ÷ 0.982)	0.17
98	Azoxyalkanes	3	1	0.667 (0.330 ÷ 0.974)	0.29
99	Trifluoromethyl pyridinone derivatives	3	1	0.667 (0.330 ÷ 0.974)	0.29
100	1,4-Diazabutadiene Derivatives	1	0	0.667 (0.224 ÷ 1.000)	0.49
101	Alkyl Xanthate Esters	1	0	0.667 (0.224 ÷ 1.000)	0.49
102	Amidoxime Esters and Amidoximes	1	0	0.667 (0.224 ÷ 1.000)	0.49
103	Antibiotic Aminoglycoside Derivatives	1	0	0.667 (0.224 ÷ 1.000)	0.49
104	Azoalkanes with Activating EWG	1	0	0.667 (0.224 ÷ 1.000)	0.49
105	Conjugated Benzoylethylene Derivatives with EWG	1	0	0.667 (0.224 ÷ 1.000)	0.49
106	Dithianes	1	0	0.667 (0.224 ÷ 1.000)	0.49
107	Fluoro bis-benzothiazole derivative	1	0	0.667 (0.224 ÷ 1.000)	0.49
108	Haloisothiazolinones	1	0	0.667 (0.224 ÷ 1.000)	0.49
109	Heterocyclic urea derevatives	1	0	0.667 (0.224 ÷ 1.000)	0.49

110	N-Hydroxyethyl Lactams	1	0	0.667 (0.224 ÷ 1.000)	0.49
111	Non-Cyclic Alkyl Phosphoramides and Thionophosphoramides	1	0	0.667 (0.224 ÷ 1.000)	0.49
112	Organic Diselenides and Ditellurides	1	0	0.667 (0.224 ÷ 1.000)	0.49
113	PAH Benzylic Alcohol Esters	1	0	0.667 (0.224 ÷ 1.000)	0.49
114	Perfluorinated Hypofluorites	1	0	0.667 (0.224 ÷ 1.000)	0.49
115	Peroxyacyl Nitrates	1	0	0.667 (0.224 ÷ 1.000)	0.49
116	S-Activated Cysteine Derivatives	1	0	0.667 (0.224 ÷ 1.000)	0.49
117	Short-Chain Alkyltin and Alkylgermanium Halides	1	0	0.667 (0.224 ÷ 1.000)	0.49
118	Tertiary Heterocyclic Amines	1	0	0.667 (0.224 ÷ 1.000)	0.49
119	Triflorometyl benzamide derivative	1	0	0.667 (0.224 ÷ 1.000)	0.49
120	Triazinone derivative	2	1	0.600 (0.228 ÷ 0.956)	0.48
121	Propyne Derivatives	3	2	0.571 (0.239 ÷ 0.895)	0.48
122	Aromatic ester hydroxylamine	0	0	N/A	N/A
123	N-Trihalomethyl Imides (Theoretical)	0	0	N/A	N/A
124	Thiadiazole-dioxide derivatives (Theoretical)	0	0	N/A	N/A
125	Thiazolidinediones (Theoretical)	0	0	N/A	N/A

1) Confidence ranges and p-value are calculated at 95% confidence level.

Detailed information for each alert such as structural boundaries, mechanisms, local training sets and references associated with each observed data is provided in ***Appendix 2***.

4.5. Algorithm and descriptor generation

The structural boundaries of the alerts are derived from the chemicals included in the local training sets (see Section 4.3). For derivation of each alert mechanistically justifiable structural fragments for interaction with DNA are identified from the chemicals having positive data in the local training set. Additional structural fragments from the other parts of the molecules which could affect (enhance or reduce) the mutagenicity effect are also introduced to complete definition of most alerts.

4.6. Software name and version for descriptor generation

TIMES, Ames mutagenicity model version 20.20

4.7. Chemicals/Descriptors ratio

Provided in Section 4.4.

Section 5. Defining the applicability domain of the model – OECD Principle 3

5.1. Description of the applicability domain of the model

The domain consists of the following sub-domain layers:

1. General parametric requirements.

The variations of molecular parameters that may affect the quality of the measured endpoint significantly are included here (such as molecular weight, etc.). The domain of general parametric includes the range of variation of hydrophobicity (log K_{OW}) and Molecular weight (MW) of chemicals in training set.

2. Structural domain.

The structural component of the model is based on the structural similarity between chemicals in the training set which were correctly predicted by the model. The structural neighborhood of atom-centered fragments (accounting for the first neighbours) extracted from correctly and incorrectly predicted parent structures from the training set is used to determine this similarity.

The target chemical could contain the following types of ACF:

- Fragments present in correctly predicted training chemicals only (i.e. correct fragments),
- Fragments found both in correctly and non-correctly predicted training chemicals (i.e. fuzzy fragments). These fragments are treated as correct fragments,
- Fragments present in non-correctly predicted training chemicals only (i.e. incorrect fragments),
- Fragments not present in the training chemicals (i.e. unknown fragments).

A chemical belongs to the structural domain of the model if it could be partitioned only on correct fragments. The user is able to analyse how important are unknown and incorrect fragments (if present in the target) and to make a decision about their effect on the quality of prediction. The distribution of structural characteristics of the target chemical and accepted thresholds is used as a criterion to determine how well the target is represented in the structural space of correctly predicted chemicals. The accepted domain thresholds for Mutagenicity are as follows:

- Correct = 100%
- Incorrect = 0%

A chemical is considered "In Domain" if it is classified to belong to all sub-domain levels. The information implemented in the applicability domain is extracted from the correctly predicted training chemicals used to build the model and in this respect the applicability domain determines practically the interpolation space of the model.

5.2. Method used to assess the applicability domain

The approach used to determine and assess the domain is described in:

Dimitrov S, Dimitrova G., Pavlov T., Dimitrova N., Patlewicz G., Niemela J., Mekenyan O., A stepwise approach for defining the applicability domain of SAR and QSAR models, *J. Chem. Inf. Model.*, 45, 839-849 (2005).

5.3. Software name and version for the applicability domain assessment

The LMC software OASIS Domain Manager v.1.13 (which is embedded in OASIS platform) is used to determine the applicability domain.
http://oasis-lmc.org/products/software/domain-manager.aspx

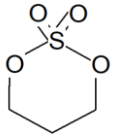
5.4. Limits of applicability

Applicability domain of the Ames model (+S9) include three sub-domain layers: general parametric requirements, structural features and alerts reliability.

- General properties requirements:

As described in the Section 5.1.1, parametric domain of the model is derived based on Log *Kow* and *MW*. Example demonstrating belonging of a training set chemical to the parametric layer of the model domain is provided below:

Example chemical:
- CAS: 1073-05-8
- Name: 1,3, 1,3,2-dioxathiane 2,2-dioxide
- 2D Depiction:



Property	Domain	Example chemical
log K_{OW}	[-18.86; 35.186]	-0.410
MW, Da	[31.024; 2368.505]	138.137

* K_{OW} is calculated by EPI Suite

The values of *logKow* and *MW* of the example chemical are within the ranges of these parameters extracted from the whole training set of the model. Hence, with respect to the general parametric requirements, the example chemical is estimated to be *In Domian*.

- Structural features
  Structural domain of the model is extracted from 4268 training chemicals contains:
  - 27 557 correct fragments,
  - 4 078 fuzzy fragments (treated as correct fragments),
  - 3 332 incorrect fragments.

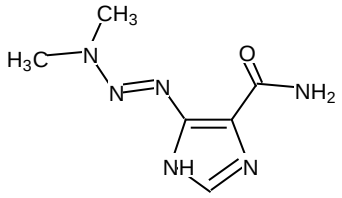
- Alerts reliability
  Reliability of alerts is estimated based on:
  - Alert performance of the local training set chemicals (AP);
  - Number of the local training sets (N);
  - Mechanistic justification (M).

According to these criteria, there are four reliability estimates for the alerts in the models:

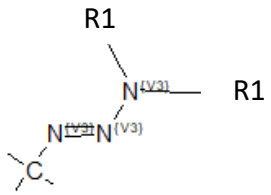
- High reliability alerts (AP>0.6, N>10, M);
- Low reliability alerts (AP<0.6, N>10, M);
- Undetermined alerts (N<10, M);
- Undetermined theoretical alerts (M).

Example chemical belonging to alert with “High reliability”.

Chemical ID:
CAS: 4342-03-4
Name: Dacarbazine
Depiction:



Belonging to alert:
Name: Acyclic Triazenes
Structural boundaries:



R_1 = H; CH_3; C_2H_5; $CH(CH_3)_2$; CH_2-C_6H_5

Reliability:
"High reliability" based on AP=1; N=14.

Currently, information for alerts reliability is provided in the model reports.

Section 6. Defining goodness-of-fit and robustness (internal validation) – OECD Principle 4

6.1. Availability of the training set

Training set of the TIMES Ames model (+S9) includes 4268 organic compounds from different chemical classes.

6.2. Available information for the training set

CAS numbers, Chemical names, SMILES, documented data, literature sources and strain information are available for each compound in the model training set.

6.3. Data for each descriptor variable for the training set

Descriptors in the models are structural alerts. The main characteristics of each alert are provided in Table 1 (Section 4.4).

6.4. Data for the dependent variable for the training set

The training set of 4268 chemicals includes:

- 2092 chemicals have positive observed Ames data
- 2176 chemicals have negative observed Ames chemicals.

Distribution of positive/negative chemicals in the training set of model is used for estimating performance and confidence range of the so-called *naïve alert* which is 0.490 (0.475 ÷ 0.505)[1)].

1) Confidence range is calculated at 95% confidence level

6.5. Other information about the training set

The training set is compiled according to the recommendations described in the OECD TG471 for availability of all five *Salmonella* strains (*E. coli*) for the Ames negative chemicals and at least one strain with positive data for the Ames positive chemicals.

6.6. Pre-processing of data before modelling

Not available

6.7. Statistics for goodness-of-fit

During the internal validation the original training set is separated many times randomly into two parts – one becomes a training set and the other becomes a test set. The model is re-derived many times using each new training set. Then, performance is estimated for the training sets and test sets. The averaged value of all training set performances is compared to the averaged value of all test set performances in order to assess the amount of optimism in the goodness-of-fit (GOF optimism) in the original model. GOF optimism is calculated as average performance over training sets minus average performance over test sets. Results are provided in Table 2.

Table 2. Performance of the original model over its training set (goodness-of-fit, GOF) vs. expected performance over set different from the training set (GOF – GOF optimism).

	Performance $_{est.}$ [1)] model	*p-value* [1)]	Performance [2) 3)] different set
All predictions (accuracy)	0.908 (0.899 ÷ 0.917)	$< 10^{-10}$	0.897
Positive chemicals (sensitivity)	0.895 (0.882 ÷ 0.908)	$< 10^{-10}$	0.866
Negative chemicals (specificity)	0.920 (0.909 ÷ 0.931)	$< 10^{-10}$	0.918

1) Confidence ranges and *p-value* are calculated at 95% confidence level.
2) Estimated performance for training set minus GOF optimism calculated from internal validation.
3) Estimation of expected performance over sets different from training sets.

Addition information including mathematical formalism underlying the above statistical measures are provided in ***Appendix 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

Method 1. *k-fold* cross-validation

In ***k-fold cross-validation*** the original training set is partitioned into *k* equally sized subsets. Each time a single subset is used as a test set and the remaining *k-1* subsets are used as training set. In this manner the process is repeated *k* times and each data from the

original training set is used once as a test data and *k-1* times as a training data. The advantage of this method is that any data is used for both training and validation and each data is used exactly once as a test data. Commonly the 10-fold cross-validation is used (90% training data, 10% test data). In addition, 4-fold cross validation (75% training data, 25% test data) is also performed and the results from both procedures are provided in Table 3.

Table 3. Results from *k-fold* (*10-fold* and *4-fold*) cross-validation.

	10-fold		**4-fold**	
	Training sets	**Test sets**	**Training sets**	**Test sets**
Unique chemicals, %	90.0 (90.0 ÷ 90.0)	10.0 (10.0 ÷ 10.0)	75.0 (75.0 ÷ 75.0)	25.0 (25.0 ÷ 25.0)
Performance $_{est.}$, all predictions (accuracy)	0.908 (0.900 ÷ 0.916)	0.893 (0.829 ÷ 0.957)	0.908 (0.894 ÷ 0.922)	0.889 (0.829 ÷ 0.950)
p-value, accuracy	$< 10^{-10}$	$< 10^{-10}$	$< 10^{-10}$	$< 10^{-10}$
Performance $_{est.}$, positive chemicals (sensitivity)	0.895 (0.879 ÷ 0.911)	0.832 (0.697 ÷ 0.968)	0.896 (0.872 ÷ 0.919)	0.852 (0.829 ÷ 0.876)
p-value, sensitivity	$< 10^{-10}$	$< 10^{-10}$	$< 10^{-10}$	1.8×10^{-10}
Performance $_{est.}$, negative chemicals (specificity)	0.920 (0.909 ÷ 0.931)	0.908 (0.3783÷ 1.033)	0.919 (0.897 ÷ 0.942)	0.924 (0.835 ÷ 1.013)
p-value, specificity	$< 10^{-10}$	5.7×10^{-8}	$< 10^{-10}$	1.8×10^{-10}

1) Confidence ranges and p-value are calculated at 95% confidence level

Method 2. *Monte Carlo* cross-validation

In ***Monte Carlo cross-validation*** the original training set is split randomly into training and test set. The advantage of this method (compared to *k-fold* cross validation) is that the proportion between training and test sets does not depend on the number of repetitions in the internal validation procedure. The ***Monte Carlo cross-validation*** (similarly to the *bootstrapping*) suppose creating a large number of new training/test sets (1000 – 10000). Results from application of this statistical method are provided in Table 5.

Table 4. Results from Monte Carlo cross-validation (1000 repetitions).

	75% training set	**63% training set**

	Training sets	Test sets	Training sets	Test sets
Unique chemicals, %	75.0 (75.0 ÷ 75.0)	25.0 (25.0 ÷ 25.0)	63.0 (63.0 ÷ 63.0)	37.0 (37.0 ÷ 37.0)
Performance $_{est.}$, all predictions (accuracy)	0.908 (0.903 ÷ 0.913)	0.902 (0.886 ÷ 0.917)	0.908 (0.901 ÷ 0.915)	0.901 (0.889 ÷ 0.913)
p-value, accuracy	$< 10^{-10}$	$< 10^{-10}$	$< 10^{-10}$	$< 10^{-10}$
Performance $_{est.}$, positive chemicals (sensitivity)	0.895 (0.887 ÷ 0.903)	0.882 (0.858 ÷ 0.907)	0.895 (0.884 ÷ 0.905)	0.881 (0.862 ÷ 0.900)
p-value, sensitivity	$< 10^{-10}$	$< 10^{-10}$	$< 10^{-10}$	$< 10^{-10}$
Performance $_{est.}$, negative chemicals (specificity)	0.920 (0.914 ÷ 0.927)	0.919 (0.899 ÷ 0.938)	0.920 (0.912 ÷ 0.929)	0.919 (0.905 ÷ 0.934)
p-value, specificity	$< 10^{-10}$	$< 10^{-10}$	$< 10^{-10}$	$< 10^{-10}$

1) Confidence ranges and *p-value* are calculated at 95% confidence level.

6.10. Robustness - Statistics obtained by Y-scrambling

Not performed

6.11. Robustness - Statistics obtained by bootstrap

In bootstrapping a newly derived training sets is populated from the original training set of the model by random sampling with replacement until the size of the new training set reaches the size of the original training set. The data not selected for the new training set becomes the new test set. On average, about 63% of original training set data goes into the new training set (some data appear more than once) and 37% remains in the new test set. One of the advantages of this method is that the new training sets and the original training set are equally sized. The process is repeated many times and the average results are provided in Table 5.

Table 5. Results from bootstrapping (1000 repetitions).

	Training sets	Test sets
Unique chemicals, %	63.2 (62.2 ÷ 64.2)	36.8 (35.8 ÷ 37.8)
Performance $_{est.}$, all predictions (accuracy)	0.908 (0.899 ÷ 0.917)	0.901 (0.890 ÷ 0.913)
p-value, accuracy	$< 10^{-10}$	$< 10^{-10}$

Performance $_{est.}$, positive chemicals (sensitivity)	0.895 (0.882 ÷ 0.908)	0.881 (0.864 ÷ 0.899)
p-value, sensitivity	$< 10^{-10}$	$< 10^{-10}$
Performance $_{est.}$, negative chemicals (specificity)	0.917 (0.909 ÷ 0.932)	0.920 (0.904 ÷ 0.935)
p-value [1)], Specificity	$< 10^{-10}$	$< 10^{-10}$

1) Confidence ranges and *p-value* are calculated at 95% confidence level.

6.12. Robustness - Statistics obtained by other methods

Not performed

6.13. Comment on the internal validation of the model

The first evident observation from above results is that averaged estimations are practically unchangeable, no matter what kind of sampling we use for the internal validation. The variability of averaged performances is 0.0033 and below even for test sets. Also their p-values are extremely low, which show very high reliability of these estimations.
The difference between performances of training and test sets - which is a measure for optimism in goodness-of-fit, - is around 0.006 for all predictions, 0.011 for positive chemicals (sensitivity) and 0.002 for negative chemicals (specificity). These values are very low and show that the model is very well balanced and provides high quality for both positive and negative chemicals. A similar quality is also expected for predictions of external chemicals.

Section 7. Defining predictivity (external validation) – OECD Principle 4

7.1. Availability of the external validation set

12140 external chemicals are available to examine performance of the model.

7.2. Available information for the external validation set

According to the OECD TG 471, the external validation set addresses the five *Salmonella* strains (TA100, TA90, TA1535, TA1537 and *E. coli* WP2 uvrA).

7.3. Data for each descriptor variable for the external validation set

Not available

7.4. Data for the dependent variable for the external validation set

Not available

7.5. Other information about the external validation set

The list with 12140 chemicals are provided by the Division of Genetics and Mutagenesis of National Institute of Health Sciences of Japan. Details for the data used in the current external validation are available in the corresponding publication:

M. Honma, A. Kitazawa, A. Cayley, R. Williams, C. Barber, T. Hanser, R. Saiakhov, S. Chakravarti, G. Myatt, K. Cross, E. Benfenati, G. Raitano, O. Mekenyan, P. Petkov, C. Bossa, R. Benigni, C. Battistelli, A. Giuliani, O. Tcheremenskaia, C. DeMeo, U. Norinder, H. Koga, C. Jose, N. Jeliazkova, N. Kochev, V. Paskaleva, C. Yang, P. Daga, R. Clark, J. Rathman. 2019. Improvement of quantitative structure-activity relationship (QSAR) tools for predicting Ames mutagenicity: outcomes of Ames/QSAR International Challenge Project. *Mutagenesis*, Vol. 34, pp. 3-16.

7.6. Experimental design of test set

The external validation set contains substances from three categories:

- Class A: Strong positive (672 chemicals)
- Class B: Positive (1085 chemicals)
- Class C: Negative (10383 chemicals)

7.7. Predictivity – Statistics obtained by external validation

Performance for the entire list with chemicals which belong and do not belong to model domain (*In Domain* and *Out of domain)*:

- Sensitivity of the Class A: 61%
- Sensitivity of Class B: 49%
- Specificity of Class C: 80%

Performance for the chemicals which belong to model domain, i.e. "*In Domain":*

- Sensitivity of the Class A: 82%
- Sensitivity of Class B: 50%
- Specificity of Class C: 90%

7.8. Predictivity – Assessment of the external validation set

The study reports of the Ames tests were peer reviewed by the ANEI-HOU committee comprising several Ames experts from academia and National Institutes and the results were authorised.

7.9. Comment on the external validation of the model

Performance of the TIMES Ames model (+S9) with respect to the strong mutagens from Class A and non-mutagenic chemicals from Class C (belonging to the model domain) is consistent with accuracy of the experimental Ames data (~85%). In turn, performance of the model with respect to Class B mutagens is lower (about 50%) as compared with Class A mutagens probably due to dose-dependency of the obtained experimental data.

Section 8. Providing a mechanistic interpretation – OECD Principle 5

8.1. Mechanistic basis of the model

Only alerts extracted from the local training sets having clear interpretation of the molecular mechanism causing the mutagenicity effect are included in the model. Mechanistic rational of each alert is provided by experts based on significant reference support from the literature. Additional information about mechanisms of alerts is provided in **Section 4.4**.

8.2. *A priori* or *a posteriori* mechanistic interpretation

The model building followed the traditional approach:

a. Building a hypothesis for the modelled event,
b. Defining the alerting groups based on parent structures,
c. Fitting of model variable to the observed data,
d. Verification of model quality,
e. Depending on the results found in step *d* model building could continue with step *a, b* or *f,*
f. Determination of the applicability domain and practical application of the model.

8.3. Other information about the mechanistic interpretation

Additional information about the mechanistic interpretation could be fund in Section 2 (2.7).

Section 9. Miscellaneous information

9.1. Comments

Model predictions are fully transparent. The user is able to analyse the whole prediction process and to verify whether it concise with his/her knowledge or purposes.
For other related models, see Section 1 (1.2).

9.2. Bibliography

Additional references are not provided.

9.3. Supporting information

Additional supporting information is not provided.