(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: *In vitro* Chromosomal Aberrations v.21.21
Platform version: OASIS TIMES 2.34.1
Name: *In vitro* Chromosomal Aberrations
Author: LMC, University "Prof. As. Zlatarov", Bourgas, Bulgaria
Date: 02 June 2025
E-mail: ovanes.mekenyan@oasis-lmc.org
www: http://www.oasis-lmc.org/

Section 1. QSAR identifier

1.1. QSAR identifier (title)

In vitro Chromosomal Aberrations with S9 metabolic activation v.21.21

1.2. Other related models

In vitro Ames mutagenicity with S9 metabolic activation

1.3. Software coding of the model

Model version: *In vitro* Chromosomal Aberrations v.21.21
Platform version: OASIS TIMES 2.34.1
Name: *In vitro* Chromosomal Aberrations
Developer: LMC, University "Prof. As. Zlatarov", Bourgas, Bulgaria
Coding language: Delphi 10.2

Section 2. General information

2.0. Abstract

In vitro chromosomal aberrations (CA) model identifies chemicals causing structural chromosome aberrations in cultured mammalian cells [1]. Structural aberrations could be of two types: chromosomal or chromatid. Majority of chemical mutagens induce aberrations of the chromatid type, but chromosome-type aberrations also occur.

The TIMES system integrates in a same modelling platform metabolic simulation of chemicals and their interaction with target macromolecules [2]. 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 direct binding to DNA is one of the underlying mechanisms which is responsible for eliciting the in vitro CA effect. Given the limited number of CA data and assuming similarity of interactions with DNA, the DNA-binding alerts from the Ames model are directly transferred to the CA model. Disturbance of protein synthesis due to inhibition of topoisomerases and interaction of chemicals with nuclear proteins, such as histone proteins, are identified as additional mechanisms leading to the CA effect. This imposed introduction of protein-binding alerts in the model. The explicit generation of metabolites allows DNA and/or protein-binding alerts 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 documented metabolic maps for mammalian liver metabolism consisting of 563 documented metabolic maps. 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 of the model includes 930 chemicals with experimental CA data separated in three groups: 444 chemicals positive as parents only (17 are proprietary), 102 chemicals positive as parents and metabolites (6 are proprietary), 384 chemicals negative as parents and metabolites (147 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

02 June 2025

2.2. QMRF author(s) and contact details

Name: Laboratory of Mathematical Chemistry
Affiliation: Laboratory of Mathematical Chemistry, University "Prof. As. Zlatarov", "Yakimov" St. #1, 8010 Bourgas, Bulgaria
URL: http://www.oasis-lmc.org
E-mail: ovanes.mekenyan@oasis-lmc.org

2.3. Date of QMRF update(s)

21 November 2014; 12 June 2015; 12 May 2016; 12 July 2016; 31 August 2016; 30 May 2017; 23 July 2018; 22 Jan 2020; 1 December 2021; 8 March 2023; 31 March 2024; 02 June 2025.

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 2.5** Model developer(s) and contact details; **Sections 2.8.** Availability of information about the model; **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 6.7** 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

Affiliation: Laboratory of Mathematical Chemistry, University "Prof. As. Zlatarov", "Yakimov" St. #1, 8010 Bourgas, BULGARIA

URL: http://www.oasis-lmc.org

E-mail: ovanes.mekenyan@oasis-lmc.org

2.6. Date of model development and/or publication

Date of the model development: 2007/2012

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

1. Evans, H.J. (1976). Cytological Methods for Detecting Chemical Mutagens. In: Chemical Mutagens, Principles and Methods for their Detection, Vol. 4, Hollaender, A. (ed) Plenum Press, New York and London, pp. 1-29
2. O. Mekenyan; M. Todorov; R. Serafimova; S. Stoeva; A. Aynur; R. Finking; E. Jacob. Identifying the structural requirements for chromosomal aberration by incorporating molecular flexibility and metabolic activation of chemicals. Chem Res Toxicol, 1927-1941, (2007).
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.

2.8. Availability of information about the model

In vitro CA 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: ovanes.mekenyan@oasis-lmc.org.

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/chromosomal-aberrations.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 CA model are collected according to the recommendation in the OECD technical guideline 473 addressing: Chinese Hamster Ovary (CHO), Chinese Hamster lung V79 and Chinese Hamster Lung (CHL)/IU, TK6.

3.2. Endpoint

In vitro Mammalian Chromosome Aberration Test

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

3.3. Comment on endpoint

The purpose of the *in vitro* chromosomal aberration (CA) test is to identify substances that cause structural chromosomal aberrations in cultured mammalian cells. Structural aberrations may be of two types, chromosome or chromatid which are detected by the *in vitro* CA test. Polyploidy could arise in chromosome aberration assays *in vitro*. While aneugens can induce polyploidy, polyploidy alone does not indicate aneugenic potential and can simply indicate cell cycle perturbation or cytotoxicity. This test is not designed to measure aneuploidy.

3.4. Endpoint units

Qualitative – positive/ negative

3.5. Dependent variable

Obs. chromosomal aberrations with S9

3.6. Experimental protocol

OECD technical guideline 473: *in vitro* mammalian chromosomal aberration test
http://www.oecd-ilibrary.org/environment/test-no-473-in-vitro-mammalian-chromosomal-aberration-test_9789264224223-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 alert based model

4.2. Explicit algorithm

Prediction of Chromosomal aberrations is based on modelling of the two events deemed to be crucial for the effect – interaction of the chemicals with DNA/proteins and their activation as a result of liver S9 metabolism.

CA 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 (165) 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 563 chemicals. 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 boundaries associated with alerting groups related to interactions with DNA/proteins. Alerts in the TIMES Chromosomal aberrations (+S9) constitute expertly-derived sets of structural fragments incorporating knowledge for the interactions of chemicals (parents and metabolites) with DNA/proteins. 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/proteins. 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 section

Table 1 summarizes the main characteristics of each DNA and protein alerts in TIMES_CA (+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 <u>High Reliability</u> 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 and protein alerts in the TIMES_CA model (+S9).

No.	Alert name	Correct	Incorrect	Performance	p-value
1	N-Nitroso Compounds	35	0	0.973 (0.920 ÷ 1.000)	1.4E-8
2	Hydrazine Derivatives	43	1	0.957 (0.898 ÷ 0.999)	4.5E-9
3	Diazenes	39	1	0.952 (0.889 ÷ 0.999)	3.1E-8
4	Alkylated nitrosoureas and nitrosoguanidines	18	0	0.950 (0.854 ÷ 1.000)	0.0001
5	Heterocyclic Aromatic Amines	15	0	0.941 (0.829 ÷ 1.000)	0.0004
6	N-Alkyl-N-nitrosocarbamates	15	0	0.941 (0.829 ÷ 1.000)	0.0004
7	Fused-Ring Primary Aromatic Amines	13	0	0.933 (0.807 ÷ 1.000)	0.0011
8	alpha,beta-Unsaturated Carboxylic Acids and Esters	26	1	0.931 (0.841 ÷ 0.998)	1.4E-5
9	Hydroxylated phenols	49	3	0.926 (0.856 ÷ 0.986)	1.9E-8
10	Arenesulphonamides	10	0	0.917 (0.762 ÷ 1.000)	0.0050
11	Vicinal Dihaloalkanes	10	0	0.917 (0.762 ÷ 1.000)	0.0050
12	N-Substituted Aromatic Amines	20	1	0.909 (0.792 ÷ 0.997)	0.0004
13	Alkylphosphates, Alkylthiophosphates and Alkylphosphonates	8	0	0.900 (0.717 ÷ 1.000)	0.015
14	Arenecarbonyl Compounds	8	0	0.900 (0.717 ÷ 1.000)	0.015
15	Polarized Haloalkene Derivatives	8	0	0.900 (0.717 ÷ 1.000)	0.015
16	Quinoid compounds	43	4	0.898 (0.813 ÷ 0.973)	1.3E-6
17	Substituted Anilines	51	5	0.897 (0.818 ÷ 0.967)	1.9E-7
18	Quinoneimines protein binding	16	1	0.895 (0.761 ÷ 0.997)	0.0017
19	N-Nitrosoamine Derivatives	7	0	0.889 (0.688 ÷ 1.000)	0.024
20	Nitrogen Mustards	7	0	0.889 (0.688 ÷ 1.000)	0.024
21	Polycyclic Aromatic Hydrocarbon, Naphthaleneimide and Carbazole Derivatives	7	0	0.889 (0.688 ÷ 1.000)	0.024
22	Haloalkane Derivatives with Labile Halogen	29	3	0.882 (0.775 ÷ 0.976)	0.0001
23	Carbamates	22	2	0.885 (0.764 ÷ 0.986)	0.0005

24	Arenediazonium and Diazonium Salts	27	3	0.875 (0.762 ÷ 0.974)	0.0002
25	Halogenated Vicinal Hydrocarbons	13	1	0.875 (0.719 ÷ 0.996)	0.0065
26	Arenecarboxylic Acid Esters	6	0	0.875 (0.652 ÷ 1.000)	0.041
27	Sulfonates and Sulfates DNA binding	6	0	0.875 (0.652 ÷ 1.000)	0.041
28	alpha-Activated Haloalkanes	19	2	0.870 (0.735 ÷ 0.983)	0.0018
29	Pyrimidines and Purines	12	1	0.867 (0.701 ÷ 0.996)	0.010
30	Dialkyl Alkylphosphonates	5	0	0.857 (0.607 ÷ 1.000)	0.070
31	Sulfonates and Sulfates protein binding	5	0	0.857 (0.607 ÷ 1.000)	0.070
32	Haloalkane Derivatives Containing Chain Heteroatom	17	2	0.857 (0.711 ÷ 0.981)	0.001045
33	Epoxides, Aziridines, Thiiranes and Oxetanes	45	7	0.852 (0.757 ÷ 0.939)	2.4E-5
34	alpha,beta-Unsaturated Carbonyls and Related Compounds	21	3	0.846 (0.709 ÷ 0.967)	0.0028
35	C-Nitroso compounds protein binding	53	9	0.844 (0.754 ÷ 0.927)	1.0E-5
36	N-Hydroxylamines	57	10	0.841 (0.754 ÷ 0.922)	6.8E-6
37	Substituted Phenols	51	9	0.839 (0.754 ÷ 0.927)	8.8E-6
38	Epoxides, Aziridines and Thiiranes	45	8	0.836 (0.738 ÷ 0.928)	0.0001
39	Benzoquinolines and Acridines derivatives	9	1	0.833 (0.632 ÷ 0.994)	0.039
40	Conjugated Nitroalkenes and Five-Membered Nitro- and Amino Heterocycles	4	0	0.833 (0.549 ÷ 1.000)	0.11
41	Fused-Ring Nitroaromatics	4	0	0.833 (0.549 ÷ 1.000)	0.11
42	Isothiocyanates	4	0	0.833 (0.549 ÷ 1.000)	0.11
43	Nitrogen and Sulfur Mustards	4	0	0.833 (0.549 ÷ 1.000)	0.11
44	Pyrazolone and Pyrazolidine Derivatives	4	0	0.833 (0.549 ÷ 1.000)	0.11
45	Quinoline Derivatives	4	0	0.833 (0.549 ÷ 1.000)	0.11
46	C-Nitroso Compounds	44	8	0.833 (0.734 ÷ 0.926)	0.0001
47	Quinoneimine, Thionine and Phenoxazinium Derivatives	8	1	0.818 (0.602 ÷ 0.993)	0.061
48	Nitroaniline Derivatives	12	2	0.813 (0.627 ÷ 0.973)	0.034
49	Haloalcohols	19	4	0.800 (0.645 ÷ 0.941)	0.014

50	N-Nitrosamines	7	1	0.800 (0.568 ÷ 0.991)	0.097
51	alpha-Activated benzyls	3	0	0.800 (0.473 ÷ 1.000)	0.20
52	Carboxylic acid Anhydrides	3	0	0.800 (0.473 ÷ 1.000)	0.20
53	Formaldehyde Releasers	3	0	0.800 (0.473 ÷ 1.000)	0.20
54	Gallic Acid Esters	3	0	0.800 (0.473 ÷ 1.000)	0.20
55	Heterocyclic N-Hydroxylamines	3	0	0.800 (0.473 ÷ 1.000)	0.20
56	Heterocyclic nitro compounds	3	0	0.800 (0.473 ÷ 1.000)	0.20
57	Quinone Methides	3	0	0.800 (0.473 ÷ 1.000)	0.20
58	Single-ring Substituted Primary Aromatic Amines	44	11	0.789 (0.683 ÷ 0.890)	0.0010
59	(Thio)Phosphates	10	2	0.786 (0.579 ÷ 0.968)	0.074
60	Dicarbonyl Compounds	9	2	0.786 (0.579 ÷ 0.968)	0.071
61	Quinones and Trihydroxybenzenes	10	2	0.786 (0.579 ÷ 0.968)	0.074
62	Specific Imine and Thione Derivatives	9	2	0.769 (0.550 ÷ 0.964)	0.107
63	alpha,omega-Dihaloalkanes	2	0	0.750 (0.368 ÷ 1.000)	0.34
64	Aminoacridine DNA Intercalators	2	0	0.750 (0.368 ÷ 1.000)	0.34
65	Bipyridilium Herbicides	2	0	0.750 (0.368 ÷ 1.000)	0.34
66	Ethenyl Pyridines	2	0	0.750 (0.368 ÷ 1.000)	0.34
67	Hexahydrotriazine Derivatives	2	0	0.750 (0.368 ÷ 1.000)	0.34
68	N-Haloacylamides	2	0	0.750 (0.368 ÷ 1.000)	0.34
69	Polynitroarenes	2	0	0.750 (0.368 ÷ 1.000)	0.34
70	Propargyl Derivatives	2	0	0.750 (0.368 ÷ 1.000)	0.34
71	Propyne Derivatives	2	0	0.750 (0.368 ÷ 1.000)	0.34
72	Thiols	2	0	0.750 (0.368 ÷ 1.000)	0.348
73	Nitroazoarenes and p-Monosubstituted Azobenzene Derivatives	2	0	0.750 (0.368 ÷ 1.000)	0.348
74	Carboxylic Acid Amides	9	3	0.714 (0.486 ÷ 0.926)	0.205
75	Acyl Halides	4	1	0.714 (0.409 ÷ 0.982)	0.31

76	Sterically Hindered Piperidine Derivatives	4	1	0.714 (0.409 ÷ 0.982)	0.31
77	Nitrophenols, Nitrophenyl Ethers and Nitrobenzoic Acids	11	4	0.706 (0.495 ÷ 0.903)	0.19
78	Isocyanates and Diisocyanates	6	2	0.700 (0.432 ÷ 0.946)	0.28
79	(Thio)Acyl and (thio)carbamoyl halides, cyanides, azides, etc.	10	4	0.688 (0.467 ÷ 0.895)	0.24
80	Geminal Polyhaloalkane Derivatives	11	5	0.667 (0.454 ÷ 0.869)	0.29
81	Alpha,Beta-Unsaturated Aldehydes	5	2	0.667 (0.379 ÷ 0.935)	0.39
82	Acridone, Thioxanthone, Xanthone, Phenazine and Other Fused-Ring Heterocyclic DNA Intercalators	3	1	0.667 (0.330 ÷ 0.974)	0.45
83	4,4’-Bipyridinium Salts and N-Oxides	1	0	0.667 (0.224 ÷ 1.000)	0.58
84	Aminophenoxazinone derivative	1	0	0.667 (0.224 ÷ 1.000)	0.58
85	Benzofuranyl carbamate derivatives	1	0	0.667 (0.224 ÷ 1.000)	0.58
86	Bleomycin and Structurally Related Chemicals	1	0	0.667 (0.224 ÷ 1.000)	0.58
87	Cyanohydrins	1	0	0.667 (0.224 ÷ 1.000)	0.58
88	DNA Intercalators with Carboxamide and Aminoalkylamine Side Chain	1	0	0.667 (0.224 ÷ 1.000)	0.58
89	Flavonoids	1	0	0.667 (0.224 ÷ 1.000)	0.58
90	Four-and Five-Membered Lactones	1	0	0.667 (0.224 ÷ 1.000)	0.58
91	Halofuranones	1	0	0.667 (0.224 ÷ 1.000)	0.58
92	Hypoxanthine Derivatives	1	0	0.667 (0.224 ÷ 1.000)	0.58
93	N-Acetoxyamines	1	0	0.667 (0.224 ÷ 1.000)	0.58
94	N-Aryl-N-Acetoxy(Benzoyloxy) Acetamides	1	0	0.667 (0.224 ÷ 1.000)	0.58
95	N-Oxicarbonyl amides, N-Acyloxy-N-alkoxyamides	1	0	0.667 (0.224 ÷ 1.000)	0.58
96	Nitroalkanes	1	0	0.667 (0.224 ÷ 1.000)	0.58
97	Non-Cyclic Alkyl Phosphoramides and Thionophosphoramides	1	0	0.667 (0.224 ÷ 1.000)	0.58
98	Organic Azides	1	0	0.667 (0.224 ÷ 1.000)	0.58
99	Pyrrolizidine derivatives	1	0	0.667 (0.224 ÷ 1.000)	0.58
100	Specific 5-Substituted Uracil Derivatives	1	0	0.667 (0.224 ÷ 1.000)	0.58

101	Specific Acetate Esters	1	0	0.667 (0.224 ÷ 1.000)	0.58
102	Sultones	1	0	0.667 (0.224 ÷ 1.000)	0.58
103	Sultones protein binding	1	0	0.667 (0.224 ÷ 1.000)	0.58
104	Amino Anthraquinones	2	1	0.600 (0.228 ÷ 0.956)	0.62
105	Azoxyalkanes	2	1	0.600 (0.228 ÷ 0.956)	0.62
106	Hydroxamic acid	3	1	0.667 (0.330 ÷ 0.974)	0.457
107	p-Substituted Mononitrobenzenes	2	1	0.600 (0.228 ÷ 0.956)	0.62
108	Nitroarenes with Other Active Groups	3	2	0.571 (0.239 ÷ 0.895)	0.65
109	Haloalkene Derivatives with Electron-Withdrawing Groups	3	3	0.500 (0.184 ÷ 0.816)	0.805
110	N,N-Dialkyldithiocarbamate Derivatives and Azaarene Dithiocarbamates	1	1	0.500 (0.094 ÷ 0.906)	0.82
111	Monohaloalkanes	1	3	0.333 (0.026 ÷ 0.670)	0.97
112	Hydroxybenzophenone Derivatives	0	1	0.333 (0.000 ÷ 0.776)	1.00
113	Organic Peroxy Compounds	0	1	0.333 (0.000 ÷ 0.776)	1.00
114	Quinolone Derivatives	0	1	0.333 (0.000 ÷ 0.776)	1.00
115	p-Aminobiphenyl Analogs	0	2	0.250 (0.000 ÷ 0.632)	1.00
116	1,4-Diazabutadiene Derivatives	0	0	N/A	N/A
117	Acyclic Triazenes	0	0	N/A	N/A
118	Alkyl Xanthate Esters	0	0	N/A	N/A
119	Alkylnitrites	0	0	N/A	N/A
120	Alpha-Beta Conjugated Alkene Derivatives with Geminal Electron-Withdrawing Groups	0	0	N/A	N/A
121	Alpha-Haloethers	0	0	N/A	N/A
122	Amidoxime Esters and Amidoximes	0	0	N/A	N/A
123	Anthrones	0	0	N/A	N/A
124	Antibiotic Aminoglycoside Derivatives	0	0	N/A	N/A
125	Aromatic ester hydroxylamine	0	0	N/A	N/A
126	Azoalkanes with Activating EWG	0	0	N/A	N/A
127	Benzanthrone Derivatives	0	0	N/A	N/A
128	Benzoyl cyclohexanedione derivatives	0	0	N/A	N/A
129	Chlorinated Diphenylmethane and Benzophenone Derivatives	0	0	N/A	N/A
130	Conjugated Benzoylethylene Derivatives with EWG	0	0	N/A	N/A

131	Coumarins and Thiocoumarins	0	0	N/A	N/A
132	Diazoalkanes	0	0	N/A	N/A
133	Dichlorophosphine and Dichlorophosphonium Derivatives	0	0	N/A	N/A
134	Dithianes	0	0	N/A	N/A
135	Fluoro bis-benzothiazole derivative	0	0	N/A	N/A
136	Fused-Ring Conjugated Lactones	0	0	N/A	N/A
137	Haloalkene Cysteine S-Conjugates	0	0	N/A	N/A
138	Haloazaarene and Fused-Ring Haloquinoline Derivatives	0	0	N/A	N/A
139	Halogenated Oxetanes and Haloepoxides	0	0	N/A	N/A
140	Haloisothiazolinones	0	0	N/A	N/A
141	Heterocyclic Nitroso compounds	0	0	N/A	N/A
142	Heterocyclic urea derevatives	0	0	N/A	N/A
143	N-Acyloxy(Alkoxy) Arenamides	0	0	N/A	N/A
144	N-Alkylindolinium and N-Alkylbenzothiazolium Salts	0	0	N/A	N/A
145	N-Hydroxyethyl Lactams	0	0	N/A	N/A
146	N-methylol derivatives	0	0	N/A	N/A
147	N-Trihalomethyl Imides (Theoretical)	0	0	N/A	N/A
148	Nitrobiphenyls and Bridged Nitrobiphenyls	0	0	N/A	N/A
149	Non-Aromatic Hydroxylamine Derivatives	0	0	N/A	N/A
150	Organic Diselenides and Ditellurides	0	0	N/A	N/A
151	PAH Benzylic Alcohol Esters	0	0	N/A	N/A
152	Perfluorinated Hypofluorites	0	0	N/A	N/A
153	Peroxyacyl Nitrates	0	0	N/A	N/A
154	Polyethylene Polyamines	0	0	N/A	N/A
155	Quinoxaline-Type 1,4-Dioxides	0	0	N/A	N/A
156	S-Activated Cysteine Derivatives	0	0	N/A	N/A
157	Short-Chain Alkyltin and Alkylgermanium Halides	0	0	N/A	N/A
158	Substituted Benzoindoline and Indole Derivatives	0	0	N/A	N/A
159	Substituted Chlorophenylalkylurea Derivatives	0	0	N/A	N/A
160	Substituted Nitropyridines, Aminopyridines and N-Oxides	0	0	N/A	N/A
161	Sulfonyl Halides	0	0	N/A	N/A
162	Tertiary Heterocyclic Amines	0	0	N/A	N/A
163	Thiadiazole-dioxide derivatives	0	0	N/A	N/A
164	Thiazolidinediones	0	0	N/A	N/A
165	Tri-Methylindole derivatives	0	0	N/A	N/A

166	Triarylimidazole and Structurally Related DNA Intercalators	0	0	N/A	N/A
167	Triazinone derivative	0	0	N/A	N/A
168	Triflorometyl benzamide derivative	0	0	N/A	N/A
169	Trifluoromethyl pyridinone derivatives	0	0	N/A	N/A

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 and/or proteins 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 Chromosomal aberrations model version 21.21

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.

https://oasis-lmc.org/products/software/domain-manager.aspx

5.4. Limits of applicability

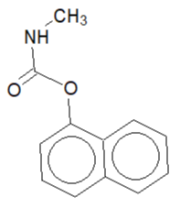
Applicability domain of the CA model (+S9) include three sub-domain layers: general parametric requirements, structural features and alerts reliability.

- 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: 63-25-2
- Name: Carbaryl
- 2D Depiction:



Property	Domain	Example chemical
log KOW	[-13.164; 29.679]	2.350
MW, Da	[44.06; 1514.086]	201.211

* *KOW* 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 extracted from 930 training chemicals contains:

- 2600 correct fragments,
- 321 fuzzy fragments (treated as correct fragments),
- 399 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).

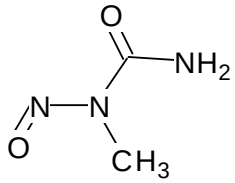
Example chemical belonging to alert with “High reliability”.

Chemical ID:

CAS: 684-93-5

Name: 1-methyl-1-nitrosourea

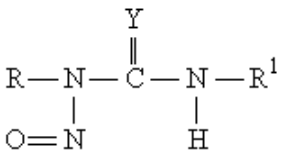
2D depiction:



Belonging to alert:

Name: Alkylated nitrosoureas and nitrosoguanidines

Structural boundaries:



where:

R = (Csp^3)n – acy at n ≥ 1, preferably linear or branched C1–C5 alkyl groups; R may also include an acyl group C(=O)Csp^3 (acy) and a nitro group;

R1 = H atom; (Csp^3)n – acy at n ≥ 1; C(=O)Csp^3 (acy), Csp^2 (aryl), nitro group, etc.;

Y = O and NH.

Reliability:

"High reliability" based on AP=1; N=18 and M.

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 CA model (+S9) includes 930 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 930 chemicals include:

- 546 chemicals having positive observed CA data
- 384 chemicals having negative observed CA data.

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 0.587

(0.555 ÷ 0.618)[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 TG473.

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)	0.843 (0.820 ÷ 0.866)	$< 10^{-10}$	0.832
Positive chemicals (sensitivity)	0.867 (0.838 ÷ 0.895)	$< 10^{-10}$	0.812
Negative chemicals (specificity)	0.806 (0.769 ÷ 0.847)	$< 10^{-10}$	0.760

[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 external 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 (74.9 ÷ 75.1)	25.0 (24.9 ÷ 25.1)
Performance $_{est.}$, all predictions (accuracy)	0.843 (0.820 ÷ 0.867)	0.828 (0.629 ÷ 1.028)	0.843 (0.793 ÷ 0.894)	0.831 (0.694 ÷ 0.968)
p-value, accuracy	$< 10^{-10}$	1.1×10^{-7}	$< 10^{-10}$	$< 10^{-10}$
Performance $_{est.}$, positive chemicals (sensitivity)	0.866 (0.846 ÷ 0.886)	0.763 (0.478 ÷ 1.048)	0.863 (0.815 ÷ 0.908)	0.750 (0.448 ÷ 1.055)
p-value, sensitivity	$< 10^{-10}$	0.014	$< 10^{-10}$	0.0001
Performance $_{est.}$, negative chemicals (specificity)	0.806 (0.756 ÷ 0.861)	0.672 (0.310 ÷ 1.033)	0.807 (0.702 ÷ 0.918)	0.699 (0.388 ÷ 1.011)
p-value, specificity	$< 10^{-10}$	3×10^{-7}	$< 10^{-10}$	3×10^{-7}

[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, %	75.1 (75.1 ÷ 75.1)	24.9 (24.9 ÷ 24.9)	63.0 (63.0 ÷ 63.0)	37.0 (37.0 ÷ 37.0)
Performance $_{est.}$, all predictions (accuracy)	0.843 (0.829 ÷ 0.857)	0.834 (0.791 ÷ 0.877)	0.843 (0.826 ÷ 0.861)	0.832 (0.802 ÷ 0.863)
p-value, accuracy	$< 10^{-10}$	$< 10^{-10}$	$< 10^{-10}$	$< 10^{-10}$
Performance $_{est.}$, positive chemicals (sensitivity)	0.868 (0.850 ÷ 0.883)	0.848 (0.797 ÷ 0.900)	0.866 (0.844 ÷ 0.888)	0.846 (0.806 ÷ 0.886)
p-value, sensitivity	$< 10^{-10}$	1.7×10^{-8}	$< 10^{-10}$	$< 10^{-10}$
Performance $_{est.}$, negative chemicals (specificity)	0.808 (0.785 ÷ 0.831)	0.807 (0.737 ÷ 0.877)	0.808 (0.778 ÷ 0.838)	0.809 (0.758 ÷ 0.859)
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, %	36.7 (34.8 ÷ 38.7)	63.3 (61.3 ÷ 65.2)
Performance $_{est.}$, all predictions (accuracy)	0.834 (0.802 ÷ 0.866)	0.843 (0.819 ÷ 0.866)
p-value, accuracy	$< 10^{-10}$	$< 10^{-10}$
Performance $_{est.}$, positive chemicals (sensitivity)	0.848 (0.808 ÷ 0.887)	0.867 (0.838 ÷ 0.895)
p-value, sensitivity	$< 10^{-10}$	5.5×10^{-7}
Performance $_{est.}$, negative chemicals (specificity)	0.810 (0.759 ÷ 0.861)	0.807 (0.769 ÷ 0.845)
p-value, 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 model shows good predictive performance for positive chemicals (Sensitivity) – 87% for training set, around 84% expected Sensitivity for chemicals different from the training set. On the contrary, prediction performance for negative chemicals (Specificity) is not that high (~81%) indicating for possible over-prediction. Lower Specificity could be due to the fact that the model training set is relatively small and not balanced – 60% of the training chemicals are observed positive which favours training of positive chemicals rather than negative ones. One should also address that *in vitro* CA tests is known to provide false positive predictions due to cytotoxicity.

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

7.1. Availability of the external validation set

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

7.2. Available information for the external validation set

The external validation set includes pesticide chemicals from the EFSA database. Details of the EFSA database are available in the website of the agency:
https://data.europa.eu/euodp/en/data/dataset/database-pesticide-genotoxicity-endpoints

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 EFSA genotoxicity database is biased towards negative chemicals. This justifies the fact that Sensitivity of the model is examined based on a small number of chemicals as compared with the substances used for estimating Specificity of the model. Details about the external validation set are available in:

P.I. Petkov, T. W. Schultz, M. Honma, T. Yamada, E. Kaloyanova, O. Mekenyan. 2019. Validation of the performance of TIMES genotoxicity models with EFSA pesticide data. *Mutagenesis*, Vol. 34, pp. 83-90.

7.6. Experimental design of test set

The external validation set of 93 pesticides contains:

- Positive data (10 chemicals)
- Negative data (83 chemicals)

7.7. Predictivity – Statistics obtained by external validation

Performance of the chemicals which belong and does not belong to the model domain (*In domain* and *Out of domain*) is:

- Sensitivity = 70% (7 of 10 chemicals)
- Specificity = 43% (36 of 83 chemicals)

Lower Specificity indicated that the *in vitro* liver S9 metabolic simulator associated with the model has to be modified with respect to the pesticide chemicals. These and other modifications of the metabolic simulator are performed in the latest version of the model.

7.8. Predictivity – Assessment of the external validation set

The EFSA genotoxicity database for pesticide chemicals is assumed to be a high quality database. According to the description of the database, all endpoints are evaluated using standard tests conditions as described in the corresponding tests guidelines.

7.9. Comment on the external validation of the model

Performance of the CA model (+S9) in terms of Sensitivity is reasonable accounting for the fact that all 10 chemicals with positive CA data do not belong to the model domain. Lower Specificity indicates that modifications of some alerts and/or *in vitro* S9 metabolic simulator associated with the model are needed. Such modifications are performed in the latest versions of the model.

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.

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.