Genotox-iS™

Pharmaceutical Impurities: Learn about the in silico evaluation steps

Altox
ALTERNATIVE TOXICOLOGY

Due to the fact that the ICH Q3A(R2), ICH Q3B(R2) and ICH Q3C(R3) guidelines do not contemplate the evaluation of genotoxic impurities, in 2014 the **ICH M7** was published setting criteria for the establishment of **acceptable limits for mutagenic impurities or mutagenic degradation products that result in an acceptable level of risk.**

1 Alert analysis

Under the ICH M7 criteria, the first step of *in silico* evaluation is an analysis of potential structural alerts.

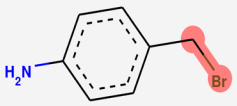
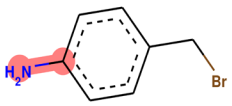
Impurities or degradation products containing structural alerts can be classified as potentially mutagenic.

Genotox-IS highlights the fragments related to positive alert which are considered potentially mutagenic.

Genotox-iS™

Structural identification of the groups with mutagenic potential, allow visualization of the molecule and the nomenclature of the grouping.

Elucidation from robust literature reviews and in vitro mutagenicity data.

Category	Alert	Alert ID	References
in vitro mutagenicity (Ames alert) alerts by ISS		Aliphatic halogens	Bolt, H. M. and Gansewendt, B. (1993). Mechanisms of Carcinogenicity of Methyl Halides. Crit.Rev.Toxicol. 23, 237-253. Guengerich, F. P. (1991). Oxidation of Toxic and Carcinogenic Chemicals by Human Cytochrome P-450 Enzymes. Chem.Res.Toxicol. 4, 391-407.
in vitro mutagenicity (Ames alert) alerts by ISS		Primary aromatic amine,hydroxyl amine and its derived esters	Benigni, R., Giuliani, A., Franke, R., and Gruska, A. (2000). Quantitative structure-activity relationships of mutagenic and carcinogenic aromatic amines. Chem.Revs. 100, 3697-3714. Woo, Y. T. and Lai, D. Y. (2001). Aromatic amino and nitro-amino compounds and their halogenated derivatives. In 'Patty's Toxicology. Vol. 4.' (Eds E. Bingham, B. Cohrssen, and C. H. Powell.) pp. 969-1105. (John Wiley and Sons, Inc: New York.)

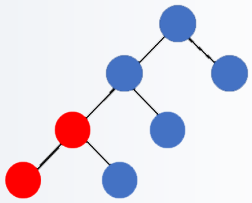
2 Statistical modeling

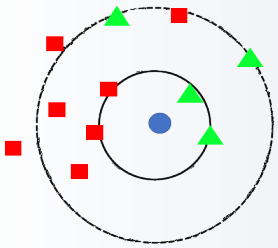
However, it is not enough for the molecule to have a reactive group to be considered potentially mutagenic. In step 2, the statistical model can evaluate the influence of other fragments that can attenuate, neutralize, or potentiate the reactivity of the molecule as a whole.

Genotox-iS statistical modeling is based on a statistical algorithm of rigorous consensus based on three powerful individual models:

Random Forest (RF)

It is a method that builds a multitude of possibilities and establishes rules for decision-making. The algorithm will create a structure composed of “nodes” where a condition is checked and flows through the branches according to the similarities.



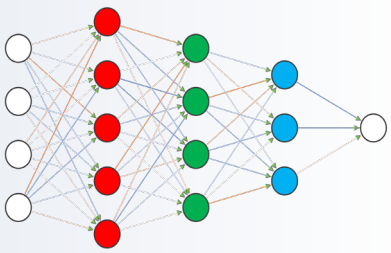


K-Nearest Neighbors (KNN)

This algorithm classifies the sample according to the characteristics of other structurally similar molecules. If the nearest neighbors are mostly mutagenic, the sample in question will be classified as mutagenic, for example.

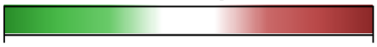
Deep Learning

It is a model trained to make inferences of structural patterns related to the presence and absence of mutagenicity, according to weights and biases, within a set of neural networks.

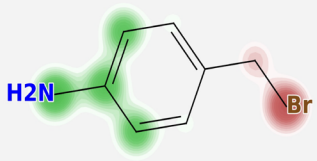
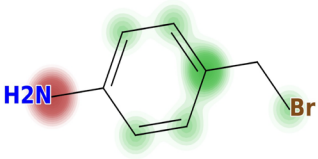
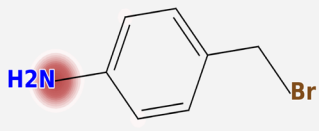


The advantage of applying the statistical battery is that it increases the predictivity of the evaluation, with reliable data and visual indication of the fragments related to mutagenic potential.

Probability Map

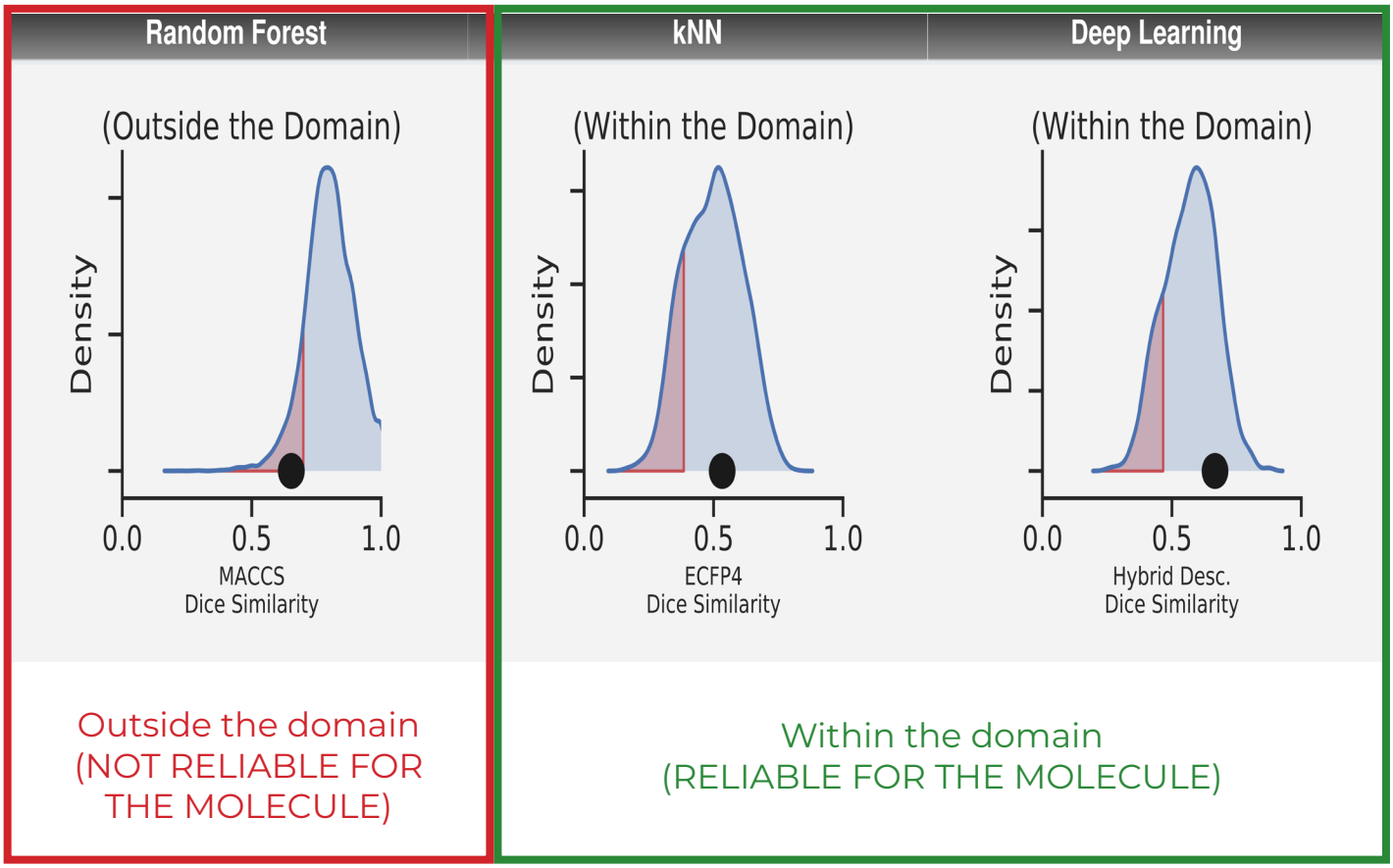


Non-Mutagen (-) Mutagen (+)

Algorithm	Prediction (Confidence)	Probability Mapping (SAR)
Random Forest Machine learning decision model implemented with the 2D MACCS fingerprint	Non-Mutagen (-) (60.9%)	
kNN k-nearest neighbors decision model implemented with the 2D Extended Connectivity Fingerprint	Mutagen (+) (71.4%)	
Deep Learning Deep Learning categorical model implemented with hybrid descriptors (ECFP6 fingerprint and physicochemical properties: MW, TPSA, $logK_{ow}$, logD)	Mutagen (+) (99.3%)	

3 Expert Review, Applicability Domain Inspection and similar molecules

The applicability domain is the chemical space in which the model makes predictions with a given confidence.



It is extremely important to establish the scope and limitations of the models.

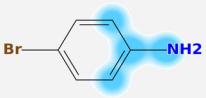
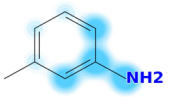
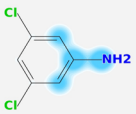
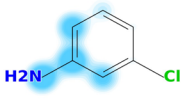
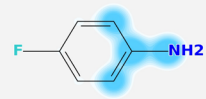
In addition, the software statistically evaluates the predictive ability of the models with a set of 10 most similar compounds.

This evaluation allows you to determine which models were truly capable of correctly predicting the mutagenic potential.

Performance for the 10-most similar molecules

Similarity Map

Low — High

Molecule (Similarity)	Experimental Data	Random Forest Prediction (Confidence)	kNN Prediction (Confidence)	Deep Learning Prediction (Confidence)
 (0.86)	REAL Non-Mutagen	Non-Mutagen (63.2%)	Non-Mutagen (71.4%)	Mutagen (73.2%)
 (0.8)	Non-Mutagen	Mutagen (53.0%)	Non-Mutagen (71.4%)	Non-Mutagen (66.2%)
 (0.79)	Non-Mutagen	Non-Mutagen (90.8%)	Non-Mutagen (71.4%)	Non-Mutagen (84.1%)
 (0.79)	Non-Mutagen	Non-Mutagen (96.7%)	Non-Mutagen (85.7%)	Non-Mutagen (73.7%)
 (0.79)	Mutagen	Non-Mutagen (74.0%)	Mutagen (57.1%)	Mutagen (69.4%)

4 Final Result

A highly predictive final result is generated based on the rule-based and statistically valid models:

Algorithm	Prediction (Confidence)	Applicability Domain
Model 1 – Rule-based model		
Structural Alerts	**Mutagen (+)**	-
Model 2 - Statistical battery		
Random Forest Machine learning decision model implemented with the 2D MACCS fingerprint	**Non-Mutagen (-)** **(60.9%)**	Within 
kNN k-nearest neighbors decision model implemented with the 2D Extended Connectivity Fingerprint	**Mutagen (+)** **(71.4%)**	Within 
Deep Learning Deep Learning categorical model implemented with hybrid descriptors (ECFP6 fingerprint and physicochemical properties: MW, TPSA, $\log K_{ow}$, logD)	**Mutagen (+)** **(99.3%)**	Within 
Statistical consensus (rigorous)	**Mutagen (+)**	-

5 Conclusion

According to ICH M7, the lack of structural alerts in the analysis with the two mutually complementary methodologies (a rule-based model and a statistical model) is sufficient to conclude that the impurity is not of concern for mutagenic potential and no additional testing is recommended.

> Impurities classified as **non-mutagenic** are treated as ordinary impurities and can be controlled according to the criteria in ICH Q3A/B and RDC 53/2015.

> Impurities classified as **mutagenic or potentially mutagenic** must be controlled according to ICHM7 criteria and using the appropriate Threshold of Toxicological Concern (TTC) (Classes 2 and 3).

Adress: Av. Dr. Vital Brasil, 305, conj. 803, Butantã, São Paulo, SP.
E-mail: atendimento@altox.com.br
Phone: +55 (11) 3777-4820
www.altox.com.br