In Silico Predictive Toxicology

Genotoxic Impurities Case Study

ADeBIOTeCH
THINK TANK ONE HEALTH

Seminar
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Harmonic Pharma

- Harmonic Pharma is a deeptech company providing the pharmaceutical, chemistry, biotechnology, cosmetics and nutraceutical industry with customized services during compound development projects.

- Harmonic Pharma has a long term experience in designing *in silico* methods using artificial intelligence - e.g. machine learning - for investigating any chemicals.

- Harmonic is the Seqens'Lab partner for *in silico* predictive toxicology with regards to genotoxic impurities (GTIs).

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Risk Assessment

Investigating GTIs using *in silico* methodologies

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Principles and procedures for implementation of ICH M7 recommended QSAR* analyses

- QSAR models should follow the 5 principles given by the Organisation for Economic Co-operation and Development (OECD).
- Two QSAR prediction methodologies are required : statistical-based and expert rule-based.
- If warranted, the outcome of *in silico* analysis can be reviewed with the support of an expert toxicologist:
  - Accepting/refuting a positive
  - Accepting/refuting a negative
  - Out-of-domain
  - Indeterminates
- Altogether, the aim is to provide a rationale to support the final conclusion on the relevance of any positive, negative, conflicting, or inconclusive prediction.

*QSAR stands for Quantitative Structure-Activity Relationship


EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

25 August 2015
EMA/CHMP/ICH/83812/2013
Committee for Human Medicinal Products

ICH guideline M7(R1) on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk

Step 5

https://www.ema.europa.eu/en/ich-m7-assessment-control-dna-reactive-mutagenic-impurities-pharmaceuticals-limit-potential#current-effective-version--section

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Two complementary *in silico* methodologies

Two different methodologies to predict the results of a bacterial mutagenesis

A –STATISTICAL-BASED QSAR

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- Mutagenic and no mutagenic compound datasets (7,164 compounds) derived from public databases with regards to experimental Ames tests
- Alert is generated based on a selection of molecular descriptors
- Compliance with the domain of applicability is checked prior to any prediction
- The output is the probability that a compound will result in a positive test in Ames mutagenicity assay

B – RULE-BASED QSAR

Leadscope®

- Alert knowledge base built from publicly cited alerts and knowledge derived from public and proprietary databases (8,412 compounds)
- Confidence score for each alert provided with each positive or negative call
- Predictions only made for compounds within the domain of applicability
- Provides access to citations, mechanisms, and all examples with full study reports

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A – STATISTICAL-BASED QSAR

To predict mutagenic potential of impurities

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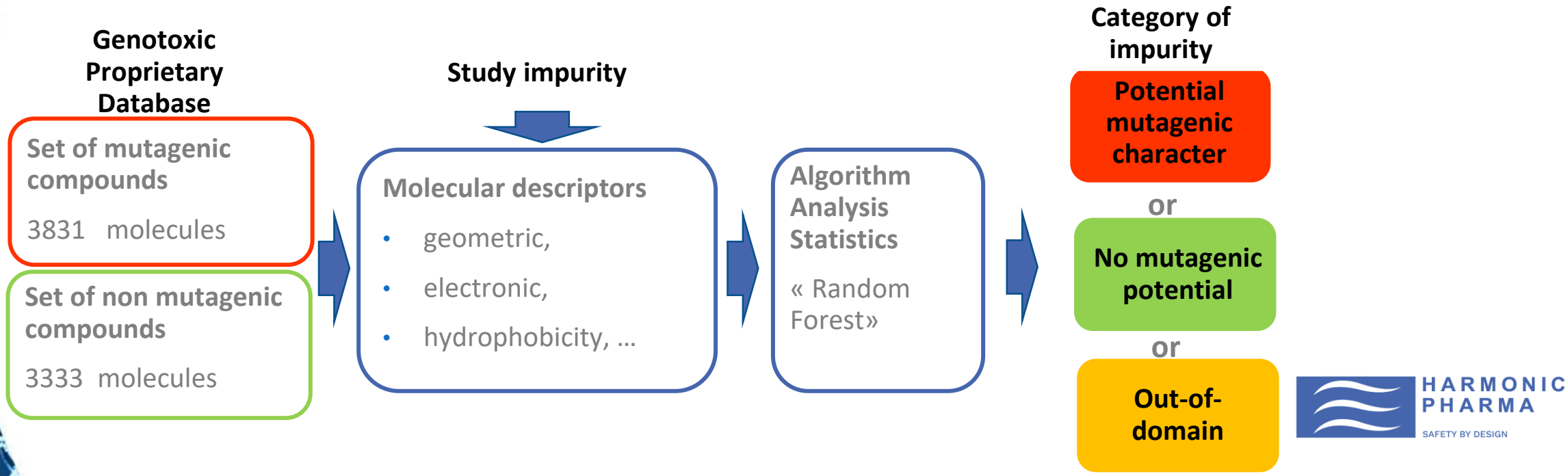
HPH_QSAR_Genotoxicity v1.0

- It is based on Random Forest statistical algorithm
- The prediction is the probability that a compound will result in a positive test in Ames mutagenicity assay
- Process used for any impurity:
  - Molecular descriptors are calculated and compared with those of reference molecules from the two datasets,
  - The model generates a probability value reflecting the category of the impurity within le domain of applicability
  - If probability < 0.44, the impurity is considered as non mutagenic
  - If probability > 0.44, the impurity is considered as mutagenic



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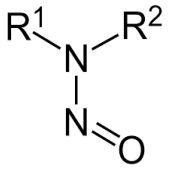
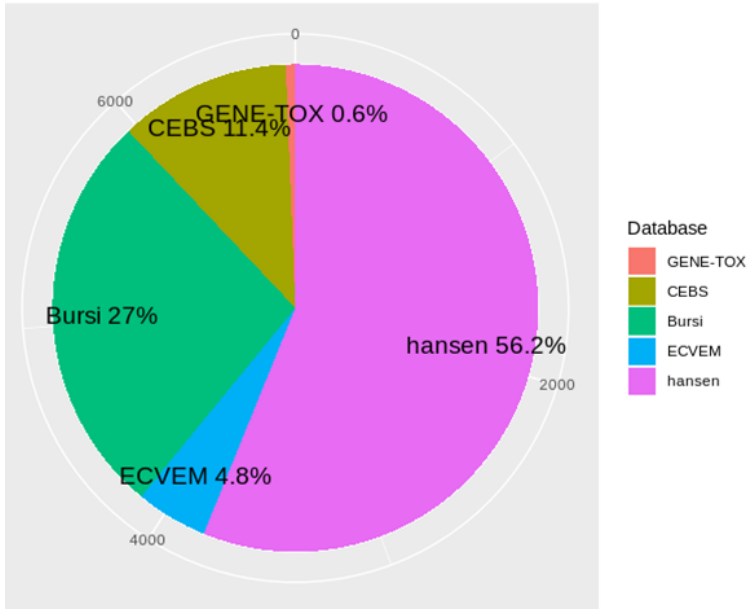
Genotoxic proprietary database

- The model is based primarily on data from the Ames test conducted following standard test protocol (OECD TG471).
- Modelling was performed using a standardized Ames genotoxicity dataset containing 7164 compounds compiled from five public databases:
  - Hansen *et al.* [1],
  - Bursi *et al.* [2],
  - CEBS [3],
  - Genetic Toxicology Data Bank (GENETOX) [4],
  - EURL-ECVAM [5,6]



- The database contains 213 nitrosamine molecules.
  - 187 known to be mutagenic
  - 26 known to be non mutagenic



[1] Katja Hansen *et al.* (2009) Benchmark Data Set for in Silico Prediction of Ames Mutagenicity, J. Chem. Inf. Model., 49, 2077–2081.
[2] Kazius J, Mcguire R & Bursi R (2005) Derivation and validation of toxicophores for mutagenicity prediction. Journal of Medicinal Chemistry, 48(1), 312-320.
[3] Isabel A. Lea *et al.* (2016) CEBS : a comprehensive annotated database of toxicological data , Nucleic Acids Research, 45, D964-D971.
[4] GENE-TOX mutagenicity studies (NIH), https://pubchem.ncbi.nlm.nih.gov/bioassay/1259408.
[5] Federica Madia (2018) « EURL ECVAM Genotoxicity and Carcinogenicity Consolidated Database of Ames Positive Chemicals. European Commission, Joint Research Centre (JRC
[6] Federica Madia *et al.* (2020) « EURL ECVAM Genotoxicity and Carcinogenicity Database of Substances Eliciting Negative Results in the Ames Test : Construction of the Database », Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 854-855, p. 503199.

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Compliance and performance

HPH_QSAR_Genotoxicity v1.0 is in line with the OECD principles* for the QSAR validation, namely :

- A well defined category of toxicity : Ames positive/Ames negative
- An unambiguous algorithm : Random Forest
- A well defined domain of applicability : MW, HBA, HBD, RotB, logP, TPSA
- Internal validation : 10-fold cross validation
- External validation : CCRIS dataset

Performance	Statistics
AUC[1]	0.88
Sensitivity[2]	0.81
Specificity[3]	0.81

(1) AUC - i.e. Area Under the Curve - characterizes the performance of the model. The closer to the value 1 the more robust. That means that the model is suitable to discriminate between toxic compounds and non toxic ones.
(2) Sensibility : capability to predict the category of a toxic molecule correctly.
(3) Specificity : capability to predict the category of a non toxic molecule correctly.

Regulatory QMRF (QSAR Model Reporting Format) file available upon request

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* https://www.oecd.org/chemicalsafety/risk-assessment/37849783.pdf

External validation

- Experimental validation set from the CCRIS* dataset is composed of 711 molecules with 372 mutagenic and 339 non mutagenic molecules.

Performance	Statistics
AUC	0.82
Sensitivity	0.76
Specificity	0.75

Predictivity	Number of molecules
True Positive	281 (75%)
True Negative	253 (75%)
False Negative	91(25%)
False Positive	86 (25%)

CRRIS dataset contains 25 nitrosamines known as mutagenic and which are correctly predicted as True Positive by the HPH_QSAR_Genotoxicity model.

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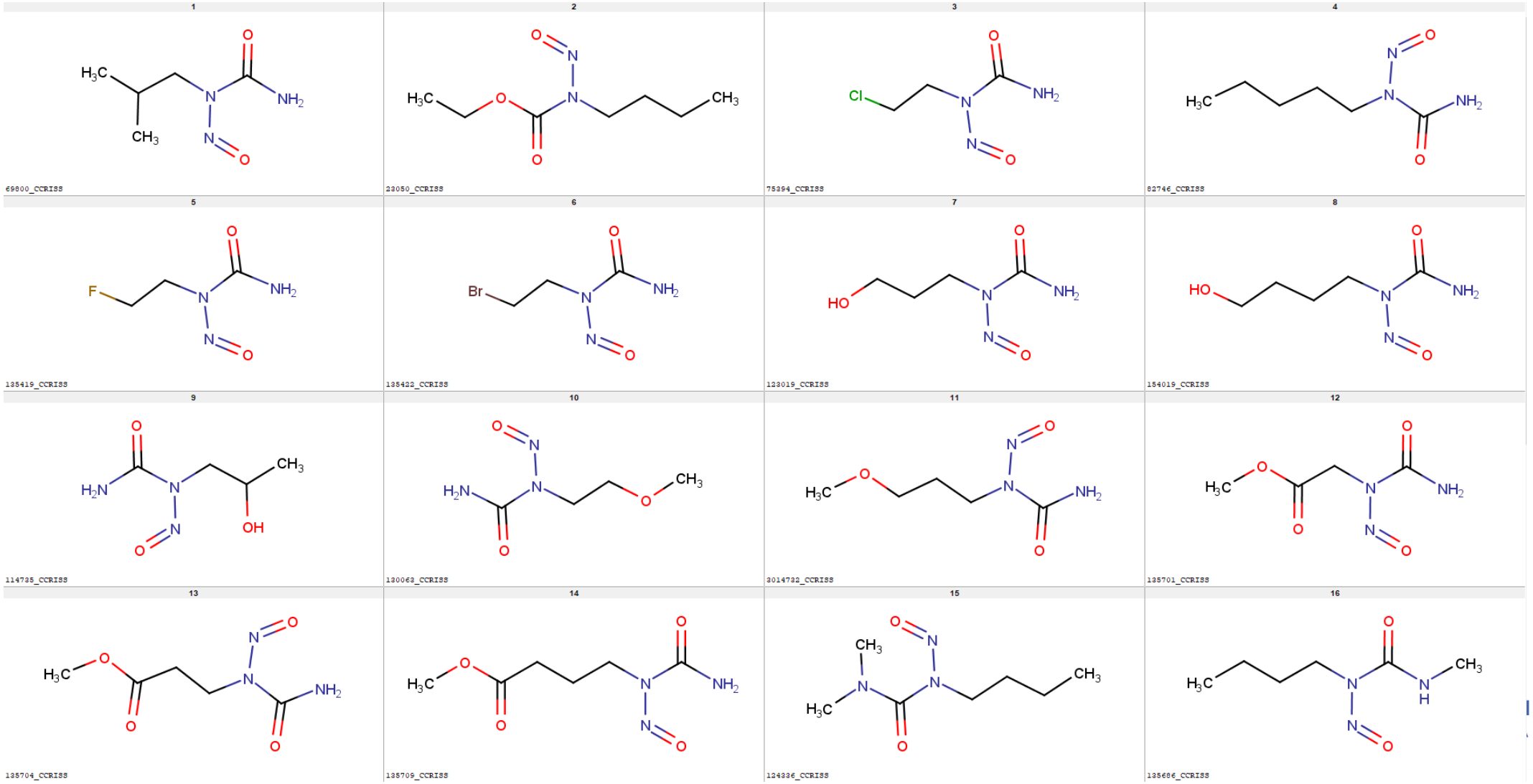
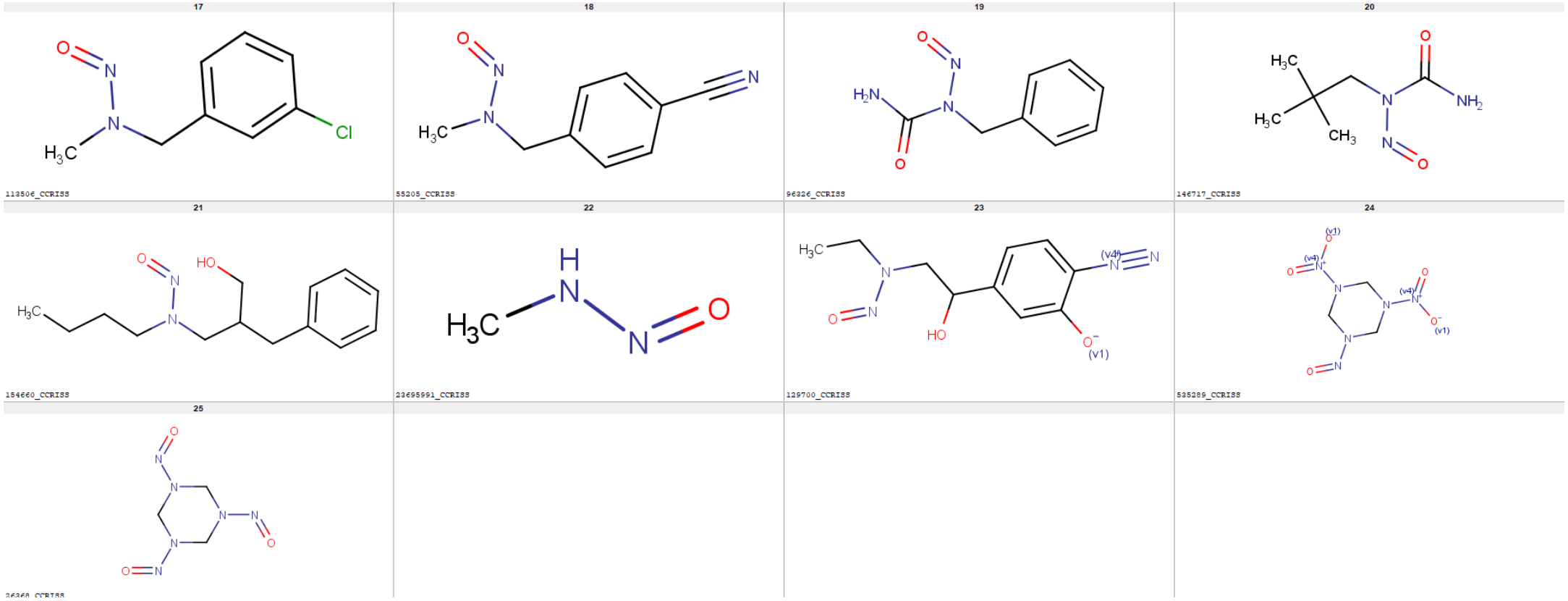
*Chemical Carcinogenesis Research Information System (CCRIS), National Cancer Institute (NCI), https://pubchem.ncbi.nlm.nih.gov/source/22070

Nitrosamines known as mutagenic (16/25)



Nitrosamines known as mutagenic (9/25)



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B – RULE-BASED QSAR

To predict mutagenic potential of impurities

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Example of Leadscope®

- Leadscope now part of Instem provides a genotox expert alert suite
- It is an expert rule-based system designed to support the ICH M7 guideline on impurities
- It complements the first statistical method by providing the structural alerts for mutagenicity that are molecular functional groups or substructures that were mainly derived from existing mechanistic knowledge of their link to the mutagenic activity of chemicals.

www.leadscope.com

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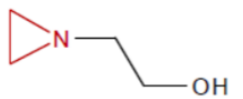
Expert rule-based QSAR

- Examples of positive and negative calls

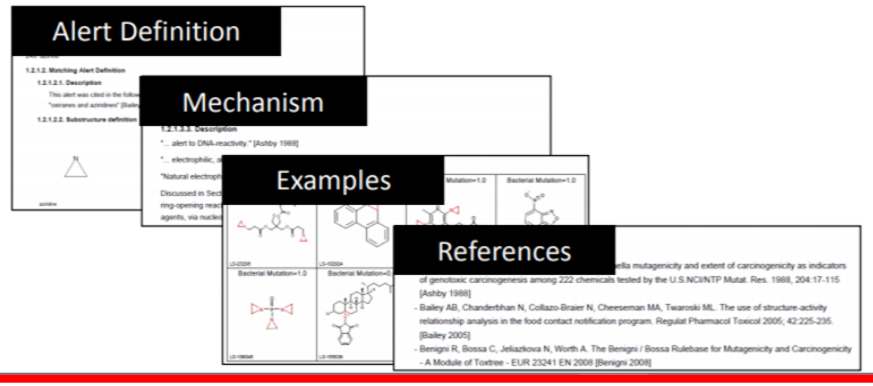
Test chemical



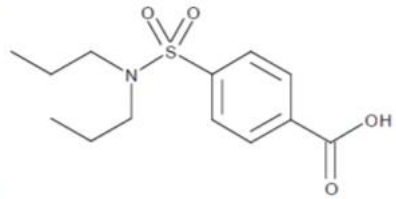
Results

Call:	**Positive**
Precision:	**0.97**
Matched Alert(s):	**24: aziridine**

Explanation "24: aziridine"



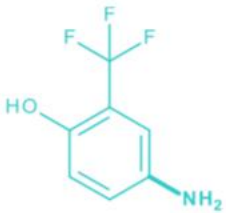
Test chemical



Results

Call:	**Negative**
Precision:	**0.15**
Matched Alert(s):	**No alerts**

Test chemical



Results

Call:	**Negative**
Precision:	**0.15**
Matched Alert(s):	**No alerts**

Source : from Leadscope data

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Concluding Remarks

To secure active pharmaceutical ingredients (APIs) and comply with regulatory requirements

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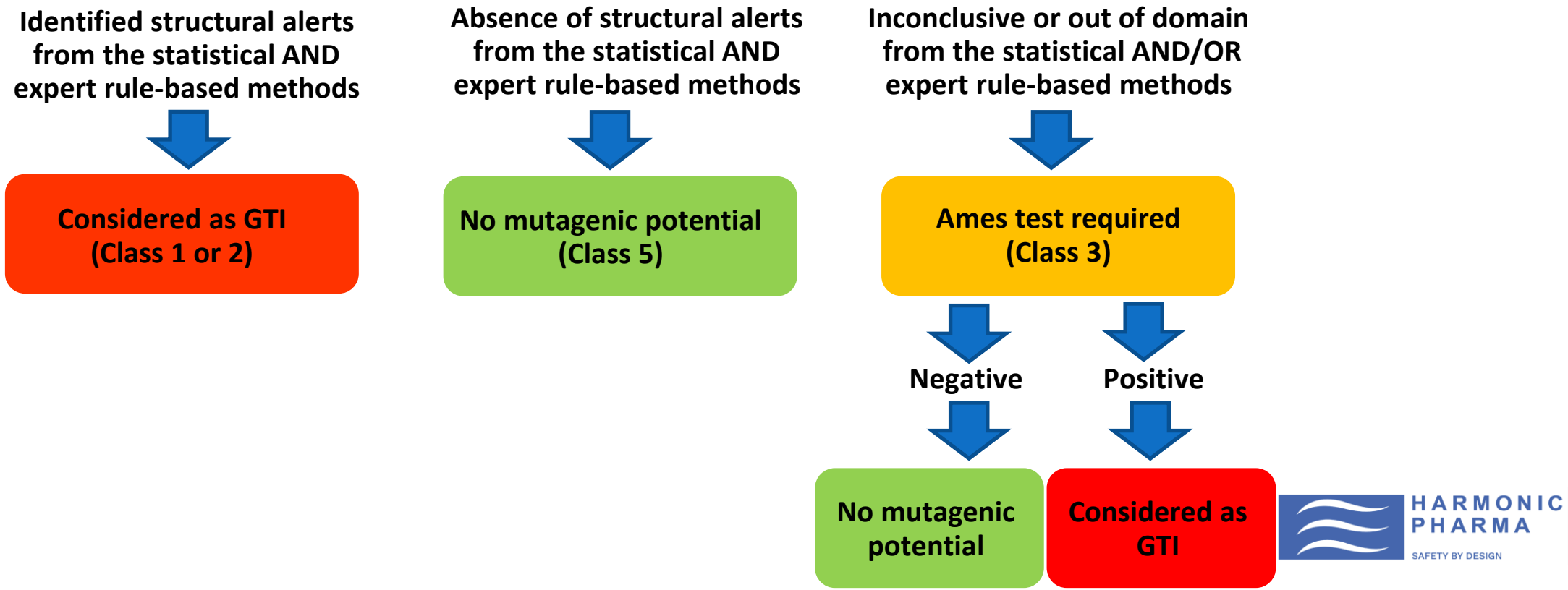
QSAR analyses for impurities

Overall, the two QSAR methodologies can be used for classifying nitrosamines and beyond i.e. they are suitable for diverse chemical structures.

The *in silico* analysis may be reviewed by an expert toxicologist to support the results with regards to ICH M7 assignment classes based on analogs search and bibliographic data.



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Integrated solution

As part of the Seqens'Lab ecosystem, we provide you with a suitable solution to investigate the mutagenic potential of impurities and secure your development according to ICH M7 guidelines

Identification of impurities along the synthesis of APIs

SEQENS 'Lab

https://www.seqens.com

in silico analysis from chemical structure of impurities

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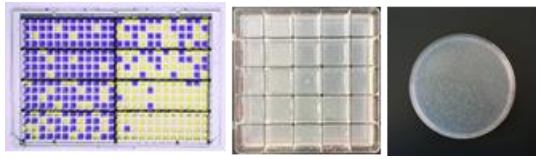
www.harmonicpharma.com

https://gti.harmonicpharma.com/

In vitro Ames test : Ames/NanoAMES – non GLP or GLP compliant

GENEVOLUTION



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