Mutagenic impurities: predicting alerting structures using *in silico* tools

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About Lhasa Limited

- Not-for-profit company and educational charity
- Established 1983, headquarters in Leeds

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- Not-for-profit company and educational charity
- Established 1983, headquarters in Leeds
- Purpose is to facilitate data and knowledge sharing in chemistry and the life sciences
- Develops database and *in silico* prediction systems
- Worldwide membership including academia, government agencies and industry

Lhasa Limited Products

Derek Nexus - Toxicity Prediction in Mammals and Bacteria

Meteor Nexus - Predicts the Metabolic Fate of Chemicals in Mammals

Vitic Nexus - Chemically Intelligent Toxicity Database

Zeneth - Predicting Forced Chemical Degradation Pathways

Sarah Nexus - (Q)SAR methodology to predict mutagenicity

Mirabilis - Assesses the relative purging of synthetic impurities

Summary

- Why are alerts sufficient to identify (or rule out) mutagenic impurities?
  - What evidence supports their usage?
- Using two complementary *in silico* systems
  - Expert and statistical
- The importance of expert review
- How *in silico* systems can assist further

ICH M7

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

ASSESSMENT AND CONTROL OF DNA REACTIVE (MUTAGENIC) IMPURITIES IN PHARMACEUTICALS TO LIMIT POTENTIAL CARCINOGENIC RISK

M7

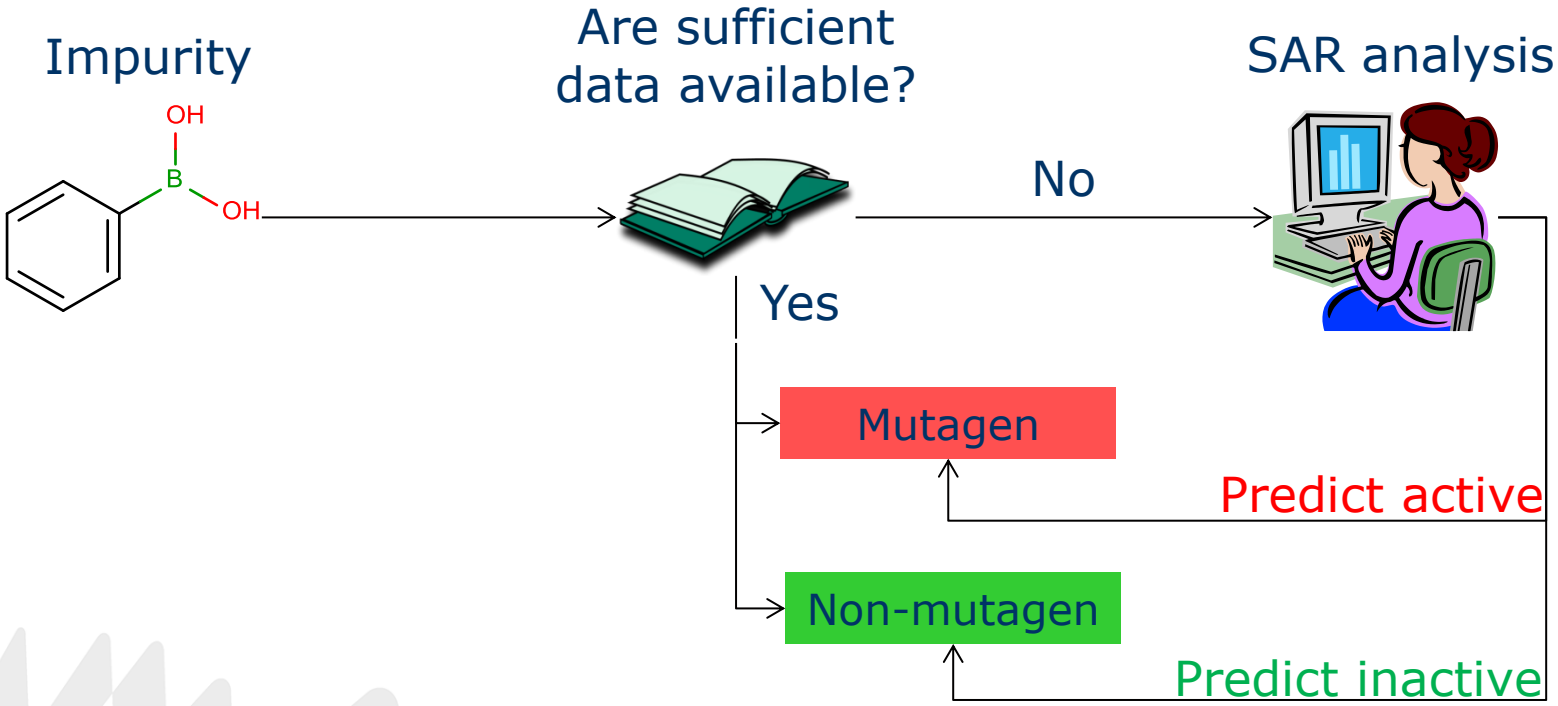
Current *Step 4* version

dated 23 June 2014

SAR Analysis in ICH M7

6. HAZARD ASSESSMENT ELEMENTS

Hazard assessment involves an initial analysis of actual and potential impurities by conducting database and literature searches for carcinogenicity and bacterial mutagenicity data in order to classify them as Class 1, 2, or 5 according to Table 1. If data for such a classification are not available, an assessment of Structure-Activity Relationships (SAR) that focuses on bacterial mutagenicity predictions should be performed. This could lead to a classification into Class 3, 4, or 5.

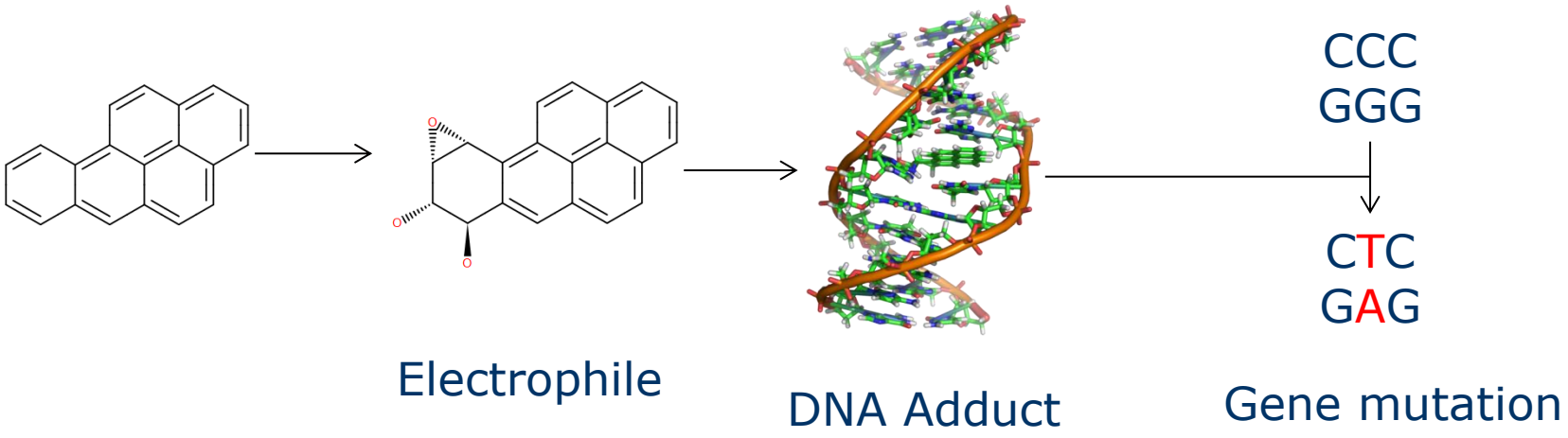


Acceptance of *in silico* mutagenicity predictions

- The hazard posed by mutagens has been well-characterised
- Exposure (and therefore risk) are relatively low
  - Impurities are present at low levels in pharmaceutical formulations

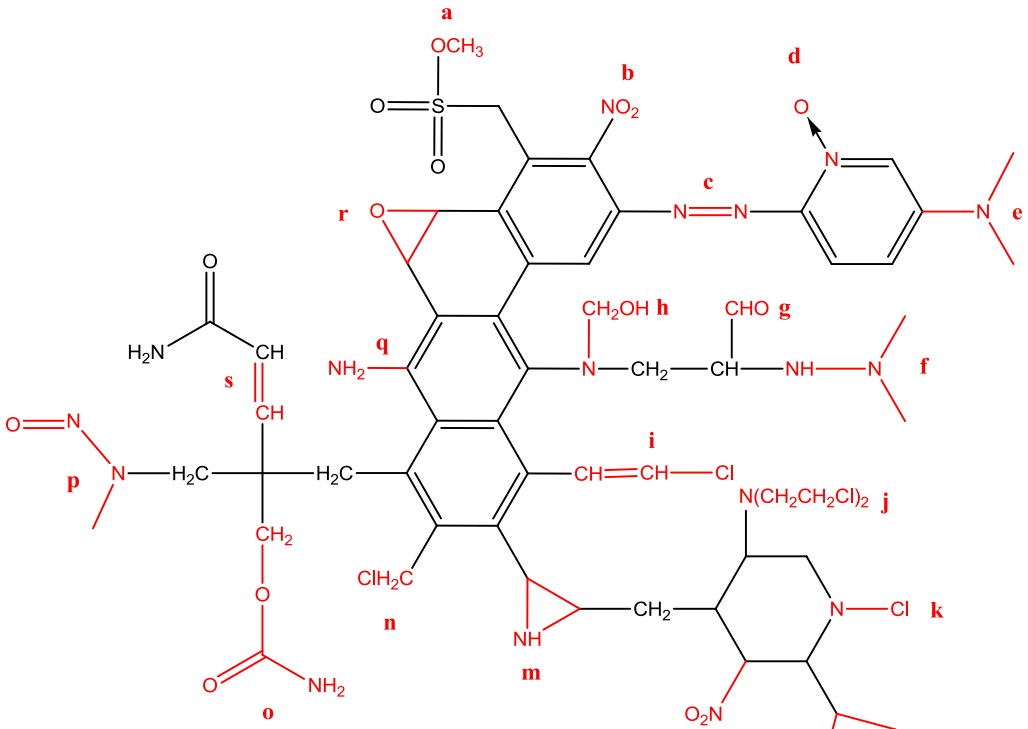
Hazard

- Mutagenicity is generally driven by a well understood molecular initiating event (MIE)



- Observed in a robust, reliable *in vitro* assay system (bacterial reverse mutation assay, aka the Ames test)

Predicting Hazard

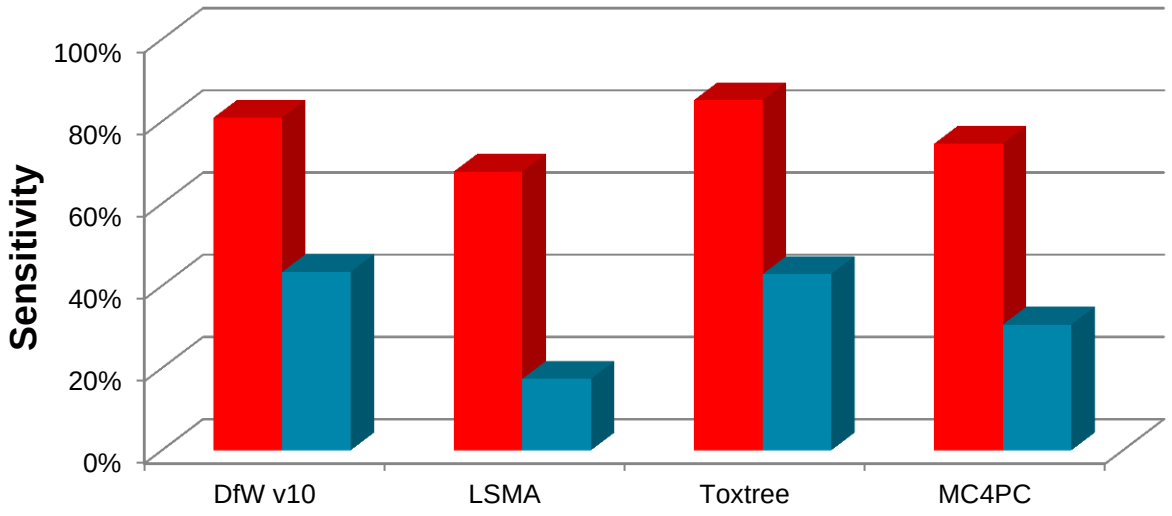


The Ashby-Tennant polycarcinogen

Tennant and Ashby

Predictive performance

- The performance of *in silico* mutagenicity models varies by data set
- Generally, models can predict data in public domain, but often struggle to predict proprietary data
  - Assisted by using proprietary data in models

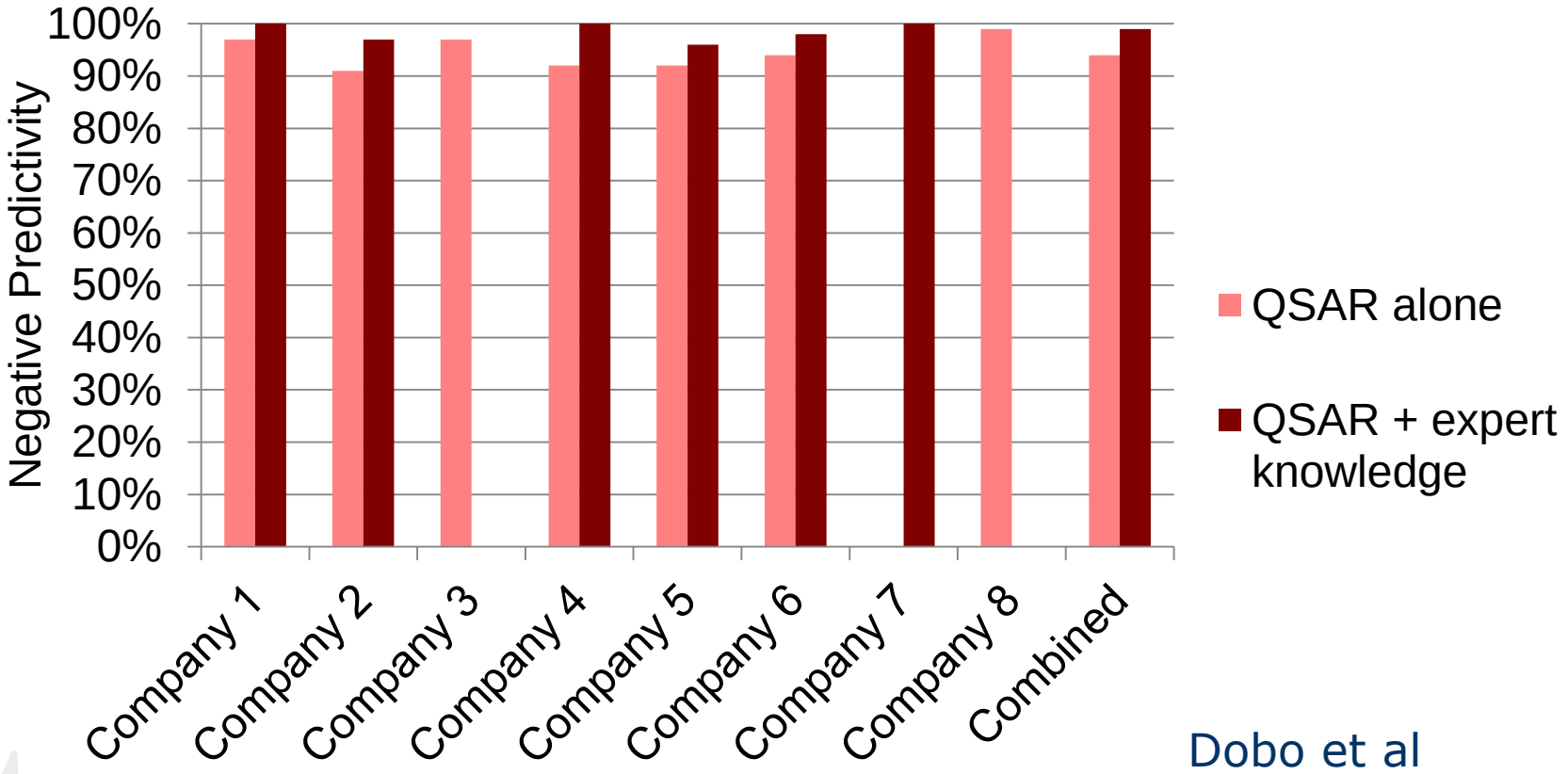


TP/(TP+FN)

Hillebrecht et al

Predictive performance for pharmaceutical impurities

- Impurities are more like well-predicted public compounds (than poorly predicted APIs)

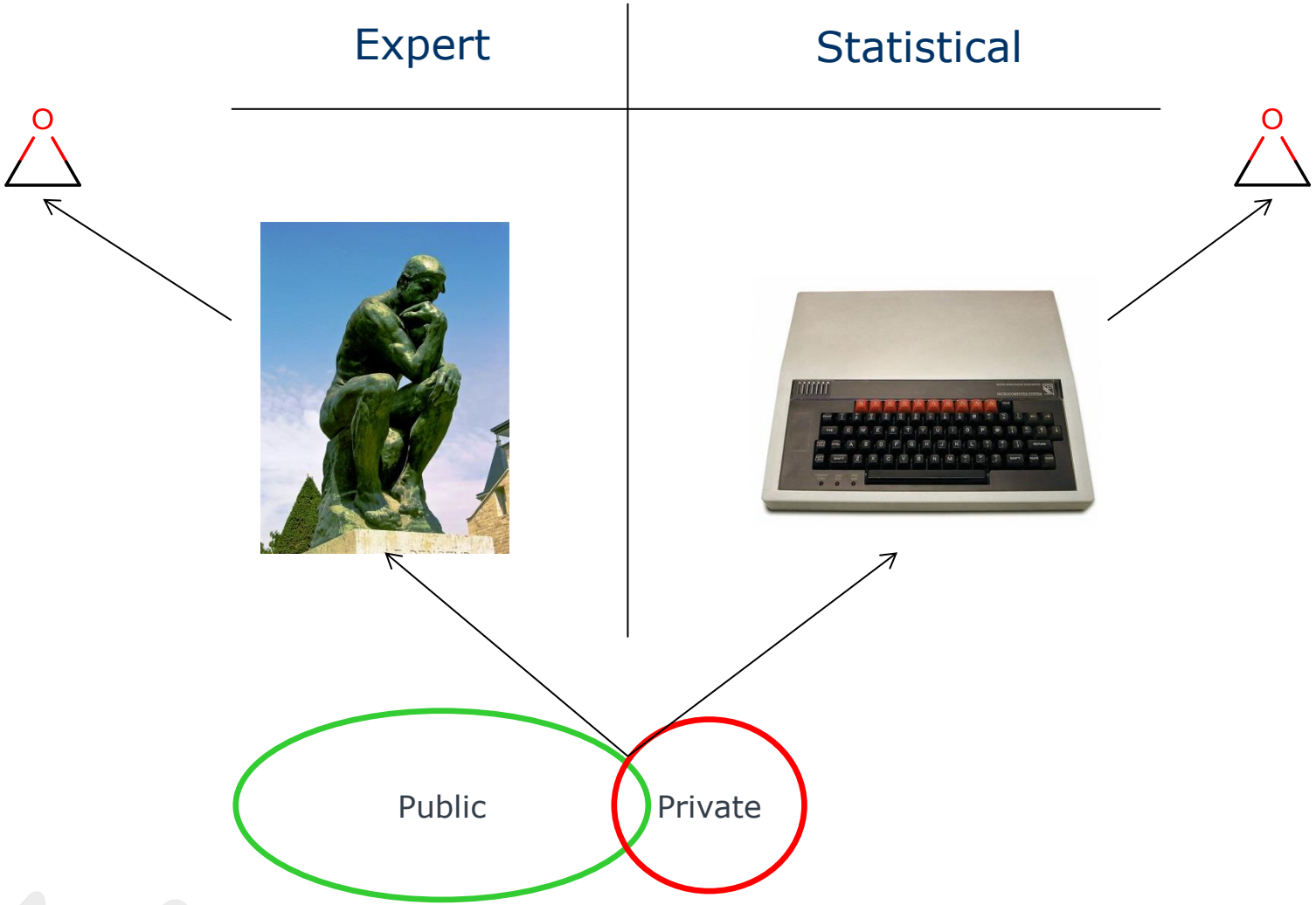


Dobo et al

Two systems required, one expert, one statistical

A computational toxicology assessment should be performed using (Q)SAR methodologies that predict the outcome of a bacterial mutagenicity assay (Ref. 6). Two (Q)SAR prediction methodologies that complement each other should be applied. One methodology should be expert rule-based and the second methodology should be statistical-based. (Q)SAR models utilizing these prediction methodologies should follow

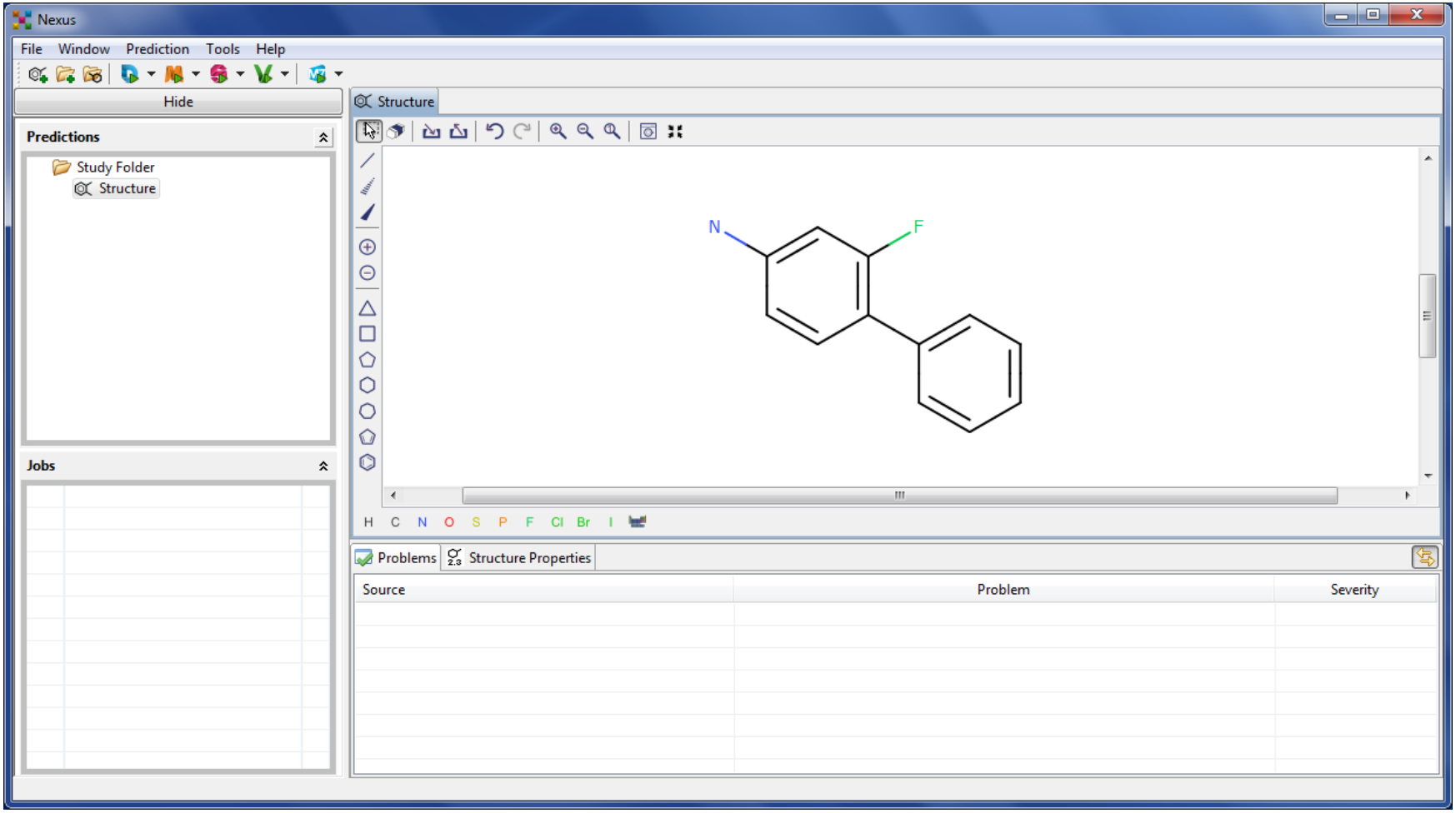
Expert and statistical systems



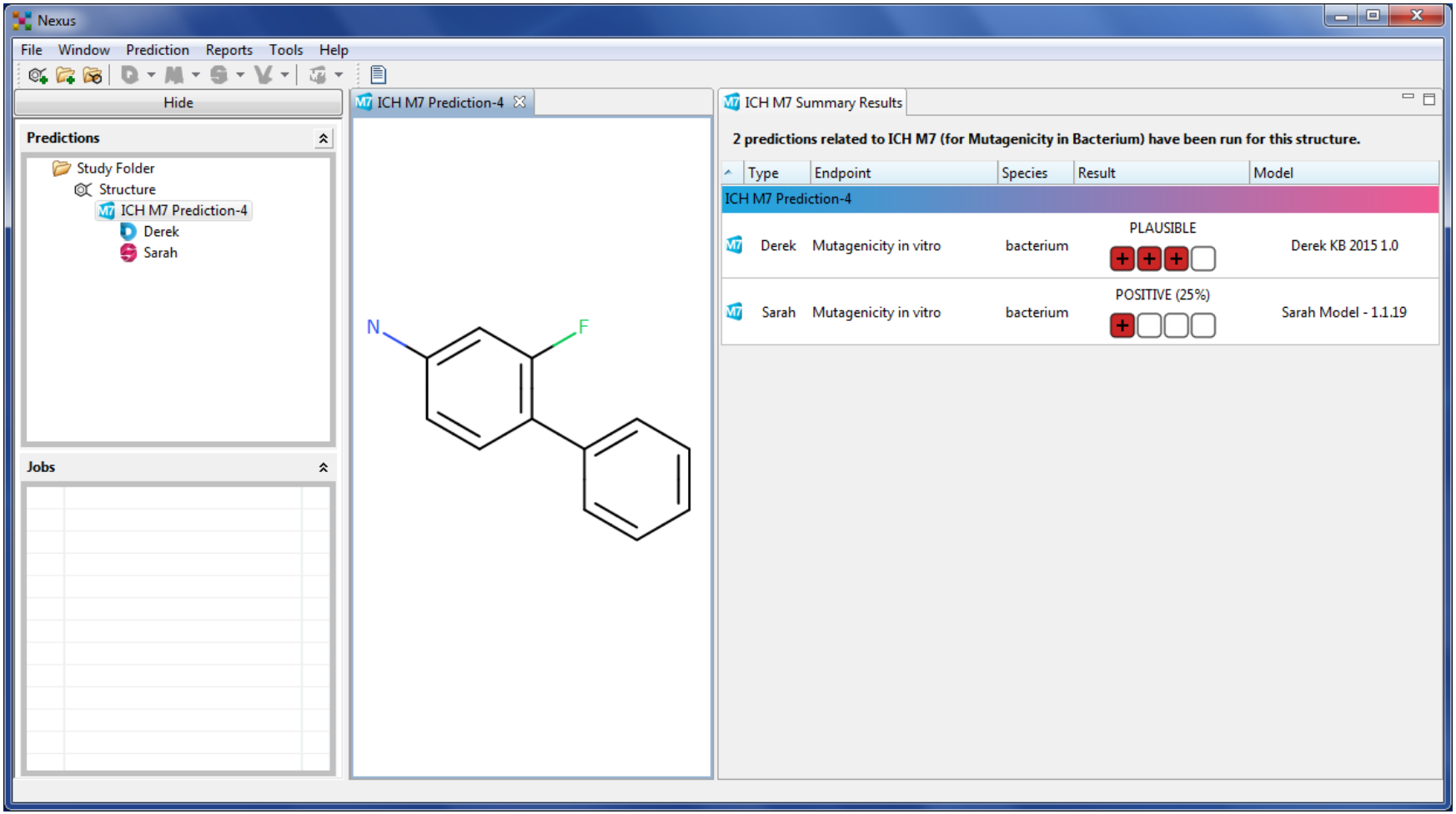
Commonly used *in silico* mutagenicity models

Sutter 2013

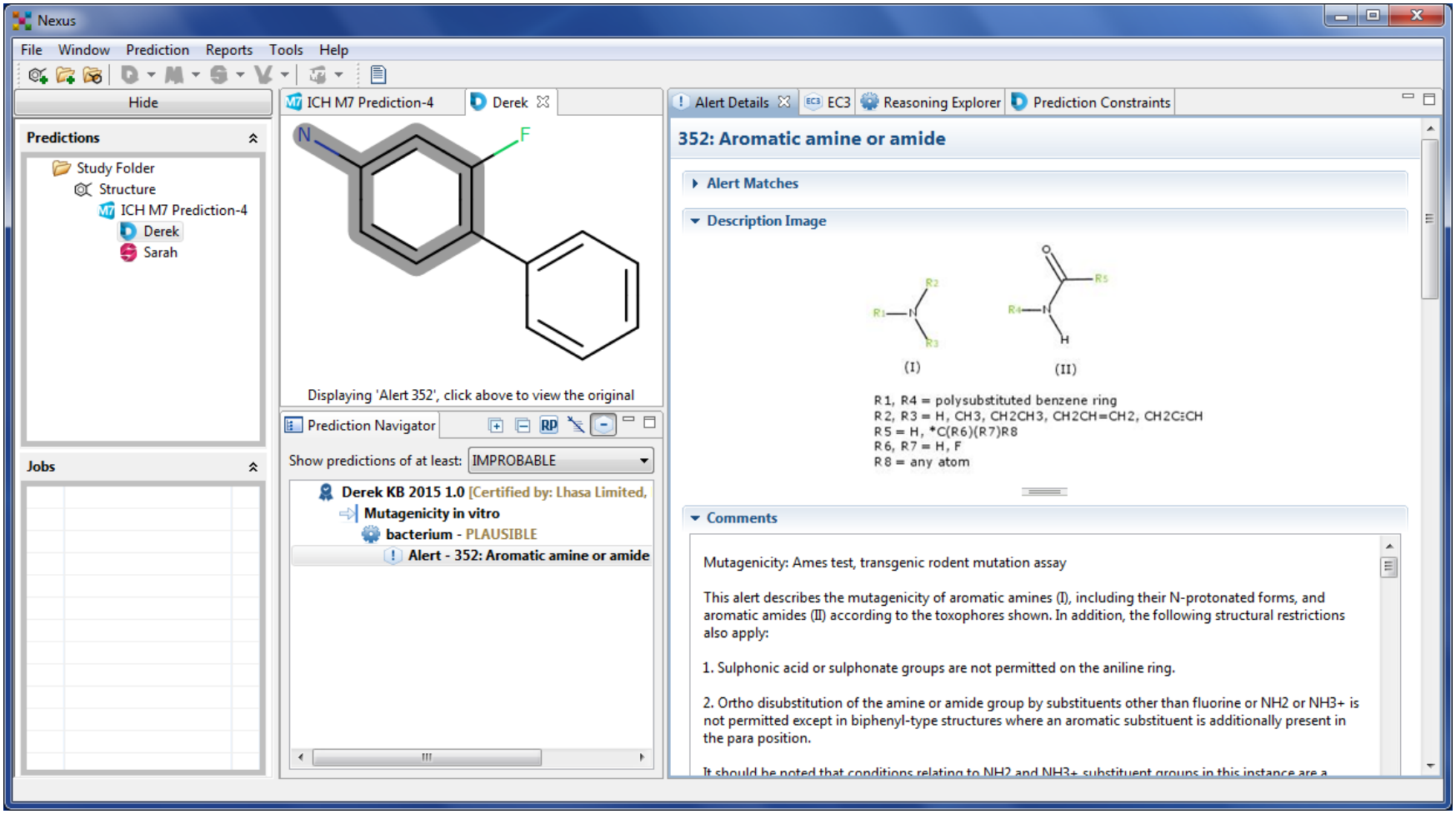
Using two systems



Using two systems



Expert system



Expert system

Comments

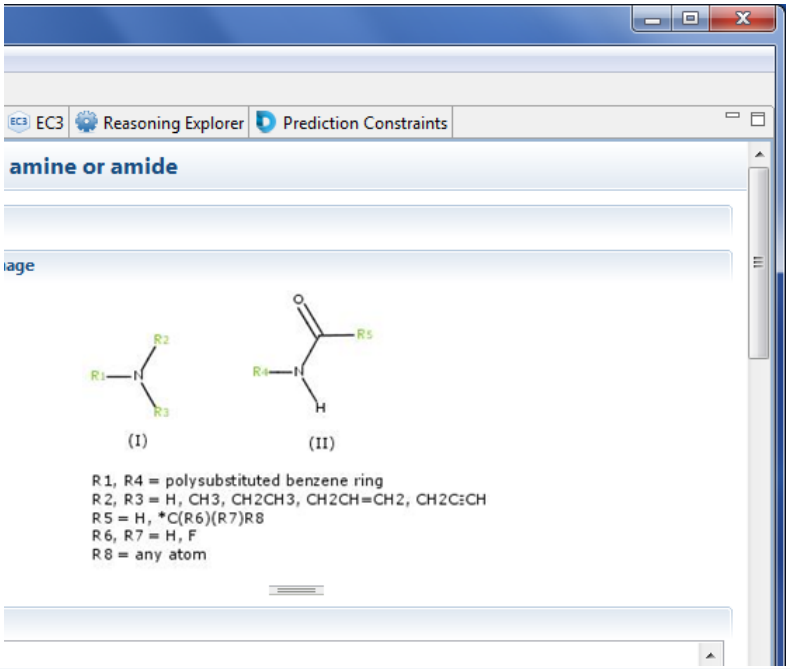
Many aromatic amines exhibit mutagenicity in the Ames test, notably in Salmonella typhimurium strains TA98 and TA100 in the presence, but not absence, of S9 mix [Debnath et al, Benigni et al 1998, Benigni et al 1994]. The mechanism of action is generally considered to involve N-hydroxylation, typically mediated by cytochrome P450 1A2, and subsequent O-esterification [Colvin et al]. The resulting esterified product may then give rise to a reactive nitrenium ion which is capable of binding to cellular nucleophiles such as DNA.

Secondary aromatic amides may likewise exhibit activity in the Ames test in Salmonella typhimurium strains TA98 and TA100 in the presence of S9 mix [Trieff et al]. In such cases, deacylation of the amide may take place prior to N-hydroxylation.

The basis for the structural restrictions of the current alert may be summarised as follows:

1. Although non-polycyclic aromatic amines generally exhibit a lower mutagenic potency than polycyclic aromatic amines [Benigni et al 1998], a literature survey suggests that polysubstituted anilines with at least one free ortho position are generally mutagenic in the Ames test [Kugler-Steigmeier et al]. Aromatic amides are in general less mutagenic than their corresponding primary aromatic amines, possibly as a result of the need for initial N-deacylation to take place prior to N-hydroxylation [Trieff et al].

2. In order for secondary or tertiary aromatic amines to exhibit mutagenic activity, N-dealkylation must first occur in order to allow bioactivation of the amine to take place [Lai et al]. It has been demonstrated that N-methyl and N-ethyl substituents are more readily cleaved by N-dealkylation than larger alkyl groups [Testa], and it is therefore assumed that secondary and tertiary aromatic amines which bear such small alkyl substituents are more likely to exhibit mutagenic activity than those in which larger N-alkyl substituents are present. This is consistent with the view of Ashby and Tennant that aromatic amines with N-alkyl substituents of three or more carbons in size should not be considered structurally alerting for DNA reactivity [Ashby and Tennant]. In addition to saturated methyl and ethyl groups, small unsaturated substituents such as allyl and propargyl are also readily removed by N-dealkylation [Testa], and are therefore also considered within the scope of the alert. It should be noted that by restricting the substituent types permitted on the aromatic amine nitrogen in this way, structures in which the aromatic amine nitrogen is contained within a ring are effectively discounted. This is supported by several reports of aromatic amine mutagenicity which has been reduced or eliminated by confining the aromatic amine nitrogen to a ring [Ashby et al 1983, Ashby et al 1982].



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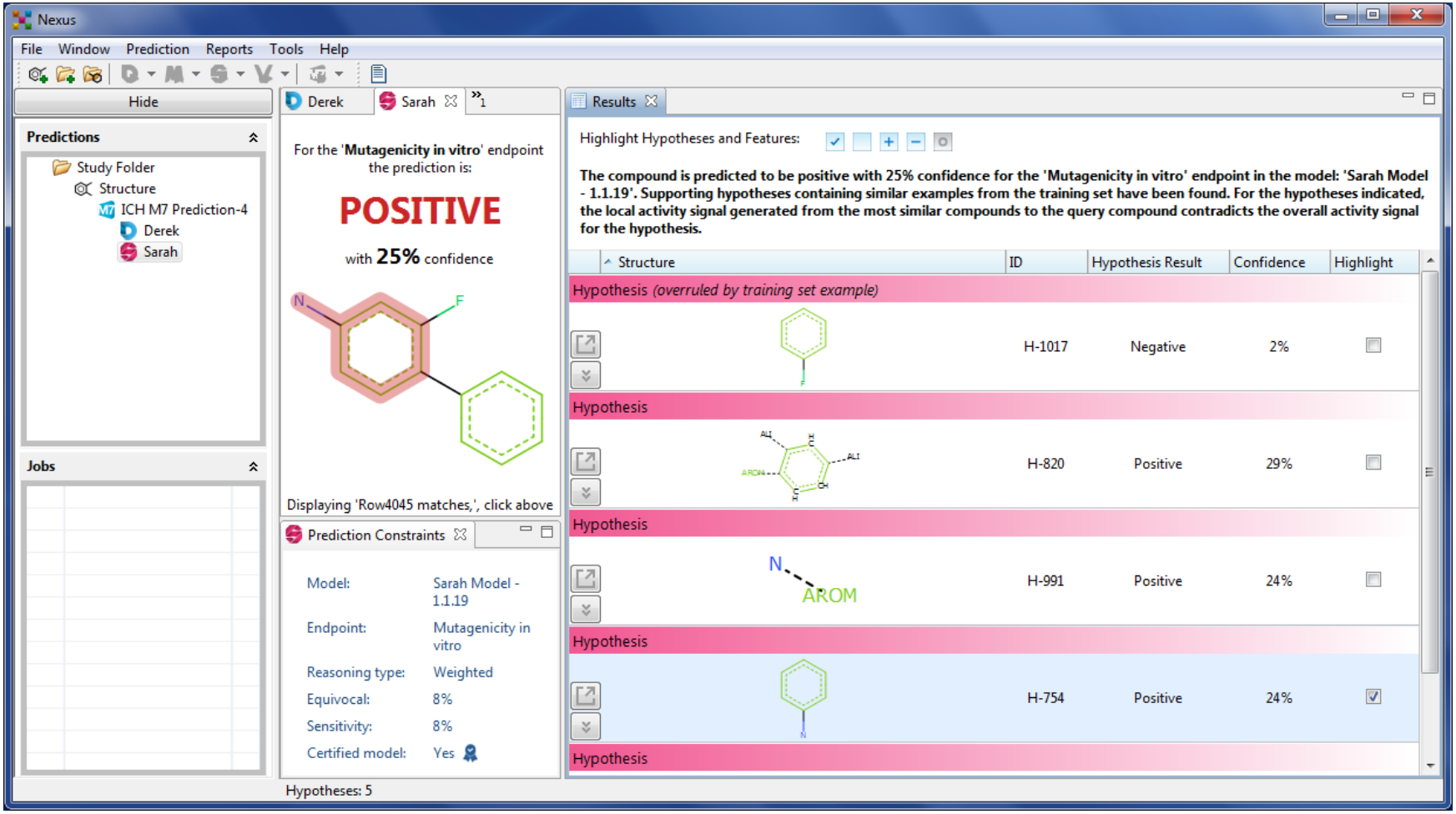
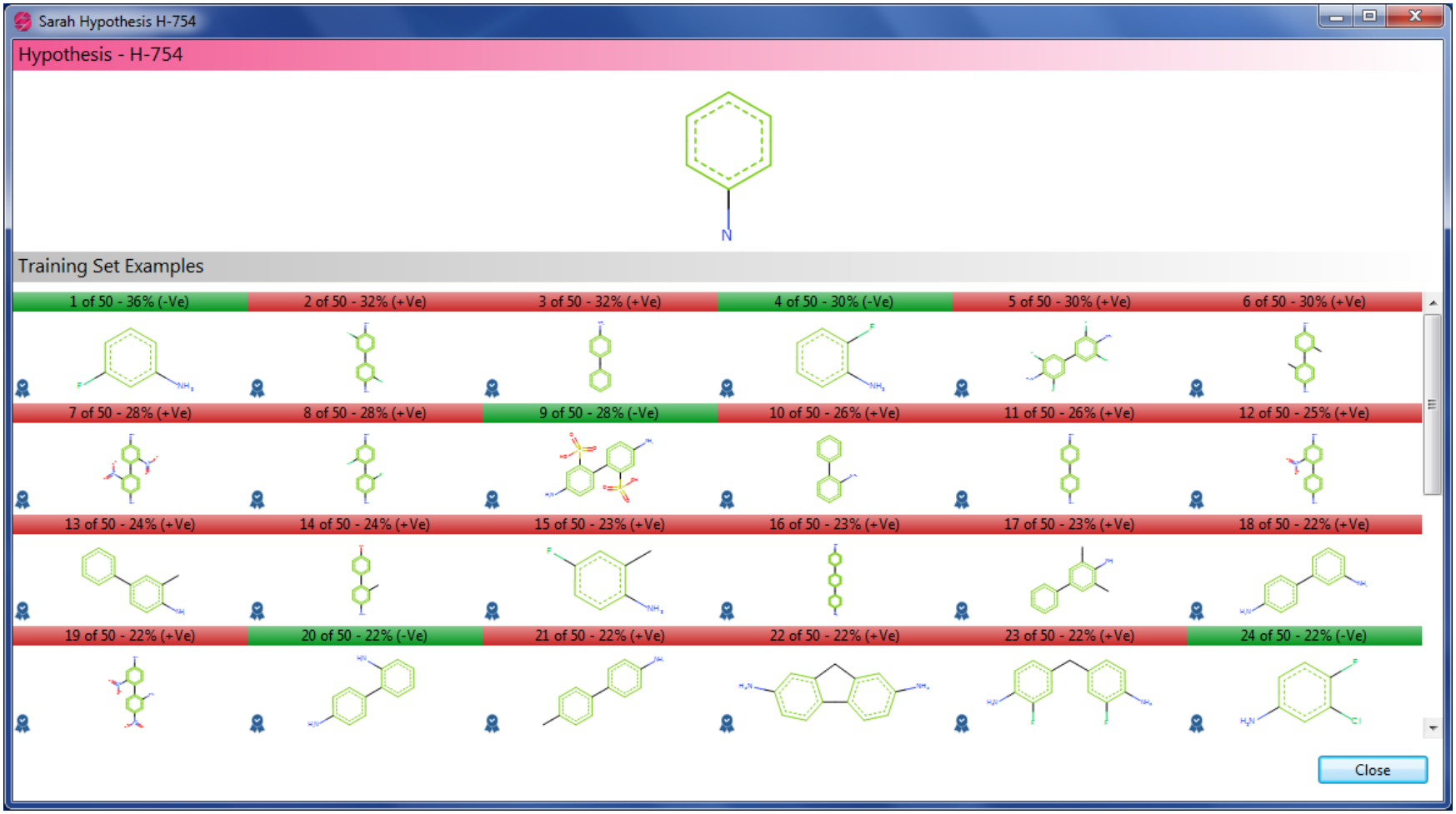
ID	Title	Author
467	Salmonella mutagenicity tests: V. Results from the testin	Zeiger E, Anderson B, Haworth S, Lawlor T ar
468	Salmonella mutagenicity tests: III. Results from the testin	Zeiger E, Anderson B, Haworth S, Lawlor T, M
470	Salmonella mutagenicity test results for 250 chemicals.	Haworth S, Lawlor T, Mortelmans K, Speck W
470	Salmonella mutagenicity tests: IV. Results from the testin	Zeiger E, Anderson B, Haworth S, Lawlor T ar
471	Chemical structure, Salmonella mutagenicity and extent	Ashby J and Tennant RW.
474	Genotoxicity of aniline derivatives in various short-term t	Kugler-Steigmeier ME, Friederich U, Graf U, L
475	Evaluation of two suggested methods of deactivating or	Ashby J, Paton D, Lefevre PA, Styles JA and R
475	Aromatic amines and acetamides in Salmonella typhimu	Trieff NM, Biagi GL, Ramanujam VMS, Conn
492	Monooxygenase-catalyzed N-C cleavage.	Testa B.
492	Cancer risk reduction through mechanism-based molec	Lai DY, Woo YT, Argus MF and Arcos JC.
493	Cyclic amines as less mutagenic replacements for dimetl	Ashby J, Paton D and Lefevre PA.
500	A QSAR investigation of the role of hydrophobicity in reg	Debnath AK, Debnath G, Shusterman AJ and
501	QSAR models for both mutagenic potency and activity: a	Benigni R, Andreoli C and Giuliani A.
501	QSAR models for discriminating between mutagenic and	Benigni R, Passerini L, Gallo G, Giorgi F and C

Statistical results



Statistical results

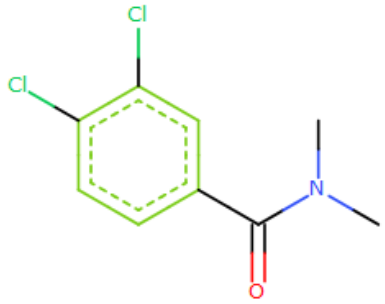


Possible outcomes from Sarah Nexus

For the '**Mutagenicity in vitro**' endpoint the prediction is:

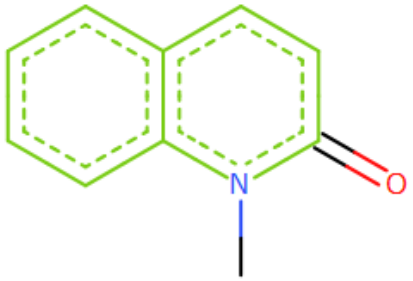
NEGATIVE

with **42%** confidence



For the '**Mutagenicity in vitro**' endpoint the prediction is:

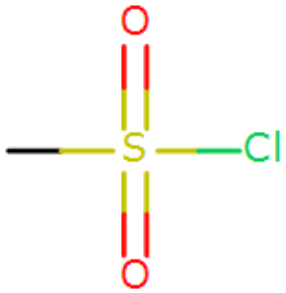
EQUIVOCAL



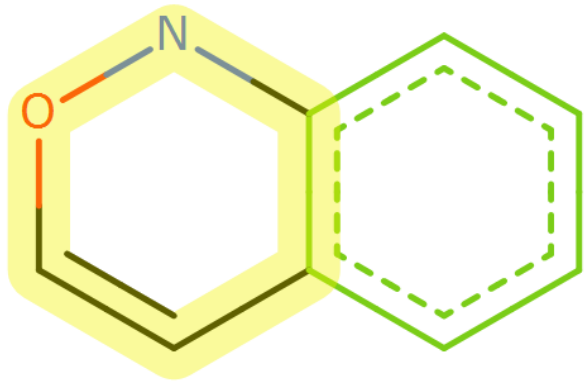
For the '**Mutagenicity in vitro**' endpoint the prediction is:

POSITIVE

with **100%** confidence

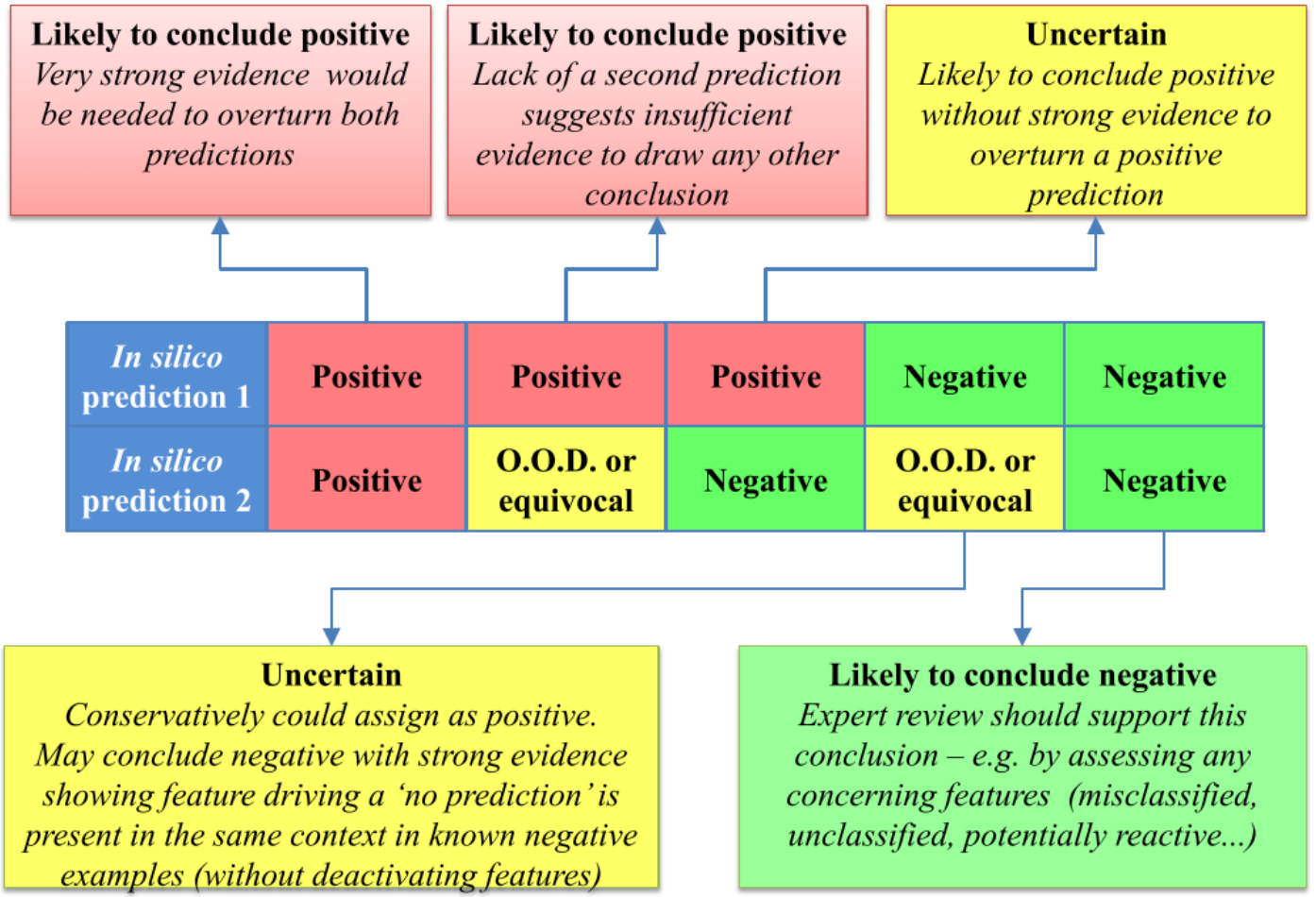


OUTSIDE DOMAIN



Displaying 'outside domain features,', click above to view the original structure

Combining two systems



O.O.D. = out of domain

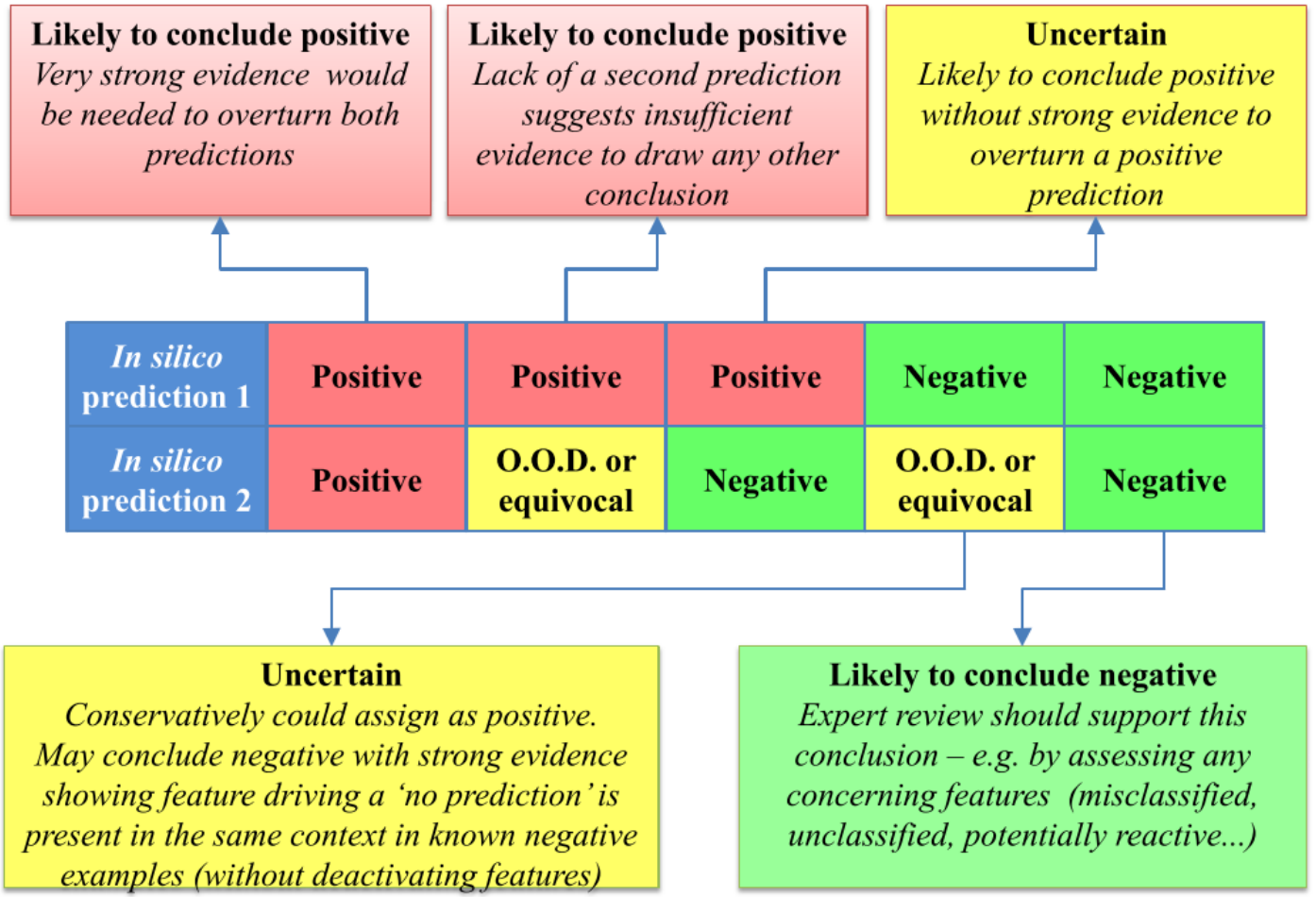
Barber et al

Expert review of *in silico* predictions

- M7 guidelines
  - “If warranted, the outcome of any computer system-based analysis can be reviewed with the use of expert knowledge..”

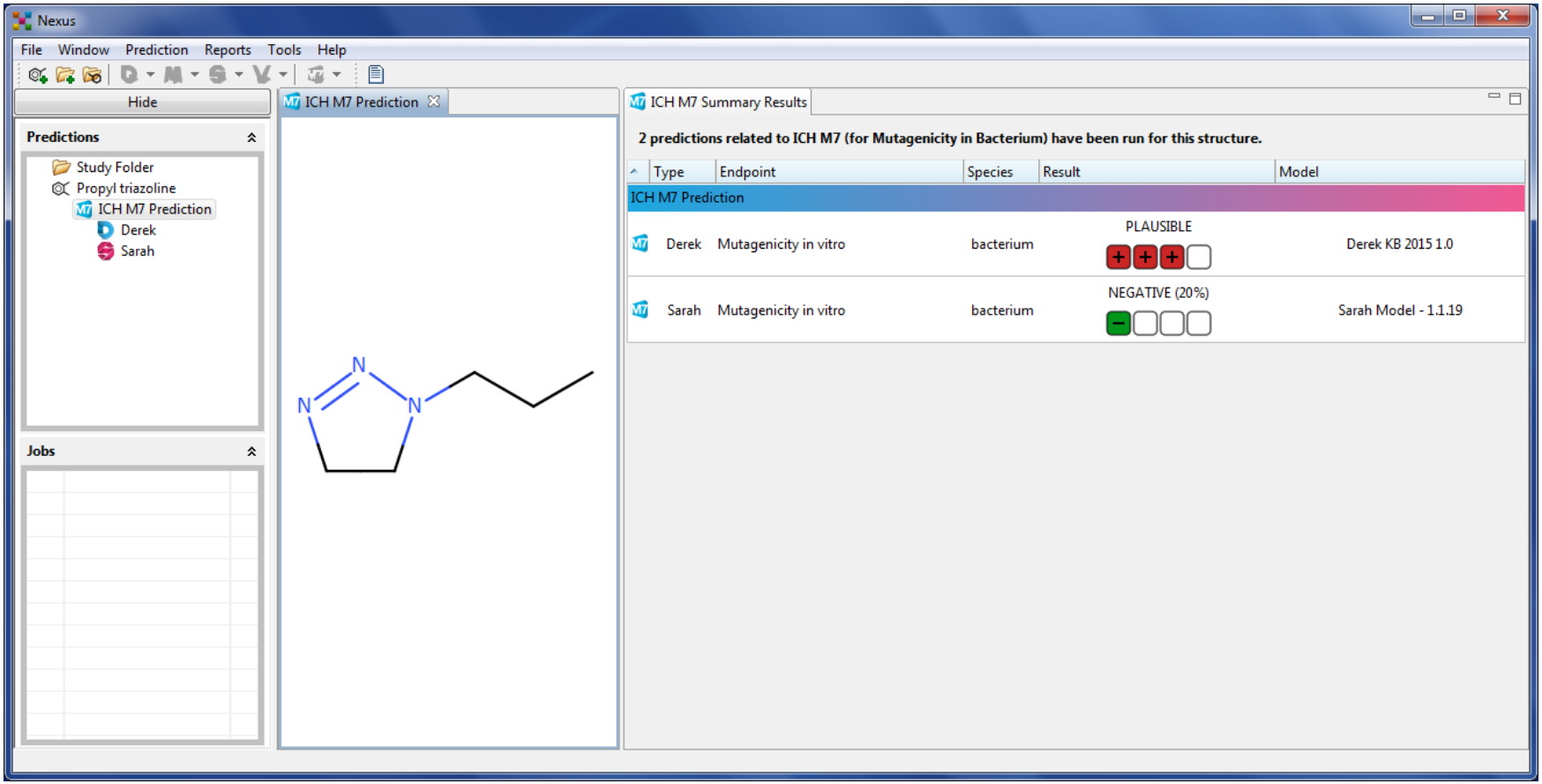
- We recommend that you always do some review….
  - Be guided by the software and your knowledge…
    - Use confidence measures if they are proven to indicate accuracy
    - Use software that is transparent and highlights areas of concern
    - Use the expert commentary, mechanism and references
    - Look at relevant close examples from the models & databases
  - Depth of your analysis and the detail you report will vary
    - …from a cursory analysis to a well-supported argument

Combining two systems

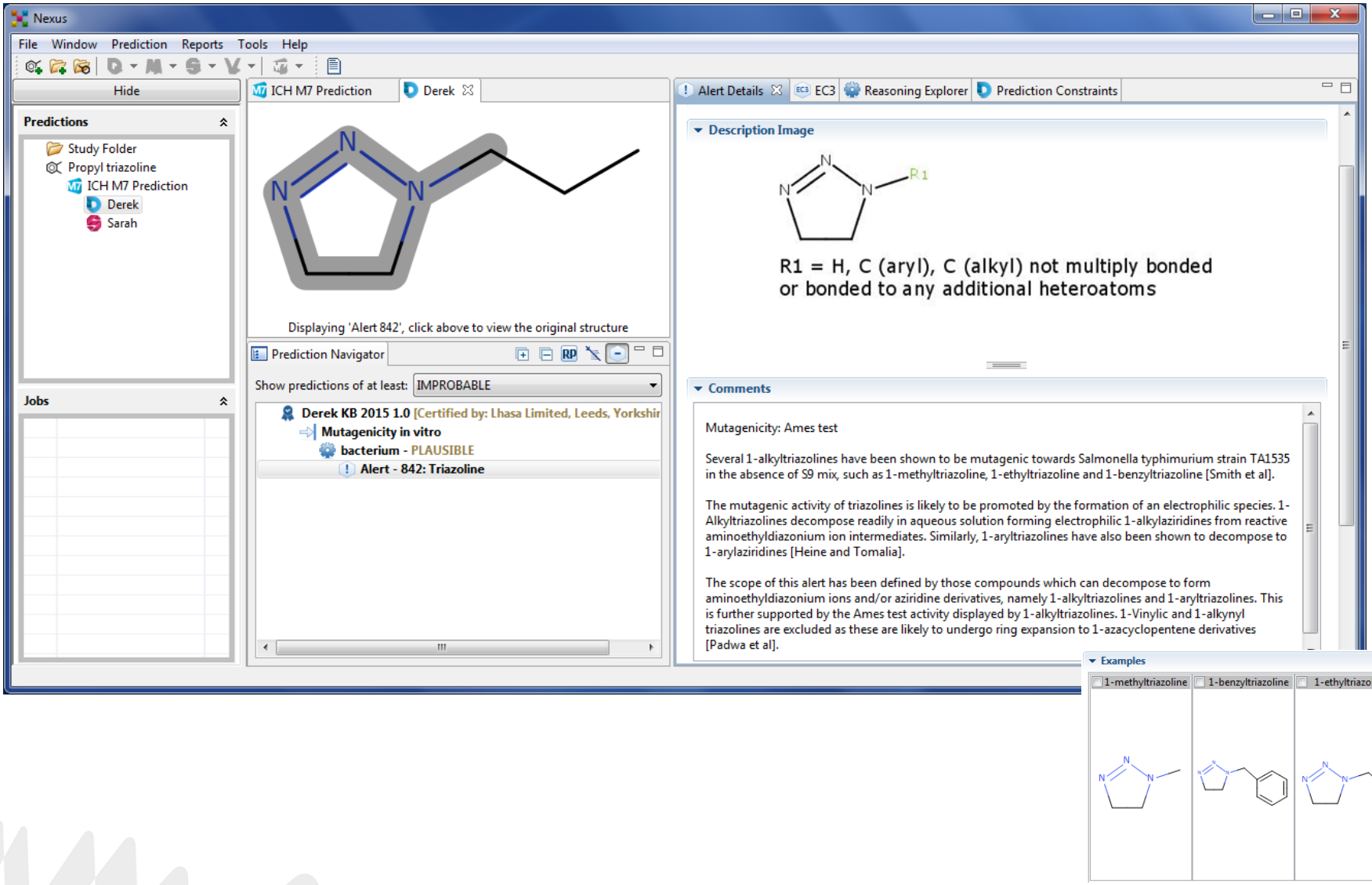


O.O.D. = out of domain

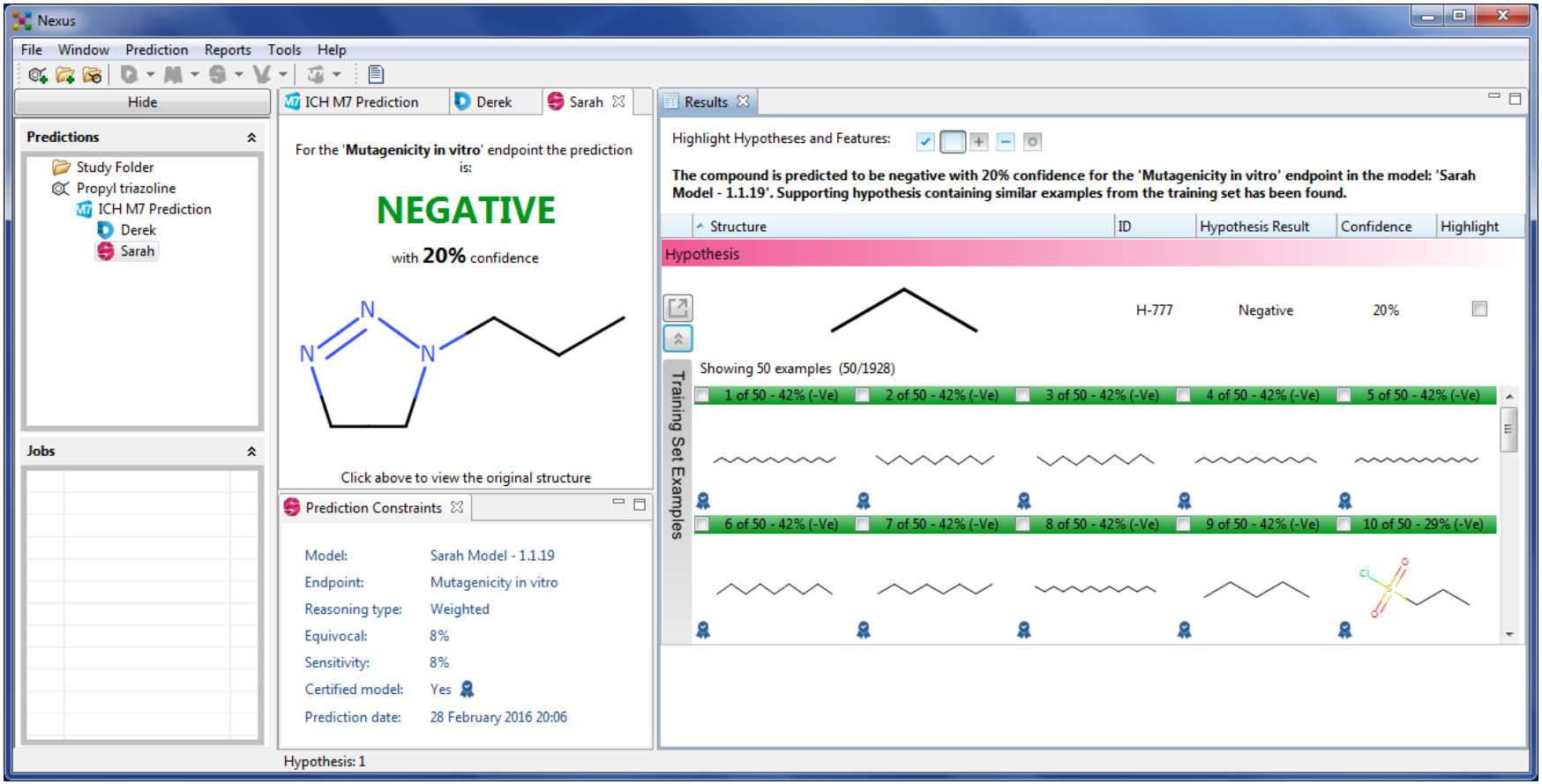
Example – propyl triazoline



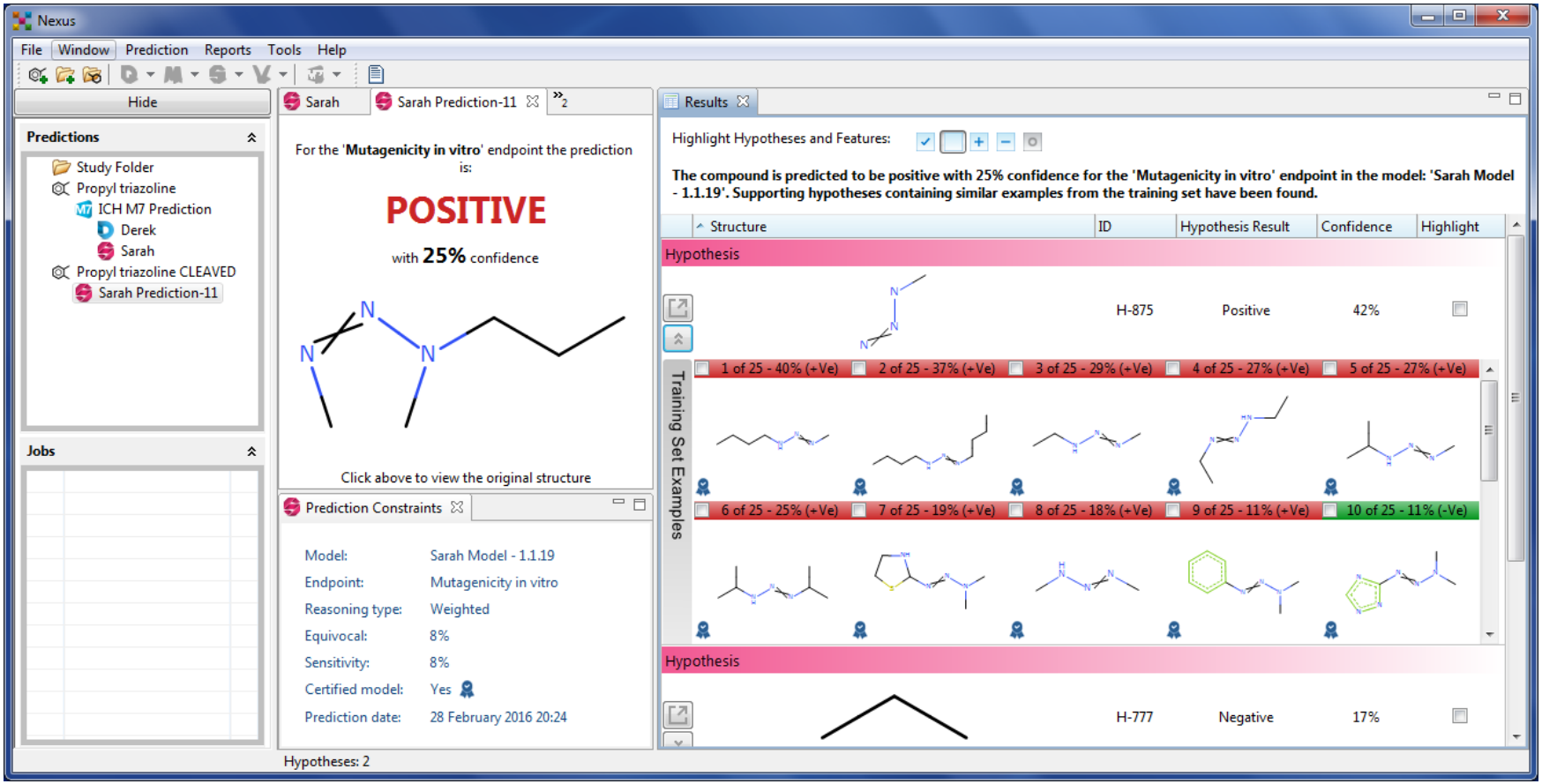
Example – propyl triazoline (Derek)



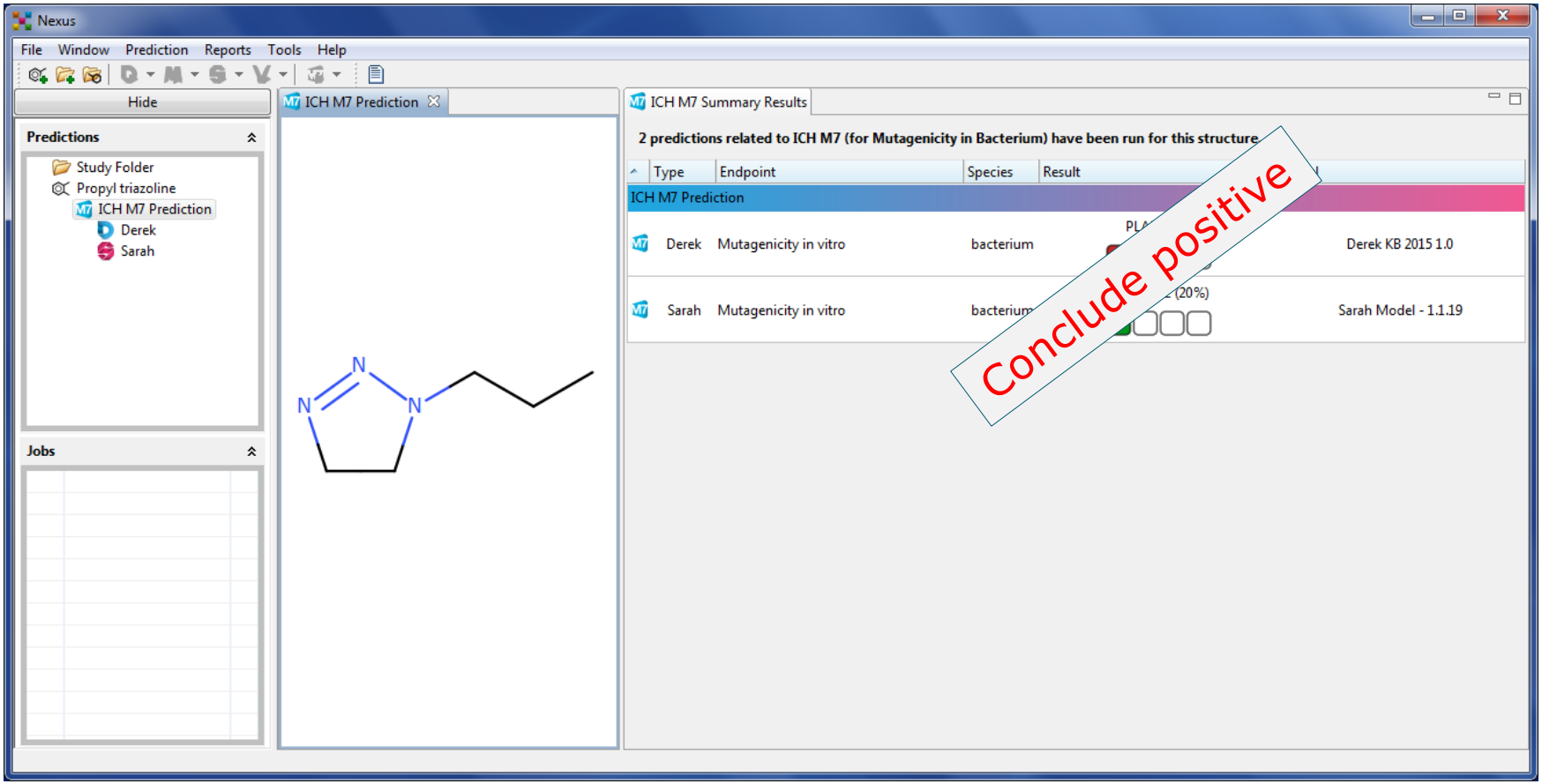
Example – propyl triazoline (Sarah)



Example – propyl triazoline analogue (Sarah)



Example – propyl triazoline conclusion



Summary so far

- Theoretical and empirical evidence supports the use of *in silico* toxicology tools for the genotoxicity risk assessment of pharmaceutical impurities
  - The usage is enshrined in ICH M7 guidance
- The guidance indicates that two complimentary tools should be used (with expert review, as required)
  - Increases probability that mutagens will not be missed
  - Leads to a multiplicity of outcomes
    - Human expert review becomes more important with higher levels of computational uncertainty

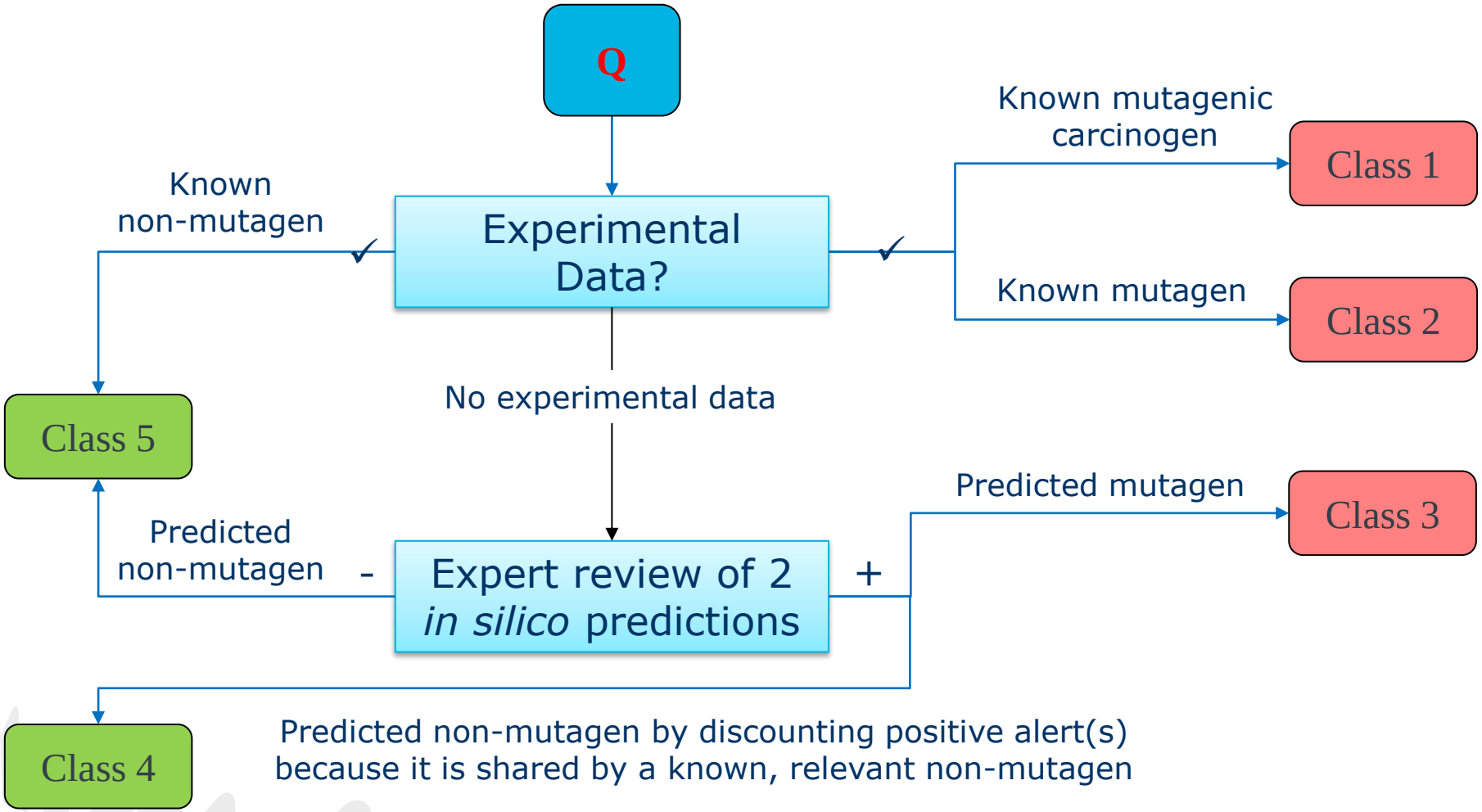
ICH M7 Classifications

Class	Definition
1	Known mutagenic carcinogens.
2	Known mutagens with unknown carcinogenic potential (bacterial mutagenicity positive, no rodent carcinogenicity data).
3	Alerting structure, unrelated to the structure of the drug substance, no mutagenicity data.
4	Alerting structure, same alert in drug substance which have been tested and are non-mutagenic.
5	No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity.

M7 Classification

http://www.ich.org/products/guidelines/multidisciplinary/article/multidisciplinary-guidelines.html

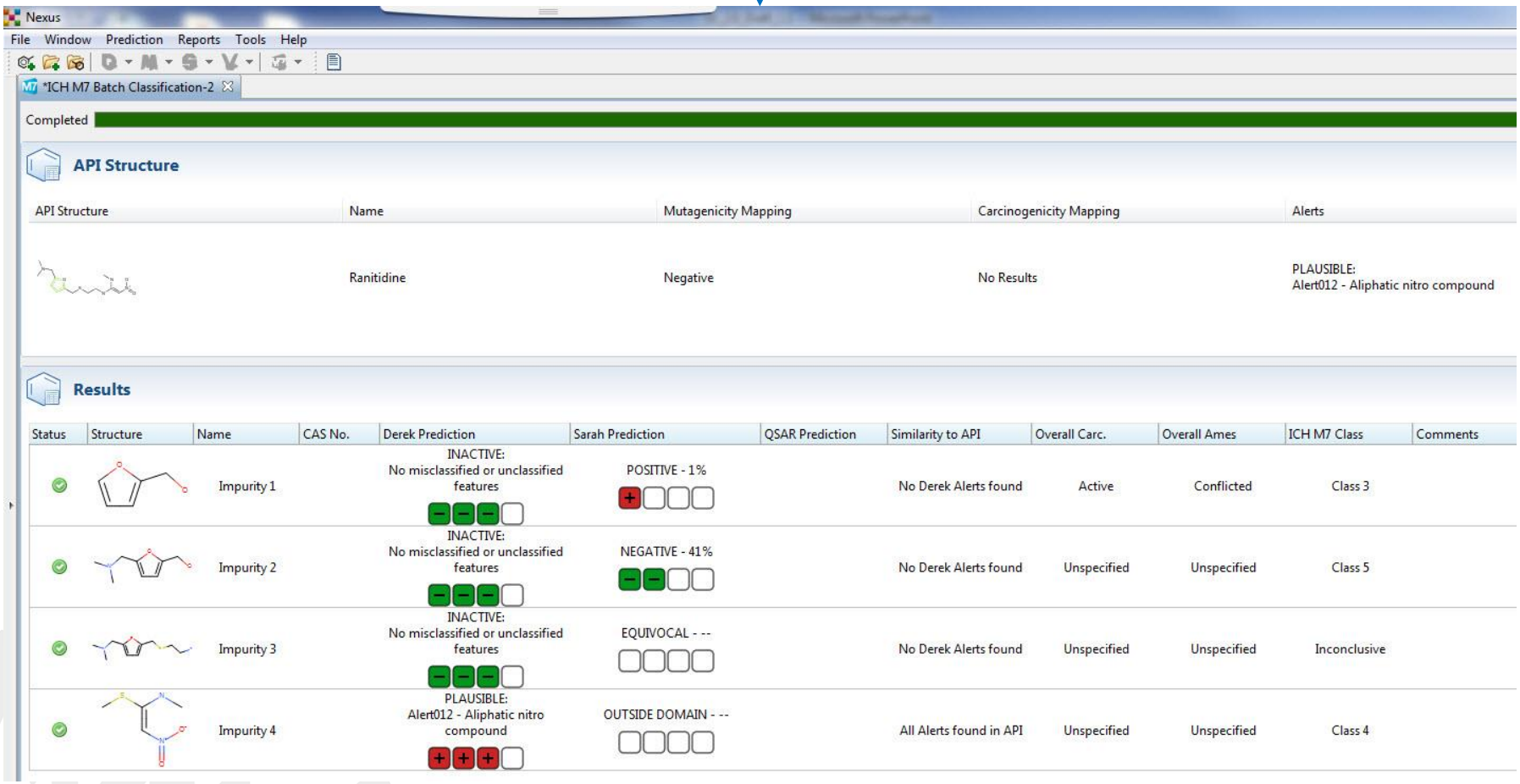
- M7 classification helps define how to control impurities…



Batch process against M7 settings

.sdf file
API impurities

- User can add additional data
- Searches for carc' and mut' data from Lhasa and custom database



ICH M7 class generated and report produced

Overall Carc.	Overall Ames
Active	Conflicted
Unspecified	Unspecified
Unspecified	Unspecified
Unspecified	Unspecified

ICH M7 Class
Class 3
Class 5
Inconclusive
Class 4

CPDB Carc.	Lhasa Ames	User Carcinogenicity	User Ames
Single-Cell Activity: Active Multi-Cell Activity: Active	Conflicted	Unspecified	Unspecified
Unspecified	Unspecified	Unspecified	Unspecified
Unspecified	Unspecified	Unspecified	Unspecified
Unspecified	Unspecified	Unspecified	Unspecified

Each impurity is classified according to whether there is Ames or Carcinogenicity information in addition to the Derek and Sarah Predictions

Users can also input experimental results for mutagenicity or carcinogencity which updates the ICH M7 Class

Conclusion

- Theoretical and empirical evidence supports the use of *in silico* toxicology tools for the genotoxicity risk assessment of pharmaceutical impurities
  - The usage is enshrined in ICH M7 guidance
- The guidance indicates that two complimentary tools should be used (with expert review, as required)
  - Increases probability that mutagens will not be missed
  - Leads to a multiplicity of outcomes that needs to be resolved by human expert
- The scope of these tools will increase to cover more of the workflow and assist expert review

References

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