

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

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Leaders in the development of expert chemoinformatic systems
and trusted curators of proprietary data.


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

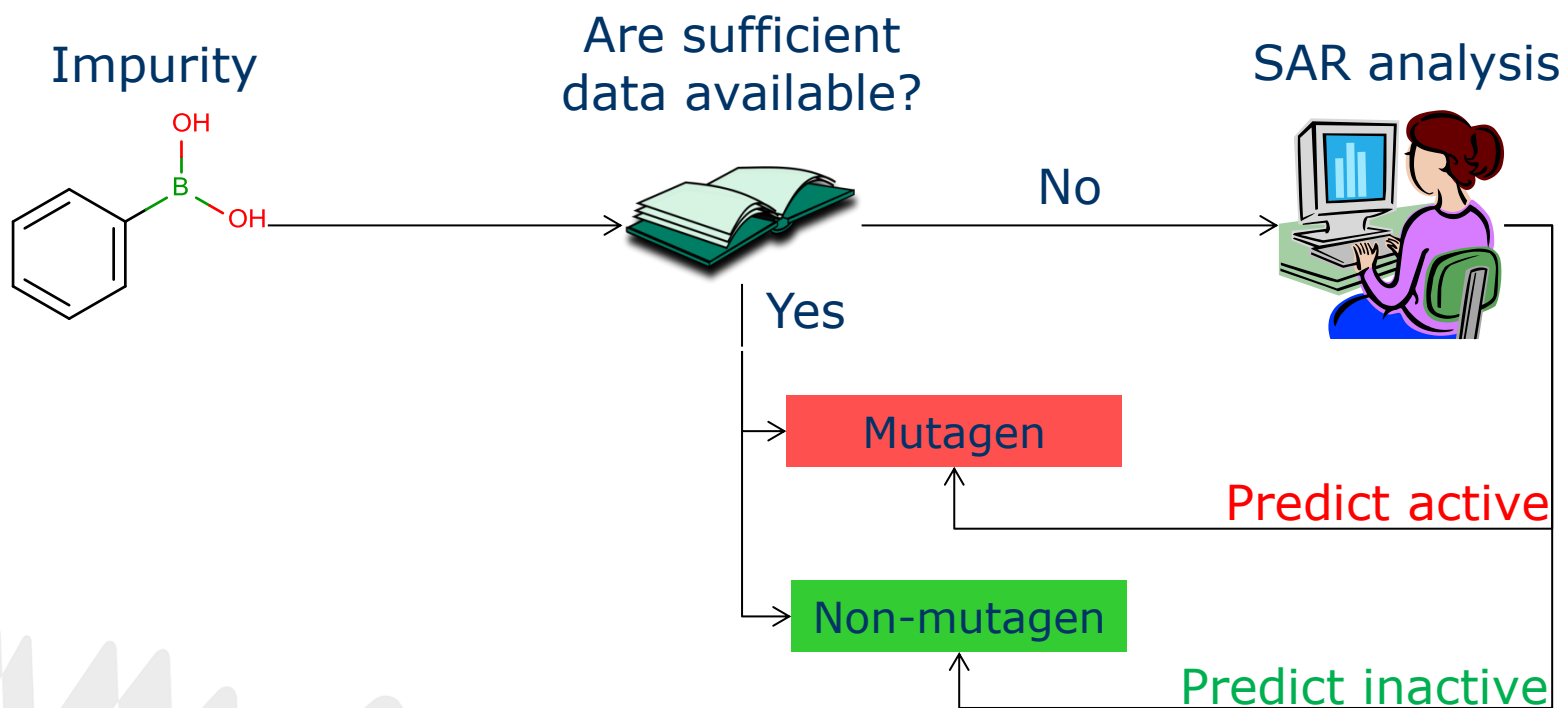
dated 23 June 2014



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.



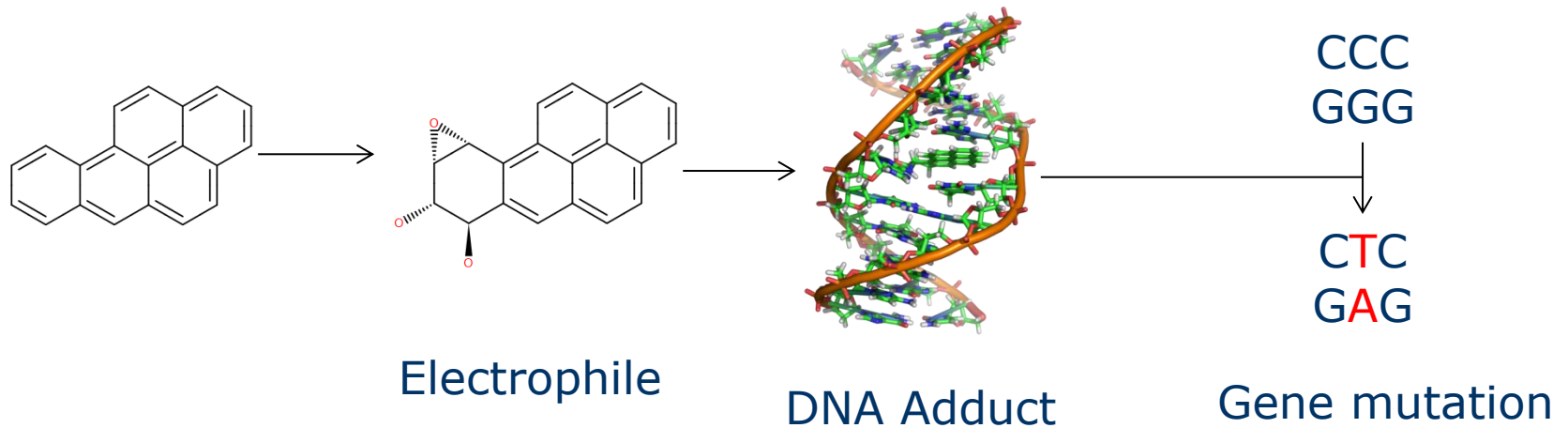


Acceptance of *in silico* mutagenicity predictions

- 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
- 

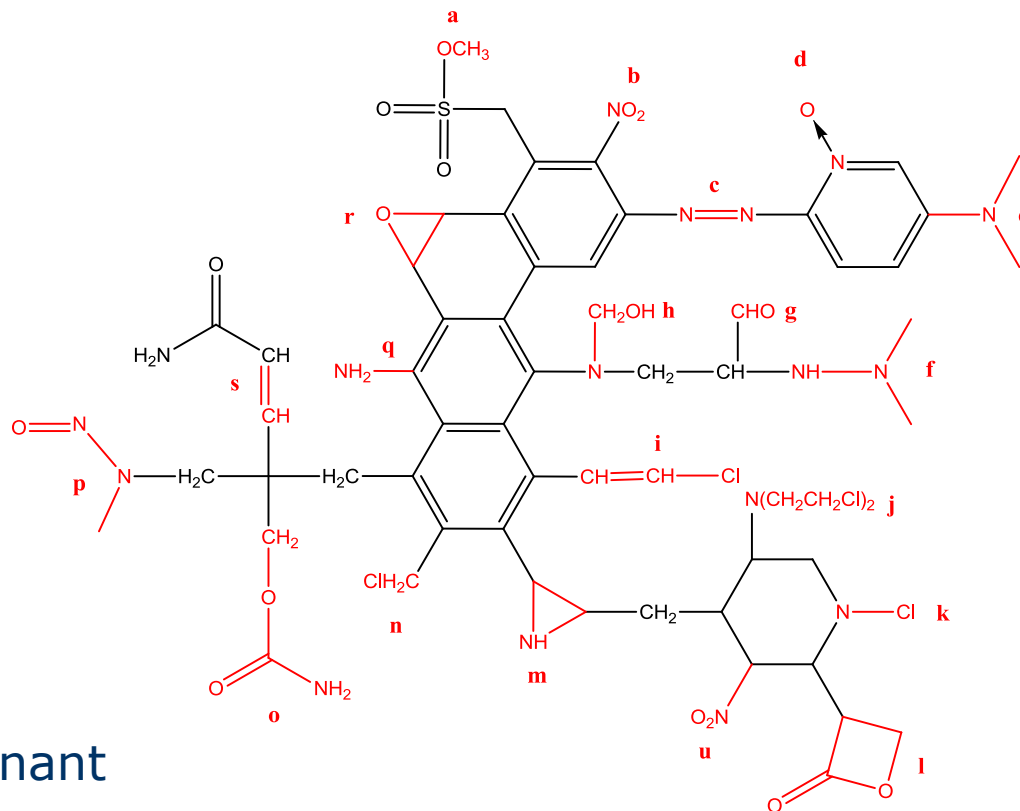
Hazard

- 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

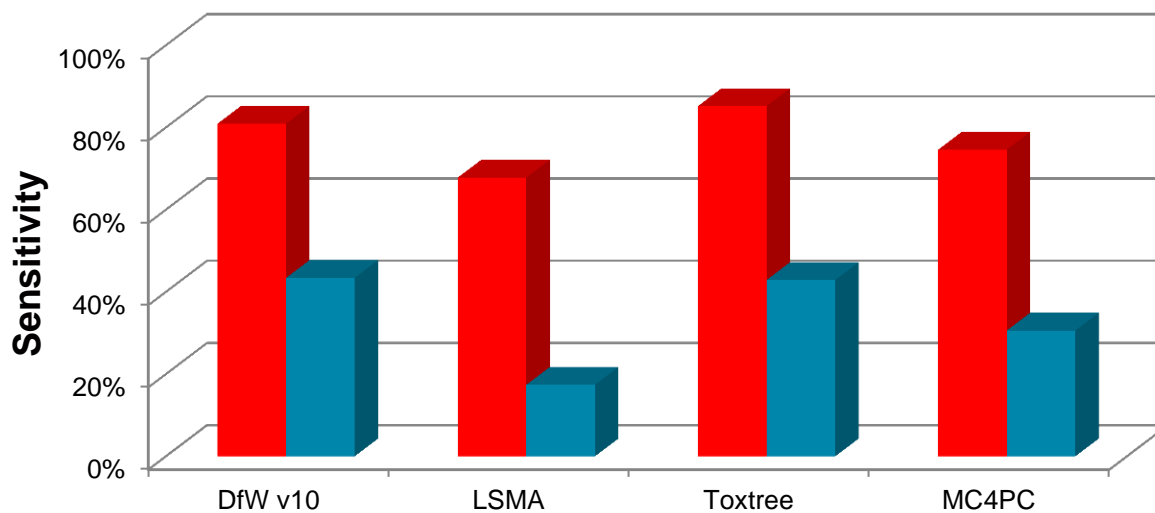


The Ashby-Tennant
polycarcinogen

$C(X)_4^t$
X= H, F, Cl, Br, I (in any combination)

Predictive performance

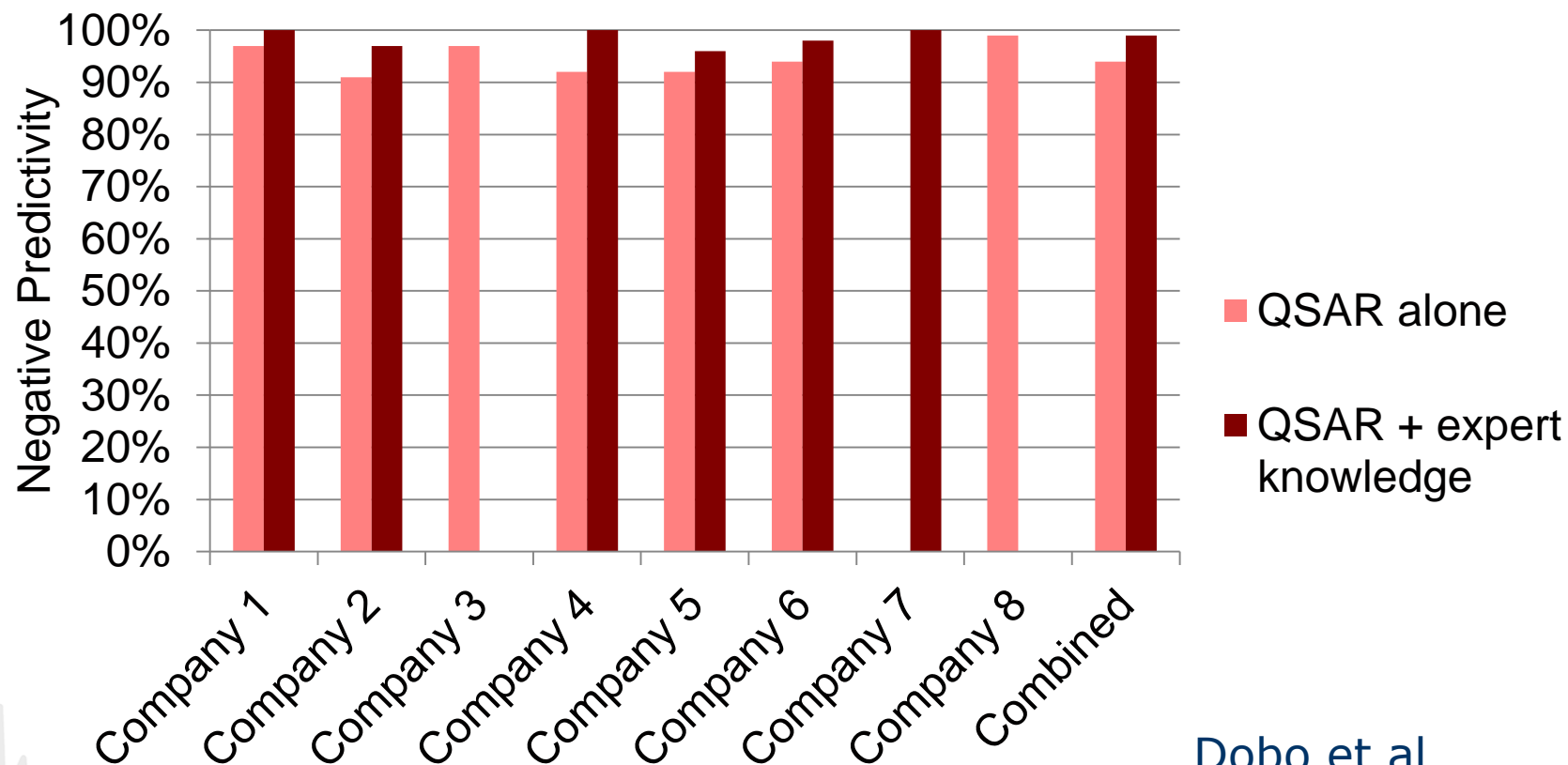
- The performance of *in silico* mutagenicity models varies by data set
- Generally, models can predict data in public domain, but often struggle to predict proprietary data
 - Assisted by using proprietary data in models



TP/(TP+FN)

Predictive performance for pharmaceutical impurities

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




Dobo et al

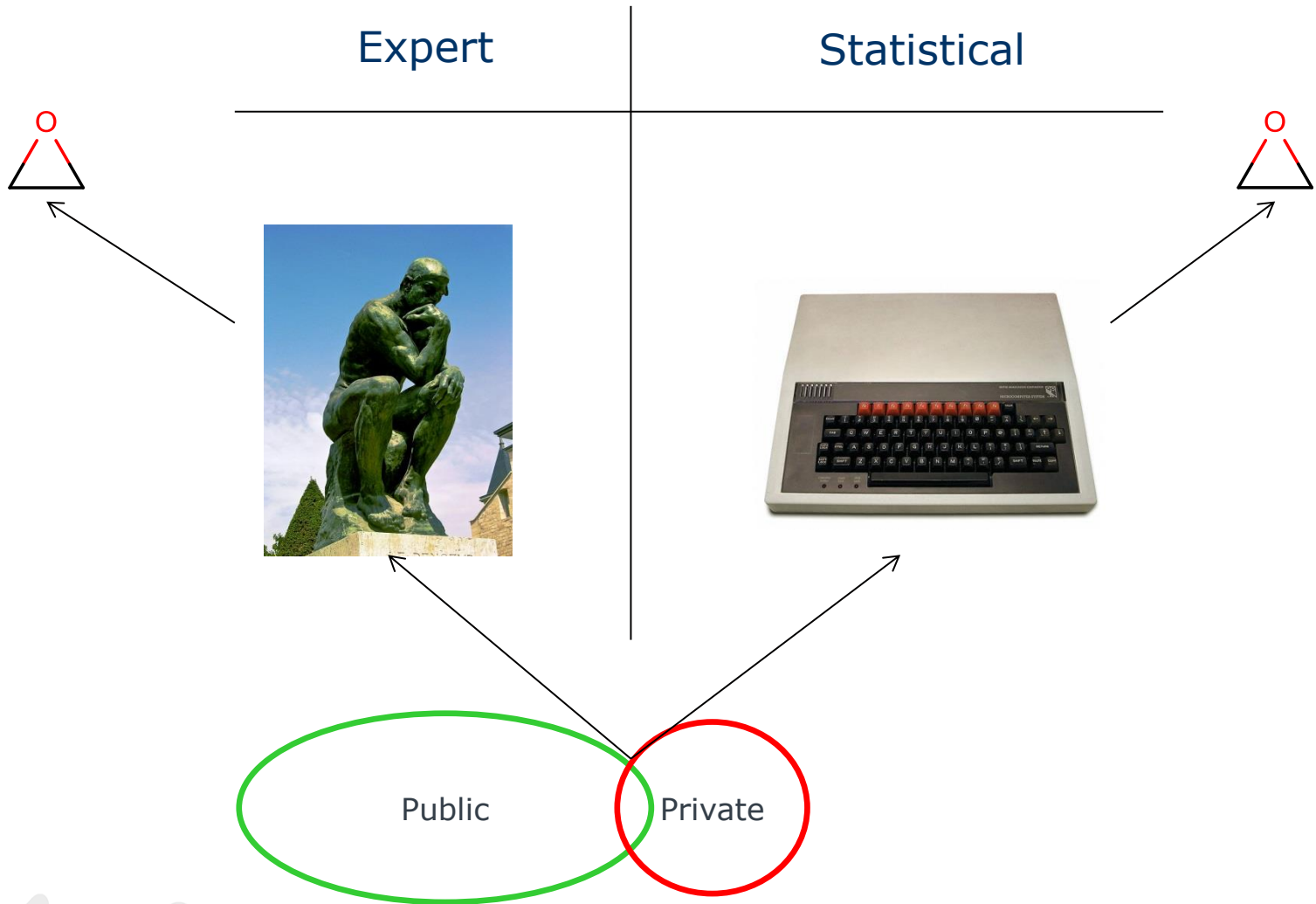


Two systems required, one expert, one statistical

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



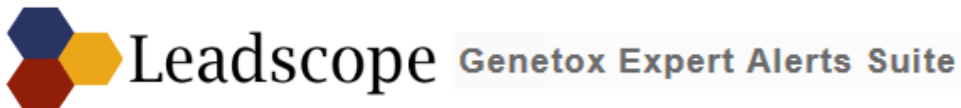
Expert and statistical systems



Commonly used *in silico* mutagenicity models

Expert

Statistical



Non-human Genetic Toxicity Suite



GT_EXPERT

GT1_A7B
GT1_AT_ECOLI
PHARM_SALM
PHARM_ECOLI

Using two systems

The screenshot displays the Nexus software interface. On the left, a 'Predictions' sidebar shows a tree view with 'Study Folder', 'Structure', and 'ICH M7 Prediction-4' (subdivided into 'Derek' and 'Sarah'). Below this is a 'Jobs' section with a grid. The central pane shows the chemical structure of 4-fluorophenylbenzene, with an 'N' atom at the para position and a 'F' atom at the ortho position. The right pane, titled 'ICH M7 Summary Results', contains a table of predictions.

2 predictions related to ICH M7 (for Mutagenicity in Bacterium) have been run for this structure.

| Type | Endpoint | Species | Result | Model |
|----------------------------|-----------------------|-----------|---------------------|----------------------|
| ICH M7 Prediction-4 | | | | |
| M7 Derek | Mutagenicity in vitro | bacterium | PLAUSIBLE + + + | Derek KB 2015 1.0 |
| M7 Sarah | Mutagenicity in vitro | bacterium | POSITIVE (25%) + | Sarah Model - 1.1.19 |

Expert system

The screenshot displays the Nexus software interface. The main window shows a chemical structure of a biphenyl derivative with a fluorine atom and an amine group. The interface includes a menu bar (File, Window, Prediction, Reports, Tools, Help), a toolbar, and several panels:

- Predictions Panel:** Shows a tree view with 'Study Folder', 'Structure', 'ICH M7 Prediction-4', 'Derek', and 'Sarah'.
- Jobs Panel:** A table for tracking prediction jobs.
- Prediction Navigator:** A dropdown menu set to 'IMPROBABLE'.
- Alert Details Panel:** Displays 'Alert 352: Aromatic amine or amide' with a description and structural restrictions.

Chemical Structure: A biphenyl system where the first ring has a fluorine atom at the 2-position and an amine group at the 1-position. The second ring is attached at the 4-position of the first ring.

Alert 352: Aromatic amine or amide

Description Image: Shows two chemical structures labeled (I) and (II). Structure (I) is an aromatic amine with substituents R1, R2, and R3 on the nitrogen. Structure (II) is an aromatic amide with substituents R4 and R5 on the nitrogen and a carbonyl group.

Structural Restrictions:

- R1, R4 = polysubstituted benzene ring
- R2, R3 = H, CH3, CH2CH3, CH2CH=CH2, CH2C≡CH
- R5 = H, *C(R6)(R7)R8
- R6, R7 = H, F
- R8 = any atom

Comments:

Mutagenicity: Ames test, transgenic rodent mutation assay

This alert describes the mutagenicity of aromatic amines (I), including their N-protonated forms, and aromatic amides (II) according to the toxophores shown. In addition, the following structural restrictions also apply:

1. Sulphonic acid or sulphonate groups are not permitted on the aniline ring.
2. Ortho disubstitution of the amine or amide group by substituents other than fluorine or NH2 or NH3+ is not permitted except in biphenyl-type structures where an aromatic substituent is additionally present in the para position.

It should be noted that conditions relating to NH2 and NH3+ substituent groups in this instance are a

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].

amine or amide

Image

(I) (II)

R1, R4 = polysubstituted benzene ring
R2, R3 = H, CH3, CH2CH3, CH2CH=CH2, CH2C≡CH
R5 = H, *C(R6)(R7)R8
R6, R7 = H, F
R8 = any atom


References

| 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 | Monoxygenase-catalyzed N-C cleavage. | Testa B. |
| 492 | Cancer risk reduction through mechanism-based molecu | Lai DY, Woo YT, Argus MF and Arcos JC. |
| 493 | Cyclic amines as less mutagenic replacements for dimet | Ashby J, Paton D and Lefevre PA. |
| 500 | A QSAR investigation of the role of hydrophobicity in re | 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 I, Gallo G, Gianni F and C |

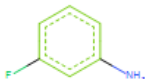


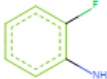
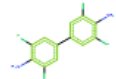



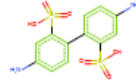



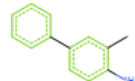

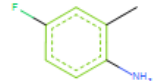

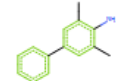
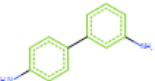

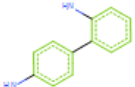
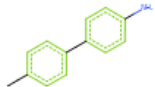
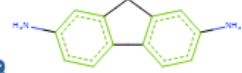
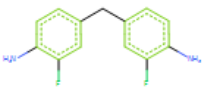
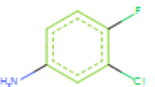
Statistical results

Sarah Hypothesis H-754

Hypothesis - H-754



Training Set Examples

| | | | | | |
|---|---|---|--|---|---|
| 1 of 50 - 36% (-Ve) | 2 of 50 - 32% (+Ve) | 3 of 50 - 32% (+Ve) | 4 of 50 - 30% (-Ve) | 5 of 50 - 30% (+Ve) | 6 of 50 - 30% (+Ve) |
|  |  |  |  |  |  |
| 7 of 50 - 28% (+Ve) | 8 of 50 - 28% (+Ve) | 9 of 50 - 28% (-Ve) | 10 of 50 - 26% (+Ve) | 11 of 50 - 26% (+Ve) | 12 of 50 - 25% (+Ve) |
|  |  |  |  |  |  |
| 13 of 50 - 24% (+Ve) | 14 of 50 - 24% (+Ve) | 15 of 50 - 23% (+Ve) | 16 of 50 - 23% (+Ve) | 17 of 50 - 23% (+Ve) | 18 of 50 - 22% (+Ve) |
|  |  |  |  |  |  |
| 19 of 50 - 22% (+Ve) | 20 of 50 - 22% (-Ve) | 21 of 50 - 22% (+Ve) | 22 of 50 - 22% (+Ve) | 23 of 50 - 22% (+Ve) | 24 of 50 - 22% (-Ve) |
|  |  |  |  |  |  |

Close

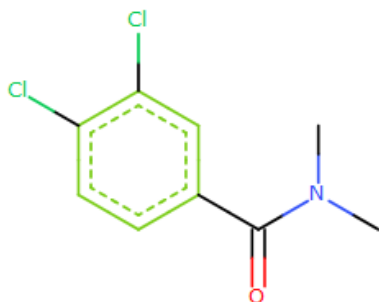


Possible outcomes from Sarah Nexus

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

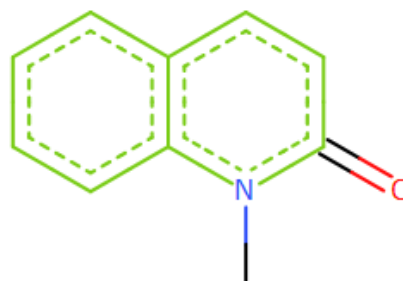
NEGATIVE

with **42%** confidence



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

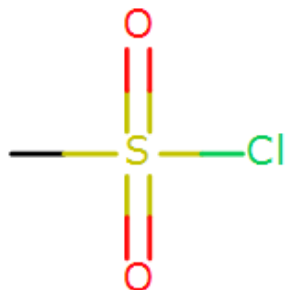
EQUIVOCAL



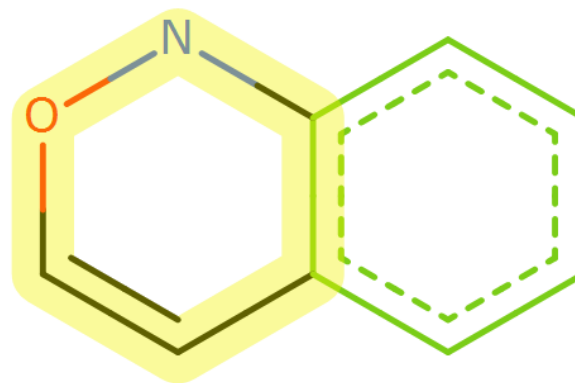
For the 'Mutagenicity in vitro' endpoint the prediction is:

POSITIVE

with **100%** confidence

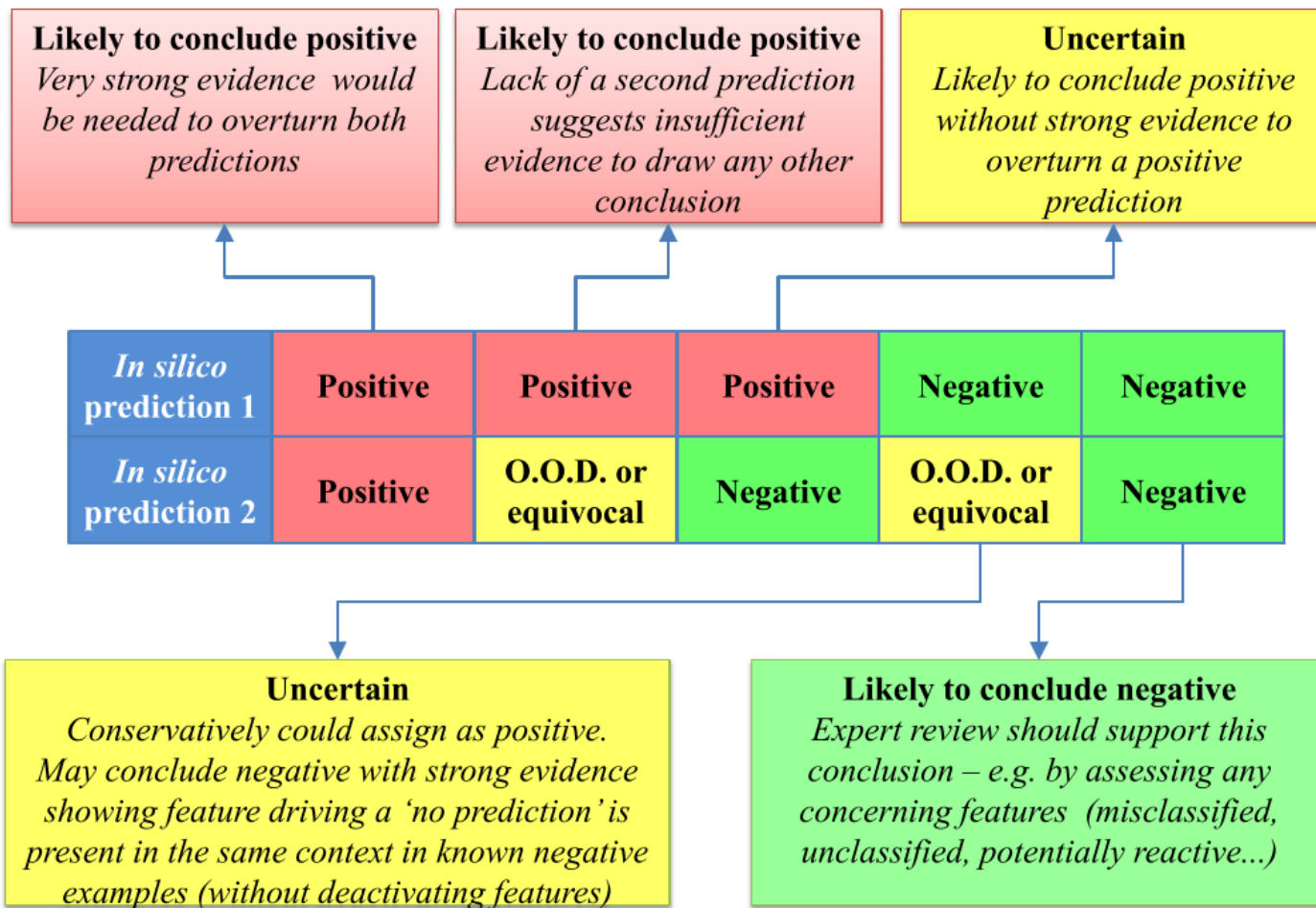


OUTSIDE DOMAIN



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

Combining two systems

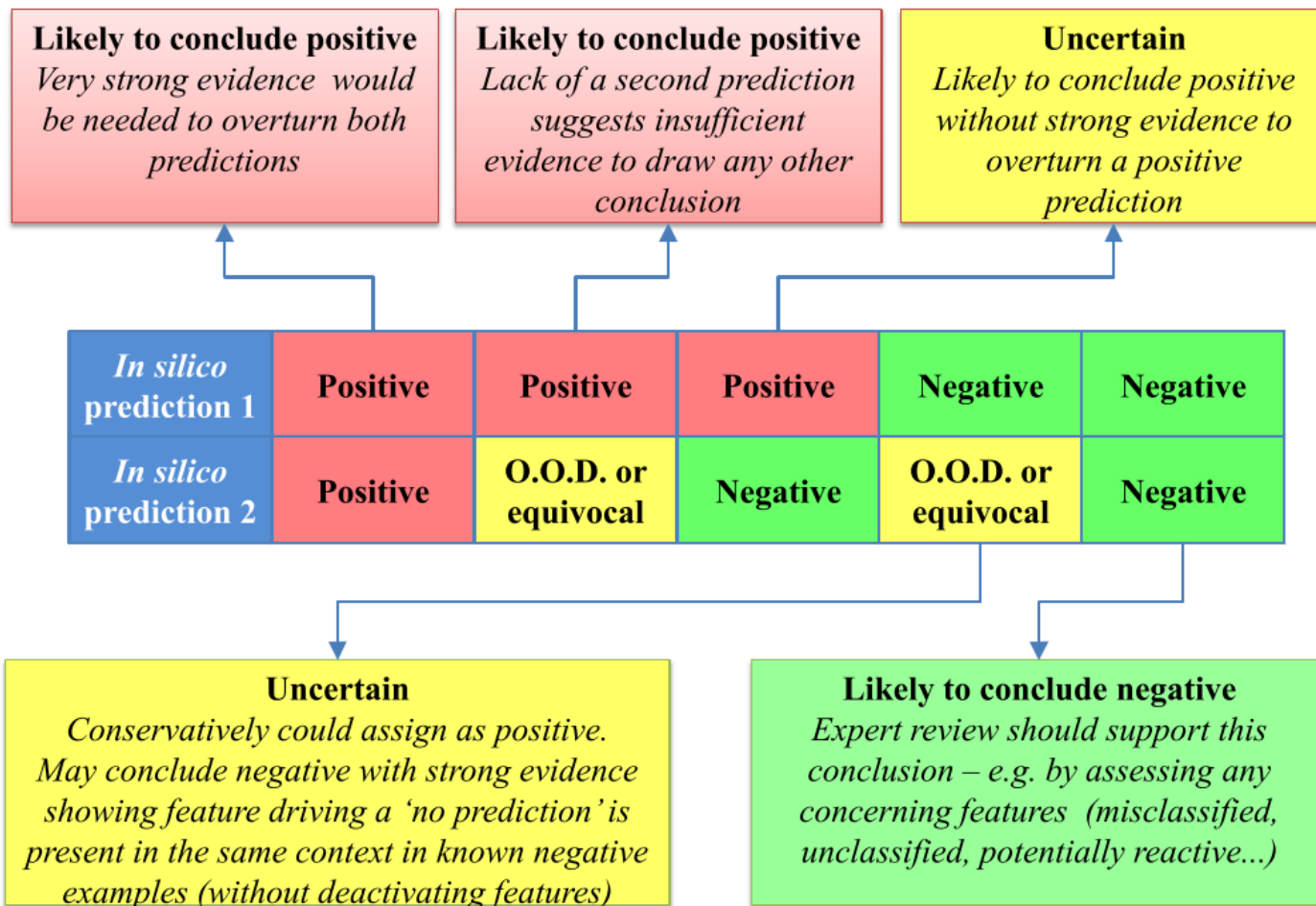


O.O.D. = out of domain

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

The screenshot displays the Nexus software interface for ICH M7 predictions. The central panel shows the chemical structure of propyl triazoline. The right panel, titled "ICH M7 Summary Results", provides a table of predictions.

| Type | Endpoint | Species | Result | Model |
|--------------------------|-----------------------|-----------|---------------------|----------------------|
| ICH M7 Prediction | | | | |
| M7 Derek | Mutagenicity in vitro | bacterium | PLAUSIBLE +++ | Derek KB 2015 1.0 |
| M7 Sarah | Mutagenicity in vitro | bacterium | NEGATIVE (20%) - | Sarah Model - 1.1.19 |

Example – propyl triazoline (Derek)

Alert Details

Description Image

R1 = H, C (aryl), C (alkyl) not multiply bonded or bonded to any additional heteroatoms

Comments

Mutagenicity: Ames test

Several 1-alkyltriazolines have been shown to be mutagenic towards Salmonella typhimurium strain TA1535 in the absence of S9 mix, such as 1-methyltriazoline, 1-ethyltriazoline and 1-benzyltriazoline [Smith et al].

The mutagenic activity of triazolines is likely to be promoted by the formation of an electrophilic species. 1-Alkyltriazolines decompose readily in aqueous solution forming electrophilic 1-alkyltriazolines from reactive aminoethyldiazonium ion intermediates. Similarly, 1-aryltriazolines have also been shown to decompose to 1-arylaziridines [Heine and Tomalia].

The scope of this alert has been defined by those compounds which can decompose to form aminoethyldiazonium ions and/or aziridine derivatives, namely 1-alkyltriazolines and 1-aryltriazolines. This is further supported by the Ames test activity displayed by 1-alkyltriazolines. 1-Vinyl and 1-alkynyl triazolines are excluded as these are likely to undergo ring expansion to 1-azacyclopentene derivatives [Padwa et al].

Examples

| | | |
|---|---|--|
| <input type="checkbox"/> 1-methyltriazoline | <input type="checkbox"/> 1-benzyltriazoline | <input type="checkbox"/> 1-ethyltriazoline |
| | | |

Example – propyl triazoline (Sarah)

Predictions

Study Folder
Propyl triazoline
ICH M7 Prediction
Derek
Sarah

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

NEGATIVE
with **20%** confidence

CCCN1=CN=CN1

Click above to view the original structure

Prediction Constraints

Model: Sarah Model - 1.1.19
Endpoint: Mutagenicity in vitro
Reasoning type: Weighted
Equivocal: 8%
Sensitivity: 8%
Certified model: Yes
Prediction date: 28 February 2016 20:06

Hypothesis: 1

Results

Highlight Hypotheses and Features:

The compound is predicted to be negative with 20% confidence for the 'Mutagenicity in vitro' endpoint in the model: 'Sarah Model - 1.1.19'. Supporting hypothesis containing similar examples from the training set has been found.

| Structure | ID | Hypothesis Result | Confidence | Highlight |
|-----------|-------|-------------------|------------|--------------------------|
| | H-777 | Negative | 20% | <input type="checkbox"/> |

Showing 50 examples (50/1928)

Training Set Examples

- 1 of 50 - 42% (-Ve)
- 2 of 50 - 42% (-Ve)
- 3 of 50 - 42% (-Ve)
- 4 of 50 - 42% (-Ve)
- 5 of 50 - 42% (-Ve)
- 6 of 50 - 42% (-Ve)
- 7 of 50 - 42% (-Ve)
- 8 of 50 - 42% (-Ve)
- 9 of 50 - 42% (-Ve)
- 10 of 50 - 29% (-Ve)

Example – propyl triazoline analogue (Sarah)

Predictions

- Study Folder
- Propyl triazoline
- ICH M7 Prediction
- Derek
- Sarah
- Propyl triazoline CLEAVED
- Sarah Prediction-11

Jobs

| Job Name | Status |
|---------------------|-----------|
| Sarah | Completed |
| Sarah Prediction-11 | Completed |

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

POSITIVE

with 25% confidence

CCCN1=NN=C1

Click above to view the original structure

Prediction Constraints

Model: Sarah Model - 1.1.19
Endpoint: Mutagenicity in vitro
Reasoning type: Weighted
Equivocal: 8%
Sensitivity: 8%
Certified model: Yes
Prediction date: 28 February 2016 20:24

Hypotheses: 2

Results

Highlight Hypotheses and Features:

The compound is predicted to be positive with 25% confidence for the 'Mutagenicity in vitro' endpoint in the model: 'Sarah Model - 1.1.19'. Supporting hypotheses containing similar examples from the training set have been found.

| Structure | ID | Hypothesis Result | Confidence | Highlight |
|------------------------------|----------------------|-------------------|------------|--------------------------|
| | H-875 | Positive | 42% | <input type="checkbox"/> |
| Training Set Examples | | | | |
| | 1 of 25 - 40% (+Ve) | | | <input type="checkbox"/> |
| | 2 of 25 - 37% (+Ve) | | | <input type="checkbox"/> |
| | 3 of 25 - 29% (+Ve) | | | <input type="checkbox"/> |
| | 4 of 25 - 27% (+Ve) | | | <input type="checkbox"/> |
| | 5 of 25 - 27% (+Ve) | | | <input type="checkbox"/> |
| | 6 of 25 - 25% (+Ve) | | | <input type="checkbox"/> |
| | 7 of 25 - 19% (+Ve) | | | <input type="checkbox"/> |
| | 8 of 25 - 18% (+Ve) | | | <input type="checkbox"/> |
| | 9 of 25 - 11% (+Ve) | | | <input type="checkbox"/> |
| | 10 of 25 - 11% (-Ve) | | | <input type="checkbox"/> |
| | H-777 | Negative | 17% | <input type="checkbox"/> |

Example – propyl triazoline conclusion

The screenshot displays the Nexus software interface for ICH M7 predictions. The central panel shows the chemical structure of propyl triazoline. The right panel, titled 'ICH M7 Summary Results', indicates that two predictions were run for this structure. The results are as follows:

| Type | Endpoint | Species | Result | Model |
|-------------------|-----------------------|-----------|-----------|----------------------|
| ICH M7 Prediction | | | | |
| Derek | Mutagenicity in vitro | bacterium | PLA (20%) | Derek KB 2015 1.0 |
| Sarah | Mutagenicity in vitro | bacterium | 0% | Sarah Model - 1.1.19 |

A red stamp with the text "Conclude positive" is overlaid on the results table.

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

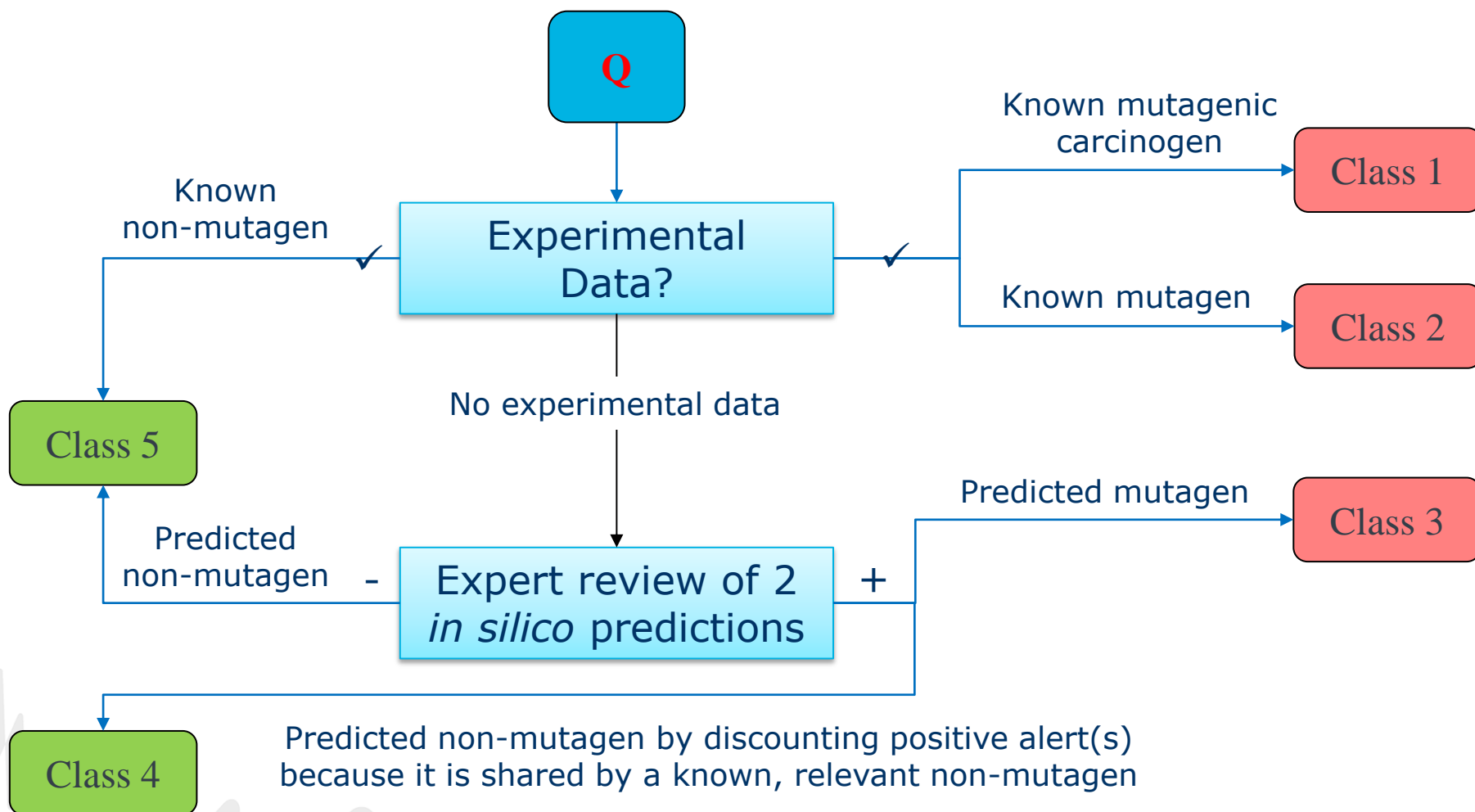
| Class | Definition |
|-------|--|
| 1 | Known mutagenic carcinogens. |
| 2 | Known mutagens with unknown carcinogenic potential (bacterial mutagenicity positive, no rodent carcinogenicity data). |
| 3 | Alerting structure, unrelated to the structure of the drug substance, no mutagenicity data. |
| 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

Nexus

File Window Prediction Reports Tools Help

*ICH M7 Batch Classification-2

Completed

API Structure

| API Structure | Name | Mutagenicity Mapping | Carcinogenicity Mapping | Alerts |
|---------------|------------|----------------------|-------------------------|---|
| | Ranitidine | Negative | No Results | PLAUSIBLE: Alert012 - Aliphatic nitro compound |

Results

| Status | Structure | Name | CAS No. | Derek Prediction | Sarah Prediction | QSAR Prediction | Similarity to API | Overall Carc. | Overall Ames | ICH M7 Class | Comments |
|--------|-----------|------------|---------|--|---|-----------------|-------------------------|---------------|--------------|--------------|----------|
| ✓ | | Impurity 1 | | INACTIVE: No misclassified or unclassified features [Green][Green][Green][White] | POSITIVE - 1% [Red+][White][White][White] | | No Derek Alerts found | Active | Conflicted | Class 3 | |
| ✓ | | Impurity 2 | | INACTIVE: No misclassified or unclassified features [Green][Green][Green][White] | NEGATIVE - 41% [Green][Green][White][White] | | No Derek Alerts found | Unspecified | Unspecified | Class 5 | |
| ✓ | | Impurity 3 | | INACTIVE: No misclassified or unclassified features [Green][Green][Green][White] | EQUIVOCAL - -- [White][White][White][White] | | No Derek Alerts found | Unspecified | Unspecified | Inconclusive | |
| ✓ | | Impurity 4 | | PLAUSIBLE: Alert012 - Aliphatic nitro compound [Red+][Red+][Red+][White] | OUTSIDE DOMAIN - -- [White][White][White][White] | | All Alerts found in API | Unspecified | Unspecified | Class 4 | |



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 carcinogenicity 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

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