ICH M7 Expert Review Workshop

Resolving common prediction scenarios using automated arguments in Nexus 2.3

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Agenda

- *In silico* workflow under ICH M7

- Features of expert review
  - Common prediction scenarios & expert review arguments
  - How Lhasa has approached this with Nexus 2.3

- Expert review workshop
  - Highlighting scenarios where Nexus 2.3 can help expert review

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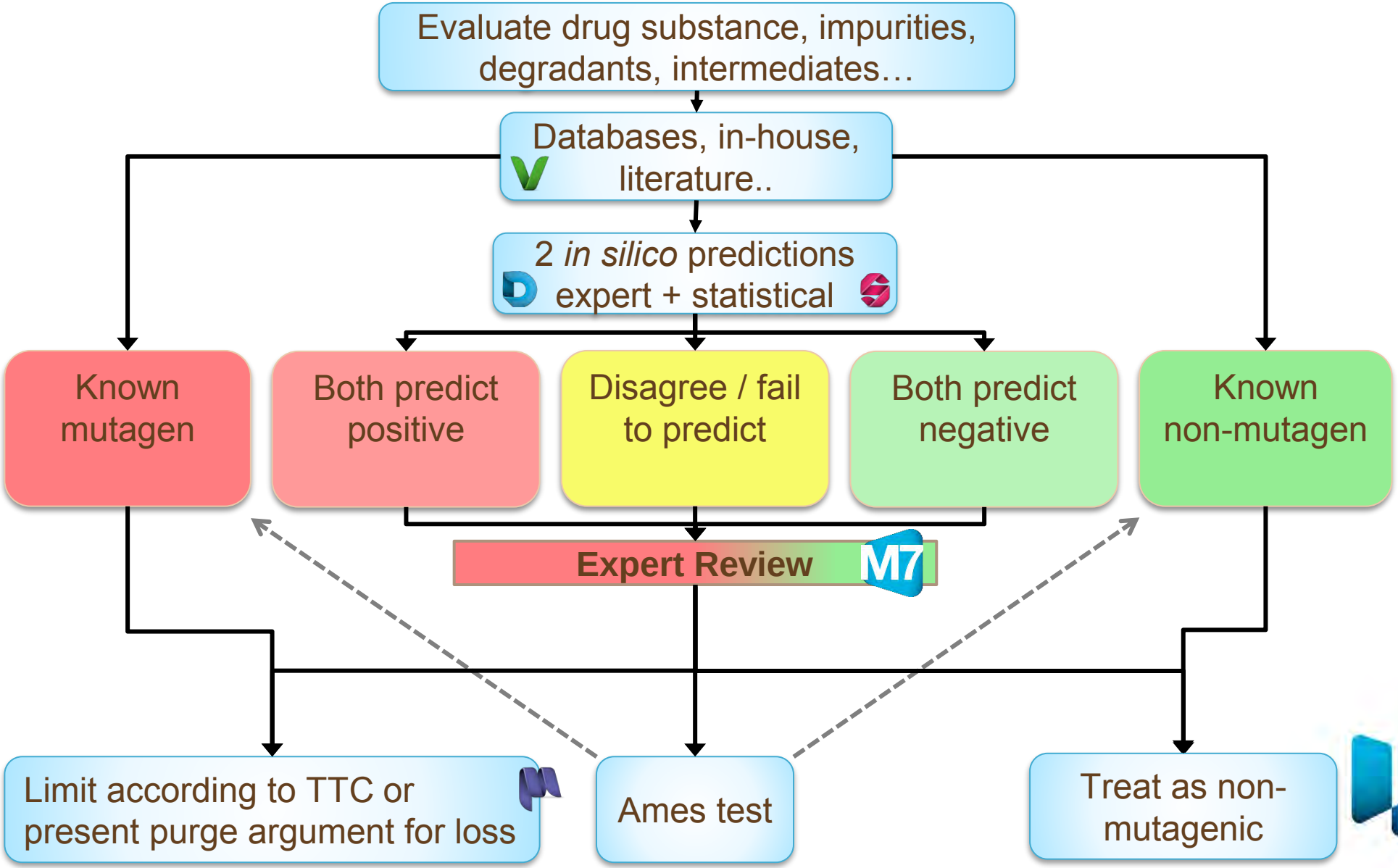
In silico workflow under ICH M7



Expert review is…

Regulatory Toxicology and Pharmacology
Volume 67, Issue 1, October 2013, Pages 39-52

Use of *in silico* systems and expert knowledge for structure-based assessment of potentially mutagenic impurities

Andreas Sutter [a], Alexander Amberg [b], Scott Boyer [c], Alessandro Brigo [d], Joseph F. Contrera [e], Laura L. Custer [f], Krista L. Dobo [g], Veronique Gervais [h], Susanne Glowienke [i], Jacky van Gompel [j], Nigel Greene [g], Wolfgang Muster [d], John Nicolette [k], M. Vijayaraj Reddy [l], Veronique Thybaud [m], Esther Vock [n], Angela T. White [o], Lutz Müller [d]

Regulatory Toxicology and Pharmacology
Volume 73, Issue 1, October 2015, Pages 367-377

Establishing best practise in the application of expert review of mutagenicity under ICH M7 ☆

Chris Barber [a], Alexander Amberg [b], Laura Custer [c], Krista L. Dobo [d], Susanne Glowienke [e], Jacky Van Gompel [f], Steve Gutsell [g], Jim Harvey [h], Masamitsu Honma [i], Michelle O. Kenyon [d], Naomi Kruhlak [j], Wolfgang Muster [k], Lidiya Stavitskaya [j], Andrew Teasdale [l], Jonathan Vessey [a], Joerg Wichard [m]

Regulatory Toxicology and Pharmacology
Volume 102, March 2019, Pages 53-64

Principles and procedures for handling out-of-domain and indeterminate results as part of ICH M7 recommended (Q)SAR analyses ☆

Alexander Amberg [a], Roxanne V. Andaya [b], Lennart T. Anger [a], Chris Barber [c], Lisa Beilke [d], Joel Bercu [e], Dave Bower [f], Alessandro Brigo [g], Zoryanna Cammerer [h], Kevin P. Cross [f], Laura Custer [i], Krista Dobo [j], Helga Gerets [k], Véronique Gervais [l], Susanne Glowienke [m], Stephen Gomez [n], Jacky Van Gompel [o], James Harvey [p] … Glenn J. Myatt [f]

Regulatory Toxicology and Pharmacology
Volume 71, Issue 2, March 2015, Pages 295-300

(Q)SAR assessments of potentially mutagenic impurities: A regulatory perspective on the utility of expert knowledge and data submission

Mark W. Powley

Regulatory Toxicology and Pharmacology
Volume 77, June 2016, Pages 13-24

Principles and procedures for implementation of ICH M7 recommended (Q)SAR analyses ☆

Alexander Amberg [a], Lisa Beilke [b], Joel Bercu [c], Dave Bower [d], Alessandro Brigo [e], Kevin P. Cross [d], Laura Custer [f], Krista Dobo [g], Eric Dowdy [c], Kevin A. Ford [h], Susanne Glowienke [i], Jacky Van Gompel [j], James Harvey [k], Catrin Hasselgren [d], Masamitsu Honma [l], Robert Jolly [m], Raymond Kemper [n], Michelle Kenyon [g] … Glenn J. Myatt [d]

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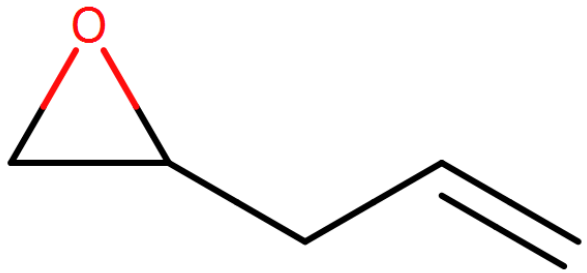
Expert review is…

- …required for *in silico* predictions under ICH M7 & is essential for each impurity that is processed
  - Used to ensure predictions are relevant & accurate
  - Used to conclude assessment of activity based on predictions

- …often straightforward



"*Derek & Sarah have both produced strong predictions for bacterial mutagenicity based on the same toxicophore & there is no reason to doubt these predictions. Therefore, we conclude this impurity is positive & assigned ICH M7 Class III.*"

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

Type	Endpoint	Species	Result	Model
ICH M7 Prediction				
Derek	Mutagenicity in vitro	bacterium	PLAUSIBLE (+++)	Derek KB 2020 1.0
Sarah	Mutagenicity in vitro	bacterium	POSITIVE (64%) (++)	Sarah Model - 2020.1

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Expert review is…

- …required for *in silico* predictions under ICH M7 & is essential for each impurity that is processed
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- …often straightforward, but some situations are harder to resolve
  - How do I conclude if Derek and Sarah disagree?
  - How do I find relevant information from the software to support my conclusion?
  - How do I document this in a concise way for a regulator?

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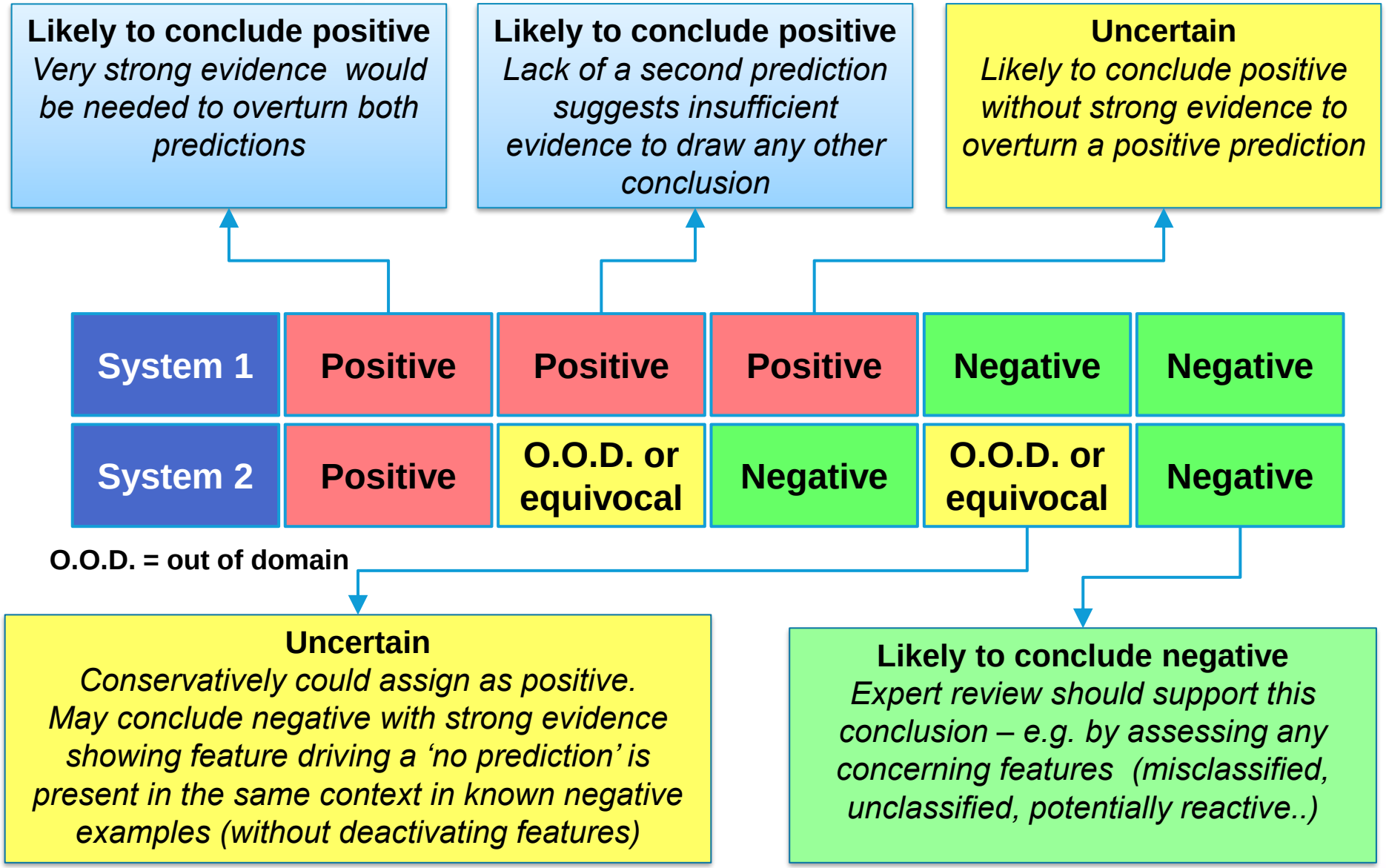

Establishing best practise in the application of expert review of mutagenicity under ICH M7
Regulatory Toxicology and Pharmacology 2015, 73, 367-377

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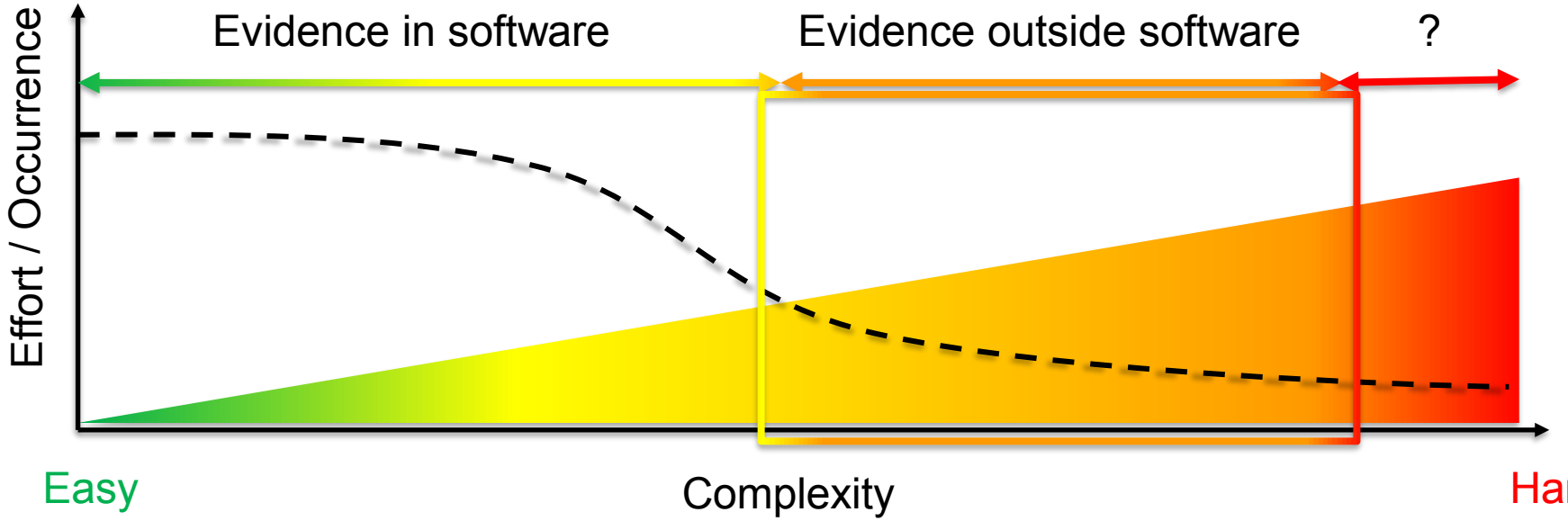

Predictions Agree High Confidence Relevant Hypotheses Relevant NN Reliable Data	Predictions Agree Lower Confidence Relevant Hypotheses Less Relevant NN Reliable Data Other, more relevant NN available	Predictions Disagree Low Confidence NN Not Relevant Predictions can be resolved by considering common limitations and additional data	Predictions Disagree Low Confidence NN Not Relevant

NN = nearest neighbours

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Expert review is…

- …required for *in silico* predictions under ICH M7 & is essential for each impurity that is processed
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  - Used to conclude assessment of activity based on predictions

- …often straightforward, but some situations are harder to resolve
  - How do I conclude if Derek and Sarah disagree?
  - How do I find relevant information from the software to support my conclusion?
  - How do I document this in a concise way for a regulator?

- …often completed with recycled arguments for common prediction scenarios
  - How can I make expert review consistent and efficient to save time?

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Common arguments to resolve predictions

- Adequate Ames data is available
- Ames test does not assess the hazard caused by the compound class adequately
- Toxicophore identified by one system has not been adequately assessed by the other
- Toxicophore identified by one system is not causative of activity
- Toxicophore identified by one system is not negated by negative features
- Data available for nearest neighbours is not of sufficient quality to make prediction
- Nearest neighbours are not adequately similar enough to make a prediction

61 arguments written for possible prediction scenarios

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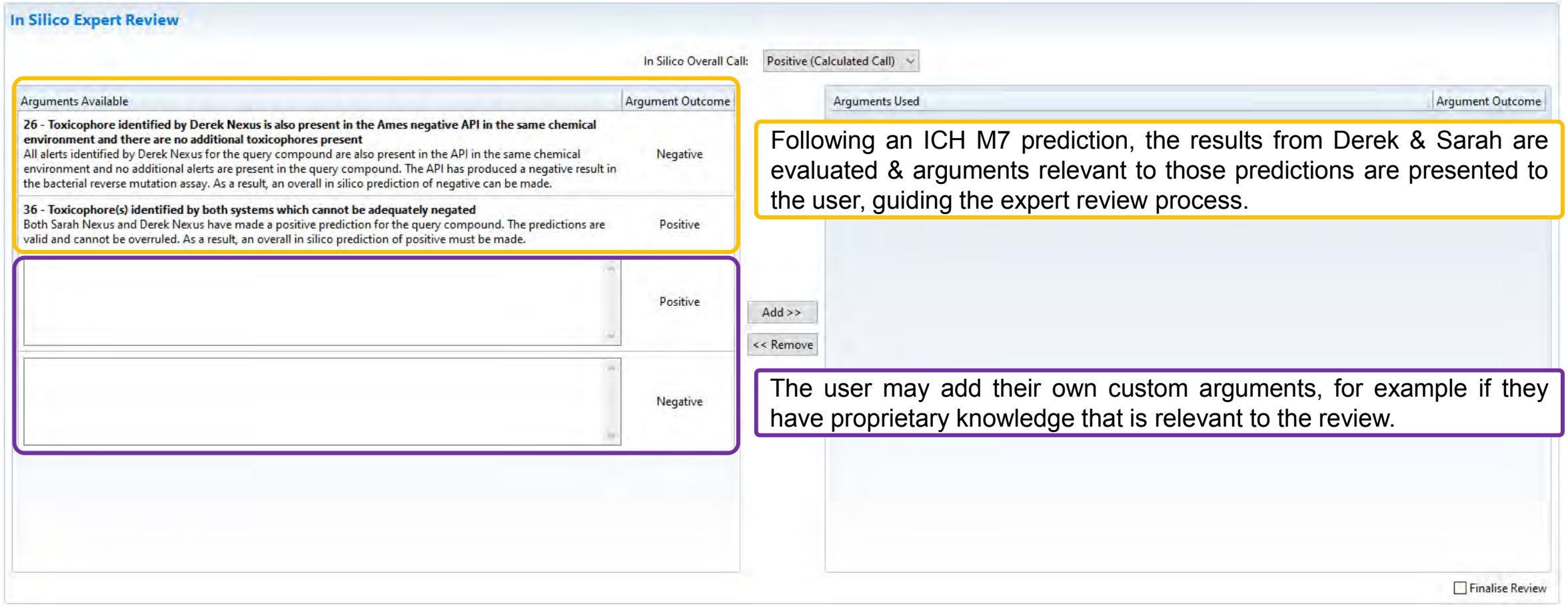
Nexus 2.3 – selected arguments



Following an ICH M7 prediction, the results from Derek & Sarah are evaluated & arguments relevant to those predictions are presented to the user, guiding the expert review process.

The user may add their own custom arguments, for example if they have proprietary knowledge that is relevant to the review.

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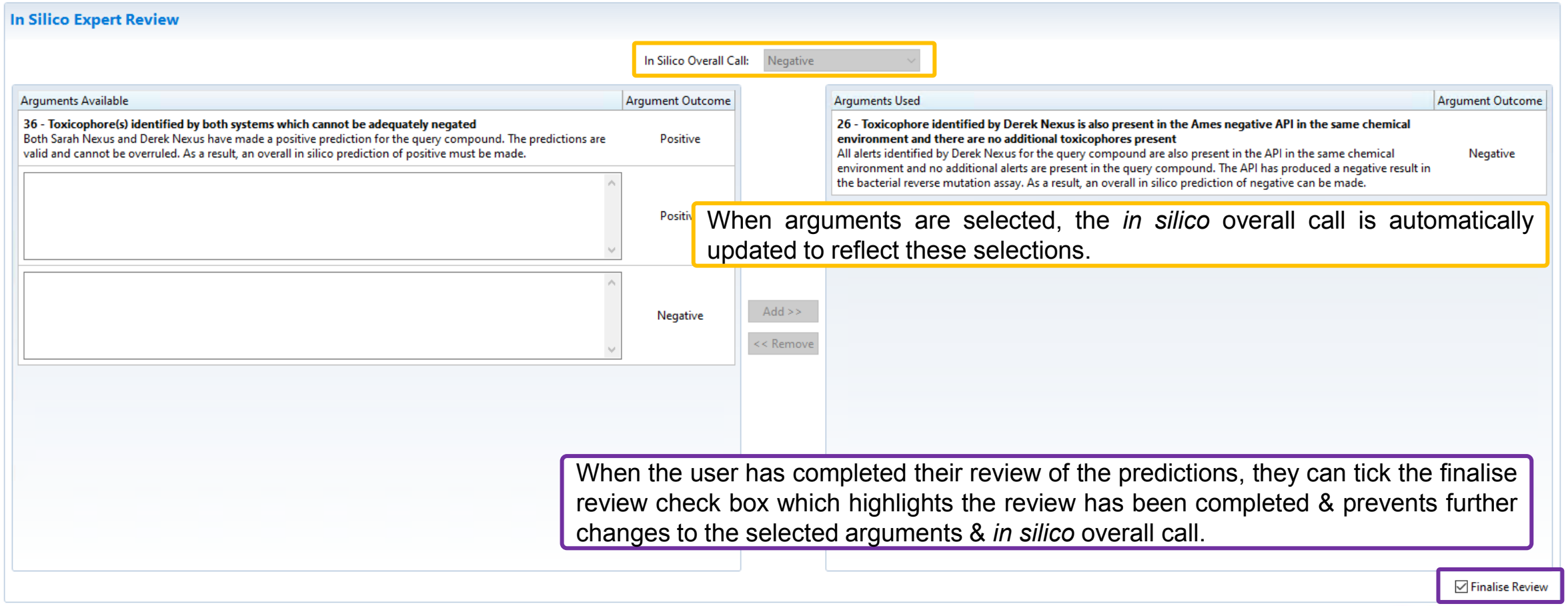
Nexus 2.3 – selected arguments



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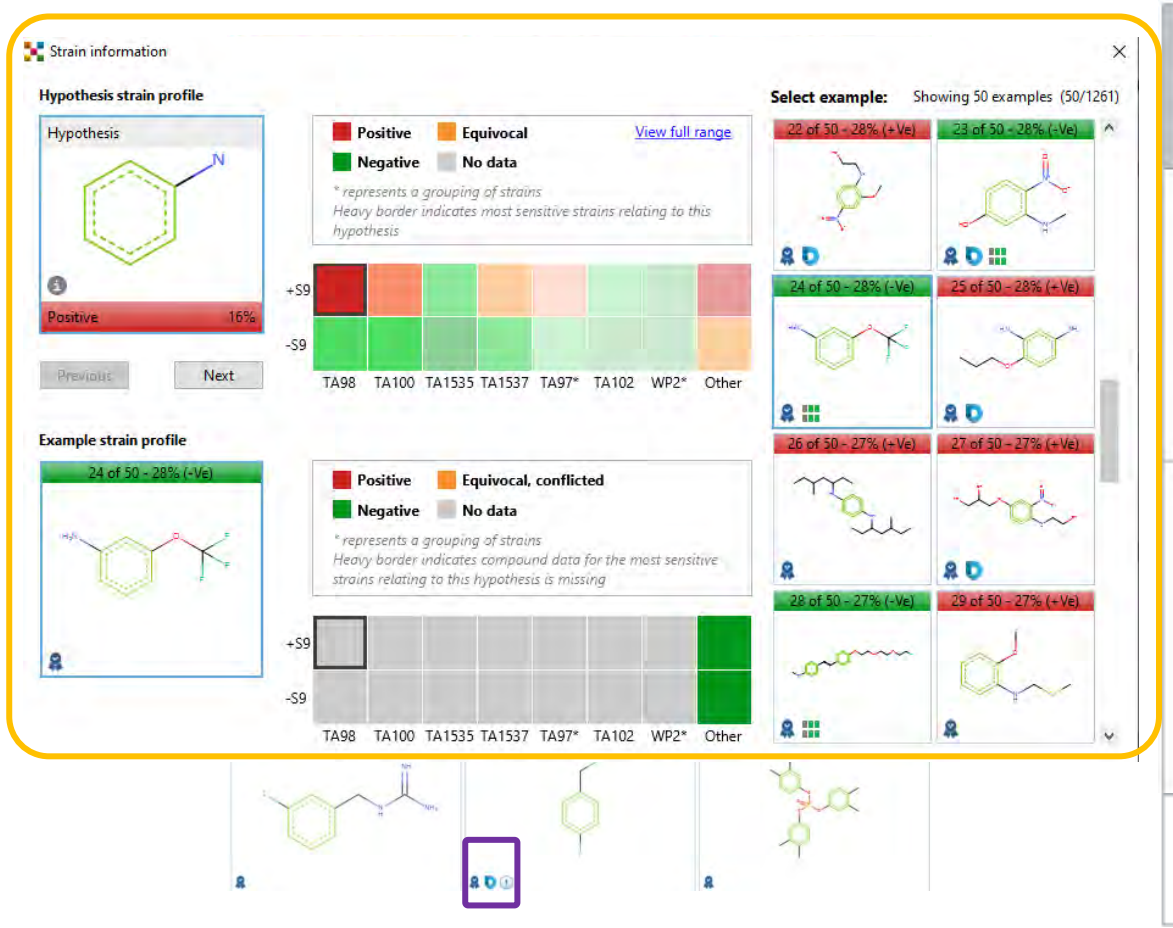
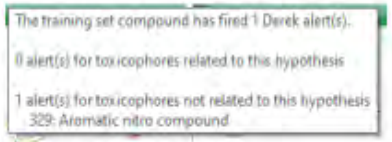
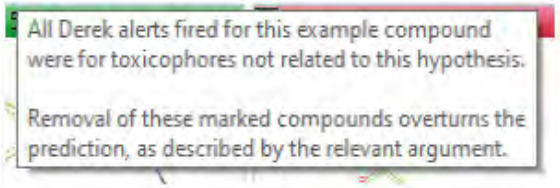
Nexus 2.3 – integrating Derek & Sarah



Icon shown with Sarah Training Set Compound	Meaning
D	The training set example has fired one or more Derek alerts for Ames mutagenicity. The tool tip displays the alert number(s) and whether the alert is associated with toxicophores related to the hypothesis or not. 
!	All the Derek alerts fired for the training set compound were for toxicophores not related to the hypothesis. Removing these compounds overturns the prediction. An argument corresponding to this will be shown on the Expert Review screen. 
▦	The training set compound has incomplete strain information and is missing data for the most sensitive strains of the hypothesis. Clicking the icon opens the strain profile window.

When an ICH M7 prediction is run, specific information relating to Derek & Sarah is highlighted in the Sarah prediction results:

- Do the Sarah training examples activate Derek mutagenicity *in vitro* alerts?
- Do the Sarah hypotheses relate to any activated Derek mutagenicity *in vitro* alerts?
- Have the Sarah training examples which are non-mutagenic been tested in the most appropriate strains?

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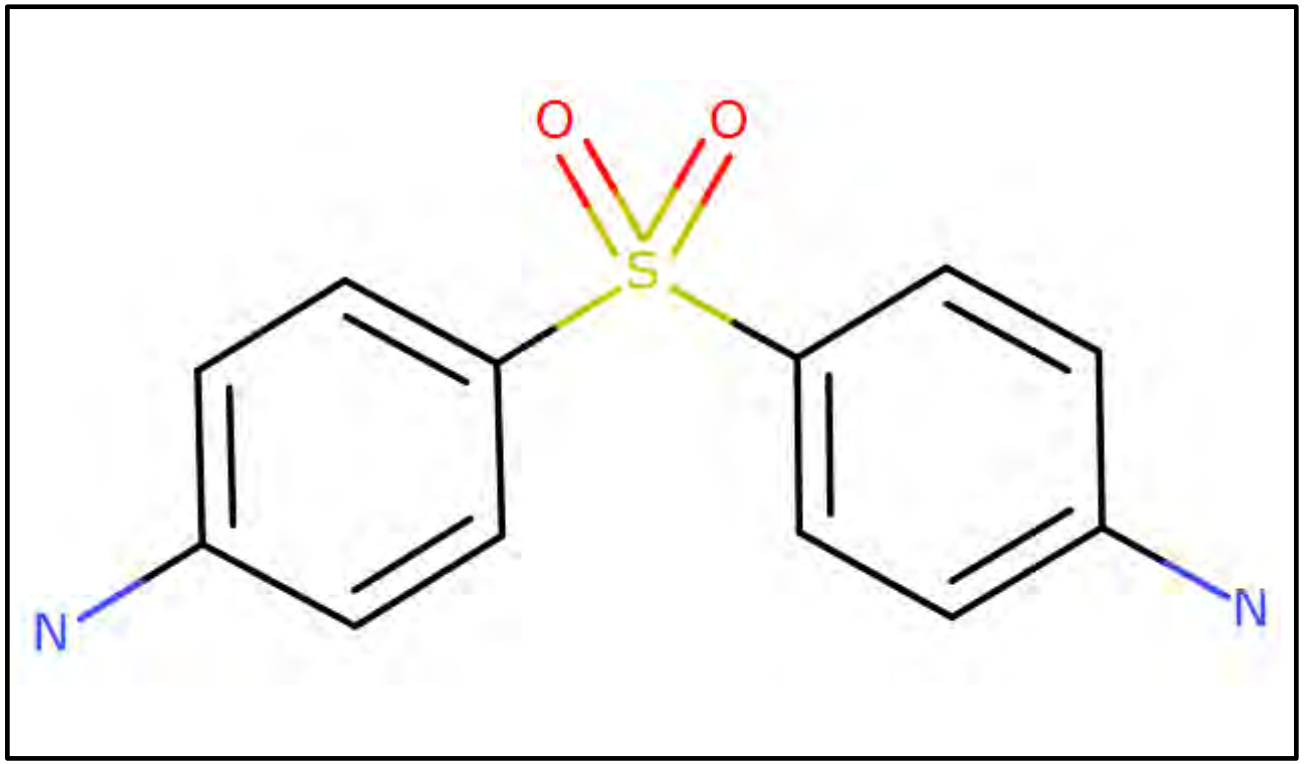
Worked examples

Example 1



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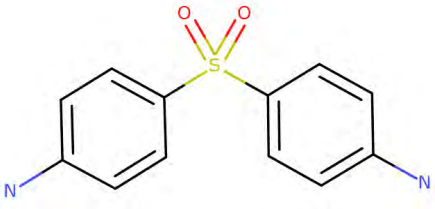
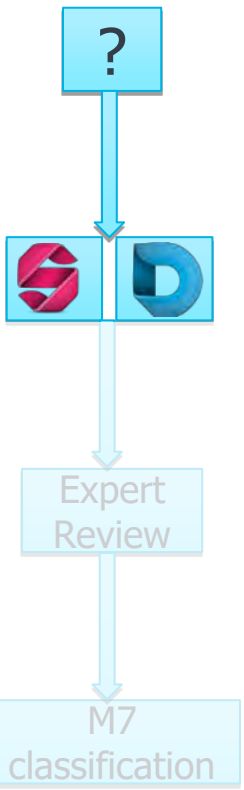
Review high level predictions





ICH M7 Summary Results

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

Type	Endpoint	Species	Result	Model
ICH M7 Prediction-2				
Derek	Mutagenicity in vitro	bacterium	INACTIVE	Derek KB 2020 1.0
Sarah	Mutagenicity in vitro	bacterium	NEGATIVE (100%)	Sarah Model - 2020.1

In Silico Expert Review

In Silico Overall Call: Negative (Calculated Call)

Arguments Available	Argument Outcome
37 - No relevant toxicophore has been identified by either system Both Sarah Nexus and Derek Nexus have made a negative prediction for the query compound. There is no reason to doubt these predictions. As a result, an overall in silico prediction of negative can be made.	Negative
61 - Adequate negative Ames test data used to support prediction. The query compound is an exact match with an example compound associated with negative Ames test data, captured in one or both of the systems. The data relating to this compound are adequate to support the negative prediction. As a result, an overall in silico prediction of negative is supported by these data.	Negative
	Positive
	Negative

Add >>

<< Remove

Arguments Used	Argument Outcome

Finalise Review

Derek & Sarah agree

Derek: inactive result suggests high confidence in negative prediction.

Sarah: 100% confidence shows chemical is known in Sarah training set.

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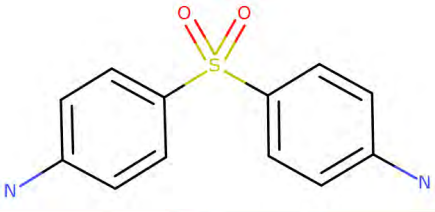
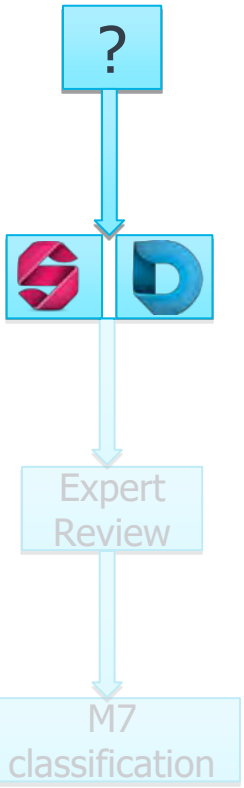
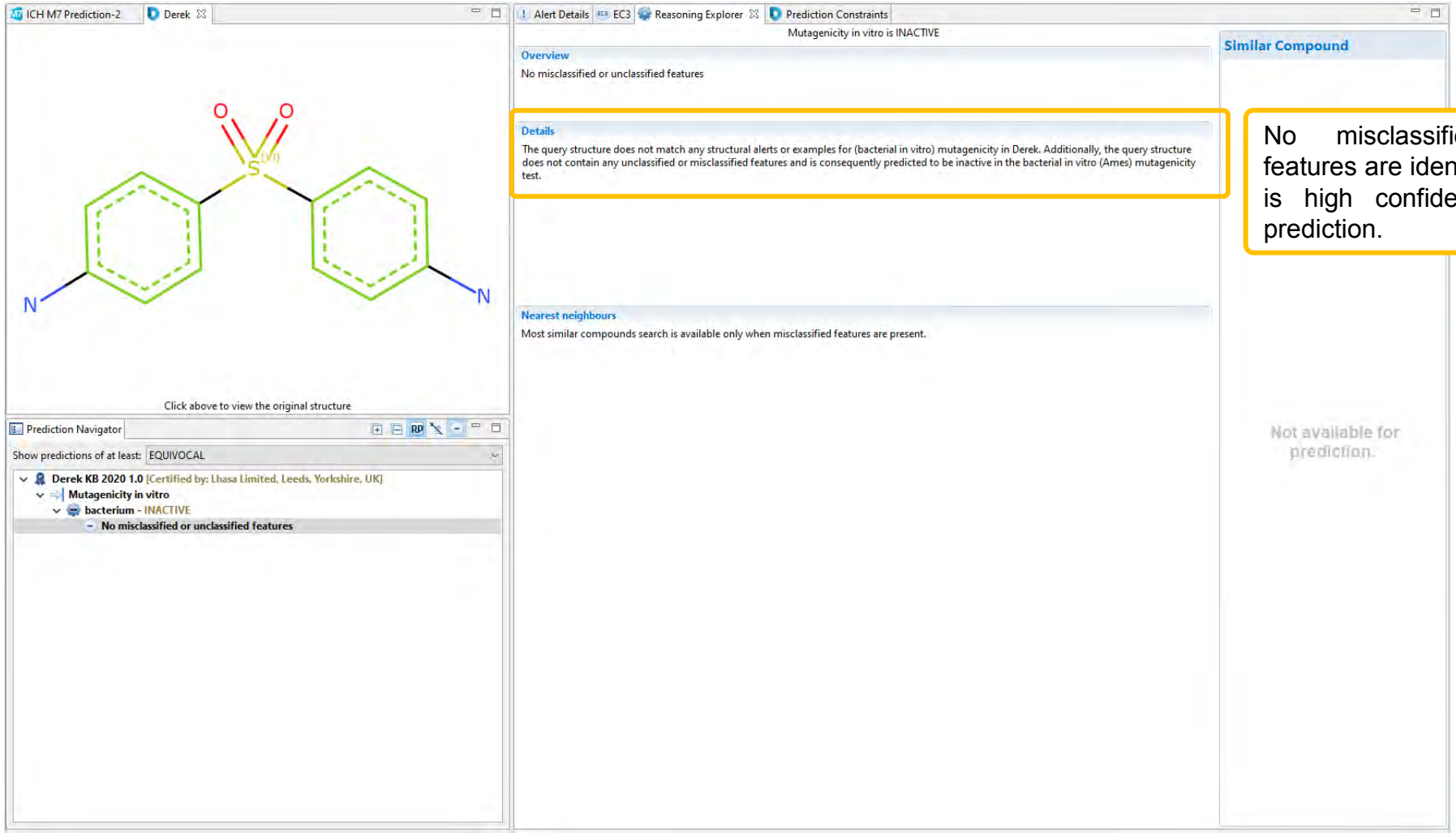
Review the expert prediction



Derek nexus





No misclassified or unclassified features are identified, suggesting there is high confidence in the negative prediction.

No misclassified or unclassified features raises no doubt in the negative prediction made by Derek.

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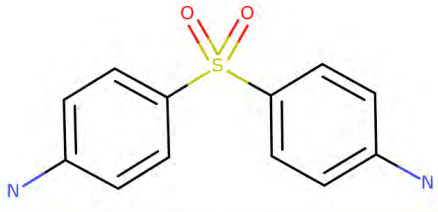
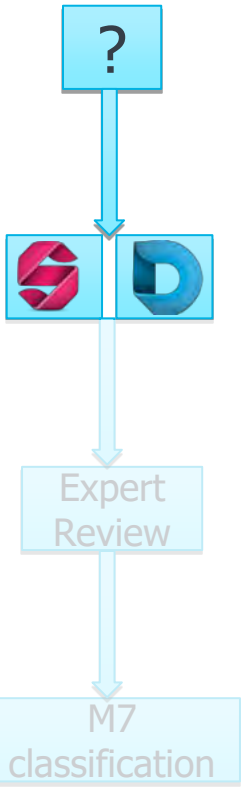
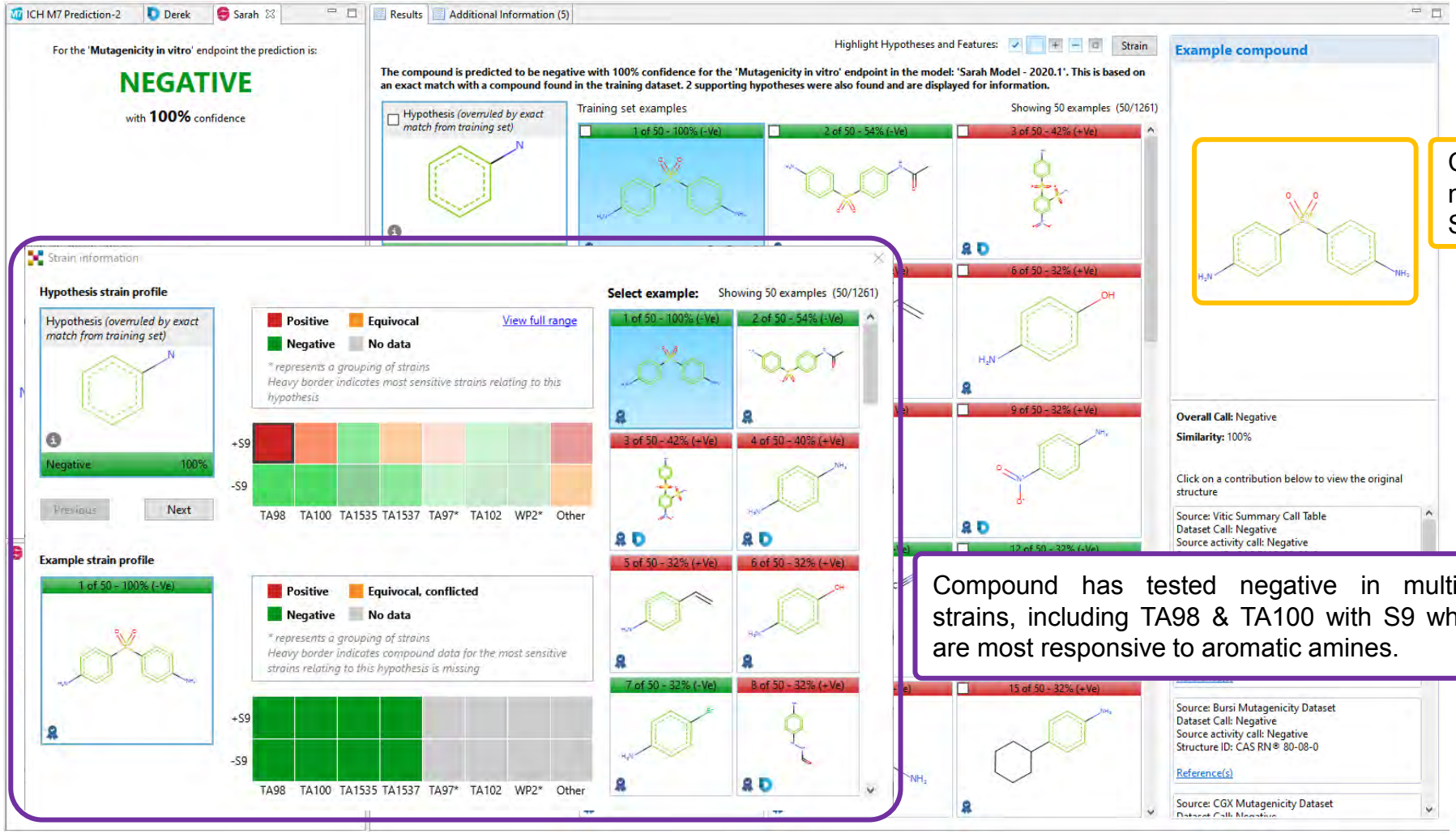
Review the statistical prediction



Sarah nexus





Compound is a known non-mutagen in the Sarah training set.

Compound has tested negative in multiple strains, including TA98 & TA100 with S9 which are most responsive to aromatic amines.

Compound is a known non-mutagen in the Sarah training set & has been tested adequately, hence there is no reason to disagree with this negative prediction.

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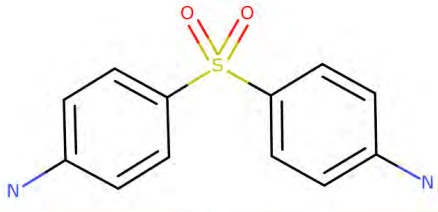
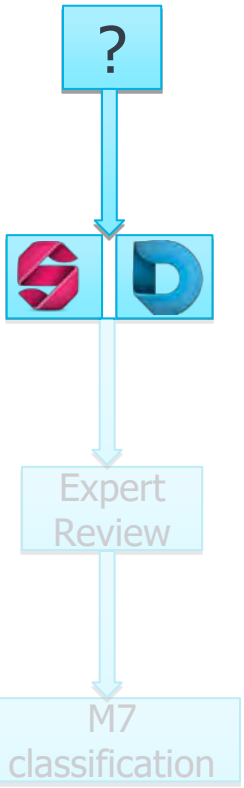
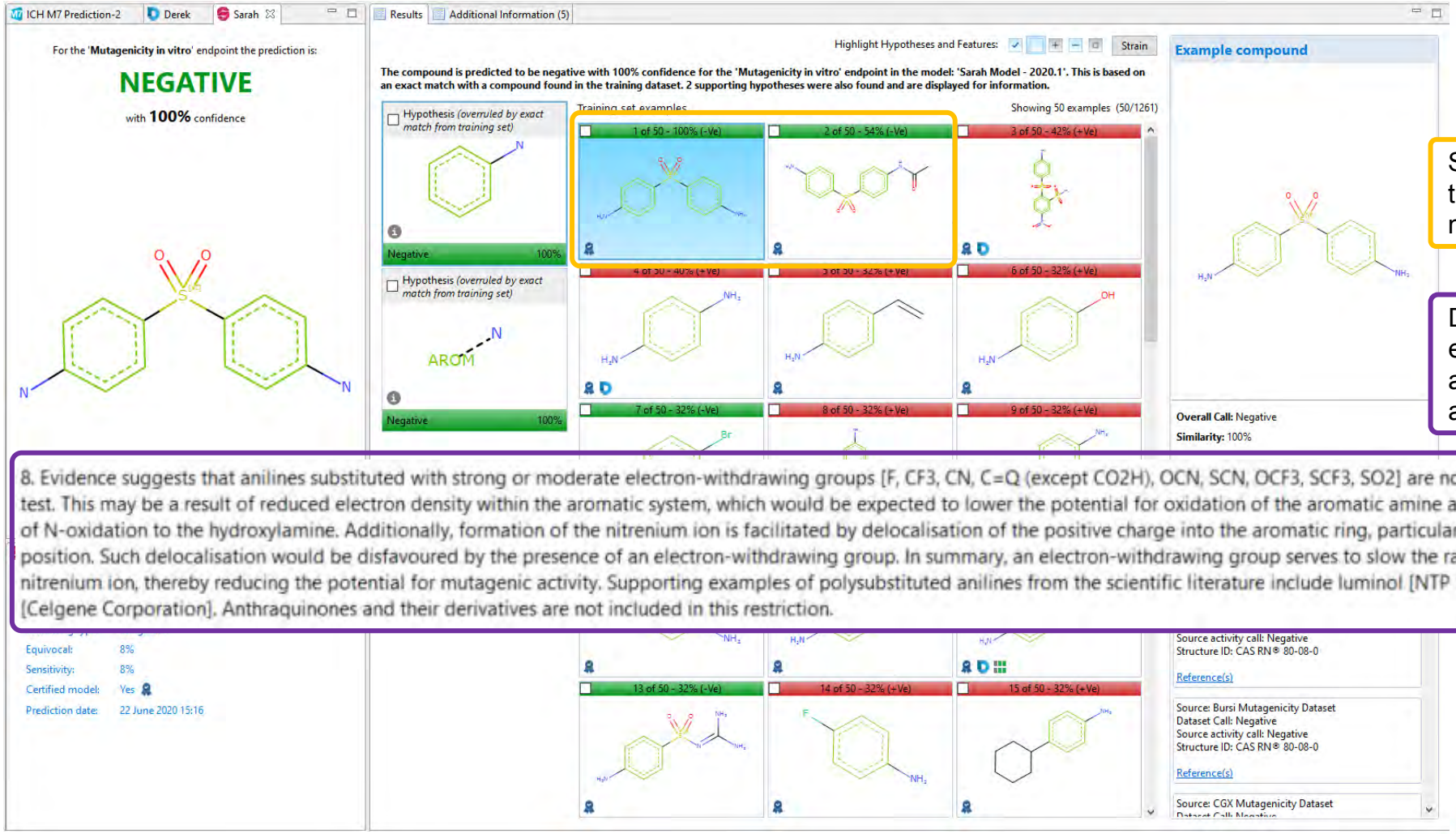
Review the statistical prediction



Sarah nexus





Sulfone compounds in the training set are non-mutagens.

Derek alert comments explain such compounds are excluded from aromatic amine alerts.

8. Evidence suggests that anilines substituted with strong or moderate electron-withdrawing groups [F, CF3, CN, C=Q (except CO2H), OCN, SCN, OCF3, SCF3, SO2] are not mutagenic in the Ames test. This may be a result of reduced electron density within the aromatic system, which would be expected to lower the potential for oxidation of the aromatic amine and therefore reduce the rate of N-oxidation to the hydroxylamine. Additionally, formation of the nitrenium ion is facilitated by delocalisation of the positive charge into the aromatic ring, particularly at the ortho and para position. Such delocalisation would be disfavoured by the presence of an electron-withdrawing group. In summary, an electron-withdrawing group serves to slow the rate of formation of the nitrenium ion, thereby reducing the potential for mutagenic activity. Supporting examples of polysubstituted anilines from the scientific literature include luminol [NTP 1986] and lenalidomide [Celgene Corporation]. Anthraquinones and their derivatives are not included in this restriction.

Compound is a known non-mutagen in the Sarah training set & has been tested adequately, hence there is no reason to disagree with this negative prediction. In addition, aromatic amines with strong electron withdrawing groups such as SO_2 are excluded from Derek aromatic amine alerts.

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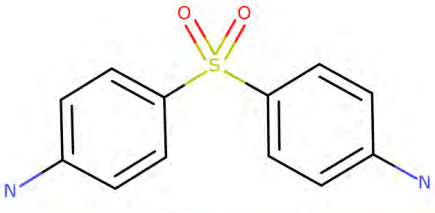
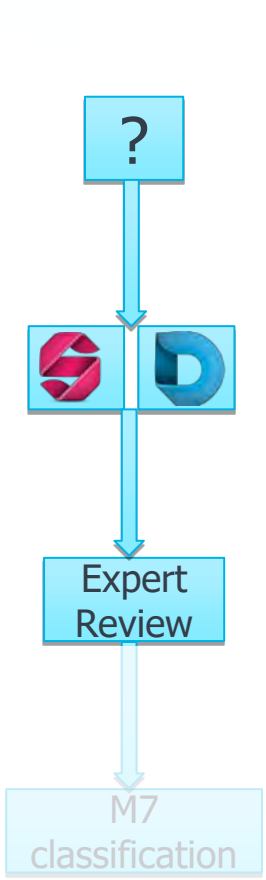
Expert review





Derek nexus

INACTIVE

- Inactive prediction has no misclassified or unclassified features that would reduce confidence in the prediction
- Alert comments discuss sulfones inactivating aromatic amines

Sarah nexus

NEGATIVE (100%)

- Compound is a known non-mutagen in the Sarah training set that has been tested adequately

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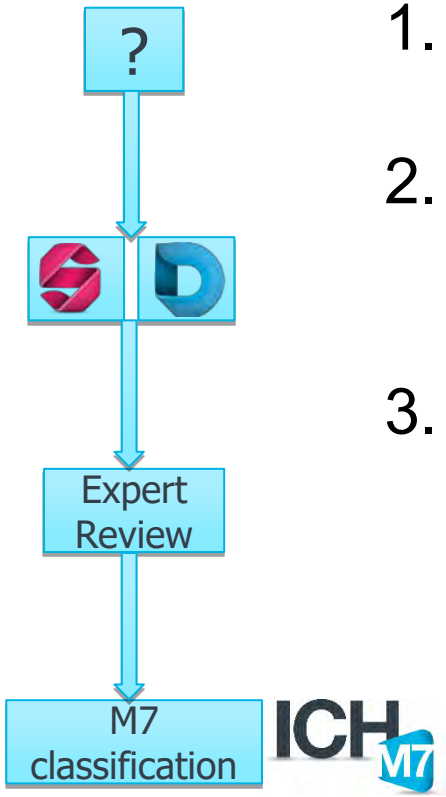
Please make your selection



1. Class 3 – Alerting structure
2. Class 5 – No alerts or alerting with sufficient data to demonstrate lack of mutagenicity
3. Unsure

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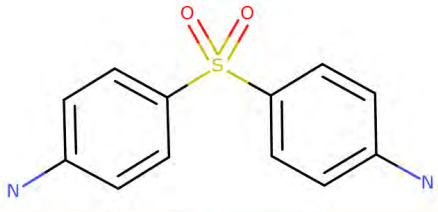
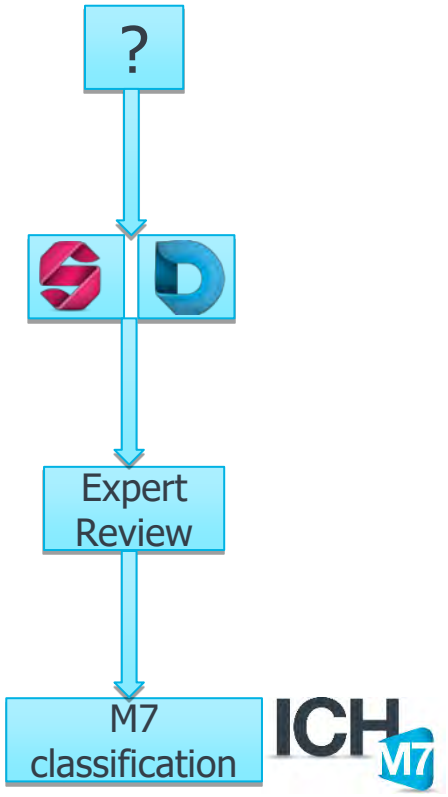
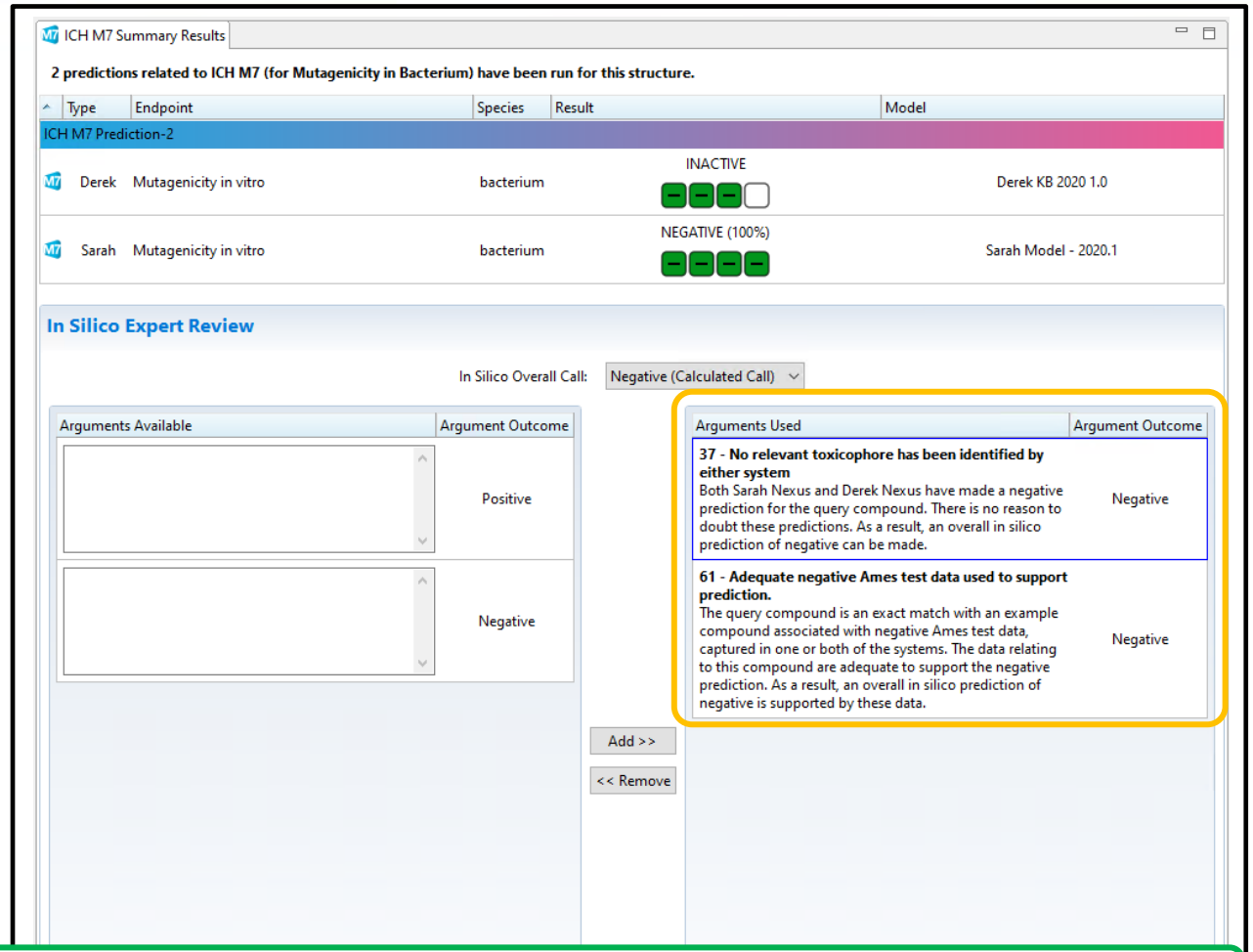
ICH M7 classification







Class 5

There is no reason to doubt either prediction & compound is a known non-mutagen that has been adequately tested.

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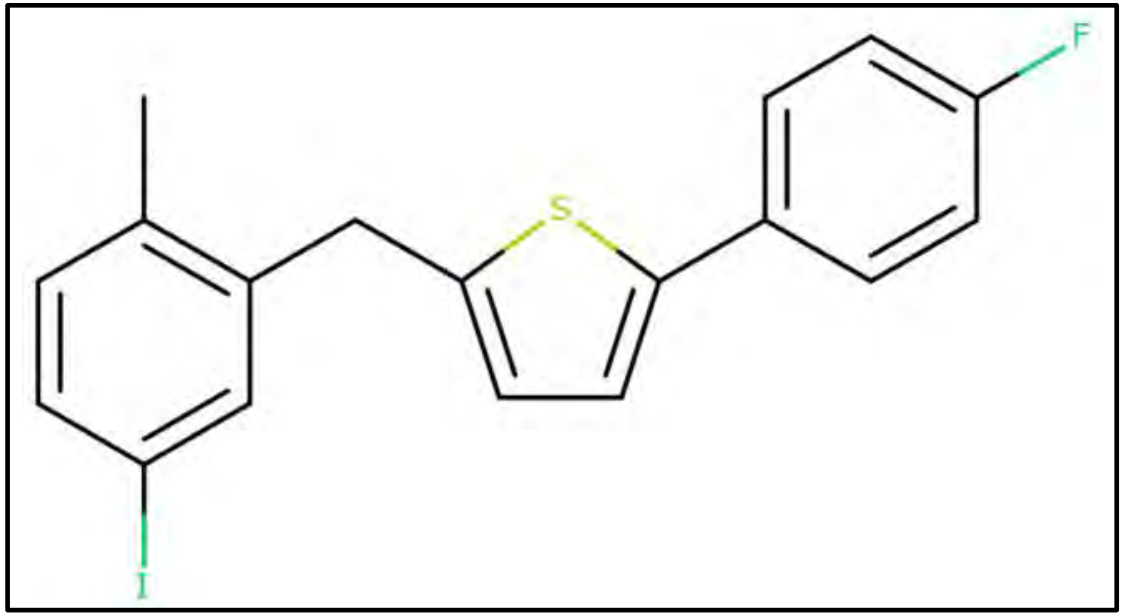
Example 2



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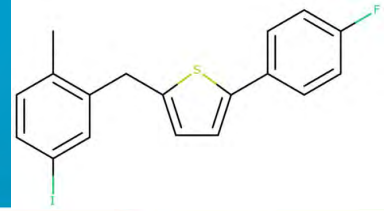
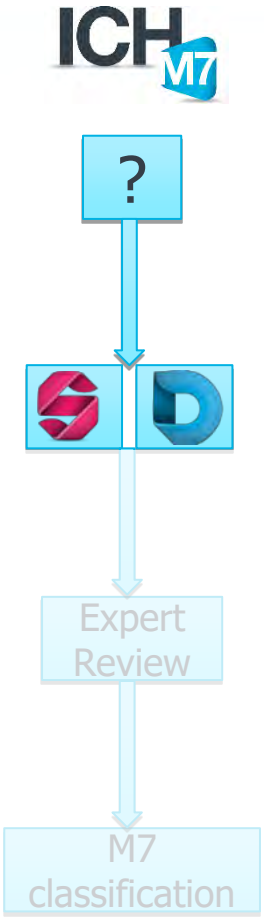
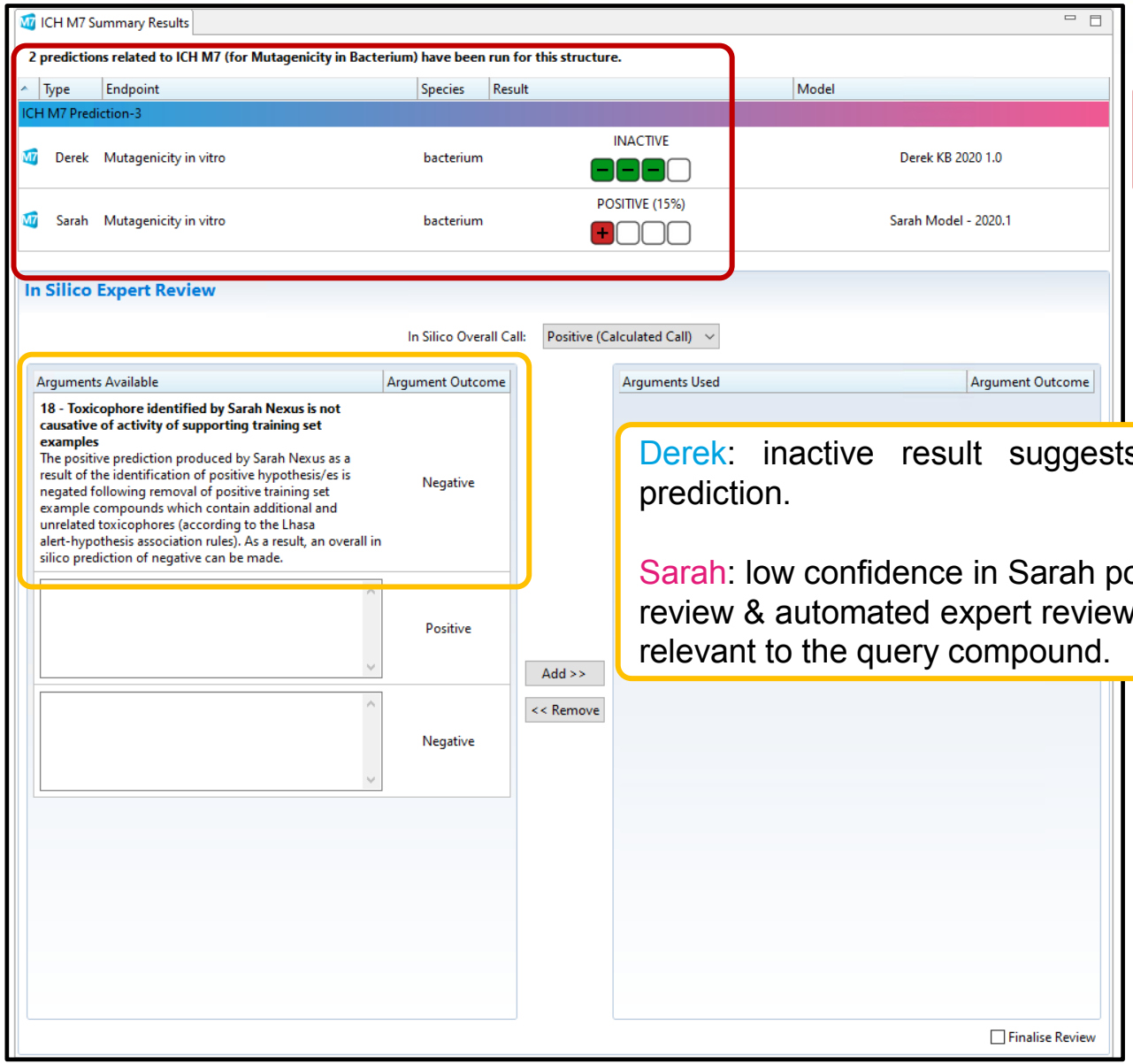
Review high level predictions







Derek & Sarah
disagree

Derek: inactive result suggests high confidence in negative prediction.

Sarah: low confidence in Sarah positive suggests examples require review & automated expert review argument notes they may not be relevant to the query compound.

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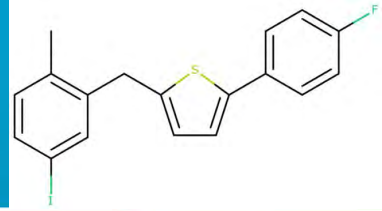
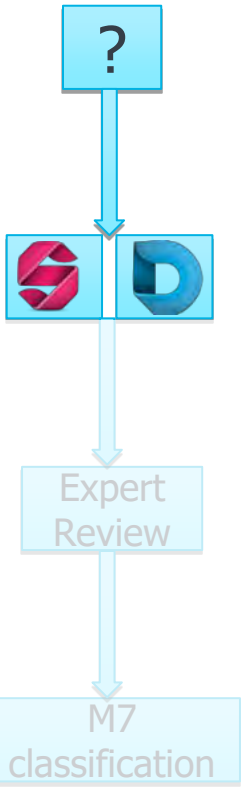
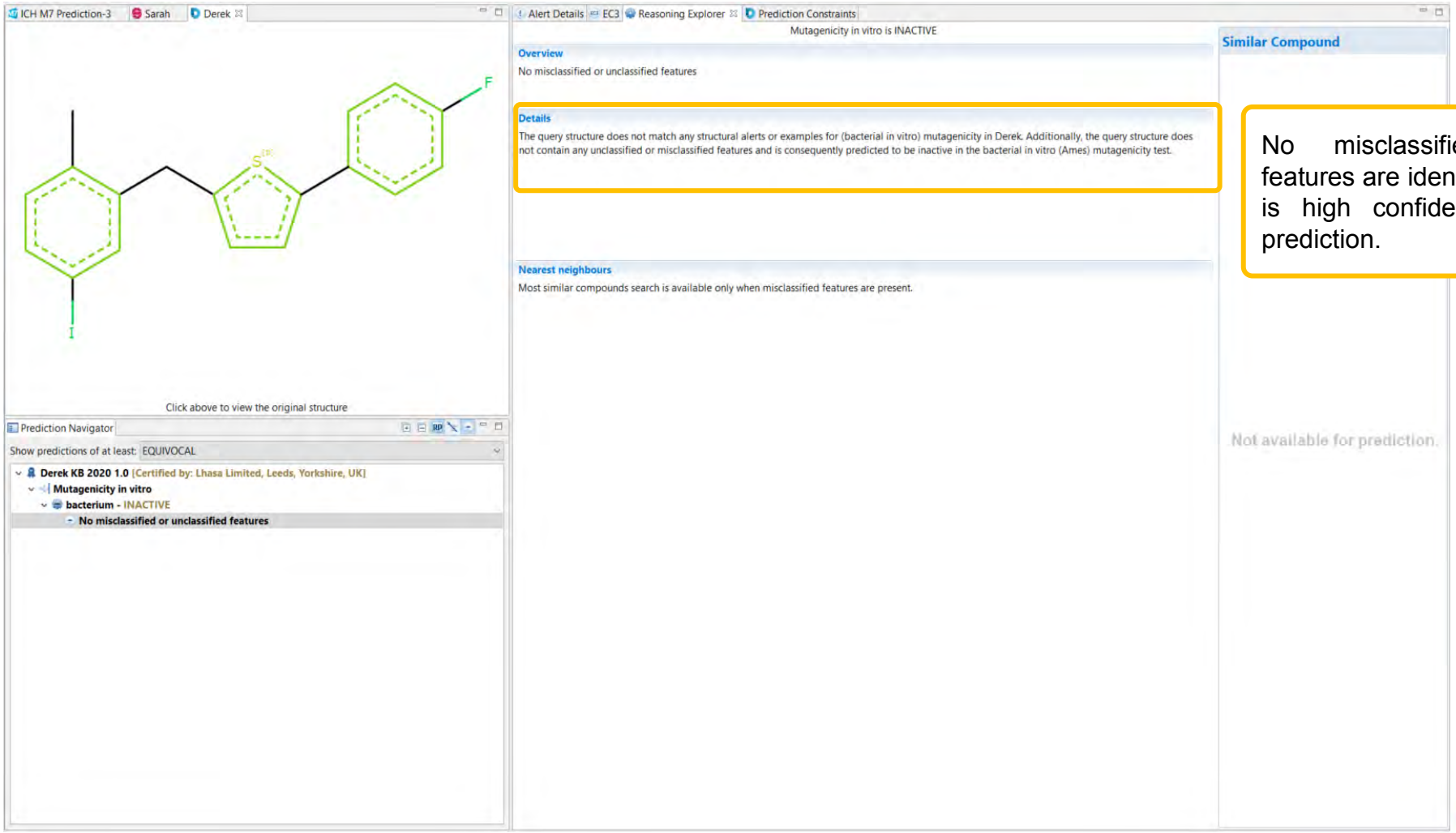
Review the expert prediction



Derek nexus





No misclassified or unclassified features are identified, suggesting there is high confidence in the negative prediction.

No misclassified or unclassified features raises no doubt in the negative prediction made by Derek.

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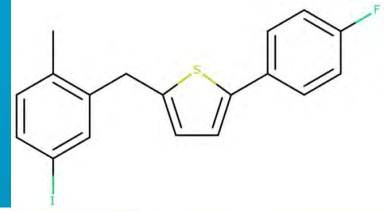
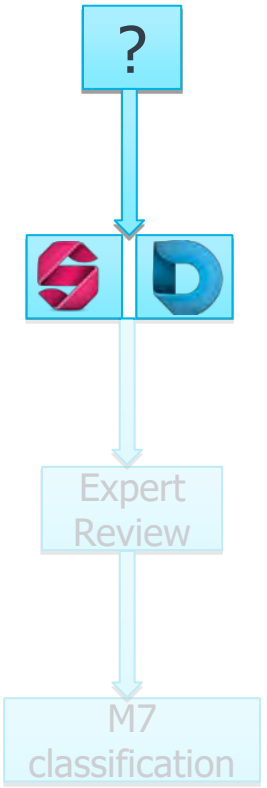
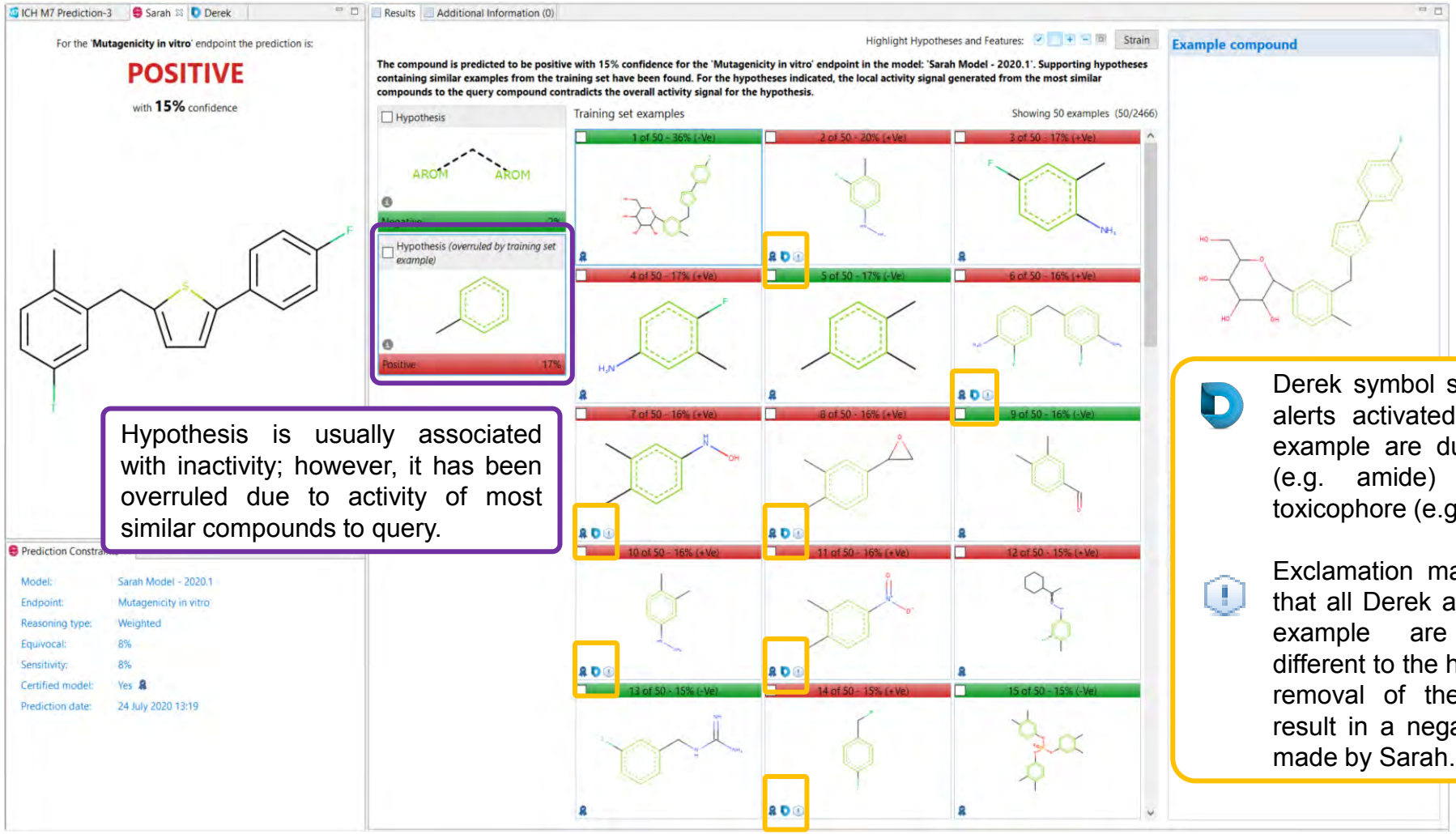
Review the statistical prediction



Sarah nexus





Hypothesis is usually associated with inactivity; however, it has been overruled due to activity of most similar compounds to query.

Derek symbol shows whether Derek alerts activated by the training set example are due to the hypothesis (e.g. amide) or an alternative toxicophore (e.g. aromatic amine).

Exclamation mark symbol highlights that all Derek alerts activated by the example are for toxicophores different to the hypothesis. Therefore, removal of these examples would result in a negative prediction being made by Sarah.

Although Sarah provides a positive prediction, the positive hypothesis is a result of training set examples demonstrating activity as a result of activating features which are not in the query compound or hypothesis. Removal of these examples instead results in a negative prediction being made.

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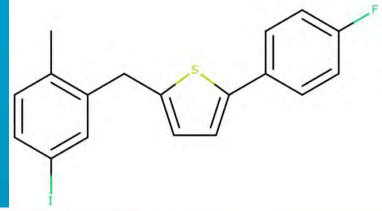
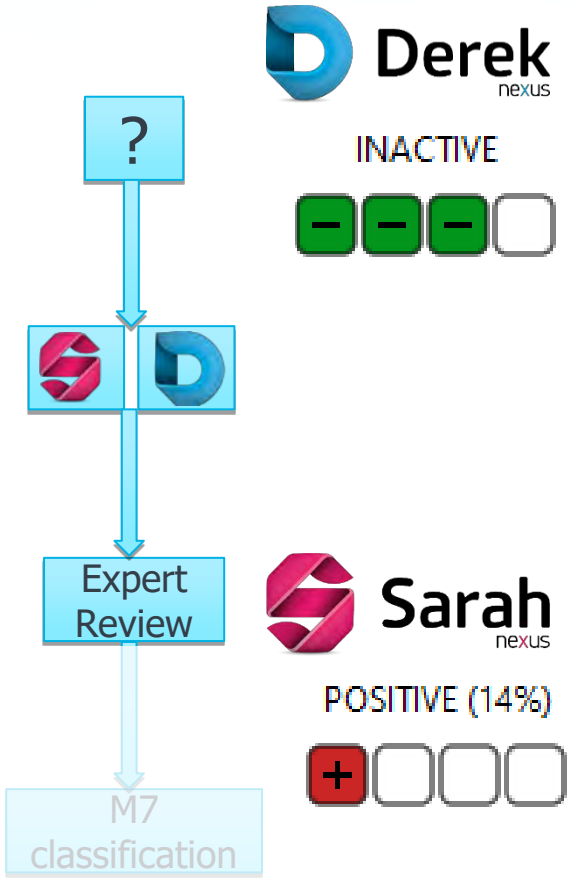
Expert review



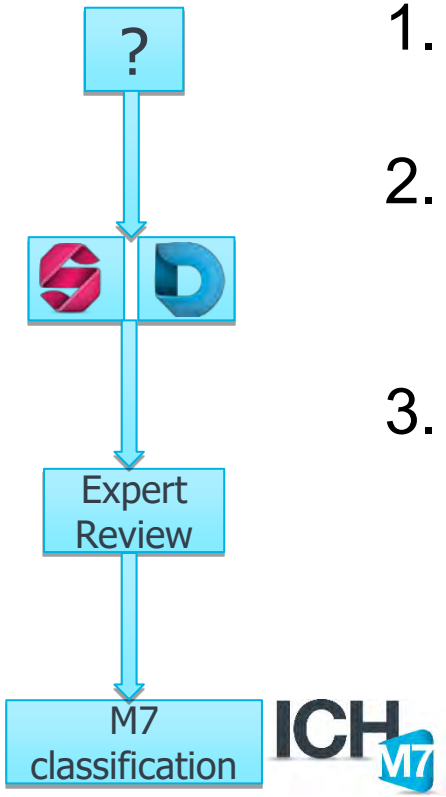


- Inactive prediction has no misclassified or unclassified features that would reduce confidence in the prediction

- Positive prediction is supported by 1 hypothesis, although it is an overruled negative hypothesis & overall confidence is relatively low (15%)
- Mutagens in the training set are active due to activating groups not present in the query compound & their removal from prediction gives a negative prediction

Lhasa Limited

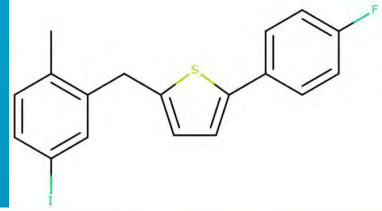
Please make your selection

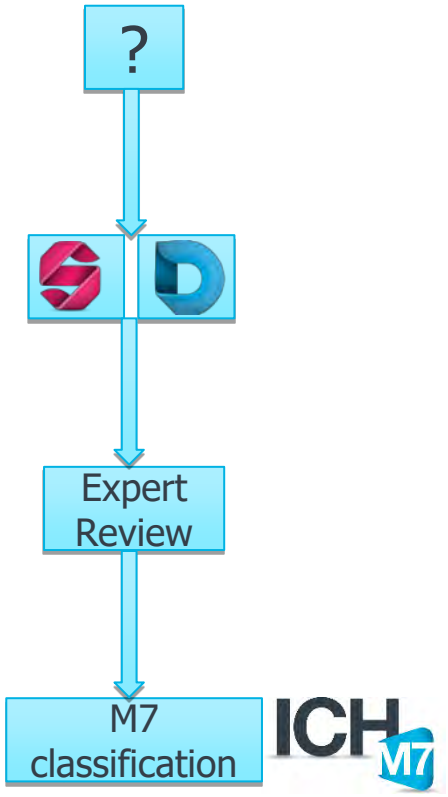


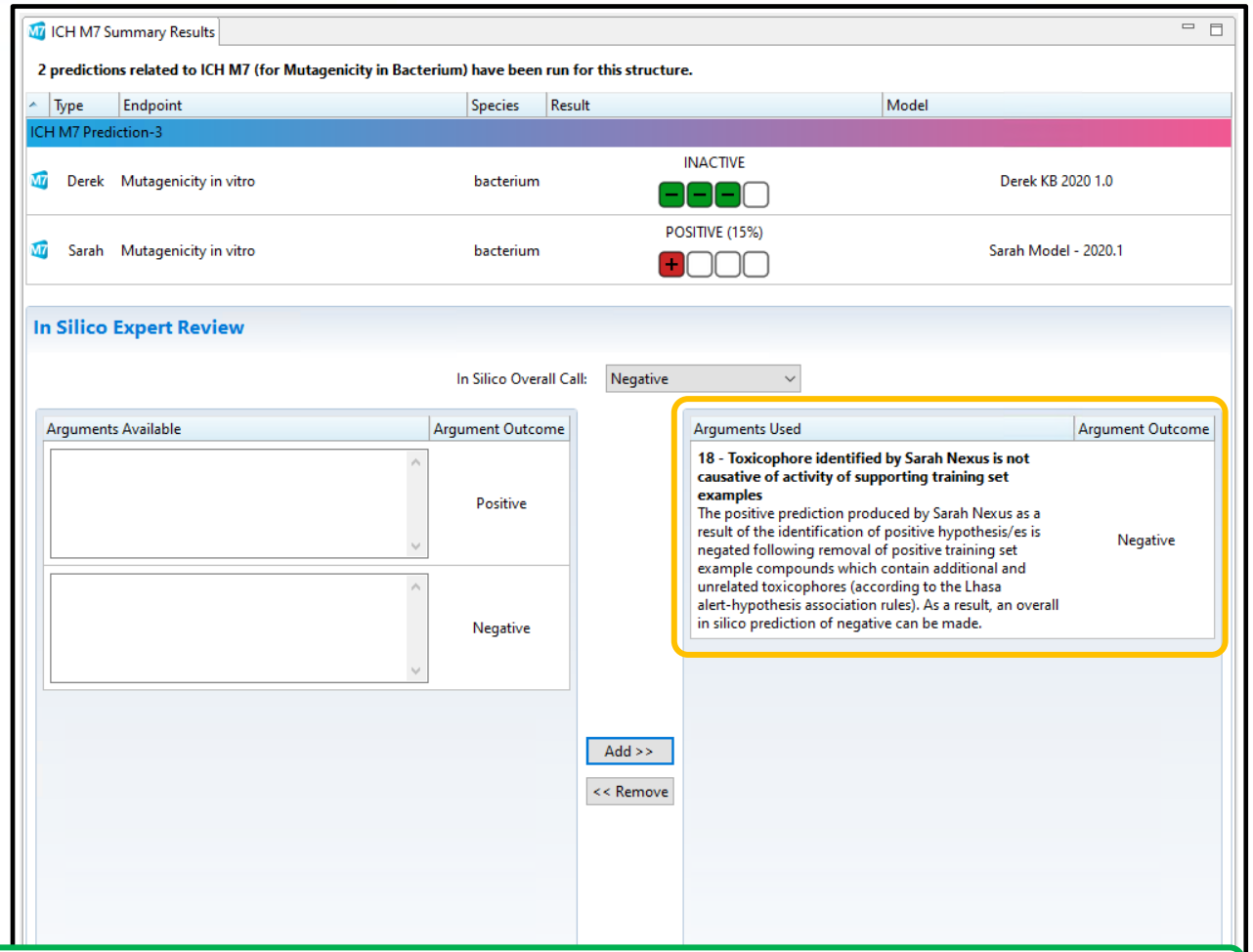
1. Class 3 – Alerting structure
2. Class 5 – No alerts or alerting with sufficient data to demonstrate lack of mutagenicity
3. Unsure

Lhasa Limited

ICH M7 classification







Class 5

The positive prediction made by Sarah is based on compounds which have different toxicophores & match different Derek alerts to the query compound, hence it is reasonable to overrule & accept the negative prediction made by Derek.

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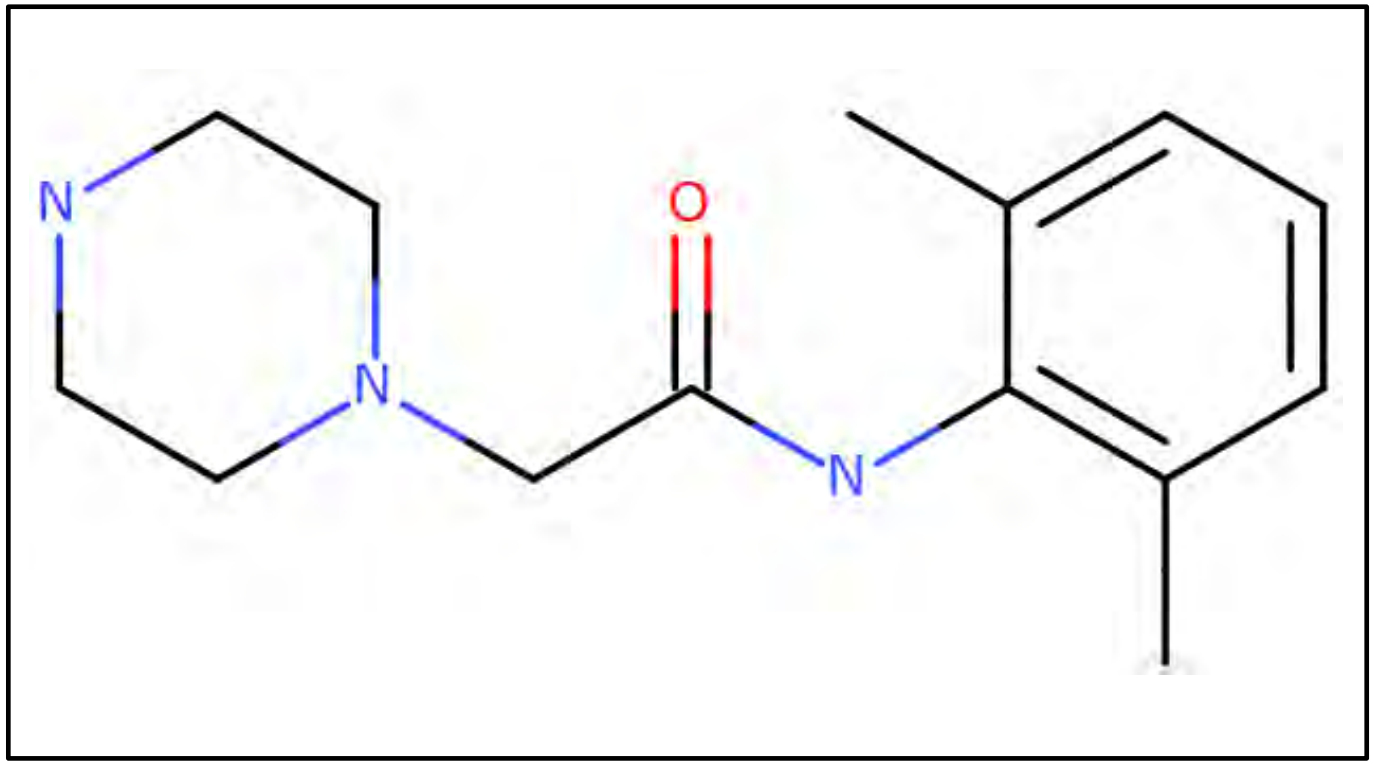
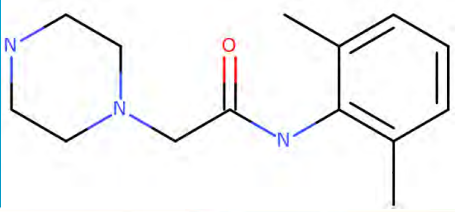
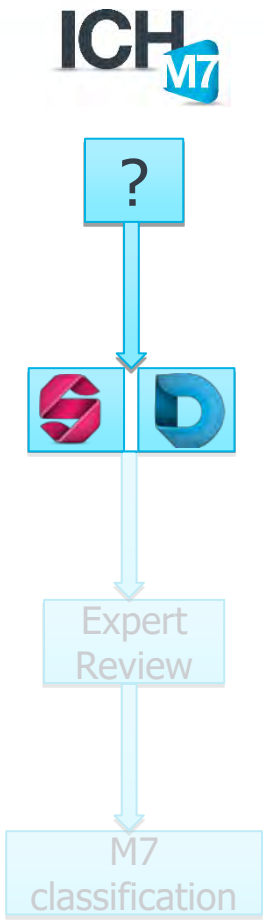
Example 3



Lhasa Limited

Review high level predictions





ICH M7 Summary Results

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

Type	Endpoint	Species	Result	Model
ICH M7 Prediction				
Derek	Mutagenicity in vitro	bacterium	PLAUSIBLE	Derek KB 2020 1.0
Sarah	Mutagenicity in vitro	bacterium	NEGATIVE (27%)	Sarah Model - 2020.1

In Silico Expert Review

In Silico Overall Call: Positive (Calculated Call)

Arguments Available	Argument Outcome
11 - Toxicophore identified by Derek Nexus has not been adequately assessed by Sarah Nexus At least one alert identified by Derek Nexus does not correspond to a related hypothesis in Sarah Nexus and has not been adequately assessed by Sarah Nexus. As a result, an overall in silico prediction of positive must be made.	Positive
41 - Toxicophore(s) identified by Derek Nexus can be adequately negated by most similar compounds in Sarah Nexus Sarah Nexus has produced a negative prediction overall and no positive hypotheses have been identified for the query compound. The most similar compounds to the query structure used to make the prediction are adequate to negate all the hazards identified in Derek Nexus. As a result, the Derek Nexus positive prediction can be overruled and an overall in silico prediction of negative can be made.	Negative
	Positive
	Negative

Add >>

<< Remove

Arguments Used	Argument Outcome

Finalise Review

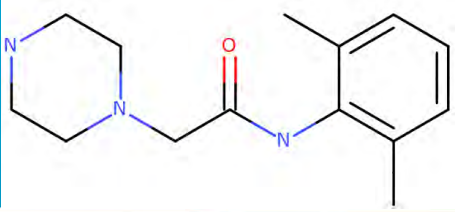
Derek & Sarah
disagree

Derek: plausible result suggests good confidence in positive prediction.

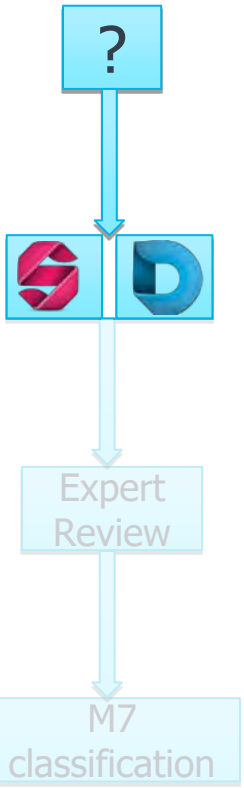
Sarah: example compounds need to be reviewed to ascertain relevance to the toxicophore identified by Derek.

Lhasa Limited

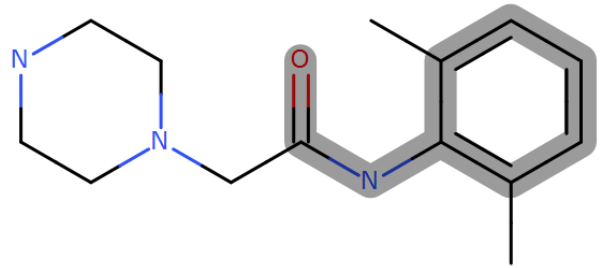
Review the expert prediction



Derek nexus



ICH M7 Prediction | Derek | Sarah



'Alert 352' selected, click above to view the original structure

Prediction Navigator

Show predictions of at least: EQUIVOCAL

- Derek KB 2020 1.0 [Certified by: Lhasa Limited, Leeds, Yorkshire, UK]
  - Mutagenicity in vitro
    - bacterium - PLAUSIBLE
      - Alert - 352: Aromatic amine or amide

Alert Details | EC3 | Reasoning Explorer | Prediction Constraints

352: Aromatic amine or amide

Alert Matches

Description Image

Comments

4. The presence of sulphonic acid or sulphonate groups has been shown to inhibit the mutagenic activity of aromatic amines [Jung et al].

5. Ortho disubstitution has been shown to reduce or eliminate the mutagenic activity of some aromatic amines as a result of steric inhibition of N-hydroxylation [Garner et al, Kugler-Steigmeier et al, Lai et al, Ashby et al 1982], although fluorine substituents, being small in size, do not exhibit this effect [Garner et al]. In a systematic study of increasing ortho substituent size it was observed that the mutagenic potential is reduced, though still present, until diisopropylaniline is reached which has been observed to be non-mutagenic in TA100 in the presence of S9 [Kugler-Steigmeier et al]. 2-Amino-3-chlorobenzoic acid is an exception to this trend since, despite having small substituents, a recent study using a highly-purified sample reported it to be negative in five strains, contrary to previous reports [Gunther et al]. 3,5-Dimethyl-4-aminobiphenyl is also a reported exception to the general trend of a reduction in mutagenic activity since it continues to show significant mutagenic activity, particularly when using a liquid pre-incubation protocol [Ashby et al 1982]. It has been proposed that this may be a consequence of an alternative mechanism of activation, since the related ortho-disubstituted structure 3,3',5,5'-tetramethylbenzidine gives a negative response in the Ames test [Ashby et al 1982]. On the basis that attenuation of mutagenic activity appears not to occur for all members of this biphenyl-type structure class, structures with an additional aromatic ring in the para position are omitted from the ortho disubstitution restrictions described.

6. Disubstituted anilines bearing only halogen and/or trifluoromethyl substituents on the aromatic ring generally give a negative response in the Ames test. Zeiger et al (1987, 1988, 1992) and Zimmer et al have tested a number of dihaloanilines (substituents = F, Cl, Br) in Salmonella typhimurium strains TA98, TA100, TA97 and TA1535 and have found them to be negative in the presence and absence of rat liver S9 mix (although some activity was observed for 2,4-dichloroaniline and 2,4-difluoroaniline in the presence of hamster liver S9 mix). Data for a further seven disubstituted anilines in this class (substituents = F, Cl, Br, CF3) contributed by Novartis Pharma also showed no activity in TA98 and TA100. 2,4,6-Trichloro- and 2,4,6-tribromo-anilines have also been reported to give a negative response in the Ames test but currently there are insufficient reports for non-ortho-disubstituted trihaloanilines to generalise this observation [Zeiger et al 1992, Zimmer et al]. In general, aromatic amines or amides containing one halogen or trifluoromethyl group and one alkyl group are also inactive in the Ames test. Examples include 3-chloro-p-toluidine and 5-chloro-o-toluidine [Haworth et al] and compounds containing other halogens contributed by GlaxoSmithKline.

7. Aromatic amides may potentially deacylate or N-hydroxylate as a first step in their activation to DNA-reactive species. However, there is evidence that neither deacylation [Testa and

Validation Comments

Mutagenicity: Ames test

The alert has demonstrated the following predictive performance:

1) Proprietary data set 1: 94 compounds activate this alert of which 41 are reported positive (positive predictivity: 44%)
2) Proprietary data set 2: 4 compounds activate this alert of which 2 are reported positive (positive predictivity: 50%)
3) FDA CFSAN data set: 249 compounds activate this alert of which 202 are reported positive (positive predictivity: 81%)

Endpoints

ID	Name	Parent
4086	Mutagenicity	Mutagenicity (ALL)

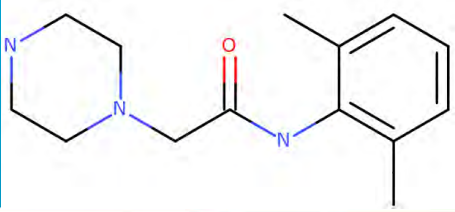
Alert comments explain bis-*ortho*-substituted aromatic amines are excluded due to steric inhibition of the required *N*-hydroxylation. Although 2,6-dimethylphenyl substitution is allowed, it is worth reviewing activity based on these comments.

Variable PPV, moderate (44%) to high (81%) for alert.

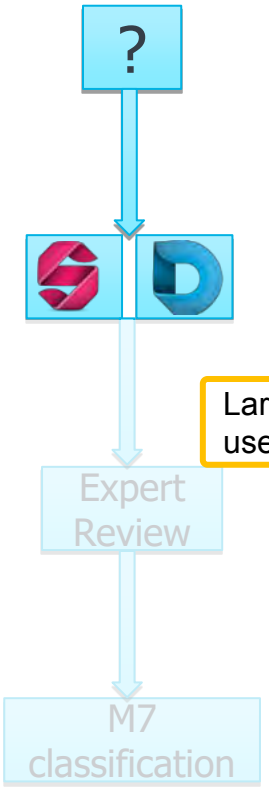
The restriction of bis-*ortho*-substituted aromatic amines where a substituent is “large” does not exclude the 2,6-dimethylphenyl, it warrants additional review into this positive activity predicted by Derek as it may considered a near miss for the exclusion.

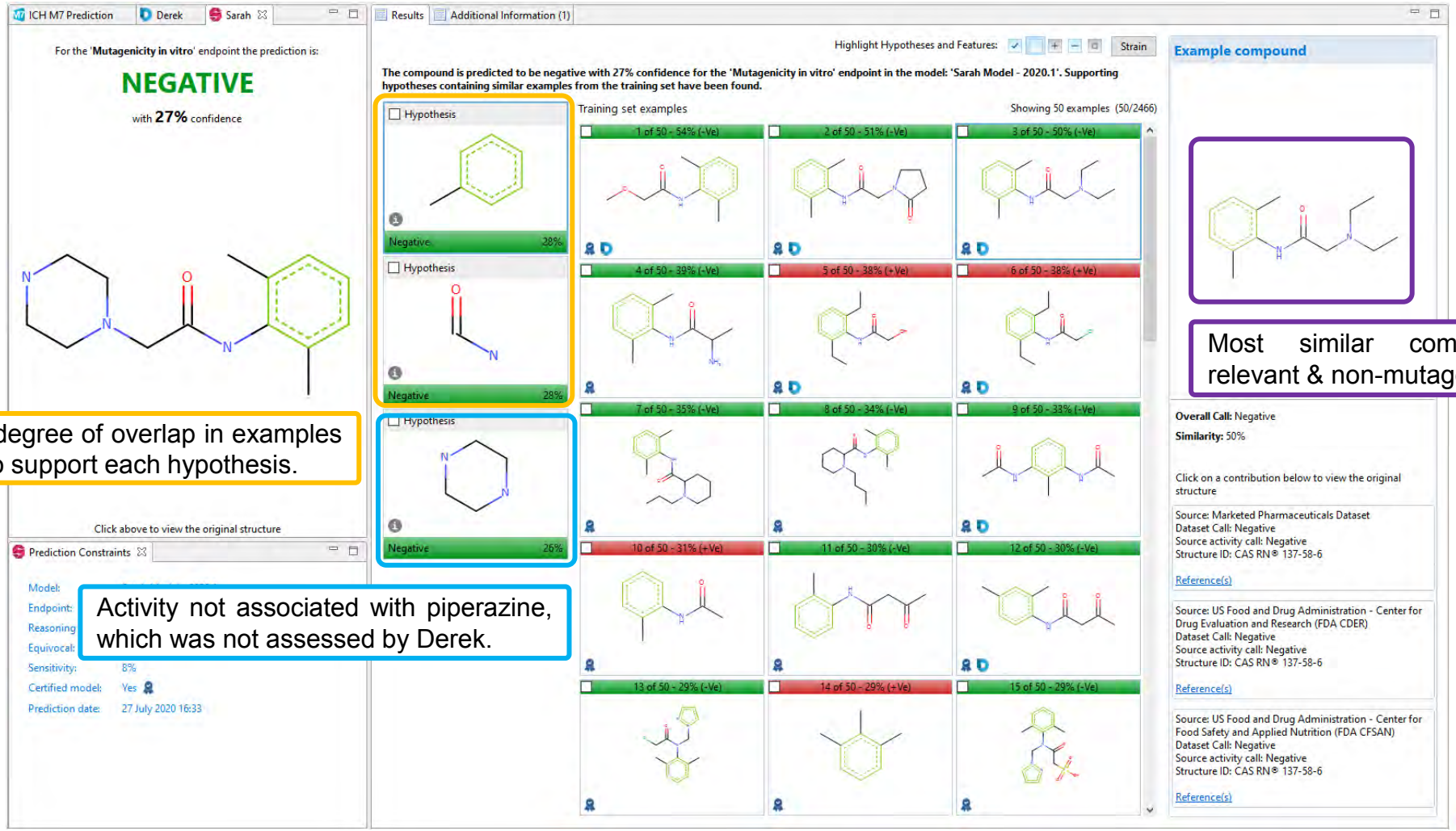
Lhasa Limited

Review the statistical prediction



Sarah nexus





Large degree of overlap in examples used to support each hypothesis.

Activity not associated with piperazine, which was not assessed by Derek.

Most similar compounds are relevant & non-mutagenic.

There are several compounds similar to the query in Sarah which assess the aromatic amine identified by Derek as well as the piperazine. Moreover, the most similar examples are similar to the query so it is reasonable to consider this to be an acceptable negative prediction.

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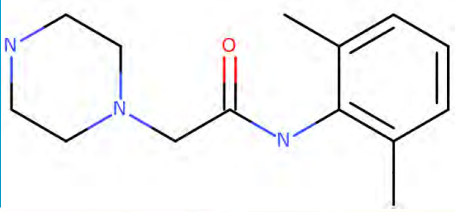
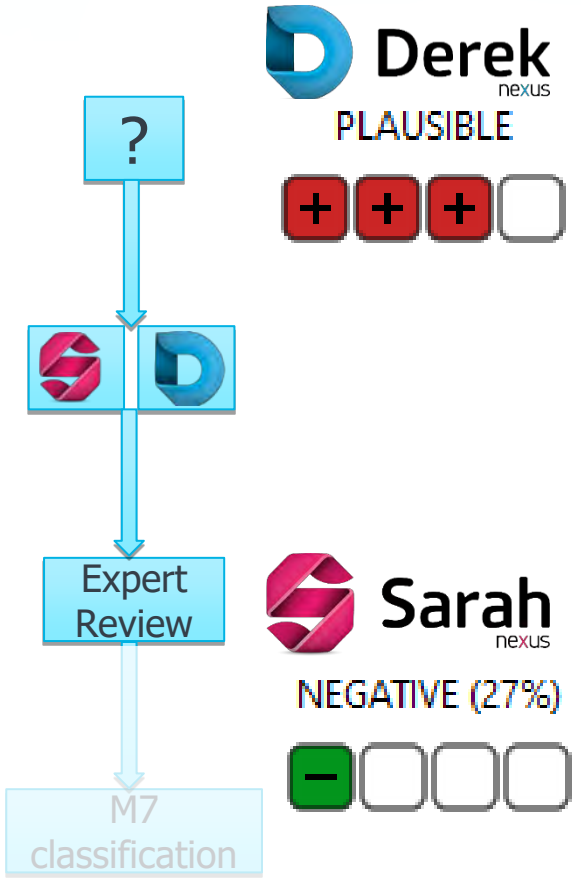
Expert review





Derek nexus

PLAUSIBLE

- Matches alert for aromatic amine
- Comments discuss exclusion of aromatic amines with bis-*ortho*-substituents where one is not “small”, hence this may be considered a near-miss for this exclusion

Sarah nexus

NEGATIVE (27%)

- Negative prediction with good confidence is supported by 3 hypotheses
- Several similar examples which are relevant to use for read-across

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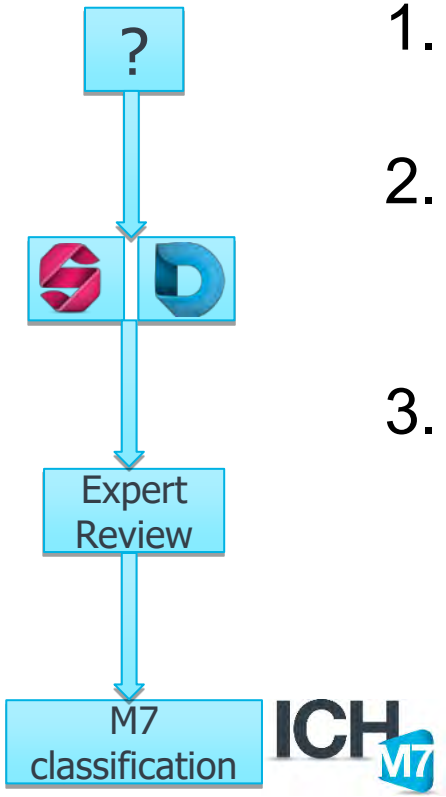
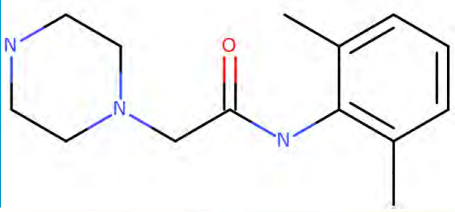
Please make your selection

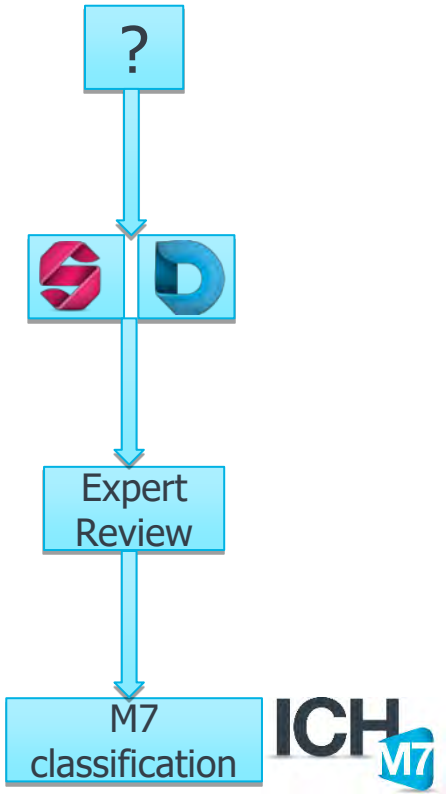


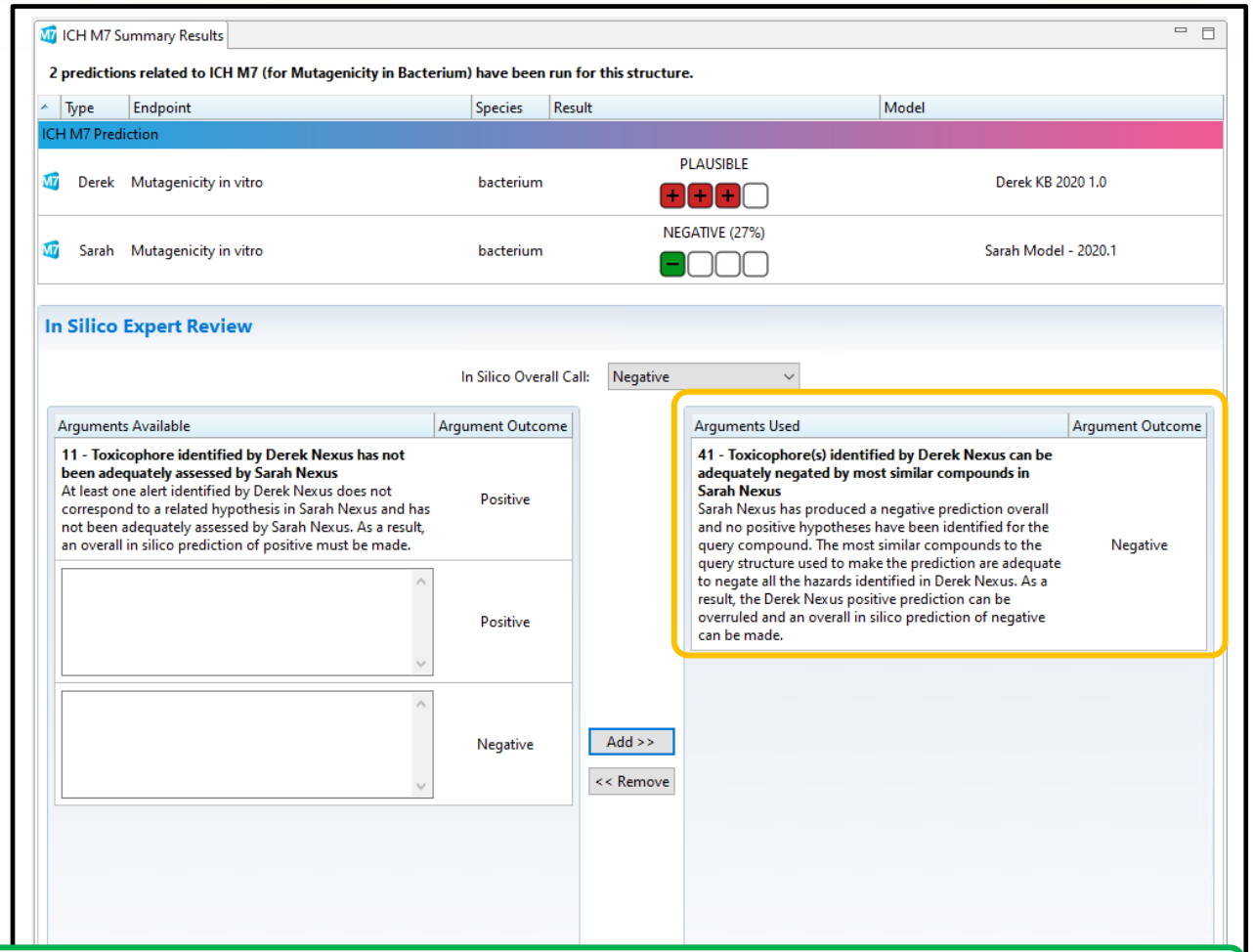
1. Class 3 – Alerting structure
2. Class 5 – No alerts or alerting with sufficient data to demonstrate lack of mutagenicity
3. Unsure

Lhasa Limited

ICH M7 classification





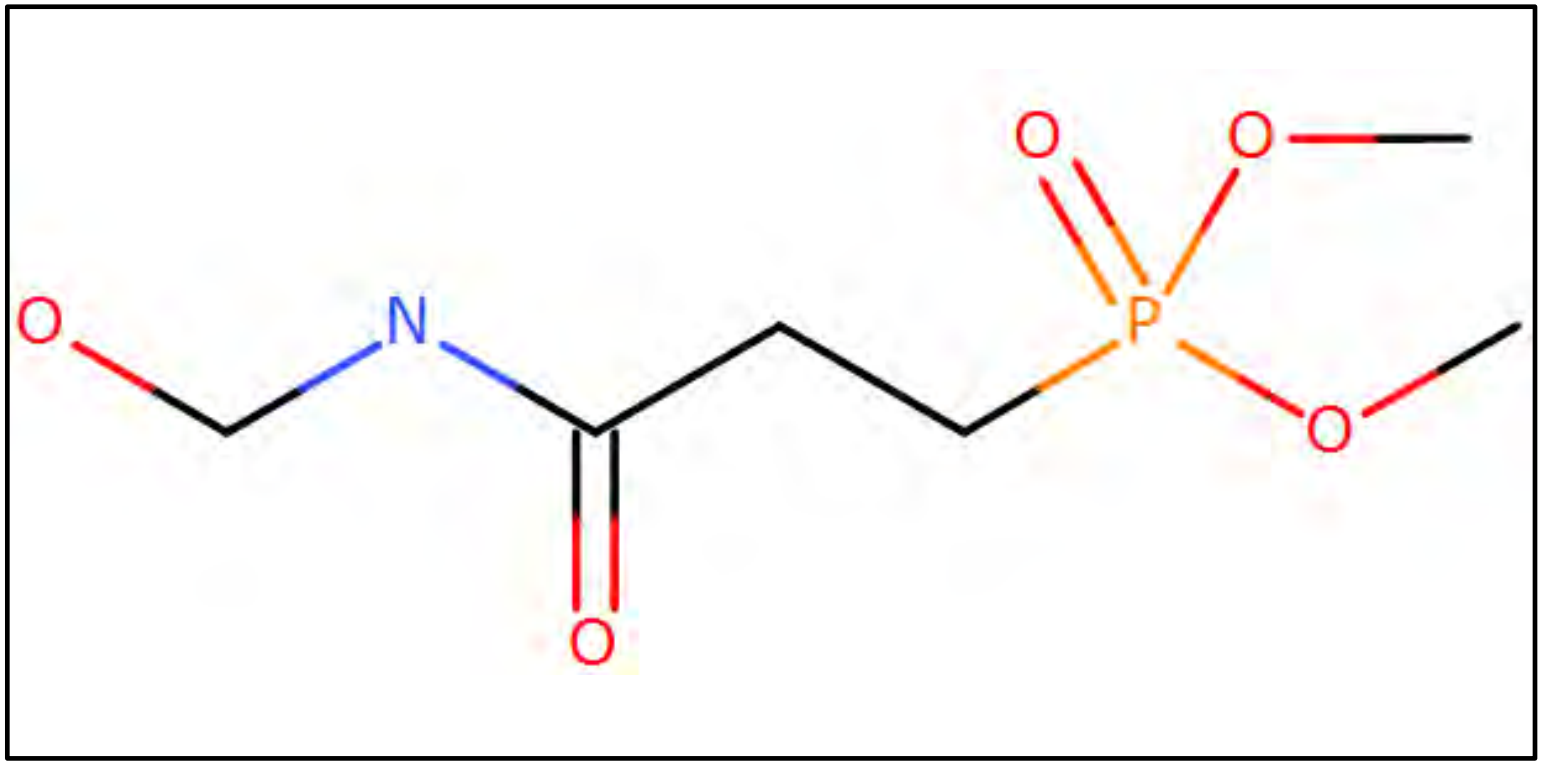


Class 5

Positive prediction made by Derek details an exclusion for which the query is a near-miss, reducing confidence in the prediction. The similarity & relevance of non-mutagens in Sarah support overturning the Derek prediction.

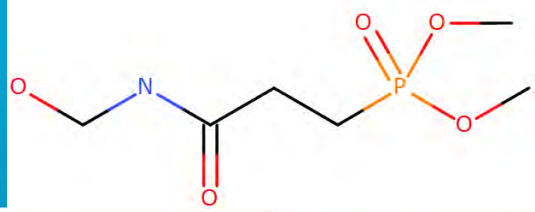
Lhasa Limited

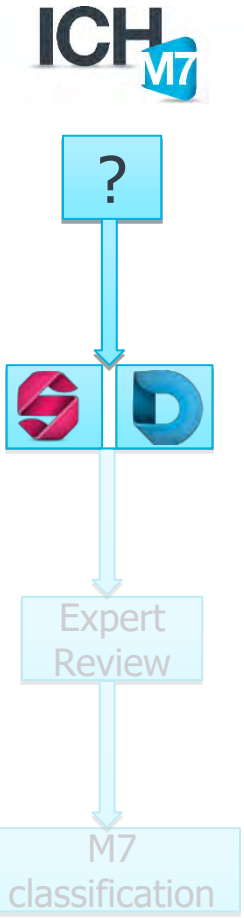
Example 4



Lhasa Limited

Review high level predictions





ICH M7 Summary Results

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

Type	Endpoint	Species	Result	Model
ICH M7 Prediction-3				
Derek	Mutagenicity in vitro	bacterium	EQUIVOCAL	Derek KB 2020 1.0
Sarah	Mutagenicity in vitro	bacterium	NEGATIVE (32%)	Sarah Model - 2020.1

In Silico Expert Review

In Silico Overall Call: Positive (Calculated Call)

Arguments Available	Argument Outcome
11 - Toxicophore identified by Derek Nexus has not been adequately assessed by Sarah Nexus At least one alert identified by Derek Nexus does not correspond to a related hypothesis in Sarah Nexus and has not been adequately assessed by Sarah Nexus. As a result, an overall in silico prediction of positive must be made.	Positive
38 - Adequate negative Ames test data from Sarah Nexus additional information used to overrule prediction The query compound is an exact match with a compound present in the additional information supplied with the Sarah Nexus training set. An overall call could not automatically be assigned for this compound but review of the available data indicates it produces negative results in the Ames test. As a result, an overall in silico prediction of negative can be made.	Negative
39 - Adequate positive Ames test data from Sarah Nexus additional information used to overrule prediction The query compound is an exact match with a compound present in the additional information supplied with the Sarah Nexus training set. An overall call could not automatically be assigned for this compound but review of the available data indicates it produces positive results in the Ames test. As a result, an overall in silico prediction of positive must be made.	Positive
41 - Toxicophore(s) identified by Derek Nexus can be adequately negated by most similar compounds in Sarah Nexus Sarah Nexus has produced a negative prediction overall and no positive hypotheses have been identified for the query compound. The most similar compounds to the query structure used to make the prediction are adequate to negate all the hazards identified in Derek	Negative

Add >>

< Remove

Arguments Used	Argument Outcome

Finalise Review

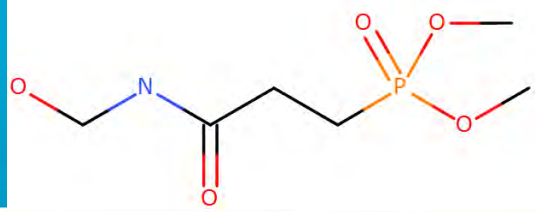
Derek & Sarah
disagree

Derek: the equivocal result warrants analysis as it's considered to be a positive result with low confidence.

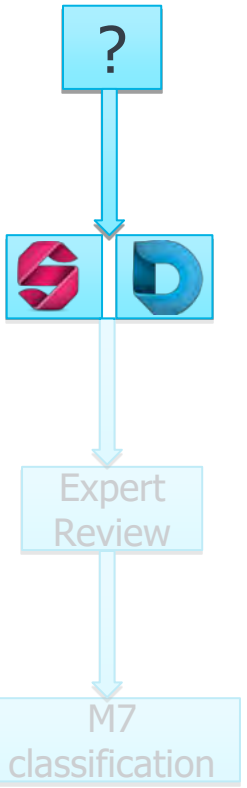
Sarah: example compounds, including those in the additional information tab which are not used by the hypothesis, need to be reviewed to ascertain relevance to the toxicophore identified by Derek.

Lhasa Limited

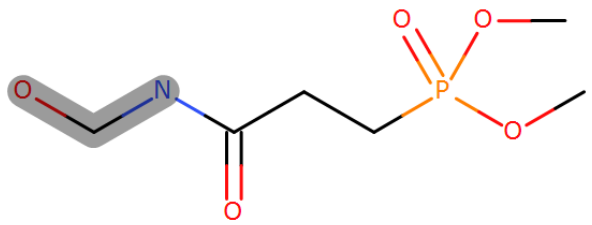
Review the expert prediction



Derek nexus



ICH M7 Prediction-3 | Derek | Sarah



'Alert 307' selected, click above to view the original structure

Prediction Navigator

Show predictions of at least: EQUIVOCAL

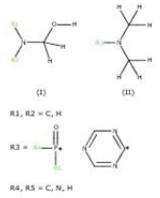
- Derek KB 2020 1.0 [Certified by: Lhasa Limited, Leeds, Yorkshire, UK]
  - Mutagenicity in vitro
    - bacterium - EQUIVOCAL
      - Alert - 307: N-Methylol compound or precursor

Alert Details | EC3 | Reasoning Explorer | Prediction Constraints

307: N-Methylol compound or precursor

Alert Matches

Description Image



Comments

This alert originates from the identification of the N-methylol group (toxicophore I) as a DNA reactive centre through hydrolysis to formaldehyde [Ashby and Tennant] but positive results in bacterial mutagenicity tests are frequently not obtained [Overton et al 1986, Ashby et al 1985b, Lander et al]. In some cases N-methyl groups (toxicophore II) are considered to be metabolised to the N-methylol group and these have also been included.

Examples include 9-hydroxymethyl-N-carbazole which tested positive in Salmonella typhimurium TA100 without metabolic activation [LaVoie et al], hexamethylphosphoramide (HMPA) [Sarrif et al] and hexamethylmelamine (HEMLA) [Ashby et al 1985a] which tested positive in Salmonella typhimurium TA100, but only with non-standard protocols (suspension assays) or in the presence of high concentrations of metabolic activation (30% S9). 4-Chloro-N-(hydroxymethyl)benzamide and its N-acetoxymethyl derivative [Overton et al 1986], N-methylolacrylamide [Tennant and Ashby], chloroacetamide N-metholol [Ashby et al 1985b] and 7-hydroxymethyl theophylline [Lander et al] all tested negative in Salmonella typhimurium TA100.

The predominantly reported mechanism is hydrolysis of the N-methylol to the amine and formaldehyde which reacts with DNA [LaVoie et al]. Covalent DNA binding has been reported for many N-methylol derivatives [Ashby et al 1985b, Ames et al, Lander et al]. For the putative N-methylol precursor HMPA metabolic oxidative demethylation yields formaldehyde via an unstable N-methylol intermediate [Sarrif et al, Ashby et al 1985b]. However, alternative mechanisms for HMPA cannot be excluded [Ziljstra et al]. For one specific compound HEMLA and its analogues, generation of electrophilic methyleneiminium or methyleneimine species from an intermediate N-methylol group has also been suggested, based on DNA binding studies of HEMLA radiolabelled in the ring [Ames et al, Overton et al 1985]. DNA cross-linking and DNA-protein interstrand cross-linking, mediated through both the iminium intermediate and formaldehyde have been described [Ames et al, Coley et al]. Conjugation of the imine or iminium intermediates from HEMLA by the electron withdrawing triazine group may increase their electrophilic reactivity and allow this alternative mechanism to occur. Studies of the non-mutagenic chloroacetamide-N-methylol also suggested DNA binding additional to that of the methylol carbon, but the mechanism is unclear and the level of binding low [Ashby et al 1985b].

Validation Comments

Mutagenicity: Ames test

The alert has demonstrated the following predictive performance:

1) Proprietary data set 1: 1 compound activates this alert of which 0 are reported positive (positive predictivity: 0%)
2) Proprietary data set 2: 0 compounds activate this alert
3) FDA CFSAN data set: 9 compounds activate this alert of which 5 are reported positive (positive predictivity: 56%)

Endpoints

ID	Name	Parent
4086	Mutagenicity	Mutagenicity (ALL)

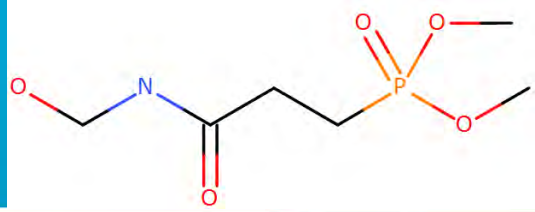
Comments highlight methylols exhibit weak activity in Ames test; however, results are inconsistent. Mechanistic discussion suggests mechanism requires metabolism to formaldehyde which reacts with DNA. Therefore, activity is dependent on (1) the ability of metabolic system used & (2) weak mutagenicity of formaldehyde as ultimate mutagen.

Moderate (56%) PPV for alert suggests chemical class expected to have mixture of activity.

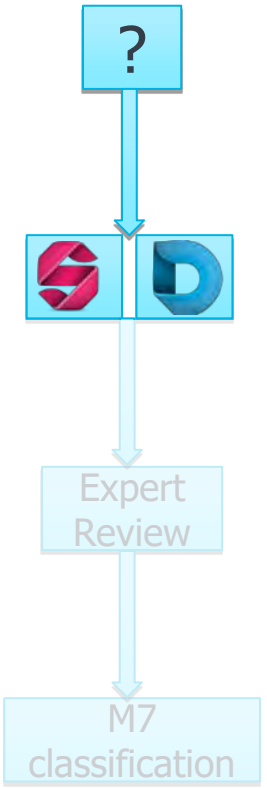
Alert comments suggest methylols are weakly mutagenic as they require metabolism to formaldehyde which is a weak mutagen itself. Therefore, it is necessary for the Ames test protocol to use a metabolic system that is competent otherwise it may be that the Ames test is not suitable for this class, which is consistent with the inconsistent results observed. It is reasonable to treat this as a positive, albeit one that warrants further review.

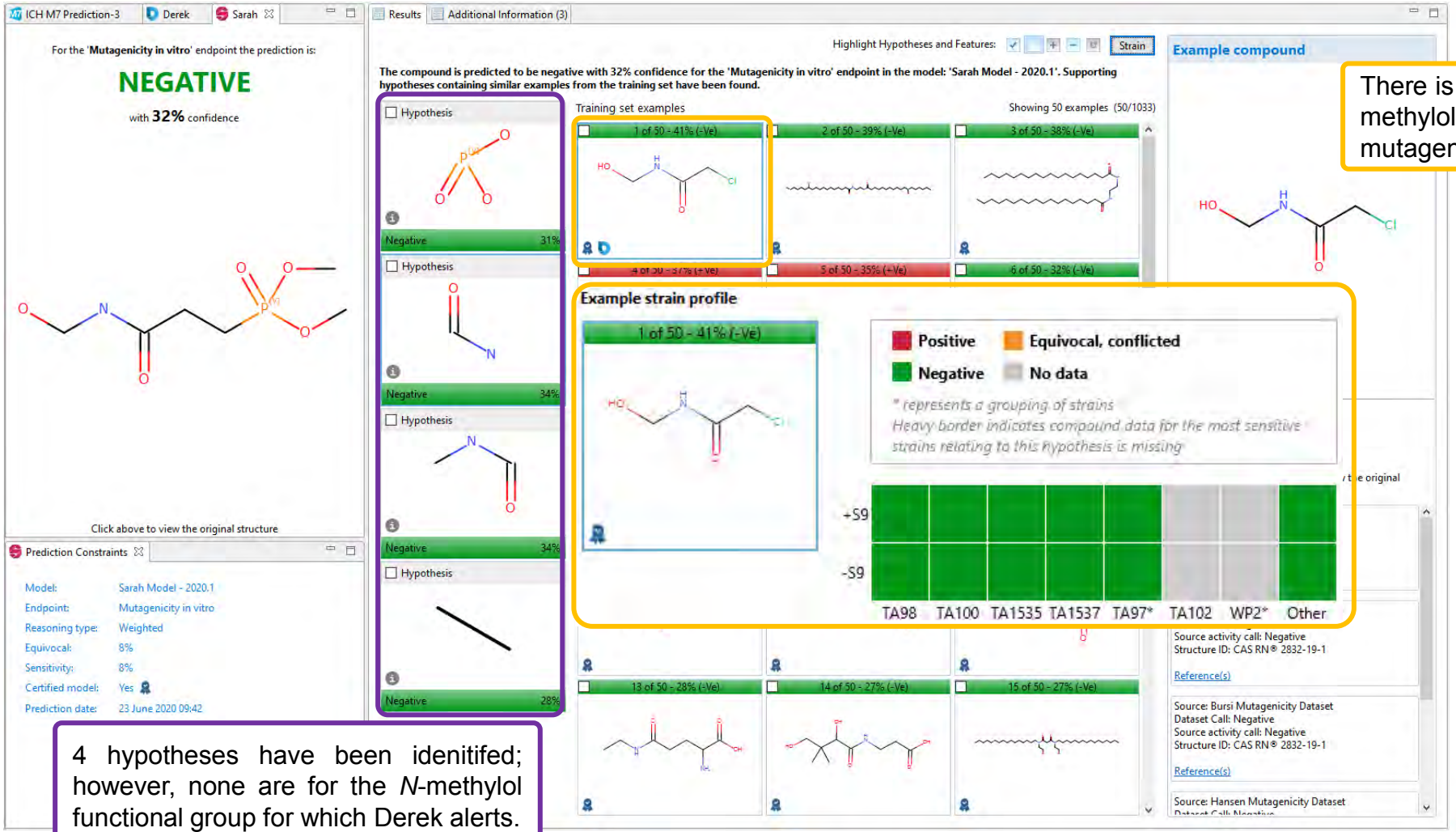
Lhasa Limited

Review the statistical prediction



Sarah nexus





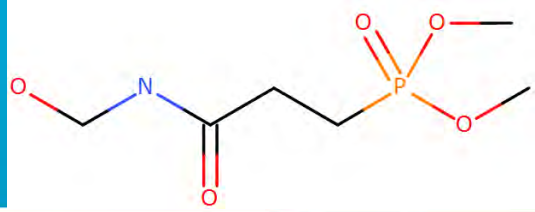
There is a single example of a *N*-methylol which is similar & non-mutagenic in all tested strains.

4 hypotheses have been idenitifed; however, none are for the *N*-methylol functional group for which Derek alerts.

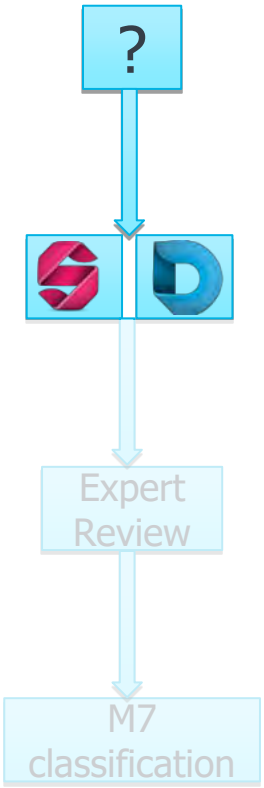
Sarah identifies 4 hypotheses; however, none are for the *N*-methylol specifically. There is a non-mutagenic *N*-methylol in the training set which is relevant & provides confidence in the negative prediction.

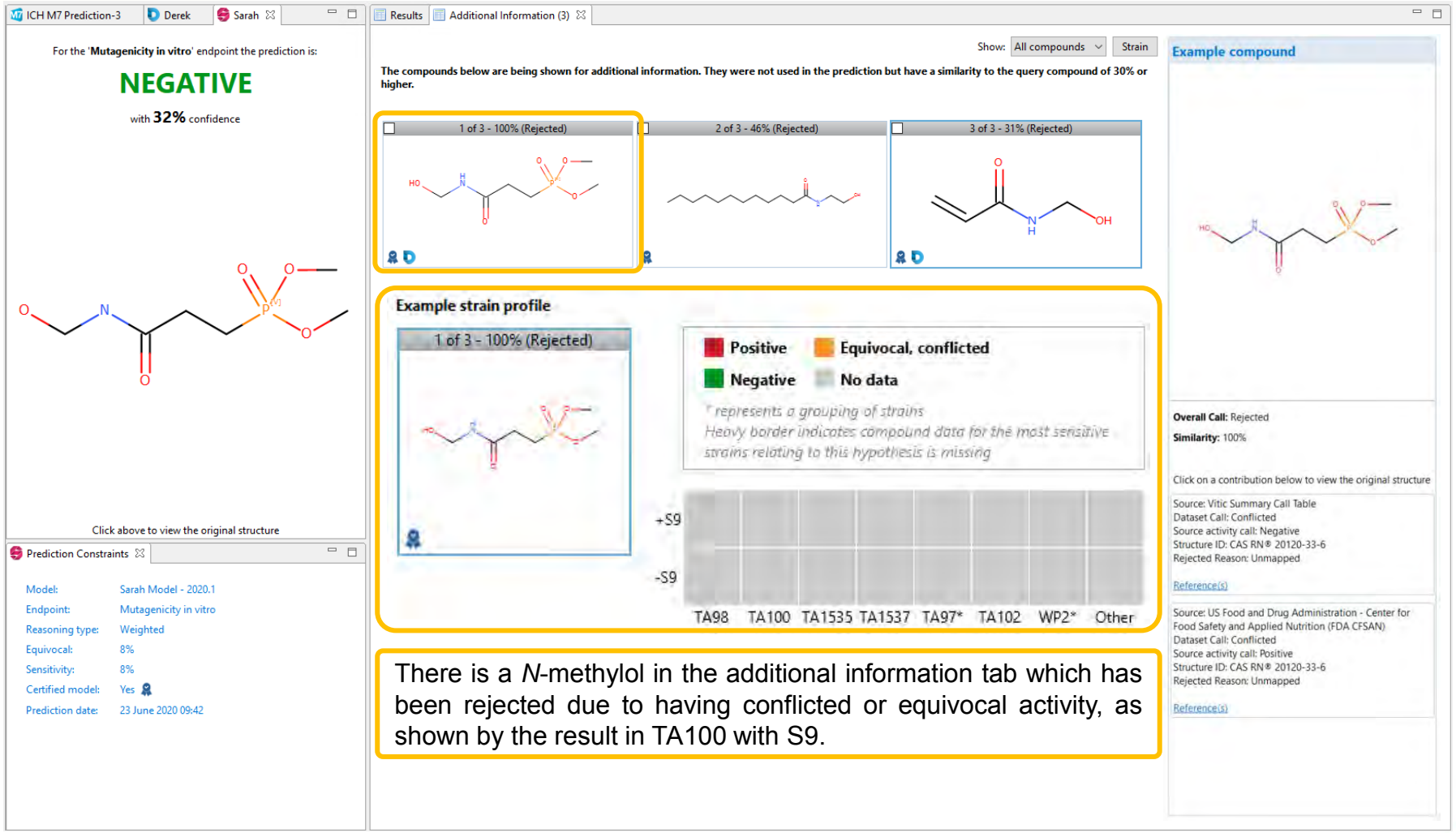
Lhasa Limited

Review the statistical prediction



Sarah nexus



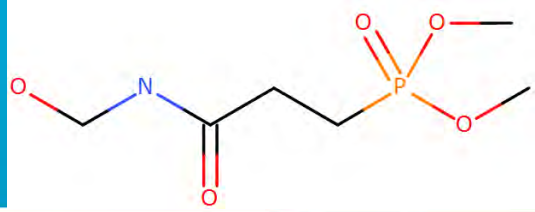


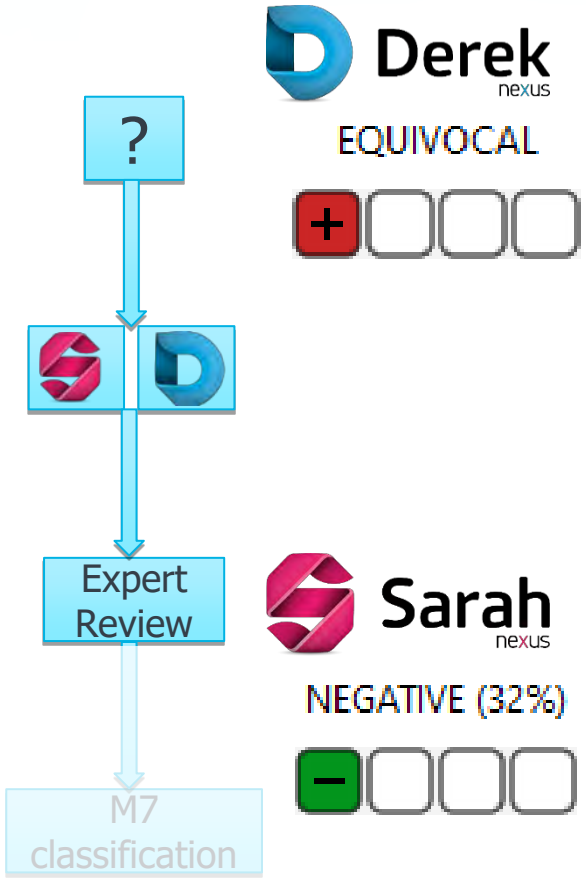
There is a *N*-methylol in the additional information tab which has been rejected due to having conflicted or equivocal activity, as shown by the result in TA100 with S9.

The impurity is in the additional information tab in the Sarah training set as it has been rejected for having a conflicted call. There is no strain information available to help any potential resolution.

Lhasa Limited

Expert review





Derek nexus

EQUIVOCAL

- Matches alert for *N*-methylol
- Comments suggest chemical class is expected to be weakly mutagenic & inconsistent results may be obtained in the Ames test due to metabolic incapability & the fact that formaldehyde, the ultimate mutagenic species, is weakly mutagenic itself

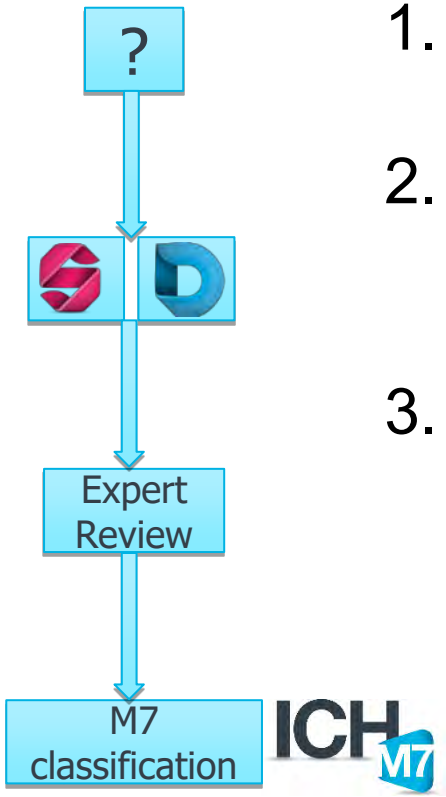
Sarah nexus

NEGATIVE (32%)

- Negative prediction is made & supported by 4 hypotheses, although none are specifically for the *N*-methylol functional group
- Training set includes a negative example that may be used for read-across; however, the query itself is in the additional information tab showing conflicted or equivocal activity

Lhasa Limited

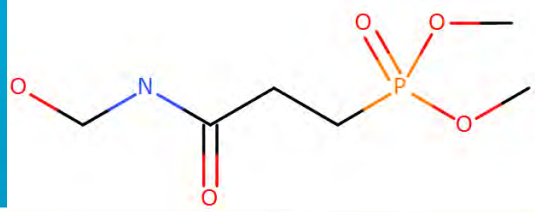
Please make your selection

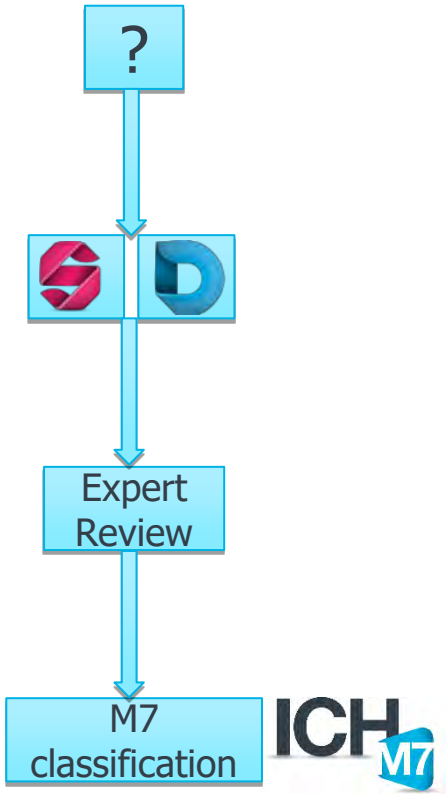


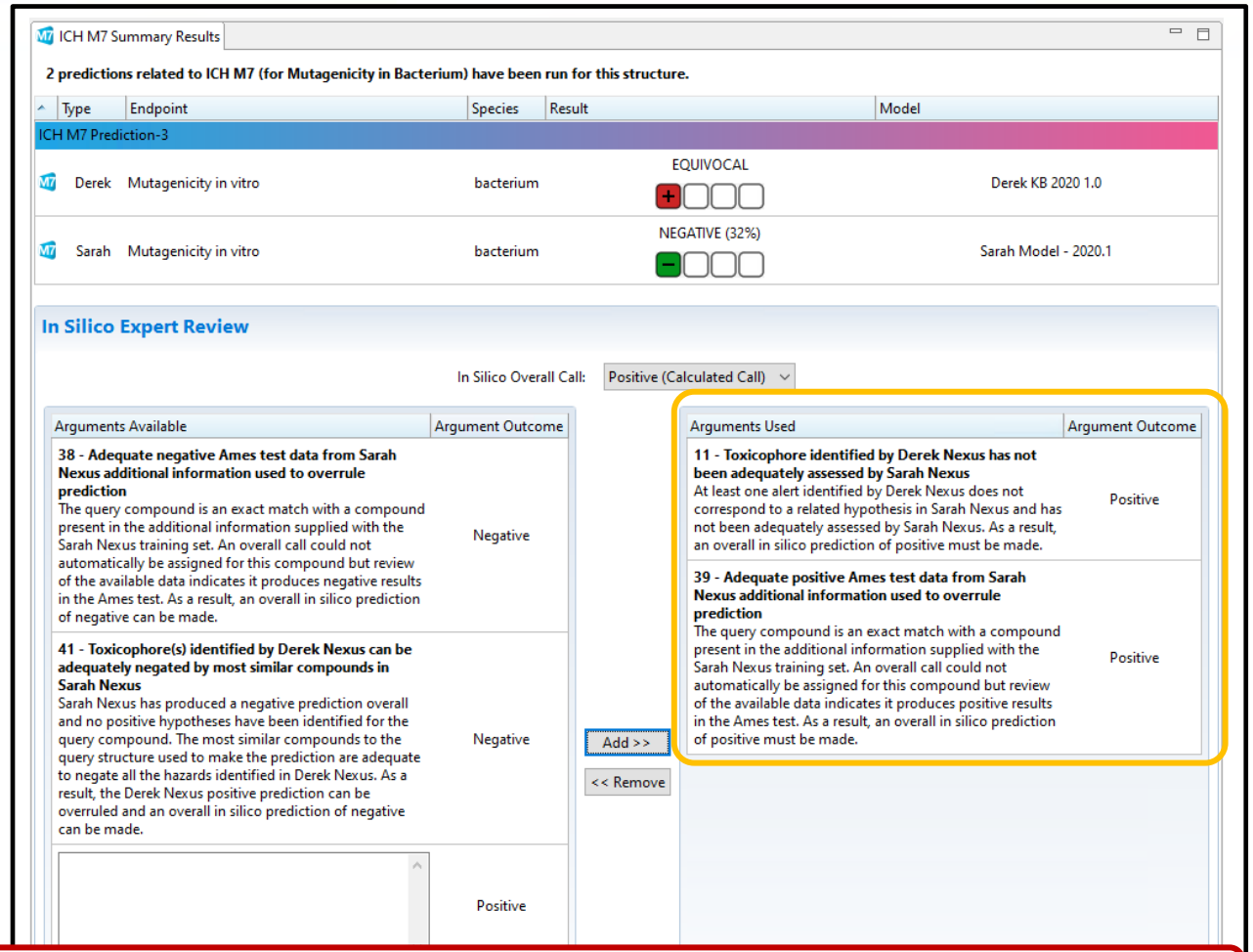
1. Class 3 – Alerting structure
2. Class 5 – No alerts or alerting with sufficient data to demonstrate lack of mutagenicity
3. Unsure

Lhasa Limited

ICH M7 classification





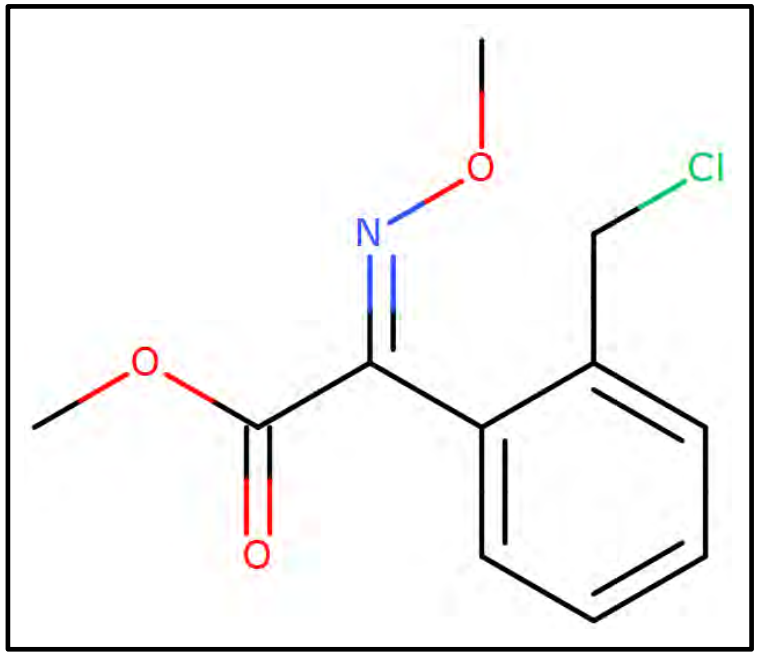


Class 3

There is low confidence in the positive prediction by Derek; however, Sarah has not specifically assessed the *N*-methylol functionality & there is conflicting results for the query itself in the training set. There is not enough evidence to support the negative prediction made by Sarah & it is advised to test, although it is important to consider the Ames test may require certain protocols to confirm (in)activity for this class.

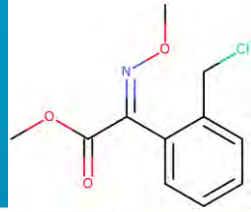
Lhasa Limited

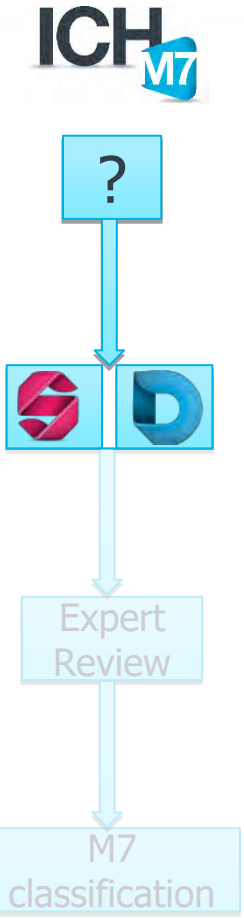
Example 5

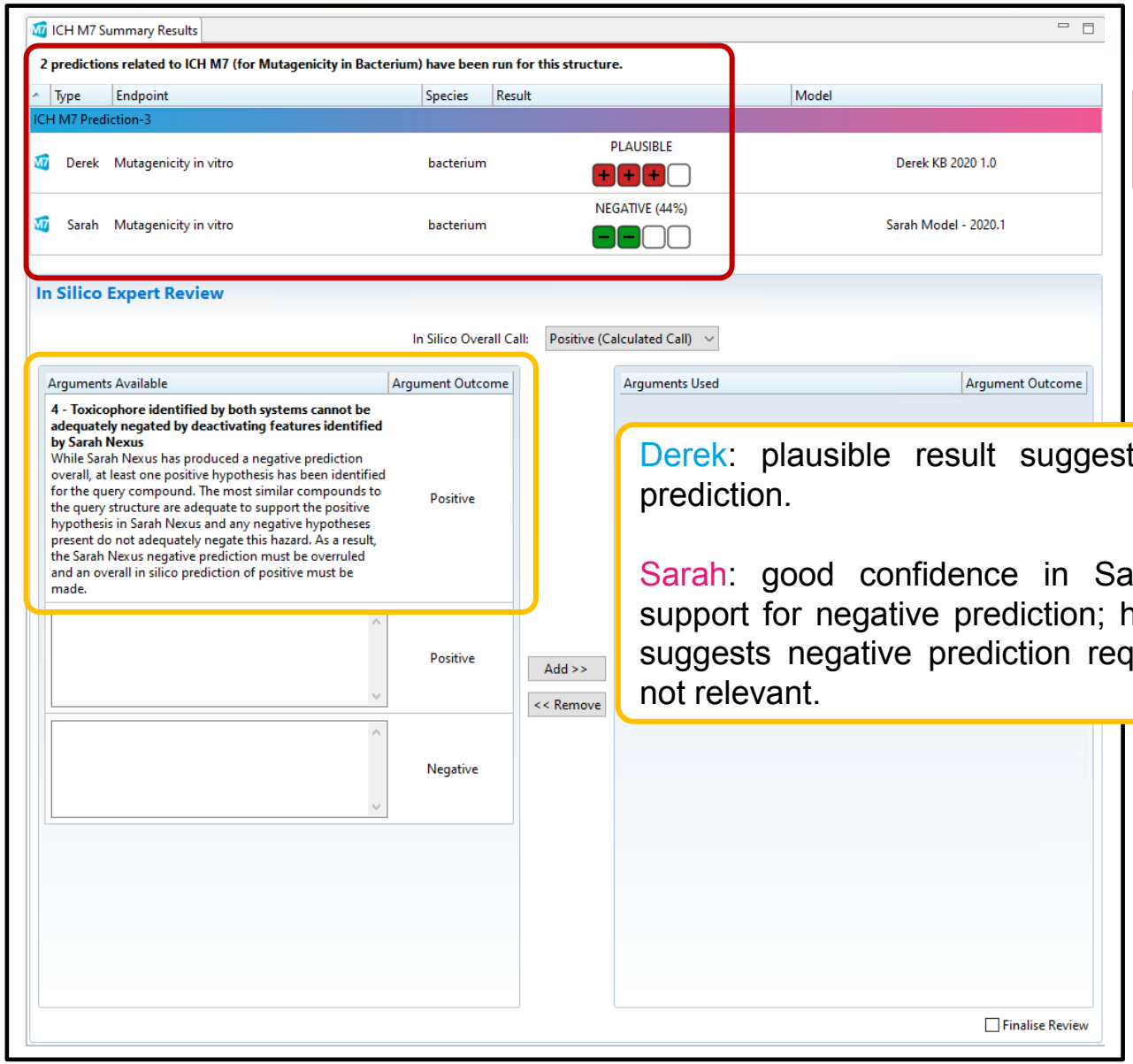


Lhasa Limited

Review high level predictions







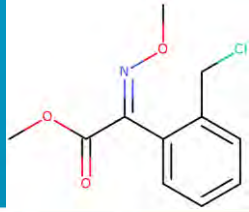
Derek & Sarah
disagree

Derek: plausible result suggests good confidence in positive prediction.

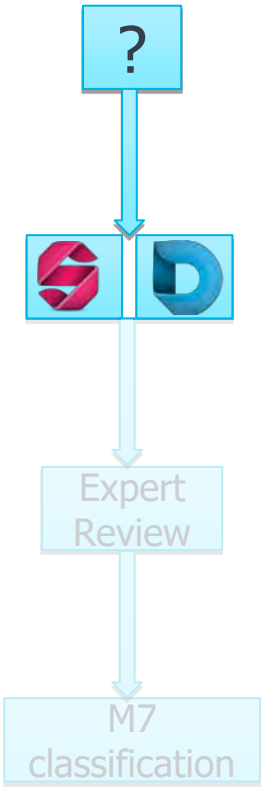
Sarah: good confidence in Sarah prediction (44%) suggests support for negative prediction; however, expert review argument suggests negative prediction requires review as compounds are not relevant.

Lhasa Limited

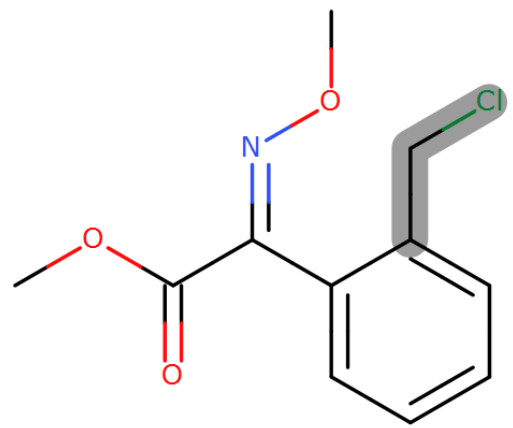
Review the expert prediction



Derek nexus



ICH M7 Prediction-3 | Derek



'Alert 027' selected, click above to view the original structure

Prediction Navigator

Show predictions of at least: EQUIVOCAL

- Derek KB 2020 1.0 [Certified by: Lhasa Limited, Leeds, Yorkshire, UK]
  - Mutagenicity in vitro
    - bacterium - PLAUSIBLE
      - Alert - 027: Alkylating agent

Alert Details | EC3 | Reasoning Explorer | Prediction Constraints

027: Alkylating agent

Alert Matches

Description Image

R1 = Cl, Br, I, OS(=O)$_n$R4
R2, R3 = any (with exclusions as specified in alert description)
R4 = any except OH, NH2, CF3
n = 1, 2

Comments

This alert describes the genotoxicity of alkylating agents where the carbon bearing the functional group is a primary or secondary alkyl carbon atom. In addition to alkyl halides, it includes alkyl sulphinates, sulphonates and sulphates [Tan et al].

Alkyl halides are electrophilic species that are capable of directly alkylating DNA. Consequently, many compounds are mutagenic in the Ames test in the presence and absence of S9 mix, notably in Salmonella typhimurium strains TA100 and TA1535 [Barber et al, Eriksson et al]. In general, alkyl chlorides are less mutagenic than their bromo and iodo counterparts, and given the non-mutagenicity reported for n-butyl chloride [Barber et al, Zeiger et al 1987] and n-dodecyl chloride [Zeiger et al 1992], longer chain alkyl chlorides may not give a positive result in the Ames test [Barber et al]. Shorter chain alkyl chlorides such as methyl chloride [Andrews et al], ethyl chloride [Zeiger et al 1992] and 2-propyl chloride [Eriksson et al, Simmon et al] are, in contrast, known to be mutagenic.

There is also some evidence to suggest that the mutagenicity of some benzyl halides may not be observed in the Ames test [Ball et al]. This may be due, in part, to their high cytotoxicity and inability to be tested at high doses. Benzyl chloride has been reported weakly mutagenic but only in the presence of S9 mix [Zeiger et al 1987]. When the test bacteria are exposed to vapours in a desiccator, however, strong activity is observed in both the presence and absence of S9 mix [Simmon]. For secondary benzyl halides, the lack of a mutagenic response has been attributed to the extra stability of the benzyl cation and formation of unstable DNA adducts which spontaneously cleave prior to replication [Ball and Young]. In contrast, corresponding biphenyl compounds, such as 4-(chloromethyl)biphenyl [Ashby et al 1981, Trueman and Callander] and polyaromatic compounds such as 9-chloromethylanthracene [Azuma et al] and 1-chloromethylpyrene [Ball and Young] do give strong positive responses in the Ames test despite their toxicity. Heterocyclic analogues, such as 2-(chloromethyl)pyridine [Claxton et al], have also been reported to be mutagenic in the Ames test.

The following structural classes are excluded from the alert:

Validation Comments

Mutagenicity: Ames test

The alert has demonstrated the following predictive performance:

1) Proprietary data set 1: 151 compounds activate this alert of which 68 are reported positive (positive predictivity: 45%)
2) Proprietary data set 2: 6 compounds activate this alert of which 3 are reported positive (positive predictivity: 50%)
3) FDA CFSAN data set: 437 compounds activate this alert of which 345 are reported positive (positive predictivity: 79%)

Endpoints

ID	Name	Parent
4086	Mutagenicity	Mutagenicity (ALL)

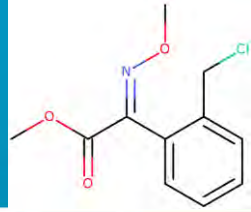
Comments provide good evidence of activity for benzyl chlorides & compound does not match any of the listed exclusions in the alert.

Moderate (45%) to good (79%) PPV for alert.

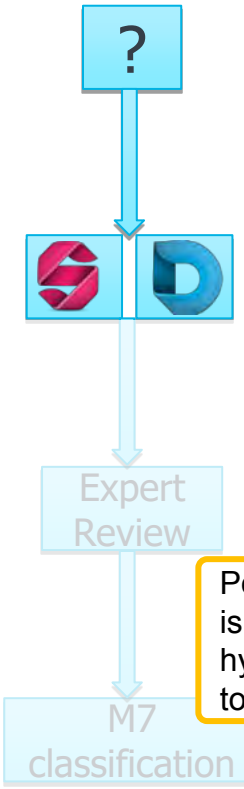
Derek provides a positive prediction with plausible level of reasoning which has good evidence of activity for this chemical class & has good PPV in validation, hence good confidence in the prediction.

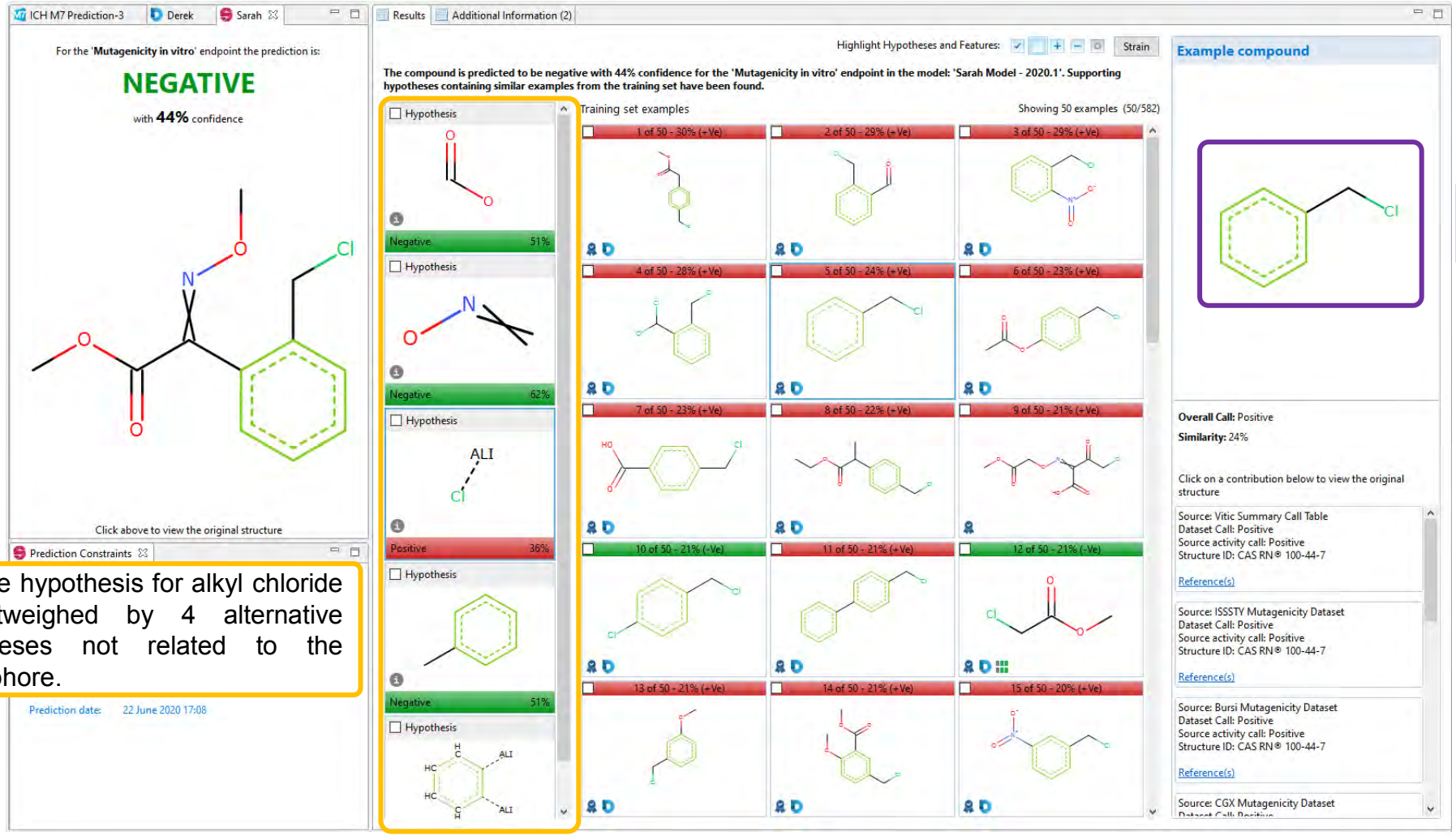
Lhasa Limited

Review the statistical prediction



Sarah nexus





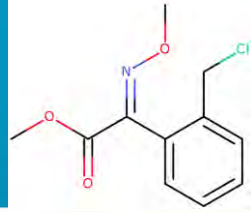
Positive hypothesis for alkyl chloride is outweighed by 4 alternative hypotheses not related to the toxicophore.

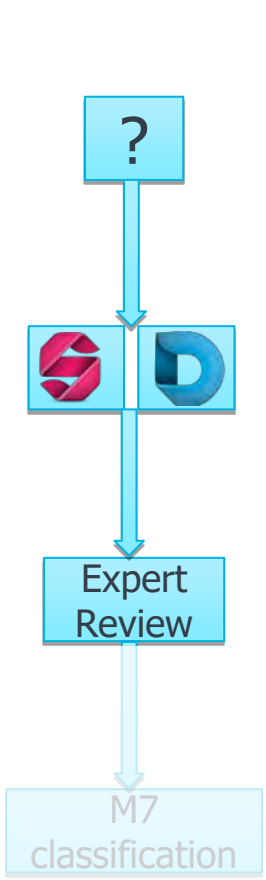
Benzyl chloride is a known mutagen.

Sarah provides a negative prediction with good confidence (44%) & 4/5 hypotheses are negative. However, the positive hypothesis is the aliphatic chloride which is the toxicophore identified by Derek in the compound & this is supported by several mutagenic benzyl chlorides. Therefore, the non-negating negative hypotheses are swamping the positive hypothesis & should be overruled.

Lhasa Limited

Expert review





Derek nexus

PLAUSIBLE

- Matches alert for alkylating agent for the benzyl chloride moiety
- Comments provide good evidence of activity for benzyl chlorides & compound does not match any of the listed exclusions in the alert

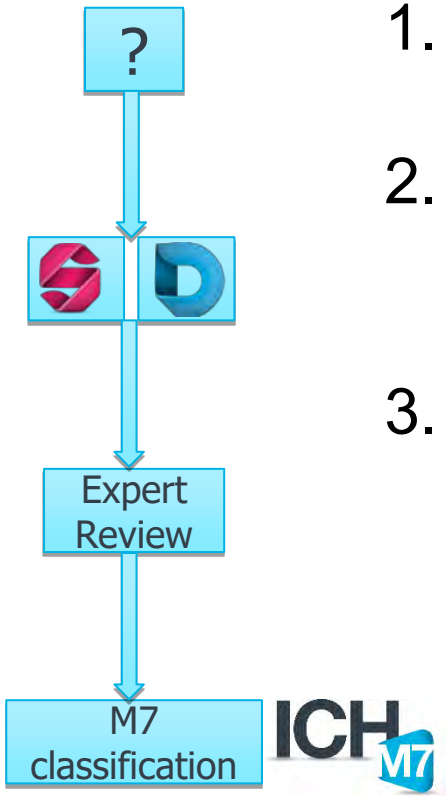
Sarah nexus

NEGATIVE (44%)

- Negative prediction with good confidence is supported by 4 negative hypotheses
- 1 positive hypothesis for aliphatic chlorides contains several examples of mutagenic benzyl chlorides whereas the 4 negative hypotheses can be considered as non-negating features

Lhasa Limited

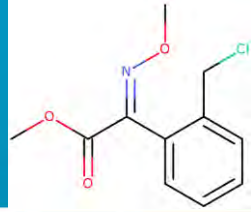
Please make your selection

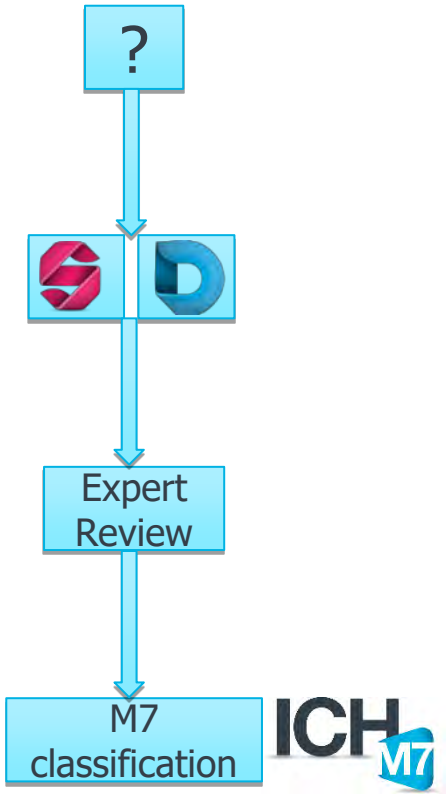


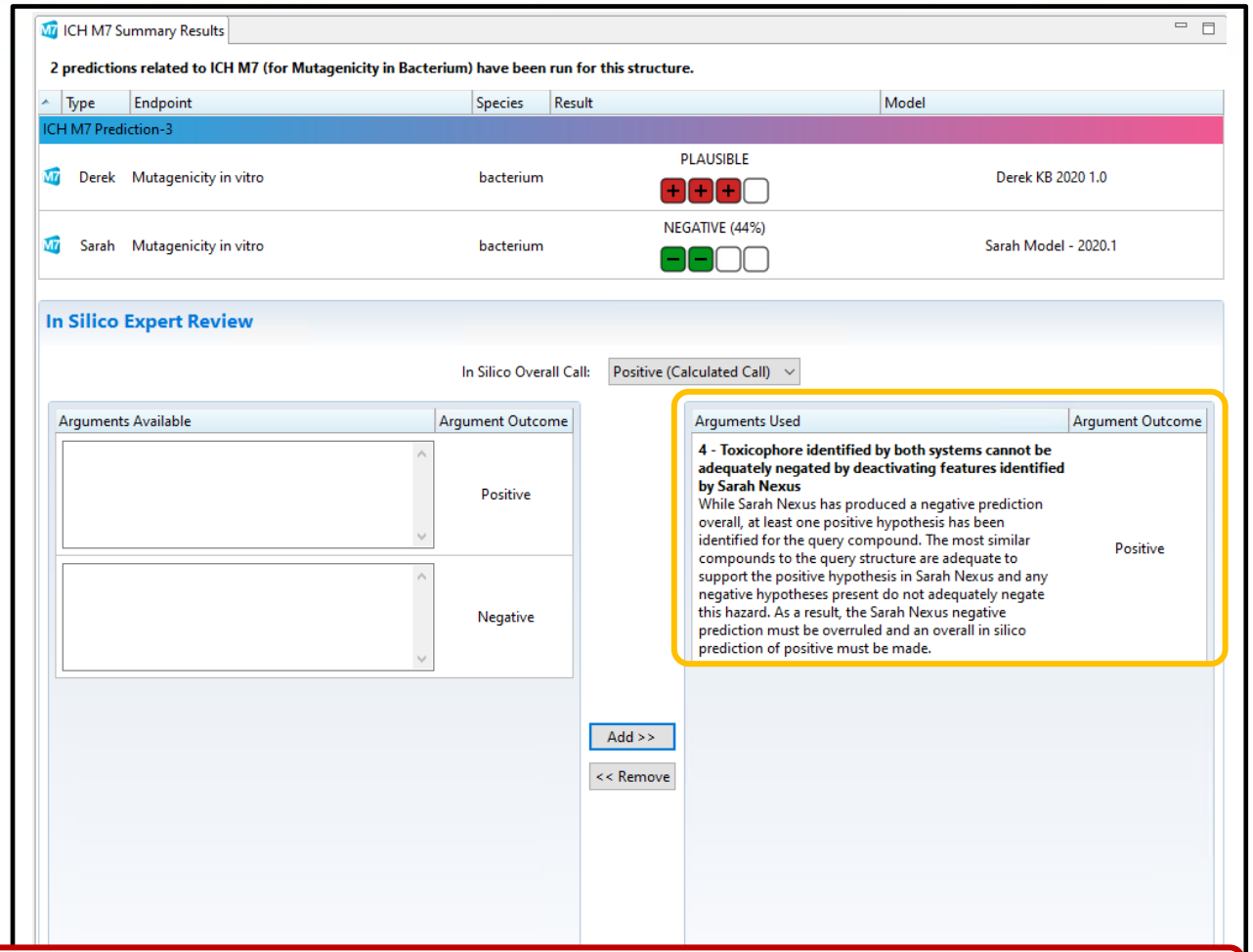
1. Class 3 – Alerting structure
2. Class 5 – No alerts or alerting with sufficient data to demonstrate lack of mutagenicity
3. Unsure

Lhasa Limited

ICH M7 classification





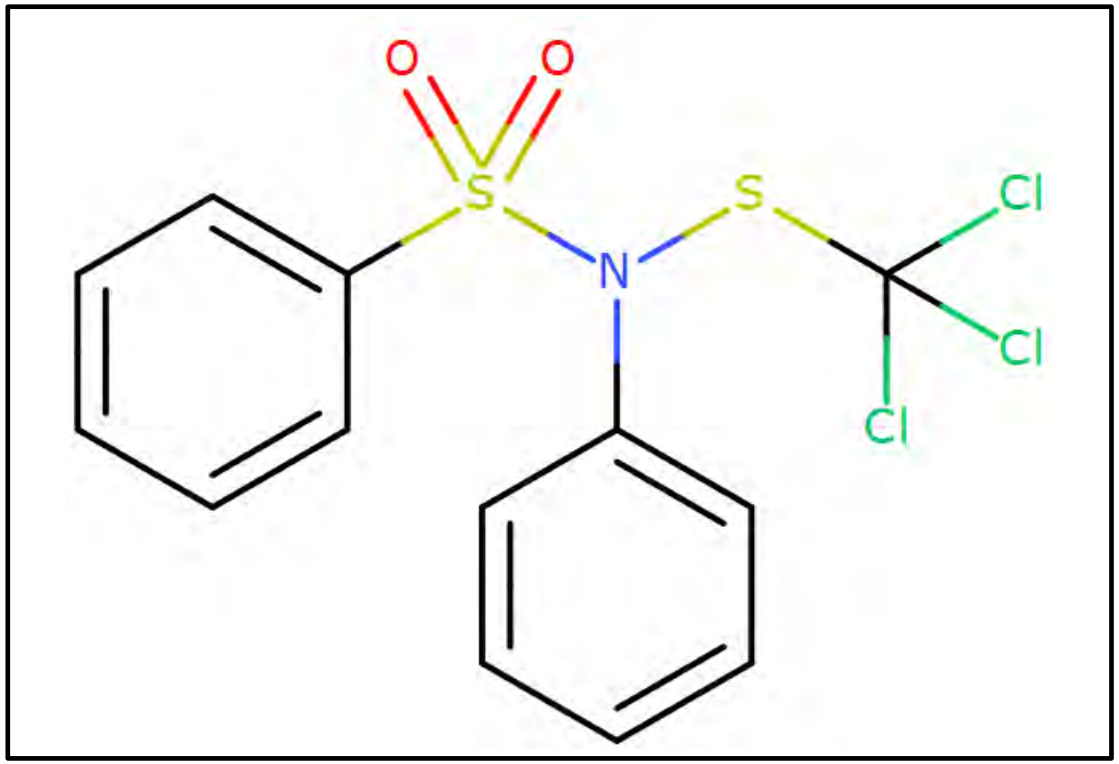


Class 3

Positive prediction made by Derek & Sarah has identified the same toxicophore; however, in Sarah it has been swamped by non-negating features.

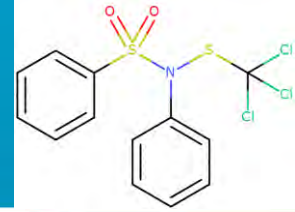
Lhasa Limited

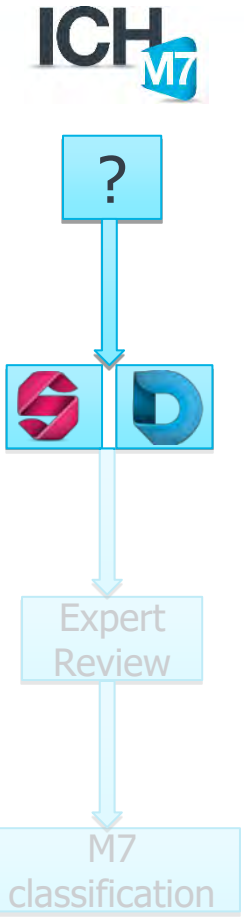
Example 6

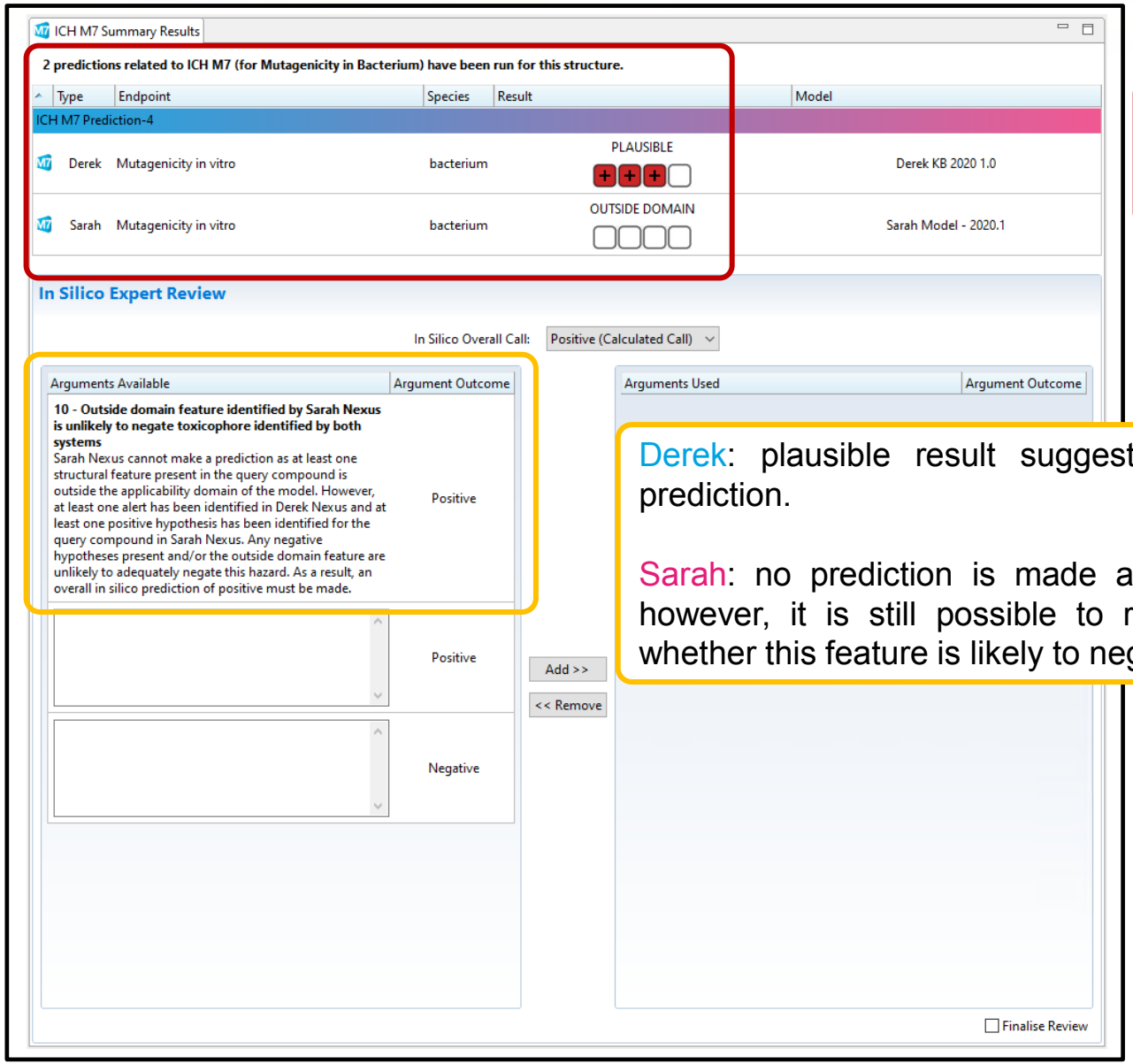


Lhasa Limited

Review high level predictions







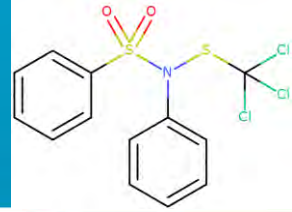
Derek & Sarah
Inconclusive
Sarah makes no prediction

Derek: plausible result suggests good confidence in positive prediction.

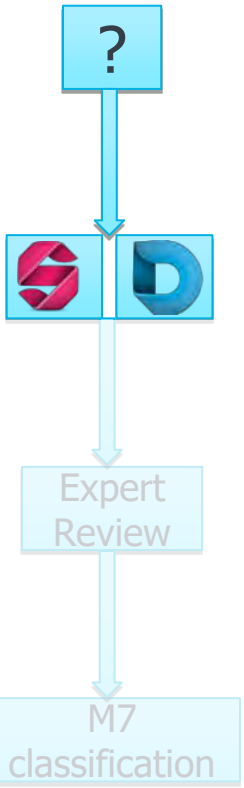
Sarah: no prediction is made as the query is outside domain; however, it is still possible to review the compound & assess whether this feature is likely to negate the Derek prediction.

Lhasa Limited

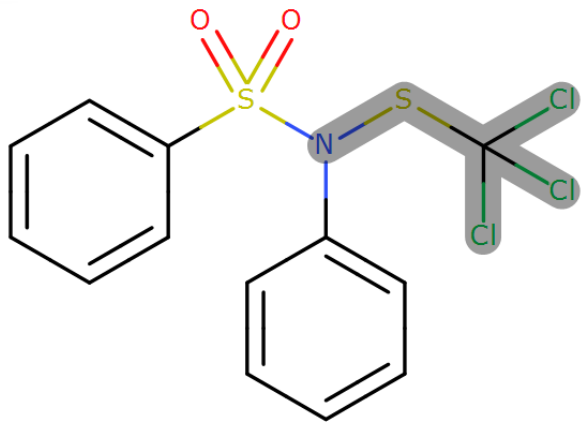
Review the expert prediction



Derek nexus



ICH M7 Prediction-4 | Derek



'Alert 521' selected, click above to view the original structure

Prediction Navigator

Show predictions of at least: EQUIVOCAL

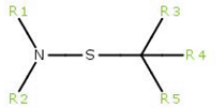
- Derek KB 2020 1.0 [Certified by: Lhasa Limited, Leeds, Yorkshire, UK]
  - Mutagenicity in vitro
    - bacterium - PLAUSIBLE
      - Alert - 521: N-Polyhaloalkylthio compound

Alert Details | EC3 | Reasoning Explorer | Prediction Constraints

521: N-Polyhaloalkylthio compound

Alert Matches

Description Image



R1, R2, R5 = any
R3, R4 = F, Cl, Br, I

Comments

This alert describes the genotoxic activity of compounds containing the N-polyhaloalkylthio group in the in vitro chromosome aberration test and Ames test. Examples of compounds which have been reported to produce positive results in at least one of the tests include captan [Meyer, Sofuni, McCann et al], captafol [Sofuni, Ruiz and Marzin], dichlofluanid [ACP], tolylfluanid [Logan] and folpet [US EPA, Larsen, Hour et al]. Activity may be observed in both the presence and absence of S9 mix, although in several reports the responses were reduced by the addition of S9 mix [Sofuni, Meyer, Larsen, Ruiz and Marzin].

There are several mechanisms through which N-polyhaloalkylthio compounds may promote a positive response in the chromosome aberration test. Such compounds are known to react with thiols [Bernard and Gordon, Kramer], and their mechanism of action may result from covalent interaction with proteins containing the thiol group. For example, captan and folpet are reported to inhibit important enzymes in the ADP-ATP cycle [Kramer], which can result in disruption of cellular ATP production [Kaars Sijpesteijn]. Captan and captafol have also been shown to inhibit topoisomerase II [Rahden-Staron], and inhibitors of this enzyme are likely to be clastogenic [Ferguson]. Additionally, the N-trihalomethylthio group may also generate thiophosgene or thiocarbonyl halide analogues [Bernard and Gordon, Provan et al], which may also contribute to the observed activity. The fact that these compounds can undergo rapid hydrolysis at the N-S bond to generate highly reactive halomethylthio derivatives which can directly attack DNA, has also been proposed as a possible mechanism for their mutagenic activity [Hour et al, Bernard and Gordon].

The available evidence indicates that these compounds generally give negative results in in vivo cytogenetic tests. For instance, negative results have been reported for folpet [Arce et al], tolylfluanid [Logan] and dichlofluanid [ACP] in the mouse bone marrow micronucleus test, following oral administration. Tolylfluanid [Logan] and dichlofluanid [ACP] have both given negative results in the hamster bone marrow chromosome aberration test, although a positive response was observed for dichlofluanid in an earlier study [ACP]. Results for captan in in vivo cytogenetic tests are mixed. The compound was reported positive in the mouse bone marrow micronucleus test and chromosome aberration test [Feng and Lin], however, some errors in reporting make the data from this study difficult to interpret and negative results in these tests, using mouse and rat bone marrow, have been reported in other studies [Tezuka et al, Arce et al]. Captan [Chidiac and Goldberg] and folpet [Arce et al] gave negative results in the micronucleus test in the mouse using duodenal crypt cells, while a positive result has been reported for captafol in the rat micronucleus test using kidney cells [Robbiano et al].

Validation Comments

Mutagenicity: Ames test

The alert has demonstrated the following predictive performance:

1) Proprietary data set 1: 0 compounds activate this alert
2) Proprietary data set 2: 0 compounds activate this alert
3) FDA CFSAN data set: 5 compounds activate this alert of which 4 are reported positive (positive predictivity: 80%)

Endpoints

ID	Name	Parent
4086	Mutagenicity	Mutagenicity (ALL)

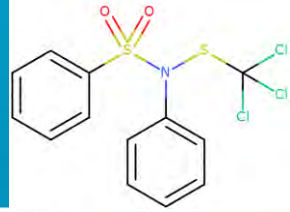
Comments discuss multiple examples of *N*-polyhaloalkylthio compounds which are active in the Ames test & mechanisms for the genotoxic activity.

High PPV (80%) for alert.

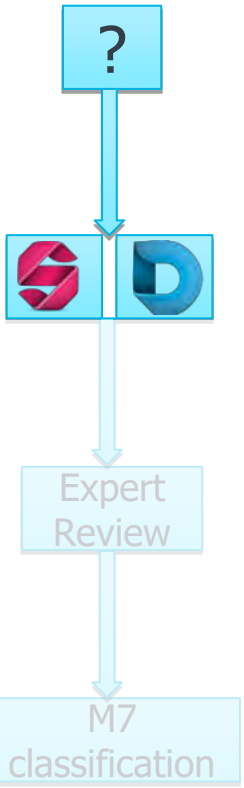
Derek provides a positive prediction with plausible level of reasoning which has good evidence of activity for this chemical class & has good PPV in validation, hence good confidence in the prediction.

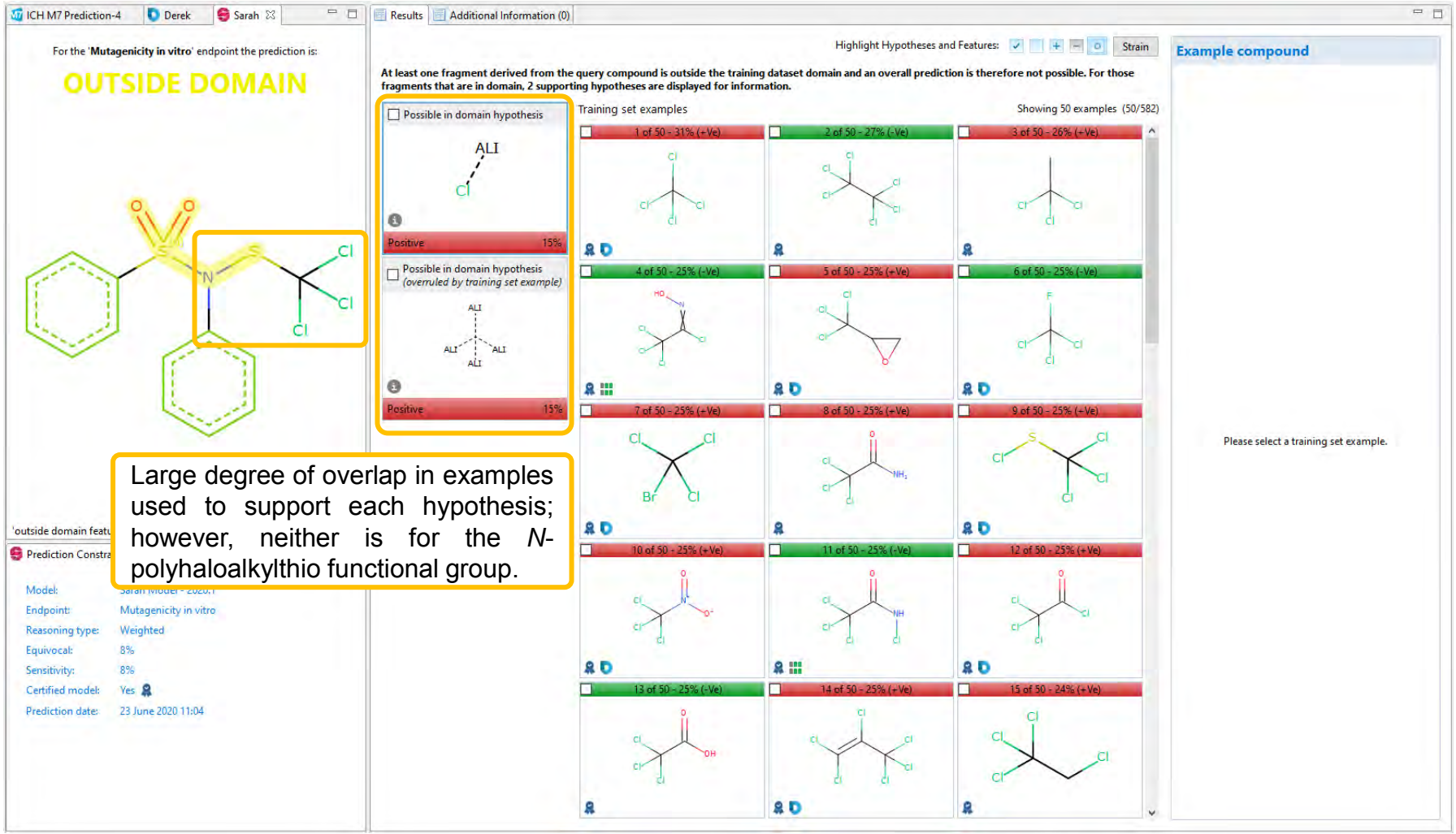
Lhasa Limited

Review the statistical prediction



Sarah nexus



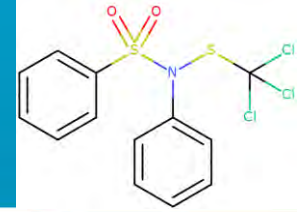


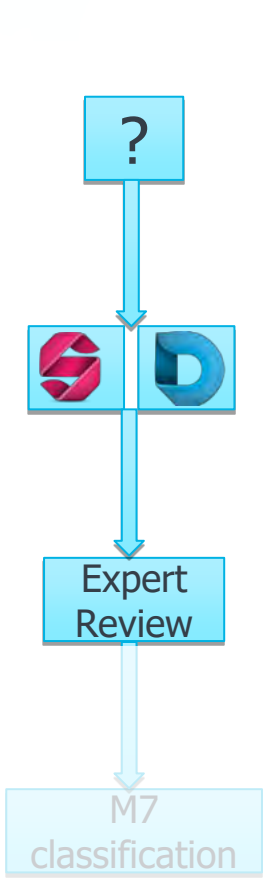
Large degree of overlap in examples used to support each hypothesis; however, neither is for the *N*-polyhaloalkylthio functional group.

The query is outside domain, although the specific feature (*N*-thio-*N*-sulfonyl) is not the same as the toxicophore identified by Derek (*N*-polyhaloalkylthio). Therefore, it would be possible to assess the activating feature if present in the training set examples; however, this is not the case. Alternative hypotheses identified by Sarah are supporting of activity for polyhaloalkyl compounds.

Lhasa Limited

Expert review





Derek nexus

PLAUSIBLE

- Matches alert for *N*-polyhaloalkylthio compound
- Comments provide good evidence of activity for *N*-polyhaloalkylthio compounds & multiple mechanisms are expected to contribute to genotoxicity

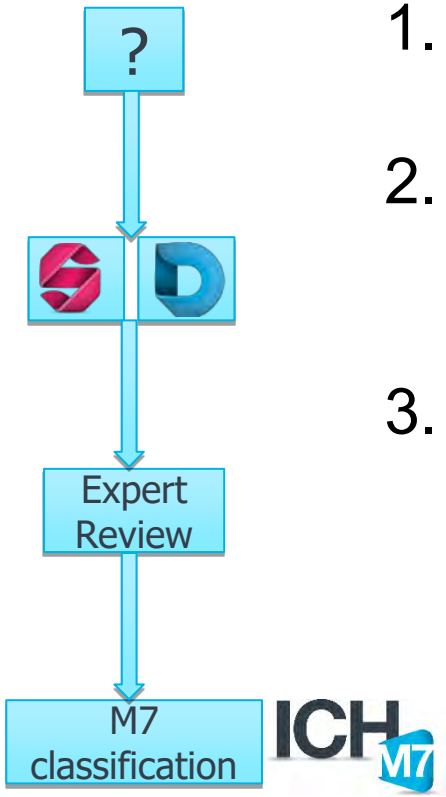
Sarah nexus

OUTSIDE DOMAIN

- Outside domain feature (*N*-thio-*N*-sulfonyl) prevents Sarah making a prediction
- Outside domain feature is different to the toxicophore identified by Derek & there are no examples of *N*-polyhaloalkylthio compounds in the training set
- Unable to conclude mutagenic potential of *N*-polyhaloalkylthio compounds but polyhaloalkyl compounds considered mutagenic

Lhasa Limited

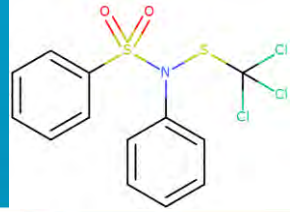
Please make your selection

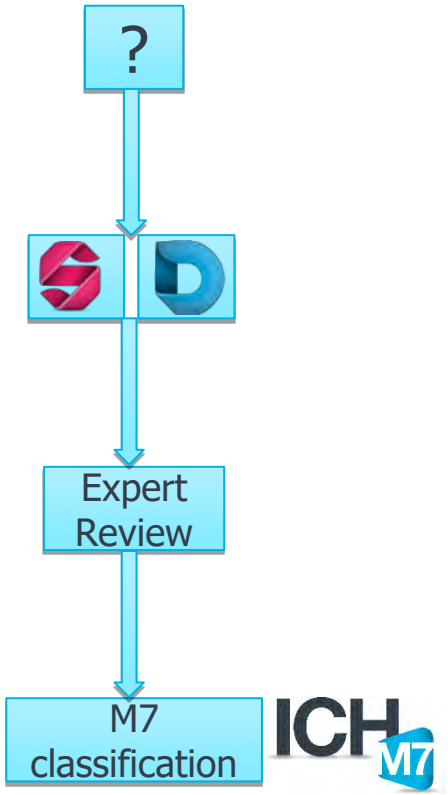


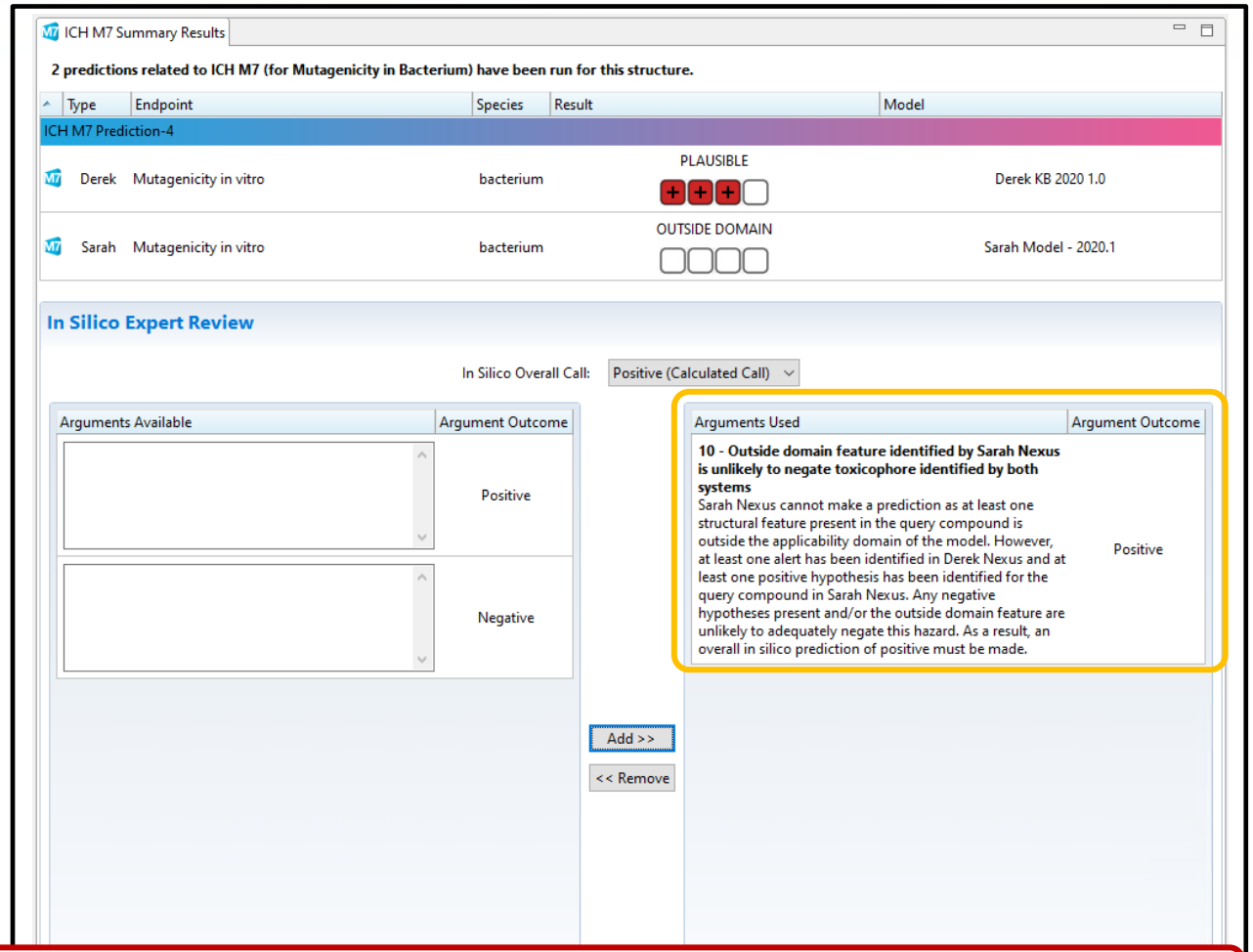
1. Class 3 – Alerting structure
2. Class 5 – No alerts or alerting with sufficient data to demonstrate lack of mutagenicity
3. Unsure

Lhasa Limited

ICH M7 classification







Class 3

No prediction is made by Sarah as a feature is outside domain; however, this is not the same functional group as that identified by Derek. Sarah does not assess the same activating feature but does provide further evidence for polyhaloalkyl compounds being mutagenic. Although the query is outside Sarah's domain, the evidence presented does not give any reason to doubt the Derek prediction.

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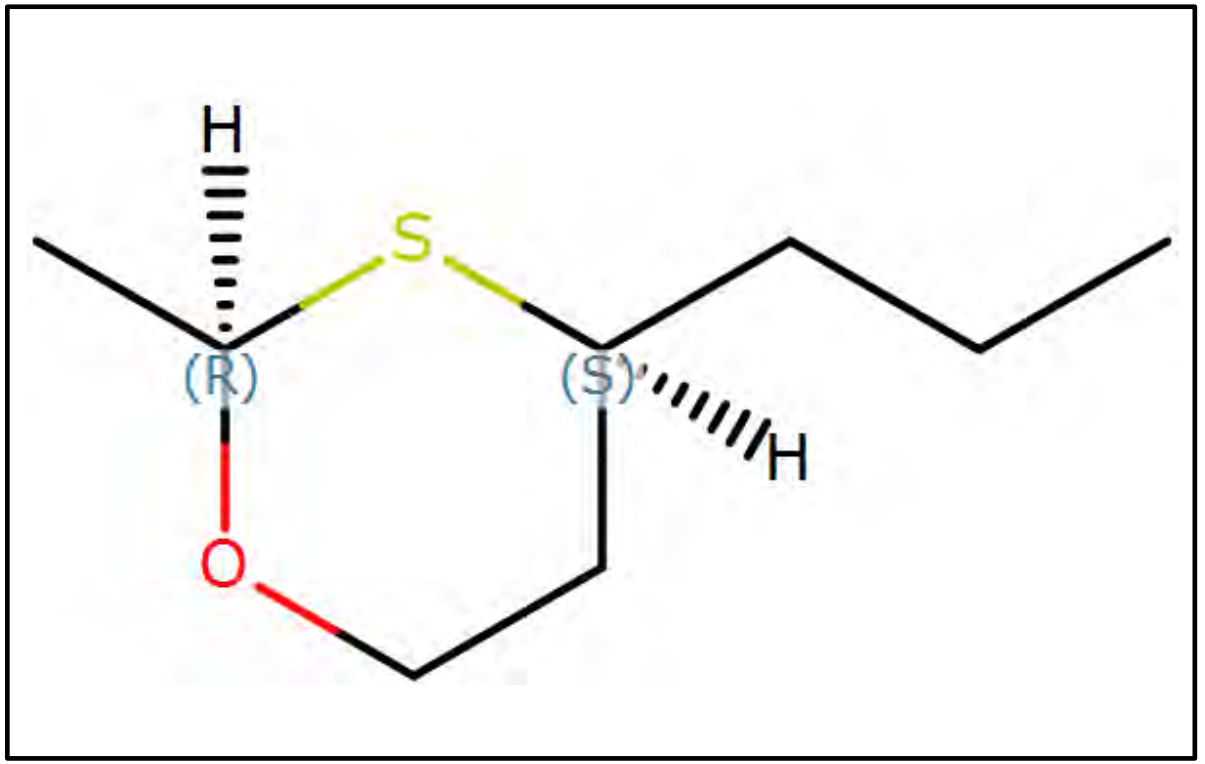
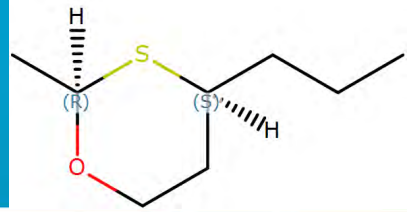
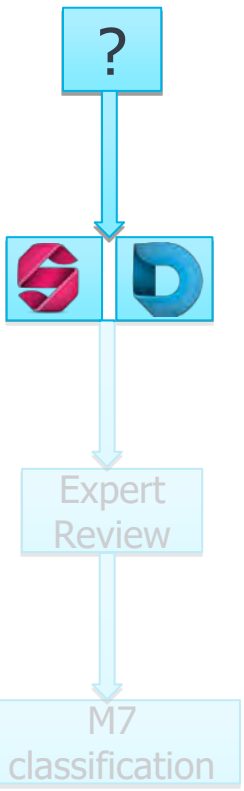
Example 7



Lhasa Limited

Review high level predictions





ICH M7 Summary Results

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

Type	Endpoint	Species	Result	Model
ICH M7 Prediction-2				
Derek	Mutagenicity in vitro	bacterium	INACTIVE *	Derek KB 2020 1.0
* Contains unclassified features				
Sarah	Mutagenicity in vitro	bacterium	OUTSIDE DOMAIN	Sarah Model - 2020.1

In Silico Expert Review

In Silico Overall Call: Inconclusive

Arguments Available	Argument Outcome
23 - No toxicophore has been identified by either system and the outside domain feature identified by Sarah Nexus does not pose a hazard Sarah Nexus cannot make a prediction as at least one structural feature present in the query compound is outside the applicability domain of the model. No structural alerts have been identified by Derek Nexus. Available evidence suggests that the out of domain feature does not pose a hazard and no additional positive hypotheses have been identified by Sarah Nexus. As a result, an overall in silico prediction of negative can be made.	Negative
24 - Outside domain feature identified by Sarah Nexus cannot be dismissed as a potential toxicophore Sarah Nexus cannot make a prediction as at least one structural feature present in the query compound is outside the domain of the model. No structural alerts have been identified by Derek Nexus. However, available evidence suggests that the out of domain feature may pose a hazard or additional positive hypotheses have been identified by Sarah Nexus that cannot be negated. As a result, an overall in silico prediction of positive must be made.	Positive
	Positive

Add >>

<< Remove

Finalise Review

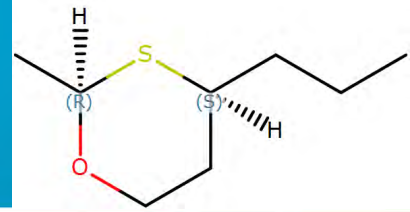
Derek & Sarah
Inconclusive
No prediction is made by Sarah

Derek: unclassified feature reduces confidence in negative prediction; however, it is still possible to review the compound & assess the mutagenic potential of this feature.

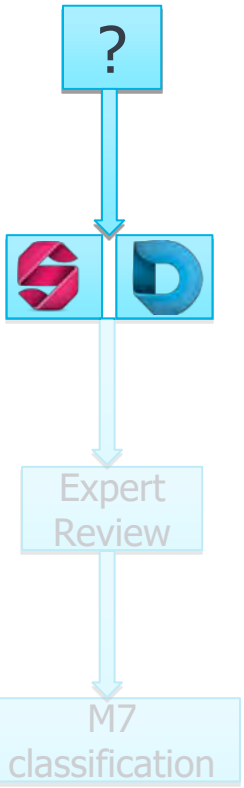
Sarah: no prediction is made as the query is outside domain; however, it is still possible to review the compound & assess whether this feature is likely to (dis)agree with the Derek prediction.

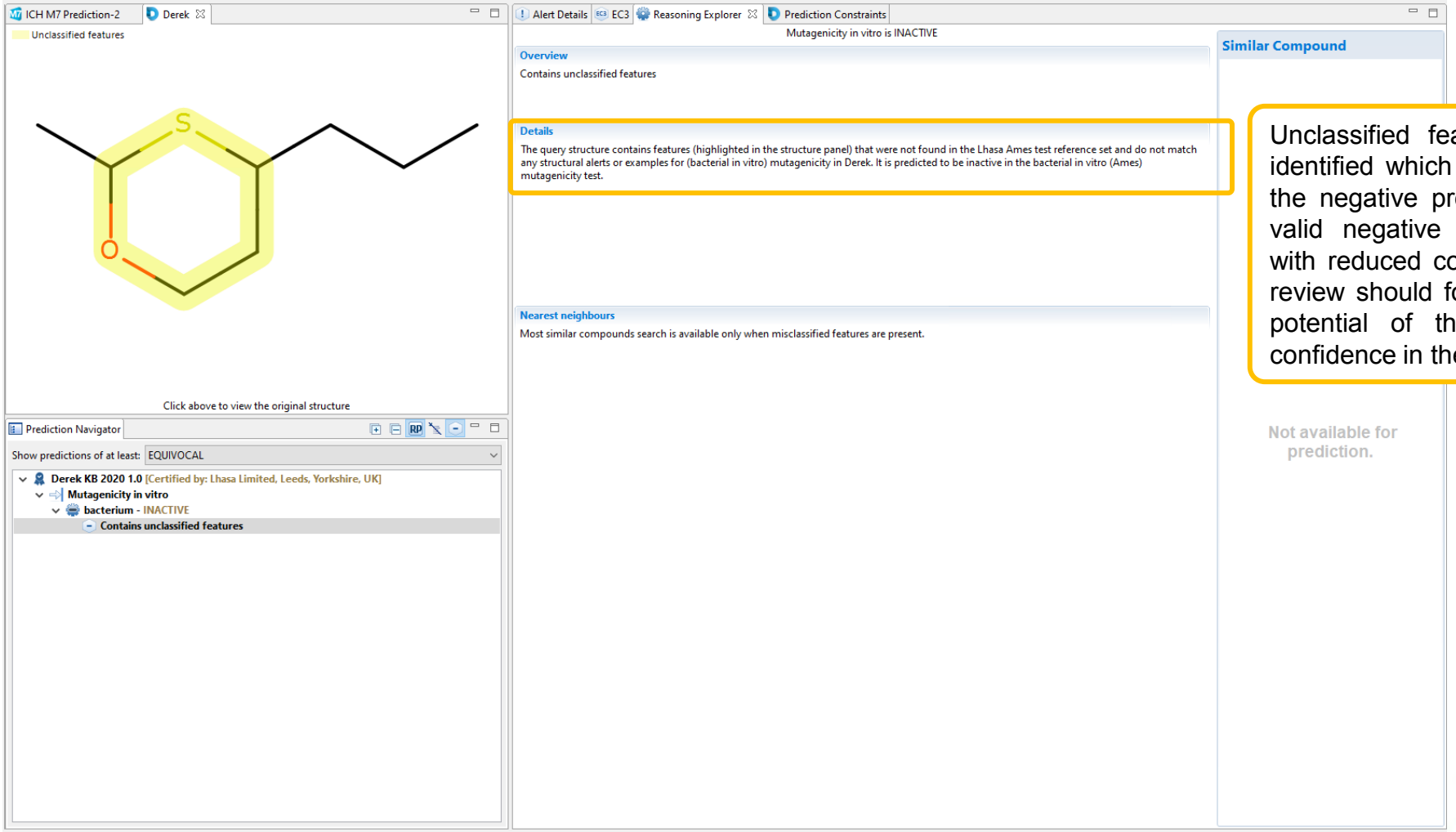
Lhasa Limited

Review the expert prediction



Derek nexus



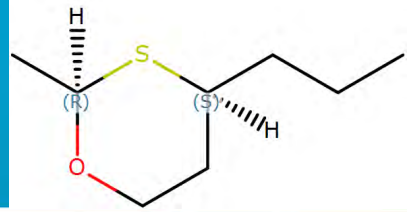


Unclassified feature (1,3-oxathine) is identified which reduces confidence in the negative prediction. This is still a valid negative prediction, albeit one with reduced confidence where expert review should focus on the mutagenic potential of this feature to ensure confidence in the prediciton.

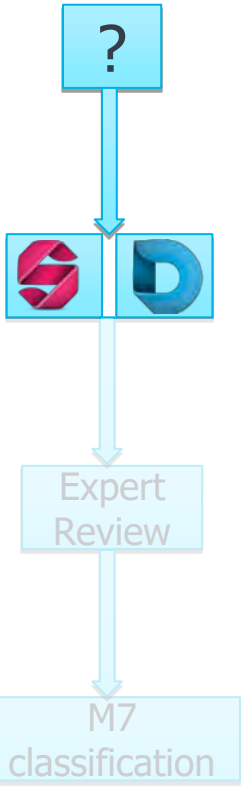
Derek provides a negative prediction; however, the 1,3-oxathiane is unclassified meaning that it is not present in the Lhasa Ames test reference set. This reduces confidence in the negative prediction & warrants further investigation.

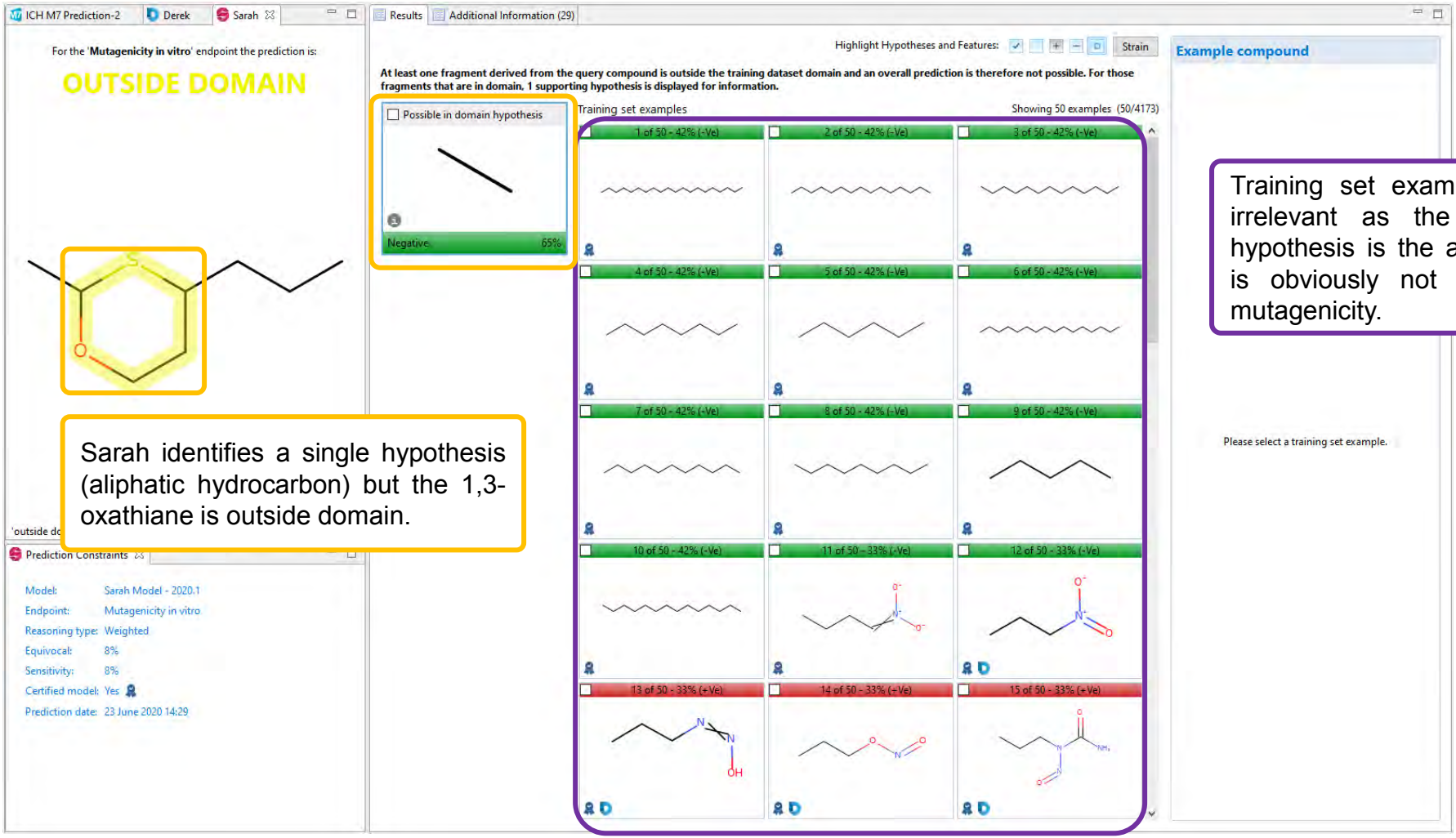
Lhasa Limited

Review the statistical prediction



Sarah nexus





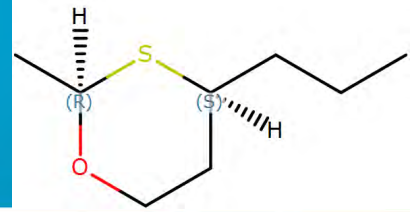
Sarah identifies a single hypothesis (aliphatic hydrocarbon) but the 1,3-oxathiane is outside domain.

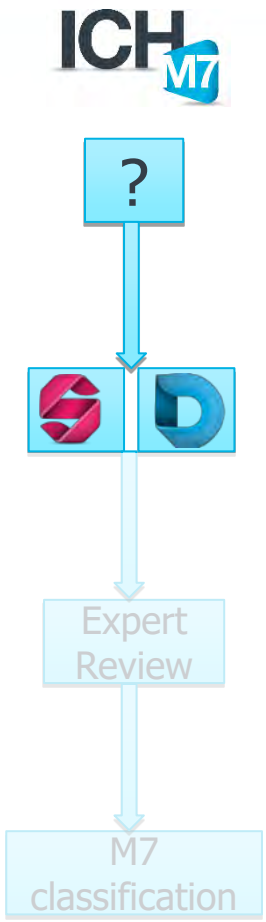
Training set examples are largely irrelevant as the only identified hypothesis is the alkyl chain which is obviously not associated with mutagenicity.

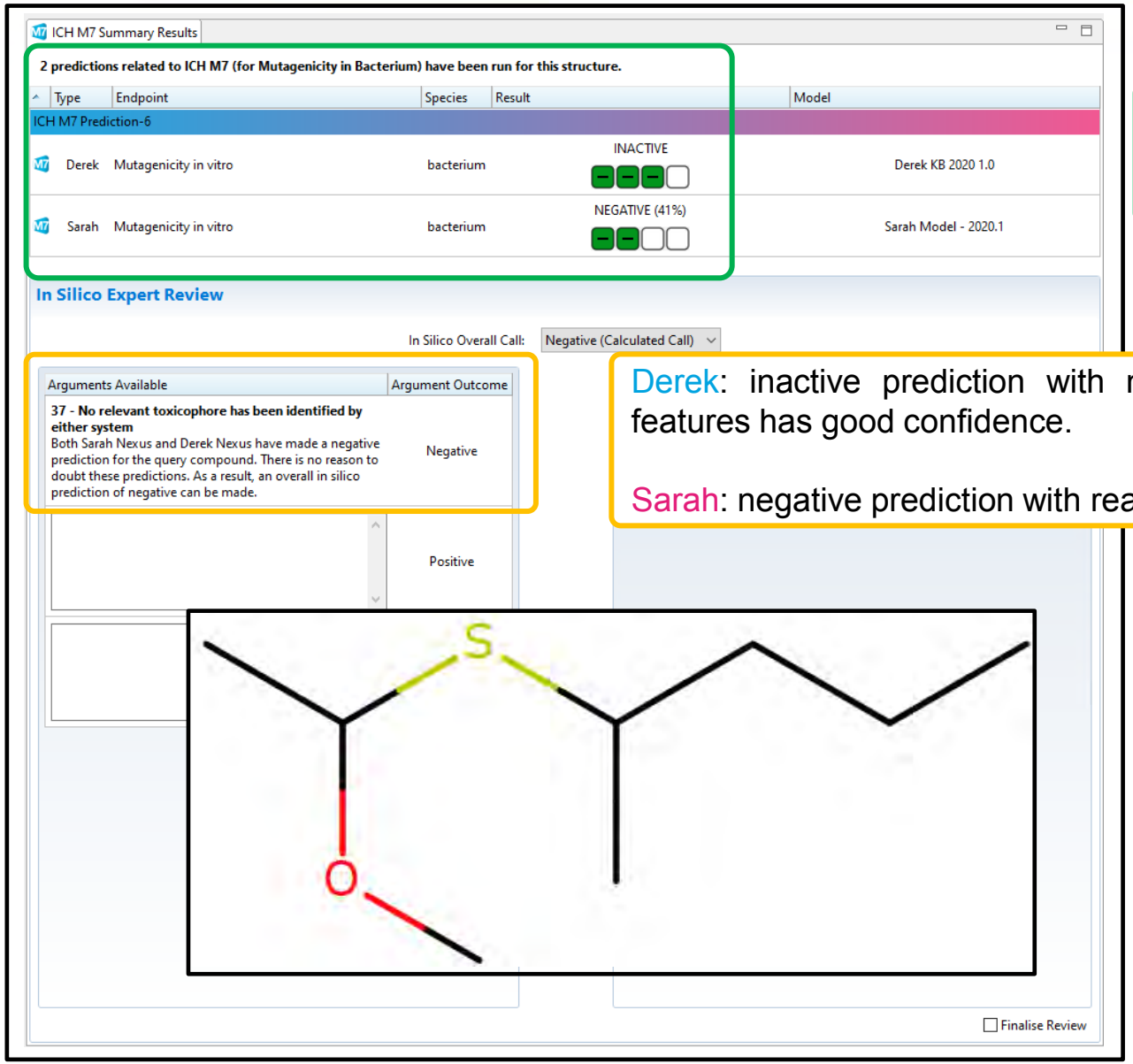
Sarah identifies the aliphatic chain, which is obviously not associated with mutagenicity; however, Sarah provides no formal prediction as the 1,3-oxathiane is outside domain.

Lhasa Limited

Review high level predictions







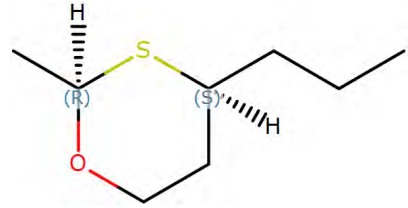
Derek & Sarah
Agree

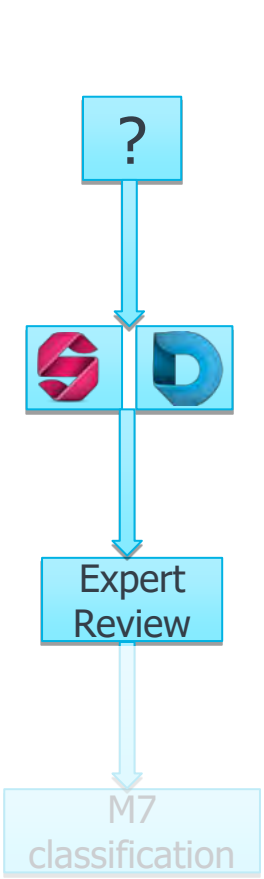
Derek: inactive prediction with no misclassified or unclassified features has good confidence.

Sarah: negative prediction with reasonable (41%) confidence.

Lhasa Limited

Expert review





Derek nexus

INACTIVE *

- Negative prediction contains unclassified feature
- 1,3-Oxathiane is not present in the Lhasa Ames reference test set, reducing confidence in the negative prediction
- Opening the ring retains the (1-alkoxyethyl)(alkyl)sulfane function & returns an inactive prediction with no misclassified or unclassified features
- Prediction of metabolic pathways provided by Meteor does not suggest ring opening to release acetaldehyde, a suspected mutagen, is likely to occur

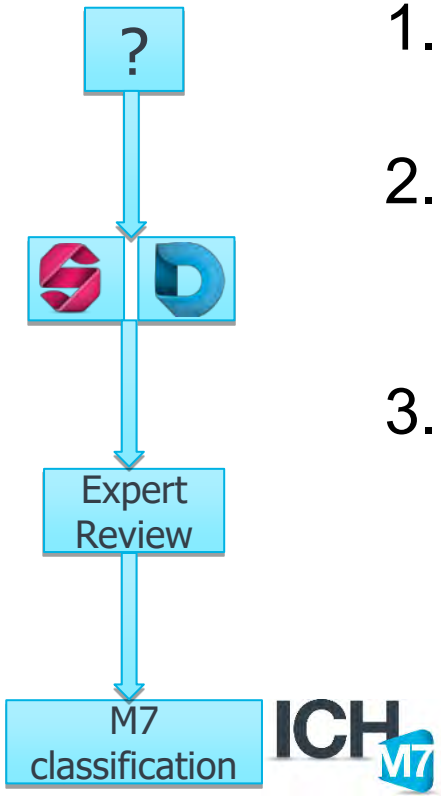
Sarah nexus

OUTSIDE DOMAIN

- Outside domain feature (1,3-oxathiane) prevents Sarah making a prediction
- Outside domain feature is a (1-alkoxyethyl)(alkyl)sulfane contained in a ring system, hence opening the ring is a way to assess mutagenic potential in this scenario & Sarah returns a negative prediction although no example compounds specifically contain this feature

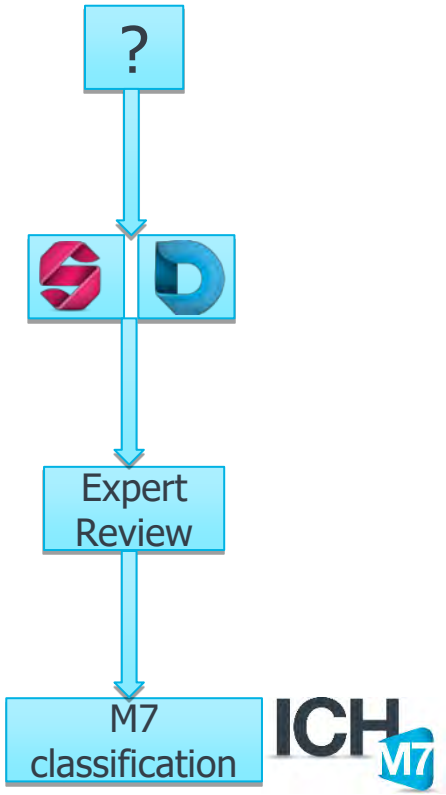
Lhasa Limited

Please make your selection



1. Class 3 – Alerting structure
2. Class 5 – No alerts or alerting with sufficient data to demonstrate lack of mutagenicity
3. Unsure

ICH M7 classification



ICH M7 Summary Results

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

Type	Endpoint	Species	Result	Model
ICH M7 Prediction-6				
Derek	Mutagenicity in vitro	bacterium	INACTIVE *	Derek KB 2020 1.0
* Contains unclassified features				
Sarah	Mutagenicity in vitro	bacterium	OUTSIDE DOMAIN	Sarah Model - 2020.1

In Silico Expert Review

In Silico Overall Call: Negative

Arguments Available	Argument Outcome
24 - Outside domain feature identified by Sarah Nexus cannot be dismissed as a potential toxicophore Sarah Nexus cannot make a prediction as at least one structural feature present in the query compound is outside the domain of the model. No structural alerts have been identified by Derek Nexus. However, available evidence suggests that the out of domain feature may pose a hazard or additional positive hypotheses have been identified by Sarah Nexus that cannot be negated. As a result, an overall in silico prediction of positive must be made.	Positive
	Positive
	Negative

Add >>

<< Remove

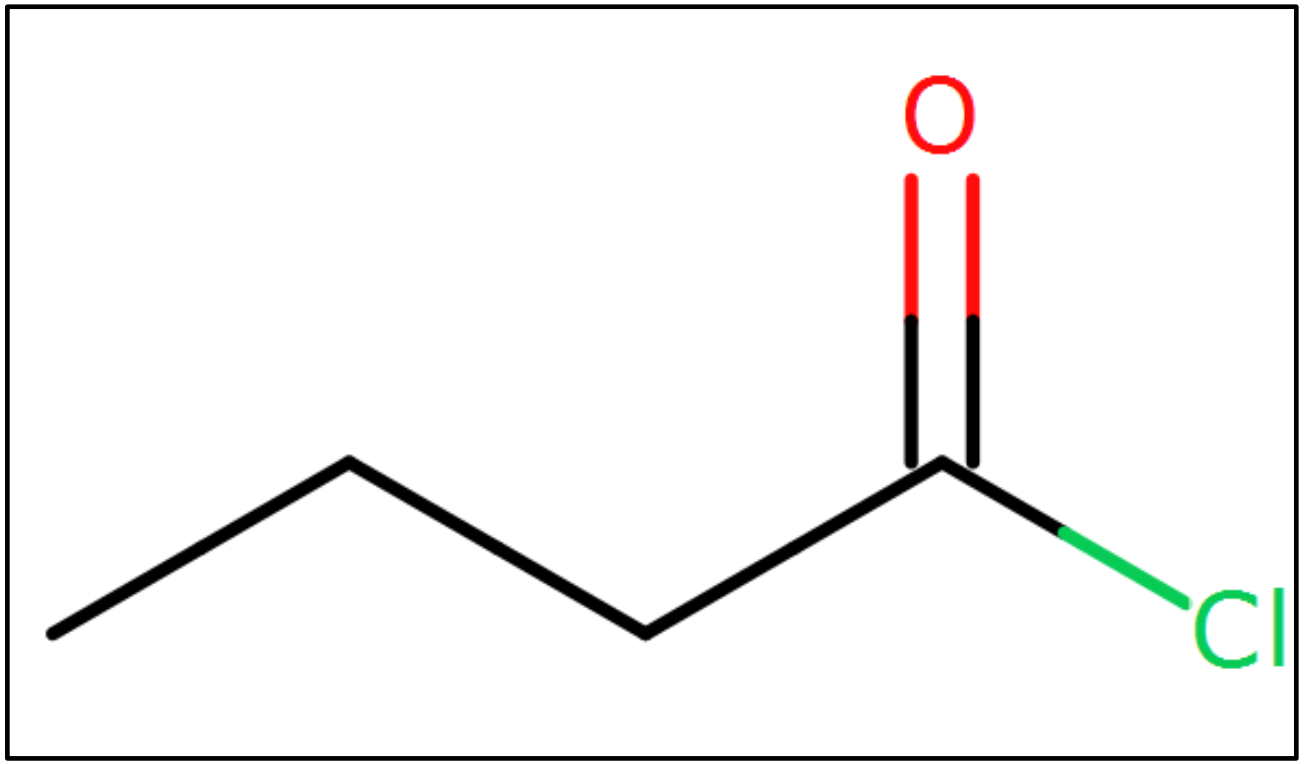
Arguments Used	Argument Outcome
23 - No toxicophore has been identified by either system and the outside domain feature identified by Sarah Nexus does not pose a hazard Sarah Nexus cannot make a prediction as at least one structural feature present in the query compound is outside the applicability domain of the model. No structural alerts have been identified by Derek Nexus. Available evidence suggests that the out of domain feature does not pose a hazard and no additional positive hypotheses have been identified by Sarah Nexus. As a result, an overall in silico prediction of negative can be made.	Negative

Class 5

In this instance, opening the ring system while retaining the (1-alkoxyethyl)(alkyl)sulfane function may be accepted as a method of addressing the unclassified & outside domain feature in Derek & Sarah respectively. In doing so, a negative prediction is returned. It is not expected that the compound will be active; however, as Sarah doesn't have any examples of the (1-alkoxyethyl)(alkyl)sulfane function, it may still be advisable to test.

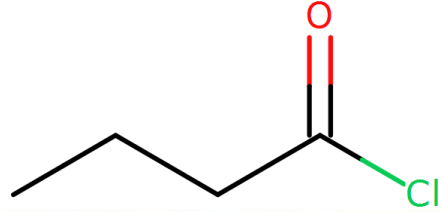
Lhasa Limited

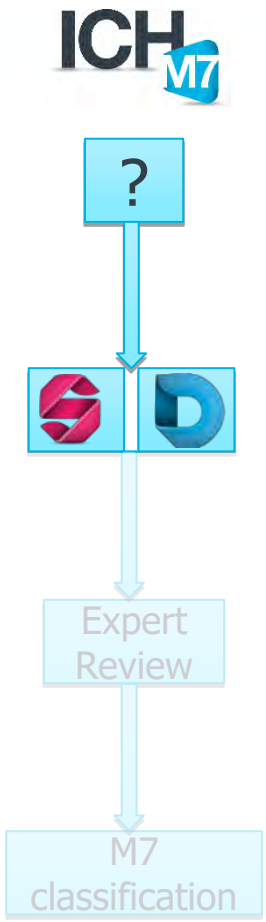
Example 8

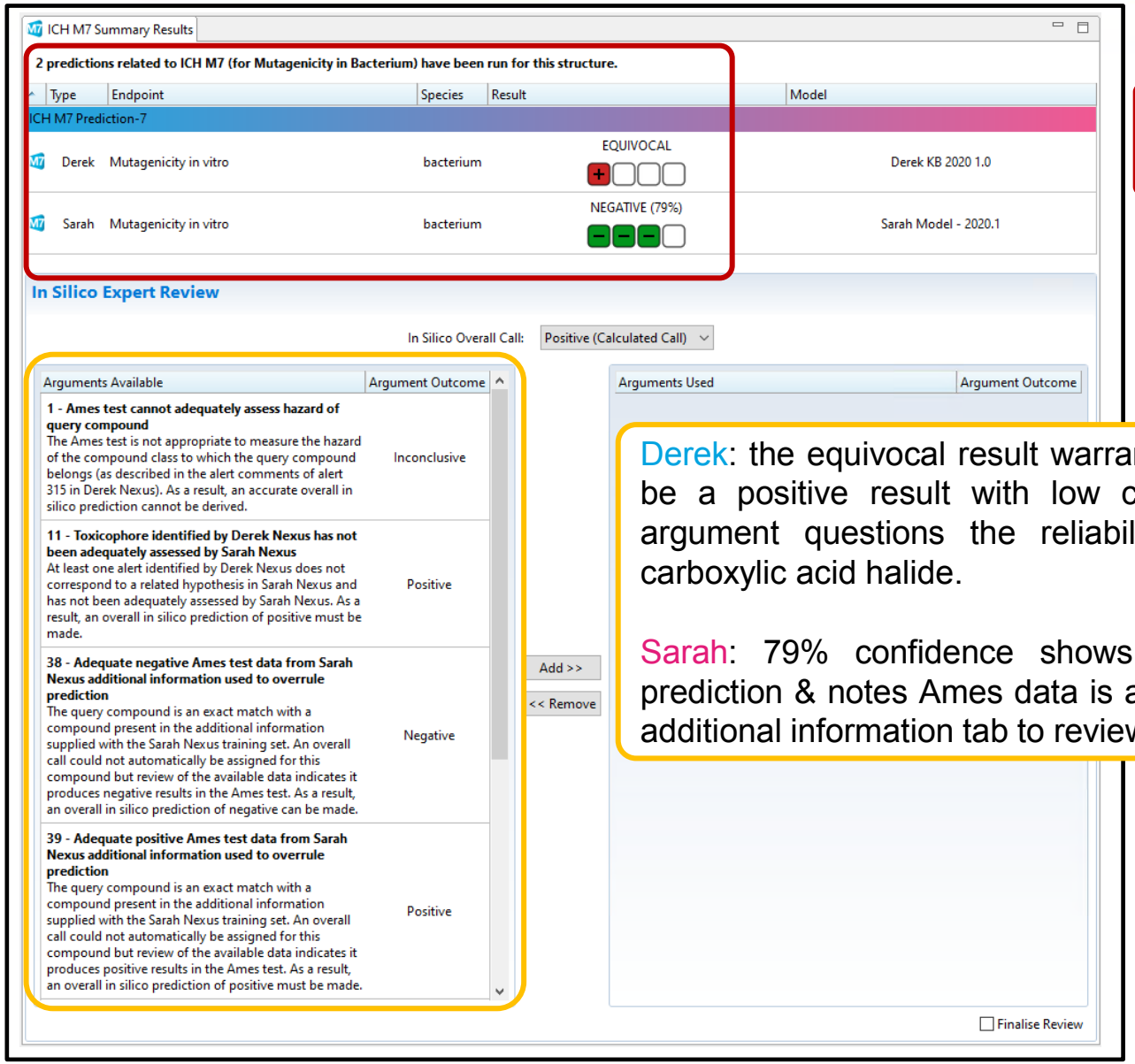


Lhasa Limited

Review high level predictions







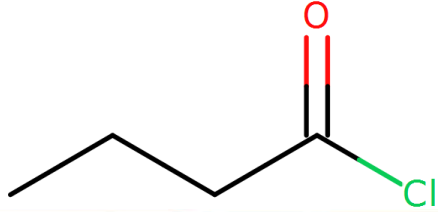
Derek & Sarah
disagree

Derek: the equivocal result warrants analysis as it's considered to be a positive result with low confidence & the expert review argument questions the reliability of the Ames test for this carboxylic acid halide.

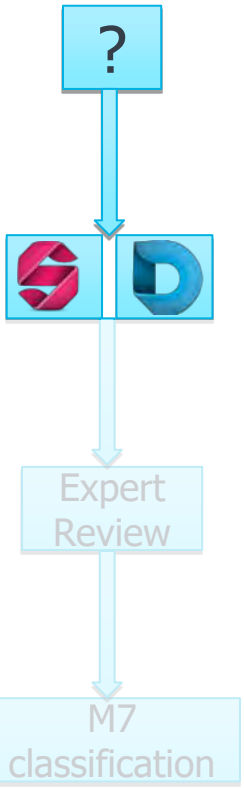
Sarah: 79% confidence shows good confidence in negative prediction & notes Ames data is available for the compound in the additional information tab to review.

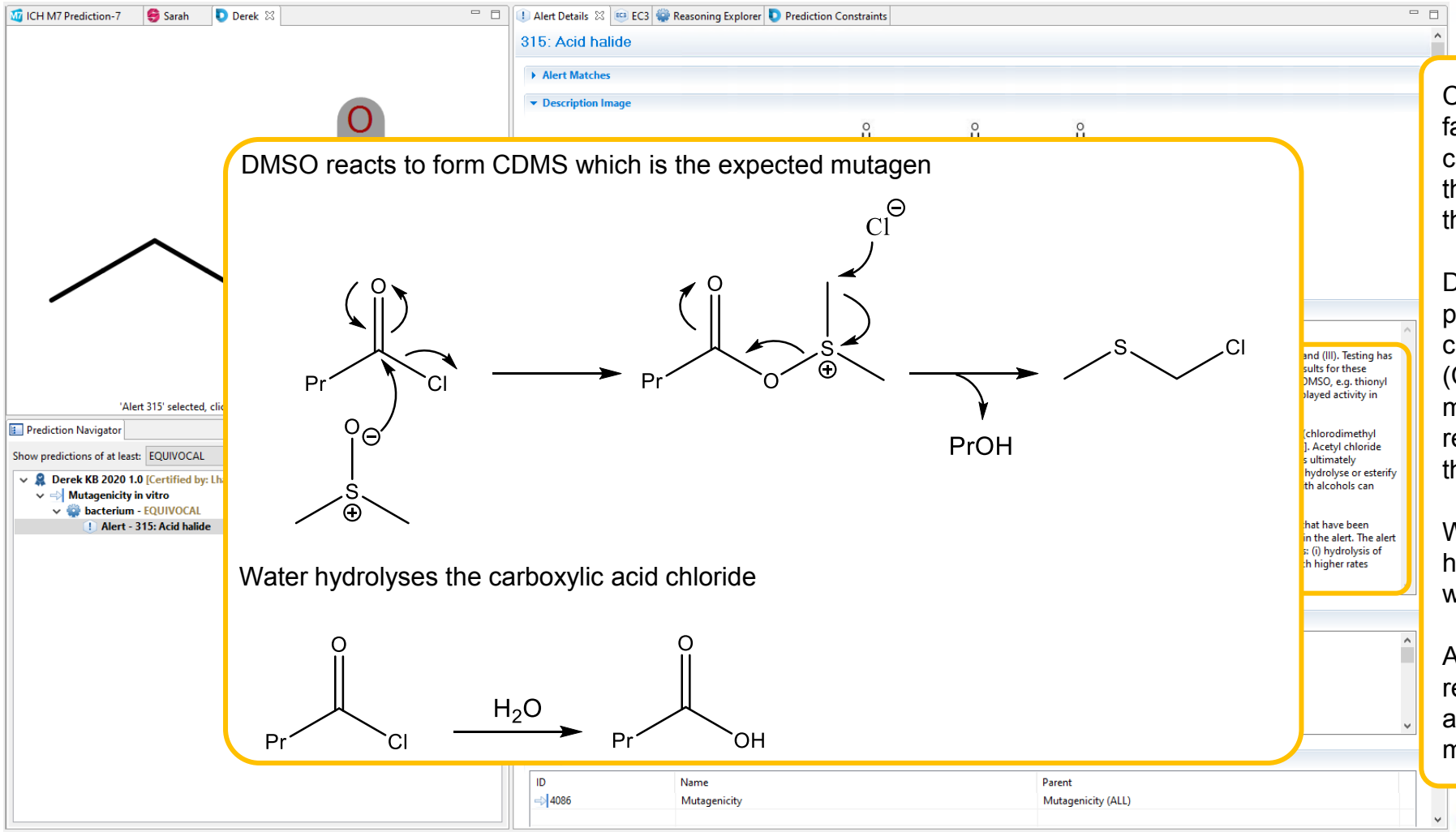
Lhasa Limited

Review the expert prediction



Derek nexus





DMSO reacts to form CDMS which is the expected mutagen

Water hydrolyses the carboxylic acid chloride

Comments discuss the fact that results for carboxylic acid halides in the Ames test depend on the choice of solvent.

DMSO provides false positive responses as chlorodimethyl sulfide (CDMS), an expected mutagen, is formed by reaction of DMSO with the acid chloride.

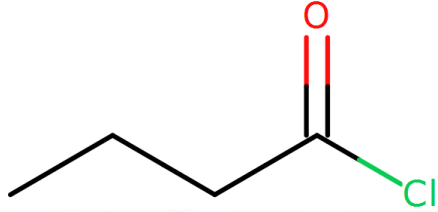
Water hydrolyses acid halides to the acids which are inactive.

Acetonitrile & other non-reactive organic solvents are thought to be the most appropriate media.

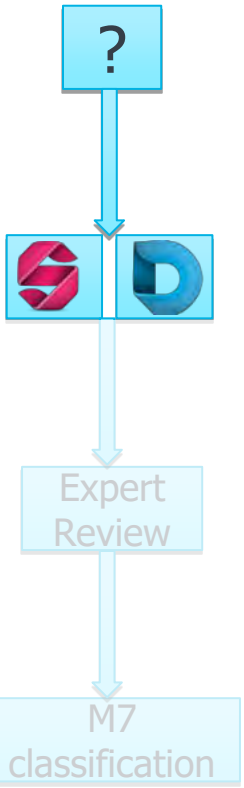
Derek provides a positive prediction for the carboxylic acid halide; however, the alert is set at the equivocal level of reasoning as there is evidence for & against so it requires review. The alert comments detail that activity is often dependent on the choice of solvent, hence carboxylic acid halides require review on a case by case basis. It is reasonable to consider the positive prediction with low confidence.

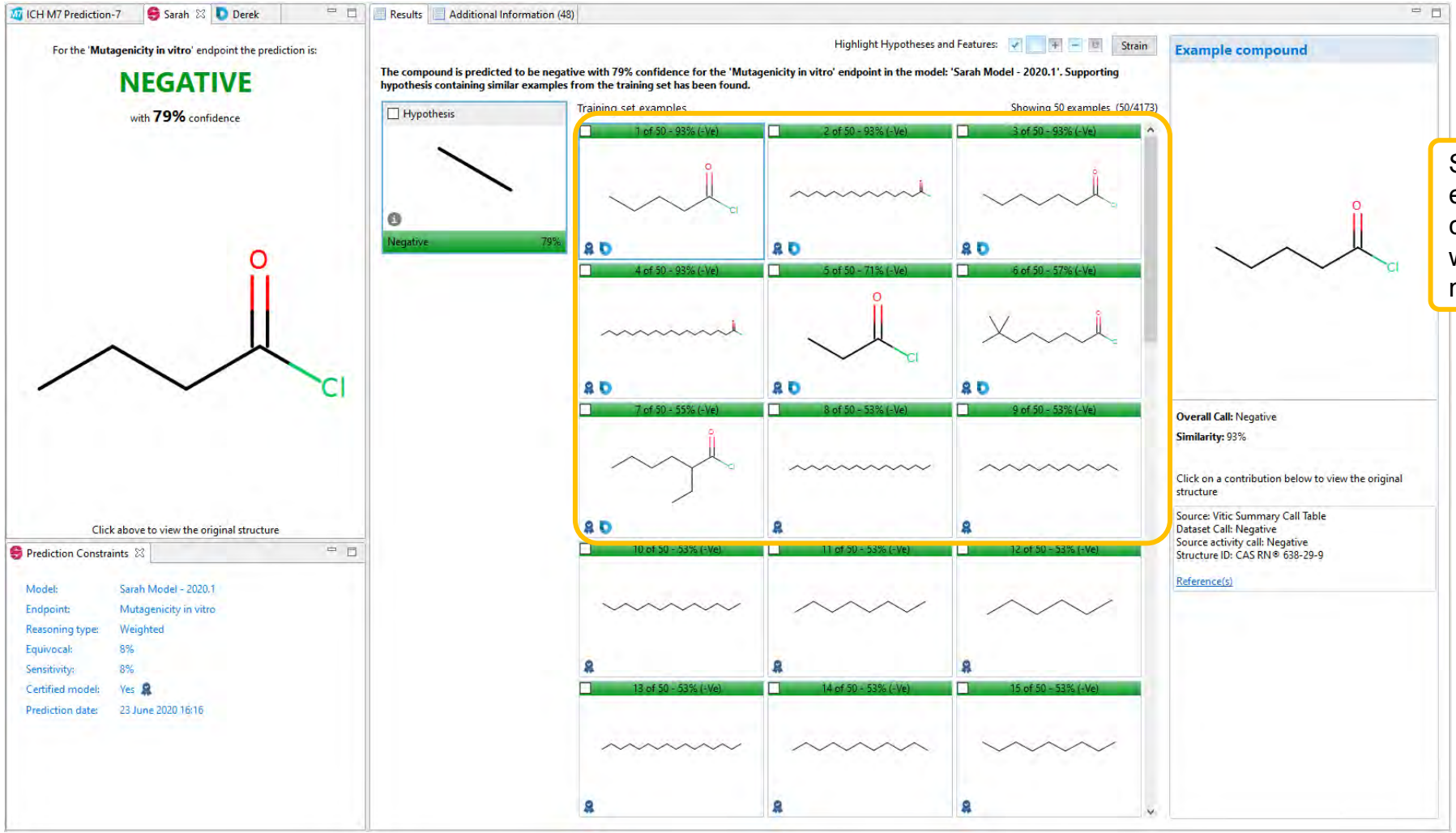
Lhasa Limited

Review the statistical prediction



Sarah nexus



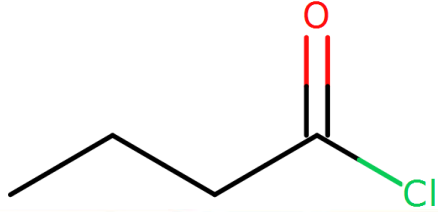


Sarah has many examples of similar carboxylic acid halides which are all non-mutagenic.

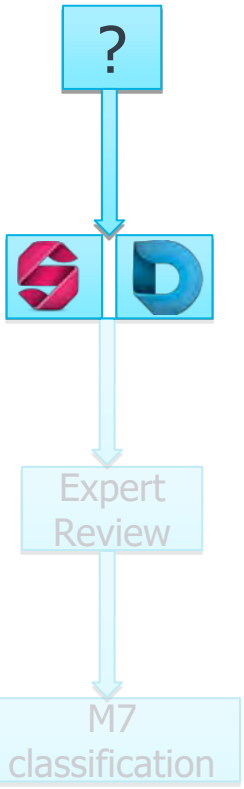
Many similar carboxylic acid halides in the Sarah training set are non-mutagenic, providing confidence in the prediction; however, based on Derek comments, full review of the test protocols is required.

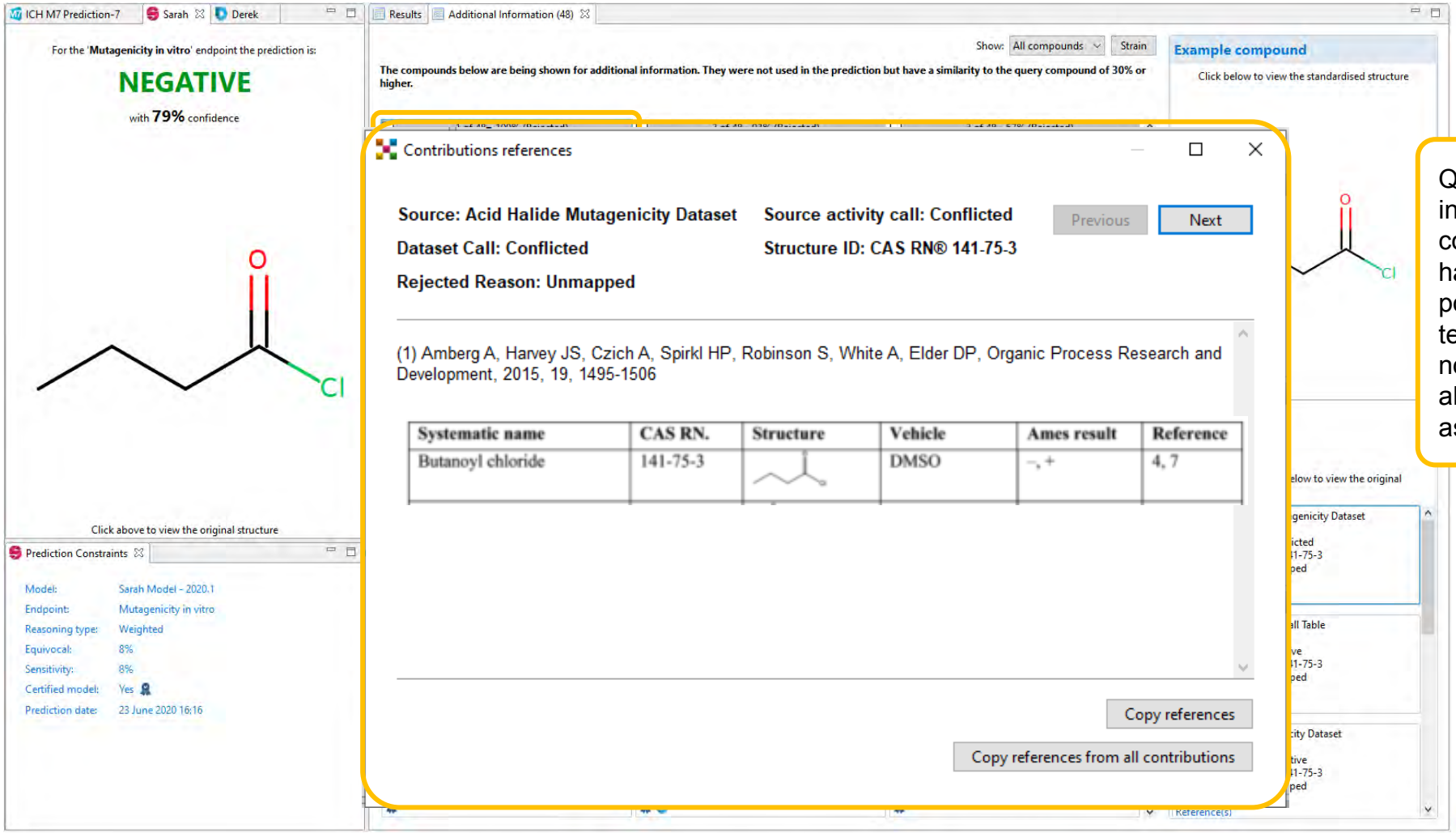
Lhasa Limited

Review the statistical prediction



Sarah nexus



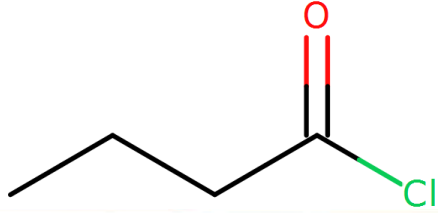


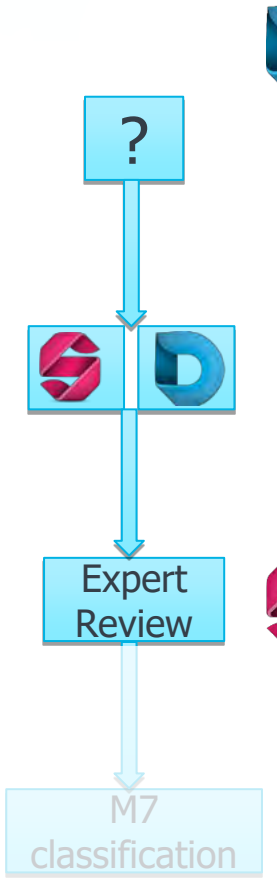
Query is in the additional information tab with conflicted results as it has been reported to be positive & negative when tested in DMSO. It has not been tested in alternative solvents to assess these results.

Query has been reported as positive & negative in the Ames test, albeit having only been tested in DMSO. Considering the comments in Derek, it is likely that the positive result is a result of formation of CDMS; however, this cannot be concluded without testing in other solvents simultaneously.

Lhasa Limited

Expert review





Derek nexus

INACTIVE

- Matches alert for carboxylic acid ester
- Comments discuss fact that activity is expected to be dependent on the solvent used as reaction with DMSO yields the expected mutagen CDMS whereas water hydrolyses the carboxylic acid halide

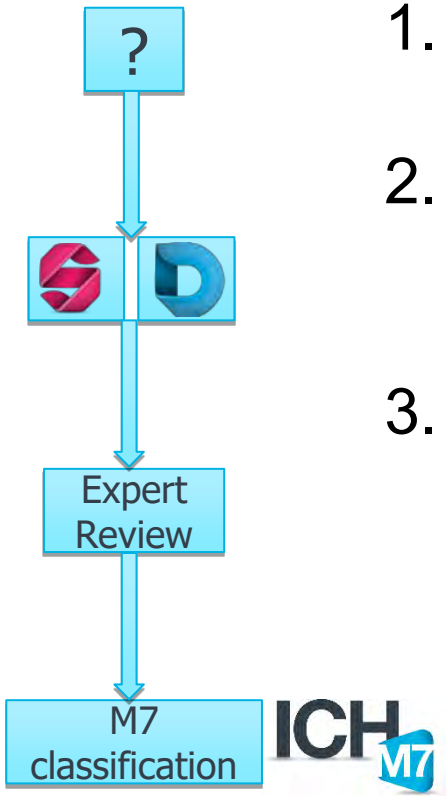
Sarah nexus

NEGATIVE (79%)

- Compound is known to Sarah training set; however, it is not included as conflicted results have been obtained using DMSO & no tests in other solvents are available to resolve this

Lhasa Limited

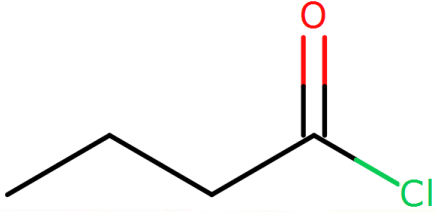
Please make your selection

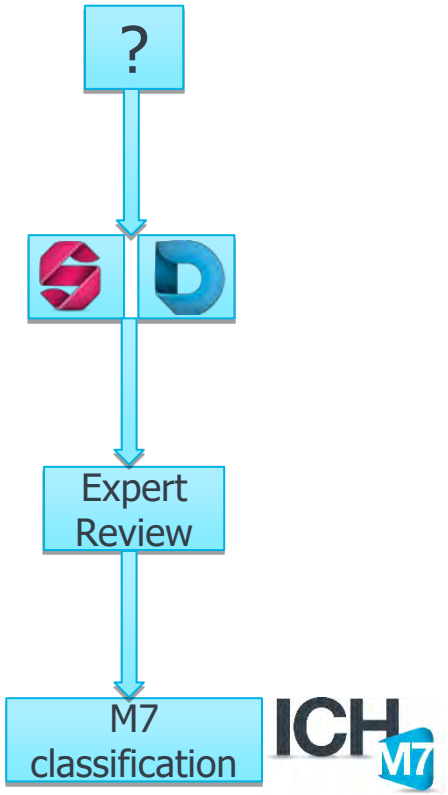


1. Class 3 – Alerting structure
2. Class 5 – No alerts or alerting with sufficient data to demonstrate lack of mutagenicity
3. Unsure

Lhasa Limited

ICH M7 classification





ICH M7 Summary Results

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

Type	Endpoint	Species	Result	Model
ICH M7 Prediction				
Derek	Mutagenicity in vitro	bacterium	EQUIVOCAL	Derek KB 2020 1.0
Sarah	Mutagenicity in vitro	bacterium	NEGATIVE (79%)	Sarah Model - 2020.1

In Silico Expert Review

In Silico Overall Call: Inconclusive

Arguments Available	Argument Outcome
11 - Toxicophore identified by Derek Nexus has not been adequately assessed by Sarah Nexus At least one alert identified by Derek Nexus does not correspond to a related hypothesis in Sarah Nexus and has not been adequately assessed by Sarah Nexus. As a result, an overall in silico prediction of positive must be made.	Positive
38 - Adequate negative Ames test data from Sarah Nexus additional information used to overrule prediction The query compound is an exact match with a compound present in the additional information supplied with the Sarah Nexus training set. An overall call could not automatically be assigned for this compound but review of the available data indicates it produces negative results in the Ames test. As a result, an overall in silico prediction of negative can be made.	Negative
39 - Adequate positive Ames test data from Sarah Nexus additional information used to overrule prediction The query compound is an exact match with a compound present in the additional information supplied with the Sarah Nexus training set. An overall call could not automatically be assigned for this compound but review of the available data indicates it produces positive results in the Ames test. As a result,	Positive

Add >>

<< Remove

Arguments Used	Argument Outcome
1 - Ames test cannot adequately assess hazard of query compound The Ames test is not appropriate to measure the hazard of the compound class to which the query compound belongs (as described in the alert comments of alert 315 in Derek Nexus). As a result, an accurate overall in silico prediction cannot be derived.	Inconclusive

Unclassified

Based on available evidence & conflicted results for the compound, it is not possible to conclude mutagenic potential. There is reason to doubt the activity of carboxylic acid halides in the Ames test; however, they contain a functional group that could potentially react with DNA. Alternatively, it is considered they may be hydrolysed rapidly & present no concern. It is also possible that their reactivity results in purge during synthesis allowing control under section 8 of ICH M7 instead.

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Conclusions

- *In silico* predictions under ICH M7 require, & benefit from, expert review
- Expert review varies for each prediction scenario
  - ...but some scenarios are more frequent & common arguments can be applied
- Scientific knowledge from multiple disciplines is required for expert review
- Expert review will be aided by…
  - …understanding how *in silico* predictions work
  - …understanding activity of specific chemical classes in the Ames test
  - …making associations between different models more visible
  - …presenting likely arguments to guide areas requiring review

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Thank you

shared **knowledge** • shared **progress**

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