ICH M7 – Best Practise in Assessing the Mutagenic Potential of Impurities using \textit{in Silico} Methodologies

Symposium on Streamlining Drug Discovery
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Agenda

- About Lhasa
- Background
- *In silico* systems and ICH M7 workflow
- Applying expert review
- Worked Examples
Introduction to Lhasa Limited

- Established in 1983
- HQ located in Leeds, United Kingdom
- Not-for-profit & Educational Charity
- Facilitate collaborative data sharing projects in the chemistry-related industries
- Controlled by our members
- Creators of knowledge base, statistical and database systems
ICH M7

• “Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk”
• ‘Global’ guidelines – America, Europe and Japan

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*Or other relevant positive mutagenicity data indicative of DNA-reactivity related induction of gene mutations (e.g., positive findings in in vivo gene mutation studies)
ICH M7 – Permits the use of *in silico* predictions

- You may use the Ames (*in vitro*) assay
  - Or use *in silico* predictions in its place
- If you submit *in silico* predictions, you will need:
  - Two predictions – one expert rule-based and one statistical-based
  - To undertake expert review
    - To provide additional evidence for any prediction
    - To support the final conclusion
In silico systems and ICH M7 workflow
**In silico workflow under ICH M7**

1. **Evaluate drug substance, impurities, degradants, intermediates…**
   - Databases, in-house, literature.
   - 2 *in silico* predictions expert + statistical

   - Known mutagen
   - Both predict positive
   - Disagree / fail to predict
   - Both predict negative
   - Known non-mutagen

   **Expert Review**

   - Limit according to TTC or present purge argument for loss
   - Ames test
   - Treat as non-mutagen
Using *in silico* predictions

- “The absence of structural alerts from both is sufficient to conclude that the impurity is of no mutagenic concern”

- Expert review can provide
  - Additional supportive evidence
  - Reason to dismiss an *in silico* prediction
  - Rationale to support the final conclusion
In silico systems should give you

- A prediction
  - ‘Out-of-domain’ or ‘indeterminate’ is **not** a prediction
    - Is there enough information to make an expert call in such cases?
    - Is the scope of the alert/applicability domain clearly defined?
    - How good is the coverage of your chemical space?

- Accuracy
  - You should assess against your chemical space (not public data)

- A measure of the model’s confidence in a prediction
  - Is it meaningful? Has it been shown to correlate with accuracy
    - It should tell you how much to worry and why
In silico systems should be

• Regularly updated with new data or knowledge
  • Chemical space is changing – models need to keep up
  • Public vs proprietary chemical space

• Known to regulatory authorities
  • Not essential but expect lots of questions if:
    • They don’t understand the approach
    • They have not seen the training data
    • They haven’t evaluated the performance
    • They don’t get enough supporting data
**In silico** systems should give you

- A transparent prediction
- Supporting information (data, explanation)
- The most important criteria
- The ability to defend or challenge every prediction
  - This may be hard if the model automates the conclusion or does not say why
  - A regulator may not accept an automated decision and ask you to explain
Choosing your *in silico* systems

✓ Performance
  ✓ Accuracy
  ✓ Coverage (out of domain or indeterminate is not a prediction)

✓ Transparent
  ✓ Explanation of how/why each prediction is made
  ✓ Clear applicability domain (and methodology for it)
  ✓ Relevant measure of confidence for each prediction
    ✓ highlights and explains any uncertainty

✓ Sufficient information to support or challenge a prediction
  ✓ Can see the underlying data and/or rationale

✓ Robust and broad training set
  ✓ Curated
  ✓ Sight of confidential data
  ✓ Regularly updated

✓ Is used and understood by regulators
ICH M7 says…..

• “If warranted, the outcome of any computer system-based analysis can be reviewed with the use of expert knowledge in order to provide additional supportive evidence on relevance of any positive, negative, conflicting or inconclusive prediction and provide a rationale to support the final conclusion.”

What is Expert Review

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

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d Pfizer, Drug Safety Research and Development, Groton, CT, USA
e Novartis Institutes for Biomedical Research, Department of Preclinical Safety, Basel, Switzerland
f Janssen, Drug Safety Sciences, Beere, Belgium
g Unilever, Safety and Environmental Assurance Centre, Colworth, Beds, UK
h GlaxoSmithKline, Computational Toxicology, Ware, Herts, UK
i National Institute of Health Sciences, Tokyo, Japan
j FDA Center for Drug Evaluation and Research, Silver Spring, MD, USA
k F. Hoffmann-La Roche Ltd., Pharma Research and Early Development, Basel, Switzerland
l AstraZeneca, Macclesfield, Cheshire, UK
m Bayer, HealthCare, Genetic Toxicology, Berlin, Germany
Expert Analysis step-by-step

1. **Databases**
   - Enter query compound(s)

2. **(Q)SAR**
   - Generate statistical and expert predictions

3. **Review**
   - Expert review

4. **Conclusion**
   - (Optionally) source further supporting data

5. **Report**

(Q)SAR = Quantitative Structure-Activity Relationship
Likely to conclude positive
Very strong evidence would be needed to overturn both predictions

Likely to conclude positive
Lack of a second prediction suggests insufficient evidence to draw any other conclusion

Uncertain
Likely to conclude positive without strong evidence to overturn a positive prediction

System 1
Positive  Positive  Positive  Negative  Negative

System 2
Positive  O.O.D. or equivocal  Negative  O.O.D. or equivocal  Negative

O.O.D. = out of domain

Uncertain
Conservatively could assign as positive. May conclude negative with strong evidence showing feature driving a ‘no prediction’ is present in the same context in known negative examples (without deactivating features)

Likely to conclude negative
Expert review should support this conclusion – e.g. by assessing any concerning features (misclassified, unclassified, potentially reactive..)

Establishing best practice in the application of expert review of mutagenicity under ICH M7
Regulatory Toxicology and Pharmacology 2015, 73, 367-377
Dealing with out of domains

- Dealing with Out of Domain (Q)SAR Predictions for ICH M7: A Regulatory and Industrial Perspective
  - Dr. Naomi Kruhlak – FDA
  - Michelle Kenyon – Pfizer
Anecdotal evidence suggests new drug applicants routinely encounter a significant number of out of domain results (10% to 50%)

- Consequence of novel chemistry: Many APIs are out of domain, so highly-similar, late-stage impurities also out of domain
- Models constructed from public data, represent public chemical space
- Review of new drugs approved in 2016 and 2017 by Dr. Mark Powley, formerly of CDER’s Office of New Drugs:

<table>
<thead>
<tr>
<th>Summary</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approved NMEs with (Q)SAR</td>
<td>18</td>
</tr>
<tr>
<td>Approved NMEs with detailed (Q)SAR</td>
<td>13</td>
</tr>
<tr>
<td>Total impurities evaluated by (Q)SAR</td>
<td>488</td>
</tr>
<tr>
<td>Out of domain results</td>
<td>86</td>
</tr>
</tbody>
</table>
## NME Regulatory Strategies by Applicants

<table>
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<tr>
<th>Strategy</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow up with 3&lt;sup&gt;rd&lt;/sup&gt; model</td>
<td>5</td>
</tr>
<tr>
<td>Apply additional model</td>
<td></td>
</tr>
<tr>
<td>Comparison with experimentally negative analogue(s)</td>
<td>6</td>
</tr>
<tr>
<td>Steric hindrance (based on expert knowledge)</td>
<td>5</td>
</tr>
<tr>
<td>Apply expert knowledge</td>
<td></td>
</tr>
<tr>
<td>Comparison with (Q)SAR negative analogue</td>
<td>4</td>
</tr>
<tr>
<td>Class 4 (positive prediction in presence of unknown fragments)</td>
<td>7</td>
</tr>
<tr>
<td>“Class 4-type” conclusions</td>
<td>38</td>
</tr>
<tr>
<td>Chemistry covered by experimentally negative API with identical (Q)SAR profile (i.e., negative prediction in 1&lt;sup&gt;st&lt;/sup&gt; model + OOD in 2&lt;sup&gt;nd&lt;/sup&gt; model)</td>
<td></td>
</tr>
<tr>
<td>Experimental Ames assay</td>
<td>12</td>
</tr>
<tr>
<td>Test/control</td>
<td></td>
</tr>
<tr>
<td>Control as class 3 impurity – positive prediction in one model</td>
<td>5</td>
</tr>
<tr>
<td>Control as class 3 impurity – negative prediction in one model</td>
<td>1</td>
</tr>
<tr>
<td>Assign class 5 impurity with no further explanation</td>
<td>3</td>
</tr>
<tr>
<td>Requires follow-up</td>
<td></td>
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<td>Total</td>
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OODs – Regulatory conclusions

• OOD results are generated for different reasons by different software
  • Important to have an understanding of why a structure is OOD so it can be handled appropriately

• There are several acceptable strategies for addressing an out of domain
  • An OOD is not a valid prediction and does not contribute to a Class 5 assignment – needs to be followed-up
  • Standard internal practice is to run a 3rd model
  • Using experimental data (and/or predictions) from structural analogues sharing uncovered attributes has been successful
  • Application of expert knowledge can resolve many ambiguous outcomes, including OODs

• Adequate documentation is critical
  • Regulatory (Q)SAR submissions still vary significantly in quality
  • OODs addressed with expert knowledge held to high standard—need a well-documented rationale
  • Inadequately documented submissions may result in additional review cycles
An expert knows.....

- What (s)he needs to know
- How to apply that knowledge
- Where there is uncertainty
- Who to ask for help
Mutagenicity is driven by the chemical structure
• Mutagenicity is predicted by the Ames assay
Essential knowledge of an expert (metabolism)

- Many compounds become active through metabolic activation
Skills of an expert or an expert team

Chemist
- Process chemistry
- Analytical chemistry
- Chemical reactivity
- Functional groups
- Similarity
- Impurity profile

Drug Metabolist
- (Q)SAR
- Reactive metabolites
- Metabolic profile

Toxicologist
- Mechanisms of activity
- Protocol and limitations of Ames assay
- Interpretation of strain data
- Supporting data
- How in silico systems work strengths/limitations

Where to focus
Skills of an expert or an expert team

• It is unlikely that a single person will be expert in everything
• Many companies have a team that make these assessments

• The choice of software is important
  • It must give you enough information to trust a prediction
  • ....and to challenge it
Worked Examples
Example 1

<table>
<thead>
<tr>
<th>Expert rule-based</th>
<th>Negative</th>
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<tr>
<td>Statistical-based</td>
<td>Positive</td>
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Conflicting Predictions!
Example 1

Epoxide moiety concerning to the expert system
The right systems help you with expert review!

- **Performance**
  - ✓ Accuracy
  - ✓ Coverage (out of domain or indeterminate is not a prediction)
- **Transparent**
  - ✓ Explanation of how/why each prediction is made
  - ✓ Clear applicability domain (and methodology for it)
  - ✓ Relevant measure of confidence for each prediction
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  - ✓ Sufficient information to support or challenge a prediction
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- **Robust and broad training set**
  - ✓ Curated
  - ✓ Sight of confidential data
  - ✓ Regularly updated
- ✓ Is used and understood by regulators
Expert Review – Expert System

• Well supported alert
  • No reason to immediately dismiss the positive prediction

R1-R4 = any atom but with exclusions including glycicyl-type compounds, cyclohexyl epoxides with aliphatic ring fusions, tri- and tetra- alkyl or aryl substituted epoxides, spiroalkyl epoxides, and 1,2-diacid/ester/amide epoxides

Epoxides are electrophilic compounds that readily bind to DNA [Citti et al, Sugiura and Goto]. As a consequence, they may exhibit mutagenicity in the Ames test, generally in strains TA100 and TA1535 without S9 mix [Canter et al, von der Hude et al, Sugiura and Goto, Tamura et al, Wade et al]. The effect of S9 mix on the mutagenic response varies depending, for example, on the susceptibility of the test chemical to detoxification by epoxide hydrolases and glutathione S-transferase present in the S9 mix [Castelain et al].
Expert Review – Statistical System

- Overall prediction negative
- But model aware of epoxide moiety
- Close training set examples are positive
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<th>Equivocal</th>
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<tr>
<td>Statistical-based</td>
<td>Positive (low confidence)</td>
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One equivocal and one weakly positive
Example 2

Acid chloride moiety concerning
Positive results are not driven by the acid chloride but by the solvent.
Expert Review – Statistical System

- Weakly positive prediction
- Lack of relevant examples
- Other reasons for activity
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Conflicting Predictions!
Expert Review – Expert System

• Clear and unambiguous negative prediction
Expert Review – Statistical System

- Positive prediction can be overturned by the expert
- Other reasons for activity or weak positive evidence
## Expert Review Conclusion

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  - *Regulatory Toxicology and Pharmacology, 2015, 73, 367–377*

- Use of *in silico* systems and expert knowledge for structure-based assessment of potentially mutagenic impurities.
  - *Regulatory Toxicology & Pharmacology, 2013, 67, 39–52*

- (Q)SAR assessments of potentially mutagenic impurities: A regulatory perspective on the utility of expert knowledge and data submission.
  - *Regulatory Toxicology & Pharmacology, 2015, 71, 295–300*

  - *Regulatory Toxicology & Pharmacology, 2012, 62, 449-55*

- A practical application of two in silico systems for identification of potentially mutagenic impurities.
  - *Regulatory Toxicology & Pharmacology, 2015, 72, 335-349*

  - *Regulatory Toxicology & Pharmacology, 2015, 71, 388-397*
Acknowledgements

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Thank you!

Questions?