In silico strategies to assess potentially mutagenic impurities under ICH M7

SOT 2017

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In silico strategies to assess potentially mutagenic impurities under ICH M7

**ICH M7**

- **Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk**

**Description**

The tripartite harmonised ICH Guideline reached Step 4 of the ICH process in June 2014. This Guideline offers guidance on analysis of Structure Activity Relationships (SAR) for genotoxicity. Furthermore, it is intended to resolve questions such as whether impurities with similar alerts that potentially have similar mechanism of action should not be combined in calculating a Threshold of Toxicological Concern (TTC) and whether the TTC may differ based on differences in the approved duration of use.

**Implementation**

- **EC**: Adopted by CHMP, 25 September 2014, issued as EMA/CHMP/ICH/83812/2013
- **MHLW/PMDA**: Adopted 10 November 2015, PFSB/ELD Notification No. 111
- **FDA**: Published in the Federal Register, 28 May 2015, Vol. 80, No 102, p. 30465
- **Health Canada**: Implemented 21 January 2016 File #: 15-114011-514
- **Swissmedic**: Please refer to the press release on Swissmedic’s website for information on implementation

**Finalised Guideline:**

June 2014

- **Concept Paper**
- **Business Plan**
- **Audio Presentation**

**Adopted worldwide**

**Supports the use of in silico models in decision-making**

**Enables fast, safe decision-making**
An integrated solution to ICH M7
..developed through collaboration

ICH M7

Impurity identification

Mutagen?

Purged?

No
Yes

No
Yes

Treat as non-mutagenic impurity
Control to limits in API
No further action
An integrated solution to ICH M7
devolved through collaboration

• The ability to assess an impurity as non-mutagenic or not present in the final API can offer significant efficiencies without compromising safety.

• *In silico* tools can provide a robust and cost-efficient solution provided they are fit-for-purpose.


  • Distinguishing between expert and statistical systems for application under ICH M7 Barber *et. al.* Reg. Tox. and Pharm. 2017, 84, 124

  • Establishing Good Computer Modelling Practice (GCMP) in the Prediction of Chemical Toxicity Judson *et. al.* Mol. Inf. 2015, 34, 276
An integrated solution to ICH M7
..developed through collaboration

ICH M7

Impurity identification

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No

Yes

No

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..developed through collaboration

ICH M7

Impurity identification

Mutagen?  Purged?

Database search  In silico  Test

Expert system  Statistical system

Expert Assessment

Classification

Control

Report
Identifying potential mutagenic impurities

ICH Harmonised Tripartite Guideline

Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk

M7

Impurity identification

ICH M7

Observed impurities

Synthesis-derived

Expected impurities

Degradation-derived

Starting material

Reagent

Catalyst / ligand

Intermediates

By-products

Decomposition

Reaction with
- Packaging
- Excipients
Identifying impurities from degradation

**Degradation Product:** A molecule resulting from a chemical change in the drug molecule brought about over time and/or by the action of light, temperature, pH, water, or by reaction with an excipient and/or the immediate container/closure system.

…observed during manufacture or stability studies

“potential degradation pathways” including from interaction with excipients and/or packaging

‘relevant stress conditions’
- Light
- Heat
- Humidity
- Acid/base hydrolysis
- Oxidation
Identifying impurities from degradation

- Light
- Heat
- Humidity
- Acid/base hydrolysis
- Oxidation

‘relevant stress conditions’

Expert review

- Photochemical (37)
- Rearrangement (31)
- Elimination (49)
- Addition (77)
- Hydrolysis (80)
- Oxidation (126)

Zeneth includes predictions of reaction of the API under different conditions in the presence of excipients, solvents and degradants
Undertaking a database search

- Database searching
  - Public data
  - Proprietary data

Exact match

Substructure / similarity search

Further supporting information for expert review

Class 1
- Mutagen
- Carcinogen

Class 2
- Mutagen
- Carcinogen: ?

Class 5
- Non-Mutagen

M7 (Muller) classification
Undertaking a database search

Vitic Nexus – an authoritative source of data

- In-house data
- Public data
- Pre-competitive data
- Sources include:
  - FDA
  - NTP
  - ISSTY
  - Kirkland
  - Hansen, Bursi
  - MPDB
  - Literature
- In vitro genetox
  - 164,001 | 10,246
- In vivo genetox
  - 10,595 | 2,723
- Overall-call genetox
  - 17,322 | 8,932
- Carcinogenicity
  - 16,419 | 3,865
- In Vitro Stimulation

- Aromatic amines
  - 3,639 | 424
- Intermediates
  - 16,971 | 1,088
- Excipients
  - 2,435 | 975
- Consortia of Lhasa members

- Vitic contains raw, summary, and Lhasa overall call data + literature references
- Currently 400K records, 20K compounds across a wide range of tox endpoints
Lhasa Carcinogenicity Database

- Searchable repository of 6529 long-term carcinogenicity studies covering 1529 chemicals
- Builds upon work by Lois Swirsky Gold et al.
- Recalculates TD$_{50}$ values following the non-lifetable statistical method using a new R script
  - Will be published this year
- Data will be expanded upon in the future
Captan

Summary

<table>
<thead>
<tr>
<th>Species</th>
<th>Low Dose (mg/kg/day)</th>
<th>High Dose (mg/kg/day)</th>
<th>Result</th>
<th>Sex</th>
<th>Tumour sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>1.010</td>
<td>2.110</td>
<td>POSITIVE</td>
<td>Female</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Rat</td>
<td>1.410</td>
<td>2.680</td>
<td>POSITIVE</td>
<td>Male</td>
<td>Small intestine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Female</td>
<td>Uterus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>Kidney</td>
</tr>
</tbody>
</table>

Chemical structure

CAS number: 133-06-2
Chemistry unique identifier: 123-05-2
Chemistry name: Captan
Synonym(s):
- 3a,4,7,7a-Tetrahydro-2-[[(trichloromethyl)thio]-1H-isoindole-1,3(2H)diene; N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide; N-Trichloromethylthio tetrahydrophthalimide; Orthocid; N-Trichloromethylthio-1,2,5,6-tetrahydrophthalimide

Molecular weight: 300.59
Molecular formula: C9H8ClENO2S
SMILES: C1=C(C=C(C(=O)(C(=O)Cl)Cl)Cl)C(=O)Cl;
## Study details and citations

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>Strain</th>
<th>Route</th>
<th>Exposure time</th>
<th>Experiment time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td></td>
<td>CD1</td>
<td>Diet</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Tumour Site - Small intestine
- **Tumour Type**: More than one tumour type
  - **Dose (mg/kg/day)**: 0, 70, 1180, 1850
  - **Incidence**: 3/80, 18/80, 22/80, 30/80
  - **Probability**: <= 0.0005
  - **Lhasa LD50**: 900
  - **Gold LD50**: 2890

### Tumour Site - Small intestine
- **Tumour Type**: Carcinoma
  - **Probability**: <= 0.0005
  - **Lhasa LD50**: 1450
  - **Gold LD50**: 3500

### Tumour Site - Small intestine
- **Tumour Type**: Adenoma
  - **Probability**: <= 0.002
  - **Lhasa LD50**: 8290

### Literature reference(s)

### Notes (exposure, histopathology, mortality)

---

### Additional details

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>Strain</th>
<th>Route</th>
<th>Exposure time</th>
<th>Experiment time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Female</td>
<td>CD1</td>
<td>Diet</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Tumour Site - Small intestine
- **Tumour Type**: Adenoma
  - **Probability**: <= 0.035
  - **Lhasa TD50**: 13300

### Tumour Site - Small intestine
- **Tumour Type**: Carcinoma
  - **Probability**: <= 0.0005
  - **Lhasa TD50**: 1329
  - **Gold TD50**: 2119

### Tumour Site - Small intestine
- **Tumour Type**: Carcinoma
  - **Probability**: <= 0.0005
  - **Lhasa TD50**: 1329
  - **Gold TD50**: 2119
Derek Nexus - an expert *in silico* prediction system

- Key endpoints of relevance for M7

- Carcinogenicity
  - Carcinogenicity
  - Photocarcinogenicity
- Genotoxicity
  - Chromosome damage
  - Mutagenicity
    - Mutagenicity
      - in vitro
      - in vivo
    - Photomutagenicity
  - Non-specific genotoxicity
- Irritation
Sarah Nexus – a statistical model for mutagenicity

- Supplied with model built with Lhasa-curated public data
- Optimised to learn mutagenicity…
  - Fragmentation designed for reactivity-driven endpoints
  - Self-organising Hierarchical Network to maximise information gain
  - Decision-tree to reduce the chance of coincidence
- Explicit applicability domain
- Confidence score is provided for each prediction
- Predictions are transparent and therefore interpretable
M7 Classification

- M7 classification helps define how to control impurities…

How Lhasa ICH M7 classification can help

- User can add additional data
- Searches for carcinogenic and mutagenic data from Lhasa and custom database
### ICH M7 class generated and report produced

Each impurity is classified according to whether there is Ames or Carcinogenicity information in addition to the Derek Prediction.

Users can also input experimental results for mutagenicity or carcinogenicity which updates the ICH M7 Class.
Mirabilis – supporting expert assessment of purge

- Concept is part of the M7 guidelines

Risk of potential mutagenic impurity

Ames or 2 in silico models...

Present a purge argument for absence...

Risk mitigated

-ve

Treat as a non-mutagen

Control & monitor in final API

+ve

Risk assessed


An integrated solution to ICH M7
..developed through collaboration

ICH M7

Impurity identification

Mutagen?

Carc-DB
Database search

In silico

Test

Expert system

Statistical system

In silico

In silico

Purged?

Measure

Expert Assessment

Classification

Report

Control
A case study

- Selective 5HT2a inverse agonist
- Non-dopaminergic anti-psychotic for Parkinson sufferers
- Approved by the FDA in 2016 (Acadia Pharmaceuticals)

WO 2016/141003A1

Pimavanserin
4-hydroxybenzaldehyde

- Processed through Derek Nexus and Sarah Nexus
  - Both report a negative prediction (in Sarah’s training set)

5 strains reported in Vitic (ref 1996)
A case study

- Selective 5HT2a inverse agonist
- Non-dopaminergic anti-psychotic for Parkinson sufferers
- Approved by the FDA in 2016 (Acadia Pharmaceuticals)

Pimavanserin

- \( \text{Br} \) to \( \text{H} \)
- \( \text{K}_2\text{CO}_3, \text{KI}, \text{DMF} \)

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{O} & \quad \text{O} \\
\end{align*}
\]

\[
\begin{align*}
\text{K}_2\text{CO}_3, \text{KI}, \text{DMF} & \quad \text{HONH}_2 \\
\text{H} & \quad \text{O} \\
\text{O} & \quad \text{O} \\
\text{O} & \quad \text{O} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{O} & \quad \text{O} \\
\end{align*}
\]

- \( \text{i. } \text{H}_2, \text{Raney-Ni} \)
- \( \text{ii. } \text{Acetic acid} \)
- \( \text{iii. } \text{NaOH} \)

- \( \text{Cl} \) to \( \text{O} \)
- \( \text{Cl} \)

- \( \text{K}_2\text{CO}_3, \text{KI}, \text{DMF} \)

- \( \text{L-tartaric acid} \)

Pimavanserin

- free base
- hemi-tartrate
1-Bromo-2-methylpropane (iso-Butyl Bromide)

- Search in Vitic → exact match
1-Bromo-2-methylpropane (iso-Butyl Bromide)

- Search in Vitic → exact match
  - Reference quite old (1977) – pre-dates OECD guidelines
  - No pre-incubation
  - Concentration was not reported
- Was tested in 5-strains +/- S9
  - ..but not using e. coli or TA102 [not that relevant for alkyl halides]
- Was tested in a desiccator (volatile compound)
- Read-across?
  - Close analogues tested in the same paper were active

- Activity cliff – would retest or consider as mutagenic!
A case study

- Selective 5HT2a inverse agonist
- Non-dopaminergic anti-psychotic for Parkinson sufferers
- Approved by the FDA in 2016 (Acadia Pharmaceuticals)
Phosgene

- Processed through Derek Nexus and Sarah Nexus
Phosgene

- Test result in Vitic

**Vitic Search Results**

<table>
<thead>
<tr>
<th>Structures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Select All</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Report</th>
<th>Structure</th>
<th>Name</th>
<th>CAS Number</th>
<th>Formula</th>
<th>Weight</th>
<th>Similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><img src="image.png" alt="Phosgene" /></td>
<td>Phosgene</td>
<td>75-44-5</td>
<td>CCl₂O</td>
<td>98.91</td>
<td>1.000000</td>
</tr>
</tbody>
</table>

- Tested negative in TA98/100 but unlikely to survive conditions
- Expect it to be Ames negative but treat as ‘mutagenic’
A case study

- Selective 5HT2a inverse agonist
- Non-dopaminergic anti-psychotic for Parkinson sufferers
- Approved by the FDA in 2016 (Acadia Pharmaceuticals)
Benzyl isocyanate

- Processed through Derek Nexus and Sarah Nexus
Benzyl isocyanate

- Processed through Derek Nexus and Sarah Nexus
- Derek has a strong alert – detailed expert analysis, lots of supporting publications…
Benzyl isocyanate

- Processed through Derek Nexus and Sarah Nexus
  - Derek has a strong alert – detailed expert analysis, lots of supporting publications...
  - Sarah does not have a specific hypothesis for isocyanates
Benzyl isocyanate

- Processed through Derek Nexus and Sarah Nexus
  - Derek has a strong alert – detailed expert analysis, lots of supporting publications...
  - Sarah does not have a specific hypothesis for isocyanates
    - But a range of analogues known – majority are inactive

- Conservative expert review is positive
A case study

- Selective 5HT2a inverse agonist
- Non-dopaminergic anti-psychotic for Parkinson sufferers
- Approved by the FDA in 2016 (Acadia Pharmaceuticals)
For larger sets of compounds, use batch mode...

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
<th>Endpoint</th>
<th>Species</th>
<th>Derek Result</th>
<th>Sarah Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="benzaldehyde" /></td>
<td>1 benzaldehyde</td>
<td>Mutagenicity in vitro</td>
<td>bacterium</td>
<td>INACTIVE</td>
<td>NEGATIVE - 100%</td>
</tr>
<tr>
<td><img src="image" alt="bBuBr" /></td>
<td>2 bBuBr</td>
<td>Mutagenicity in vitro</td>
<td>bacterium</td>
<td>PLAUSIBLE</td>
<td>NEGATIVE - 100%</td>
</tr>
<tr>
<td><img src="image" alt="phosgene" /></td>
<td>5 phosgene</td>
<td>Mutagenicity in vitro</td>
<td>bacterium</td>
<td>INACTIVE</td>
<td>OUTSIDE DOMAIN</td>
</tr>
<tr>
<td>* Contains unclassified features</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="isocyanate" /></td>
<td>6 isocyanate</td>
<td>Mutagenicity in vitro</td>
<td>bacterium</td>
<td>PLAUSIBLE</td>
<td>NEGATIVE - 36%</td>
</tr>
<tr>
<td><img src="image" alt="Pimavanserin" /></td>
<td>8 Pimavanserin</td>
<td>Mutagenicity in vitro</td>
<td>bacterium</td>
<td>INACTIVE</td>
<td>NEGATIVE - 35%</td>
</tr>
<tr>
<td><img src="image" alt="benzaldehyde-ether" /></td>
<td>3 benzaldehyde-ether</td>
<td>Mutagenicity in vitro</td>
<td>bacterium</td>
<td>INACTIVE</td>
<td>NEGATIVE - 24%</td>
</tr>
<tr>
<td><img src="image" alt="benzylamine-ether" /></td>
<td>4 benzylamine-ether</td>
<td>Mutagenicity in vitro</td>
<td>bacterium</td>
<td>INACTIVE</td>
<td>NEGATIVE - 52%</td>
</tr>
<tr>
<td><img src="image" alt="secondary benzylamine" /></td>
<td>7 secondary benzylamine</td>
<td>Mutagenicity in vitro</td>
<td>bacterium</td>
<td>INACTIVE</td>
<td>NEGATIVE - 30%</td>
</tr>
</tbody>
</table>
Can Mirabilis help assess the risk of the impurity surviving synthesis?

Pimavanserin

free base

hemi-tartrate

L-tartaric acid

Chemical reactions:
1. K$_2$CO$_3$, KI, DMF
2. H$_2$, Raney-Ni
3. Acetic acid
4. NaOH
Can Mirabilis help assess the risk of the impurity surviving synthesis?
1. Enter the route

1-chloro-4-nitrobenzene + 1,3-dibromo-2-propanol → 4-nitrocinnamic acid

K2CO3, KI, DMF
2. Confirm the reaction classes
2. Confirm the reaction classes
3. Assign the impurities to be tracked
3. Assign the impurities to be tracked
4. Establish purge factors
4. Establish purge factors

Predicted purge factor: 100  
User defined purge factor: 100

Comments:
Benzyl bromide was found to react with 10% sodium hydroxide in water, 21% sodium ethoxide in ethanol and 1,8-Diazabicyclo[5.4.0]undec-7-ene in acetonitrile when tested at various temperatures. No reaction was observed between benzyl bromide and aqueous sodium hydrogen carbonate when tested at various temperatures in water.
Mirabilis knowledge base supports expert assessment

“A consortium-driven framework to guide the implementation of ICH M7 Option 4 control strategies”  <manuscript in preparation>
5. Obtain total purge across full route

Table:

<table>
<thead>
<tr>
<th>Impurity</th>
<th>Stage</th>
<th>Reactivity</th>
<th>Solubility</th>
<th>Volatility</th>
<th>Other</th>
<th>Theoretical</th>
</tr>
</thead>
<tbody>
<tr>
<td>iButyl-Br</td>
<td>Stage 1</td>
<td>100</td>
<td></td>
<td>10</td>
<td></td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>Stage 2</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Stage 3</td>
<td>100</td>
<td>3</td>
<td></td>
<td>1</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>Stage 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stage 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1000</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td></td>
<td>3×10^8</td>
</tr>
</tbody>
</table>
6. Establish whether below threshold of concern.

M7 specifies acceptable limits of mutagenic impurities in final drug product

Are you below this?

Measured or assumed levels during synthesis
Purge Value from Mirabilis = Expected level in final API

“A consortium-driven framework to guide the implementation of ICH M7 Option 4 control strategies.” <manuscript in preparation>
### Options depend upon the predicted / measured levels

<table>
<thead>
<tr>
<th>Option</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Option 1</td>
<td>Analysis show below limit in API</td>
</tr>
<tr>
<td>Option 2</td>
<td>Analysis shows below limit in precursor / intermediate…</td>
</tr>
<tr>
<td>Option 3</td>
<td>Analysis shows above limit in precursor / intermediate - but with knowledge of purge can be assured that level in the API is below the acceptable limit without the need for any additional testing</td>
</tr>
<tr>
<td>Option 4</td>
<td>Prediction of purge with sufficient confidence to be below acceptable limit in API such that no analytical testing is recommended. [impurity not in specification]</td>
</tr>
</tbody>
</table>
Mirabilis is built on an approach accepted by regulators:

- Risk assessment of genotoxic impurities in new chemical entities: Strategies to demonstrate control. Teasdale..

  - Org. Process Res. Dev. 2010, 14, 943

- Evaluation and Control of Mutagenic Impurities in a Development Compound: Purge Factor Estimates vs Measured Amounts. Mclaughlin..
  - Org. Process Res. Dev. 2015, 19, 1531
Mirabilis was built through a pre-competitive collaboration

• Currently there is a consortium of 19 companies
  • Designed to fit agreed ‘best workflow’
  • Purge factor values
    • Initial values from expert elicitation
    • Literature review to support/evaluate
    • Wet chemistry undertaken to address knowledge gaps
  • Performance evaluation on-going
  • Suggested safety margins and levels of supporting information proposed
    • “A consortium-driven framework to guide the implementation of ICH M7 Option 4 control strategies” <manuscript in preparation>
  • Engagement with regulators on-going
## Conclusions

<table>
<thead>
<tr>
<th>Chemical Structure</th>
<th>Conclusion</th>
</tr>
</thead>
</table>
| ![Non-mutagen (Vitic) accepted](image) | - Non-mutagen (Vitic) accepted  
  - Class 5 non-mutagen |
| ![Non-standard (negative) Ames data discounted by expert](image) | - Non-standard (negative) Ames data discounted by expert  
  - Class 3 mutagen  
  - High purge ($3 \times 10^8$) predicted by Mirabilis M7 Option 4 (not specified in submission) |
| ![Derek contains unclassified : Sarah out of domain](image) | - Derek contains unclassified : Sarah out of domain  
  - Treated as Class 3 mutagen  
  - High purge ($1 \times 10^{11}$) predicted by Mirabilis M7 Option 4 (not specified in submission) |
| ![Derek positive : Sarah negative.](image) | - Derek positive : Sarah negative.  
  - Treated as Class 3 mutagen  
  - Low purge predicted by Mirabilis  
  - Evaluate Options 1 or 2 (test in API or precursor) |
An integrated solution to ICH M7
..developed through collaboration

ICH M7

Impurity identification

Mutagen?

In silico

Test

Carc-DB

Database search

Expert Assessment

Expert system

Statistical system

In silico

Expert Assessment

Purged?

Measure

In silico

Mirabilis

Classification

Control

Report
Questions