American College of Toxicology Presentation

Leadscope Submission Tool
Agenda

• Overview of ICH M7 *in silico* analysis and implementation  
  Glenn Myatt

• Case study perspective  
  Dan Benz

• Demonstration of the regulatory submission tool  
  Kevin Cross
Overview of ICH M7 *In Silico* Analysis and Implementation

Glenn Myatt
ICH M7 Background

- “Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk” (ICH, 2014)
- An *in silico* analysis may be able to predict the mutagenic potential of an impurity

<table>
<thead>
<tr>
<th>Class</th>
<th>Definition of the ICH M7 Hazard Classifications</th>
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<tbody>
<tr>
<td>1</td>
<td>Known mutagenic carcinogens</td>
</tr>
<tr>
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<td>Known mutagens with unknown carcinogenic potential</td>
</tr>
<tr>
<td>3</td>
<td>Alerting structure, unrelated to the structure of the drug substance; no mutagenicity data</td>
</tr>
<tr>
<td>4</td>
<td>Alerting structure, same alert in drug substance or compounds related to the drug substance (e.g., process intermediates) which have been tested and are non-mutagenic</td>
</tr>
<tr>
<td>5</td>
<td>No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity</td>
</tr>
</tbody>
</table>

ICH, 2014. ICH M7 – assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk. 
Assessing Available Data

• “...database and literature searches...” (ICH, 2014)
  - Bacterial mutagenicity databases (e.g. Leadscope SAR Genetox Database)
  - Rodent carcinogenicity databases (e.g. Leadscope SAR Carcinogenicity Database)

• The adequacy of any Ames data used in both the class 2 or class 5 assignments should be reviewed

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• Leadscope databases can be used to identify historical studies

  - Commercial genetic toxicity and rodent carcinogenicity databases from numerous sources (including US FDA CDER product approval reviews, FDA CFSAN, NTP, CCRIS, etc.), as well as ongoing data harvesting from the literature

  - The database currently includes genetic toxicity data for 11,028 compounds and 179,732 test results, and rodent carcinogenicity data for 3,598 compounds and 11,538 test results

• Results can be used to assess adequacy of tests performed
Generating an *In Silico* Prediction

- Two complementary methodologies:
  - “expert rule-based” (*e.g.*, Leadscope genetox experts alerts)
  - “statistical-based” (*e.g.*, Leadscope Statistical QSAR suite)
- “...should follow the general validation principles set forth by the Organisation for Economic Co-operation and Development (OECD).” (ICH, 2014)

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<td>5</td>
<td>No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity</td>
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</table>
In silico methodologies adhering to OECD principles should generate the following prediction results:

- Positive (predicted to be mutagenic)
- Negative (predicted to be non-mutagenic)
- Calls where the model is unable to generate a positive or negative prediction (e.g., inconclusive or out-of-domain)

Predictions from the two methodologies will need to be combined to generate an overall prediction.
“...additional supportive evidence on relevance of any positive, negative, conflicting or inconclusive prediction and provide a rationale to support the final conclusion.” (ICH, 2014)

“(1) maximize confidence in a (Q)SAR prediction, (2) provide rationale to supersede a positive or negative (Q)SAR prediction, or (3) provide a basis for assessing mutagenicity in absence of a (Q)SAR prediction.” (Powley, 2015)
Clear Negative

- High sensitivity from using two recommended *in silico* methodologies
- Negative from both *in silico* methodologies
- Visually inspection of the results may be performed
Positive Prediction

• Positive (mutagenic) prediction from any methodology is positive

• Possible to refute prediction based on expert opinion:
  ▪ ICH M7 Class 4 (shared alert with known negative)
  ▪ Mechanism
  ▪ Relevance of statistical-based model features
  ▪ Analogs
Inconclusive / Out-of-Domain

Expert Opinion

• Inconclusive/out-of-domain
  ▪ Visual inspection by expert
  ▪ Strength of single prediction
  ▪ Mechanism
  ▪ Chemical analogs

• Out-of-domain
  ▪ Addition of non-reactive group
• Materials and methods
• Summary of results and conclusions
• Supporting opinions
• Appendices
Upcoming Publication on ICH M7 In Silico Implementation

- **Title:**
  - Principles and procedures for implementation of ICH M7 recommended (Q)SAR analyses

- **Authors:**
  - Sanofi, Toxicology Solutions, Gilead, Leadscope, Roche, Bristol Myers Squibb, Pfizer, Genentech, Janssen, GlaxoSmithKline, Vertex, FDA Center for Drug Evaluation and Research, Novartis, Eli Lilly and Company, AbbVie, Merck, KRKA, Sanofi, AstraZeneca, Janssen, Bayer
• **Model Applier**
  - Robust
  - Options
  - Expertise
  - Research: Databases and QSAR Models
  - In-depth
  - Think

• **Regulatory Submissions ("Turbotox")**
  - Improved
  - Latest thinking
  - Buy-in
Case Study Perspective

Model Applier

Test Sets
- Import a test set
- Manage test sets

Predictions
- Review models / alerts
- Apply models / alerts
- Review prediction results
- Regulatory submissions
Case Study Perspective

ICH M7 Submission - ACT Case Studies - ICH M7 Submission (9)

Summary Spreadsheet

Steps:
Select test set
Select API structure
Select Impurities
Select in silico
Select submission name

Summary Spreadsheet
Assess structures
ACT Case Study 5 (API)
  ✓ ACT Case Study 1
  ✓ ACT Case Study 2
  ✓ ACT Case Study 3
  ✓ ACT Case Study 4
  ✓ ACT Case Study 5 (Impurity)
Generate Submission

API Structure
Negative in silico results have been initially assigned to class 5. Laboratory data may be added or an opinion created that agrees with or refutes the in silico results. Each positive/inconclusive result requires a refining opinion or limit definition.

Add API Data

ACT Case Study 5 (API)
Summary of impurity classifications (ICH M7 classes 1 to 5)

<table>
<thead>
<tr>
<th>#</th>
<th>Impurities</th>
<th>Laboratory Data</th>
<th>Expert rule-based</th>
<th>Matching Alerts</th>
<th>QSAR Salmonella</th>
<th>Salmonella Probability</th>
<th>QSAR E.coli/TA-102</th>
<th>E.coli/TA-102 Probability</th>
<th>In silico consensus assessment</th>
<th>M7 Class assignment</th>
<th>Additional supportive evidence and</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Available (not currently used)</td>
<td>Negative</td>
<td>No Alerts</td>
<td>Negative</td>
<td>0.0374</td>
<td>Negative</td>
<td>Negative</td>
<td>0.315</td>
<td>Negative</td>
<td>5</td>
<td>Accepted negative in silico result. The impurity lacks obvious reactive potential.</td>
</tr>
</tbody>
</table>

Select a single impurity for possible actions: Add Data | Create Opinion | Establish Limit/Control | Delete Impurity

Save | Save As... | Summary Spreadsheet | Generate Submission
## Case Study Perspective

<table>
<thead>
<tr>
<th>#</th>
<th>Inhibitor</th>
<th>Laboratory Data</th>
<th>Matching Alerts</th>
<th>QSAR</th>
<th>Salmonella</th>
<th>QSAR E.coli/TA102</th>
<th>E.coli/TA102</th>
<th>In silico consensus assessment</th>
<th>MT Class assignment</th>
<th>Additional supportive evidence and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>[Chemical Structure]</td>
<td>Available (not currently used)</td>
<td>Negative</td>
<td>No Alerts</td>
<td>Negative</td>
<td>0.0374</td>
<td>Negative</td>
<td>0.115</td>
<td>Negative</td>
<td>Accepted negative in silico result. The inhibitor lacks obvious reactive potential.</td>
</tr>
<tr>
<td>2</td>
<td>[Chemical Structure]</td>
<td>Available (not currently used)</td>
<td>Negative</td>
<td>No Alerts</td>
<td>Negative</td>
<td>0.18</td>
<td>Negative</td>
<td>0.0066</td>
<td>Negative</td>
<td>Accepted negative in silico result. The inhibitor lacks obvious reactive potential.</td>
</tr>
<tr>
<td>3</td>
<td>[Chemical Structure]</td>
<td>Available (not currently used)</td>
<td>Positive</td>
<td>Positive</td>
<td>0.639</td>
<td>Negative</td>
<td>0.0974</td>
<td>Positive</td>
<td>Accepted negative in silico result. The inhibitor lacks obvious reactive potential.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>[Chemical Structure]</td>
<td>Available (not currently used)</td>
<td>Negative</td>
<td>No Alerts</td>
<td>Negative</td>
<td>0.0002</td>
<td>0.0128</td>
<td>Negative</td>
<td>Accepted negative in silico result. Some ACT Case Study 5 (inhibitor) activity was observed.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>[Chemical Structure]</td>
<td>N/A</td>
<td>Indeterminate</td>
<td>Negative</td>
<td>0.136</td>
<td>Negative</td>
<td>0.0005</td>
<td>Negative</td>
<td>Accepted negative in silico result. See ACT Case Study 5 Appendix for details.</td>
<td></td>
</tr>
</tbody>
</table>
Case Study 1: Assess Available Data

### Summary Spreadsheet

**Steps:**
- Select test set
- Select API structure
- Select impurities
- Select in silico
- Select submission name

**Assess structures**
- ACT Case Study 5 (API) (API)
  - ACT Case Study 1
- ACT Case Study 2
- ACT Case Study 3
- ACT Case Study 4
- ACT Case Study 5 (impurity)

**Generate submission**

---

**Data is available, but you have not chosen to include it in the assessment.**

There are 3 studies from the Leadscope databases.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Escherichia coli (also known as 4191); Mutagenicity test with 4104 in the Salmonella/Aeromonas/mammalian/mutagenesis reverse mutation assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study type</td>
<td>bacterial mutagenesis</td>
</tr>
<tr>
<td>Source</td>
<td>ES</td>
</tr>
<tr>
<td>Species</td>
<td>Salmonella typhimurium (7), Escherichia coli (2)</td>
</tr>
<tr>
<td>Strains</td>
<td>TA1535, TA1537 (2), TA55 (2), TA100 (2), WP2uvrA (2)</td>
</tr>
<tr>
<td>Metabolic activation</td>
<td>Present (5); Absent (4)</td>
</tr>
<tr>
<td>Metabolic activation system</td>
<td>S9 Ret Liver Acrisol 1254 (5)</td>
</tr>
<tr>
<td>Test calls</td>
<td>Non mutagenic (9)</td>
</tr>
<tr>
<td>Study Report</td>
<td>Leadscope DB Study Report</td>
</tr>
</tbody>
</table>

Do you wish to use this study as part of the assessment?

- Yes
- No
## Case Study 1: Assess Available Data

![Chemical Structure](image1.png)

<table>
<thead>
<tr>
<th>Study call</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title</td>
<td>Ro 64-0796 (also known as 4101): Mutagenicity test with 4104 in the Salmonella-Escherichia/mammalian-microsome reverse mutation assay</td>
</tr>
<tr>
<td>Reference</td>
<td>Ro 64-0796 (also known as 4101): Mutagenicity test with 4104 in the Salmonella-Escherichia/mammalian-microsome reverse mutation assay</td>
</tr>
<tr>
<td>Study type</td>
<td>bacterial mutagenesis</td>
</tr>
<tr>
<td>Source</td>
<td>cdcr</td>
</tr>
<tr>
<td>Species</td>
<td>Salmonella typhimurium (7); Escherichia coli (2)</td>
</tr>
<tr>
<td>Strains</td>
<td>TA1535; TA1537 (2); TA98 (2); TA100 (2); WP2uvrA (2)</td>
</tr>
<tr>
<td>Metabolic activation</td>
<td>Present (5); Absent (4)</td>
</tr>
<tr>
<td>Metabolic activation system</td>
<td>S9 Rat Liver Acclor 1254 (5)</td>
</tr>
<tr>
<td>Test calls</td>
<td>Non mutagenic (9)</td>
</tr>
</tbody>
</table>
Case Study 2: Clearly Negative

Steps:
- Select test set
- Select API structure
- Select impurities
- Select in silico
- Select submission name
- Summary Spreadsheet

Assess structures
  - ACT Case Study 2
    - Assess available data
      - Assess in silico results
        - Review in silico results
        - Summarize conclusions

Generate submission

The results from the in silico analysis for ACT Case Study 2 are:

- **Export Alert: Bacterial Mutation**
  - **Prediction methodology:** Genetic Toxicity Bacterial Mutation Alerts v2 (System: Leadscope Model Applier v2.0.4.1)
  - **Prediction results:** Negative
  - **Laboratory data:** Negative
  - **Alerts fired:** No Alerts
  - **Alerts precision:** 0.1666

- **QSAR: Salmonella**
  - **Prediction methodology:** Genetic Toxicity Salmonella Model v3 (System: Leadscope Model Applier v2.0.4.1)
  - **Prediction results:** Negative
  - **Prediction probability:** 0.18

- **QSAR: E.coli/TA102**
  - **Prediction methodology:** Genetic Toxicity E-Coli/A-T Model v1 (System: Leadscope Model Applier v2.0.4.1)
  - **Prediction results:** Negative
  - **Prediction probability:** 0.0096
  - **Laboratory data:** Negative

ACT Case Study 2

Additional expert knowledge can be used to support or even refute the negative in silico results. Do you wish to provide such an opinion?

- Yes
- No (the impurity lacks obvious reactive potential)

Comments:
Case Study 2: Clearly Negative

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Impurities</th>
<th>Expert rule-based</th>
<th>QSAR Salmonella</th>
<th>QSAR E.coli/TA102</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Chemical Structure" /></td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

2015 ACT Case Study 2
Case Study 3: Clearly Positive

Summary Spreadsheet

Steps:
- Select test set
- Select API structure
- Select Impurities
- Select in silico
- Select submission name

Summary Spreadsheet

Assess structures
- ACT Case Study 5 (API) (API)
  - ACT Case Study 1
  - ACT Case Study 2
  - ACT Case Study 3
- Assess available data
- Assess in silico results
- Establish limit/control
- Summarize conclusions

- ACT Case Study 4
- ACT Case Study 5 (impurity)

Generate submission

The following summarizes the conclusions on ACT Case Study 3

- Laboratory data: Available (not currently used)
- Expert rule-based: Positive
- QSAR Salmonella: Positive
- QSAR E.coliTA102: Negative
- In silico consensus assessment: Positive

ICH M7 category: Class 3 - Alarming structure, unrelated to the structure of the drug substance; no mutagenicity data

Analysis summary:
- Accepted positive in silico result.
- Matching alerts: 207, aromatic amine (N=H2) (strong activating anilines) (0.01)

Exposure limit/control summary:

Comments:
# Case Study 3: Clearly Positive

![Chemical Structure](image.png)

<table>
<thead>
<tr>
<th>Impurities</th>
<th>Expert rule-based</th>
<th>QSAR Salmonella</th>
<th>QSAR E.coli/TA102</th>
<th>Additional supportive evidence and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image.png" alt="Chemical Structure" /></td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td><strong>Accepted positive <em>in silico</em> result.</strong> Matching alerts: 267: aromatic amine(NH₂) (strong activating anilines) (0.91)</td>
</tr>
</tbody>
</table>

2015 ACT Case Study 3
Case Study 4: Handling Out-of-Domains

The results from the in silico analysis for ACT Case Study 4 are:

**Expert Alerts: Bacterial Mutation**  
Prediction methodology: Genetic Toxicity Bacterial Mutation Alerts v2 (System: Leadscope Model Applier v2:0.5.2)  
Prediction results: Negative  
Laboratory data: Negative  
Alerts fired: No Alerts  
Alerts precision: 0.1616

**QSAR: Salmonella**  
Prediction methodology: Genetic Toxicity Salmonella Model v3 (System: Leadscope Model Applier v2:0.5.2)  
Prediction results: Negative  
Prediction probability: 0.0002

**QSAR: E.coli/TA102**  
Prediction methodology: Genetic Toxicity E-Coli/TA102 Model v1 (System: Leadscope Model Applier v2:0.5.2)  
Prediction results: Out of Domain  
Prediction probability: 0.0130
## Case Study 4: Handling Out-of-Domains

<table>
<thead>
<tr>
<th>Impurities</th>
<th>Expert rule-based</th>
<th>QSAR Salmonella</th>
<th>QSAR E.coli/TA102</th>
<th>Additional supportive evidence and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Impurity" /></td>
<td>Negative</td>
<td>Negative</td>
<td>Out of Domain</td>
<td><strong>Accepted negative <em>in silico</em> result. The impurity lacks obvious reactive potential.</strong></td>
</tr>
</tbody>
</table>

2015 ACT Case Study 4
Case Study 5: Handling Indeterminates

Steps:
- Select test set
- Select API structure
- Select Impurities
- Select in silico
- Select submission name

Summary Spreadsheet

Assess structures
✓ ACT Case Study 1
✓ ACT Case Study 2
✓ ACT Case Study 3
✓ ACT Case Study 4
✓ ACT Case Study 5

ACT Case Study 5 (Impurity)
- Assess available data
- Assess in silico results
- Review in silico results
- Opinion regarding negative in silico result
- Summarize conclusions

Generate submission

The results from the in silico analysis for ACT Case Study 5 (impurity) are:

Expert Alerts: Bacterial Mutation
- Prediction methodology: Genetic Toxicity Bacterial Mutation Alerts v2 (System: Leadscope Model Applier v2.0.4.1)
- Prediction results: Indeterminate
  - Alerts: A323 (benzene, 1-hydroxy, 2-nitro and benzene, 1-hydroxy, 4-nitro) (Indeterminate)
  - Alerts precision: 0.5835

QSAR: Salmonella
- Prediction methodology: Genetic Toxicity Salmonella Model v3 (System: Leadscope Model Applier v2.0.4.1)
- Prediction results: Negative
  - Prediction probability: 0.305

ACT Case Study 5 (impurity)

QSAR: E.coli/TA102
- Prediction methodology: Genetic Toxicity E.coli/TA1 Model v1 (System: Leadscope Model Applier v2.8.4.1)
- Prediction results: Out of Domain
  - Prediction probability: 0.005

Additional expert knowledge can be used to support or even refute the negative in silico results. Do you wish to provide such an opinion?
- Yes
- No (the impurity lacks obvious reactive potential)

Comments: API has the same alert in the same environment. The API was shown to be negative in laboratory testing.
## Case Study 5: Handling Indeterminates

### 2015 ACT Case Study 5 (impurity)

<table>
<thead>
<tr>
<th>Impurities</th>
<th>Expert rule-based</th>
<th>QSAR Salmonella</th>
<th>QSAR E.coli/TA102</th>
<th>Additional supportive evidence and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Impurity Structure" /></td>
<td>Indeterminate</td>
<td>Negative</td>
<td>Out of Domain</td>
<td>Accepted negative <em>in silico</em> result. See '2015 ACT Case Study 5 (impurity) Opinion'</td>
</tr>
</tbody>
</table>
Case Study 5: Opinion
Case Study 5: Opinion

- Alerting structure is fully contained in the same environment in the API which tested negative in the laboratory: class 4 opinion
Summary: Regulatory Submission

ACT Presentation

Sponsor organization:
Prepared By: Dan Benz
Organization that prepared report:
Expert opinion provided by: OmnyCorp
Version: Dan Benz

Date: November 01, 2015
Summary: Regulatory Submission

Materials and methods
An assessment aligned with ICH Q2(R1) was performed on the actual and potential impurities listed in the table below using the following QSAR methodologies and systems:

| Expert rule-based methodology and parameters: | Leadscope genetox expert alerts v2 (System: Leadscope Model Applier v2.0.0.2); the domain assessment was turned on |
| Statistical-based methodology and parameters: | Leadscope Salmonella statistical-based QSAR model v3, Leadscope E. coli TA102 statistical-based QSAR model v1 (System: Leadscope Model Applier v2.0.0.2); probabilities above 0.8 set to positive; probabilities below 0.4 set to negative and domain assessment was turned on |
| Genetic toxicity database used for searching: | Leadscope SAR genetox 2015 |
| Mutagenicity database used for searching: | Leadscope SAR carcinogenicity 2015 |

These in silico methodologies follow the general validation principles set forth by the Organisation for Economic Co-operation and Development (OECD). The Leadscope statistical-based methodologies and the Leadscope SAR databases were developed through a research collaboration agreement with the US FDA.

Results
The following table and notes summarize the results of this QSAR analysis for 5 impurities. Structural alerts and significant model features are highlighted. Alerts and positive model features in red, negative model features in blue-green, and indeterminate in gray.

<table>
<thead>
<tr>
<th>#</th>
<th>Impurity Structure</th>
<th>Laboratory Data</th>
<th>Expert rule-based system</th>
<th>Statistical-based system</th>
<th>Overall assessment</th>
<th>MT Class assignment</th>
<th>Additional supportive evidence and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="https://example.com/impurity1.png" alt="Image" /></td>
<td>None</td>
<td>Negative</td>
<td>Negative</td>
<td>Non-mutagenic</td>
<td>5</td>
<td>Accepted negative in silico result. The impurity lacks obvious reactive potential.</td>
</tr>
<tr>
<td>2</td>
<td><img src="https://example.com/impurity2.png" alt="Image" /></td>
<td>None</td>
<td>Positive</td>
<td>Positive</td>
<td>Predicted Mutagenic</td>
<td>3</td>
<td>Accepted positive in silico result. Matching alerts: 207; aromatic amine (NH)2 (strong activating amine) (0.31)</td>
</tr>
<tr>
<td>#</td>
<td>Impurity Structure</td>
<td>Laboratory Data</td>
<td>Expected non-specific signal</td>
<td>Statistical-based system</td>
<td>Overall assessment</td>
<td>MF Class assignment</td>
<td>Additional supportive evidence and comments</td>
</tr>
<tr>
<td>----</td>
<td>-------------------</td>
<td>----------------</td>
<td>-----------------------------</td>
<td>--------------------------</td>
<td>------------------</td>
<td>---------------------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>2</td>
<td><img src="image1.png" alt="Impurity 1" /></td>
<td>None</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Non-mutagenic</td>
<td>Absorbed negative in plasmid result. The impurity does not cause a toxicity potential.</td>
</tr>
<tr>
<td>4</td>
<td><img src="image2.png" alt="Impurity 2" /></td>
<td>None</td>
<td>Intermediates</td>
<td>Negative</td>
<td>Negative</td>
<td>Non-mutagenic</td>
<td>Absorbed negative in plasmid result. The impurity does not cause a toxicity potential.</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3.png" alt="Impurity 3" /></td>
<td>None</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Non-mutagenic</td>
<td>Absorbed negative in plasmid result. The impurity does not cause a toxicity potential.</td>
</tr>
</tbody>
</table>

2015 ACT Case Study 5 (Impurity) #5 - Opinion supporting negative result - evaluation of suitable analogs.

2015 ACT Case Study 6 (Impurity) 2015 ACT Case Study 6 (Impurity)
Demonstration of the Regulatory Submission Tool

Kevin Cross