Toxicological Qualification of Impurities

Klaus Olejniczak,
Non-clinical Regulatory Consultant,
Berlin
klausolejniczak@yahoo.de
Assessment of Impurities

- Relevant Information
- Impurities in new substances and marketed substances
- Genotoxic Impurities
IMPURITIES  Relevant Information

• Common Technical Document Nonclinical Overview
• ICH Guideline Q3A Impurities in New Drug Substances
• ICH Guideline Q3B Impurities in New Drug Products
• ICH Guideline Q3C Impurities: Residual Solvents
• ICH Guideline Q3D: Residues of Metals (In Development)
• ICH Guideline M7: Limits for Genotoxic Impurities (In Development)
• EU Guideline: Limits for Genotoxic Impurities
An assessment of the impurities and degradants present in the drug substance and product should be included along with what is known of their potential pharmacologic and toxicologic effects.

This assessment should form part of the justification for proposed impurity limits in the drug substance and product, and be appropriately cross-referenced to the quality documentation.
Consider the proposed impurity limits in relation to:

- toxicology of the impurity in relation to the active substance
- route of administration
- daily dose
- target population
- duration of therapy
- proposed indication
"An important point to remember is that the test material in toxicology tests should optimally be less pure than that to be used in the clinic: the toxicologists should be asking for a supply of characterised bulk medicinal product, taken from the manufacturing process before its final recrystallisation."

The Regulatory Affairs Journal, May 1996
### Example

**2.3.7.4 Toxicology**

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Purity (%)</th>
<th>Specified Impurities</th>
<th>Test Article:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>PROPOSED</td>
<td>&gt;95</td>
<td>≤0.1</td>
<td>≤0.2</td>
</tr>
</tbody>
</table>

**SPECIFICATION:**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>LN125</td>
<td>98.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>94007</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>94008</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96718</td>
</tr>
<tr>
<td>94NA103</td>
<td>99.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96046</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96050</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>94214</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>97634</td>
</tr>
<tr>
<td>95NA215</td>
<td>97.3</td>
<td>0.1</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96047</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96037</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>94211</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>97028</td>
</tr>
<tr>
<td>95NB003</td>
<td>94.6</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>94019</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>97012</td>
</tr>
<tr>
<td>96NB101</td>
<td>99.0</td>
<td>0.4</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>94018</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95012</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95013</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95013</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96208</td>
</tr>
<tr>
<td>Batch No.</td>
<td>Purity (%)</td>
<td>Specified Impurities</td>
<td>Study Number</td>
<td>Type of Study</td>
</tr>
<tr>
<td>-----------</td>
<td>------------</td>
<td>----------------------</td>
<td>--------------</td>
<td>---------------</td>
</tr>
<tr>
<td>95NB003</td>
<td>94.6</td>
<td>0.2</td>
<td>0.3</td>
<td>94019</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>97012</td>
</tr>
<tr>
<td>96NB101</td>
<td>99.0</td>
<td>0.4</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95013</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96208</td>
</tr>
</tbody>
</table>

PROPOSED SPECIFICATION: Justified?
<table>
<thead>
<tr>
<th>Batch</th>
<th>Identified Impurity (%)</th>
<th>Field of Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 0,01</td>
<td>Mutagenicity/</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carcinogenicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rat</td>
</tr>
<tr>
<td>2</td>
<td>0,2</td>
<td>Carcinogenicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mouse</td>
</tr>
<tr>
<td>3</td>
<td>0,5</td>
<td>Humans</td>
</tr>
</tbody>
</table>
ICH Q3 A

Safety assessment studies to qualify an impurity should compare the drug substance containing a representative amounts of the new impurity with previously qualified material.

Safety assessment studies using a sample of the isolated impurity can also be considered.
**Illustration of Reporting Impurity Results for Identification and Qualification in an Application (Attachment 2 / ICH Q3A Guideline)**

<table>
<thead>
<tr>
<th>'Raw' Results (%)</th>
<th>Reported Results (%)</th>
<th>Action</th>
<th>Identification (Threshold 0.10%)</th>
<th>Qualification (Threshold 0.15%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.066</td>
<td>0.07</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>0.0963</td>
<td>0.10</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>0.12</td>
<td>0.12</td>
<td>Yes</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>0.1649</td>
<td>0.16</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Example: Maximum Daily Dose (Drug Substance) < 2g
Attachment 3: Decision Tree for Identification and Qualification

1. Is impurity greater than identification threshold?
   - Yes
     - Structure identified?
       - Yes
         - Reduce to not more than (≤) identification threshold?
           - Yes
             - No further action
           - No
             - Consider patient population and duration of use and consider conducting:
               - Genotoxicity studies (point mutation, chromosomal aberration)
               - General toxicity studies (one species, usually 14 to 90 days)
               - Other specific toxicity endpoints, as appropriate
             - Reduce to safe level
             - Any clinically relevant adverse effects?
               - Yes
                 - Qualified
               - No
                 - No action
           - Greater than qualification threshold?
             - Yes
               - Reduce to safe level
             - No
               - No action
       - No
         - No action
   - No
     - Any known human relevant risk?
       - Yes
         - Reduce to safe level
       - No
         - No action
Consider need for:

1. Genotoxicity studies (point mutation, chromosomal aberration)
2. General toxicity studies (one species, min. 14 days, max. 90 days)
3. Other specific toxicity endpoints, as appropriate
• Q3A: „Acceptance criteria should be set no higher than the level that can be justified by safety data, and should be consistent with the level achievable by the manufacturing process and the analytical capability.“

• It is not indicated which levels of genotoxic impurities can be justified by safety data
The guideline does not need to be applied retrospectively to authorised products unless there is a specific cause for concern. What might constitute "a cause-for-concern" in terms of application to currently marketed products?

If a manufacturing procedure for API remains essentially unchanged a re-evaluation with respect to the presence of potentially genotoxic impurities is generally not needed. However, new knowledge may indicate a previously unknown cause for concern.
ICH Q3 A

Safety assessment studies to qualify an impurity should compare the new drug substance containing a representative amounts of the new impurity with previously qualified material.

Safety assessment studies using a sample of the isolated impurity can also be considered for genotoxic impurity?
Power of testing for detection of genotoxic impurities is limited

Examples:
Ethyl methane sulfonate (EMS)
Ames test max. concentration: 5000 µg/plate
LOEC for EMS: 1500 µg/plate,

Consequences to detect a genotoxic effect
30% EMS in drug substance

Standard genotoxicity testing of drug substance very unlikely to detect genotoxic impurities when content is < 1500 ppm (0.15%)
LOEC: Lowest Observed Effect Concentration
Regulators Recommendation

Genotoxicity studies using a sample of the isolated impurity **must** be considered
Structure-Activity-Relationship (SAR)

• TOX expert: generic rule-based decision approach DEREK
• CHEM/PHYS expert: topology-based structural descriptors (charge, electron density, etc.), QSAR oriented
  TOPKAT, QSARIS, TOXSYS, TOXSCOPE
• CHEM/TOX expert: all possible substructures associated with various toxicities, includes influence of “deactivating” structures
  ➔ MULTICASE (used by the FDA!)
DEREK (Deductive Estimation of Risk from Existing Knowledge) marketed by LHASA at University of Leeds
Salmonella Mutagenicity for BENZO(A)PYRENE:
This molecule appears to be the same as 976 of activity 39.00 entered under the name :Benzo(a)pyrene

Experimentally, the molecule is found to be inactive

The molecule contains the Biophore (number of copies = 1):

```
C." -CH
/       \
CH =C.    CH
\      /
CH =CH
```

33 out of the known 40 molecules (82%) containing such Biophore are SALMONELLA MUTAGENS with an average activity of 33 (c.l.=100%)

Constant is 35.8

The molecule also contains the Biophore:

```
C. =CH -CH =CH -C. =CH -CH =C. -
```

The probability that this molecule is a SALMONELLA MUTAGEN is 81.0% increased to 90.0% due to the presence of the extra Biophore

The projected SALMONELLA MUTAGENIC activity is 36.0 CASE units
Genotoxic Impurities
Threshold or Non-threshold

„On the other hand“

Practical: Threshold exist
Exposure from drugs is neglectable in comparison to exposure from enviroment, food etc.

Theoretical: Non-threshold
From a regulatory point of view: All unnecessary risks should be avoided
Genotoxic compound with evidence of threshold

**Permitted Daily Exposure (PDE) Calculation**

- Interaction with spindel apparatus
- Topoisomerase inhibition
- Inhibition of DNA synthesis
- Overloading of defense mechanisms
- Metabolic overload
- Induction of erythropoeisis
- Hyper-hypothermia
Permitted Daily Exposure (PDE) Calculation

\[
PDE \text{ (mg/day)} = \frac{\text{NOEL or LOEL (mg / kg)} \times \text{Weight adjustment (50 kg)}}{\text{Modifying factors: } F_1 \times F_2 \times F_3 \times F_4 \times F_5}
\]

F1: Interspecies differences (surface area : body weight ratio for man compared to testing species; rat = 5, mouse = 12)

F2: Inter-individual differences (10)

F3: Duration of exposure (1-10)

F4: Nature of toxicity, for a threshold genotoxic comp. >10??

F5: Quality of data (1-10)
Genotoxic compound without evidence of threshold

• In general, pharmaceutical measurements should be guided by a policy of controlling levels to “as low as reasonably practicable” (ALARP principle), where avoiding is not possible.
Genotoxic compound without evidence of threshold

If the level of a mutagenic impurity is below the threshold of toxicological concern (equivalent to a clinical dose ≤1.5 μg/day) it is not necessary to apply ALARP considerations unless it is a structure of very high concern, e.g. N-nitroso, aflatoxins-like and azoxy-compounds.
Genotoxic compound **without** evidence of threshold

- ALARP principle
  Residual Ethylen Oxide should not exceed a limit of 1ppm. This limit is based on the current limit of detection
Genotoxic compound **without** evidence of threshold

**Toxicological Assessment**

- Procedures for the derivation of acceptable risk levels are considered in the Appendix 3 of the Q3C Note for Guidance on Impurities: Residual Solvents for Class 1 solvents.

- However, these approaches require availability of adequate **data from long-term carcinogenicity studies.**
Genotoxic carcinogens

Example:Benzene (Solvent)

• From the data of human leukemia and exposure concentrations of benzene, it was calculated that a daily intake of 0.02 mg was associated with a lifetime excess cancer risk of $10^{-5}$ (Integrated Risk Information System (IRIS), US EPA 1990)
Genotoxic compound **without** evidence of threshold

Toxicological Assessment

Application of a **Threshold of Toxicological Concern**

- The **TTC**, originally developed as a “threshold of regulation” at the FDA for food-contact materials (Rulis 1989, FDA 1995) was established based on the analysis of 343 carcinogens from a carcinogenic potency database (Gold et al. 1984) and was repeatedly confirmed by evaluations expanding the database to more than 700 carcinogens (Munro 1990, Cheeseman et al. 1999, Kroes et al. 2004)
Genotoxic compound **without** evidence of threshold

Toxicological Assessment
Application of a Threshold of Toxicological Concern

- Analysis of high potency carcinogens led to the suggestion a **TTC of 0.15 µg/day** are needed for chemicals with structural alerts that raise concern for potential genotoxicity (Kroes et al. 2004).
- For application of a TTC in the assessment of acceptable limits of genotoxic impurities in drug substances a value of **1.5 µg/day**, corresponding to a 10-5 lifetime risk of cancer can be justified as for pharmaceuticals a **benefit** exists
Daily intake of genotoxic impurity <1.5 µg
No safety concern!

1.5 µg/day predicted of not exceeding $10^{-5}$ cancer lifetime risk
TTC translated into ppm
impurity in drug

Limit of Impurity \[\text{ppm}\] = \[
\frac{1.5 \mu g}{\text{Daily Dose of Drug [g]}}\]

Analytical control:
- readily achievable
- technically challenging
- impractical?
• TTC not accepted for structural groups with high potency of carcinogenic risk
  • Aflatoxin-like compounds
  • N-nitroso-like compounds
  • Azoxy-like compounds
TTC

• TTC value higher than 1.5 microgram/day may be accepted?
  • Short-term use
  • Patient population very small
  • Life-threatening condition (safer alternatives not available)
  • Human exposure from other sources (e.g. food) much greater
Guidance for Industry
Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches

DRAFT GUIDANCE

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
December 2008
Pharmacology and Toxicology
Guidance for Industry
## Staged TTC

### EU: Duration of exposure

<table>
<thead>
<tr>
<th>Allowable daily intake (µg/day)</th>
<th>Single dose</th>
<th>&lt; 1 months</th>
<th>&lt; 3 months</th>
<th>&lt; 6 months</th>
<th>&lt; 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>60</td>
<td>20</td>
<td>10</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

### FDA: Duration of clinical trial exposure

<table>
<thead>
<tr>
<th>Genotoxic and carcinogenic impurity threshold (µg/day)</th>
<th>&lt; 14 days</th>
<th>14 days to 1 mo</th>
<th>1 mo to 3 mos</th>
<th>3 mos to 6 mos</th>
<th>6 mos to 12 mos</th>
<th>&gt; 12 mos</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>60</td>
<td>20</td>
<td>10</td>
<td>5</td>
<td>1.5</td>
<td></td>
</tr>
</tbody>
</table>
### A few typical daily exposures to carcinogens

<table>
<thead>
<tr>
<th>Source of carcinogen</th>
<th>Carcinogen</th>
<th>Average daily human exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indoor air</td>
<td>Formaldehyde</td>
<td>598 µg</td>
</tr>
<tr>
<td></td>
<td>Benzene</td>
<td>155 µg</td>
</tr>
<tr>
<td>Tap water</td>
<td>Bromodichloromethane</td>
<td>13 µg</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>17 µg</td>
</tr>
<tr>
<td>Celery</td>
<td>8-methoxy psoralen</td>
<td>4.9 µg</td>
</tr>
<tr>
<td>Coffee</td>
<td>Catechol</td>
<td>1.3 mg</td>
</tr>
<tr>
<td></td>
<td>Hydroquinone</td>
<td>333 µg</td>
</tr>
<tr>
<td></td>
<td>Caffeic acid</td>
<td>23.9 mg</td>
</tr>
<tr>
<td>Lettuce</td>
<td>Caffeic acid</td>
<td>7.9 mg</td>
</tr>
<tr>
<td>Brown mustard</td>
<td>Allyl isothiocyanate</td>
<td>62.9 µg</td>
</tr>
</tbody>
</table>

David Jacobson-Kram, FDA
Genotoxic Impurities
Example

\[
\begin{align*}
\text{H}_2\text{C} & \quad \text{N} = \text{H} \\
\text{H}_2\text{C} & \quad \text{H}_2\text{C}
\end{align*}
\]

Aziridine
Aziridine

• This can arise from:
  – Use of alkylating agents in the synthesis
  – Use of aziridine / substitute aziridine in the synthesis
  – Use of polyethylenimine as flocculant
  – Degradation of Triazolines
Δ²-1,2,3-Triazolines

2-Aminoalkyldiazenium Ion

CHEMICAL DEGRADATION OF TRIAZOLINES

Aziridines
Are unidentified impurities < 0.1 % (0.10%) safe?

<table>
<thead>
<tr>
<th>DRUG</th>
<th>IMPURITY</th>
<th>ADVERSE REACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug xy</td>
<td>Dimethyl sulphide *</td>
<td>Garlic taste/odour</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>&quot;peak E&quot; ? *</td>
<td>Eosinophilia-Myalgia syndrome (EMS)</td>
</tr>
</tbody>
</table>

* << 0.1%

Tryptophan: More than 60 minor impurities were identified in EMS-associated batches. The specific impurity responsible for the toxic effects was never established.
Acknowledgement

Lutz Müller, Roche

Peter Kasper, BfArM
THANK YOU