N-Nitrosamines Status / Implications

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**Background**

Initial cause - change in synthesis around Tetrazole functionality in 'pseudo-generic' Valsartan

- Change notified in 2012
- July 2018 Valsartan recalled by Bfarm-EMA, FDA (NDMA)
- August 2018 Valsartan, Losartan, Irbesartan- (NDEA)
- EMA Article 31 triggered July 2018, extended in August – 'Nitrosamine free synthesis (30ppb LOQ). Finalised April 2019 - phased in over 2 years
- FDA 'non-detected' - currently at c.5ppb, SwissMedic 30ppb all Bx from May
- Pharm Eur monograph adopted for Nitrosamine testing in 5 Sartans- released in July 2019
- Health Canada mandates NDMA, NDEA, NMBA NDIPA and NDEIPA- co-ordinate with FDA and EMA July 2019

<table>
<thead>
<tr>
<th>R₁</th>
<th>R₂</th>
<th>Compound</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me</td>
<td>Me</td>
<td>NDMA</td>
<td>Di-methylamine from DMF</td>
</tr>
<tr>
<td>Et</td>
<td>Et</td>
<td>NDEA</td>
<td>Di-ethyl amine from TEA or DMF</td>
</tr>
<tr>
<td>IPA</td>
<td>IPA</td>
<td>NDIPA</td>
<td>Di-isopropyl amine from DIPEA</td>
</tr>
<tr>
<td>Et</td>
<td>IPA</td>
<td>NDEIPA</td>
<td>Ethyl-isopropyl amine from DIPEA</td>
</tr>
<tr>
<td>Me</td>
<td>O</td>
<td>NMBA</td>
<td>4 -(methylamino) Butanoic acid from NMP</td>
</tr>
</tbody>
</table>

- Me: methyl
- Et: ethyl
- IPA: isopropyl
- O: oxygen

**Chemical Structures**

![Chemical Structures](image-url)
Background – Scale of Impact

The AT II antagonists (Sartans) group is part of the “WHO Model List of Essential Medicines”

4 key impacted manufacturers, ZHP, ZT (China), Mylan, Hetero and Aurobindo (India) - supply many MAH

Global recall – >1100 batches impacted (‘61% of German Valsartan tested had detectable Nitrosamines’)

Initial cause- related to change in synthesis of Tetrazole functionality in ’pseudo-generics

\[
\text{Candesartan Cilexetil}
\]

\[
\text{HO} \quad \text{N} \quad \text{N} \quad \text{O} \quad \text{CH}_3 \quad \text{N} \quad \text{N} \quad \text{N} \quad \text{NH} \quad \text{OOH}
\]

\[
\text{Candesartan Cilexetil}
\]
Valsartan Recall – Key Points

• Issue arose due to a change in the manufacturing process
  • The exact change was not initially reported.

• However the issue arose during the manufacture of the tetrazole ring
  • Usually manufactured using an azide + nitrile
    • e.g. tributyl tin azide + R-CN
Modified Synthesis of Valsartan
(used by Zhejian Huahai Pharma Co)

Novartis originally used tributyltin azide to form the key tetrazole in valsartan (top), while a 2012 route from ZHP used sodium azide instead (bottom).
N-Nitrosodimethylamine (NDMA)

CYP2E1

\[
\begin{align*}
\text{H}_3\text{C} & - \text{N} - \text{CH}_3 \quad \text{NDMA} \\
\text{NO} & \rightarrow \text{oxidation to intermediate methyl radical} \\
& \text{and } \alpha\text{-hydroxylation} \\
\text{H}_3\text{C} & - \text{N} - \text{CH}_2\text{-OH} \quad \alpha\text{-hydroxy-NDMA} \\
\text{NO} & \rightarrow \text{HCHO} \\
\text{H}_3\text{C} & - \text{N} = \text{N} - \text{OH} \quad \text{Diazohydroxid} \\
\text{H}_3\text{C} & - \text{N} = \text{N}^* \quad \text{Methyldiazoniumion}
\end{align*}
\]

<table>
<thead>
<tr>
<th>Species</th>
<th>TD50 (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>0.0959\text{m,v}</td>
</tr>
<tr>
<td>Mouse</td>
<td>0.189\text{m}</td>
</tr>
</tbody>
</table>
Valsartan Recall – Key Points

- To generate NDMA requires the presence of Dimethylamine + Nitrite

  Where do they come from?

  - Zhjiang Patent – NaNO₂ used in process

- A third factor is suitable conditions e.g. acid to generate Nitrosyl cation etc
Valsartan Recall – Key Points

Preliminary Investigation – Establish the Root Cause

- To generate NDMA: requires the presence of **Dimethylamine** + Nitrite *where do they come from?*

- DMF from the azide step may contain **dimethylamine** which carries into the NaNO₂ step.

- Disproportionation of DMF to dimethylamine and CO is known to be catalysed by acids and bases so the ZnCl₂ may also lead to dimethylamine under the conditions over the 13 hours at 80°C.

Why use NaNO₂ ?

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**Chemical reactions** [edit]

In the laboratory, sodium nitrite can be used to destroy excess sodium azide.[39][40]

\[
2 \text{NaN}_3 + 2 \text{NaNO}_2 + 4 \text{H}^+ \rightarrow 3 \text{N}_2 + 2 \text{NO} + 4 \text{Na}^+ + 2 \text{H}_2\text{O}
\]
Valsartan recall – Key Points

• The potential formation of N-Nitrosamines is understood

• The actual nitrosation reagent is the nitrosyl cation, NO⁺ which is formed in situ:

• The nature of the product depends on the nature of the initial amine
• Primary alkyl or aryl amines yield diazonium salts (hence the diazotisation reaction)

• Alkyl diazonium salts are very unstable and yield carbocation-derived products by loss of the very good leaving group, N₂:

• Secondary alkyl or aryl amines yield N-nitrosoamines:
Not all processes – even to sartans’ are equally at risk
Example Risk assessment

• The following slides represent Candesartan
• Although it contains the same tetrazole ring as Valsartan, the synthesis is **very different:**
  • DMF not used in tetrazole stage
  • Tetrazole stage **multiple stages** from API
• Risk Assessment followed the principles of M7.
  • Identified compounds of concern (Nitrite, secondary / tertiary amines)
    • **Purge calculations**
    • Control strategy
Candesartan Cilexetil

- The candesartan cilexetil manufacturing process consists of a total of 9 steps.

- The tetrazole ring is formed as an intermediate in Step 5 (MET), which is 4 steps away from candesartan cilexetil.

WHAT IF ANY RISK EXISTS?

- In step 2 dimethyl formamide and triethylamine are employed in the process.

- These materials offer a potential source of secondary amines.

- The theoretical maximum level of any potential source of secondary amines in these materials is typically controlled by specification for any individual unspecified impurity, 0.2% maximum.

- Between step 2 in which dimethylformamide and triethylamine are employed, and the tetrazole ring formation step (MET) there are 3 steps.

- In these 3 steps there are multiple processing operations all of which will reduce levels of any secondary amine or potential source of secondary amines.
The principle of relating the physico-chemical properties of the mutagenic impurity to the chemical process is defined in the concept of purge factor calculations.
ICH M7 Option 4 – Control Options

• Section 8 – Control
• Option 4
  – So reactive – no testing required

• Option 3
  – Test at intermediate stage with a higher limit + understanding of process capacity.

• Option 2
• Test for the impurity in the specification for a raw material, starting material or intermediate at permitted level

• Option 1
• Test for the impurity in the drug substance

These options can be assessed using Mirabilis (purge calculations)
Purge Calculations

• Original principle of concept now developed into an in silico tool
• Developed by an industry consortium (over 20 companies) with Lhasa over a 3 year period this provides a number of advantages over the paper based approach:
  • Systematic layout – consistent approach
  • Knowledge base linked to Impurity and reaction recognition
    • Access to detailed information that makes / assists predictions
  • Reporting tools.
Mirabilis – general process / principles
Goal: establish framework to leverage purge predictions to inform selection of control strategy during development, which in turn informs both data collection and regulatory reporting recommendations
Mirabilis (P)MI Purge Prediction Decision Tree

Key premise: purge excess dictates data collection needs and regulatory reporting practices

- Impurity requires management as (P)MI

  Determine Purge Ratio (PR) in current API route for (P)MI

  \[
  \text{Purge Ratio} = \frac{\text{Predicted purge factor for (P)MI}}{\text{Required purge factor to achieve TTC or PDE for (P)MI}}
  \]

- Select initial ICH M7 control strategy for (P)MI during development based on Purge Ratio. Implement recommended experimental data collection and regulatory reporting strategies based upon Purge Ratio (next slide)

- Does final data package support commercial ICH M7 Option 4 strategy?

  - Yes
    - Select ICH M7 Option 4 commercial strategy
  
  - No
    - Select ICH M7 Option 1, 2 or 3 commercial strategy, as appropriate
## Control options correlation to ratio

<table>
<thead>
<tr>
<th>If PR &gt; 1000x</th>
<th>If 1000 &gt; PR &gt; 100x</th>
<th>If PR &lt; 100x</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Data Collection Recommendations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collection of additional experimental data not recommended</td>
<td>Collection of additional non-trace experimental data (solubility, reactivity, and volatility) recommended. Collection of additional trace PMI analysis not necessary to support scientific rationale</td>
<td>Additional data are expected to support an Option 4 control strategy when PMI Purge Ratio &lt;&lt;100x. Detailed experimental fate &amp; purge studies are expected to support a commercial Option 4 control strategy</td>
</tr>
<tr>
<td><strong>Regulatory Reporting Recommendations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Provide a summary of key elements of predicted purge factor calculations and Purge Ratio</td>
<td>Provide a summary of key elements of predicted purge factor calculations, Purge Ratio, and supporting non-trace data on purge properties in regulatory submissions</td>
<td>Provide a summary of predicted purge factor calculations, Purge Ratio, supporting trace and non-trace fate and purge data for commercial API routes in regulatory submissions</td>
</tr>
</tbody>
</table>
Candesartan - Example calculations

- Registered Starting Material
  - Stage 1
  - Stage 2
  - Stage 3
  - Stage 4
- Tetrazole Formation
  - Stage 5
  - Stage 6
  - Stage 7
  - Stage 8
  - Stage 9
- API

PF = Theoretical purge factor
*if formed

- Et₃N
- DMF
- *DMA/DEA

PF > 1×10¹⁵
PF > 1×10¹⁵
PF > 1×10²⁰

- NaNO₂
- PF > 1×10⁶

- *NDEA
- *NDMA

PF ~ 1×10³
PF > 1×10⁴
Conclusion at no point in the process could there be both secondary amines and nitrite present
Outcome of testing

- Initially > 40 batches of API tested – NDMA not detected Limit 150ppb
- DMA Not detected in Stage 5 (tetrazole) <100ppb
- Nitrite not detected in Stage 5

**Now:**

- Option 4 backed up by testing upstream of API for both Nitrosamines and Nitrite,
  - Subsequently confirmed by over 85 Bx analysis at nmt 5 ppb for NDMA and NDEA
  - including 65 Bx for 5 nitrosamines
The story continues…

Claim of hundreds of thousands of ppb, impact effective withdrawal even though validity of results later challenged.
N-Nitrosamines – Future Implications

In September 2019 EMA issue a request to extend the evaluation of risk posed by N-Nitrosamines to ALL products
• **What is being requested?**

• In summary – Issued instruction

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**Steps companies should take**

- Evaluate possibility of nitrosamines being present in **every concerned medicine within 6 months**

- Prioritise evaluations, starting with medicines more likely to be at risk of containing nitrosamines

- Take into account findings from CHMP’s review of sartans

- Notify authorities of outcome of risk evaluations

- Test products at risk of containing any nitrosamines

- Immediately report detection of nitrosamines to authorities

- Apply for necessary changes to **marketing authorisations to address nitrosamine risk**

- Complete all steps within 3 years, prioritising high risk products
What are the potential risk factors?

- **Synthesis / process related**
  - Intrinsic – chemical reaction
  - Extrinsic risk factors – cross contamination

- **Product related**
  - Drug Product – excipient related
  - Packaging Systems
Implications

• Scale is huge and if reliant on testing alone, effectively insurmountable

• Industry attempting to find a pragmatic way forward, focused on identified risk:
  
  • Propose to only test if a actual risk is identified
  
  • ICH M7 control options and purge calculations are therefore a vital component of such an approach
  
  • Mirabilis provides an ideal platform to determine risk as shown by Candesartan
Current analytical testing methodology and capacity

• It is good to have analytical methodology available and regulatory efforts to support suitable methods being available is appreciated.

• Currently ~ 20 published methods (September 2019) developed by OMCLs.
  • 9 European (including 1 from Swissmedic) and 6 FDA
  • Canada, Taiwan and Korea have also published methods

• It would be good to rationalise to a limited number of sample prep and introduction, chromatography and then detection
  • limits set for sartans challenge current technology
Current analytical testing methodology and capacity

- In the current situation appropriate specificity best achieved by MS/MS data

- Trade off between sensitivity and specificity
  - False positives described in official methods and literature
  - Impact of false positive results is very high

- Small m/z ions prone to chemical interference from the matrices
  - Small target molecules falls into the region of chemical background
  - In GCMS EI Single Ion Monitoring m/z 74 >10,100 library hits as top 30% ion
Analytical Capacity

• It will be important to **focus** analytical testing capacity on the most important / highest priority testing
  - Risk assessment should be capable of focusing the analytical testing needed – this should be based on ICH M7.
  - Universal blanket testing of APIs (and for what?) should not be necessary and there may well not be sufficient analytical capacity to conduct such testing on a routine basis.

• When addressing the analytical effort necessary to measure trace levels of nitrosamines, it is critical to focus testing based **probability of presence of certain risks**.

• Is there sufficient analytical capacity to allow product manufacturers / MAH to routinely test every batch of API, solvent, excipient or even drug products by GC-MS/MS or HPLC-MS/MS?
Process related risk - How to address this?

INTRINSIC RISK

Should be relatively straight forward:

- Examine syntheses – especially the registered synthesis
  Are there any potential sources of Nitrite?

- IF yes
  Where in the synthesis?
  Are there any potential sources of $2^o$ amines?

- Based on this approach combined with, where necessary, analysis, a risk assessment can be made and if necessary a control strategy introduced.
Process related risk - How to address this?

INTRINSIC RISK

- Analogous to Sulphonate esters we can start to understand formation and fate.

- Through Mirabilis – establish relevant purge factors and understand conditions to destroy any N-Nitrosamines present

- ALREADY STARTED TO DO THIS
Scientific Understanding - Nitrosation by N\textsubscript{2}O\textsubscript{3}

- Consists of two equilibria coupled with a fast reaction.
- Hydrolysis of N\textsubscript{2}O\textsubscript{3} back to HNO\textsubscript{2} is usually fast relative to nitrosation, which gives rise to third order kinetics
  
  \[
  \text{Rate} = kK [R_2\text{NH}]_T \cdot f_N \cdot [\text{HNO}_2]_T \cdot f_{\text{HA}}
  \]

- Both the available concentration of the reacting secondary amine and available concentration of nitrous acid are pH dependent
- The rate of nitrosation therefore varies with pH with a maximum at pH 3.15
Kinetics of Nitrosation

• Extensive studies of the kinetics and mechanism of nitrosation have been undertaken.

• In aqueous solution nitrosation is primarily via NO\(^+\) derived from nitrous acid, with a range of species being capable of acting as carriers of NO\(^+\) at low acidities (<0.1 M).

• Nitrous acid is a weak acid with a pK\(_a\) of 3.2 meaning that a degree of acidity is required for an effective reaction.

Nitrosation by trace nitrite in water ([HNO2]T = 6.5×10\(^{-5}\) M)

• Considering trace contamination by dimethylamine (1 mM)
  • 0.35% of NO\(_2^-\) converted in 1×10\(^7\) seconds (115 days)
**Process related risk - How to address this?**

- Potentially a far more significant issue – SCOPE.
- Must develop a systematic approach
- **Possible options:**
  - Develop understanding of fate of N-Nitrosamines in solvent recycling – where are there risks. *Would this allow you to define stages where recycling is acceptable, based on proximity to API stage?*
  - **GMP** – examine how solvents are recycled
    - Within a process OR
    - Pooled

**ETHYL ACETATE**
- Simulations of the NDMA / NDEA / ethyl acetate system using COSMOtherm found no evidence of the existence of an azeotrope.
  - **It should therefore be possible to distil ethyl acetate and leave any contaminating nitrosamine behind.**
Product related risk

• Certain Excipients have been reported to contain traces of Nitrite

• Is this significant?

• Investigation under way

• Is it realistic to test until we know the risk?
**Suggested DP Prioritisation Risk Assessment Workflow**

**Step 1**

**Risk Assessment considering**

Is a secondary amine motif present in DS or as specified impurity?  
Y/N

**Step 2**

**Prioritisation of Risk Assessment**

- Is there a known risk of nitrosating agents in the excipient or API?  
- Is it a solution, topical or oral solid dose formulation?  
- Do the conditions in the processing or formulation add risk? (e.g. water or solvent addition, drying, heat, pH etc.)  

Y/N

**Step 3**

**High Risk**

Test for identified nitrosamine  
Use data to inform future assessments

No further action at this time; await further data / understanding of risk factors
Packaging

Statement for Industry/Industry Associations:

Novartis develops a changeover plan for lidding foil with nitrocellulose as printing primer

Schematics of lidding foil:

• A new potential NDMA/NDEA source has been identified which is linked to the use of lidding foil containing nitrocellulose as printing primer in combination with ink containing amines.

• These are standard materials commonly used in the pharmaceutical industry for the primary packaging of solid oral products.
Conclusions

• To investigate the risk it was first vital to understand the root cause.

• ICH M7 provides a systematic, risk based approach to assess the risk.

• N-Nitrosamine formation is only possible if BOTH Secondary amines and a Nitrosating agent is present AND there are favourable conditions (low pH / concentration)
  • Mechanism has been extensively studied

• Principles within M7 remain relevant – including risk assessment principles, including purge calculations.

• Purge calculations and Mirabilis, fully aligned to the principles of ICH M7 conclusively showed that there was no risk in the case of Candesartan.

• They can equally play a pivotal role in current risk assessments.