Controlling potential genotoxic impurities encountered during API synthesis

Utilising expert knowledge of chemical properties to manage risk

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Outline

• Background
  ▪ Potential mutagenic impurities in synthesis
  ▪ Analytical implications
  ▪ Purge calculations
  ▪ Mirabilis consortium and regulatory advocacy

• Mirabilis
  ▪ Impurity carry-over workflow
  ▪ Origins
  ▪ Workflow for ICH M7

• Theoretical case study

• Ongoing developments
The threat posed by potential mutagenic impurities (PMI) in drug substances arises, for example, from the use of reagents such as alkylating agents within the synthesis.

What makes them useful reagents in synthesis, high reactivity, is often what makes them PMIs.

Virtually all syntheses will involve the use of mutagenic or potentially mutagenic reagents or possess potential risk arising from a PMI formed in the process.

Any synthetic drug therefore may have a latent PMI-related risk.
Evaluating Risk Posed by Mutagenic Impurities

• Fundamentally there is a need to assess the risk posed by mutagenic impurities

• *Are there any mutagenic impurities present in the final product at levels of concern?*

• Historically the emphasis has been to analytically test for every MI
  ▪ Significant analytical challenges
  ▪ Takes little account of knowledge of intrinsic reactivity / physico-chemical parameters
Each project will have to deal with 5 PMIs introduced in the synthetic route
  - Estimated at 4.1 by Elder and Teasdale\(^1\)
  - However recent experience at AZ suggests this is closer to ~5

---

Analytical Workflow

**Phase I**
- Develop analytical methods
- Analyse batches

**Phase II**
- Re-develop analytical methods
- Analyse batches

**Phase III**
- Re-develop analytical methods
- Analyse batches

**Additional studies e.g. solubility**
- Spike/Purge and fate studies

**Post Approval**
- Analyse batches

Additional studies e.g. solubility
- Spike/Purge and fate studies

**Analytical Workflow**
In Practice – Annual Analysis Effort (Mid/large company)

Based on the scale of operations suggested, annual analytical effort for PMIs could involve:

- 50 analytical methods
- 7 re-developed analytical methods
- 4 spike/purge and fate studies
- 4 additional studies
- 250 analytical methods conducted

Total hours spent = 10,944

Question Posed – Could a systematic way be found that takes into account factors that reduce the risk of potential carryover, incorporating knowledge of intrinsic reactivity / physico-chemical parameters?
Purge Factor Calculation – Basic Principles

- The following **key factors** were defined in order to assess the potential carry-over of a PMI:
  - Reactivity, solubility, volatility and any additional physical process designed to eliminate impurities e.g. chromatography

- A **score** is assigned on the basis of the **physicochemical properties** of the PMI relative to the process conditions
  - These are then simply multiplied together to determine a ‘purge factor’ (for each stage)

- The overall purge factor is the product of the factors for individual stages

- Predicted purge is then compared to required purge (this being based on the safety limit and initial level introduced into the process)
Original Purge Prediction Scoring System

- The original scoring system was built on basic principles – referred to as a ‘paper’ assessment because it’s not automated (manual calculation via spreadsheet)
  - Reactivity shown to have largest effect
  - Other factors especially solubility would also influence purging

<table>
<thead>
<tr>
<th>Physicochemical parameters</th>
<th>Purge factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactivity</td>
<td>Highly reactive = 100</td>
</tr>
<tr>
<td></td>
<td>Moderately reactive = 10</td>
</tr>
<tr>
<td></td>
<td>Low reactivity/unreactive = 1</td>
</tr>
<tr>
<td>Solubility</td>
<td>Freely soluble = 10</td>
</tr>
<tr>
<td></td>
<td>Moderately soluble = 3</td>
</tr>
<tr>
<td></td>
<td>Sparingly soluble = 1</td>
</tr>
<tr>
<td>Volatility</td>
<td>Boiling point &gt;20 °C below that of the reaction/process solvent = 10</td>
</tr>
<tr>
<td></td>
<td>Boiling point within ±20 °C of that of the reaction/process solvent = 3</td>
</tr>
<tr>
<td></td>
<td>Boiling point &gt;20 °C above that of the reaction/process solvent = 1</td>
</tr>
<tr>
<td>Ionisability</td>
<td>Ionisation potential of GTI significantly different from that of the desired product</td>
</tr>
<tr>
<td>Physical processes: chromatography</td>
<td>Chromatography: 10–100 based on extent of separation</td>
</tr>
<tr>
<td>Physical processes: <em>e.g.</em>, other scavenger resins</td>
<td>Evaluated on an individual basis.</td>
</tr>
</tbody>
</table>

- Scoring system originally designed to be conservative
  - *On validation this was experimentally observed*
  - *It was decided that this should be retained rather than seeking absolute parity*
  - *Urquhart et al recently demonstrated the approach for Atovaquone*
Paper Assessment – Case Study – AZD9056

AZD9056 Aldehyde → 3-aminopropan-1-ol → AZD9056 Imine → H₂/Pt → AZD9056 Free Base

isopropyl chloride (by-product)

AZD9056 HCl → HCl in IPA → AZD 9056 Chloride (minor by-product)

MeOH / water → pure
Comparison of the overall predictions with the experimental results shows that the predicted purge factor is in good correlation with each impurity

- Under-predicts the purge capacity of the process
- Clearly demonstrates risk of carry over to be low
- Predictions indicated where formation of an impurity needed to be regulated through process control, rather than relying on the ability of the process to eliminate it.

<table>
<thead>
<tr>
<th>Predicted</th>
<th>Measured</th>
<th>Predicted</th>
<th>Measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,000</td>
<td>112,000</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>(Solubility, Reactivity)</td>
<td>(Solubility)</td>
<td>(Solubility, Volatility)</td>
<td></td>
</tr>
</tbody>
</table>
Is this simply about avoiding analytical testing?

- **Three Step reaction**
  - Starting material contains an aromatic N-oxide
  - Alcohol converted to alkyl halide
  - Coupled to a thiol
  - Oxidation step

- **Only final step isolated**
  - Impurity is un-reactive/highly soluble/non-volatile
  - No purge predicted in steps 1 and 2

- **Spiking experiment 3000ppm**
  - Only reduced to 2000ppm

Purge calculations showed that control at starting material is required.
Mirabilis Consortium
- Achaogen
- Astra Zeneca
- Abbvie
- Bayer
- Celgene
- Excella
- Esteve
- Galapagos
- GSK
- Janssen

Mirabilis Members
- Genentech
- USV
- Inovat
- Aligos
- Gilead

The Mirabilis Community
Mirabilis

- Mirabilis project established to take basic principles of paper-based predictions and augment them

- Establish an industry-wide framework which all parties agree to adhere to, incorporating industry and regulatory bodies

- Key concepts:
  - Use of an *in silico* template allows for greater consistency in terms of how predictions are structured and reported (*reproducibility*)
  - Predictions by Mirabilis are informed from a **knowledge base**, the basis of these is clearly visible (**objectivity and transparency**)
  - Knowledge management & pre-competitive knowledge sharing (**supports further development**)
Regulatory Advocacy

• Purge prediction principles and scoring rules are well established and used in paper assessments.

• ICH M7 provides framework for control strategy options.

• Mirabilis provides a partially automated and living knowledge base to assist scientists and regulators in making and reporting purge predictions.

• The Mirabilis consortium developed a framework to implement this technology consistently into PMI workflows throughout development and commercialisation.

• Goal: consistent application and presentation of purge prediction science to promote and support broad regulatory acceptance.
A consortium-driven framework to guide the implementation of ICH M7 Option 4 control strategies

Chris Barber a, Vincent Antonucci b, Jens-Christoph Baumann c, Roland Brown d, Elizabeth Covey-Crump a, David Elder e, Eric Elliott f, Jared W. Fennell g, Fabrice Gallou h, Nathan D. Ide i, Guido Jordine h, Jeffrey M. Kallemeyn i, Dirk Lauwers j, Adam R. Looker k, Lucie E. Lovelle h, Mark McLaughlin b, Robert Molzahn l, Martin Ott a, Didier Schils m, Rolf Schulte Oestrich c, Neil Stevenson n, Pere Talavera o, Andrew Teasdale p, Michael W. Urquhart n, David L. Varie g, Dennie Welch i

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 PMI Purge Prediction Decision Tree

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

**Impurity requires management as PMI**

**Determine Purge Ratio (PR) in current API route for PMI**

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

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

**Select ICH M7 Option 4 commercial strategy**

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

**Yes**

**Select ICH M7 Option 1,2 or 3 commercial strategy, as appropriate**

**No**
Purge Ratio prediction of PMI “X” (a process reagent)

- Assume TTC is 100 ppm
- Assume charge (initial conc.) is 1 eq or 10^6 ppm
- 10^4 purge factor (10^6 / 100 ppm) needed to achieve TTC
- Therefore to achieve a 10^3 Purge Ratio (i.e. three order magnitude more purge predicted than required to achieve TTC), Mirabilis must predict a 10^7 cumulative purge factor

\[
\text{Purge Ratio} = \frac{\text{Predicted purge factor for PMI}}{\text{Required purge factor to achieve TTC or PDE for PMI}}
\]
When Purge Ratio > 1000…

<table>
<thead>
<tr>
<th>Data Collection Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection of additional experimental data not necessary to support scientific rationale for non-commercial or commercial API routes.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Regulatory Reporting Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Report “unlikely to persist” or cumulative predicted purge factor and Purge Ratio for non-commercial API routes in regulatory submissions.</td>
</tr>
<tr>
<td>Replace with summary of key elements of predicted purge factor calculations and Purge Ratio for commercial API routes in regulatory submissions.</td>
</tr>
</tbody>
</table>

Option 4 recommended
Example presentation in regulatory dossier when Purge Ratio > 1000 in commercial route

<table>
<thead>
<tr>
<th>Point of introduction</th>
<th>Stage 2 of 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>(P)MI TTC</td>
<td>50 ppm</td>
</tr>
<tr>
<td>Assumed initial concentration and rationale for selection</td>
<td>$10^6$ ppm at start of Stage 2 because “X” charge is 1 equivalent</td>
</tr>
<tr>
<td>Required Purge Factor to achieve TTC</td>
<td>$2 \times 10^4 = 10^6$ ppm initial conc. / 50 ppm TTC</td>
</tr>
<tr>
<td>Predicted Purge Factor</td>
<td>$2 \times 10^8$ (source Mirabilis software vx.x)</td>
</tr>
<tr>
<td></td>
<td>Key factors: 1000x purge in Stage 2 driven by reactivity and solubility, purge in Stages 3-5 driven by solubility</td>
</tr>
<tr>
<td>Purge Ratio</td>
<td>$1 \times 10^4 = 2 \times 10^8 / 2 \times 10^4$</td>
</tr>
<tr>
<td>Control Strategy</td>
<td>Option 4</td>
</tr>
</tbody>
</table>

No supporting experimental data collection recommended when Purge Ratio is large
When Purge Ratios > 100x and <1000x

### Data Collection Recommendations

Collection of additional non-trace experimental data (solubility, reactivity, and volatility) recommended to support scientific rationale for both non-commercial and commercial API routes.

Collection of additional trace PMI analysis not necessary to support scientific rationale for non-commercial or commercial API routes.

### Regulatory Reporting Recommendations

Report the cumulative predicted purge factor and Purge Ratio for non-commercial API routes in regulatory submissions.

Replace with summary of key elements of predicted purge factor calculations, Purge Ratio, and supporting non-trace data on purge properties for commercial API routes in regulatory submissions.

**Option 4 recommended**
Example presentation in regulatory dossier when Purge Ratio > 100x and <1000x in commercial route

<table>
<thead>
<tr>
<th>Point of introduction</th>
<th>Stage 3 of 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>(P)MI TTC</td>
<td>50 ppm</td>
</tr>
</tbody>
</table>
| Assumed initial concentration and rationale for selection | 10⁶ ppm at start of Stage 3  
1 eq. added to reaction |
| Required Purge Factor to achieve TTC | 2 x 10⁴ [10⁶ ppm initial/50 ppm TTC] |
| Predicted Purge Factor | 6 x 10⁶ (source Mirabilis software vx.x)  
Key factors: 1000x purge in Stage 3 driven by reactivity and solubility, purge in Stages 4-5 driven by solubility |
| Purge Ratio           | 300          |
| Supporting experimental data | (P)MI solubility in solvent x (Stage 3 crystallisation solvent) is 50 mg/mL; PMI “X” measured in isolated product Y (Stage 3) as ND<200 ppm; additional 6000x purging below this level predicted in Stage 4 + Stage 5 |
| Control Strategy      | Option 4     |

For moderate Purge Ratios, some supporting data recommended but not necessarily trace level measurements.
When Purge Ratios <100x

Data Collection Recommendations
For non-commercial API routes, experimentally measure PMI purging, including trace PMI analyses as appropriate, to support scientific rationale.

Note: Additional data are expected to support an Option 4 control strategy when PMI Purge Ratio <100x. For commercial API routes, detailed experimental fate & purge studies are expected to support a commercial Option 4 control strategy.

Regulatory Reporting Recommendations
Report summary of key elements of predicted purge factor calculations, Purge Ratio, and supporting non-trace or trace data for non-commercial API routes in regulatory submissions.

Replace with complete summary of predicted purge factor calculations, Purge Ratio, supporting trace and non-trace fate and purge data for commercial API routes in regulatory submissions.

Option 4 possible with strong data package, otherwise Options 1, 2, or 3 are recommended
Example presentation in regulatory dossier when Purge Ratio <100x in commercial route

<table>
<thead>
<tr>
<th>&lt;insert chemical structure of (P)MI “X”&gt;</th>
<th>Point of introduction</th>
<th>Stage 4 of 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>(P)MI TTC</td>
<td></td>
<td>50 ppm</td>
</tr>
<tr>
<td>Assumed initial concentration and rationale for selection</td>
<td>10^6 ppm at start of Stage 4</td>
<td>1 eq. added to reaction</td>
</tr>
<tr>
<td>Required Purge Factor to achieve TTC</td>
<td>2 x 10^4 [10^6 ppm initial/50 ppm TTC]</td>
<td></td>
</tr>
<tr>
<td>Predicted Purge Factor</td>
<td>1 x 10^6 (source Mirabilis software vx.x)</td>
<td>Key factors: 1000x purge in Stage 4 driven by reactivity and solubility, purge in Stage 5 driven by solubility</td>
</tr>
<tr>
<td>Purge Ratio</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Supporting experimental data</td>
<td>(P)MI solubility in solvent x (Stage 4 crystallisation solvent) is 50 mg/mL; PMI “X” measured in Y commercial batches of API at ND&lt;5 ppm</td>
<td></td>
</tr>
<tr>
<td>Control Strategy</td>
<td>Option 4</td>
<td></td>
</tr>
</tbody>
</table>

For lower Purge Ratios, trace supporting data package required to justify an Option 4 strategy, otherwise select Option 1, 2, or 3 as appropriate
### In Practice – Time Saved (Mid/large company)

<table>
<thead>
<tr>
<th>No Purge Approach</th>
<th>Purge Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>Re-developed analytical methods</td>
<td>1.75</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Spike/purge and fate studies</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Additional studies</td>
<td>2</td>
</tr>
<tr>
<td>250</td>
<td>130</td>
</tr>
<tr>
<td>Analytical methods conducted</td>
<td>4,252 hours pa.</td>
</tr>
<tr>
<td>10,944 hours pa.</td>
<td>4,252 hours pa.</td>
</tr>
</tbody>
</table>
Further Regulatory Advocacy

• Lhasa and the consortium are also actively engaging directly with regulators to discuss purge factors and Mirabilis

• To date, informal discussions have been held with numerous regulators across North and South America, Europe and Asia

• Aims are;
  ▪ To establish the requirements for submissions, including; information disclosed, level of detail
  ▪ Address/mediate concerns held by either regulators or industry regarding any aspect of the process
  ▪ Develop a best practice for application of the purge argument within a regulatory context
Impurity Carry-over Workflow

API synthesis

Knowledge of physicochemical properties

Reacting functionality

Mutagenic?

Experimental toxicity

Unknown

In-silico toxicity prediction

Mutagenic

Impurity Control

Assess likelihood of impurity persisting

Purged

Testing unnecessary

Option 3 or 4

Plan

Implement

High utility
Efficient syntheses

Test for impurity

Mutagenic

Option 1 or 2

Not Purged

Option 3

Non-mutagenic

Teasdale et al's scoring approach to purify prediction

Mirabilis

Mutagenic

In-vitro toxicity

Analytical challenge
Time consuming
Expensive

Option 1 or 2

Mutagenic

Testing unnecessary

Not Purged

Option 3
Origins of Mirabilis Knowledge Base

- Common alerting impurity types and popular chemical transformations identified by the Mirabilis consortium
  - 15 impurity types
  - 58 transformation types

- Each impurity type is analysed against every transformation to assess potential purge, generating a reactivity matrix

- Further development of the knowledge base is ongoing and has now expanded to 25 impurity types and 80 transformation types
• A consortium collaboration exercise resulted in an “expert elicitation” for each reactivity purge factor
  ▪ Each member was given the reaction grid and asked to give their expert opinion on whether they agreed or disagreed with the proposed reactivity purge factors
  ▪ Lhasa collated the results and modified the grid accordingly
    • Where members agreed on a reactivity purge factor then a consensus call was made.
    • For those without consensus, a conservative call was made

• The consensus model results in conservative purge values incorporating the knowledge and experience from multiple chemists, as opposed to the single viewpoint present in the paper-based approach
The reactivity purge factor for this impurity class in this reaction has been assigned a value of 100 based on the expert elicitation of seven pharmaceutical companies in 2014. A consensus between experts was reached, where all companies agreed on the reactivity purge factor for this scenario.
Augmenting the elicitation

- Lhasa scientists continue to augment the elicitation using published literature (Journals, Books, Patents):
  - Scientific comments detailing mechanisms of purge, scope and limitations
  - Provision of examples which highlight the expected purge outcome
  - References to support the purge/scientific comments
  - Identifying gaps in existing knowledge
  - Highlighting possible generation of new PMIs as a result of purge (e.g. hydrazine → hydrazide)
  - Adjusting the elicitation purge values where the weight of published evidence indicates a change is required (with a focus on conservatism)
Aromatic amines are expected to react under the conditions for N-acylation of amine to form amide. They may remain unreacted if a more reactive amine is present.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Range</th>
<th>Effect on reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>0 - 60</td>
<td>no change</td>
</tr>
<tr>
<td>Time (h)</td>
<td>0 - 24</td>
<td>no change</td>
</tr>
<tr>
<td>Solvents</td>
<td>Aprotic organic solvents: DCM, THF, MeCN, PhMe; H₂O</td>
<td>no change</td>
</tr>
<tr>
<td>Reagents</td>
<td>Non-nucleophilic base: Et₃N, C₂H₅N, Dipea; alkali: NaOH, K₂CO₃, NaHCO₃</td>
<td>no change</td>
</tr>
</tbody>
</table>

Primary and secondary aromatic amines are expected to react with acid halides/anhydrides to form amides. Aromatic amines can be a reactant in the N-acylation of amines reaction under standard reaction conditions described (E0495). However, if an aromatic amine is present in the reaction as an impurity, there may be another more reactive amine present in the reaction mixture (either as a reactant or additional impurity) that could react preferentially (E0496, E0497).
Mirabilis workflow

1. Establish API treatment length
2. Define API daily dose
3. Define initial impurity level
4. Identify impurity
5. Identify transformation
6. Add all unit operations (e.g., Work-up, extraction, filtration)
7. Identify purge relationship
8. Return relevant purge and supporting info
9. Add purges and justification for each unit operation
10. Review reactivity purge assignment
11. Combine purges to achieve total purge per stage
12. Calculate purge ratio to establish control option 4 suitability and regulatory requirements
13. Calculate purge required to reach acceptable intake level
14. Define API daily dose
15. Define initial impurity level
16. Identify purge relationship
17. Return relevant purge and supporting info
18. Review reactivity purge assignment
19. Combine purges to achieve total predicted purge
20. Repeat for each stage of the synthesis

Start

Synthetic scheme

Calculate purge ratio to establish control option 4 suitability and regulatory requirements

Establish API treatment length

Identify acceptable intake from ICH M7 (e.g. >1 - 10 years = 10 μg/day)

Combine all stage purges to achieve total predicted purge

Identify purge required to reach acceptable intake level

Review reactivity purge assignment

Combine purges to achieve total purge per stage

Add purges and justification for each unit operation

Add all unit operations (e.g., Work-up, extraction, filtration)
Theoretical Case Study

Utilising Mirabilis in an impurity assessment workflow
Imatinib

- Imatinib is an anti-cancer drug with a maximum daily dose of 800mg for up to 3 years

- Hopkin *et al* published a 3-stage synthesis to Imatinib
  - Additional steps include basic work up/extraction (stages 1 and 3), precipitation and wash (stage 2), and column chromatography (stage 3)

- Six reactants/intermediates plus reagents and solvents in the synthesis need to be analysed for potential ICM M7 control*

- ICH M7 control limit of 10 μg for any PMIs based on dose and duration of treatment

* ICH M7 does not actually apply to anti-cancer drugs

http://www.glivec.com/dosing/
Known Toxicities

Reactants, Intermediates and API

Reagents

Solvents

- PDE = 62.5 mg/day
- PDE = 6.0 mg/day
- PDE = 8.8 mg/day
- PDE = 3.8 mg/day
Which structures are PMIs?
Known Toxicities + \textit{in silico} predictions

Reactants, Intermediates and API

Reagents

Solvents
ASSIGN IMPURITY CLASS

Multiple matches have been found.

Select classes to assign:

Matches:
- Acid halide
- Primary alkyl chloride

Custom classes:

Assigned class(es):
- M Acid halide
- M Primary alkyl chloride

Molecular formula: C₈H₆Cl₂O
Molecular weight: 189.04
Boiling point: [°C]
N-Acylation of amine - Acid halide

Reactivity: 100
Solubility: 
Volatile: 
Other: 
Predicted: 100
Measured: 100

Overall: 100
The reactivity purge factor for this impurity class in this reaction has been assigned a value of 100 based on the expert elicitation of seven pharmaceutical companies in 2014. A consensus between experts was reached, where all companies agreed on the reactivity purge factor for this scenario.
PREDICTED REACTIVITY DETAILS - 2

Predicted purge factor: 10
User defined purge factor: 10

Impurity class: Aromatic amine
Reaction class: N-Alkylation of aliphatic amine

COMMENTS

KB comments:
- Aromatic amines react with alkylating agents in the presence of base at a moderate rate.
- The rate of reaction of alkylating agents with alkyl amines is faster than the reaction between alkylating agents and aromatic amines, however the impurity should react if there is not a large excess in the reaction mixture.
- In the event that the aromatic amine impurity reacts with a reactant (organohalide or sulfonate) of the amine N-alkylation reaction, a new impurity of the same class is formed.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Range</th>
<th>Effect on reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>0 - 100</td>
<td>Potential change</td>
</tr>
<tr>
<td>Time (h)</td>
<td>0 - 48</td>
<td>No change</td>
</tr>
<tr>
<td>Solvents</td>
<td>MeCN, DMF, THF</td>
<td>No change</td>
</tr>
<tr>
<td>Reagents</td>
<td>Base: K₂CO₃, Na₂CO₃, NaHCO₃; KI</td>
<td>Potential change</td>
</tr>
</tbody>
</table>

The N-alkylation of aromatic amines is usually carried out by...
Analysis of purge predictions

<table>
<thead>
<tr>
<th>Stage</th>
<th>Reactivity</th>
<th>Solubility</th>
<th>Volatility</th>
<th>Other</th>
<th>Predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>1000</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1000</td>
</tr>
<tr>
<td>Stage 2</td>
<td>100</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>1000</td>
</tr>
<tr>
<td>Stage 3</td>
<td>1000</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>100000</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$1 \times 10^{11}$</td>
</tr>
</tbody>
</table>
## Analysis of purge predictions

### Cumulation of predicted purges, indicating relative contributions

<table>
<thead>
<tr>
<th>Stage</th>
<th>Reactivity</th>
<th>Solubility</th>
<th>Volatility</th>
<th>Other</th>
<th>Predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>1000</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1000</td>
</tr>
<tr>
<td>Stage 2</td>
<td>100</td>
<td>10</td>
<td>1</td>
<td>1</td>
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<tr>
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<td>×10^11</td>
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<th>Solubility</th>
<th>Volatility</th>
<th>Other</th>
<th>Predicted</th>
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<th>Volatility</th>
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</tbody>
</table>

---

### Graph

- **Purge Ratio = 1000**
- **Purge Ratio = 100**
- **Purge ratio = 1**
- **Other**
- **Solubility**
- **Reactivity**
## Analysis of purge predictions

<table>
<thead>
<tr>
<th>Stage</th>
<th>Reactivity</th>
<th>Solubility</th>
<th>Volatility</th>
<th>Other</th>
<th>Predicted</th>
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<td>100</td>
<td>$1 \times 10^{11}$</td>
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<table>
<thead>
<tr>
<th>Stage</th>
<th>Reactivity</th>
<th>Solubility</th>
<th>Volatility</th>
<th>Other</th>
<th>Predicted</th>
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</thead>
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<tr>
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<td>1</td>
<td>100</td>
<td>1000</td>
</tr>
<tr>
<td>Overall</td>
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<td>1</td>
<td>1</td>
<td>100</td>
<td>$1 \times 10^6$</td>
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</table>
Analysis of purge predictions

<table>
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<tr>
<th>Stage</th>
<th>Reactivity</th>
<th>Solubility</th>
<th>Volatility</th>
<th>Other</th>
<th>Predicted</th>
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</table>

Cumulation of predicted purges, indicating relative contributions

Stage of the synthesis

- Purge Ratio = 1000
- Purge Ratio = 100
- Purge ratio = 1
- Other
- Solubility
- Reactivity
### Analysis of purge predictions

<table>
<thead>
<tr>
<th>Stage</th>
<th>Reactivity</th>
<th>Solubility</th>
<th>Volatility</th>
<th>Other</th>
<th>Predicted</th>
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<th>Solubility</th>
<th>Volatility</th>
<th>Other</th>
<th>Predicted</th>
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</tr>
<tr>
<td>Stage 3</td>
<td>10</td>
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<td>1</td>
<td>100</td>
<td>1000</td>
</tr>
<tr>
<td>Overall</td>
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<th>Solubility</th>
<th>Volatility</th>
<th>Other</th>
<th>Predicted</th>
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<tbody>
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<td>Stage 2</td>
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<td>1</td>
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<td>Stage 3</td>
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<td>1</td>
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<td>1000</td>
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<tr>
<td>Overall</td>
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<td></td>
<td></td>
<td></td>
<td>30000</td>
</tr>
</tbody>
</table>
## Analysis of purge predictions

### Stage 1
- Reactivity: 1
- Solubility: 1
- Volatility: 1
- Other: 1
- Predicted: 1000

### Stage 2
- Reactivity: 100
- Solubility: 10
- Volatility: 1
- Other: 1
- Predicted: 1000

### Stage 3
- Reactivity: 1000
- Solubility: 1
- Volatility: 1
- Other: 100
- Predicted: 10000

### Overall
- Reactivity: 1
- Solubility: $1 \times 10^{11}$
- Volatility: 1
- Other: 1
- Predicted: 1

---

### Cumulation of predicted purges, indicating relative contributions

<table>
<thead>
<tr>
<th>Stage 2</th>
<th>Stage 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactivity</td>
<td>10</td>
</tr>
<tr>
<td>Solubility</td>
<td>1</td>
</tr>
<tr>
<td>Volatility</td>
<td>1</td>
</tr>
</tbody>
</table>

**Stage of the synthesis**
Regulatory Requirements

**Required purge**

\[
\text{Required purge} = \frac{\text{Starting level (μg/g)}}{\frac{\text{Acceptable intake (μg)}}{\text{Daily dose (g)}}} = PR\times 10^6
\]

**Impurity requiring management as PMI*** Determine Purge ratio (PR) of PMI in the API synthesis

\[
PR = \frac{\text{Predicted purge factor}}{\text{Required purge}}
\]

**Control options**

- Option 1 – Test the API
- Option 2 – Test an intermediate
- Option 3 – Test an intermediate then use purge argument
- Option 4 – Purge argument

**Barber et al., Regul. Toxicol. Pharmacol., 2017, 90, 22-28**
Impurity Carry-over

Conclusion

API synthesis

Knowledge of physicochemical properties

Reacting functionality

Mutagenic?

Experimental toxicity

Unknown

In-silico toxicity prediction

Mutagenic

Impurity Control

Assess likelihood of impurity persisting

Purged

Testing unnecessary

Subject to evidence package

Teasdale et al's scoring approach to purge prediction

In-vitro toxicity

Mutagenic

Option 1 or 2

Not Purged

Mutagenic

Option 3

Option 1 or 2

Test for impurity

Non-mutagenic
Impurity Carry-over
Conclusion

API synthesis

Knowledge of physicochemical properties

Plan

Implement

Reactive functionality

Mutagenic?

Experimental toxicity

Unknown

In-silico toxicity prediction

Mutagenic

Impurity Control

Assess likelihood of impurity persisting

Option 3 or 4

Mutagenic? Not Purged

Non-mutagenic

In-vitro toxicity

Option 1 or 2

Test for impurity

Mutagenic

Purged

Testing unnecessary

Teasdale et al’s scoring approach to purge prediction

Option 3

Vitic

Vitic intermediate

Vitic active

Vitic
Suitable for Purge ratio > 1000

---

Suitable for Purge ratio < 1000

---

**Suitable for Purge ratio > 1000**

**Suitable for Purge ratio < 1000**
Supports a data package for purge ratio < 1000

Stage: Stage 3
Buchwald-Hartwig amination; Aromatic amine (2)

Knowledge Base Comments
- Primary and secondary aromatic amines can react with aromatic halide reagents by Buchwald-Hartwig amination.
- Tertiary aromatic amines will not be affected by Buchwald-Hartwig amination conditions.
- In the event that the primary/secondary aromatic amine impurity reacts with a reactant (halide/halide) of the Buchwald-Hartwig reaction, a new impurity of the same class is formed.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Range</th>
<th>Effect on reactivity</th>
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</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>60 - 140</td>
<td>No change</td>
</tr>
<tr>
<td>Time (h)</td>
<td>0 - 24</td>
<td>No change</td>
</tr>
<tr>
<td>Solvents</td>
<td>PhMe, dioxane, THF, DMF</td>
<td>No change</td>
</tr>
<tr>
<td>Reagents</td>
<td>Base: ligand: phosphine, N-Heterocyclic carbene (catalyst: Pd Source)</td>
<td>No change</td>
</tr>
</tbody>
</table>

Primary and secondary aromatic amines are common reagents in the Buchwald-Hartwig amination reaction, resulting in the formation of further substituted aromatic amines.

Although disputed, in part, the mechanism is believed to involve oxidative insertion of a palladium(0) species between the carbon and hydrogen of an alkyl or aryl halide. In the second and third steps, the favoured theory involves coordination of the amine to the resulting palladium(II) species followed by deprotonation of the palladium amido species. However, there is evidence to suggest that steps two and three can be reversed. The catalytic cycle is completed by reductive elimination to afford palladium(0) and the new cross-coupled amine.

The Buchwald-Hartwig reaction is compatible with a vast array of amino compounds, both aliphatic and aromatic. The degree of reactivity of the amine group is determined through a combination of electronic and steric effects [Hartwig], [Yang and Buchwald] (E0024), [Crawford et al] (E0025), [Hernandez-Perez et al] (E0026).

Whilst steric effects largely dominate the amine binding to the palladium complex (the more hindered the amine the lower the binding affinity), electronic effects play a crucial role in the determination of its selectivity, an indicator of both the binding affinity and the deprotonation. In competition reactions between isomeric amines, aromatic amines display increased levels of cross coupling as binding affinities towards the palladium complex increase. Reactivity is generally higher for electron rich anilines with high $\delta^+$ values, where lone pair binding interactions with the palladium are stronger. Conversely, aliphatic amines display an increased reactivity as $\delta^-$ reduces, indicating a preference towards deprotonation prior to palladium coordination [Biscoe et al]. As such they are generally less prone to cross coupling than anilines under comparable conditions. The use of pre-metalated amines can switch the reaction selectivity through elimination of the amine binding and deprotonation steps of the catalytic cycle, resulting in a dependency on the relative nucleophilicities of the metal amides present [Biscoe et al] (E0027, E0028). Thus, the precise choice of conditions may have a large influence on the reactivity of a given aromatic amine [Crawford et al].

Tertiary aromatic amines are unaffected by the Buchwald-Hartwig amination conditions. The reactivity suggested herein does not account for the scenario of the aromatic amine moiety as a primary reactant, where excess aromatic amine is commonly included.

Knowledge Base Supporting Data
None.

Knowledge Base References


Knowledge Base Example Reactions
**Additional Mirabilis Developments in Progress**

**Reactivity**
- Continuously increasing coverage of transformations and impurities
- Reaction mining to aid reactivity purge assignment
  - Machine learning from a database of patented reactions
  - Provide the user with supporting examples when purge is assigned
  - Aid expert assessment with data in absence of transformation recognition
  - Aid Lhasa scientist assessments in identifying conditions (and relationships) resulting in purge

**Solubility – Identifying possible areas of solubility purging**
- Predict solubility of structures in a given solvent
- Differences between impurities and products may infer a possible purge related to liquid-liquid extraction
- Prime the user to consider whether a solubility purge may be a viable argument

**Volatility – automatic look-up of common boiling points**
- Comparison of known impurity boiling points against process temperatures
- Purge could be automatically assigned (subject to user confirmation)
Questions
References


• Urquhart, M.W.J.; Bardsley, B; Edwards, A.J.; Giddings, A; Griva, E; Harvey, J; Hermitage, S; King, F; Leach, S; Lesurf, C; McKinlay, C; Oxley, P; Pham, T.N.; Simpson, A; Smith, E; Stevenson, N; Wade, C; White, A; Wooster, N. Managing emerging mutagenicity risks: Late stage mutagenic impurity control within the atovaquone second generation synthesis. Regul. Toxicol. Pharmacol. 2018, 99, 22-32. DOI: 10.1016/j.yrtph.2018.08.004
Screenshots with different resolution