Progress on Building a Tool for Predicting the Purging of Mutagenic Impurities During Synthesis

A Teasdale

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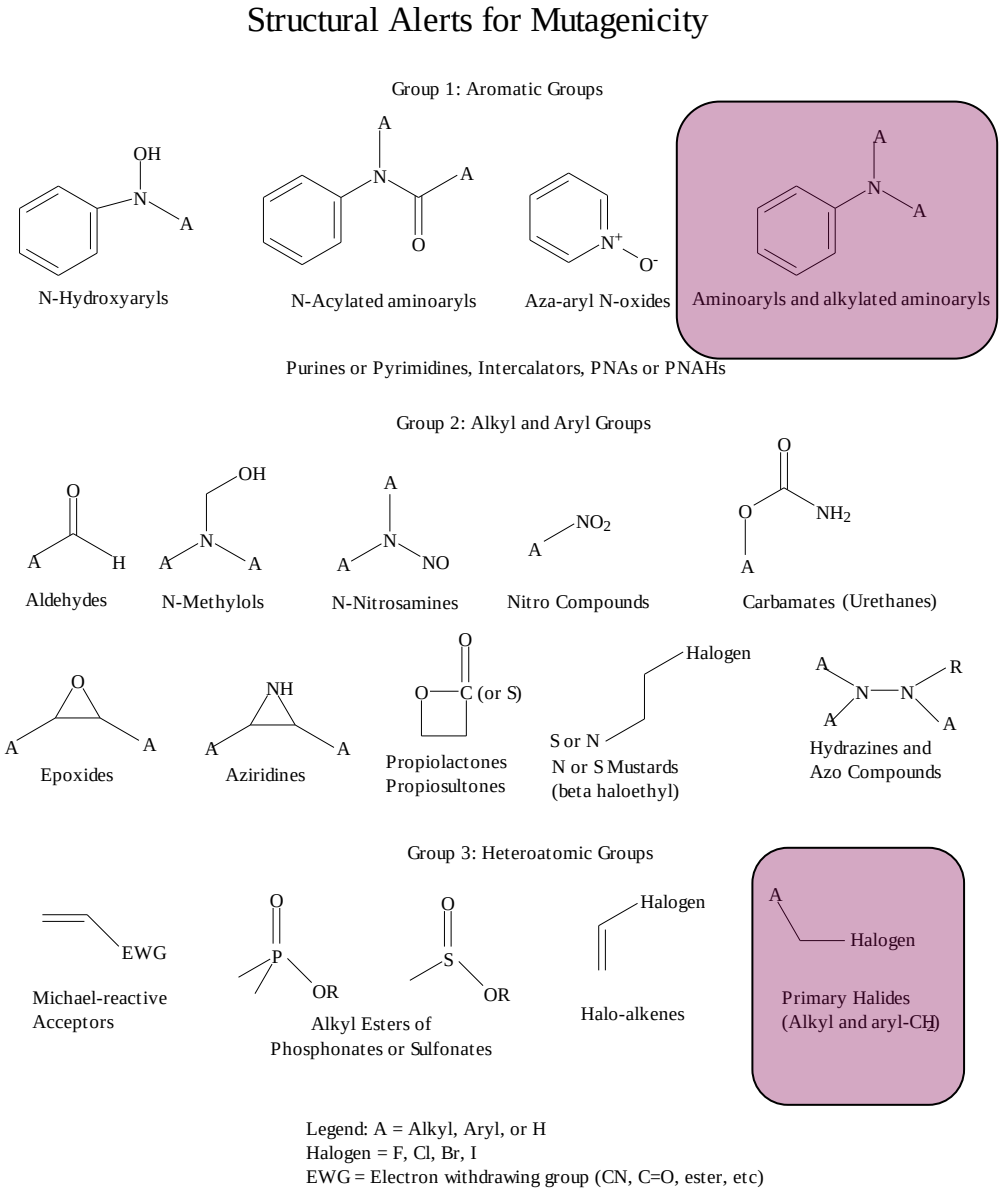
Areas covered:

1. **Background**
2. **Relationship to ICH M7 – Mutagenic Impurities Guideline.**
3. **Purge Tool – Principles**
4. **Regulatory Experience**
5. **Development of in silico tool**
    1. Overview of system
    2. Development of Knowledge Base
    3. Solubility predictions
    4. The future

Background

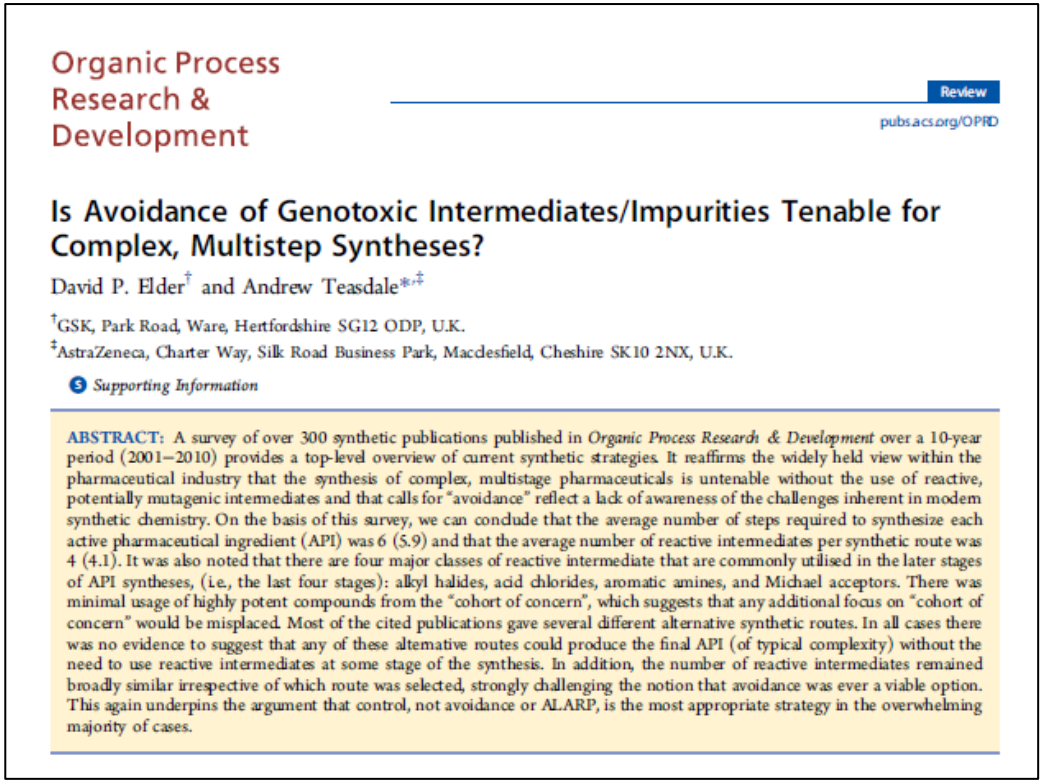
- The threat posed by mutagenic impurities (MIs) in drug substances generally arises from the use of electrophilic agents (alkylating agents) within the synthesis.
- Used in the build up of the molecular structure
  - E.g. through carbon-carbon and carbon-nitrogen bond formation

  - ubiquitous, given the current methodology
- Suggests that any synthetic drug therefore possesses a latent MI-related risk.



Background Continued

- Most synthesis will involve use of a mutagenic reagent or possess potential risk arising from an impurity formed in the process.
  - ORIGINAL APPROACH WAS TO TEST FOR ALL IMPURITIES
- Very simplistic
  - Fails to take into account the inherently reactive nature of the agent of concern and its likely fate.



Organic Process Research & Development

Review

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Is Avoidance of Genotoxic Intermediates/Impurities Tenable for Complex, Multistep Syntheses?

David P. Elder[†] and Andrew Teasdale*[,‡]

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[‡]AstraZeneca, Charter Way, Silk Road Business Park, Macclesfield, Cheshire SK10 2NX, U.K.

Supporting Information

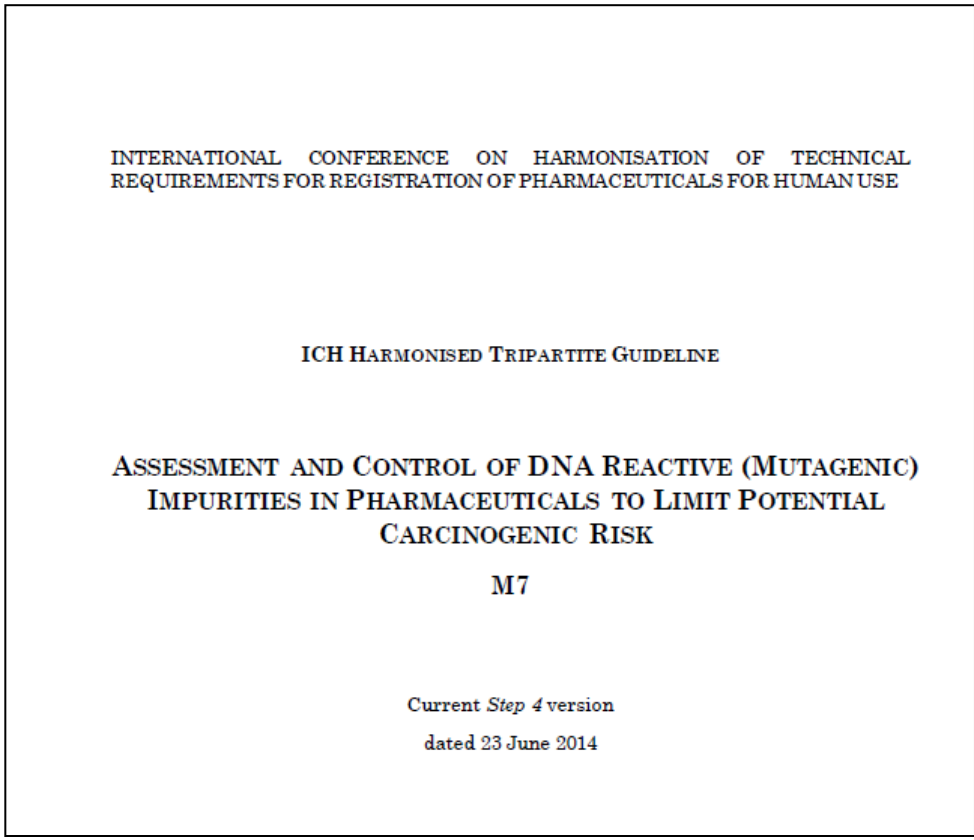
ABSTRACT: A survey of over 300 synthetic publications published in *Organic Process Research & Development* over a 10-year period (2001−2010) provides a top-level overview of current synthetic strategies. It reaffirms the widely held view within the pharmaceutical industry that the synthesis of complex, multistage pharmaceuticals is untenable without the use of reactive, potentially mutagenic intermediates and that calls for "avoidance" reflect a lack of awareness of the challenges inherent in modern synthetic chemistry. On the basis of this survey, we can conclude that the average number of steps required to synthesize each active pharmaceutical ingredient (API) was 6 (5.9) and that the average number of reactive intermediates per synthetic route was 4 (4.1). It was also noted that there are four major classes of reactive intermediate that are commonly utilised in the later stages of API syntheses, (i.e., the last four stages): alkyl halides, acid chlorides, aromatic amines, and Michael acceptors. There was minimal usage of highly potent compounds from the "cohort of concern", which suggests that any additional focus on "cohort of concern" would be misplaced. Most of the cited publications gave several different alternative synthetic routes. In all cases there was no evidence to suggest that any of these alternative routes could produce the final API (of typical complexity) without the need to use reactive intermediates at some stage of the synthesis. In addition, the number of reactive intermediates remained broadly similar irrespective of which route was selected, strongly challenging the notion that avoidance was ever a viable option. This again underpins the argument that control, not avoidance or ALARP, is the most appropriate strategy in the overwhelming majority of cases.

It is a paradox that the very reactivity that renders the agent a concern from a safety perspective is the same property that will generally ensure its effective removal in the downstream process

ICH M7- Mutagenic Impurities

Section 8 - CONTROL

- Greater flexibility in terms of mechanism to prove absence.
  - Options other than to simply test for presence in final API.
  - Ability to more widely use chemical / process based arguments to assess purging.
- Expressed in terms of a series of control options



INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

ASSESSMENT AND CONTROL OF DNA REACTIVE (MUTAGENIC) IMPURITIES IN PHARMACEUTICALS TO LIMIT POTENTIAL CARCINOGENIC RISK

M7

Current *Step 4* version

dated 23 June 2014

ICH M7 – Mutagenic Impurities

Section 8 - Control

Defines a series of control options

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

What is the right order?

ICH M7

Section 8 Control – examination of Option 4

- *What does the guideline state?*

- Where the control strategy relies on process controls in lieu of analytical testing must understand how the process chemistry and process parameters impact levels of mutagenic impurities.

- .The risk assessment can be based on physicochemical properties and process factors that influence the fate and purge of an impurity

  - This includes chemical reactivity, solubility, volatility, ionizability and any physical process steps designed to remove impurities.

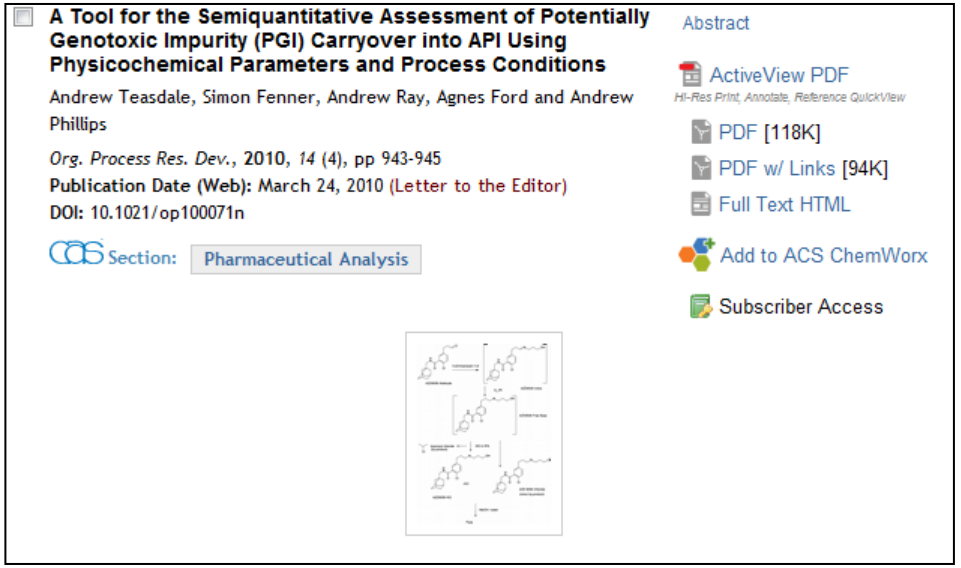
Matches wording used to describe the purge tool

Control Option 4

How do I apply this in practice?

- 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.

- OPR&D paper referenced directly in ICH M7



A Tool for the Semiquantitative Assessment of Potentially Genotoxic Impurity (PGI) Carryover into API Using Physicochemical Parameters and Process Conditions

Andrew Teasdale, Simon Fenner, Andrew Ray, Agnes Ford and Andrew Phillips

Org. Process Res. Dev., **2010**, *14* (4), pp 943-945
Publication Date (Web): March 24, 2010 (Letter to the Editor)
DOI: 10.1021/op100071n

Section: Pharmaceutical Analysis

Abstract
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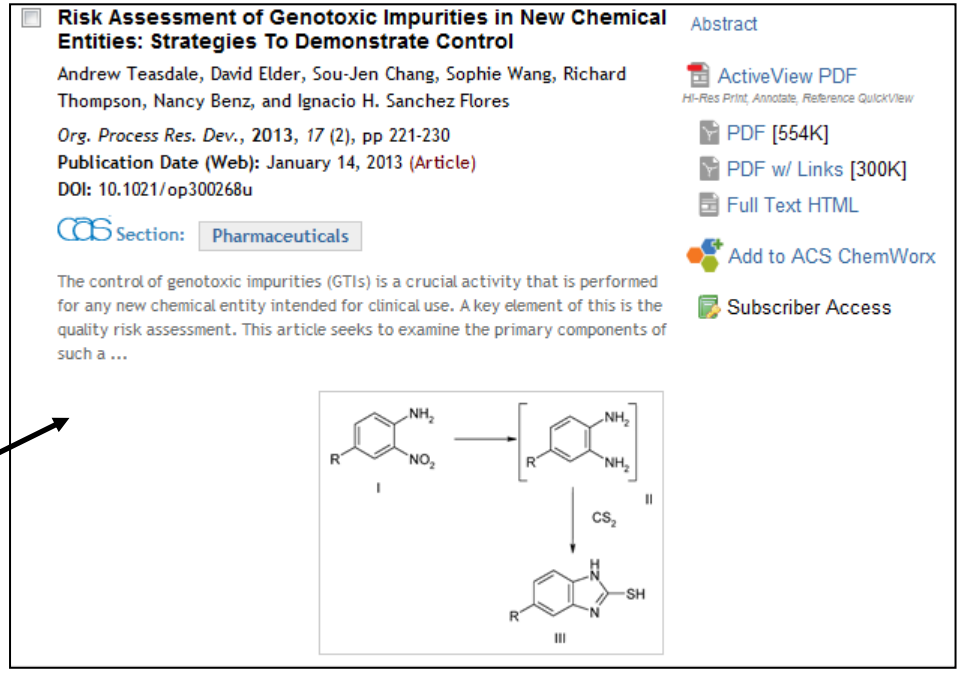

Risk Assessment of Genotoxic Impurities in New Chemical Entities: Strategies To Demonstrate Control

Andrew Teasdale, David Elder, Sou-Jen Chang, Sophie Wang, Richard Thompson, Nancy Benz, and Ignacio H. Sanchez Flores

Org. Process Res. Dev., **2013**, *17* (2), pp 221-230
Publication Date (Web): January 14, 2013 (Article)
DOI: 10.1021/op300268u

Section: Pharmaceuticals

The control of genotoxic impurities (GTIs) is a crucial activity that is performed for any new chemical entity intended for clinical use. A key element of this is the quality risk assessment. This article seeks to examine the primary components of such a ...

Abstract
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Control Option 4

Purge Factor Calculation – Basic principles

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

- **Score** assigned on the basis of the physicochemical properties of the MI relative to the process conditions.
  - These are then simply multiplied together to determine a 'purge factor' (for each stage)

- The overall purge factor is a multiple of the factors for individual stages.

Control Option 4

Purge Factor Calculation – Basic principles

Physicochemical Parameters	Purge Factor
Reactivity	Highly Reactive = 100
	Moderately reactive = 10
	Low Reactivity / un-reactive = 1
Solubility	Freely Soluble = 10
	Moderately soluble = 3
	Sparingly Soluble = 1
Volatility	Boiling point >20^{0}C <u>below</u> that of the reaction/ process solvent = 10
	Boiling point +/- 10^{0}C that of the reaction/ process solvent. = 3
	Boiling point >20^{0}C <u>above</u> that of the reaction/ process solvent = 1
Ionisability – relates to liquid / liquid extraction	Ionisation potential of GI significantly different to that of the desired product [2]
Physical Processes – chromatography	Chromatography – GI elutes prior to desired product = 100
	Chromatography – GI elutes after desired product = 10
	Others evaluated on an individual basis.

Control Option 4
Practical Use of Purge Tool

- Calculations are quick and simple
  - Conduct an assessment for all MIs.
- This is a risk assessment tool, used to identify risk.
- Using this approach helps to focus effort on those MIs that pose an actual risk.

Example Calculation

3 MIs of concern

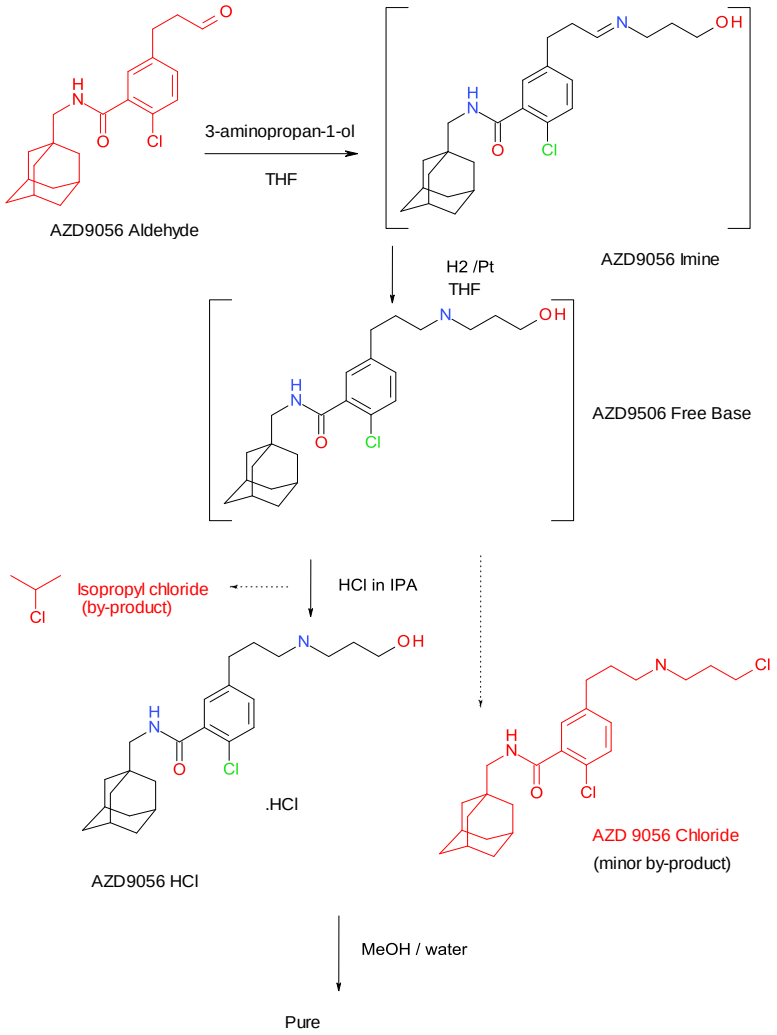
Example 1 – AZ9056 Aldehyde

Step 1 – reductive amination:

- Reactivity = 100 – based on in process control.
- Solubility = 1 – not isolated – no purging
- Volatility = 1 – not volatile

Step 2 – Isolation of HCl salt :

- Reactivity = 1
- Solubility = 10 – desired product isolated, residual Aldehyde remains in solution.
- Volatility = 1



	Step 1 (Predicted)				Step 2 (Predicted)				Step 3 (predicted)				Pure Stage	
	Reactivity	Solubility	Volatility	Predicted Purge Factor	Reactivity	Solubility	Volatility	Predicted Purge Factor	Reactivity	Solubility	Volatility	Predicted Purge Factor	Predicted Purge Factor	Measured Purge Factor
AZD9056 Aldehyde	100	1	1	**100**	1	10	1	**10**	1	10	1	**10**	**10,000**	112,000
AZD9056 Chloride	Not present	Not present	Not present	N/A	1	1	1	**1**	1	3	1	**3**	**3**	10
Isopropyl Chloride	Not present	Not present	Not present	N/A	1	10	10	**100**	1	10	10	**100**	**10,000**	38,500

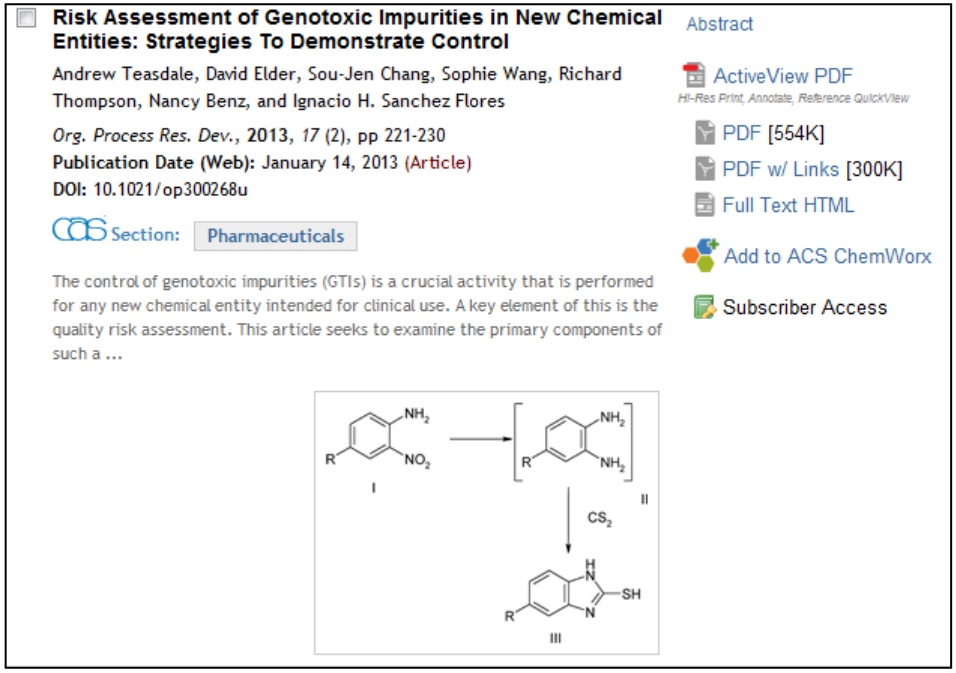
Purge Tool

How do predicted values compared to actual measured ?

- In example after example both within AZ and other companies system shows a systematic bias

- It under-predicts – typically by a factor of around 10.

- This is important ! To gain acceptance it must not over-predict.



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Relates to solubility

Control Option 4
Practical Use of the Purge Tool

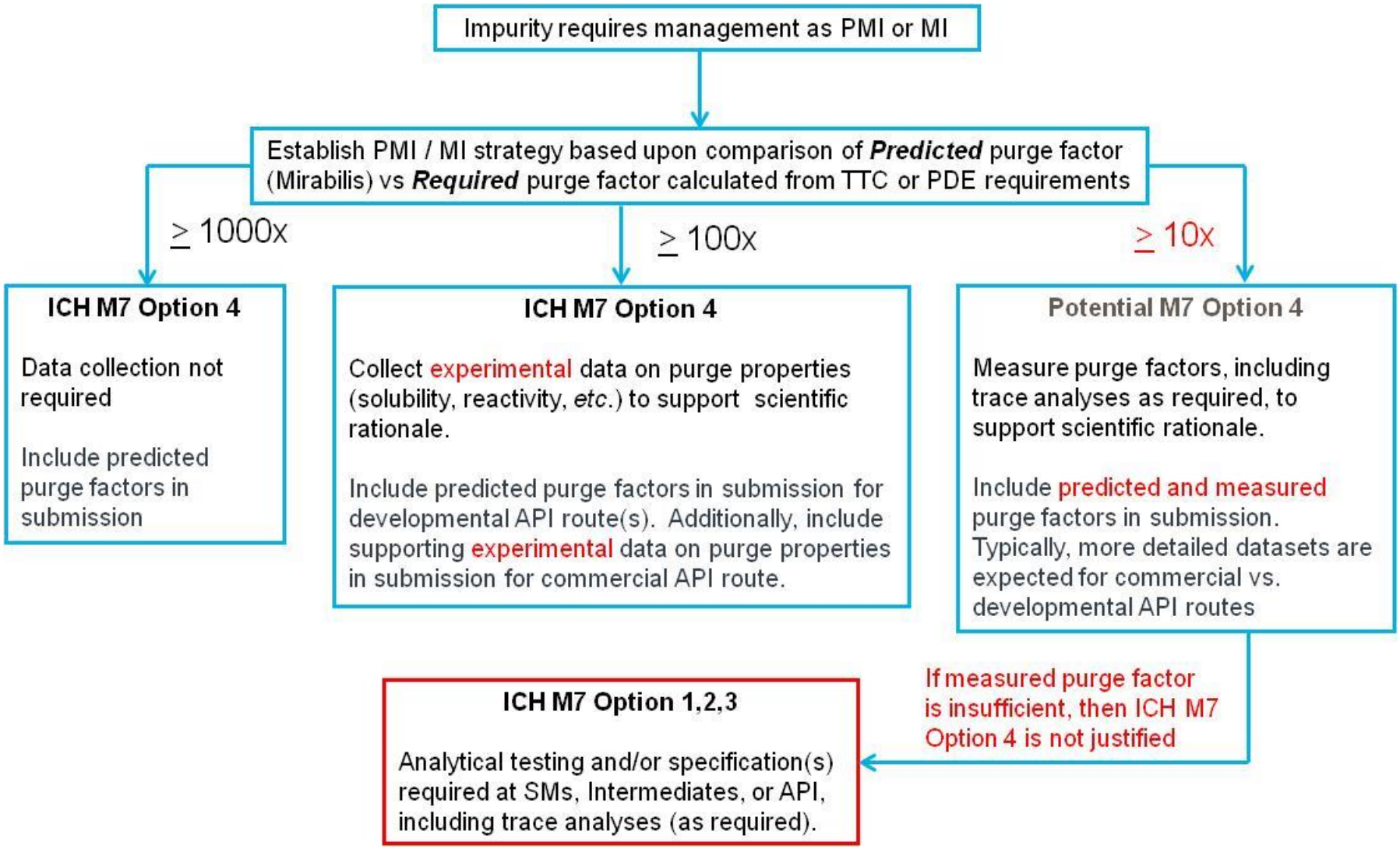
- Next Step once the calculation has been performed is to relate to the theoretical purge to the required purge.

- Examined case by case but would expect theoretical purge to be at least 10x, preferably 100x greater than required purge.
  - Even though purge tool systematically under-predicts.

- *What if predicted purge is lower than required?*
  - Analytical Testing
    - Proximity to point of introduction
    - Spike / Purge Studies

Required purge = Starting concentration / permitted concentration (based on permitted limit)

Control Option 4
Potential Decision Tree – Mirabilis Consortia



Application of purge approach and regulatory submissions – Experience

- Many organisations have applied or started applying semi-quantitative approach using purge factors as described in Teasdale et al *
- Others have used scientific rationale without explicit purge factors or purely provide analytical data to support submissions.
- Many have had risk assessments based on on the use of purge factors accepted

* *Org. Process Res. Dev.* 2013, **17**, 221-230

Purge Tool
Next Steps

- **Industry Consortia established**

- **Lhasa press release**

- *On the 17th of March 2014 the first face to face meeting took place at Burlington House, home of the Royal Society of Chemistry, in London between Lhasa Limited and the initial partners (which include AbbVie, AstraZeneca, Hoffman-La Roche, Novartis and Pfizer). Building on the approach taken by Dr. Andrew Teasdale at AstraZeneca, Lhasa limited and its partners will steer the development of the software whilst providing their expertise and data. The project is expected to last for three years and will result in the delivery of fully functional software.*

Progress on Building a Tool for Predicting the Purging of Mutagenic Impurities During Synthesis

General Approach

Aim to replicate simplicity of paper based approach but enhance consistency.

- Step 1: User enters full synthetic scheme leading to the drug substance (API)
- Step 2: Operations performed during the synthesis are organised in stages and steps
  - A step is any operation: reaction, work-up, purification
  - A stage consists of one reaction step, optionally followed by one or more work-up and/or purification steps
- Step 3: Each step can be assigned purge factors from reactivity, solubility, etc.
- Step 4 - The software will Calculate purge factors
  - For each impurity of concern
  - At each step/stage
  - Give overall purge factor for the entire synthetic route

- The calculation of purge values **based on knowledge** is the key factor and advantage of the in silico system over paper based calculations

The Consortium

- For this project, Lhasa Limited are closely working with the pharmaceutical industry AND regulators
- Working with them to:
  - Standardise of how purge factors are calculated
  - Identify gaps in knowledge
    - Provide data where possible
  - Prioritise work for building predictive models
  - Test and using the software prototypes
  - Engage with regulators

Reactivity predictions - Developing the Reaction Grid

- AstraZeneca put together a "reaction grid" to help aid in the assignment of the reactivity purge factor internally
- Based on expert knowledge of the reactivity of common classes of mutagenic impurities under well used reaction conditions
  - Colours indicate confidence in predictions:
  - Green – Well understood transformation / considerable data
  - Red – No knowledge, where no knowledge reactivity = 1

Alkylation

Reaction	Anticipated reagent (bench mark - high reactivity)	Anticipated conditions	Comments	Residual impurity class of concern: Primary alkyl iodides	Primary alkyl bromides	Primary alkyl chlorides	N- or S-Mustards	Halo-alkenes	Aryl-boronic acids	Epoxides	Acyl chlorides	Hydrazines	Hydrazides	Aliphatic aldehydes	Aromatic amino	Aromatic nitro	Sulfonate esters	Michael-reactive acceptors
N-Alkylation	Primary alkyl bromide and aliphatic amine	Excess R-X with respect to aliphatic amine, solvent		H	H	M	H	H	?	M	H	H	H	H	M	L	H	?
Reductive N-alkylation	Aldehyde and borohydride	Aliphatic amine		M	M	M	M	H	?	M	H	H	H	H	M	L	?	?
N-Arylation (Buchwald)	Aryl bromide and catalyst (Pd-PR3)			H	H	M	H	M	?	H	H	H	H	H	H	L	H	?
Amide N-alkylation	Base and primary alkyl bromide			H	H	M	H	H	?	H	H	H	H	H	M	L	H	?
Aniline N-alkylation	Primary alkyl bromide			H	H	M	H	H	?	H	H	H	H	H	H	L	H	?
O-Alkylation (ether formation)	Base and primary alkyl bromide			H	H	M	H	H	?	H	H	H	H	H	M	L	H	?
S-Alkylation (thioether formation)	Base (NaOH) and primary alkyl bromide			H	H	M	H	H	?	H	H	H	H	M	M	L	H	?

Evidence		Based on evidence from published literature and/or experimental data
		Limited evidence
		Little or no evidence (current assumption is no reaction)
		Reactive, but yields a mutagenic by-product
Reactivity	H	High reactivity - at least as reactive as reagent used in benchmark reaction
	M	Medium reactivity - reactive, but less so than benchmark reaction
	L	Low or no reactivity

59 different reactions – based on detailed literature review

Developing the Reaction Grid

- For the first version we wanted to include this in the tool to aid in decision making
- Collaborated with the consortium to do this through a method called "expert elicitation"
  - Each member was given the AZ reaction grid and asked to give their expert opinion on whether they agree or disagree with the proposed reactivity purge factors
  - Lhasa collated the results and modified the grid accordingly
    - If five or more members agreed on a reactivity purge factor then a consensus call was made
    - For those without consensus, a conservative call was made

Developing the Reaction Grid

- For example

Reactivity = 100	Reactivity = 10	Reactivity = 1	Call
6	1	0	100
1	1	5	1
4	3	0	10
1	3	3	1

- The first two rows illustrate a consensus call
- The third and fourth rows show disagreement and thus a conservative call would be made

Developing the Reaction Grid

- Unknown reactivities
  - There are some gaps in expert knowledge on how some mutagenic impurity classes react in various reactions
    - Aryl boronic acids
    - Hydrazine

  - If we don't know we don't guess – we assume un-reactive

Developing the reaction grid – desired state

- Ultimate aim is for reaction grid to become a Knowledge base
  - One where purge values are built on experimental data and published literature.

- Where there are gaps in knowledge
  - Experimental work being undertaken by the consortium
  - Protocol has been developed to measure the reaction kinetics of a representative impurity in a variety of reaction conditions
  - Classes being looked at:
    - Arylboronic acids
    - Alkyl bromides (proof of concept)
    - Hydrazines (on-going)
    - Aromatic amines (completion Q1 2016)

Reaction Grid - Experimental Work

- Example – reaction of phenylboronic acid under various reaction conditions

Observations

	Reaction Type	Reagent	Solvent	Reactive?
1	Reduction	H2 Pd/C	Dioxane	No
2		NaBH4	MeOH, THF, DCM	No
3		LiAlH4	THF	No
4		DIBAL-H	THF, DCM	No
5	Oxidation	H2O2	DCE, DCM, CH3CN	Yes
6		Peracetic Acid	DCM	Yes*
7		Oxone	CH3CN, H2O, H2O:CH3CN	Yes**
8		TEMPO	DCM	Yes***
9	Acids	Aq HCl	CH3CN, THF	No
10		Conc. H2SO4	H2O	No
11		Aq H2SO4	H2O, Dioxane, CH3CN	No
12		HBr/HOAc	DCM	No
13	Bases	Aqueous NaHCO3	CH3CN	No
14		10% NaOH	CH3CN, Dioxane, H2O	No
15		50% NaOH	H2O	Yes
16		DBU	CH3CN, DCE	No
17	Amide Bond Formation	CDI (with benzoic acid)	DCM	No
18		EDAc/HOPO (with benzoic acid)	DMF	No
19		Benzoyl chloride	THF	No
20	Nucleophiles	MeOH	THF	No
21		Benzyl amine	THF	No
22	Other Reagents	SOCl2	DCE	No
23		NCS	DCE	No
24		NCS/TEA	DCE	No
25		NBS	DCE	Yes****
26		Boc2O/TEA	THF	No
27		TMSCl/TEA	THF	No
28	Cross-Coupling	RuPhos-Pd complex (25 mol%), K2CO3, THF/H2O		?
29		Pd2dba3 (12.5 mol%), PtBu3HBF4 (25 mol%), TEA, THF		?

***Reaction was complete within 5 minutes at -78°C**
****Reaction was complete within 5 minutes at 2.5°C**
*****Reaction was complete within 5 minutes at 2.8°C**
******Reaction was complete within 5 minutes at 3.2°C**

Reaction Grid - Experimental Work

Organic Process Research & Development

Article

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A Kinetics-Based Approach for the Assignment of Reactivity Purge Factors

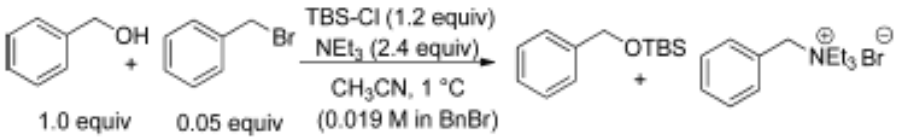
Rick C. Betori, Jeffrey M. Kallemeyn,* and Dennie S. Welch*

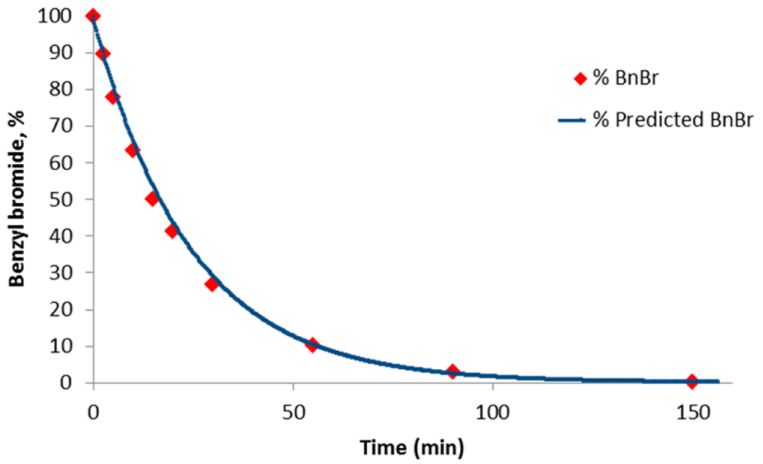
Process Chemistry, AbbVie Inc., 1 N. Waukegan Rd., North Chicago, Illinois 60064, United States

Supporting Information

ABSTRACT: The control of mutagenic impurities is of crucial interest to pharmaceutical companies and regulatory agencies alike. One risk-based methodology to assess the likelihood of impurity carryover to drug substance entails evaluation of the physicochemical properties of the entity against the parameters of the chemical process to which it is exposed. This article details a simple experimental approach that utilizes kinetic analyses to facilitate the assignment of reactivity purge factors. These reactivity purge factors are important values in the semiquantitative risk assessment for impurity carryover to drug substance.

Scheme 1. TBS Protection of Benzyl Alcohol in the Presence of Benzyl Bromide Impurity





The alignment between the rate constants and half-lives of the reaction of benzyl bromide with triethylamine in isolation and as a low-level impurity in the TBS protection of benzyl alcohol establishes the proof of concept that the kinetic information obtained from the stand-alone reaction can be used to predict impurity conversion in a more complex reaction matrix.

Reaction Grid - Experimental Work

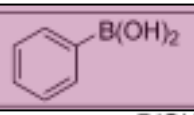
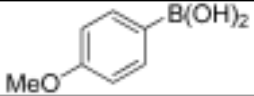
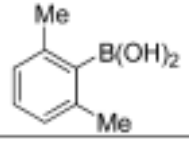
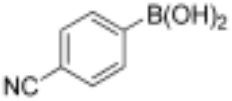
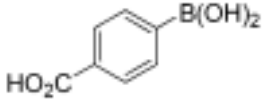
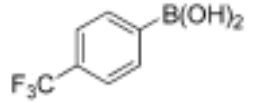
- Data from reactions can be used to determine purge factors i.e. want to normalise to match scoring system

$$\text{Purge factor} = \frac{1}{e^{-(kt)}} \qquad (7)$$

where t is the time of reaction and k is the rate constant of impurity; or

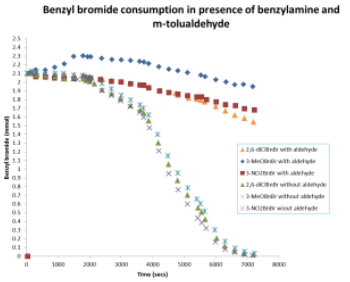
$$\text{Purge factor} = \frac{1}{e^{-(\ln(2)\times t/h)}} \qquad (8)$$

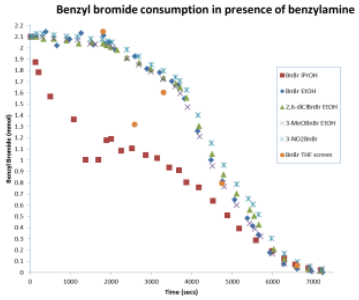
Table 6. Purge Factor Analysis for Arylboronic Acids at 95% Conversion of PhSMe at 21 °C

entry	arylboronic acid	predicted purge factor (in absence of PhSMe)	experimentally derived purge factor (in presence of PhSMe)	standardized reactivity purge factor assignment
1		2.1×10^5	1.4×10^4	100
2		3.3×10^8	6.5×10^6	100
3		1.2	1.1	1
4		153	5058	100
5		1253	9170	100
6		1640	506	100

Further Development

Reduction	$NaBH_4$	1	THF	5	0 – 60	No reaction	1
	$LiAlH_4$	1	THF	5	0 – 60	No reaction	1
	DIBAL-H	1	Toluene	5	0 – 60	No reaction	1



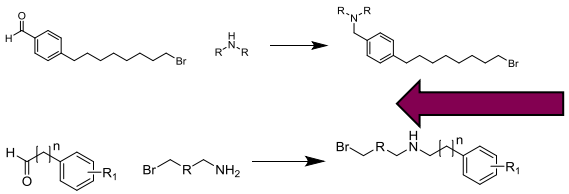


Experimental examples and supplementary info

Literature examples and supplementary info

Bromide	Solvent	Linearisation		Dynochem	
		k_{obs} (Temp) [1/s]	E_a [kJ/mol]	k_{obs} (Temp) [1/s]	E_a [kJ/mol]
Benzyl bromide	EtOH	1.3E-04 (2°C) 7.7E-04 (20°C) 2.5E-03 (34°C)	66	7.5E-04 (20°C)	63
Benzyl bromide	iPrOH	1.7E-04 (2°C) 7.5E-04 (18°C) 2.0E-03 (32.5°C)	56	9.0E-04 (20°C)	76
3-Methoxybenzyl bromide	EtOH	1.2E-04 (0.6°C) 8.1E-04 (20°C) 2.3E-03 (34.4°C)	60	7.9E-04 (20°C)	66
3-Nitrobenzyl bromide	EtOH	1.1E-04 (0.7°C) 6.1E-04 (20°C) 1.8E-03 (35°C)	57	6.0E-04 (20°C)	58
2,6-dichlorobenzyl bromide	EtOH	1.2E-04 (0.6°C) 6.9E-04 (20°C) 2.2E-03 (34.5°C)	60	6.8E-04 (20°C)	61

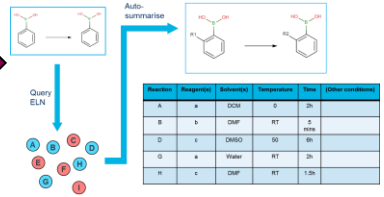
Reaction mining database summary and supplementary info



Literature references

Purge Factor = 1(10, 100)

- **Why the purge factor has been assigned**
- **Summary of data from literature, experiments etc**
  - **Impurity reaction with individual components**
  - **Impurity reaction in real scenario**
  - **Mechanisms?**
  - **Products?**
- **Scope and effects (eg temp, solvent, structural)**



Links to other models?

Solubility Predictions

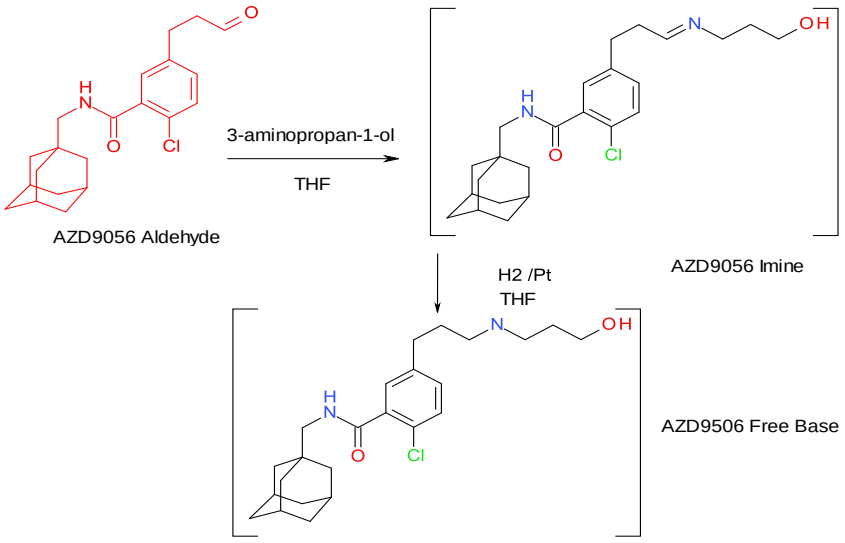
- Initial thought to base on Pharmacopeial definition of Solubility.
  - In practice this is rarely achievable even for reactants
  - Such an Approach more suited to pharmaceuticals in simple solvents.

- Approaches:
  - Option 1: Base on experimental data

    - Measured values or Reaction System
      - Applicable where mutagenic impurity is a reagent.

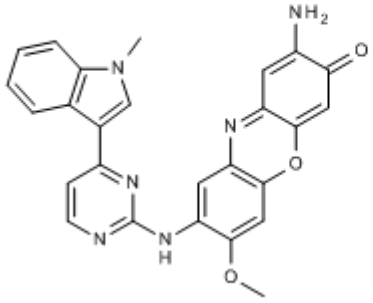
    - Important to factor in - initial solubility and required solubility at end of reaction.

In reality at point of isolation of desired product level of Mutagenic reagent may be <1% of initial level. Thus if intrinsically soluble at start of reaction small changes in solvent system unlikely to affect solubility.

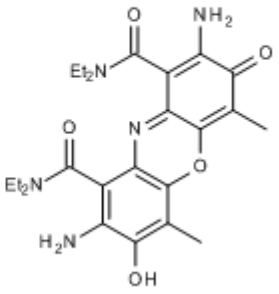


Solubility Predictions

- Approaches:
  - Extrapolation
    - It is possible to extrapolate solubility from one solvent to another
    - It is even possible to do '*ab initio*' calculations.
  - Surrogate data
    - May be possible to find data for similar structures in the literature at least for chemical transformations.



Theoretical impurity (level <<0.1%)



Literature data – range of solvents >10g/l

Conclusions

- The purge tool concept provides a quick and effective way of assessing the risk posed by an MI.

- **CRITICALLY** - The development of this as an *in silico* tool provides the basis for a systematic approach based on **knowledge**, one aligned directly with principles defined in ICH M7.