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

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ABSTRACT: 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 strategy, focusing specifically on the effective use of *in silico* assessment tools to augment this process, in particular the calculation of theoretical purge factors based on the physicochemical properties of a specific GTI and its interrelationship to the process.

1. INTRODUCTION

Synthetic processes used to produce pharmaceutical drug substances typically require the use of electrophilic agents to facilitate carbon−carbon, carbon−nitrogen, carbon−oxygen, and carbon−sulfur bond formation. Examples include alkylating agents, benzyl halides and Michael acceptors. Some of these agents may also possess the ability to react with biological substrates such as DNA, which would raise concerns about their potential carcinogenicity. Any residues of a confirmed DNA-reactive electrophilic reagent or intermediate in a drug substance would be categorized as genotoxic impurities (GTIs). Existing regulatory guidelines such as ICH Q3A (R2)/Q3B (R2)/Q3C (R4)[1−3] do not adequately address the requirements for controlling trace levels of GTIs.

In 2004 the EU issued a draft guidance followed by a finalized version in 2006 that addressed controlling GTIs in marketing applications for pharmaceuticals.[4] This European Medicines Agency (EMA) guideline recommended that, in cases where specific safety data were unavailable for a GTI, the GTI should be controlled to a level based on a threshold of toxicological concern (TTC). The guideline recommended the TTC as a default limit of daily exposure to the patient of 1.5 μg on the basis of an increased cancer risk of 10^{-5}.

Also in 2006, a PhRMA task force issued its white paper proposing a staged TTC wherein exposure to higher levels of GTIs would be acceptable in cases where duration of exposure is limited, as is the case during clinical development.[5] The higher limits were based on an extrapolation from daily lifetime exposure to short-term exposures of less than 12 months[6] but with an additional safety factor applied, so the calculated excess risk of cancer was 10^{-6} instead of 10^{-5}, since early clinical trials often include healthy volunteers, and even for patients the pharmacological benefit of the drug may not yet have been established. The white paper also classified impurities into five categories ranging from those with no structural alerts for genotoxicity to those that are known to be carcinogenic. The principles of structural alerts were originally proposed by Ashby and Tennant[7] on the basis of structure/Ames mutagenicity/carcinogenicity correlations for compounds containing an electrophilic functional group (or a compound that could be metabolised to one containing such a reactive entity, e.g. some aromatic amines).

In 2008, the EMA adopted the staged TTC approach in its published Q&A document, while reducing the allowed limits to half of those proposed by the PhRMA task force.[8] As an example, the EMA guidance allowed a limit of daily exposure of 60 μg for clinical trials lasting less than 30 days instead of 120 μg as proposed by the PhRMA task force. A similar staged TTC strategy was proposed by the FDA in their draft guideline issued later in 2008.[9]

GTIs may be generated during drug substance synthesis, drug product manufacture, or during storage of drug substance or drug product. In many cases, GTIs can be controlled during the drug substance synthetic process provided that the GTIs in question are not generated as either a drug substance degradation product or produced during drug product manufacturing or storage. In cases where the GTI is generated through interactions with manufacturing processing agents (e.g., excipients, residual solvents, etc.) or during storage during normal conditions, controlling the levels of the GTI in the drug product may also be required. Many of the principles implemented for the control of GTIs in drug substance will

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Table 1. PhRMA survey results on GTIs

areas of general consensus	areas of diversity
evaluation of the API synthetic pathway for GTIs at the preclinical stage	point in the synthetic process to begin monitoring for GTIs
consider the number of steps back from the final API that the GTI originates when providing a rationale for not testing the GTI	use of limit tests vs quantitative reporting of GTIs
use of scientific justification in lieu of testing when a GTI is introduced early in the process and is reactive enough to be eliminated in the downstream chemistry or process	degree of validation of methods used for control of GTIs

also apply for drug product. However, the drug product process is often constrained in its ability to purge (or remove) GTIs.

A critical tenet of drug substance process development is to ensure that GTIs introduced or generated during the process are controlled to acceptable levels. On the basis of EMA guidance[4] these limits are currently based on the staged TTC, TTC, or compound specific safety data associated with the impurity(ies) concerned. GTI control may be executed through implementation of simple process operations such as washes and crystallization steps and in many cases indirectly through further downstream synthetic reactions. Thus, the development of the drug substance process includes considerations that are based not only on scale-up, safety, economic and environmental factors but also on the production of an API with impurity levels that are in compliance with regulatory standards.

The evaluation and control of GTIs in a synthetic process is a multidisciplinary activity. Considerations from toxicological, processing, and analytical perspectives are involved and must align with regulatory requirements. While current regulatory guidance does provide clear expectations regarding the allowed limits of GTIs during clinical development and at marketing application, there are still a number of areas open to interpretation. Examples of GTI regulatory gray areas include defining the scope of the search for GTIs (number of chemical steps back from the final drug substance in the synthesis and consideration of hypothetical byproducts), how scientific justification (based on chemical expertise and knowledge of the chemistry of the synthetic process) can be used in lieu of analytical testing, expectations for type of analytical methodology and required level of validation, and universal understanding of specific controls as a function of stage of clinical development. A benchmarking survey, issued by Pharmaceutical Research and Manufacturers of America (PhRMA) in 2009,[10] reflects that many of these areas are addressed differently by industry and regulatory agencies (Table 1).

The proposals described in this paper expand upon the EMA guideline and FDA draft guidance to describe strategic approaches for evaluation of GTIs throughout the various stages of pharmaceutical development. It details risk assessment strategies that can be implemented to evaluate which GTIs should be measured analytically and where testing can be eliminated or reduced on the basis of scientific rationale.

2. A QUANTITATIVE RISK ASSESSMENT FOR EVALUATION OF GENOTOXIC IMPURITY CARRYOVER

2.1. Scope of Evaluation. As outlined in the Introduction, the majority of GTIs arise from the synthetic process itself. Synthetic drug substances are typically constructed through systematic modification of a compound's molecular framework through the formation and reconfiguration of carbon−carbon, carbon−nitrogen, carbon−oxygen, and carbon−sulfur bonds. Current synthetic methodology is such that the formation of such bonds is predominantly achieved through the use of electrophilic reagents. Their inherent reactive nature and potential to react with nucleophiles (including DNA) may raise concerns from the perspective of carryover into the final drug substance. However, highly reactive electrophilic reagents should be effectively purged from the API synthesis by any subsequent downstream chemistry. Therefore, an evaluation of the risk posed by such impurities is required. The assessment of GTI carryover involves identifying the potential presence/removal of such entities, as the synthetic reaction proceeds to the final API. It is critical that such an assessment balances the risk of observing the GTI in the final drug substance with the probability of its removal (purge) based on knowledge of the chemistry used in the synthetic process. It is impractical to evaluate/identify every conceivable impurity; hence, such an assessment therefore needs to be based on process understanding of likely/probable impurities. Indeed the EMA guideline advocates such an approach.[4]

2.2. The Evaluation Process. Evaluation of genotoxic potential is generally performed initially through a comparison of structures of reagents/starting materials/intermediates in the synthetic scheme with those of known genotoxins, either through simple comparison with a known alerting functionality, e.g. Ashby−Tennant alerts,[7] through searches of published information, or through assessing structures in a (quantitative) structure/activity relationship SAR/QSAR software database such a DEREK (deductive estimation of risk from existing knowledge) or MCASE for *in silico* evaluation. Use of other sources of toxicological data, e.g. TOXNET, can also be useful, this being particularly true when addressing relatively common reagents for which specific safety data may already exist.

It is critical that any such assessment is augmented by human knowledge and expertise. For example, the data set underpinning the *in silico* system should be evaluated in order to ensure that there is sufficient database coverage to allow the reviewer to have confidence in either a positive or a negative result from the SAR analysis.

The structures assessed typically include starting materials, reagents, intermediates, and known process impurities. This is often further augmented during the development process by the inclusion of additional structures that are derived from either increased knowledge of the synthetic process (in terms of impurities associated with the process) and/or identified degradation products of the drug substance (and product where applicable).

Compounds that do not contain structural alerts for genotoxicity are treated as conventional impurities and are controlled in accordance with ICHQ3A/3B/3C.[1−3] Compounds with structural alerts for genotoxicity require further action. These compounds can be tested for mutagenicity through *in vitro* methods, typically Ames testing. If the Ames test is negative, then the impurity can be treated as a conventional impurity and managed as per ICH Q3A/3B/3C guidelines.

2.3. Risk Assessment of GTI Carryover. If a starting material, reagent, intermediate, or a byproduct is identified as a possible GTI, it may appear that the best way to control it would be to eliminate its presence through an alternate synthesis route. However, in most cases, this is an impractical approach because an alternate synthesis may produce lower yields, have poorer overall quality control, and ultimately may not be economically feasible. More importantly, it is highly likely that any alternate synthesis will contain other impurities that require similar control at the TTC level, and significant additional effort will be required to discharge the risk of those new impurities, without materially improving the safety of the API or reducing the overall risk to patients. Control of GTIs rather than elimination is therefore strongly advocated. Similar considerations apply to ALARP (as low as reasonably practicable) and were accepted by EMA in the Q&A supplements.[8]

To demonstrate control, an assessment of the potential risk of carryover of a GTI at a level exceeding staged TTC or TTC levels to the drug substance is made. This is aligned with quality by design (QbD) and the risk assessment principles enshrined in ICHQ8 and Q9.[11,12] A seemingly simple but potentially myopic way to address this is to develop a sensitive analytical method and test for the possible GTI in question at the point after introduction, at the final drug substance, or at some intermediate point, i.e. Quality by Testing (QbT). This approach, however, can be a technically challenging and resource-intensive activity, especially when applied to all the GTIs that are associated with the synthetic process and runs contrary to the tenants of QbD. Moreover this approach fails to recognize that reactive GTIs will often be destroyed or eliminated in the subsequent process steps leading to the final drug substance.

The use of scientific understanding in relation to evaluation of GTI-related risk is growing. Several articles[13,14] have outlined the use of QbD principles in the assessment of GTI-related risk, these being centered on the use of spiking/purge fate studies to prove the ability of the synthetic process in question to remove specific GTIs. Such approaches are extremely useful when applied in the context of a fixed process.

Work conducted by the Product Quality Research Institute (PQRI) relating to sulfonate esters[15,16] (Teasdale et al.) compellingly demonstrated how a good scientific understanding of the properties of a compound, combined with a risk assessment of the impact of process conditions, can be used effectively to control, or even eliminate the potential GTI risk posed.

Such approaches as those described above, although comprehensive, are impractical to apply to all possible GTIs within each and every synthetic process. What is sought instead is a risk-based mechanism whereby the potential risk of carryover can be quickly and simply assessed. For many impurities, chemistry-based arguments alone may be sufficient to conclude that an impurity would not reside in the final API without the need for laboratory experimentation. This approach is often used when processing conditions for the downstream purge or destruction of the impurity are obvious. A consistent framework for this type of risk-based assessment whereby the potential for carryover can be assessed through consideration of the physicochemical properties of the GTI and the relevant processing conditions is recommended, the potential benefits to regulatory reviewers being obvious. Such an approach is not a new concept. Both Dobo[17] and Pierson[18] have described empirical approaches to such assessments based on the point of introduction of the GTI in question and the number of stages removed from the API. Such an approach, euphemistically referred to as 'stage counting', has unfortunately not been universally accepted by regulatory authorities. Several regulatory agencies continue to ask the Sponsor to provide analytical data even in cases where compelling scientific arguments would suggest proof of absence. Such arguments should be sufficient, particularly when the assessment addresses the chemistry (reactions and purifications) specific to each step that is being "counted" in a particular process. Nonetheless, it would be useful if there were an alternative approach that contained a quantitative (or semiquantitative) element, as is presented and described in the following section.

It is possible to take such an approach to risk assessment on the basis of the specific physicochemical properties of a GTI, along with an understanding of the process conditions to which it is exposed, to quantitatively assess the risk. These risk-based assessments rely on the concept of evaluating purge factors which can be defined as the ability of a synthetic process to purge (eliminate) a particular GTI.[19,20]

2.4. Purge Factors. The principle supporting the calculation of a purge factor is a simple one. It is to identify the key physicochemical parameters that influence the removal of a specific GTI in a process (in QbD terms: critical quality attributes (CQAs)) and to use these, in combination with the specific process conditions, to determine a purge factor, i.e. how much of the GTI is likely to be removed.

The key parameters identified are reactivity, solubility, volatility, ionisability, plus any additional physical process designed to eliminate impurities, e.g. chromatography. The latter approach is often employed in medicinal chemistry synthetic routes employed for preparing test materials for first human clinical trials. In order to ensure that a consistent approach is taken, a scoring system has been devised which is outlined in Table 2.

2.4.1. Reactivity. The purpose of this specific parameter is to assess the reactivity of the GTI in question in relation to processing conditions to which it is subsequently exposed. On the basis of chemical reactivity toward typical reagents encountered during workup or storage (e.g., other chemicals used within the process, water, acid, base, alcohols, etc.) it is proposed that GTIs could be generally placed into one of three categories (see Table 3).

It is critical, though, that any such classification must take into account the specific process conditions to which the GTI is exposed, and the attributes of the particular impurity. This is examined in more depth through two specific examples, one for a highly reactive GTI, e.g. acyl halide, and the second for a less reactive aryl nitro compound.

Thionyl chloride is typically used to convert nonreactive carboxylic acids into reactive acyl halides, which can then be further processed (e.g., formation of esters, amides, etc.).[21] However, due to their high reactivity they are very efficiently purged from the subsequent downstream chemistry reaction(s); this also applies to thionyl chloride itself which reacts violently with water.[22]

In contrast, a relatively stable nitro compound would show high reactivity, under most reduction conditions, to form an aromatic amine, but it may well be unreactive during subsequent stages of the process.

Experimental data relating to the process (e.g. in process reaction monitoring) can be used to refine this parameter. For

Table 2. Purge factors

physicochemical parameters	purge factor[a]
reactivity	highly reactive = 100
	moderately reactive = 10
	low reactivity/unreactive = 1
solubility[b]	freely soluble = 10
	moderately soluble = 3
	sparingly soluble = 1
volatility[c]	boiling point >20 °C below that of the reaction/process solvent = 10
	boiling point within ±10 °C of that of the reaction/process solvent. = 3
	boiling point >20 °C above that of the reaction/process solvent = 1
ionisability	ionisation potential of GTI significantly different from that of the desired product[d]
physical processes: chromatography	chromatography: 10−100 based on extent of separation
physical processes: e.g. other scavenger resins	evaluated on an individual basis.

[a]Purge factor = concentration before purging/concentration after purging. [b]This relates to solubility within the context of a recrystallisation process whereby the impurity in question, if highly soluble, will remain within the mother liquors and hence be purged from the desired product. [c]This refers to the deliberate removal of a solvent through solvent distillation or solvent exchange. [d]This relates to a deliberate attempt to partition the desired product/GI between an aqueous and organic layer, typically achieved through the manipulation of pH to change the ionised/un-ionised state of one of the components.

Table 3. Genotoxic compounds classified on the basis of reactivity

reactivity class	genotoxic groups
highly reactive	epoxides/aldehydes/sulfonate esters/acyl halides/aziridines/hydrazines
moderate reactivity	N or S mustards/Michael reactive acceptors/halo-alkenes, primary halides
low reactivity	amino aryls, nitro compounds, purines or pyrimidines, carbamates

example where an in-process control shows completion of reaction (<1% GTI remaining) this can be used to classify the GTI concerned as highly reactive for that stage of the process.

2.4.2. Solubility. Where a genotoxic reagent/intermediate is introduced into the synthetic process the process is generally optimized to maximize both yield and product quality. One critical factor in any process is the requirement that the intended reaction actually occurs. This generally means that the genotoxic reagent/intermediate in question is likely to be highly soluble in the solvent selected for the process in question. This solubility will mean that where the process concerned involves isolation of the product as a solid, the genotoxic reagent/intermediate should remain in the reaction mother liquors and be removed when the liquors are filtered off. Assignment of a purge factor for this parameter is thus based on the solubility of the GTI in question within the process solvent. Table 4 outlines the definitions used within the USP relating to solubility. For the purposes of the purge factor calculations these have been grouped to match the three classes defined in Table 1.

The scale used for the solubility factor has been set over a range 1−10. Experience has shown that the solubility factor could much higher, perhaps justifying a range of 1−100 as was used for reactivity. However we believe that that the more conservative scale of 1−10 should be retained to compensate for variance in process conditions such as uncontrolled crystallization, poor washing and/or inefficient deliquoring of the isolated product.

2.4.3. Volatility. Many low-molecular weight, potentially genotoxic impurities (PGIs) such as aldehydes and alkyl halides are volatile; e.g. methyl chloride formed through the reaction of methanol and HCl (during HCl salt formation) has a boiling point of −23 °C. Many synthetic processes employ solvent distillation or solvent exchange. As a result of such a process, any volatile GTI present may also be removed, depending on the volatility of the GTI relative to either the boiling point of the solvent or the temperature of the reaction process.

2.4.4. Ionisability. Where the ionisability of a GTI and that of the matrix in which it is present differ, for example an ionisable GTI within a nonionisable intermediate or a nonionisable GTI within an ionisable drug substance, the potential exists to reduce the level of the GTI through liquid/liquid extraction, by employing a two-phase system with appropriate manipulation of the pH of the aqueous phase.

The removal of an alkyl halide GTI from an ionisable drug substance provides one example of such an approach. pH adjustment of the aqueous phase could allow the drug substance to be extracted into the aqueous phase; the nonionisable alkyl halide GTI remains in the organic phase that can then simply be discarded. Thereafter, the pH of the aqueous phase can be adjusted and the drug substance back-extracted into an appropriate fresh organic solvent. In addition to classical liquid/liquid extractions, solid-phase extraction (SPE) can also be employed.

Note: A specific purge factor is only assigned in relation to this parameter where such an approach is specifically applied.

2.4.5. Chromatography. Although potentially costly in comparison to the simple processing techniques already described, chromatography nevertheless does provide a very powerful and flexible tool for removal of a GTI where required, particularly for early-phase, nonoptimized, medicinal chemistry, synthetic routes.

Table 4. Solubility definitions[23]

descriptive term	solubility class	parts of solvent required for 1 part of solute	solubility range (mg/mL)	solubility assigned (mg/mL)
very soluble	freely	<1	>1000	1000
freely soluble		1−10	100−1000	100
soluble	moderately	10−30	33−100	33
sparingly soluble	sparingly	30−100	10−33	10
slightly soluble		100−1000	1−10	1
very slightly soluble		1000−10000	0.1−1	0.1
practically insoluble or insoluble		>10000	<0.1	0.01

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Preparative HPLC, normally in a normal phase mode, is an established technique applied to the reduction or removal of impurities, at a multikilogram scale, usually at the drug substance stage. The removal of a GTI could simply be considered a subset of the standard chromatographic challenge of impurity removal.

An alternative to the use of chromatography is the use of resins. Lee et al. (2010) recently demonstrated the ability of certain nucleophilic cation-exchange resins to effectively purge sulphonate esters from the final stage of an API.[24]

Again a specific purge factor is only assigned in relation to this parameter where chromatography is specifically applied.

2.4.6. Calculation of Purge Factors. As outlined, scores for each parameter are assigned on the basis of the physicochemical properties of the GTI relative to the process conditions. The scale is based on the premise that a high purge factor equates to high GTI clearance. Thus, a high purge factor value indicates a low probability that a GTI will be observed on the basis of knowledge of product's physicochemical properties and understanding of the synthetic process.

For each stage these individual purge factors are then simply multiplied together to determine a 'purge factor' for that stage. The overall purge factor is then simply a multiple of the factors for individual stages.

Where the overall calculated purge factor would indicate the level of a GTI to be >100 times below the appropriate TTC limit, then no further action should typically be required.

Where the value of the calculated purge factor would indicate the level to be between 10 and 100 times below the appropriate limit, then it should potentially be supported by conduct of appropriate analytical testing/further process investigation. This may take the form of, for example, periodic testing or use of purge and spike experiments as confirmation for removing long-term testing.

Where the calculated purge factor indicates that the level could exceed the appropriate TTC, then analytical testing as a minimum would be required. It may also provide an indication of the need to modify the processing conditions to further reduce the risk of carryover.

How this might be applied is addressed in the following hypothetical examples.

2.4.6.1. Example 1. For a product dosed at 150 mg a day that is intended for chronic use, the TTC limit of 1.5ug/day would, in concentration terms, represent a limit of 10 ppm for any GTI present. Should there be a genotoxic intermediate, then the calculation of risk of carryover would start at a point of 100%, i.e., 1,000,000 ppm of the intermediate in the step in which it is produced. Thus, in this instance a purge factor of 100,000 would indicate that the process is likely to reduce the level of the GTI in question to a level equivalent to the TTC. (i.e., [concentration before purging, 1,000,000 ppm]/[purge factor, 100,000] = concentration after purging, (10 ppm).

Thus, in this instance calculated purge factors of >10,000,000 should preclude the need for any further investigation.

Calculated purge factors between 1,000,000 and 10,000,000 signal that further investigation may be required, and finally calculated purge factors of <100,000 would require as a minimum analytical testing of the GTI in question.

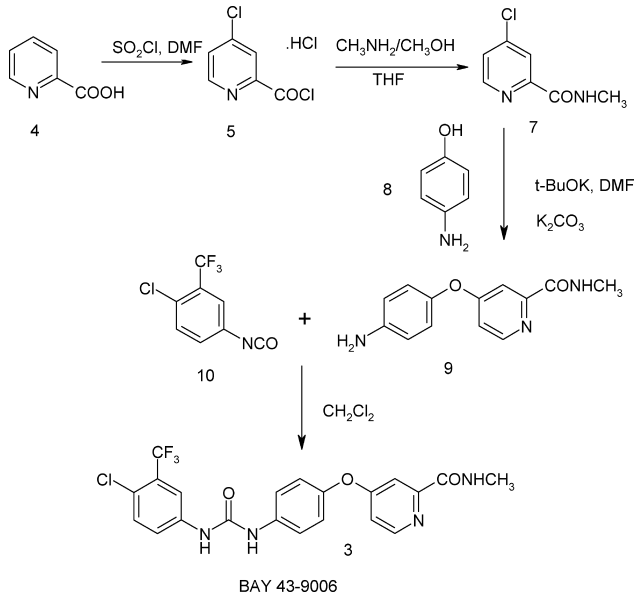
2.4.6.2. Example 2. For the same product described above, reaction monitoring has shown that during a salt formation step, a level of approximately 5% methyl chloride is formed. This measurement can be used to calculate the purge factor required and allow for adjusting the purging efficiency, if needed. In this context the required purge factor would be 5000, as based on the maximum level of methyl chloride potentially present is 5% = 50,000 ppm. Thus, in this instance a purge factor of >500,000 associated with methyl chloride should preclude the need for any further investigation.

This is explored in further detail through a series of examples.

3. RISK ASSESSMENT CASE STUDIES

The following section outlines a number of examples that serve to illustrate both the utility of the purge tool and its robustness.

Scheme 1. Synthesis of BAY 43-9006



It should be noted that the examples include both known mutagens and potential mutagens. There is no intent to infer anything about the specific status of the impurities in question, merely to use them as examples for the specific purpose of demonstrating the utility of the purge tool.

The initial case studies describe in detail the process by which purge values are selected serving to illustrate the rigor involved in this process. Case studies 3−5 focus on the accuracy of the purge factor calculation, in each case, through comparison with actual data.

3.1. Case Study 1: Purging of Highly Reactive Thionyl Chloride from Synthesis of BAY-43-9006. The synthetic scheme for the production of BAY-43-9006 is shown in Scheme 1. Thionyl chloride ($SOCl_2$) is introduced into stage 1 (of a 4-stage process) to convert nonreactive picolinic acid (**4**) into the corresponding reactive acyl halide intermediate (4-chloropyridine-2-carbonyl chloride) (**5**). Although, thionyl chloride is reported to Ames positive, there is increasing evidence that this is an artifact of the test system. Ames tests performed in DMSO give positive Ames findings, whereas the same test performed in acetonitrile is Ames negative.[25] It is well-known that sulfinyl chlorides can react with DMSO to produce chlorodimethylsulfide (CDMS), a known mutagen.[26] The CDMS is probably the agent responsible for the Ames positive result of the test article in DMSO.

The acyl halide (**5**) is reacted with methylamine to produce the corresponding amide (**7**) in 88% yield. The amide is then coupled with 4-aminophenol (**8**) in the presence of base to produce the penultimate intermediate ([4−4-aminophenoxy)-

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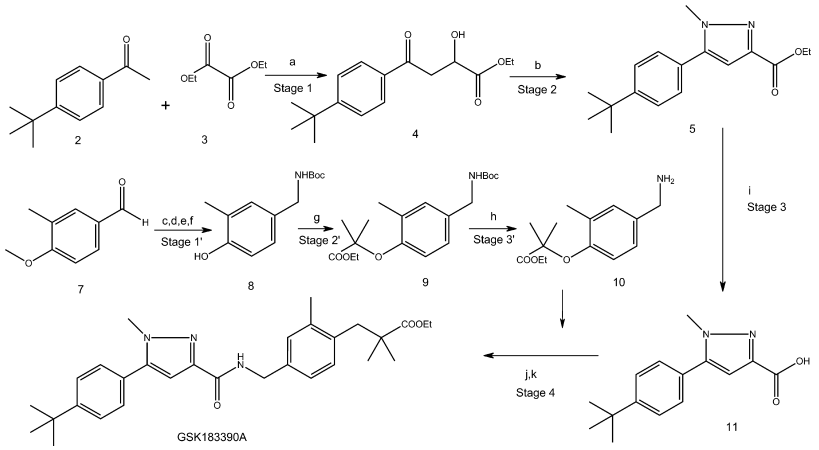
Table 5. Predicted purge factors for thionyl chloride in BAY 43-9006 process

stage	reactivity (R)	solubility (S)	volatility (V)	physical processes (PP)	comments
1	100	10	10	1	$SOCl_2$ is a very reactive intermediate (R = 100), and the high yields (89%) support a high reaction efficiency proposition. Analyte has high solubility in DMF (S = 10), and intermediate **5** is isolated, washed and dried under vacuum. Thionyl chloride boils at 79 °C (V = 10). There is no physical processing in this stage (PP = 1).
2	100	10	1	3	$SOCl_2$ will react with methylamine base and with aqueous brine (used to extract isolated intermediate) (R = 100). Analyte has high solubility in THF (S = 10). There is no specific drying step (V = 1). The isolated intermediate (**7**), though, is dissolved in ethyl acetate and washed/extracted with aqueous brine (PP = 3).
3	100	10	1	3	$SOCl_2$ will react with bases (K-*tert*-butoxide/K_2CO_3) and with aqueous brine (used to extract isolated intermediate) (R = 100). Analyte has high solubility in DMF (S = 10). There is no specific drying step (V = 1). The isolated intermediate (**7**), though, is dissolved in ethyl acetate and washed/extracted with aqueous brine (PP = 3).
4	1	10	10	1	Reactivity is predicted to be low, as the reaction is performed in a nonaqueous environment (R = 1). Analyte has a high solubility in dichloromethane (S = 10). Any residual thionyl chloride, though, is likely to be removed on drying.

Scheme 2. Synthesis of GSK183390A[a]



[a]Reactions conditions: (a) NaOEt, 0−80 °C, yield 99%; (b) CH_3NHNH_2, EtOH, 90 °C, yield 22% (unwanted isomer yield 45%); (c) $NH_2OH{\cdot}HCl$, NaOAc, EtOH, room temperature, yield 93%; (d) ammonium formate, Pd/C catalyst, reflux, MeOH, yield 50%; (e) aq HBr, yield 97%; (f) Boc_2O, CH_2Cl_2, Et_3N, yield 96%; (g) K_2CO_3, DMF, ethyl bromoisobutyrate, yield 69%; (h) TFA, CH_2Cl_2, yield 82%; (i) NaOH, room temperature, yield 98%; (j) $SOCl_2$, toluene, 80 °C, then Et_3N, **10**, room temperature, yield 96%; (k) NaOH, 80 °C, yield 73%.

Table 6. Theoretical purge factors for GTIs in PPARα/γ agonist (1) synthetic process

GTI	stage	reactivity H = 100, M = 10, L = 1	solubility F = 10, M = 3, L = 1	volatility H = 10, M = 3, L = 1	total multiple per stage	rationale for purge factor
CH_3NHNH_2	2	100	10	3	3000	bp of CH_3NHNH_2 88−90 °C, solvent removed by distillation/partial drying
	3	1	3	3	9	product isolated and dried
	4	10	10	3	300	likely reaction with SO_2Cl and also with the acyl chloride formed (step j); product isolated and dried
	ALL				**8.1×10^6**	
SO_2Cl	4 (j)	100	10	10	10000	highly reactive, volatile bp 79 °C
	4 (k)	100	10	10	10000	reacts instantaneously in aqueous environment
					1.0×10^8	

(2-pyridyl)]-*N*-methylcarboxamide) (**9**) in 87% yield. This intermediate (**9**) is then coupled with isocyanate (**10**) to yield the API (**3**).

The basis by which predicted values are selected and the associated rationales are described in Table 5.

The overall purge factor is 9×10.[12] The extremely high reactivity of thionyl chloride to high pH/aqueous conditions dominates the purging factor in the first three stages.

3.2. Case Study 2: Retrospective Evaluation of a PPARα/γ Agonist (GSK183390A). The synthetic scheme (Scheme 2) shows the original medicinal chemistry route used to manufacture a PPARα/γ agonist in drug development. From a GRA perspective there are two known genotoxic compounds involved in the synthesis:

(1) Methyl hydrazine is used in stage 2 to cyclise the diketone (**4**) to produce the pyrazole ester (**5**).
(2) Thionyl chloride is used to activate the pyrazole (**10**) via the formation of an acid chloride which is then reacted with the benzylamine (**10**) to form the API.

A purge factor assessment has been applied to this route to determine the level of risk from a genotoxic impurities perspective as part of an evaluation of the potential continued use of the procedure described (Table 6).

Empirically, the risk of carryover of GTIs/PGIs is often assessed on the basis of proximity (point of introduction in the synthesis) to the final API. Thus, thionyl chloride theoretically poses the highest risk with respect to carry over into the API as it is introduced into the final coupling stage. In practice this is

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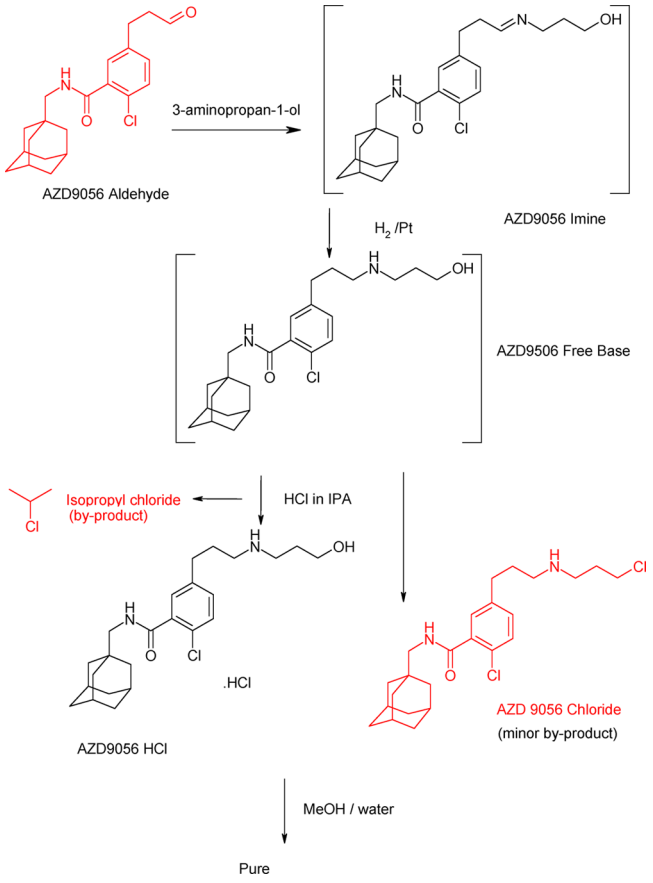
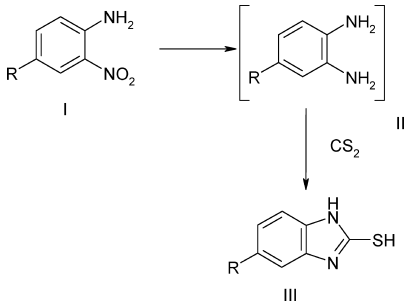
Scheme 3. Synthesis of AZD9056[19]



Scheme 4. Synthesis of starting material (III)



not the case for the following reasons. Thionyl chloride is highly reactive, both with the intended reactant (**11**) and with water (step k, Table 6); this, combined with its volatility (bp 79 °C) and its miscibility in toluene (reaction solvent), facilitates an efficient purge of any unreacted material and ensures that there will be no carryover into the final API and, importantly, no requirement to test for any residual thionyl chloride in the API. The theoretical purge factor is 1.0×10^8, which supports the contention that there will be no requirement to test for thionyl chloride in API.

Similarly, methyl hydrazine (theoretical purge 8.1×10^8) will be efficiently purged from the reaction.

3.3. Case Study 3: AZD9056. This example relates to the synthesis of a developmental drug AZD9056. Within the later stages of the synthesis three potentially genotoxic impurities were identified as being of concern (see Scheme 3).

The three potential GTIs were the following:

(1) AZD9056 aldehyde: an intermediate within the synthesis of AZD9056;
(2) isopropyl chloride: formed during the isolation stage through the reaction between isopropyl chloride and hydrochloric acid (HCl),
(3) AZD9056 chloride: formed through reaction between the API (an alcohol) and HCl.

A risk assessment was conducted for each of these in turn, and the overall results are displayed in Table 7.

3.3.1. AZD9056 Aldehyde. On the basis of a purge factor prediction of 10,000, this would indicate that the likely carryover of this compound into the final API is likely to be <100 ppm. Given its close proximity to final API, the purified API was investigated analytically to find the actual levels present. A comparison of the overall prediction with the experimental results shows that the calculated purge factor

Table 7. Comparison of predicted and measured purge factors for AZD9056 process

identity of potentially genotoxic impurity	stage	reactivity	solubility	volatility	purge factor/stage	overall purge factor	measured purge factor
AZD9056 aldehyde	crude (free base) nonisolated	10 (moderate)[a]	1[b]	1 involatile	10		
AZD9056 aldehyde	crude isolated	1 unreactive	10	1 involatile	10		
AZD9056 aldehyde	pure	1 unreactive	10	1 involatile	10		
AZD9056 aldehyde						**10 × 10 × 10 = 10000**	**112000**
AZD9056 chloride[c]	crude (free base) nonisolated	N/A	N/A	N/A	N/A		
AZD9056 chloride	crude isolated	1 unreactive	1	1 involatile	1		
AZD9056 chloride	pure	1 unreactive	3[d]	1 involatile	3		
AZD9056 chloride		1 × 3 = 3	10				
isopropyl chloride	crude (free base) nonisolated	N/A	N/A	N/A	N/A		
isopropyl chloride	crude isolated	1	10	10	100		
isopropyl chloride	pure	1	10	10	100		
isopropyl chloride						**100 × 100 = 10000**	**38500**

[a]Although conversion essentially goes to completion, the reaction is only moderately fast; hence, a conservative figure of 10 was applied (as opposed to 100). [b]Although highly soluble, the aldehyde is not purged because the crude is not isolated. [c]Chloride impurity is generated in the crude stage. [d]Solubility, although low, is greater than that of AZD9056 HCl salt.

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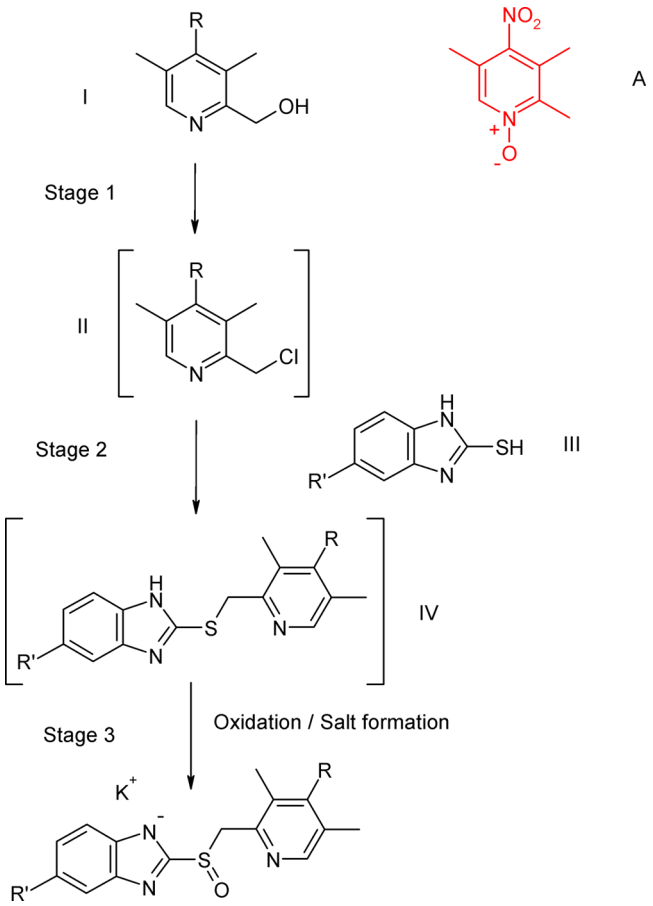
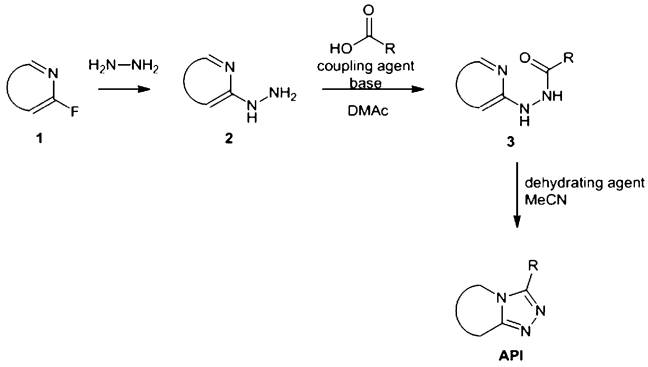
Table 8. Comparison of predicted and measured purge factors for nitro-aniline impurity (I)

identity/structure of GI of concern	stage details	reactivity (H = 100, M = 10, L = 1)	solubility (F = 10, M = 3, L = 1)	volatility (H = 10, M = 3, L = 1)	purge factor /stage	measured purge factor
nitro-aniline	reduction	100	1 nonisolated	1	100	
nitro-aniline	cyclisation	10	10	1	100	not detected <5 ppm
					overall calculated purge factor	
					10,000	

Scheme 5. Synthesis of omeprazole potassium salt



Scheme 6. Synthesis of an API involving the use of hydrazine and a substituted hydrazine intermediate (compound 2)



underpredicts the purge capacity of the process by a factor of 10. Had a less conservative value of 100 been applied to the reactive term for the reductive amination step, then the predictive value would have matched the actual observed value.

3.3.2. Isopropyl Chloride (IPC). The experimental measurements showed that levels of IPC in solution exceeded 5% w/w. Given the fact that such a high level of a PGI is formed in the latter stages of the synthesis, this would generally be a significant cause for concern. This is certainly true were a 'step counting' approach taken. Again a risk assessment was made, and this gave a predicted purge factor of 10,000 (compared to a measured purge factor of 40,000). Put in the context of the level formed (5% = 50,000 ppm) then with a predicted purge factor of 10,000, this would indicate a predicted level of <5 ppm, a prediction supported by the actual analytical results.

3.3.3. AZD9056 Chloride. This example is perhaps the most interesting as it provides testament to the ability of this risk assessment tool to gain a critical insight into the process. In this instance the impurity formed is unreactive, relatively insoluble, and nonvolatile, such that the calculated purge factor of 3 indicates that the process is therefore unlikely to effectively purge this impurity. This again tallies with the observed factor of 10. Thus, in this instance the prediction indicates the need for additional control, either to control formation of the impurity through process control or modification of the process to facilitate its removal.

3.4. Case Study 4: GI in a Registered Starting Material. The following example is derived from the synthesis of a registered starting material and relates to an assessment of the risk of GIs being present within it as a result of its synthesis (Scheme 4). The impurity of concern is the nitro precursor (I)

In this example the overall calculated purge factor was 10,000, thus indicating a low probability of carryover into the starting material (<100 ppm). This prediction was shown to be correct when the actual purge factor was measured, the

Table 9. Comparison of predicted and measured purge factors for nitropyridyl *N*-oxide impurity (A)

identity/structure of GI of concern	stage details	reactivity (H = 100, M = 10, L = 1)	solubility (F = 10, M = 3, L = 1)	volatility (H = 10, M = 3, L = 1)	total multiple per stage	measured level (ppm)
nitropyridyl (A)	stage 1: chloride formation	1	1 nonisolated	1	1	
nitropyridyl (A)	stage 2: coupling	1	1 nonisolated	1	1	2000 (based on 3000 spike)
nitropyridyl (A)	stage 3: oxidation/ salt formation	1	10	1	10	<1

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Table 10. Comparison of predicted and measured purge factors for hydrazine and substituted hydrazine intermediate (compound 2)

identity/structure of GI of concern	stage details	reactivity (H = 100, M = 10, L = 1)	solubility (F = 10, M = 3, L = 1)	volatility (H = 10, M = 3, L = 1)	total multiple per stage	measured level
hydrazine	stage 1: hydrazide formation	100	10	1-no distillation	1000	
	stage 2: final intermediate	100	3	1	300	
	stage 3: cyclisation/isolation	100	3	1	300	
	overall calculated clearance			**9×10^8**	**<0.1 ppm (LOD)**	

identity/structure of GI of concern	stage details	reactivity (H = 100, M = 10, L = 1)	solubility (F = 10, M = 3, L = 1)	volatility (H = 10, M = 3, L = 1)	total multiple per stage	measured level
intermediate 2						
	stage 2: final intermediate	100	10	1	1000	
	stage 3: cyclisation/isolation	100	3	1	300	
	overall calculated clearance			**3×10^5**	**<1 ppm (LOD)**	

impurity (I) not being detected (<5 ppm) (Table 8). As this relates to an impurity within a starting material that itself is introduced into the overall synthetic process at a number of stages away from the isolated API, it is proposed that the use of this risk assessment tool could be used to quickly evaluate the risk of carryover, in this instance into a starting material, and thus eliminate the need for specific analytical testing.

4.5. Case Study 5: GI in a Registered Starting Material (Example 2). In this example the specific concern relates again to a potential GI (A) within a starting material (I). In this instance the impurity of concern (A) is a nitropyridyl *N*-oxide derivative (Scheme 5).

What is interesting with this specific example is that the impurity of concern (A) is unreactive in both stages 1 and 2 of the process (see Table 9). Furthermore, as neither stage is isolated, then there is no opportunity to purge the impurity through removal based on solubility, and the impurity is not volatile. Hence, the calculated purge factor is 1; that is, in stages 1 and 2 of the process there is unlikely to be any significant reduction in the level of impurity A. This was mirrored experimentally when a spike of 3000 ppm (equivalent to the specification limit of 0.3%) was made into starting material (I), analysis at stage 2 within the nonisolated intermediate (IV) showed levels to have reduced to only 2000 ppm, a factor of less than 2. Subsequent analysis of the final isolated intermediate showed that levels had been reduced to <1 ppm; this was due to the high solubility of the impurity within the solvent system employed for this stage. A comparison of the experimental result to the predicted purge factor (for this stage a value of 10) illustrates the conservative nature of the solubility term when assessing the risk of carryover.

4.6. Case Study 6: Hydrazine. In this example the risk of carryover of a highly reactive (nonelectrophilic) reagent, hydrazine, and a reactive intermediate, substituted hydrazine (**2**), was assessed, as shown in Scheme 6.

This example illustrates well the advantage of this focused approach to calculation of GI-related risk. Hydrazine, a highly reactive known carcinogen, is used in this process, three stages from the API. A classical stage-counting approach based on an assumed purge of 10 per stage would indicate a 1000-fold reduction in the level. In comparison, the calculated purge factor is 9×10^8. This is borne out by the experimental results that show levels in the API to be not detected (LOD 0.1 ppm).

With respect to the reactive intermediate **2**, despite its closer proximity to the final API, the calculated purge factor is again high, 3×10^5. This is mirrored in the experimental data that showed levels to be <1 ppm at LOD level (see Table 10).

■ CONCLUSION

A thorough evaluation of the risk posed by GTIs is now a crucial part of the overall process of evaluating the quality of medicines. Such an evaluation involves assessing the potential for carryover of the GTI(s) in question at levels of concern into the API and/or drug product. This contribution describes a number of ways in which such an evaluation can be conducted, including the novel approach of purge factor calculation.

The advantage of such an approach over other empirical approaches is that it provides a quantitative assessment of the risk, based on an easily understood and standardized scale. Not only can this approach be used to provide compelling evidence to back up a chemical evaluation, it can also, as shown through the real case study examples, drive a better understanding of GTI-related risk such that attention is ultimately focused on analytical control of those specific GTIs that pose an actual rather than a theoretical risk of presence in the final drug substance.

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Notes

The authors declare no competing financial interest.

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