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GENOTOXIC IMPURITIES IN SMALL MOLECULE DRUG SUBSTANCES: IDENTIFICATION, RISK ASSESSMENT, AND CONTROL STRATEGIES

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ABSTRACT: Impurities in active pharmaceutical ingredients, especially genotoxic impurities, pose significant risks to patient safety and require stringent regulatory control. This review summarizes key regulatory expectations under ICH Q3A, Q3B, M7, and S2, covering impurity identification, qualification, and control. It discusses major risk-assessment approaches, including toxicological evaluation, genotoxic impurity classification, and acceptable intake determination using compound-specific data or the Threshold of Toxicological Concern. The review also highlights risk-based control strategies based on process understanding, analytical monitoring, and minimization of impurity levels. An integrated approach to impurity management is essential for ensuring product quality, regulatory compliance, and patient safety.

INTRODUCTION: An impurity is any component of the drug substance other than its defined chemical entity. The synthesis of an API commonly involves the use of reactive reagents that generate intermediates and by-products, which may persist as impurities in the final drug substance. Impurities can arise either from the manufacturing process (process-related impurities) or from the degradation of the API (degradation impurities) [1]. A subset of these impurities may possess mutagenic and potentially carcinogenic properties, as such impurities provide no therapeutic benefit to patients; in fact, certain chemically reactive impurities may pose unwanted toxic effects, thereby impacting patient safety as well as overall product quality.

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Regulatory authorities require drug substance manufacturers to maintain effective control over GTIs. To meet these requirements, considerable efforts are required during the development of drug substances to ensure that impurity levels are maintained within acceptable safety limits. Comprehensive and systematic evaluations are performed to identify potential GTIs that could arise at each step of the chemical synthesis process, considering the key starting materials (KSM), solvents, reagents, and raw materials used.

Impurities identified at each stage are subsequently assessed for control either through the monitoring of incoming raw materials or through a demonstrated understanding of their fate and purge, with the objective of minimizing their presence in the final drug substance. The occurrence of potential impurities in the final commercial drug substance can be anticipated based on data from stability studies, chemical development studies, and routine batch analyses [1].

Regulatory expectations emphasize that GTIs should preferably be controlled to levels that pose negligible carcinogenic risk; and where feasible, reduced to "as low as reasonably practicable" (ALARP) in the finished drug substance.

Literature Search and Selection Methodology: A systematic literature review was performed to identify relevant studies addressing the identification, risk assessment, and control of genotoxic impurities in small-molecule drug substances. Search was conducted using major electronic databases, including PubMed, Scopus, and Google Scholar. Search terms comprised combinations of keywords such as "genotoxic impurities," "GTI," "mutagenic impurities,""GTI control strategy," and "nitrosamine," linked through Boolean operators (AND, OR) to enhance the precision and comprehensiveness of the search. Publications published from 1995 to 2025 were screened to ensure coverage of both historical and contemporary advancements. Only peer-reviewed articles and relevant publications published in English were considered for inclusion.

Classification of Impurities: Impurities in drug substances are generally classified into two main categories: organic impurities (process-related and drug-related) and inorganic impurities. Organic impurities may be formed during the manufacturing process and/or during storage of the drug substance, and include starting materials, intermediates, by-products, degradation products, reagents, ligands, and catalysts. Inorganic impurities typically arise from the manufacturing process and may include reagents, ligands, catalysts, heavy metals, inorganic salts, and other materials such as filter aids or charcoal. Representative examples of process impurities include alkyl sulfonates (e.g., methyl methanesulfonate and ethyl methanesulfonate), alkyl halides, epoxides, and aromatic amines. In contrast, degradation impurities are generated during manufacturing or storage because of hydrolytic, oxidative, thermal, or photolytic degradation and may include reactive aldehydes, epoxides, α,β-unsaturated carbonyl compounds, and nitrosamines. Structural alerts commonly associated with mutagenicity include nitrosamines, alkyl sulfonates, epoxides, aziridines, alkyl halides, hydrazines, aromatic amines, and α, β-unsaturated carbonyl systems.

Thresholds for Impurities in Drug Substance: ICH Q3A(R2) establishes reporting, identification, and qualification thresholds based on the maximum daily dose of the drug substance, as summarized in **Table 1** below.

TABLE 1: THRESHOLDS FOR DRUG SUBSTANCE

Maximum Daily Dose [1]	Reporting Threshold [2, 3]	Identification Threshold [3]	Qualification Threshold [3]
≤ 2 g/day	0.05 %	0.10% or 1.0 mg/day intake (whichever is lower)	0.15% or 1.0 mg/day intake (whichever is lower)
> 2 g/day	0.03 %	0.05 %	0.05 %

[1] The amount of drug substance administered per day, [2] Higher reporting thresholds should be scientifically justified, [3] Lower thresholds can be appropriate if the impurity is unusually toxic

For non-mutagenic impurities, qualification should be performed in accordance with the thresholds specified in ICH Q3A(R2) [1] for drug substances and ICH Q3B(R2) [2] for drug products. Under these guidelines, impurities are managed based on reporting, identification, and qualification thresholds that are established according to the maximum daily dose.

Impurities present below the reporting threshold generally do not require further action, while impurities exceeding the identification and qualification thresholds should be identified and toxicologically qualified as appropriate [3]. In contrast, impurities that are known or predicted to be mutagenic are assessed according to the risk-based framework described in ICH M7, irrespective of the qualification thresholds defined in ICH Q3A/Q3B. The presence of a structural alert for mutagenicity necessitates an assessment using expert evaluation, *in-silico* methodologies, and/or experimental testing such as the bacterial reverse mutation (Ames) assay, as appropriate.

Mutagenic impurities are controlled using the Threshold of Toxicological Concern (TTC) or compound-specific acceptable intakes where available, rather than the conventional qualification thresholds applied to non-mutagenic impurities [4].

Therefore, impurities present above the identification threshold should not automatically undergo genotoxicity evaluation solely because of their level. Instead, genotoxicity assessment is warranted when a structural alert or other evidence indicates a potential mutagenic risk. For impurities lacking mutagenic concern, qualification should follow the ICH Q3A/Q3B framework. Conversely, impurities identified as mutagenic or potentially mutagenic should be evaluated and controlled in accordance with ICH M7, regardless of whether they are present above or below the qualification thresholds established for non-mutagenic impurities.

Safety Assessment of Impurities: Qualification is the process of generating and assessing data to establish the biological safety of an individual impurity or an impurity profile at specified levels. Degradation products that are significant metabolites observed in animal and/or human studies are generally considered to be qualified. Toxicokinetic parameters, such as AUC and Cmax, are useful for establishing the relationship between systemic exposure levels and the concentration of an impurity in the drug substance. The ICH Q3A(R) guideline provides a comprehensive framework for the qualification of impurities in drug substances.

To identify available toxicological data on the potential impurities, a comprehensive literature search should be conducted across multiple electronic databases, including but not limited to PubMed, OECD SIDS, NTP, BIBRA, NICNAS, ECHA dossiers, EPA, EFSA, as well as regulatory resources such as the US FDA, EMA, TGA, and PMDA. In general, process and degradation impurities lack sufficient toxicological data, and when such data exists, it is often not readily accessible. In accordance with ICH M7(R2) [3], the mutagenic potential of impurities is typically assessed using two complementary (quantitative) structure–activity relationship [(Q)SAR] methodologies, one based on expert rule-based systems and the other on statistical-based models. The use of two independent methodologies provides a more robust evaluation of bacterial mutagenicity than either approach alone. Rule-based systems identify structural features and mechanistic alerts that have been associated with DNA reactivity and mutagenicity based on expert-curated toxicological knowledge. In contrast, statistical-based models derive predictions from experimental datasets by identifying relationships between biological activity and molecular descriptors, structural fragments, and physicochemical properties using statistical and machine-learning approaches[5]. The complementary use of these methodologies enables both mechanistic interpretation and data-driven prediction of mutagenic risk. Prior to interpretation of the results, it is important to assess whether the impurity falls within the applicability domain of each model. Predictions generated outside the applicability domain may be associated with greater uncertainty and should be interpreted with caution. Commonly used QSAR platforms include commercial software such as Derek Nexus, Sarah Nexus, CASE Ultra, and Lead scope, as well as open-source tools including CAESAR, SARPY, KNN, TIMES-AMES, Toxtree, Symmetry, and ADMEWORKS. Selection of the appropriate tools should be based on regulatory acceptance, model performance, transparency of predictions, and suitability for the chemical class under evaluation.

The output from the two complementary QSAR methodologies should not be used in isolation. Rather, the predictions should undergo expert review, which considers the presence or absence of structural alerts, the reliability of the prediction, the applicability domain, relevant experimental data, published literature, and mechanistic understanding of the impurity. When both methodologies provide concordant negative predictions and no concerning structural features are identified, the impurity may generally be concluded to have no mutagenic concern. Conversely, concordant positive predictions or the presence of well-established mutagenic structural alerts typically support classification as a potential mutagenic impurity.

In cases where the two QSAR methodologies produce conflicting predictions, an expert review is required to evaluate the weight of evidence. Factors such as the strength and relevance of structural alerts, model confidence, analogue data, metabolism considerations, and available experimental results should be considered before reaching a final conclusion. Where uncertainty remains, additional investigations, including a

bacterial reverse mutation (Ames) assay, may be warranted. Based on the integrated assessment of available toxicological data, complementary (Q)SAR predictions, and expert review, impurities are classified according to the risk-based framework described in ICH M7(R2). The final outcome determines whether an impurity can be considered non-mutagenic, requires further testing, or should be controlled as a mutagenic impurity using appropriate health-based exposure limits

Based on the available toxicological data and *in-silico* predictions, the ICH Multidisciplinary Guideline M7(R2) outlines an approach for the assessment of GTIs and classifies them into five categories, as summarized in **Table 2** below.

TABLE 2: CLASSIFICATION OF IMPURITIES BASED ON THEIR MUTAGENIC AND CARCINOGENIC POTENTIAL, AND RESULTING CONTROL ACTIONS

Class	Definition	Proposed action for control
1	Known mutagenic carcinogens	Control at or below compound-specific acceptable limit
2	Known mutagens with unknown carcinogenic potential (bacterial mutagenicity positive* no rodent carcinogenicity data)	Control at or below acceptable limits (appropriate threshold of toxicological concern (TTC))
3	Alerting structure, unrelated to the structure of the drug substance; no mutagenicity data	Control at or below acceptable limits (appropriate TTC) or conduct bacterial mutagenicity assay;if non-mutagenic = Class 5; if mutagenic = Class 2
4	Alerting structure, same alert in drug substance or compounds related to the drug substance (e.g., process intermediates), which have been tested and are non-mutagenic	Treat as non-mutagenic impurity
5	No structural alerts or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity.	Treat as non-mutagenic impurity

*Or other relevant positive mutagenicity data indicative of DNA-reactivity related induction of gene mutations (e.g., positive findings in *in-vivo* gene mutation studies).

For Class 1 impurities, where compound-specific toxicological data are available, a compound-specific acceptable intake should be established. This can be derived from rodent carcinogenicity potency data, such as TD_{50} values (doses giving a 50% tumor incidence equivalent to a cancer risk probability level of 1:2). A linear extrapolation to an acceptable lifetime cancer risk of 1 in 100,000 can be performed by dividing the TD_{50} by 50,000.Alternatively, the benchmark dose lower confidence limit for a 10% response ($BMDL_{10}$), which accounts for the shape of the dose–response curve and represents a dose with 95% confidence of not exceeding a 10% tumor incidence in rodents, may be used in place of TD_{50}. In this case, linear extrapolation to a 1 in 100,000 risk level is achieved by dividing $BMDL_{10}$ by 10,000.However, evolving scientific evidence suggests that the direct application of linear low-dose extrapolation for potential carcinogens may be overly conservative and could result in an overestimation of carcinogenic risk. In the absence of compound-specific toxicological data, acceptable intake limits for GTIs are determined using the TTC concept. The TTC concept was developed to establish an intake level for unstudied chemicals that would pose a negligible risk of carcinogenicity or other toxic effects. It originated from work conducted in 1958 and 1960 and was later refined through research and analyses by the US FDA. Based on these evaluations, the FDA introduced the "threshold of regulation" (ToR) for trace substances originating from food contact materials, representing a level below which the overall risk of toxicity, including lifetime cancer risk, is considered negligible (less than 1 in 1,000,000). For potential leachables from food contact materials, a threshold of 0.5 ppb was established, corresponding to a daily intake of 1.5 µg, assuming a consumption of 3000 g of food and beverages per day. For application in pharmaceutical risk assessment, a TTC value of 1.5 µg/day for GTIs is considered appropriate, corresponding to an excess lifetime cancer risk of 1 in 100,000 (10^{-5}). However, certain high-potency mutagenic carcinogens collectively referred to as the "cohort of concern," (COC) including aflatoxin-like compounds, N-nitroso compounds, and alkyl-azoxy compounds are excluded from the application of the TTC approach.

Example 1: Derivation of Acceptable Intake from TD_{50}: *N*-Nitrosodimethylamine (NDMA) is a potent mutagenic carcinogen with a reported TD_{50} value of 0.0959 mg/kg/day. A compound-specific acceptable intake (AI) corresponding to an excess lifetime cancer risk of 1 in 100,000 can be derived using the linear extrapolation approach recommended in ICH M7.

AI = TD_{50} / 50,000 = 0.0959 mg/kg/day / 50,000 = 0.00001918 mg/kg/day. For a 50-kg adult AI = 0.00001918 mg/kg/day x 50 = 0.0000959 mg/day i.e. 95.9 ng/day. Thus, the compound-specific acceptable intake for NDMA would be 96 ng/day.

Example 2: Conversion of Acceptable Intake to a Specification Limit: To establish a specification limit for NDMA in a drug substance, the acceptable intake is divided by the maximum daily dose (MDD) of the drug. Assume that NDMA is present in valsartan and the maximum daily dose of valsartan is 80 mg/day. The specification limit (ppm) can be calculated as: AI (ng/day) / MDD (mg/day) = 96 / 80 = 1.2 ppm. Accordingly, the NDMA should be controlled at a specification limit of not more than 1.2 ppm in the Valsartan drug substance to ensure that patient exposure does not exceed the acceptable intake of 96 ng/day. Another exception to the use of the default TTC value applies to alkyl chloride compounds, which are commonly used in drug synthesis. These compounds are classified as monofunctional or multifunctional alkyl chlorides. Monofunctional alkyl chlorides are generally much less potent carcinogens compared to multifunctional alkyl chlorides, with reported TD_{50} values ranging from 36 to 1810 mg/kg/day. A TD_{50} value of 36 mg/kg/day can therefore be considered a conservative class-specific potency reference for calculating acceptable intake limits for monofunctional alkyl chlorides. This value is at least tenfold higher, indicating lower carcinogenic potency than the TD_{50} of 1.25 mg/kg/day, which is back calculated from the default lifetime TTC of 1.5 µg/day. Accordingly, this supports the justification for setting acceptable daily intake limits for monofunctional alkyl chlorides at levels up to ten times higher than the default TTC value [6]. The acceptable intake for individual impurities can be determined based on the duration of drug treatment, as outlined in the table below.

TABLE 3: ACCEPTABLE INTAKES FOR INDIVIDUAL IMPURITY

Duration of treatment	<1 Month	>1 to 12 Months	>1 to 10 Years	>10 Years to Lifetime
Daily intake (µg/day)	120	20	10	1.5

When there are three or more Class 2 and Class 3 impurities specified in drug substance specification, the total mutagenic impurities should be controlled in accordance with the limits described below.

TABLE 4: ACCEPTABLE TOTAL DAILY INTAKES FOR MULTIPLE IMPURITIES

Duration of treatment	<1 Month	>1 to 12 Months	>1 to 10 Years	>10 Years to Lifetime
Total Daily intake (µg/day)	120	60	30	5

In some cases, impurity levels may be sufficiently high so that the resulting daily patient exposure exceeds the TTC-based acceptable intake limit of 1.5 µg/day. Under such circumstances, manufacturers are required to optimize the manufacturing process to reduce impurity levels to as low as reasonably practicable (ALARP). Alternatively, genotoxicity studies may be conducted to overrule *in-silico* predictions or repeat-dose toxicity studies may be performed to qualify the impurity at higher exposure levels.

Assessment of Nitrosamines and NDSRIs: Nitrosamine risk assessment is an extensive subject that warrants comprehensive evaluation and detailed discussion. However, given the genotoxic potential of nitrosamines, a brief overview of the risk assessment approach is discussed below.

N-nitrosamines and nitrosamine drug substance-related impurities (NDSRIs) are of significant regulatory concern owing to their potential carcinogenicity and their presence in both drug substances and drug products. Regulatory guidance differentiates between small-molecule nitrosamines (e.g., NDMA, NDEA, NMPA, NDBA, NDIPA, and NIPEA) and NDSRIs (e.g., *N*-nitroso-sertraline, *N*-nitroso-benazepril), which are structurally related to the active pharmaceutical ingredient (API) and are generally formed through

nitrosation of API-derived secondary or tertiary amine functionalities. For small-molecule nitrosamines with robust carcinogenicity data, compound-specific acceptable intake (AI) limits are derived from their carcinogenic potency [7]. However, most nitrosamine drug substance-related impurities (NDSRIs) lack long-term carcinogenicity data [8]. Consequently, regulatory authorities have adopted alternative approaches for establishing acceptable intake limits, including the Carcinogenic Potency Categorization Approach (CPCA) [9] and scientifically justified read-across methodologies that utilize structurally related nitrosamines with known carcinogenic potency data. These approaches enable the estimation of carcinogenic risk and the derivation of health-based exposure limits in the absence of compound-specific carcinogenicity data.

A key component of NDSRI risk assessment is potency categorization, in which structural factors known to influence nitrosamine carcinogenicity including the microenvironment surrounding the N-nitroso moiety, the presence of α-hydrogen atoms, steric hindrance, and metabolic activation pathways are evaluated to assign a potency category and derive an appropriate acceptable intake (AI). Where suitable structural analogues exist, scientifically justified read-across approaches may be employed to support AI determination using available carcinogenic potency data. Such assessments should be supported by a comprehensive evaluation of structural and mechanistic similarities and expert scientific review.

Because some nitrosamines have demonstrated weak or inconsistent responses under standard bacterial reverse mutation (Ames) assay conditions, regulatory authorities recommend enhanced Ames test (EAT) protocol for the evaluation of nitrosamines and NDSRIs. These conditions typically include a 30-minute preincubation step, an optimized metabolic activation system comprising 30% rat liver S9 and 30% hamster liver S9 fractions, and at least two known mutagenic *N*-nitrosamines, including NDSRIs, as positive controls. These modifications improve metabolic activation and enhance assay sensitivity for the detection of nitrosamine mutagenicity. Current regulatory expectations require manufacturers to perform comprehensive nitrosamine risk assessments throughout the product lifecycle. Such assessments should include the evaluation of potential sources of nitrites and secondary or tertiary amines, as well as manufacturing processes, formulation components, packaging systems, and storage and transportation conditions that may contribute to nitrosamine formation [10]. Where a potential nitrosamine or NDSRI is identified, a science- and risk-based evaluation should be conducted to establish an appropriate acceptable intake (AI), implement suitable analytical control strategies, and develop effective mitigation measures to minimize patient exposure.

This integrated approach combines structure-based assessment, potency categorization, read-across evaluation, enhanced mutagenicity testing, and risk-based control strategies to ensure the effective identification, assessment, and management of nitrosamine-related risks in pharmaceutical products.

Genotoxicity Studies: *In-vitro* and *in-vivo* genotoxicity studies are designed to identify compounds that can cause genetic damage through various mechanisms. These assays facilitate hazard identification by detecting DNA damage and its subsequent fixation. Extensive evaluations have demonstrated that many substances that show mutagenic potential in the bacterial reverse mutation (Ames) assay are also carcinogenic in rodents. Therefore, a battery approach is recommended, as no single test can adequately detect all genotoxic mechanisms involved in tumor development.

The ICH S2B guideline recommends two alternative options for the standard genotoxicity testing battery [11]:

Option 1:

1. A test for gene mutation in bacteria.
2. A cytogenetic test for chromosomal damage (the *in-vitro* metaphase chromosome aberration test or *in-vitro* micronucleus test), or an *in-vitro* mouse lymphoma *Tk* gene mutation assay.
3. An *in-vivo* test for genotoxicity, generally a test for chromosomal damage using rodent

hematopoietic cells, either for micronuclei or for chromosomal aberrations in metaphase cells.

Option 2:

1. A test for gene mutation in bacteria.
2. An *in-vivo* assessment of genotoxicity with two different tissues, usually an assay for micronuclei using rodent hematopoietic cells and a second *in-vivo* assay. Typically, this would be a DNA strand breakage assay in liver, unless otherwise justified. *In-vivo* tests account for the absorption, distribution, metabolism, and excretion of test compounds, which can influence the genotoxic activity of a compound.

While the two options in the battery are equally suitable, choice would be based on knowledge about drug substances. For example, if systemic exposure in animal models is equal to or less than the anticipated clinical exposure, *in-vitro* assays should be employed (Option 1). On the other hand, Option 2, including a test in liver, is recommended in cases where short-lived reactive metabolites are expected to be generated in the liver.

For certain compounds, toxicokinetic or pharmacokinetic data demonstrate poor systemic absorption, indicating that they are not available at target tissues. In such cases, *in-vivo* studies are not considered useful for genotoxicity assessment. Examples of these compounds include certain radioimaging agents, aluminum-based antacids, and topically applied pharmaceutical products.

Compounds that yield negative results across all tests in Option 1 are generally considered to provide sufficient assurance for the absence of genotoxic potential. However, compounds that produce positive findings in the standard test battery may require further, more extensive evaluation, depending on their intended therapeutic indication. Also, Ames negative impurities at levels exceeding the ICH Q3A qualification threshold need to be further evaluated for genotoxicity and subchronic toxicity studies based on intended therapeutic application of drug substance. The standard battery does not include an independent test designed specifically to test for aneuploidy. This information may be derived from mitotic index, polyploidy induction, and micronucleus evaluation from *in-vitro* and *in-vivo* tests for chromosomal damage.

Drug substance where the Ames test (bacterial reverse mutation test) is not appropriate for the assessment of genotoxicity e.g., antibiotics (bactericidal compounds) and compounds known or suspected to interfere with the mammalian cell replication system (e.g., topoisomerase inhibitors, nucleoside analogues, or inhibitors of DNA metabolism). In such cases, usually two *in-vitro* mammalian cell tests (e.g. Mouse lymphoma assay (MLA), *in-vitro* micronucleus test) should be performed using two different cell types and of two different endpoints gene mutation and chromosomal damage.

If general toxicity studies are warranted, the duration of the study should be aligned with the intended duration of drug treatment. On a case-by-case basis, single-dose studies may be appropriate, particularly for drugs administered as a single dose. For repeated-dose studies, durations ranging from a minimum of 14 days up to a maximum of 90 days are generally considered appropriate. Impurity test material used in non-clinical studies should possess adequate purity, typically ≥95% as per industry practice, to ensure that any observed toxicity can be directly attributed to impurity itself.

Control of Genotoxic Impurities: A control strategy is a planned set of controls derived from current product and process understanding to ensure consistent process performance and product quality. A comprehensive understanding of the drug substance manufacturing process, including impurity formation pathways, process capability, impurity fate and purge, and stability profile, is essential for establishing appropriate controls.

Control of Process-Related Impurities: The ICH (M7) guidance provides four distinct approaches for the development of a control strategy.

Option 1:

Drug Substance Specification Control: A test for mutagenic impurity is included in the drug substance specification, with an acceptance criterion at or below the AI-based limit. This approach is typically applied when impurity is generated during the final stages of synthesis or

when available process understanding is insufficient to justify control through alternative approaches. Example: A GTI is monitored in the final API using a validated, suitably sensitive analytical method, and a specification limit is established at or below the AI derived acceptable level. Based on demonstrated process capability and manufacturing history, periodic verification (skip testing) may be implemented when impurity levels are consistently maintained below 30% of the acceptable limit for at least six consecutive pilot-scale batches or three consecutive commercial-scale batches. In the absence of such supporting data, routine testing of each batch is recommended.

Option 2:

Upstream Control through Raw Materials, Intermediates, or In-Process Testing: The impurity is controlled by including a test in the specification of a raw material, starting material, intermediate, or as an in-process control, with limits established at levels that ensure compliance with the drug substance acceptable limit. Example: A potentially genotoxic alkyl halide used as a reagent during synthesis is controlled through appropriate raw material specifications and in-process monitoring of critical intermediates. Scientifically justified acceptance criteria are established at these stages to ensure that residual levels remain within predefined limits and do not result in unacceptable levels in the final API. Such upstream controls reduce the potential for impurity carryover and minimize the need for routine end-product testing.

Option 3:

Intermediate Specification Supported by Purge Studies: A test for the impurity is included in the specification of a raw material, starting material, intermediate, or as an in-process control, with an acceptance criterion above the drug substance acceptable limit. This approach requires a demonstrated understanding of impurity fate and purge throughout the manufacturing process. Example: A potentially mutagenic epoxide impurity is detected in a process intermediate at levels above the acceptable limit for the final API. Laboratory-scale spiking studies and process development data demonstrate that subsequent hydrolysis, purification, and crystallization operations consistently purge the impurity to levels well below the AI-based limit. On the basis of the demonstrated purge factor and process robustness, a higher specification limit may be established at the intermediate stage while maintaining compliance of the final API with the acceptable intake. Such an approach should be supported by appropriate fate-and-purge studies and manufacturing process data demonstrating consistent impurity control.

Option 4:

Control Based on Process Understanding (No Routine Testing): Sufficient process understanding is established to demonstrate that impurity levels in the drug substance consistently remain below the acceptable limit without routine analytical testing or inclusion in specifications. Example: Process development and validation studies identify the factors influencing impurity formation and establish that the process design effectively prevents impurity generation or ensures its removal through downstream processing. Supporting data may include mechanistic evaluations, DoE studies, process robustness assessments, and fate-and-purge investigations demonstrating that impurity levels remain well below the AI-based limit under both routine and worst-case manufacturing conditions. Where such process understanding and control have been sufficiently established, routine testing may be omitted, provided ongoing process verification confirms the continued effectiveness of the control strategy.

Collectively, these control approaches enable manufacturers to implement science and risk-based strategies tailored to the manufacturing process. Depending on the level of process understanding and available data, controls may rely on final product specifications, upstream material controls, demonstrated impurity purge, periodic verification (skip testing), or a combination of these approaches to ensure mutagenic impurities remain below levels associated with patient risk.

Control of Degradation Products: For degradation products identified as potentially mutagenic, it is important to assess whether the degradation pathway is relevant to the drug substance manufacturing process and proposed

packaging and storage conditions. A well-designed accelerated stability study (e.g., 40°C/75% RH for 6 months) in the proposed packaging, using suitable analytical methods, is recommended to evaluate the likelihood of such degradation. Based on the results of accelerated stability studies, if the degradation product is expected to form at levels approaching the acceptable limit under the proposed packaging and storage conditions, efforts to control its formation are required. In such cases, monitoring of the degradation product in the drug substance during long-term primary stability studies under proposed storage conditions (in the intended commercial packaging) is expected, unless adequately justified otherwise. The need for a specification limit for the mutagenic degradation product will generally depend on the outcomes of these stability studies.

CONCLUSION: The control of impurities, particularly GTIs, requires a science and risk-based approach integrating process knowledge, regulatory guidance, and toxicological evaluation. Systematic identification, classification, and assessment supported by (Q)SAR tools, established thresholds, and, where necessary, experimental studies enable informed decision-making on impurity safety. By applying ICH Q3A/Q3B, M7, and S2 guidelines alongside robust control strategies and ALARP principles, manufacturers can ensure that impurity levels remain within acceptable limits, thereby safeguarding patient safety and ensuring consistent drug substance quality.

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