Ind. J. Pharm. Edu. Res., 2026; 60(1s):s42-s51.
https://www.ijper.org

Review Article

Regulatory Pathway for Genotoxic Impurities in Europe, US, Canada, India, Australia and UK

Kavyashree Kadehalli Ramegowda[1], Sangita Mishra[1], Venkatesh Madhugiri Prakash[1,2,*]

[1]Centre of Excellence in Regulatory Sciences, Department of Pharmaceutics, JSS College of Pharmacy, JSS AHER, SS Nagara, Mysuru, Karnataka, INDIA.
[2]Faculty of Pharmaceutical Sciences, UCSI University, MALAYSIA.

ABSTRACT

Genotoxic Impurities (GTIs) pose a significant risk to pharmaceutical safety due to their ability to damage DNA and cause cancer-related mutations. Regulatory agencies such as the EMA, USFDA, Heath Canada, CDSCO and TGA have established stringent frameworks to manage these risks, incorporating tools such as Structure-Activity Relationships (SAR) analysis and the Threshold of Toxicological Concern (TTC) to establish safe exposure limits. These frameworks emphasize both proactive impurity profiling and the development of sensitive analytical methods for trace-level detection. This review delves into the classification, mechanisms and control strategies for GTIs, highlighting innovations such as AI-driven predictive modeling and global efforts to improve impurity detection and management. Furthermore, it emphasizes the evolving role of risk-based approaches in addressing emerging genotoxic challenges throughout the pharmaceutical manufacturing pipeline. By combining cutting-edge science and regulatory rigor, the pharmaceutical industry continues to reduce GTI risks, ensuring the development of safer, higher-quality medicines for patients worldwide.

Keywords: Genotoxic Impurities, Pharmaceutical Safety, Regulatory Guidelines, Risk Assessment, Threshold of Toxicological Concern (TTC).

Correspondence:
Dr. Venkatesh Madhugiri Prakash
[1]Associate Professor, Centre of Excellence in Regulatory Sciences, Department of Pharmaceutics, JSS College of Pharmacy, JSS AHER, SS Nagara, Mysuru-570015, Karnataka, INDIA.
[2]Faculty of Pharmaceutical Sciences, UCSI University, MALAYSIA.
Email: venkateshmpv@jssuni.edu.in

Received: 29-03-2025;
Revised: 06-05-2025;
Accepted: 18-07-2025.

INTRODUCTION

Genotoxic impurities are chemicals or materials that can alter cellular DNA, potentially leading to mutations or cancer. Regulatory bodies, including the ICH, EMA, USFDA, Health Canada, CDSCO and TGA, have established stringent standards and restrictions to manage these impurities. Key updates in regulations have been reviewed in conducted studies and have provided significant insights into detecting and controlling genotoxic pollutants for the Pharmaceutical Research and Manufacturing Association (PhRMA).[1] The International Conference on Harmonisation (ICH) introduced its first genotoxicity guideline, S2A, in July 1995, followed by S2B in 1997. The 2013 M7 guideline focused on analyzing Structure-Activity Relationships (SAR) for genotoxicity. Subsequent revisions, including M7 (R1) in June 2015 and M7 (R2) in May 2017, aimed to update impurity limits based on new data, with the latest revision intending to refine these limits further and integrate new findings on DNA-reactive (mutagenic) impurities. Both the FDA and EMA guidelines underscore the critical role of controlling genotoxic impurities in medicinal compounds, reflecting these updates.[2,3] This review will analyze the regulatory compliance and safety measures related to Genotoxic Impurities (GTIs) according to ICH, EMA, USFDA, Health Canada, CDSCO, TGA and MHRA guidelines. It will focus on the identification, classification and management of GTIs.

Need for genotoxicity determination

Early detection and understanding of genotoxins during therapeutic development could prevent potential harm from these substances. Genetic alteration components and germ cells have significant effects even at low exposure levels. Many hereditary diseases are caused by mutations in DNA damage response genes, tumor suppressor genes, or proto-oncogenes that are brought by physical or chemical carriers. Somatic cells destroy genetic material and are also a source of degenerative diseases, including accelerated aging, immunological dysfunction, cardiovascular and neurological disorders.[4,5] Based on this idea, numerous advisory groups and regulatory agencies have released recommendations for genotoxicity testing procedures.[6]

DOI: 10.5530/ijper.20260762

Classification

Genotoxic impurities are classified as Class 1, 2, 3, 4, or 5 based on the risk potential. Mueller *et al.*, classified impurities according to their genotoxicity potentials. It is intended to determine whether a particular impurity has a genotoxicity risk and to regulate the Potential Genotoxic Impurities (PGIs) below TTC level. Class 1 contaminants are known to be both genotoxic and carcinogenic; Class 2 contaminants are genotoxic but not carcinogenic; Class 3 impurities are structural alerts (PGIs, for example) whose structures are unrelated to the API structure and with unknown genotoxic potential; Class 4 impurities are structural alerts that are part of the API's alerting structure; Class 5 impurities are governed by ICH Q3 guidelines as regular impurities; they are not structural alarms. The genotoxic impurities control and classification are illustrated in Figure 1.[7,8]

Mechanism of genotoxicity

DNA is made up of four bases: Adenine (A), Guanine (G), Cytosine (C) and Thymine (T). A and G are purines, while C and T are pyrimidines. Transitions occurs when a purine is changed with another purine (A↔G) or a pyrimidine with another pyrimidine (C↔T), usually causing less harm. Transversions, which are more disruptive, happen when a purine is swapped with a pyrimidine or vice versa (e.g., A↔C or G↔T). Harmful substances like chromium and Pyrrolizidine Alkaloids (PAs) can cause DNA damage, leading to these mutations. When cells copy this damaged DNA, mistakes may occur, leading to transversions, caused by genotoxic compounds and can contribute to cancer development. The mechanism of genotoxicity is illustrated in Figure 2.[9,10]

Assessment

Impurities are present in all drug compounds and related products. At the same time, most impurities are covered by ICH Q3A(R2) and Q3B(R2). These guidelines aim to lower potential carcinogenic risks by offering a practical framework for locating, classifying, qualifying and managing these mutagenic contaminants.[11,12] Additionally, they offer guidance regarding new drug products, novel medication substances, products containing a drug ingredient found in a previously approved product and post-approval submissions of marketed goods, but only in the following situations:

- Deviations can lead to new impurities.
- Variations to manufacturing that can affect higher acceptance criteria.
- Adjustments to the dosage schedule that can cause appreciable shifts in the acceptable cancer risk level.[13]

Two steps make up the impurity evaluation process

Step 1: The mutagenic potential of actual contaminants should be assessed.

Step 2: It should be the anticipated mutagenic potential of potential contaminants that are to be included in the finished pharmaceutical substance.[14]

Techniques

This recommendation focuses on substances that are reactive to DNA and can directly damage DNA at low concentrations, possibly resulting in mutations.[15]

a. Preventing the introduction of harmful impurities.

b. Altering pertinent process parameters to get rid of or cut these contaminants down to negligible amounts.

c. Enhancing knowledge needed to establish a specific strategy or intervention Genotoxic Impurities Testing (GIT) aims to effectively eliminate these harmful effects.

d. Conducting toxicity tests on an uncertain impurity ensures its safety even at the predicted low concentrations. The genotoxic impurity Analytical Techniques for Detection is illustrated in Table 1.[16]

Regulatory Aspects

Several position papers and regulatory guidelines concentrate on using set limits to control the amount of Potential Genotoxic Impurity (PGIs) in pharmaceuticals. Guidelines specifically addressing genotoxic impurities have been developed by various industry and regulatory authority organizations. To address their issues, R and D scientists must ensure that synthetic processes resulting in PGIs are controlled, PGIs are identified in the early stages of process development and appropriate analytical methods are developed. Regulatory Guidelines and Therapeutic Toxic Concentration Levels for Genotoxic Impurities in Various Countries are illustrated in Table 2.[17]

ICH guidelines

Drug substances and product-related impurities are governed by ICH guidelines, which also specify reporting, qualifying and identification thresholds. Three classes of residual solvents are distinguished according to possible health risks and standards for heavy metal contaminants are currently being developed. ICH guideline is focused on chemicals that may directly damage DNA and ultimately cause cancer. Impurities and residual solvents are currently covered by the accessible guidance publications Q3A(R), Q3B(R) and Q3C from the ICH.[18] The ICH M7(R2) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk (2023) guideline provides updated strategies for managing genotoxic impurities in drugs, such as new Acceptable Intake (AI) limits

(8 mg/day for formaldehyde and 32 µg/day for ethylbromide), risk-based classification and structured testing (Ames and *in vivo* studies). It emphasizes the use of validated (Q) SAR models for predictions, expert review of ambiguous cases and a globally harmonized approach to ensure patient safety. A TTC of 1.5 g/day has been justified as an acceptable limit for mutagenic contaminants in drug ingredients and drug products.[19] Refer to Table 3 for Drug Substance Impurity Thresholds.

European Medicines Agency (EMA)-Europe

Beyond ICH, Europe takes additional steps to manage genotoxic impurities, such as providing specific guidance on nitrosamines, emphasizing risk assessments, analytical methods and process optimization to prevent their formation. The European Pharmacopoeia (Ph. Eur.) establishes mandatory impurity testing standards and the EMA uses veterinary-specific guidelines such as VICH GL10 and GL11 for DNA-reactive impurities. EMA also mandates case-specific evaluations for borderline cases, working with EDQM and national agencies to ensure consistent implementation across the EU. These frameworks provide robust impurity control that goes beyond global ICH standards. EMA released the management of genotoxic impurities management guidelines for both novel and existing active substances. These recommendations divide contaminants into 2 groups according to evidence for threshold-related mechanisms. The "as low as reasonably practicable" or ALARP principle is suggested for contaminants for which there is insufficient data to support threshold mechanisms. An intake of 1.5 g/day is deemed acceptable for the TTC value. The guidelines prioritize impurity elimination, ALARP and TTC approach to reduce the need for toxicity testing and safety evaluations. Both the European Community and the Japanese Ministry of Health and Welfare have set standards for evaluating medications for genetic toxicity. Genotoxic Impurities Testing (GTIs) is divided into two groups according to the limits of genotoxic impurities guidelines of EMA. Genotoxic Impurities Testing (GTIs) explains their mechanism through the use of experimental data. These were categorized as class 2 solvents by ICH Q3C (R4). Although regulated "as low as reasonably practicable", TTC values of 1.5 g/day of GTI intake were taken into consideration. Current experimental evidence is insufficient to establish or determine the GTIs inside the allowed range.[20]

United States Food and Drug Administration (US-FDA)-US

The US FDA expands on ICH guidelines for managing genotoxic impurities, including stricter limits for vulnerable populations like paediatrics, staged Threshold of Toxicological Concern (TTC) levels based on exposure duration (e.g., up to 120 µg/day for studies under 14 days) and flexibility in applying ALARP principles. It focuses on computational tools such as QSAR models to exempt impurities from additional testing and offers specialized assessments for complex products such as herbal medicines. These refinements improve safety and flexibility in managing impurities, while promoting efficient drug development. The management of potential genotoxic or carcinogenic contaminants is outlined in this guidance with particular recommendations. It offers methods for assessing and reducing the cancer risks connected to these contaminants. The recommended approaches are similar to those outlined in the EMA guidelines. To minimize risks, the FDA suggests several key steps: First, modify the drug synthesis or purification process to reduce the formation of and enhance the removal of harmful impurities. Second, aim for a general target of no more than 1.5

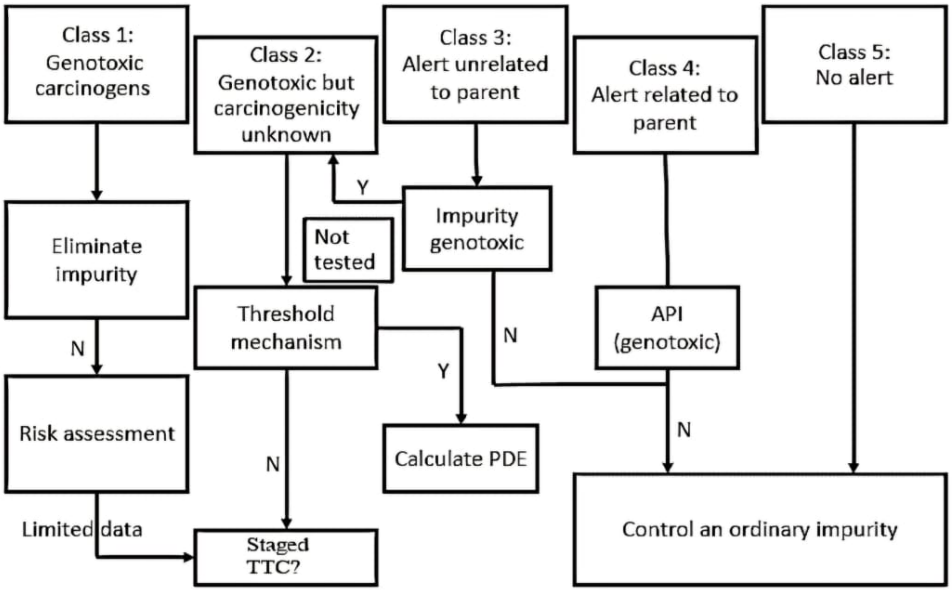

Figure 1: Genotoxic impurities control and classification strategy.[45]

Table 1: Analytical Techniques for Detection.

Technique	Instrumentation	Features and Advantages	References
HPLC	LC Systems	- Ease of use, cost-effective instrumentation. - Ultra Performance HPLC for reduced analysis time. - Max-Light cartridge cell for ultra-sensitive detection. - Extensive LC column family for analytical separations. - Scalability across laboratory settings with global service and support.	37
GC-MS (Gas Chromatography-Mass Spectrometry).	Headspace Sampler, 7890 GC system, 5977A GC/MSD System.	- Widely accepted for analyzing genotoxic impurities. - Headspace method for residual solvent analysis following 3 ICH Q3C guidelines. - Comprehensive range of GC accessories. - MSD Productivity ChemStation and MSD Security ChemStation for varied laboratory needs.	38
Liquid Chromatography Mass Spectrometry.	Serie Systems, 6500 Series AccurateMass Q-TOF, 6400 Series Triple Quadrupole Mass Spectrometers.	- Mass spectrometry offers high specificity and sensitivity. -Advanced qualitative and quantitative software for data analysis. - IBCS technology for maintaining resolution and mass accuracy. - Triggered Multiple Reaction Monitoring (MRM) for faster analysis times.	39
NMR (Nuclear Magnetic Resonance).	MR System	- NMR for specific information about bonding and stereochemistry. - Non-destructive and non-invasive nature. - Useful for characterizing impurities and degradants at low levels. - Quantitative output for impurity profiling.	40

Table 2: TCC level in Genotoxic Impurity.[41]

Country	regulatory authority	Genotoxic Impurity Guideline	Therapeutic Toxic Concentration
India	CDSCO	ICH guideline M7	1.5 µg/day
Australia	TGA	ICH Q3A(R) and ICH Q3B(R2)	1.0 mg/day
UK	MHRA	ICH Q3 guidelines	0.1 µg/day
Canada	Health Canada	ICH M7(R1)	10 mg/day
Europe	EMA	Q3C	1.5 µg/day
US	FDA	ICH Q3 guideline	1.5 µg/day

Table 3: Drug Substance Impurity Threshold.[42]

Maximum daily dose	Identification Threshold[b,c]	Identification Threshold[c]	Qualification Threshold[c]
≤2 g/day	>2 g/day	0.10% or 1.0 mg/day (Whichever is lower).	0.15% or 1.0 mg/day (Whichever is lower).
>2 g/day	0.03%	0.05%	0.05%

The amount of drug substance administered per day.Higher reporting threshold should be scientifically justified.A lower threshold can be appropriate if the impurities are unusually toxic.

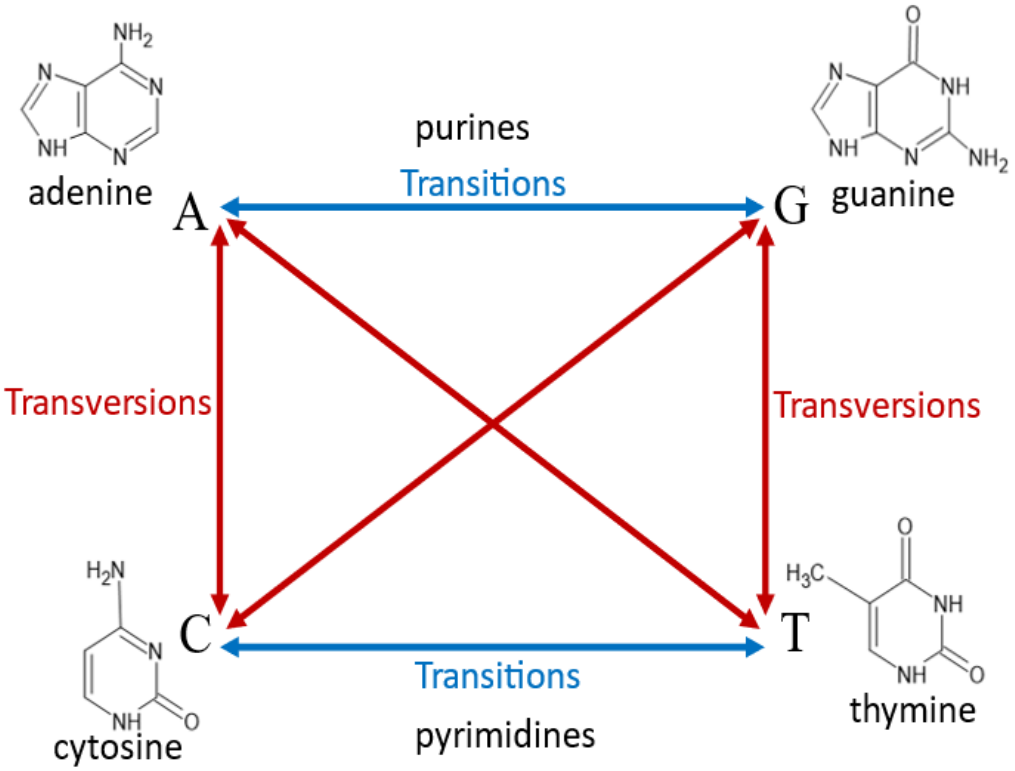

Figure 2: Mechanisms Underlying Genotoxic Effects.

µg per day of the impurity for daily exposure. Finally, further evaluate the genotoxic and carcinogenic risks to determine appropriate impurity limits, which may be adjusted based on the risk assessment.[21]

Health Canada-Canada

Health Canada also focuses on advanced therapies, using specific frameworks like S12 for gene therapy products. Stakeholder engagement through consultations and notices promotes transparency, while phased implementation allows manufacturers to comply with new standards. Independent risk assessments supplement ICH recommendations to address local public health needs while maintaining a strong and adaptable regulatory framework and it provides a comprehensive framework for the identification, classification, qualification and control of DNA-reactive (mutagenic) impurities in pharmaceuticals, aiming to minimize the risk of carcinogenic effects. This is relevant for new drug substances and products during both clinical development and post-approval changes that might introduce or increase the levels of mutagenic impurities. A key component of the guideline is the use of the Threshold of Toxicological Concern (TTC) to establish safe exposure levels. It recommends employing bacterial mutagenicity assays to assess the mutagenic potential of impurities. While the guideline is not retroactive, it requires that any new data or emerging impurities be carefully evaluated to ensure continued patient safety. The process of impurity assessment in drug substances and products is critical and involves two stages: identifying and evaluating the mutagenic potential of actual impurities and assessing potential impurities that may arise during synthesis, manufacturing, or storage. This includes evaluating synthetic impurities, degradation products and those formed during long-term storage using methodologies like Structure-Activity Relationships (SAR). Throughout a lifetime of daily exposure, the TTC-based recommended dosage of 10 mg/day is anticipated to provide protection. The appropriate increasing lifetime dosage is evenly distributed throughout the total number of exposure days during Less Than Lifetime (LTL) exposure to address LTL exposures to mutagenic chemicals in medications.[22]

Central Drugs Standard Control Organization (CDSCO)-India

CDSCO's electronic platform, SUGAM, simplifies approvals and ensures efficient oversight of impurity control in Active Pharmaceutical Ingredients (APIs) and drug products. These include requirements for detailed impurity profiling during regulatory submissions, as well as continuous quality management. Recent conferences, such as the 2nd Annual Pharma Impurity Conclave, reflect India's ongoing efforts to address challenges in genotoxic impurity detection and compliance, focusing on advanced analytical methods and regulatory harmonization. The Central Drugs Standard Control Organization (CDSCO) in India is in charge of regulating genotoxic impurities in medications. It bases its regulations on global standards such as the ICH M7.

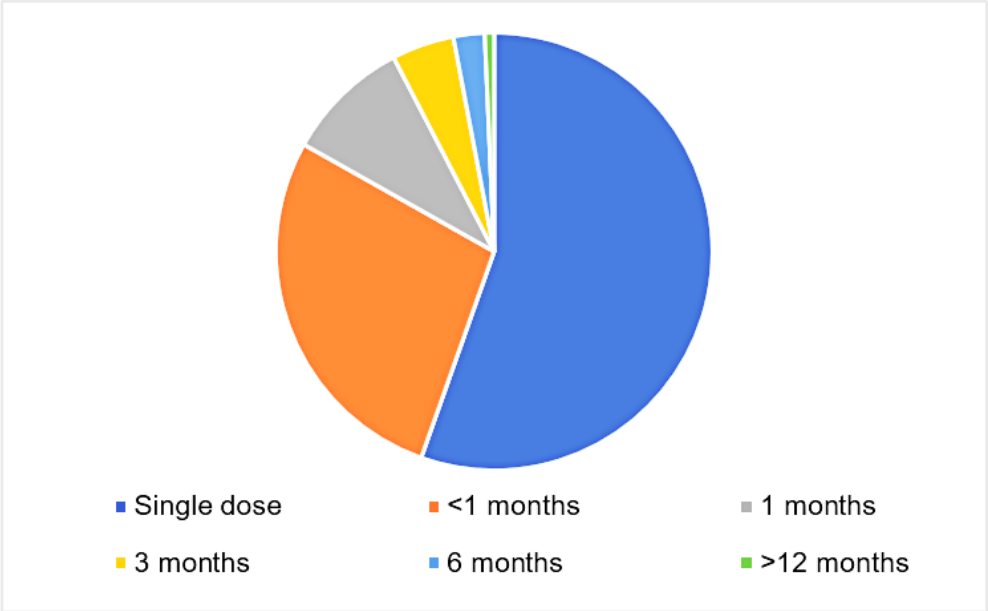

Figure 3: Acceptable limit of genotoxic impurity according to ICH.[46]

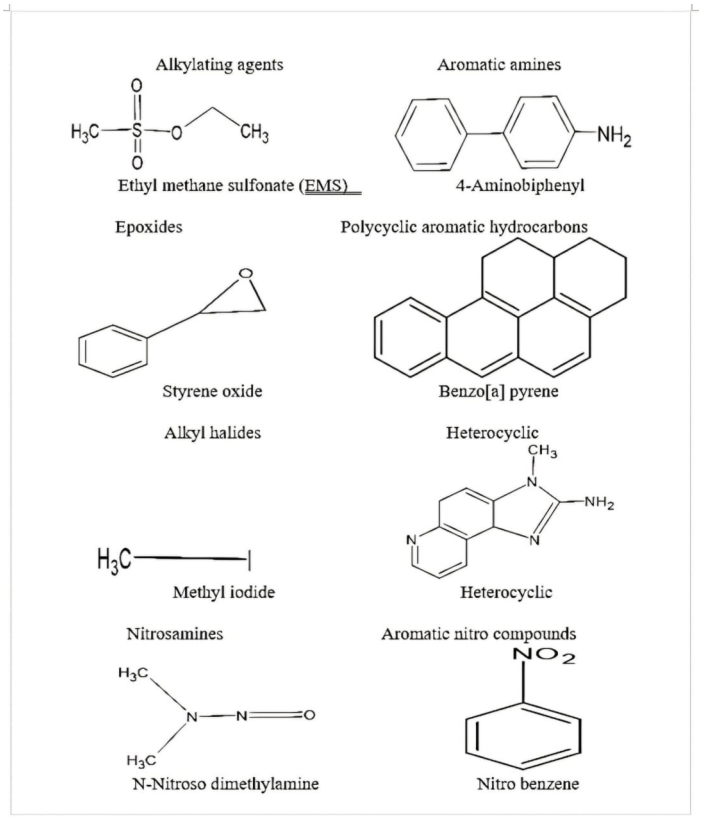

Figure 4: Structures of a few known genotoxic impurities.[43]

Drug manufacturers are required by the CDSCO to evaluate and manage genotoxic contaminants, both real and potential, according to their potential to cause cancer and mutagenesis. This is done by employing the Threshold of Toxicological Concern (TTC) approach, which uses thresholds to set permissible limits. Manufacturers are required to apply risk management techniques based on impurity classification and to furnish thorough documentation on impurity assessment and control actions. To maintain patient safety and regulatory compliance, CDSCO regularly updates its guidelines to take into account new scientific findings and technology developments. The guidelines for pharmaceutical goods (Q3B, Impurities in New Medicinal goods) or active substances define a broad concept of impurity qualification. The process of gathering and evaluating information to ascertain the biological safety of a particular impurity or impurity profile at the recommended level is known as qualification (s). It is widely acknowledged that determining appropriate dosage limits for pollutant genotoxicity is important but not specifically covered by current recommendations. Nonetheless, since medications offer benefits, using a TTC to assess the tolerated limits of genotoxic pollutants in medicinal compounds can be justified at a value of 1.5 g/day, or a 10-5 lifetime risk of cancer.[23,24]

Therapeutic Goods Administration (TGA)-Australia

The Therapeutic Goods Administration (TGA) in Australia provides detailed guidelines to control genotoxic impurities in pharmaceutical products. The TGA requires pharmaceutical companies to conduct comprehensive risk assessments to identify any potential genotoxic impurities in their products. Once identified, control strategies must be implemented to reduce these impurities to acceptable levels, often involving optimization of the manufacturing process and the use of higher-quality raw materials. The TGA follows the International ICH M7 guideline to determine acceptable limits for genotoxic impurities based on the Threshold of Toxicological Concern (TTC). This is followed to ensure that genotoxicity risk is minimized. Pharmaceutical companies must document their risk assessments, control strategies and testing results to demonstrate compliance with TGA guidelines. The TGA mandates ongoing monitoring and

post-marketing surveillance to ensure that genotoxic impurities remain within safe levels throughout the product's shelf life. Variations in manufacturing processes and formulations affecting impurity levels must be reported to the TGA and additional testing may be required. By adhering to these guidelines, companies can ensure their products are safe, effective and meet the stringent requirements for registration in the Australian Register of Therapeutic Goods (ARTG).[25,26]

The Medicines and Healthcare products Regulatory Agency (MHRA)-UK

The Medicines and Healthcare Products Regulatory Agency (MHRA) in the UK closely monitors the safety of pharmaceutical products, including the control of genotoxic impurities. Genotoxic impurities are chemical substances that can cause genetic mutations, potentially leading to cancer or other serious health issues. The MHRA follows international guidelines, such as the Threshold of Toxicological Concern (TTC) concept, to identify and prioritize these impurities for genotoxicity assessment. The TTC concept helps determine which impurities require further investigation by setting exposure limits below which the risk of genotoxicity is considered negligible. When the structure of impurity cannot be determined or exposure estimates are unavailable, a cut-off concentration of 0.1% is often used as a guideline to prioritize genotoxicity testing. The genotoxicity testing strategy adopted by the MHRA involves a combination of Quantitative Structure-Activity Relationship (Q) SAR evaluations, expert judgment and reference to genotoxicity data on chemically similar substances. (Q) SAR evaluations use computational models for predicting the genotoxic potential of impurities based on chemical structure. The MHRA ensures that pharmaceutical products in the UK meet high safety standards, minimizing risks posed by genotoxic impurities.[27]

Management of genotoxic impurity

The ICH Q3D Guideline for elemental impurities was implemented in 2021, giving a risk-based method for managing and limiting these contaminants. Elemental impurities, which are frequently harmful heavy metals, must be kept under tolerable levels to protect patients. However, applying ICH Q3D rises testing and risk assessment issues, necessitating a rigorous risk assessment to evaluate whether controls are adequate. The Acceptable limit of genotoxic impurity according to the ICH is illustrated in Figure 3. Additional efforts may include purification to reduce contaminants, selecting higher-quality components, setting specification limitations, or selecting appropriate closing mechanisms. As elemental impurities may be present at any stage of drug development, a defined management approach based on risk assessment results is critical. Changes need a re-evaluation of the risk, taking into account elements such as synthetic pathways, suppliers, materials and facilities. Regulatory consequences should be handled by making suitable changes.[28] Structures of a few known genotoxic impurities are illustrated in Figure 4. Pharmaceutical Impurities Reference Standards, which are commercially accessible, help in the identification of impurities. "VEEPRHO" the company which is a versatile supplier, specializes in the separation, synthesis and provision of impurity standards, metabolites, intermediates and APIs for the pharmaceutical sector and is dedicated to delivering products that meet industry standards, including customized synthesis.[29,30] Refer to Table 4 for a comparison of Genotoxic Impurities Assessment among the countries selected for this study.

DNA reactivity evaluation framework

Guideline M7: Assessment and Control of DNA Reactive (Mutagenic) Impurities is presently being developed by the ICH. It will have a concept paper and a business plan that will contain preliminary data and an implementation schedule.[18] It will have a broader geographic scope than the existing EMA and FDA guidelines. It should also provide clarity on other issues being debated in the industry, such as how to handle several structurally related genotoxic impurities with comparable mechanisms of action and whether or not these should be added together when determining a TTC. Furthermore, it is anticipated to reconcile discrepancies between FDA and EMA guidelines.[31,32]

Innovations and collaborative efforts

Data analysis using Artificial Intelligence (AI) can help with more effective GTI assessment. Potential usages of AI are:

Analytical system optimization.

Developing novel sorbents and sample preparation.

Increased collaboration among regulatory bodies, pharmaceutical companies and instrument manufacturers to effectively use AI.[33]

Genotoxicity control level calculation

If assays reveal genotoxic impurities, it's critical to determine the Permissible Daily Exposure (PDE). If toxicological data with a No Observable Effect Level (NOEL) are available, the following formula, which is mentioned in ICHQ3C (R4),[34] can be used to calculate:

PDE=(NOEL weight adjustment)/(F1×F2×F3×F4×F5)

F1: Allometric scale-based adjustment factor for extrapolation of animals to humans.

F2: Variability factor among individuals.

F3: Adjustment factor of the study duration.

F4: Adjustment factor based on the severity of the observed toxic effects.

F5: Variable factor in case of unestablished NOEL.[35]

Table 4: Comparison of Genotoxic Impurity by Selected Countries.

Region/ Country	Key Points on GTI Regulation	SAR	Differentiators (TTC, SAR, etc.,)	Challenges
Europe	- Specific guidance on nitrosamines. - Emphasis on risk assessments, process optimization and analytical methods.	-Extensive use of SAR, especially for nitrosamines. - VICH GL10 and GL11 for veterinary impurities.	- Applies TTC with stringent impurity-specific thresholds. - Detailed use of SAR for nitrosamines and other GTIs.	- Managing compliance across EU member states. - Incorporating local variations in regulations.
United States	- Focus on advanced therapies and gene therapies (S12). - Ongoing consultations and phased implementation for guidelines.	Strong use of QSAR and computational models. - Focus on carcinogenic risk.	- FDA combines TTC with dose-response approaches for high-risk cases. - Encourages computational toxicology tools like QSAR for risk assessment.	- Validation and standardization of computational methods. - Striking balance between regulatory flexibility and safety.
Canada	- Uses SUGAM platform for simplified approvals. - Focus on impurity profiling and quality management.	Limited use of SAR. - Strong regulatory framework for impurity identification and control.	- Uses TTC to harmonize public safety and environmental concerns. - Limited integration of SAR tools compared to US/EU.	- Implementation of newer standards. - Balancing advanced therapies with standard drug development processes.
India	CDSCO guidance, Drug and Cosmetic Act, SUGAM system.	Limited SAR use, mainly focused on qualifying impurities during synthesis and manufacturing.	- Adapts TTC thresholds with a focus on feasibility for local manufacturers. - SAR tools are underutilized due to resource constraints.	- Limited advanced testing infrastructure. - Compliance variability among local manufacturers.
Australia	- Focus on process optimization and higher-quality raw materials. - Post-marketing surveillance is emphasized.	Use of SAR in impurity evaluations. - Ongoing risk assessment and testing during the product lifecycle.	- Applies TTC tailored for local public health needs. - SAR incorporated in submissions for complex impurities.	- Ensuring global standards compliance, especially for imported APIs. - Variations in manufacturing processes affecting impurity levels.
United Kingdom	- Focus on genotoxicity testing strategy using QSAR and expert judgment. - Emphasizes TTC and Q SAR models for predicting impurity risks.	Strong emphasis on SAR for impurity evaluation. - Q SAR models to assess genotoxic potential.	- Uses TTC and integrates SAR for nitrosamine impurity testing.	- Managing post-market compliance. - Ensuring up-to-date assessment of evolving impurities.

Analytical method development

Creating an analytical technique that can identify genotoxic impurities at trace levels and well below the TTC is the fundamental goal of developing a method for detecting these impurities. To produce safe and high-quality pharmaceutical products, a series of controlled experiments should be conducted to reduce the variability of the developed analytical method. Analytical techniques for international products should meet increasingly strict global regulatory standards. The constant process of method development aims to continuously raise the caliber of the final output.[36] The comprehensive review underscores the critical importance of regulatory compliance and safety assurance in the pharmaceutical industry, particularly concerning Genotoxic Impurities (GTIs). The integration of guidelines from international regulatory bodies such as ICH, EMA, USFDA, Health Canada, CDSCO and TGA establishes a robust framework for identifying, categorizing and controlling GTIs throughout drug development.

AI in Genotoxic impurity

Detecting Genotoxic Impurities (GTIs) involves a step-by-step approach, starting with sample preparation to ensure the impurities are extracted and ready for analysis. Advanced detection techniques that identify and measure impurities, such as Nuclear Magnetic Resonance (NMR), High-Resolution Mass Spectrometry (HRMS) and Ultra-High-Performance Liquid Chromatography (UHPLC), come next. Next, more effective and

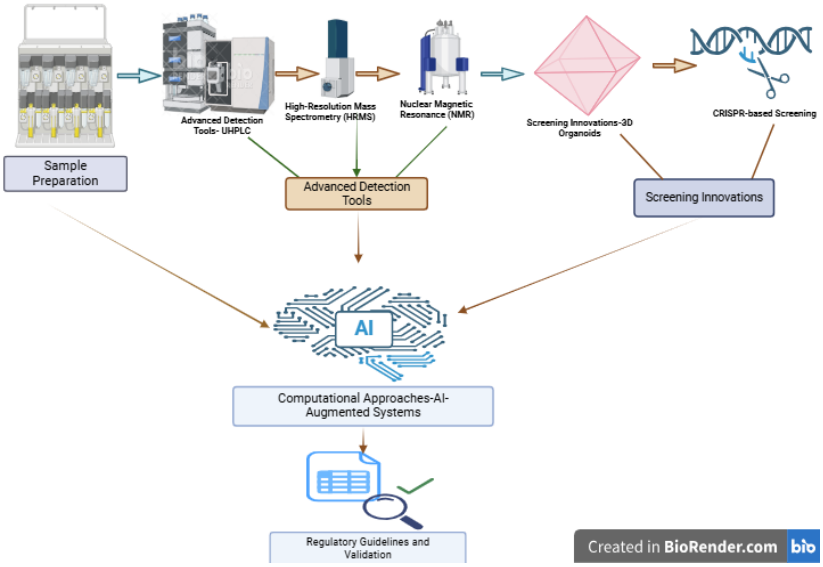

Figure 5: AI in Genotoxic impurity.[44]

predictive testing methods are provided by screening innovations like 3D organoids and CRISPR-based approaches.

These are supplemented by computational methods, such as molecular modeling and AI-augmented systems that swiftly and precisely predict and analyze impurities. Lastly, all of these procedures align with validation and regulatory requirements to guarantee pharmaceutical product safety and compliance as illustrated in Figure 5.

CONCLUSION

The comprehensive overview underscores the critical importance of regulatory compliance and safety assurance in the pharmaceutical industry, particularly concerning Genotoxic Impurities (GTIs). The integration of guidelines from international regulatory bodies such as ICH, EMEA, USFDA, Health Canada, CDSCO, TGA and MHRA establishes a framework for controlling GTIs throughout drug development. The need for genotoxicity determination is highlighted, considering the potential health consequences of DNA damage. The materials and methods section outlines a systematic approach for assessing, controlling and managing genotoxic impurities, involving evaluation of actual and potential impurities, as well as strategic approaches for risk reduction.

ACKNOWLEDGEMENT

The authors would like to acknowledge JSS College of Pharmacy, Mysuru for providing support to conduct this study.

ABBREVIATIONS

ICH: International Council for Harmonization; **TTC:** Therapeutic Toxic Concentration; **SWP:** Safety Working Party; **CPMP:** Committee for Proprietary Medicinal Products; **SAR:** Structure-Activity Relationships; **GMP:** Good Manufacturing Practice; **LTL:** Less-than-lifetime; **PDE:** Permissible daily exposure; **CDER:** Centers for Drug Evaluation and Research; **ARTG:** Australian Register of Therapeutic Goods; **EPA:** Environmental Protection Agency; **PGI:** Potential genotoxic impurity; **NOEL:** No-observed-effect level; **ALARP:** As low as reasonably practicable; **GTIs:** Genotoxic Impurities; **PhRMA:** Pharmaceutical Research and Manufacturing Association; **PGIs:** Potential Genotoxic Impurities; **QSAR:** Quantitative Structure-Activity Relationship; **NMR:** Nuclear Magnetic Resonance; **HRMS:** High-Resolution Mass Spectrometry; **UHPLC:** Ultra-High-Performance Liquid Chromatography; **EMA:** European Medicines Agency; **USFDA:** United States Food and Drug Administration; **CDSCO:** Central Drugs Standard Control Organization; **TGA:** Therapeutic Goods Administration; **MHRA:** Medicines and Healthcare products Regulatory Agency.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Genotoxic Impurities (GTIs) in pharmaceuticals pose serious risks, such as DNA damage and cancer. Regulatory bodies such as the ICH, EMA, US FDA, Health Canada, CDSCO, TGA and MHRA have developed guidelines for identifying, classifying and managing these impurities using methods such as Threshold of Toxicological Concern (TTC) and Structure-Activity Relationship (SAR) analysis. Advances in AI modeling and (Q) SAR evaluations improve impurity detection, reducing the need for additional testing. Ongoing regulatory updates refine exposure limits, prioritize vulnerable populations and emphasize process optimization and monitoring, resulting in safer, higher-quality pharmaceutical products.

REFERENCES

1. Bercu JP, Dobo KL, Gocke E, McGovern TJ. Overview of Genotoxic Impurities in Pharmaceutical Development. [cited 2024 Apr 10]; Available from: http://ijt.sagepub.com
2. Genotoxic Impurities Part 1: General Overview | Pharmaceutical Outsourcing. The Journal of Pharmaceutical and Biopharmaceutical Contract Services [Internet]. [cited 2024 Jan 3]. Available from: https://www.pharmoutsourcing.com/Featured-Articles/113769-Genotoxic-Impurities-Part-1-General-Overview/
3. Eichenbaum G, Johnson M, Kirkland D, O'Neill P, Stellar S, Bielawne J, *et al.* Assessment of the genotoxic and carcinogenic risks of p-nitrophenol when it is present as an impurity in a drug product. Regulatory Toxicology and Pharmacology. 2009; 55(1): 33-42.
4. Menz J, Götz ME, Gündel U, Gürtler R, Herrmann K, Hessel-Pras S, *et al.* Genotoxicity assessment: opportunities, challenges and perspectives for quantitative evaluations of dose-response data. Arch Toxicol [Internet]. 2023 Sep 1 [cited 2024 Jan 18];97(9): 2303. Available from: /pmc/articles/PMC10404208/
5. Liu KT, Chen CH. Determination of Impurities in Pharmaceuticals: Why and How? Quality Management and Quality Control-New Trends and Developments [Internet]. 2019 Jan 31 [cited 2024 Jan 19]; Available from: https://www.intechopen.com/chapters/65419
6. Pounikar AR, Umekar MJ, Gupta KR. Genotoxic Impurities: An Important Regulatory Aspect. Asian Journal of Pharmaceutical and Clinical Research. 2020; 10-25.
7. Reddy AVB, Jaafar J, Umar K, Majid ZA, Bin Aris A, Talib J, *et al.* Identification, control strategies and analytical approaches for the determination of potential genotoxic impurities in pharmaceuticals: A comprehensive review. J Sep Sci. 2015; 38(5): 764-79.
8. Reddy AVB, Jaafar J, Umar K, Majid ZA, Bin Aris A, Talib J, *et al.* Identification, control strategies and analytical approaches for the determination of potential genotoxic impurities in pharmaceuticals: A comprehensive review. J Sep Sci [Internet]. 2015 [cited 2024 Apr 10];38(5): 764-79. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/jssc.201401143
9. SV R. Genotoxicity: Mechanisms, Testing Guidelines and Methods. Global Journal of Pharmacy and Pharmaceutical Sciences. 2017; 1(5).
10. Genotoxicity of Drugs: Introduction, Prediction and Evaluation [Internet]. [cited 2024 Jan 31].https://www.researchgate.net/publication/334389865_Genotoxicity_of_Drugs_Introduction_Prediction_and_Evaluation
11. Preclinical Assessment of Genotoxic Impurities: An Overview of Current Regulatory Guidance, Available Assays and Data Interpretation. Pharmaceutical Industry Practices on Genotoxic Impurities. 2014; 228-53.
12. Teasdale A, Elder D, Chang SJ, Wang S, Thompson R, Benz N, *et al.* Risk assessment of genotoxic impurities in new chemical entities: Strategies to demonstrate control. Org Process Res Dev. 2013; 17(2): 221-30.
13. Shaikh T. Genotoxic Impurities and Its Risk Assessment in Drug Compounds. Drug Designing and Intellectual Properties International Journal [Internet]. 2018 Oct 23 [cited 2024 Jan 18];2(4). Available from: https://papers.ssrn.com/abstract=3958587
14. Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk M7(R1) Current Step 4 version. 2017.
15. Prakash A. Control Strategies of Genotoxic Impurities in Drug Substance and Product.
16. Genotoxic Impurity Method Development and Methodological Validation-PharmiWeb.com [Internet]. [cited 2024 Jan 3]. Available from: https://www.pharmiweb.com/article/genotoxic-impurity-method-development-and-methodological-validation
17. McGovern T, Jacobson-Kram D. Regulation of genotoxic and carcinogenic impurities in drug substances and products. TrAC Trends in Analytical Chemistry. 2006; 25(8): 790-5.
18. Sharma A, Kumar SK. Review on identification and quantification of genotoxic impurities. Int J Health Sci (Qassim) [Internet]. 2022 [cited 2024 Apr 10];6(S7):4043-65. Available from: https://sciencescholar.us/journal/index.php/ijhs/article/view/12710
19. Cayley AN, Foster RS, Brigo A, Muster W, Musso A, Kenyon MO, *et al.* Assessing the utility of common arguments used in expert review of predictions as part of ICH M7 assessments. Regulatory Toxicology and Pharmacology. 2023; 144: 105490.
20. Nadja K. Committee for Medicinal Products for Human Use (Chmp) Guideline on the Limits of Genotoxic Impurities. 2006 [cited 2024 Jan 3]; Available from: http://www.emea.eu.int
21. Genotoxic Impurities in Pharmaceutical Products.
22. Canada H, Products H, Branch F, Products Directorate T. Guidance Document Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk ICH Topic M7.
23. Pounikar AR, Umekar MJ, Gupta KR. Genotoxic Impurities: An Important Regulatory Aspect. Asian Journal of Pharmaceutical and Clinical Research. 2020; 10-25.
24. Szekely G, De Sousa MCA, Gil M, Ferreira FC, Heggie W. Genotoxic impurities in pharmaceutical manufacturing: Sources, regulations and mitigation. Chem Rev [Internet]. 2015 [cited 2024 Jan 18];115(16): 8182-229. Available from: https://pubs.acs.org/doi/abs/10.1021/cr300095f
25. Goods Administration T. Guidance 18: Impurities in drug substances and drug products. 2013 [cited 2024 Jan 3]; Available from: http://www.tga.gov.au
26. Bercu JP, Dobo KL, Gocke E, McGovern TJ. Overview of Genotoxic Impurities in Pharmaceutical Development. Int J Toxicol [Internet]. 2009 [cited 2024 Jan 19];28(6): 468-78. Available from: https://journals.sagepub.com/doi/10.1177/1091581809349195
27. COM/12/S2 Interim Guidance on A Strategy for Genotoxicity Testing and Mutagenic Hazard Assessment of Impurities in Chemical Substances (April 2012). [cited 2024 Jan 3]; Available from: http://echa.europa.eu/documents/10162/17235/substance_id_en.pdf
28. Rao RR, Kumar GR, Vardhan VM, Boddu V. Development and Validation of Three Potential Genotoxic Impurities by Liquid Chromatography Single Quad Mass Detector in Iomeprol. J Pharm Res Int. 2021; 756-67.
29. Managing the Risk of Elemental Impurities with ICH Q3D-PharmiWeb.com [Internet]. [cited 2024 Jan 3]. Available from: https://www.pharmiweb.com/article/managing-the-risk-of-elemental-impurities-with-ich-q3d
30. Lee K, Yoo W, Jeong JH. Analytical method development for 19 alkyl halides as potential genotoxic impurities by analytical quality by design. *Molecules*. 2022; 27(14): 4437. Available from: https://www.mdpi.com/1420-3049/27/14/4437/htm.
31. FDA, CDER, Deantonis DM. M7(R1) Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk. Guidance for Industry. 2018. Available from: https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm or https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm
32. Schultz TW, Carlson RE, Cronin MTD, Hermens JLM, Johnson R, O'Brien PJ, *et al.* A conceptual framework for predicting the toxicity of reactive chemicals: Modeling soft electrophilicity. *SAR QSAR Environ Res*. 2006; 17(4): 413-28. Available from: https://www.researchgate.net/publication/6866893_A_conceptual_framework_for_predicting_the_toxicity_of_reactive_chemicals_Modeling_soft_electrophilicity.
33. Bertande L. Role of analytical methods for detection of genotoxic impurities. 2023; 12: 2023.
34. Sommer S, Buraczewska I, Kruszewski M. Micronucleus assay: The state of art and future directions. *Int J Mol Sci*. 2020; 21(4). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7073234/.
35. Bobst S, Sukumar G. Genotoxic impurities in pharmaceutical products. *European Pharmaceutical Review*. 2019; 24(6): 56-58. Available from: https://www.europeanpharmaceuticalreview.com/article/108031/genotoxic-impurities-in-pharmaceutical-products/.
36. Amit G, Hussain S, Tabrez S. Genotoxic impurities and its risk assessment in drug compounds. *Drug Des Int Prop Int J*. 2018; 2(4).
37. Kumar T, Xavier N, Ramya M. A high-performance liquid chromatography method for determination of genotoxic impurity hydroxylamine in drug substances. *J Chromatogr Sci*. 2019; 57(1): 63-70. Available from: https://dx.doi.org/10.1093/chromsci/bmy082.
38. Wieder L, Pan J, Veeneman R. Application note: Residual solvent analysis of USP method <467> residual solvents on the Agilent 8890 GC system.
39. Kumar T, Ramya M, Srinivasan V, Xavier N. A simple and direct LC-MS method for determination of genotoxic impurity hydroxylamine in pharmaceutical compounds. *J Chromatogr Sci*. 2017; 55(7): 683-9. Available from: https://dx.doi.org/10.1093/chromsci/bmx019.
40. Ramachandra B. Development of impurity profiling methods using modern analytical techniques. *Crit Rev Anal Chem*. 2017; 47(1): 24-36. Available from: https://www.tandfonline.com/doi/abs/10.1080/10408347.2016.1169913.
41. More SJ, Bampidis V, Benford D, Bragard C, Halldorsson TI, Hernández-Jerez AF, *et al.* Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment. *EFSA Journal*. 2019; 17(6): e05708. Available from: https://onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2019.5708.
42. Reddy AVB, Jaafar J, Umar K, Majid ZA, Bin Aris A, Talib J, *et al.* Identification, control strategies and analytical approaches for the determination of potential genotoxic impurities in pharmaceuticals: A comprehensive review. *J Sep Sci*. 2015; 38(5): 764-79.
43. Snodin DJ. Genotoxic impurities: From structural alerts to qualification. Org Process Res Dev. 2010; 14(4): 960-76. Available from: https://pubs.acs.org/doi/abs/10.1021/op100118e
44. AI Genotoxicity Prediction | Use of Digital Technology | Eisai Co., Ltd. [cited 2024 Dec 5]. Available from: https://www.eisai.com/innovation/research/digital/genotoxicity/index.html.
45. Reddy AVB, Jaafar J, Umar K, Majid ZA, Bin Aris A, Talib J, *et al.* Identification, control strategies and analytical approaches for the determination of potential genotoxic impurities in pharmaceuticals: A comprehensive review. *J Sep Sci*. 2015; 38(5): 764-79.
46. Kalidindi VR, Somalanka BM, Seethamraju SM, Nori LP. Pharmaceutical impurities and their regulatory aspects with a special focus on genotoxic impurities. *International Journal of Pharmaceutical Research and Allied Sciences*. 2024; 13(2): 1-15.

Cite this article: Ramegowda KK, Mishra S, Prakash VM. Regulatory Pathway for Genotoxic Impurities in Europe, US, Canada, India, Australia and UK. Indian J of Pharmaceutical Education and Research. 2026;60(1s):s42-s51.