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Review on identification and quantification of genotoxic impurities

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Abstract---Genotoxic impurities can be broadly defined as those impurities that have been demonstrated to cause harmful changes in genetic material regardless of the mechanism. Globally people suffer from various health complications due to genotoxic impurities. Recent recommendations from European and United States (USA) drug regulatory bodies mandate the management of genotoxic and possibly genotoxic contaminants in pharmaceutical ingredients at per million levels. The purpose of this review is to make a critical analysis of the techniques used to comply with the prevailing rules and regulations and very strict limits on genotoxic impurities. Possible strategies to further expand the scope of currently available technologies and regulations are also to be discussed. These strategies include redesigning the synthesis of the drug substance to avoid introducing problematic impurities; modifying relevant process parameters to eliminate or reduce such impurities to negligible levels; Using process understanding to demonstrate that a particular genotoxic impurity cannot be formed or removed efficiently and by conducting toxicity studies to demonstrate that a suspected impurity is not harmful to it at low levels.

Keywords---Genotoxic, modifying relevant, pharmaceutical ingredients.

Introduction

Pharmacy and the pharmaceutical industry have progressed at a dizzying pace over the last century, from small pharmacies and dispensaries to multi-billion-dollar global corporations. Apart from the well-known R&D (research and development) of novel pharmaceutical products, the safety of medicines is becoming increasingly

important. There have been a plethora of pharmaceutical scandals in recent decades, ranging from unsafe chemicals and wrong dosage forms to purposely fortified drugs and unintentional contaminations. Today, the primary focus in treating a medical illness is to ensure patient safety and comfort. This requires high-quality drugs and treatments, as well as strict manufacturing guidelines and tactics. Such attempts by health authorities are certainly remarkable. Changing the term GMP for Good Manufacturing Practices to cGMP, where the "c" stands for "current", for example, emphasizes the ongoing commitment to GMP compliance [1]. Similarly, the US Food and Drug Administration (USFDA) declared in 2004 that it would transition from Quality by Test (QbT) to Quality by Design (QbD) [2]. The International Council for Harmonization (ICH) successfully adopted this approach, resulting in the introduction of several quality criteria [3]. The basic goal of QbD is to develop large-scale manufacturing processes around the idea of producing high-quality products with minimal variation. The term "impurity" in chemistry refers to a chemical substance that is different from the chemical composition of a limited chemical phase. [4]. Three main conditions must be met to designate a chemical compound with the property of "purity"[5]. To begin with, a pure chemical substance must thermodynamically appear in at least one chemical phase and be distinguished by its one-component phase diagram. Second, a pure chemical must demonstrate that it is homogeneous in practice (ie, it will show no change in its properties after being subjected to a wide variety of consecutive analytical chemical procedures). All attempts and tests of further separation and purification will fail in the perfect pure chemical. Finally, it must not contain any trace of any other chemical species, according to the conventional chemical definition. In reality, there are no chemicals that are 100 percent pure as there is always some level of contamination. The number of contaminants found tends to grow as detection limits decrease in analytical chemistry [6].

Although impurities are considered a nuisance in chemical synthesis, they are generally of little concern as long as their identity is clear and their amounts are under control. Chemists can accept compounds with purities of 97.5% or even less as long as these reagents perform their function [7]. From a pharmaceutical point of view, this question is considerably more complicated. Here, the result of chemical synthesis is not just a chemical substance per se, rather it is designed and manufactured to address a specific medical indication. Here impurities are particularly important, as they can – unintentionally – be administered to patients together with the substance in which they are present.

Genotoxic impurities are those that directly induce DNA damage when present in low concentrations, resulting in mutations and the potential for cancer [8]. PGIs (potentially genotoxic impurities) are those that present mutagenic structural alerts. The authorities have recently issued a series of relevant guidelines [9] in response to the potential harm of PGIs.

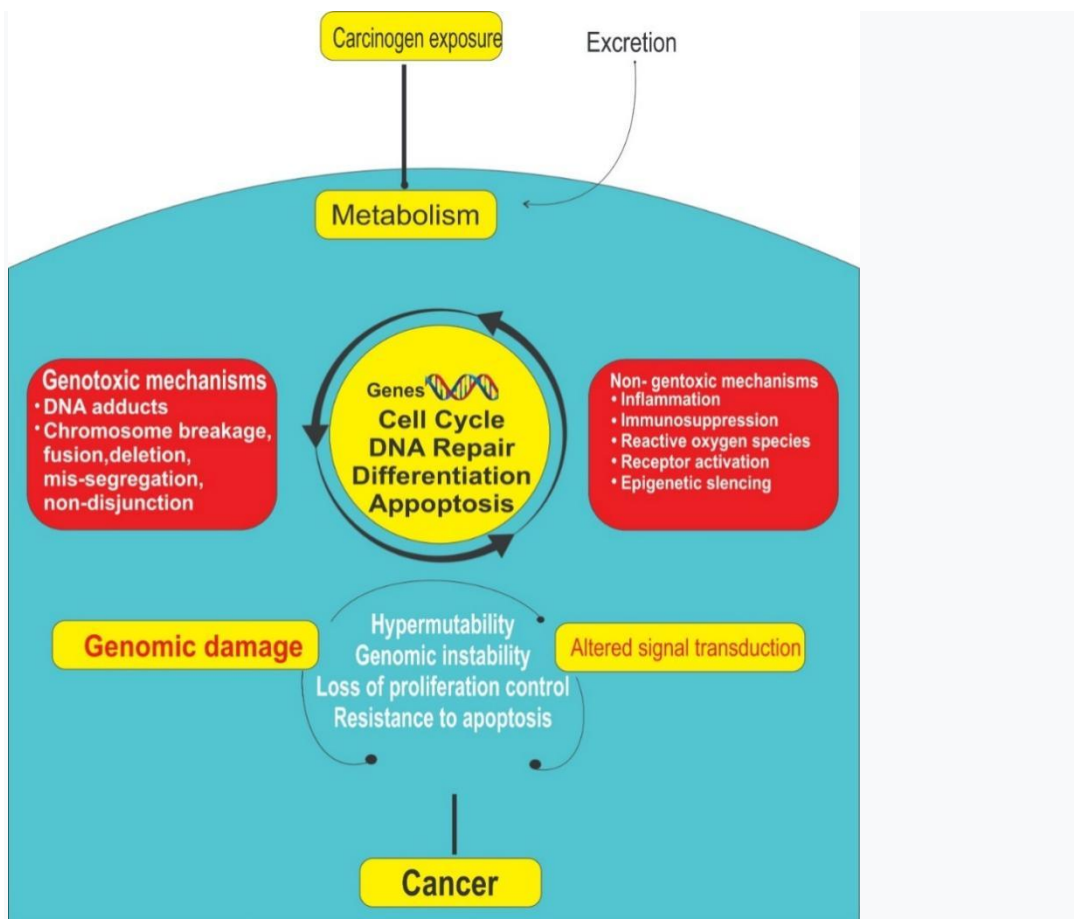


Fig-1, [10]

Separate guideline M7 was released by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) in 2014 [4]. Genotoxic impurities, according to the standards, are those that induce DNA damage directly when present at low levels, resulting in mutations and potentially causing cancer [8]. PGIs (potentially genotoxic impurities) are those that exhibit mutagenicity structural alerts. Authorities have recently recognized the possible harm caused by PGIs and issued a series of associated guidelines [9], [11]. Separate guideline M7 was released by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) in 2014 [12]. A notion of the threshold of toxicological concern (TTC) was established in the guidelines to define an appropriate daily intake that poses a negligible risk of carcinogenicity or other hazardous effects. For most medications, a TTC value of 1.5 g/day is considered the permissible daily consumption of PGIs. The ratio of TTC (g/day) to the maximum daily dose (mg/day) determines the maximum number of PGIs allowed in a pharmacological ingredient. [13]

To get to the final medicinal substance, many starting materials, process intermediates, and reagents are used in the process of synthesizing active pharmaceutical ingredients (APIs). Some of these components, intermediates, and reagents, as well as synthetic process by-products, can be harmful and exist as contaminants in the active ingredient or final medicinal product in low quantities. Toxic contaminants may cause significant health effects in people if they are present in high enough concentrations. As a result, pharmaceutical companies and regulatory agencies understand how critical it is to keep contaminants out of medication compounds and products. ICH Q3A(R2), Q3B(R2), and Q3C(R4) are regulatory guidance documents that provide advice on the identification, toxicological qualification, and derivation of acceptable limits for drug substance impurities, drug product degradants, and other contaminants. As part of the safety evaluation process, regulatory bodies all over the world seek data on the genotoxic risk of new medications. Preclinical investigations are commonly carried out to determine the fundamental toxicological profile of novel chemical entities (NCE). Toxicological data is used to analyze NCE's safety and efficacy, which will aid in anticipating the drug's anticipated risk/benefit evaluation throughout the New Drug Application (NDA) process. Genotoxicity assays have become a necessary part of the regulatory process. Furthermore, many people in India are unaware of genotoxicity, even though it is now mandated by European and US regulatory bodies to include it in drug master files. Genotoxicity testing of new chemical entities (NCE) is commonly performed to identify hazards related to DNA damage and repair. Gene mutation, structural chromosomal aberration, recombination, and numerical changes are all examples of these damages. These changes are responsible for heritable consequences, and somatic mutations have been shown to have a role in cancer. Because chemicals that test positive in these assays have the potential to be human carcinogens and/or mutagens, these tests have mostly been utilized to predict carcinogenicity and genotoxicity [17].

Methods

This review aims to provide a comprehensive understanding of genotoxic impurities and the risks associated with them. It also aims to highlight the gaps present in the current strategies available for the identification, validation, and regulation of genotoxic impurities in active pharmaceutical ingredients as well as herbal drugs. Manuscripts from reliable scientific sources in the public domain such as Google scholar, Web of Science, PubMed, etc. along with the guidelines and regulations available at the Food and Drug Association (FDA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and European Medical Agency (EMA). In this manuscript, we have attempted to present a comprehensive summary of our findings in the literature to make it easier for the reader to understand the impact of genotoxic impurities and the concerns associated with them. How this manuscript was prepared? From where the information was taken etc., the basic method of literature survey.

Classification Of Genotoxic Impurities

Chemical carcinogens have serious health consequences in many countries, and international organizations such as the World Health Organization (WHO) have

established regulations to regulate them. They are classified into five types, according to the international conference on harmonization, and assessment of GTIs in medicines M7 recommendations [18].

- **Class 1** – These contaminants are known to be genotoxic and carcinogenic, posing significant harm or risk.
- **Class 2** – Although these contaminants are known to be genotoxic, they do not have the potential to cause cancer. As a result, the "Toxicological Threshold Approaches (TTC)" must be used to manage these contaminants to some extent.
- **Class 3** – These impurities have an unusual structure that differs from the structure of the pharmacological compounds, and their genotoxic potential is uncertain. The impurities are identified for the structure-activity relationship in these groupings.
- **Class 4** – These impurities have a parent structure that is similar to that of pharmacological compounds, have an alert function, and are non-genotoxic.
- **Class 5** – These contaminants have no structural warnings. These contaminants are considered non-mutagenic.

GTIs are regulated because they offer a cancer risk to humans, and even tiny quantities of such impurities in the final active pharmaceutical ingredient might generate severe toxicological concerns (API). To ensure the community's safety, GTIs must be recognized in medications and monitored at very low doses. The purpose of this paper is to explore the analytical methodologies and problems used in pharmaceuticals to access, monitor, and control GTIs. [19]– [21].

Sources Of Genotoxic Impurities

Genotoxic impurities can enter drug compounds from a variety of sources, the most common of which is the starting material utilized in drug substance manufacturing and its impurities. Similarly, genotoxic intermediates and by-products produced during the manufacturing process may be carried forward as genotoxic contaminants in the therapeutic material. Aside from these, genotoxic contaminants in pharmacological compounds can also be found in the solvents, catalysts, and reagents utilized in the synthesis process. Impurities in pharmacological substances are produced as a result of degradation products formed during storage and shipment, as well as exposure to light, air oxidation, and hydrolysis. If a certain isomer of the drug substance is required, stereoisomers of the raw material and intermediate also contribute to the formation of chiral impurities in the drug substance. Figure 1 depicts impurity development at various phases of drug manufacturing. Extractable and leachable impurities, in addition to these excipients and their impurities, might contribute to genotoxic impurities in medicinal products [22], [23]. (Table 1)

Table 1: Genotoxic compounds in drug substances [24].

Category/Stage	Compounds
Starting material	Hydrazine, Nitroso, and acrylonitrile compounds
Intermediate	Benzaldehyde, Nitro compounds
By-product	Sulphonate esters, phosgene
Reagent	Formaldehyde, epoxides, esters of phosphate & sulphonates
Solvent	Benzene, 1,2-dichloroethane
Catalyst	Toxic heavy metals, metal phosphates
Degradation product	N-oxides, aldehydes,

Mechanism Of Genotoxicity

Through interactions with the DNA sequence and structure, genotoxic chemicals cause harm to the genetic material in cells. For instance, the transition metal chromium interacts with DNA in its highly oxidized form, causing DNA lesions that contribute to carcinogenesis. Through reductive activation, the metastable oxidation state Cr(V) is achieved. The researchers used a Cr(V)-Salen complex at a particular oxidation state to explore the interaction of DNA with carcinogenic chromium. [3] The interaction was unique to the genomic sequence's guanine nucleotide. To optimize the interaction of the Cr(V)-Salen complex with the guanine base, the researchers converted the bases to 8-oxo-G, which allows for site-specific oxidation. The interaction between the two molecules resulted in DNA lesions; guanidinohydantoin and spiroiminodihydantoin were the two lesions found at the changed base location. To further characterize the lesion location, it was discovered that polymerase had stalled at the region and that adenine had been incorrectly integrated into the DNA sequence opposite the 8-oxo-G base. As a result, these lesions are mainly G→T transversions. According to researchers, high-valent chromium acts as a carcinogen because "the mechanism of damage and base oxidation products associated with the interaction of high-valent chromium and DNA... is relevant to the in vivo formation of DNA damage leading to cancer in chromate-exposed human populations. As a result, it demonstrates how high-valent chromium may behave as a carcinogen when combined with 8-oxo-G to produce xenobiotics [25].

Pyrrrolizidine alkaloids are another example of a genotoxic chemical that causes DNA damage (PAs). These substances are primarily found in plant species and are toxic to animals, including humans; approximately half of them have been identified as genotoxic, while others have been identified as tumorigenic. After metabolic activation, the researchers found that "PAs cause DNA adducts, DNA cross-linking, DNA breaks, sister chromatid exchange, micronuclei, chromosomal abnormalities, gene mutations, and chromosome mutations in vivo and in vitro [26]. Within genes, the most frequent mutations are G: C → T: Atranversions and tandem base substitutions. The pyrrrolizidine alkaloids are carcinogenic in vivo and in vitro and are hence primarily responsible for liver carcinogenesis. Comfrey is a plant species that includes fourteen distinct phytoalexins (PAs). In liver endothelial cells and hepatocytes, the active metabolites interact with DNA, causing DNA damage, mutation induction, and cancer formation. Finally, the

researchers determined that "comfrey is mutagenic in the liver and that the PA present in comfrey seems to be responsible for the toxicity and tumor induction caused by comfrey [27].

Regulatory Guideline

Because of improved technological skills in identifying impurities and increased focus on their potential influence on human health, regulatory challenges connected to the presence of genotoxic or carcinogenic impurities have arisen more frequently. ICH guidelines Q3A(R), Q3B(R), and Q3C [28] are currently accessible guidance publications that address impurities and residual solvents. In addition, the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA) has released a draught of a suggested guideline regarding genotoxic impurity limits [29], and preliminary US regulatory considerations have been made available [30].1. ICH guidelines for industry: Q3A(R), Q3B(R) and Q3C

Impurities in drug substances and drug products are addressed in ICH Guidelines Q3A(R) and Q3B(R), respectively. Any component of the novel drug substance or product that is not the chemical entity designated as the drug substance or an excipient in the drug product is characterized as an impurity in these guidance publications. Thresholds for recognizing, reporting, and qualifying impurities are defined based on the amount of drug substance or product taken. "Such studies can be undertaken on the novel drug ingredient containing the impurities to be controlled, while isolated impurity studies can sometimes be suitable," according to Guideline Q3A(R). Guideline Q3B contains similar text (R). For several types of solvents, ICH Guideline Q3C establishes acceptable concentration limits or permitted daily exposures, however, it does not address a limitation of exposure based on concerns about genotoxic potential. Extrapolation using mathematical models should only be used to define exposure limits in circumstances when trustworthy carcinogenicity data are available, according to the guidance. The International Conference on Harmonization (ICH) is now working on guideline M7: Assessment and Control of DNA Reactive (Mutagenic) Impurities [31]. It will have a broader geographic scope than the existing EMA and FDA guidelines because it is an ICH guideline. It is also expected to clarify inconsistencies between FDA and EMA guidelines, as well as other issues currently being debated in the industry, such as how to deal with multiple structurally related genotoxic impurities with similar mechanisms of action and whether they should be added together when calculating a TTC. Fig-2 [32]

➤ **EMA proposed guidelines on limits of genotoxic impurities**

The EMA CHMP published a draught guideline on genotoxic impurity limits, which recommends categorizing genotoxic impurities into those for which there is "sufficient (experimental) evidence for a threshold-related mechanism" and those for which there is "insufficient (experimental) evidence for a threshold-related mechanism." The techniques provided in ICH Q3C for class 2 solvents would be used to regulate those genotoxic contaminants with sufficient evidence. This method determines a "permitted daily exposure" (PDE) by combining the No Observed Effect Level (NOEL) or Lowest Observed Effect Level (LOEL) from the

most relevant animal study with safety criteria. Chemicals that may fall within this category of genotoxins include:

- Interfering with the mitotic spindle can cause aneuploidy;
- Interfering with topoisomerase activity can cause aneuploidy
- Inhibiting DNA synthesis can cause aneuploidy.

The recommendation advocates a policy of regulating levels to "as low as reasonably practicable" for genotoxic contaminants for which there is insufficient evidence for a threshold-related mechanism (ALARP principle). This method stipulates that every effort should be taken to avoid the production of such contaminants during drug substance manufacturing, and if that is not possible, attempts should be made to decrease them using technical means (e.g., purification steps). Alkylating agents, intercalating agents, and free radical-generating agents are examples of compounds that interact with DNA directly or indirectly. Because all exposures to such agents theoretically carry some level of carcinogenic risk, regulatory agencies typically conduct quantitative risk assessments to calculate the increased levels of adverse events, such as cancers, that result from specific exposures and set exposure levels that result in "acceptable" risks, which are typically 1 in 10⁵ or 1 in 10⁶ additional cancers from lifetime exposures[33]. In the case of Class 1 carcinogenic solvents, the methods for these quantitative risk assessments are mentioned in ICH guidance Q3C, Appendix 3.

➤ **PhRMA genotoxic impurity task force white paper**

The Pharmaceutical Research and Manufacturing Association (PhRMA) set up a Genotoxic Impurity Task Force, which produced a White Paper and presented its recommendations in public meetings [34]. The publication lays out a technique for detecting, classifying, qualifying, and assessing the toxicological risk of potentially genotoxic contaminants in pharmaceuticals. All identified or expected contaminants, according to the Task Force, should be placed into one of five categories. It offers structural classification as well as functional group alerting. The presence of such structural moieties was previously thought to play a role in DNA mutation[35].Fig: Structure alerting functional groups[35].

➤ **FDA approach to regulation of genotoxic impurities**

The USFDA has issued draught guidelines to address GTI concerns, which aim to describe and limit the lifetime cancer risk associated with patient exposure to genotoxic and carcinogenic contaminants. The following are some of the suggested approaches:

- to Prevent the production of genotoxic and carcinogenic impurities

- Characterization of genotoxic and carcinogenic risk and exposure (allowing a maximum daily exposure target of 1.5 g/day)

- Reduction of genotoxic and carcinogenic impurity levels (allowing a maximum daily exposure target of 1.5 g/day)
- Flexible approach considerations to better enable suitable impurity standards [36].

Analysis Of Genotoxic Impurities

Analyze the impurities using sensitive, selective, and robust analytical methodologies to develop GTI control strategies that are sensible and sufficient. The methodology for analyzing GTIs is determined by the target criteria and expected levels for these impurities to comply with regulatory requirements. The analytical process should provide detection limits of 1 to 5 ppm (0.0001–0.0005 percent w/w) in the ideal case. Because a greater number of additional organic contaminants, such as excipients, may be present at lower concentration ranges, such low levels necessitate not only more sensitive analytical instruments but also higher demands on selectivity. Low-level impurities may be hampered by the comparatively substantial amount of API[37]. Gas chromatography (GC) and liquid chromatography (LC), both of which are frequently paired with mass spectrometry (MS) detection, are two analytical procedures for determining GTIs. The following are some common examples of GTIs trace analysis utilizing GC and LC. Online reaction monitoring's potential is also discussed[38].

ICH Identification Limit	Genotoxic Impurities Typical Limits		
0.1%	0.01%	0.001%	0.0001%
1000 ppm	100 ppm	10 ppm	1 ppm

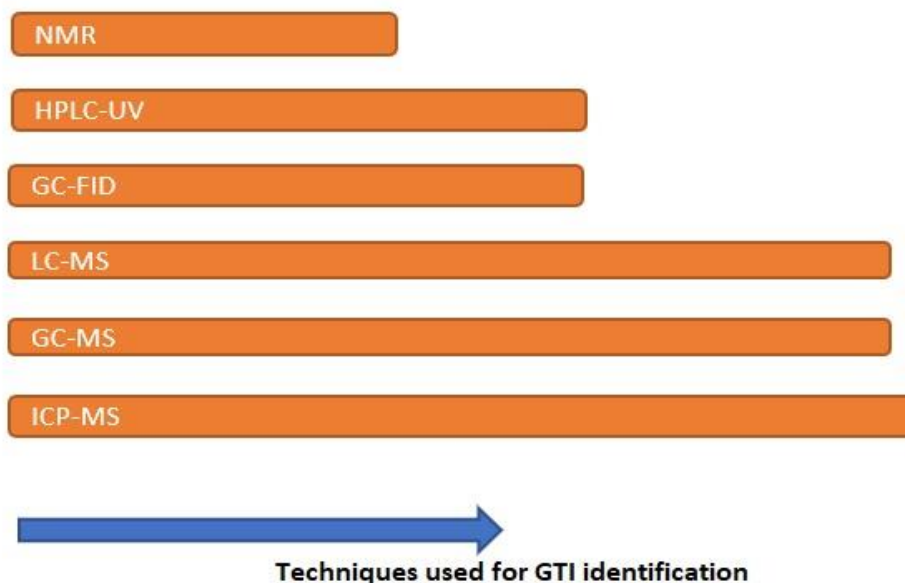


Fig [38]

The European Medicines Evaluation Agency (EMA) established guidelines on the limits of genotoxic impurities in pharmaceutical components based on the threshold of toxicological concern (TTC) concept (1). Impurities with "structural alert functionality" (2) must be quantified at levels lower than the TTC, which is equivalent to 1.5 g of daily consumption (for lifetime exposure). In practice, this means that genotoxic impurities in the drug substance or pharmaceutical product must be monitored at levels significantly below those used in traditional impurity assessments methods should typically allow for the detection of GIs (a specific solute or set of solutes) at concentrations of 10 ng/g to 1000 ng/g drug substance (10–1000 ppb). As a result, technique development for trace identification of possible genotoxic contaminants in the pharmaceutical analysis is a difficulty. HPLC (with UV/Vis detectors) and GC (with FID detectors) have traditionally been the most widely used procedures for detecting genotoxic impurities. To attain improved sensitivity and selectivity, mass spectrometers are increasingly being used as detectors in recent years[39].

Risks Assessments

In a synthetic process, evaluating and controlling GTIs is a multidisciplinary task. There are toxicological, processing, and analytical considerations to be made, all of which must be in line with regulatory criteria. While current regulatory advice sets clear expectations for GTI limits during clinical development and marketing application, there are still some grey regions. GTI regulatory grey 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 instead of analytical testing, expectations for analytical methodology and required level of validation, and universal understanding.

Electrophilic agents are commonly utilized in synthetic procedures to facilitate the creation of carbon, carbon-nitrogen, carbon-oxygen, and carbon-sulfur bonds in medicinal therapeutic compounds. Alkylating agents, benzyl halides, and Michael acceptors are examples. Some of these compounds may have the ability to react with biological substrates like DNA, raising worries about their carcinogenic potential. In a pharmacological compound, any residues of a verified DNA-reactive electrophilic reagent or intermediary would be classified as genotoxic contaminants (GTIs). The needs for managing trace levels of GTIs are not properly addressed by existing regulatory standards such as ICH Q3A (R2)/Q3B (R2)/Q3C (R4)[45]–[47]. As a result, an assessment of the danger caused by such contaminants is necessary. As the synthetic reaction progresses to the final API, the assessment of GTI carryover entails recognizing the probable presence/removal of such entities. Such an assessment must balance the risk of detecting the GTI in the final drug molecule with the likelihood of removing it (purge) based on knowledge of the synthetic chemistry. Because evaluating/identifying every possible impurity is impracticable, such an assessment must be based on a process understanding of likely/probable impurities. The EMA guidance encourages this approach[48].

Evaluation Of Genotoxic Impurities

A full description of a compound's chemical structure and physicochemical characteristics is the first step in determining its toxicological risk. In addition, the compound's creation or synthesis pathways, as well as methodologies for quantitative analysis, should be described and discussed [49]. The initial assessment of genotoxic potential is usually done by comparing the structures of reagents/starting materials/intermediates in the synthetic scheme with those of known genotoxins, either by simple comparison with a known alerting functionality, such as Ashby–Tennant alerts,[50] through searches of published information, or by assessing structures in a (quantitative) structure/activity relationship SAR/QSAR software database, such as DEREK (deductive estimation of risk from existing knowledge) or MCASE for in silico evaluation. Other toxicological data sources, such as TOXNET, can also be beneficial, especially when dealing with relatively common chemicals for which specific safety data may already exist.

Starting materials, reagents, intermediates, and known process contaminants are commonly analyzed structures. This is frequently supplemented during the development process by the addition of additional structures derived from 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, if applicable)[44].

Compounds without structural genotoxicity alarms are classified as ordinary impurities and are regulated by ICHQ3A/3B/3C [45]–[47]. Further action is required for compounds having genotoxicity structural alerts. In vitro methods, such as Ames testing, can be used to test these substances for mutagenicity. If the Ames test comes out negative, the impurity can be treated like any other and managed according to ICH Q3A/3B/3C recommendations. Exposure Assessment: The threshold of toxicological concern

The Committee for Proprietary Medicinal Products of the European Medicines Evaluation Agency (EMA) produced a draught position paper on the limits of genotoxic impurities at the end of 2002, in part as a result of this lack of clarity on unusually powerful impurities (subsequently finalized as EMA, 2006). It argued that the ICH Q3 guidelines were lacking in detail on the subject of acceptable limits for genotoxic impurities and that they relied on the frequently cited but frequently contested regulatory assumption that in vivo DNA-reactive compounds have the potential to damage DNA at any concentration, and thus there is no discernible threshold. As a result of this assumption, any degree of exposure entails a risk, hence there is no such thing as a "safe" level of exposure. As a result, defining an acceptable exposure level for these chemicals requires a different approach. The guideline then goes through the "Threshold of Toxicological Concern" (TTC), which was created by the US government. Chemicals migrating from food packaging materials are regulated by the Food and Drug Administration (FDA). The limit for the latter was set at 0.5 ppb which, assuming consumption of 3000 g/day food, translates to 1.5 µg/day. This dose was meant to be low enough to be of little danger, even if a drug exempted from regulation was later determined to be carcinogenic [51].

An assessment of the possible danger of a GTI carrying over to the drug substance at a level exceeding staged TTC or TTC thresholds is done to demonstrate control. This is consistent with the ideas of quality by design (QbD) and risk assessment contained in ICHQ8 and Q9 [52], [53]. The development of a sensitive analytical method and testing for the putative GTI in issue at the point of introduction, at the final drug substance, or an intermediate step, i.e. Quality by Testing, is a seemingly easy but possibly short-sighted strategy to address this (QbT). This approach, on the other hand, can be a technically difficult and resource-intensive operation, especially when applied to all of the GTIs involved with the synthetic process, and it goes against the QbD tenets. Furthermore, this method overlooks the fact that reactive GTIs are frequently destroyed or removed throughout the succeeding process steps that lead to the final pharmacological substance [44].

Despite these and other flaws in the cancer risk assessment model from which the TTC is derived, no other viable alternative approach acceptable to regulatory authorities has emerged, as evidenced by the number of regulatory areas in which the concept has been applied, such as food flavouring substances and indirect food additives. As a result, while recognizing this as the only model now acceptable, it is critical to understand its assumptions, limits, and over-conservatism before applying it.

Control and Mitigation of Genotoxic Impurities

Once a PGI has been identified as an actual or potential contaminant, process development chemists have four options: (1) change the synthesis route to eliminate the PGI; (2) change relevant process parameters to reduce the PGI to below a level of concern; (3) use chemical and mechanistic arguments, ideally supported by experimental evidence, to demonstrate that the PGI will not be present at significant levels; and (4) use a combination of these options.

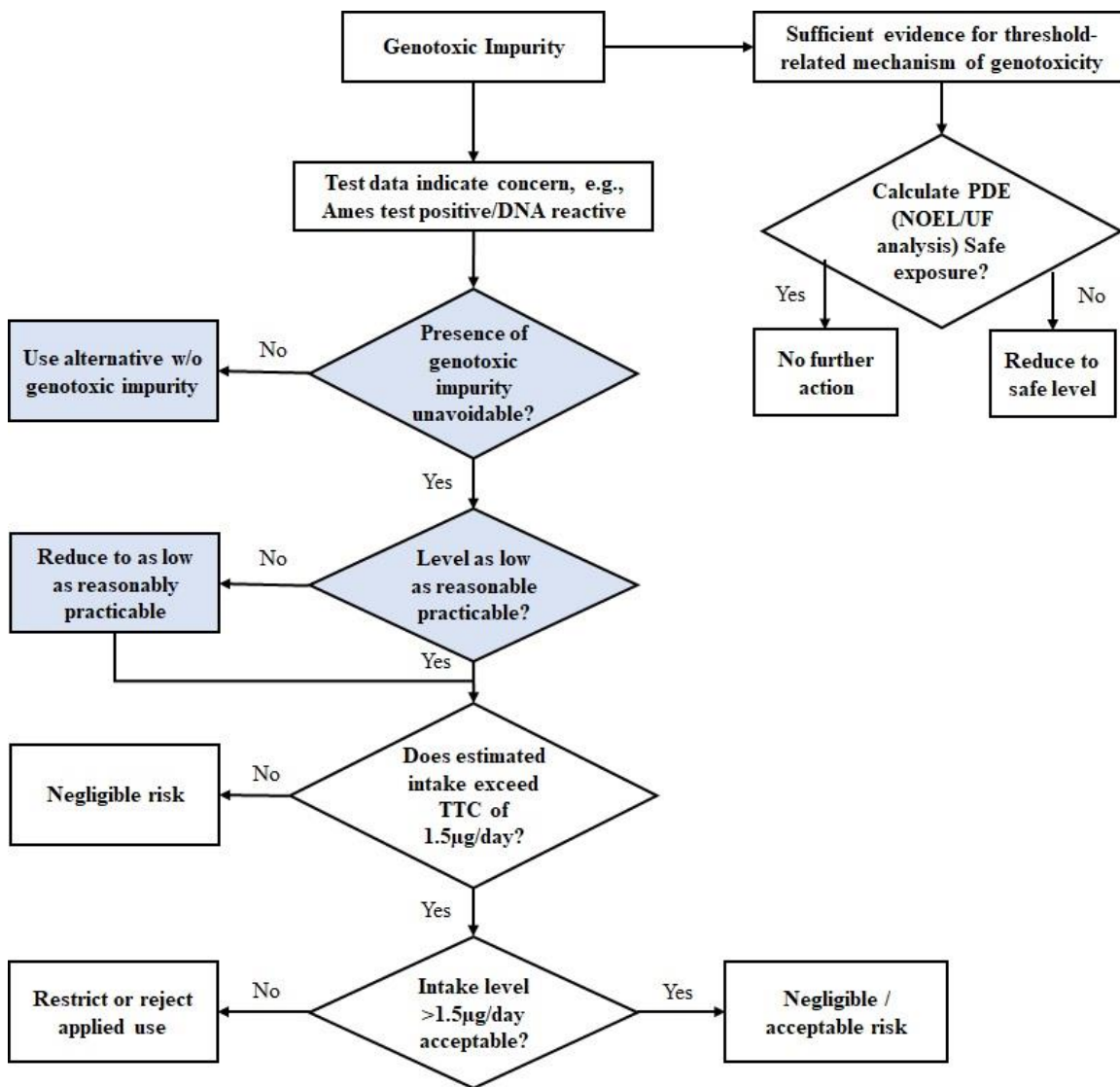
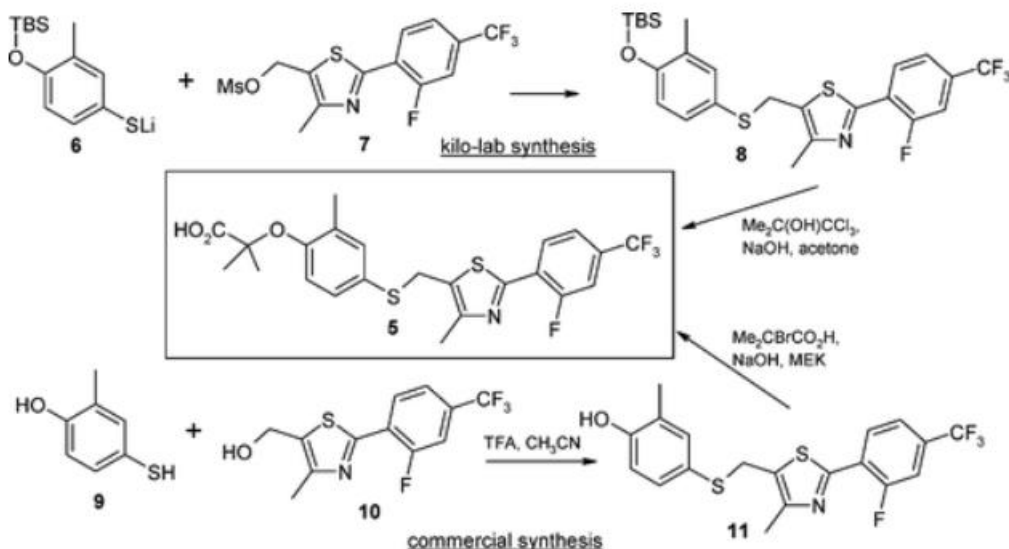


Fig: EMEA Decision tree[54]

Altering The Synthesis

The number one desired choice, according to the EMEA decision tree (Figure 1), is to eliminate PGIs. Although it is not always practical, the potential for generating genotoxic impurities is one of several factors that development chemists consider when comparing the merits of competing syntheses, and is frequently cited as a reason for switching synthetic routes during development, especially as processes scale up. Following are a few recent examples. Brown et al. (GlaxoSmithKline [GSK]) [55] identified the mesylate intermediate 7 in their kilo-lab approach as having the potential for genotoxicity when outlining the process development of sodelglitazar (5, Scheme 2), a possible type-2 diabetes medication. The difficulty

was avoided in the commercial method by using a different strategy for forming the thioether bond, which involved the nongenotoxic alcohol 10.

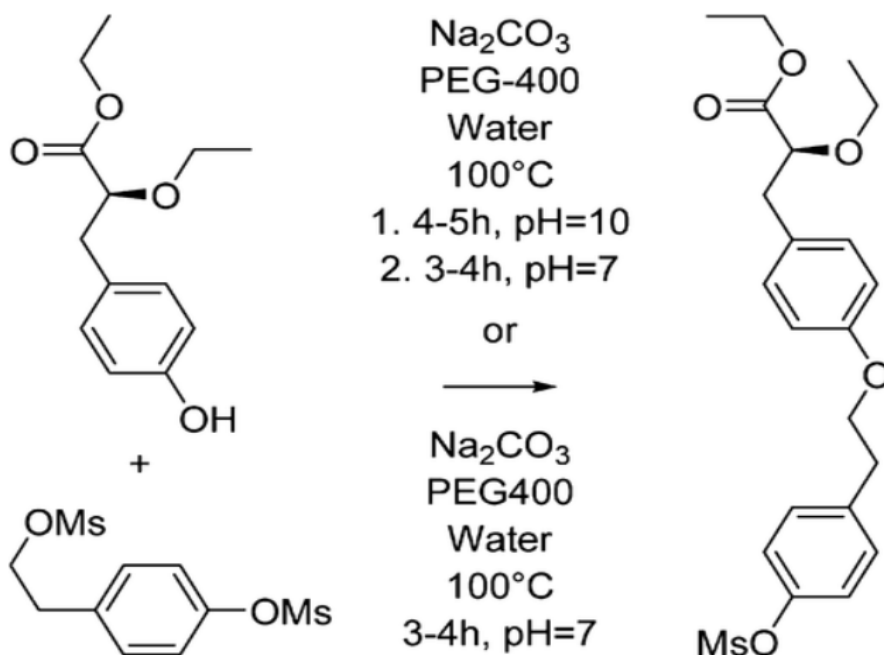


Scheme 2

Adjustment of Process Parameters

The following example demonstrates the feasibility of reducing the development of genotoxic contaminants by adjusting simple factors such as reaction time, pH, temperature, and solvent matrix.

A potentially genotoxic besylate ester is added in excess to the phenolic key intermediate to form an ether[56] at pH 10 and 100 °C for 4–5 hours while using PEG-400 as a phase-transfer catalyst in the presence of sodium carbonate in the synthesis of the AstraZeneca drug tesaglitazar for type 2 diabetes management (Scheme 86). By lowering the pH to 7 and increasing the reflux time to 8–9 hours, the alkyl sulfonate ester can be completely hydrolyzed without the carboxylate ester being hydrolyzed in the API. Other genotoxic sulfonate esters used in excess can be eliminated using a similar technique that takes advantage of different reactivities.



Scheme 86

Quality By Design

The QbD technique has been suggested for developing synthetic routes or selecting conditions for API synthesis, as well as controlling GTI creation below threshold quantities. In pharmaceuticals, QbD strives to design and construct API formulations for which the ultimate quality should be guaranteed a priori through the design of synthetic routes and the manufacturing process. QbD has four stages: defining the quality profile to be targeted; (ii) designing the product and manufacturing process to attain that quality; (iii) identifying and selecting quality attributes, process parameters, and sources of variability; and (iv) controlling quality throughout time. In the case of GTI risk management, the goal for product quality is to keep GTI below certain thresholds while maintaining high API yields[57].

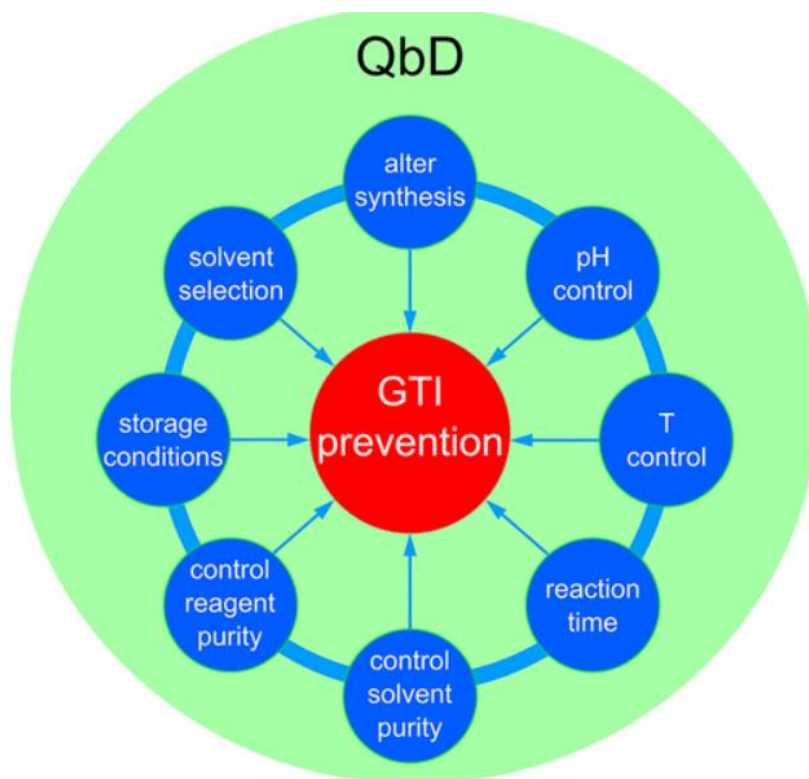


Figure 6. Quality-by-design strategy for prevention of GTI formation[57].

Herbal Medicine and Genotoxicity

Herbal remedies have a long history of usage in the prevention and treatment of illness; their use dates back to humanity's first written records, through antiquity and the medieval ages, and to current times [58]. They have been an integral component of human civilization from the dawn of time. The World Health Organization (WHO) reports that approximately 80% of the world's population continues to depend on medicinal herbs for basic health care. Herbal medications are then extensively utilized around the globe, particularly in Western countries [59]. For example, 71% of the population in Canada (IPSOS-Reid, 2005) IPSOS-Reid, 2005. Baseline Natural Health Products Survey among Consumers, and 80% in Germany [60] had used traditional medicines classified as "complementary and alternative medicine" throughout their lives. Around 19% of the adult population in the United States uses herbal medical products [61] [62], and herb supplement sales climbed by 23 percent in the United States from 2000 to 2010, reaching a market size of more than 5 billion dollars (NBJ, 2011) NBJ, 2011. NBJ Supplement Business Report. Europe imported around 400,000 tonnes of medicinal plants from Africa and Asia in 2004, with an average market worth of US\$ 1 billion[63]. Additionally, the WHO highly advocates the use of traditional herbal medicines in primary health care delivery systems in poor countries [64].

Even when herbal medical items are effective and well-documented, their toxicity is often unknown; moreover, in contrast to contemporary drug research and development, the toxicity of traditional herbal remedies is seldom investigated. The majority of the populace, on the other hand, is unconcerned, feeling that since these items have been used before, they should be safe. In the post-genome and bioinformatics age, genomics, proteomics, and metabonomics developments may be critical in determining the genotoxicity, teratogenicity, and nephrotoxicity of plant-based therapeutic products [65].

Discussion

Please bring in the lacunae in current regulations, changes needed, Indian scenario, etc,

The current regulations in the field of genotoxicity largely correspond to that of European and American agencies such as the FDA, ICH, and EMA. The guidelines issued by these agencies while highly comprehensive lack the specific requirements concerning the Indian sub-continent. India is a veritable goldmine of herbal medicines with Ayurveda at the forefront of the global medical industry. As such, there is a requirement to study the methods related to the identification of genotoxic impurities in the Indian sub-context. The biology and immunity of the Indian population are vastly different from the Caucasian population which may alter the acceptable threshold limits for particular genotoxic impurities and may have an entirely different set of effects in the case of others. Currently, Central Drugs Standards Control Organisation (CDSCO) is the governing body that issues regulations related to new drugs and cosmetics in India. ICMR has also published GCP guidelines about traditional drugs ICMR, Ethical guidelines for biomedical research on human participants. Director General, Indian Council of Medical Research, New Delhi, 2006. According to these guidelines, traditional herbal medicines have been classified into three groups: [12] 1. Traditional Herbal drugs as per Classical text, regular use, and prescribed pharmacopeia – reverse pharmacology approach 2. Traditional formulations for a new indication / new process / new combination/ new herbal or plant-based NCE – acute, subacute, and chronic toxicity data to be generated (Schedule Y of Drugs & Cosmetics Act, 1940) 3. Formulations – GMP-compliant Standardisation Department of AYUSH, ICMR, and CSIR work together to achieve safe, effective AYUSH products for the identified diseases and to develop new drugs. AYUSH's objectives are to control drug quality, lay down pharmacopeial standards, oversee the working of the Pharmacopeial Laboratory of Indian Medicines (PLIM), partner with the Quality Council of India (QCI), and oversee the functioning of Indian Medicine Pharmaceutical Company Limited (PCL). AYUSH also controls the enforcement of Good Manufacturing Practices (GMP), setting up of common facilities following the Cluster approach and implementing the scheme for Drug Quality Control. With the advent of the IPR regime, the AYUSH department has also started digitalization of traditional medicinal formulations, knowledge & manuscripts, and documentation and promotion of local health traditions.

However, it has been observed that the Indian regulations are comparatively less stringent than their European or American counterparts. Due to the lack of research facilities in India, there is simply a gap in data required for the

formulation of such regulations in India. As such Indian regulations are still at a nascent stage when compared to regulations of Europe and the US. Harmonization of regulations, like that in European Countries, could overcome the barrier to efficient trade as well as uniform standards for herbal medicinal products.

Conclusion

Genotoxins are difficult to identify and regulate in a synthetic process due to their changing nature and various entrance routes. Thus, synthetic pathways must be examined for the presence of structural warnings associated with genotoxicity. If GTIs are discovered, it is necessary to find alternative synthetic pathways that are free of these impurities. If this is not physically practicable, then safety restrictions based on the TTC idea must be established. These limitations often occur at lower concentrations and need analytical results with sufficient selectivity and sensitivity. Additionally, GTIs must be handled on an ongoing basis throughout the medication development process. If the route is altered, new intermediates must be evaluated. If the acceptable toxicological limit has changed as a result of the change in daily dosage, the process and analytical procedures must be evaluated for control at the new level. To balance risk and expense during the creation of pharmacological compounds, a multidisciplinary approach including specialists in toxicology, and synthetic and analytical chemistry is necessary. Finally, it's worth concluding our assessment with the statement that a safe product has appropriate risks about the scale of the projected benefits and the accessible alternatives.

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