Genotoxic impurities in pharmaceutical products – regulatory, toxicological and pharmaceutical considerations

Katarzyna Regulska

Greater Poland Cancer Center

https://orcid.org/0000-0002-9584-3128

Corresponding author: katarzyna.regulska@wco.pl

Marcin Michalak

Greater Poland Cancer Center

https://orcid.org/0000-0001-5583-0368

Marek Murias

Poznan University of Medical Sciences, Departament of Toxicology

https://orcid.org/0000-0002-2903-4912

Beata Stanisz

Poznan University of Medical Sciences, Departament of Pharmaceutical Chemistry

https://orcid.org/0000-0003-4611-7039

ABSTRACT

This article provides an overview of the most important aspects around the detecting and reporting of genotoxic impurities in the pharmaceutical industry. It focuses on relevant regulatory, toxicological, and pharmaceutical considerations. In this regard, the concept of Threshold of Toxicological Concern is explained and the most common genotoxic impurities are described. Furthermore, toxicological methods for genotoxic impurities screening are presented. Finally, the article emphasises several issues regarding further development.

Genotoxicity is defined as any detrimental modification of the genetic material irrespective of its causative mechanism, as per ICH guidelines ICH (S2) R1 Genotoxicity testing and data interpretation for pharmaceuticals intended for human use [1]. Screening for the genotoxicity of pharmaceuticals intended for human use is crucial with regards to safety during therapy, and it is warranted during non-clinical development by ICH M3 (R2) Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals and ICH (S2) R1 guidelines [1,2]. Moreover, according to ICH M7 Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk guideline [3,4], the impurities present in a final drug formulation, including degradation products and reaction-related impurities (i.e. starting materials, reagents, intermediates, solvents, catalysts, etc.), also require genotoxicity profiling. Specifically, mutagenic impurities are DNA reactive substances with the potential to directly damage DNA even at low concentrations (equalling ppm level), causing mutations and thus potentially leading to neoplasia. The presence of unusually toxic (e.g. DNA reactive) impurities has been of significant con-

cern to the industry and regulators for a long time, mainly because lower thresholds (below 100-1000 ppm as mandated for conventional impurities) seem relevant in their case. Thus, analytical procedures applicable to active pharmaceutical ingredients (APIs) and drug products or the most commonly encountered impurities and degradants have been found to lack the appropriate level of sensitivity for adverse mutagenic effect detection [5,6]. Therefore, although sufficient as a framework for the qualification and control of the most commonly encountered impurities and degradants, ICH Q3A(R2) Impurities in new drug substances and ICH Q3B (R2) Impurities in New Drug Productscannot be applied to genotoxic impurities. Finally, ICH guideline M7(R1) was adopted in 2014 (current effective version dated Feb 2018) to supplement ICH Q3A, Q3B and M3 (R2) and to provide a practical testing approach to support the identification, characterisation, qualification, and control of mutagenic impurities in pharmaceuticals. Its purpose is to establish acceptable limits that guarantee negligible lifetime risk of cancer [7].

The scope of application of ICH guideline M7(R1) excludes its retrospective use. In fact, it pertains only to new drug substances and new drug products which undergo the procedure of clinical development; there are several exceptions, including advanced cancer indications, biological/biotechnological, peptide, oligonucleotide, radiopharmaceutical or fermentation products, herbal products, and crude products of animal or plant origin. It also remains effective for post-approval submissions of marketed products, but only when there is new mutagenic data for the reported impurities or the introduced changes of synthesis and manufacture affect the impurity profile to the extent that the levels of existing impurities or degradants increase or unreported impurities or degradants appear [4]. Modifications of indication or dosing which significantly affect the acceptable cancer risk level will also result in the need to apply ICH M7 (R1). Importantly, the theoretical structural alerts alone will not trigger concern in marketed products unless the structure belongs to the group defined as the 'cohort of concern', which includes aflatoxin-like-, N-nitroso-, and alkyl-azoxy structures [4,7,8].

In new drug substances and new drug products, actual synthetic and degradation impurities exceeding the ICH Q3A/B reporting thresholds as well as the potential impurities which could be present in the final API or drug formulation must be evaluated for their mutagenicity. Potential impurities include starting materials, reagents, and intermediates in the route of synthesis [4,9]. As for degradation products, the requirement for mutagenicity assessment applies to all those observed above the ICH Q3A and ICH Q3B reporting thresholds during long-term storage. In addition to this, potential degradation products mandated for mutagenicity evaluation include all those observed above the ICH Q3A/B identification threshold during accelerated stability studies [9]. Initially, they must be screened for DNA-reactivity based on the literature research or an assessment of Structure-Activity Relationships, which predict bacterial reverse-mutation assay outcomes. This initial assessment is designed to establish whether individual impurities contain (or could be metabolised into) any electrophilic structural features that might constitute structural alerts for DNA-reactivity. Such compounds involve the following: alkyl esters of phosphoric or sulphonic acid, aromatic nitroso-groups, aromatic azo-groups, aromatic ring N-oxides, aromatic mono- and di-alkyl amino groups, alkyl hydrazines, alkyl aldehydes, N-methylo derivatives, monohaloalkanes, N and S mustards, propiolactones, propiosulfones, aromatic and aliphatic substituted primary alkyl halides, carbamates, alkyl N-nitrosoamines, aromatic amines and N-hydroxy derivatives, aliphatic epoxides and aromatic oxides, and aliphatic nitro group or halogenated methans [7,10-12].

One of most investigated genotoxic impurities are sulfonates. Sulfonate salts are frequently used in pharmaceutical optimisation due to their favourable physio-chemical properties, including a higher melting point, a limited tendency to form hydrates, and higher solubility [9]. For example, halperidol mesylate provides a higher dissolution rate which results in rapid onset of action. Sulfonic acid, however, can react with methanol, ethanol or isopropanol to produce sulfonate esters, which are recognised as potential alkylating agents. Methane sulfonate (MMS) and ethyl methane sulfonate (EMS) are well-established gentotoxic compounds in vitro and in vivo and, historically,

they have been reported as contamination in nelfinavir mesylate [13] and imatinib mesylate [14]. Moreover, alkyl halides, as electrophilic impurities, can be present in drug products secondary to the reaction of salt formation based on strong acid/base interactions in the presence of alcohol. Other electrophilic compounds commonly reported in drug products such as bethamethasone and atenolol are epoxides. Hydrazines, in turn, have DNA-forming adducts potential and are used as starting materials for pharmaceutical synthesis [9].

Nonetheless, at present the definition of the structural alert is not precisely settled. Thus, in some cases the correlation between the presumed structural alert existence and the real carcinogenicity might be weak [15]. For example, simple n-alkyl aldehydes, except formaldehyde, turn out to be negative in bacterial mutagenicity tests [16]. Similarly, out of acyl chlorides, acetyl chloride, chloracetyl chloride, and butyryl chloride are not mutagenic [7,17]. Furthermore, in the group of carbamates, the confirmed genotoxicity data only exists for vinyl carbamates, whereas the aromatic amines N-methylaniline and 4-aminophenol are not mutagenic [15]. This clearly indicates that the concept of structural alert applies only to selected compounds with that particular feature, giving rise to a certain proportion of false predictions. Therefore, the currently-adopted regulatory approach still demands a high level of expertise in chemistry and toxicology and it justifies a constant need for scientific endeavours to identify new mutagenicity data [15].

Based on the collected in-silico and literature data, the impurities are categorised into one of five classes. These classes, in turn, have relevant follow-up control actions: class 1 impurities are known mutagenic carcinogens; class 2 impurities are known mutagens with unknown carcinogenic potential; class 3 impurities demonstrate alerting structures (un-related to drug substance) with no supporting mutagenicity data; class 4 impurities show alerting structures (related to drug substance which is itself non-mutagenic); and class 5 impurities present no alerting structures. The impurity can be categorised as class 5 or 4 with no additional qualification studies if two complementary QSAR protocols (an expert rule-based and statistical-based) confirm no mutagenic concern or sufficient data is available to prove

that there is no mutagenicity or carcinogenicity. If a structural alert is predicted or there is carcinogenicity scientific data available (Class 1 to 3 assigned impurities), Ames testing is required as a follow up action. If compounds exhibit a so-called structural alert, it is acceptable to maintain such an impurity at levels less than the Threshold of Toxicological Concern (TTC) (discussed later) without performing Ames testing on condition that the structural alert falls outside the cohort of concern (devoid of N-nitroso, aflatoxin-like, and alkyl-azoxy groups). The single bacterial reverse mutation assay (the Ames test) is the test of choice for mutagenicity and carcinogenicity prediction as it has exhibited a relevant sensitivity for the detection of genetic changes as well as for the majority of genotoxic rodent and human carcinogens [7]. This procedure has been adequately described in ICH S2(R1) and OECD 471 guidelines [1,18]. Specifically, the Ames test is designed to detect the point mutation-inducing capacity of the analysed substances. The procedure involves the exposition of amino-acid-dependent auxotroph Salmonella typhimurium or Escherichia coli strains to increasing concentrations of the tested impurity. Pre-existing point mutations in test bacteria render them incapable of growing and forming colonies in an amino-acid-deficient medium. The exposition to a mutagenic compound (tested substance) causes base substitutions or frameshifts within the mutated-bacterialgene and may cause a reversion to amino-acid prototrophy, thus restoring the revertant bacteria's ability to grow in the medium devoid of the amino acid essential for the parent test strain [18]. Some critical considerations regarding the Ames test as the sole reference for mutagenicity involve its positive/negative predictivity of approximately 80% and 50%, respectively. This indicates that a number of potential false positive and negative results may occur. There is also substantial interpretation uncertainty with regards to the Ames-negative and mammalian-cell genotoxicity assays-positive results [18].

For impurities classified as 1, 2, and 3, the acceptable human intakes are established. When the compound-specific risk assessment confirms carcinogenicity (class 1), the compound-specific acceptable limit for human exposure should be met. For class 2 and 3, where no carcinogenicity data is available, the conception of

the staged TTC can be adapted to each individual impurity [7]. TTC defines the permissible intake of a mutagenic impurity as 1.5 µg per person per day, which is deemed as bearing a negligible cancer risk for a long-term exposure exceeding 10 years [19]. Cancer risk, however, is assumed to increase as a function of cumulative dose, meaning that lifetime exposure to mutagenic impurity can be adjusted for a shorter duration of drug use according to its indications [7]. This concept would enable the higher daily intake of mutagenic impurities for the expected restricted treatment period, referred to as less-than-lifetime (LTL) exposure. In line with this, for a treatment duration of less than one month (for example, drugs used in emergency, antidotes, anaesthesia, acute ischemic stroke), 120 µg/day exposure to mutagenic impurity is acceptable. For treatment duration ranging from one to 12 months (e.g. anti-infective drugs) the allowed exposure is defined as 20 µg/day, and for treatments lasting 1-10 years (drugs used in diseases with shorter life expectancy) the exposure limit is reduced to 10 µg/day. The structural classes belonging to the 'cohort of concern', e.g. aflotoxin-like, N-nitroso or azoxy compounds are exempted from the TTC approach due to their high carcinogenicity potential. For several impurities commonly encountered in final drug formulations, the acceptable daily intake has been individually defined as demonstrated in **Table 1** [7].

Finally, for class 1-3 impurities there is a need for the development of control strategies to maintain their allowed limit. In addition, for the control of degradation products that are potentially mutagenic, a degradation pathway must be established and validated with regards to its relevance in real-time storage [7,9].

Although the current regulatory approach appears to exhibit a reasonable level of pragmatism, some issues still seem to lack clarity and precision. Unfortunately, the announcement of these rigorous requirements for genotoxicity screening did not prevent the N-nitrosodimethylamine (NDMA) crisis in 2018 and 2019, when the N-nitroso impurities were accidentally detected in ranitidine and valsartan-containing marketed products. This occurrence led to their global recall. It was further revealed that some of these drug products contained as much as 17 µg NDMA in a single tablet; the Food and Drug Administration estimated that this would lead

Table 1. Acceptable daily intakes or permissible daily exposure of common drug impurities [7]

Impurity	µg/day
Acrylonitrile	6
Benzyl Chloride	41
Bis(chloromethyl)ether	0.004
1-Chloro-4-nitrobenzene	117
p-Cresidine	45
Dimethylcarbamoyl chloride	5
Ethyl chloride	1.810
Glycidol	4
Hydrazine	32
Methyl Chloride	1.361
Aniline	720
Hydrogen peroxide	68.000 or 0.5% whichever is lower
p-Chloroaniline	34
Dimethyl Sulfate	1.5

to one additional case of cancer for every 8,000 patients taking the drug at the highest dose [20]. In March 2020, European Medicines Agency published a questions and answers document containing "Information on nitrosamines for marketing authorisation holders" to provide marketing authorisation holders (MAH) with guidance on performing testing for nitrosamine impurity. In line with this, MAHs are obliged to handle a risk evaluation to establish whether chemically synthesised APIs bear a risk of contamination with nitrosamines by 31 March 2021. In scenarios where risk is identified, these initial evaluations must be followed with a second step of confirmatory testing by 26 September 2022 for chemical APIs [21].

This event has ultimately proven that the scientific research for mutagenic impurities and degradation pathways of well-established pharmaceuticals remains absolutely essential.

References

1. European Medicines Agency Guidelines: ICH (S2) R1 Genotoxicity testing and data interpretation for phar-

maceuticals intended for human use. https://www.ema.europa.eu/en/ich-s2-r1-genotoxicity-testing-data-interpretation-pharmaceuticals-intended-human-use. Accessed 2021 January 12.

2. European Medicines Agency Guidelines: ICH M3 (R2) Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals. https://www.ema.europa.eu/en/ich-m3-r2-non-clinical-safety-studies-conduct-human-clinical-trials-pharmaceuticals. Accessed 2021 January 12.

3. Shaikh T. Genotoxic Impurities and Its Risk Assessment in Drug Compounds. Drug Designing & Intellectual Properties International Journal. 2018 Oct 23;2(4). https://doi.org/10.32474/ddipij.2018.02.000143

4. European Medicines Agency Guidelines: ICH M7 Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk. https://www.ema.europa.eu/en/ich-m7-assessment-control-dna-reactive-mutagenic-impurities-pharmaceuticals-limit-potential. Accessed 2021 January 12.

5. Snodin D, Elder D. Genotoxic Impurities Part 1: General Overview. Pharmaceutical Outsourcing. 2012 May 25;.

6. Savale SK. Genotoxicity of Drugs: Introduction, Prediction and Evaluation. Asian Journal of Biomaterial Research. 2018;4(6):1-29.

7. European Medicines Agency Guidelines: ICH Q3A(R2) Impurities in new drug substances and ICH Q3B (R2) Impurities in New Drug Products. https://www.ema.europa.eu/en/ich-q3a-r2-impurities-new-drug-substances. Accessed 2021 January 12.

8. Müller L, Mauthe RJ, Riley CM, Andino MM, Antonis DD, Beels C, DeGeorge J, De Knaep AG, Ellison D, Fagerland JA, Frank R, Fritschel B, Galloway S, Harpur E, Humfrey CD, Jacks AS, Jagota N, Mackinnon J, Mohan G, Ness DK, O'Donovan MR, Smith MD, Vudathala G, Yotti L. A rationale for determining, testing, and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity. Regulatory Toxicology and Pharmacology. 2006 Apr;44(3):198-211. https://doi.org/10.1016/j.yrtph.2005.12.001

9. R Pounikar A, J Umekar M, R Gupta K. Genotoxic impurities: an important regulatory aspect. Asian Journal of Pharmaceutical and Clinical Research. 2020 Apr 11;:10-25. https://doi.org/10.22159/ajpcr.2020.v13i6.37370

10. Maithani M, Raturi R, Gupta V, Bansal P. Evolution of regulatory aspects of genotoxic impurities in pharmaceuticals: Survival of the fittest. Journal of Liquid Chromatography & Related Technologies. 2017 Sep 14;40(15):759-769. https://doi.org/10.1080/10826076.2017.1357574

11. Dobo KL, Greene N, Cyr MO, Caron S, Ku WW. The application of structure-based assessment to support safety and chemistry diligence to manage genotoxic impurities in active pharmaceutical ingredients during drug development. Regulatory Toxicology and Pharmacology. 2006 Apr;44(3):282-293. https://doi.org/10.1016/j.yrtph.2006.01.004

12. Elder D, Facchine KL, Levy JN, Parsons R, Ridge D, Semo L, Teasdale A. An Approach to Control Strategies for Sulfonate Ester Formation in Pharmaceutical Manufacturing Based on Recent Scientific Understanding. Organic Process Research & Development. 2012 Oct 12;16(11):1707-1710. https://doi.org/10.1021/op300216x

13. Elder DP, Snodin DJ. Drug substances presented as sulfonic acid salts: overview of utility, safety and regulation. Journal of Pharmacy and Pharmacology. 2009 Mar;61(3):269-278. https://doi.org/10.1211/jpp.61.03.0001

14. Ramakrishna K, Raman N, Rao KN, Prasad A, Reddy KS. Development and validation of GC–MS method for the determination of methyl methanesulfonate and ethyl methanesulfonate in imatinib mesylate. Journal of Pharmaceutical and Biomedical Analysis. 2008 Mar;46(4):780-783. https://doi.org/10.1016/j.jpba.2007.11.013

15. Snodin D, Elder D. Genotoxic Impurities Part 2: Toxicological Overview. Pharmaceutical Outsourcing. 2012 Sep 16;.

16. Snodin DJ. Genotoxic Impurities: From Structural Alerts to Qualification. Organic Process Research & Development. 2010 Jul 16;14(4):960-976. https://doi.org/10.1021/op100118e

17. Seifried HE, Seifried RM, Clarke JJ, Junghans TB, San RHC. A Compilation of Two Decades of Mutagenicity Test Results with the AmesSalmonella typhimuriumand L5178Y Mouse Lymphoma Cell Mutation Assays†. Chemical Research in Toxicology. 2006 May;19(5):627-644. https://doi.org/10.1021/tx0503552

18. OECD (2020), Test No. 471: Bacterial Reverse Mutation Test, OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD Publishing; . https://doi.org/10.1787/9789264071247-en

19. Kroes R, Renwick A, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, van Schothorst F, Vos J, Würtzen G. Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. Food and Chemical Toxicology. 2004 Jan;42(1):65-83. https://doi.org/10.1016/j.fct.2003.08.006

20. Scinto K. NDMA contamination in medications has caused concern among consumers, and health care professionals after WHO recalled 1159 lots of ARBs including valsartan, losartan, and irbesartan. https://www.pharmacytimes.com/view/updates-on-nmda-contaminations-in-medications. Accessed 2021 March 16.

21. European Medicines Agency: Questions and answers document on "Information on nitrosamines for marketing authorisation holders" to provide guidance on handling nitrosamine impurity testing for marketing authorization holders. https://www.ema.europa.eu/en/human-regulatory/post-authorisation/referral-procedures/nitrosamine-impurities. Accessed 2021 January 5.