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REVIEW ON GENOTOXIC IMPURITIES IN DRUG SUBSTANCES

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ABSTRACT

Genotoxic substances are those chemical compounds capable of causing genetic mutation and of contributing to the development of tumors while there are many different factors that can affect DNA,RNA and other genetic materials, the property of genotoxicity only applies to those substances that actually cause harm to the genetic information. Genotoxicity assays too insensitive to detect effects of an impurity at 0.1%; very few genotoxic compounds would be detectable at or below this level. The management of genotoxic impurities in the synthesis of pharmaceuticals is very important for the safety of use of the drug. As these impurities are considered to be carcinogenic and they can cause mutations they are to be detected even in trace level for the safety.

Keywords: DNA, Genotoxicity, Pharmaceuticals.

INTRODUCTION

Genotoxic substances are those chemical compounds capable of causing genetic mutation and of contributing to the development of tumors while there are many different factors that can affect DNA,RNA and other genetic materials, the property of genotoxicity only applies to those substances that actually cause harm to the genetic information. A substance that has a property of genotoxicity is known as a genotoxin. Genotoxins can be carcinogens, or cancer causing agents.

In most cases, genotoxicity leads to mutations in various cells and other bodily systems. Mutation can lead to host of other problems, from cancer to a wide variety of different diseases. Sometimes mutation caused by genetics is completely harmless and can go completely unnoticed. In many other cases, though, the effects of genotoxins can be deadly. Mutations can come in many different forms; genetic information duplicated, deleted, inserted.

Though there are many mechanisms by which, genotoxicity can affect; genetic information, one of the most common mechanism involves the formation of strong chemical bonds between the genotoxins and the molecules that compose genetic information, such as DNA and RNA. In some cases, these bonds do not strongly affect the existing genetic data [1].

Sources of genotoxic substances

Genotoxins are ubiquitous and can be found in numerous sources such as food products, food supplements, air and water supplies. Genotoxins may also be found in pharmaceutical products, which will be the mainly focusing. Specifically the class of genotoxins covered will be those which are electrophillic compounds that can react with DNA. An example from this class in alkylating compounds that form covalent derivatives on the N7 nitrogen of guanine. One potential result of this can lead to a mutation such as DNA base mispairing. Another example is the formation of an adduct of a nitrogen containing genotoxin [2].

Genotoxic substances add risk without any benefits, Genotoxic impurities in relation to pharmaceuticals can come from many places including starting materials, reagents, intermediates, solvents or unwanted side reactions from the API synthetic process that get carried over into the final product. In addition API itself can decompose to form Genotoxic impurities or they can form in the drug product by reaction between excipients or containers and the API. As these substances are reactive in nature they form drug complex substance,

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this reactivity however can react with DNA and damage if they are carried over into a product taken by patients.

The viracept (Nelfinavirmesylate) contamination incident is an example of a case that contributed to the heightened awareness, and potential dangers of genotoxic impurities in pharmaceutical product. Various lots of this HIV drugs distributed by Roche pharmaceuticals were pulled off the market in 2007 [3].

Strange odors were noticed from different batches of the drug product and further analysis reveled abnormally high levels of ethyl mesylate (ethyl methanesulfonate). Ethyl mesylate is an alkylating class of genotoxic impurity that can covalently bind to DNA and enhance cancer risk. The source of ethyl mesylate cleaning and methane sulfonic acid contained in the drug product. This incident prompted much discussion regarding specifications for the amounts of this genotoxic impurity as found in one particular pharmaceutical product. This highly publicized incident helped bring more attention to the issue of genotoxic impurities in pharmaceuticals.

Some of the commonly encountered potentially genotoxic structural motifs are given fig. A group of these referred alkylating agents, such as alkyl halides, alkyl sulfonates, and related structures. These molecules might be used as reagents or can be otherwise generated during chemical synthesis. For example a salt counter ion of basic molecule such as HX(X=halogen) reacts with alcohol to form an alkyl halide (fig 2). Alkyl sulfonates, including alkyl esters of sulfonate, methane sulfonicacid (mesylate), benzene sulfonicacid (besylate) and p-toluene sulfonic acid (tosylate), are commonly used as alkylation agents in chemical synthesis. For example, dimethylsulfonate and diethyl sulfonate are commonly used as methylating and ethylating agents, respectively. In addition, certain sulfonicacid are commonly used as counter ions to form API salts. Interactions of the acids with residual alcohols may lead to the generation of alkyl sulfonates, which are potential GTIs, as illustrated in Fig 3 [4].

Identification of Genotoxic impurities

The synthesis of pharmaceutical products frequently involves the use of reactive reagents and formation of intermediates and by-products. Low levels of some of these may be present in the final drug substances and drug product as impurities. Such chemically reactive impurities may have at the same time the potential for unwanted toxicities including genotoxicity and carcinogenicity and hence can have impact on product risk assessment. Degradation products and process-related substances are reviewed during the pharmaceutical assessment in order to evaluate their genotoxic potential. Starting materials, reagents and intermediates may contain functional groups that could react with the DNA. The identification of PGI or known GTI can been achieved using publicly accessible data or specialized software [4-5].

Available databases

Genotoxic and/or carcinogenic properties for a number of substances are reported by potency database, CPDB. This database reports results from technical reports issued by the National Cancer Institute for several chemicals. Information about functional groups and compounds that can react with DNA can be found in databases such as TOXNET, NIOSH and GESTIS.

STRUCTURAL ALERTS

When substance specific data on genotoxicity / carcinogenicity are not available, the impurities can be screened for the presence of structural alerts. The identification of structural alerts is important both for assessing substance related risks and for understanding the mode of action. the structural alerts are molecular substructures or functional groups related to the potential mutagenic/ carcinogenic properties of substance. They can be identified by a structure-activity relationship (SAR) analysis between the chemical structure/physicochemical properties of the compound and the biological effect. The advances in computational potential, the widespread use of computers for SAR analysis in medicinal chemistry, as well as the need for a fast first pass screening to assess toxicity, prompted the development of computational methods for toxicological assessment. These methods are based on structure similarity and quantitative structure-activity relationship (QSAR) models [6].

AMES TEST

In the 1970s a scientist named, bruceames, discovered a procedure that tests carcinogens in compounds. This procedure became to be known as the ames test studies show that carcinogens are easily detectable in microorganisms.

The Ames Test is used as the key assay for confirming the genotoxic potential of an impurity highlighted as PGI by a structural alert, since many chemicals that are mutagenic in the Ames Test are carcinogens in rodents. According to the EMAQ&A document, a positive Ames test confirms that the TLC limit must be applied, while a negative Ames test, conducted according to regulatory standards, overrules any structural alert, and the limit for the impurity can be established according to ICH Q3 criteria.

Thus genotoxic carcinogens can be identified by combining the use of structural alerts with the Ames Test. The Ames Test is considered to be predictive for genotoxicity since most carcinogens are positive in this test, with very low incidence of false negative hen the appropriate concentration of the tested substance is used. The most common exception is carbamates, genotoxicity of these compounds is not detected by Ames test and therefore, a mutagenicity test in mammalian cells is necessary [7].

Sometimes the Ames Test gives positive results which do not correspond to genotoxicity and carcinogenicity in mammals. This is due to protective or elimination mechanisms and/or to different cell control mechanisms effective in mammals but not in bacteria. Positive Ames Test results does not necessarily mean that the substance Is a mutagen in humans. In the case uncertainity, the standard battery for mutagenicity can be applied. According to the ICH S2 (R1) guideline (currently at step 3) the standard battery consists of assessment of mutagenicity in a bacterial reverse mutation assay. For this test a bacterium salmonella typhimurium was used. This organism cannot survive without the amino acid histidine in order to detect its mutation ability. This mutation ability tells us if the compound given to the bacteria is carcinogenic. If the salmonella does not mutates, then the compound is not carcinogenic [8-11].

The test uses several strains of the bacterium salmonella typhimurium that carry mutations in genes involved in histidine synthesis i.e.it is an auxotrophic mutant ,so that they require histidine for growth. The variable being tested is the mutagens ability to cause a reversion to growth on a histidine free medium. The tester strains are specially constructed to have both frame shift and point mutations in the genes required to synthesize histidine, which allows for the detection of mutagens acting via different mechanisms. Some compounds are quite specific, causing reversions in just one or two strains. The tester strains also carry mutation in the genes responsible for lipo polysaccharide synthesis, making the cell wall of the bacteria more permeable, and in the excision repair system to make the test more sensitive.

Rat liver extract is optionally added to stimulate the effect of metabolism, as some compounds, like benzo[a]pyrene, are not mutagenic themselves but their metabolic products. The bacteria are spread on an agar plate with a small amount of histidine. This small amount of histidine in the growth medium allows the bacteria to grow for an initial time and have the opportunity to mutate. When histidine is depleted only bacteria that have mutated to gain the ability to produce its own histidine will survive. The plate is incubated for 48 hours. The mutagenicity of a substance is proportional to the number of colonies observed [12-15].

Classification ofGenotoxic impurities

On the basis of available data, impurities identified during the pharmaceutical assessment can be Classified in terms of the related risk level, according to five categories:

Category 1: compounds with literature data (at least in animal models) which provide evidence for carcinogenicity.

Category 2: compounds which are known mutagens with unknown carcinogenic potential (positive in the Ames test but without evidence for in-vitro carcinogenicity)

Category 3: compounds with structural alert(not shared with the drug substance) not confirmed by the Ames test.

Category 4: compounds with a structural alert shared with the drug substance. The impurity I considered qualified in this case since genotoxicity of the drug substance is characterized.

Category 5: compounds without structural alert.

Limits for the identified and classified GIT can be established as outlined below. For compounds in

Category 1: the first action Is to eliminate them from the process. If this test is not possible the TTC limit must be applied.

Category 2: it can be decided to use the TTC limit or to do a specific toxicological risk assessment, if a threshold-related mechanism can be demonstrated the PDE limit can be calculated.

Category 3: the TTC limit can be applied or the actual genotoxic potential can be assessed by the Ames test. If the tet is positive the TTC limit can be used or a more specific toxicological assessment can be carried out.

As discussed for compounds in Category 2, if a threshold-related mechanism can be demonstrated the PDE limit can be calculated. If the Ames test is negative the Q3A criteria also apply for compounds in Category 4 or 5. When the impurity is in Category 2 or 3 , the option to apply the TTC or to stud y the toxicology of the compound more in-depth can be considered. The staged- TTC limit can be applied during clinical development, in the meanwhile the increased knowledge about the process can confirm whether to control the impurity at the TTC level is an achievable task or not. If the control of the impurity at the TTC level is turns out to be not practicable, it could be worthwhile doing a further toxicological characterization. This characterization can be done by a step-by-step approach. The confirmation of the Ames result and corresponding biological relevance are addressed firstly. Experiments aimed at assessing the GTI mode of action can be carried by out after the confirmation of genotoxicity. The limit for the impurity can then be assessed by appropriate in vivo studies, when a threshold-related mechanism is proved [16].

Regulation and guidance on Genotoxic impurities

Regulatory issues related to the presence of genotoxic or carcinogenic impurities have arisen with greater frequency due to enhanced technological capability in identifying impurities and increased focus on their potential impact on human health. Currently available guidance documents include ICH guidelines Q3A(R),Q3B(R) and Q3C, which address issues related to impurities and residual solvents. In addition, the European Medicines Agency (EMEA), Committee for Medicinal products for Human Use(CHMP) has published a draft of a proposed guideline regarding limits of genotoxic impurities and initial US regulatory considerations have been presented publicly [17-19].

ICH Guidelines for Industry

Q3A(R), Q3B(R) and Q3C ICH Guidelines address the issue of impurities in drug substance and drug products, respectively. These guidance documents define an impurity as any component of the new drug substance or product that is not the chemical entity defined as drug substance or an excipients in the drug product. Depending on the quantity of drug substance or product consumed, thresholds are set for identifying, reporting and qualifying impurities. The guidelines state that certain tests can be conducted if considered desirable; part of the battery of tests used to qualify impurities include assays to determine whether the impurity is genotoxic. Suggested assays to assess genotoxic potential include a "minimum screen" of *in-vitro* assays: a bacterial point mutation assay (Ames test) and an assay for chromosomal damage. Guideline Q3A(R) indicates that "such studies can be conducted on the new drug substance containing the impurities to be controlled, although studies using isolated impurities can sometimes be appropriate" simply stating that additional testing ,removal of the impurity or lowering the level of the impurity should be considered. ICH Guideline Q3C sets acceptable concentration limits or permissible daily exposures for various classes of solvents but does not discuss a limitation of exposure based upon concerns for genotoxic potential [20].

EMEA proposed Guideline on limits of Genotoxic impurities

EMEA guideline the limits of GTIs, classifies GTIs into two categories

GTIs with sufficient (experimental) evidence for a threshold related mechanism. These are to be regulated using methods outlined in ICH Q3A (R4) for class 2 solvents.

GTIs without sufficient (experimental) evidence for a threshold related mechanism. These are to be controlled as low as reasonably practicable (ALARP principle). Although this approach is acceptable in most instances, mechanistic data sufficient to allow for an assessment of threshold mechanism is lacking [21].

Hence, this guideline proposed the use of "threshold of toxicological concern (TTC)" that refers to a threshold exposure level to compounds which will not pose a significant risk of carcinogenicity or other toxic effects. A TTC value of 1.5 µg/day intake of GTI Is considered being associated with an acceptable risk. The concentration limit in ppm of GTI permitted in a drug substance is the ratio of TTC in µg/day and daily dose in grams/day. The TTC approach benefits consumers, industry and regulators by avoiding unnecessary toxicity testing and safety evaluations. This guideline summarizes its recommendations in the form of a decision in which the preferred option is to eliminate GTIs, second preference into apply ALARP principle and the final alternative is the TTC approach. EMEA also released "Question and Answer" document by clarifying questions arouses in its original guidance.

USFDA GUIDANCE

USFDA released draft guidance to address GTI issues. This guidance describes a variety of ways to characterize and reduce potential life time cancer risk associated with patient exposure to genotoxic or carcinogenic impurities. There commended approaches include a) prevention of genotoxic or carcinogenic impurity formation,(b) reduction of genotoxic or carcinogenic impurity levels(allowing a maximum daily exposure target of 1.5µg/day) (c) additional characterization of genotoxic or carcinogenic risk and (d) considerations for flexibility in approach to better support appropriate impurity specifications [22].

GUIDANCE FOR ONCOLOGY PRODUCTS

TTC limits may be liberalized for GTIs for oncology products. The USFDA draft guidance states value higher than 1.5µg per day may be acceptable in situations where the anticipated human exposure will be short term, for the treatment of life threatening conditions, when life expectancy is less than 5 years, or where the impurity is a known substance and human exposure will be much greater from other sources. The ICH S9 guideline on non-clinical evaluation for anticancer pharmaceuticals also states," for genotoxic impurities, several approaches have been used to set limits based on increase in life time risk of cancer. Such limits are not appropriate for pharmaceuticals intended to treat patients with advanced cancer and justifications should be considered to set higher limits" [23].

Table 1. Examples of common synthetic transformations that should be avoided according to EMEA guidelines

Functional group	**Named reaction**
Primary halides and sulfonates	Williamson ether sysnthesis
Phosphonate esters	Homer –wadsworth-emmonsolefination
Aldehydes	Aldol and claisencondeasation
Hydrazines	Fisher indole synthesis
Aminoaryl and alkylamino aryls	Common intermediates
Michael acceptors	Michael reaction
Epoxides	Sharpless asymmetric epoxidation

Halo-alkenes	Cross coupling reactions
Aromatic nitro compounds	Source of aromatic functionality

Figure 1. Representative structures of potential genotoxic impurities

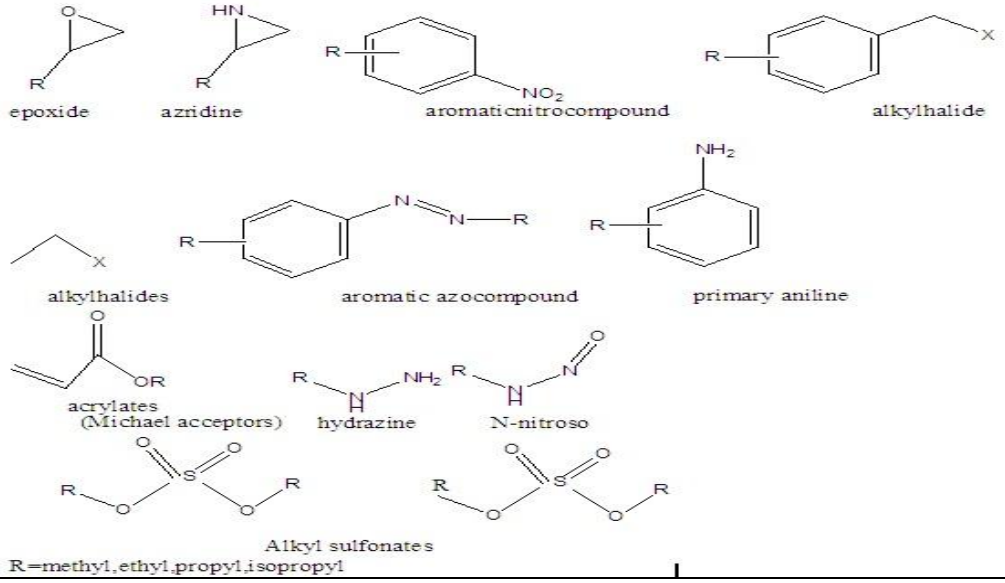


Figure 2. Generation of Alkyl halides during chemical synthesis

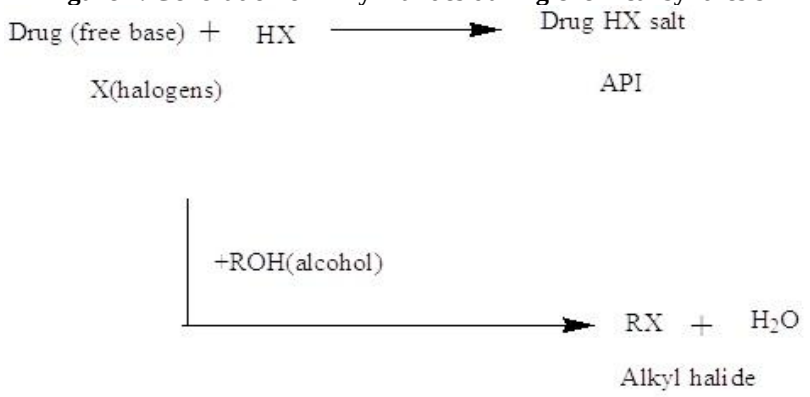


Figure 3. Generation of Alkyl sulfonates during chemical synthesis

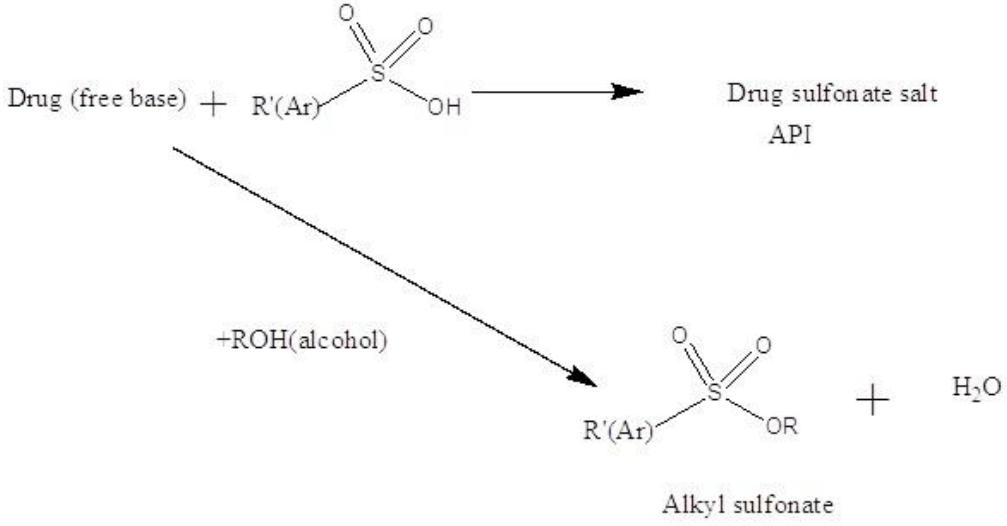
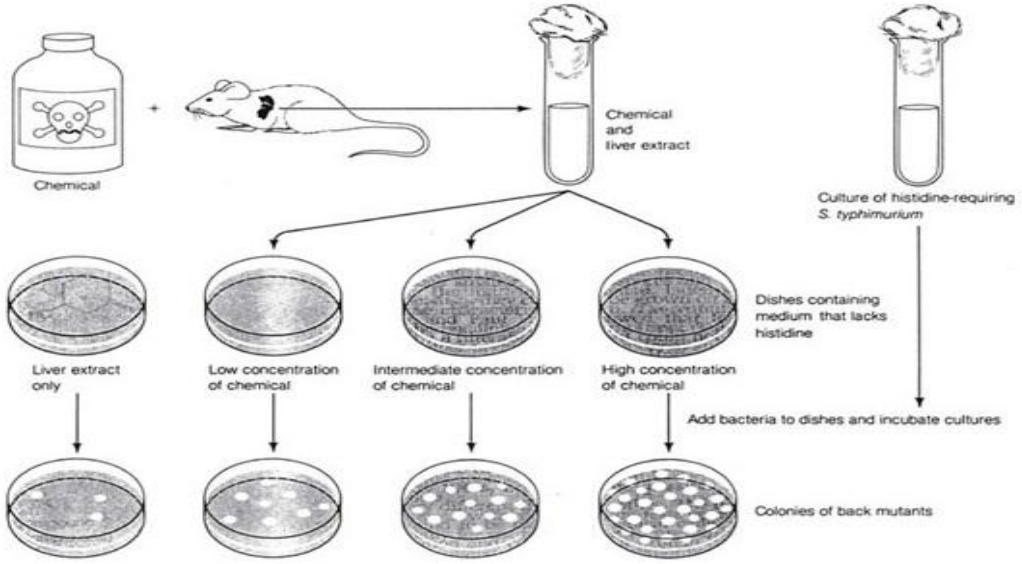


Figure 4. Representation of Ames Test procedure



CONCLUSION

Genotoxic impurities represents a special case relative to the International Conference on HarmonisationQ3A/Q3B guidance's, because genotoxicity tests used to qualify the drug substance may not be sufficient to demonstrate safety of use of the drug. The main elements of regulatory guidance on GTIs have been in place for several years, there is still considerable scientific uncertainty on many key toxicological issues. For example, structural alerts are not clearly defined or agreed, the role of data from mammalian cell assays remains unclear, the current TTC appears somewhat biased and certainly overly conservative and the methodology for its derivation is inconsistent with prior art established for non-genotoxic solvents in ICH Q3C. Furthermore, the introduction of in-silico techniques into the regulatory arena brings with it a number of critical issues on the appropriate number of independent systems, data integrity and the role of expert interpretation. Various risk assessment techniques are available for non-genotoxic and genotoxic carcinogens and it is currently unclear which are likely to be more or less acceptable. It is to be hoped that eventual ICH guidance will acknowledge such problems and permit a range of approaches provided that they are scientifically justified.

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