A BRIEF REVIEW ON CARCINOGENIC POTENTIAL OF NITROSAMINE IMPURITIES IN PHARMACEUTICALS

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Abstract:
Nitrosamine is the class of synthetic compound which is a potent genotoxic agent and considered as probable plausible human cancer-causing agent by international agency for research on cancer (IARC). Thus, N-nitroso compounds are known as potent carcinogenic and global worry according to the various authorities and also from ICH M7. Recently N-Nitrosodimethylamine (NDMA) has been detected in several pharmaceutical marketed drugs. These events have led regulatory agencies to require that N-nitrosamines risk assessments be performed on all marketed medical products. The need for these assessments is driven by the high carcinogenic potency of several N-nitrosamines in rodents, thus making these substances a significant regulatory concern. These impurities formed in drug products due to solvent, catalyst, raw materials are used in manufacturing process. Control of N-Nitroso drug substance related impurities is challenge for the pharmaceutical companies and regulatory agencies.

Keywords: Genotoxic, *NDMA, Acceptable intake, Limit, DNA, Secondary amine, Nitrous acid, NDSRI, Carcinogenocity, Muragenic*

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Background

Nitrosamines are a class of chemical compounds that include the nitroso group. They can be found in water, food, tobacco, pesticides, and plastics, but they gained widespread notice in the middle of 2018 when they were discovered in pharmaceuticals.

The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) recommendation on evaluation and oversight of DNA reactive (mutagenic) contaminants in pharmaceuticals to limit possible carcinogenic risk1 refers to N-nitrosamines as the "cohort of concern" due to their potency as mutagenic carcinogens. The International Agency for Research on Cancer (IARC) has classed the two most important nitrosamines, N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA), as probable human (class 2A) carcinogens, but they are also genotoxic (3, 4). Once activated by microsomal liver enzymes, N-nitrosamines may react with DNA base pairs to generate unstable α-hydroxyalkylnitrosamines and release alkyl diazonium ions, that alkylate DNA bases and cause a carcinogenic response.

Discovery of Nitrosamines in medicinal products

The Chinese active pharmaceutical ingredient (API) company Zhejiang Huahai Pharmaceutical (ZHP) found NDMA contamination in the valsartan production process on June 6, 2018. Findings demonstrated that nitrosamine pollutants arose in the ZHP facility after July 2012, when the manufacture of valsartan was altered to boost yields and decrease waste. Particularly, the producer altered the synthetic procedure for tetrazole ring synthesis by substituting tributyltin azide with a more hazardous anhydrous sodium azide and using dimethylformamide (DMF) as the solvent. Sodium nitrite, which creates nitrous acid in an acidic media, was employed to neutralise excess sodium azide, resulting in the formation of NDMA by the nitrosation of dimethylamine (DMA) impurity in dimethylformamide.(Figure 1). Production changes that caused formation of NDMA in the Zhejiang Huahai Pharmaceutical plant.[1]

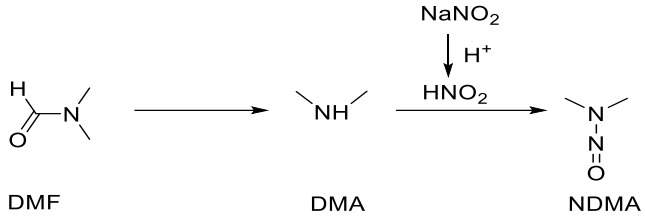


Figure 1:- Formation of NDMA from N, N-Dimethylformamide

Regulatory Response

In reaction to this revelation, the medicines agencies of the member states of the European Union (EU) recalled a variety of ZHP-manufactured medications comprising valsartan API. In accordance with Article 31 of Directive 2001/83/EC on the Community code relating to medicinal products for human use, the European Commission (EC) delegated to the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency the responsibility of assessing valsartan medicines in the EU that contained the ZHP-manufactured active pharmaceutical ingredient (API) on 5 July 2018. (7). In the weeks that followed, the existence of another nitrosamine, NDEA, was verified in a number of items. In addition, studies revealed that N-nitrosamine contamination was not restricted to the ZHP facility and valsartan API manufacturing, but also impacted other sartans having tetrazole rings (Figure 2). This discovery expanded the evaluation to include all sartans with a tetrazole ring. In 2019, NDMA and NDEA were detected in ranitidine and metformin as well. When quality testing revealed that some ranitidine medicines contained NDMA in higher concentrations than the acceptable daily intake limit, the European Medicines Agency (EMA) initiated a second review of medicinal products containing ranitidine in September 2019, which led to the conclusion that MAHs could perhaps expand their risk assessment to include all medicinal products containing a chemically synthesised active substance.

[1] Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk- ICH M7

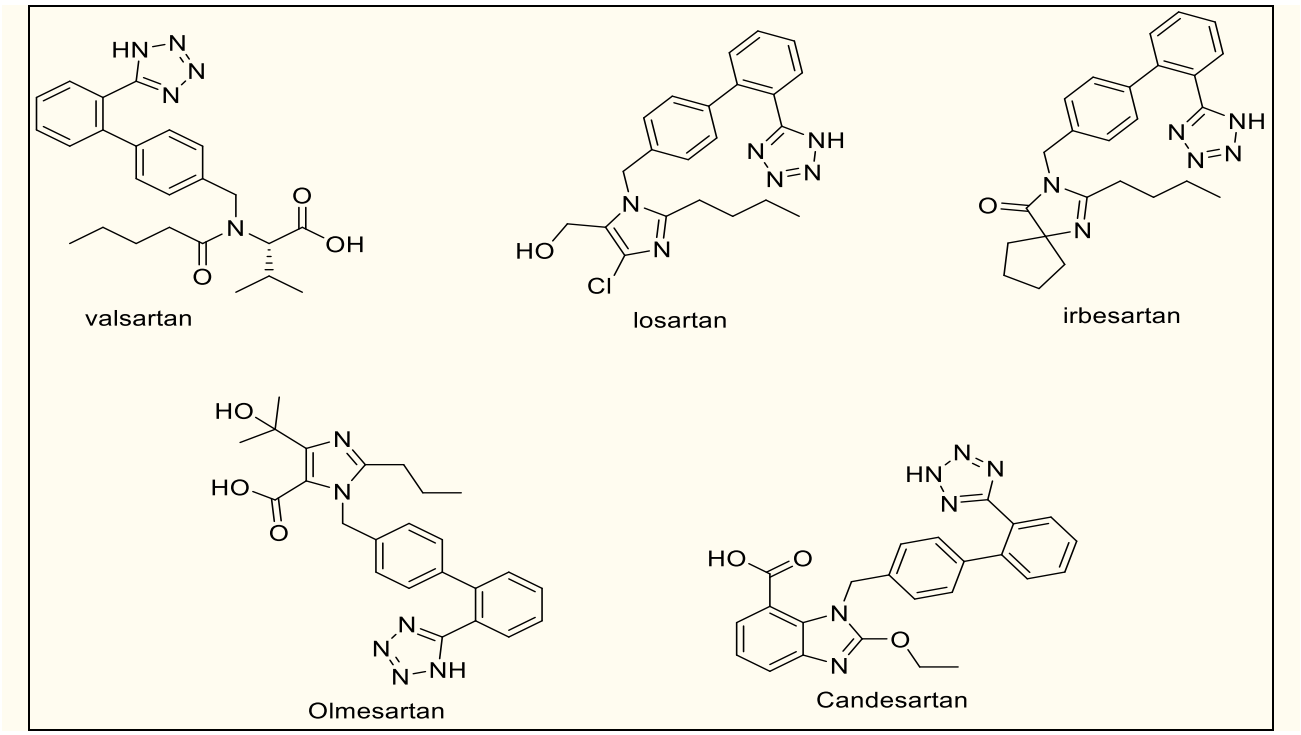


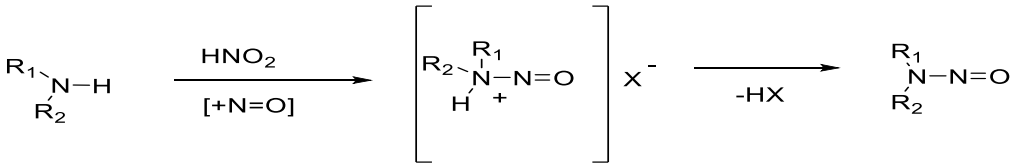
Figure 2 Sartans with a tetrazole ring contaminated with impurities

The finding of nitrosamines in some types of prescription goods prompted the FDA and other international agencies to perform a comprehensive examination of these impurities in impacted active pharmaceutical ingredients (APIs) and medication products. 8, 9 This guidance analyses the possible fundamental causes of nitrosamine establishment and advises API and drug product manufacturers to (I) perform risk evaluation of their authorised or commercialised products and products with pending applications, and (II) take suitable steps to minimize or inhibit the presence of nitrosamines in APIs and drug products. Although nitrosamine contaminants have been identified in a limited number of drug products, and batches of those products have been recalled due to unacceptable levels10 of these impurities, nitrosamine impurities may exist in other APIs and drug products due to the use of susceptible procedures and supplies that can produce nitrosamine impurities. Thus, all chemically produced APIs fall inside the scope of these guidelines. In addition to the drug products specified in FDA notifications, they also apply to drug products using chemically synthesized APIs and drug products at risk owing to additional circumstances indicated in this advice (see sections II.B and C).

What are nitrosamine impurities?

Nitrosamines more correctly N-Nitrosamines are a class of mutagenic impurities produced by the reaction of a secondary or tertiary amine with a nitrosating agent under acidic conditions. Nitrosamine impurities such as NDMA and N-nitrosodiethylamine (NDEA) are of concern due to their potential to cause cancer.

Representative Reaction to Form Nitrosamines:



The International Agency for Research on Cancer classifies NDMA and NDEA as probable human carcinogens (Group 2A). When there is scant evidence of carcinogenicity in people and significant evidence of carcinogenicity in experimental animals, this category is used. Despite the lack of concrete evidence that these chemicals cause cancer in people, their presence in pharmaceuticals is judged inappropriate.

When secondary amine is exposed to nitrite during food processing or preservation, NDMA can

develop. Beer, fish and fish products, dairy products including cheese, dried milk, meat, cereals, and vegetables are dietary sources of NDMA.

NDMA, N-nitrosodiethylamine (NDEA), N-nitroso-N-methyl-4-aminobutanoic acid (NMBA), N-nitrosoisopropylethyl amine (NIPEA), N-nitrosodiisopropylamine (NDIPA), N-nitrosodibutylamine (NDBA), and N-nitrosomethylphenylamine (NMPA) are seven nitros (Figure 2). Five have been discovered in drug substances or drug products (NDMA, NDEA, NMBA, NIPEA, and NMPA).

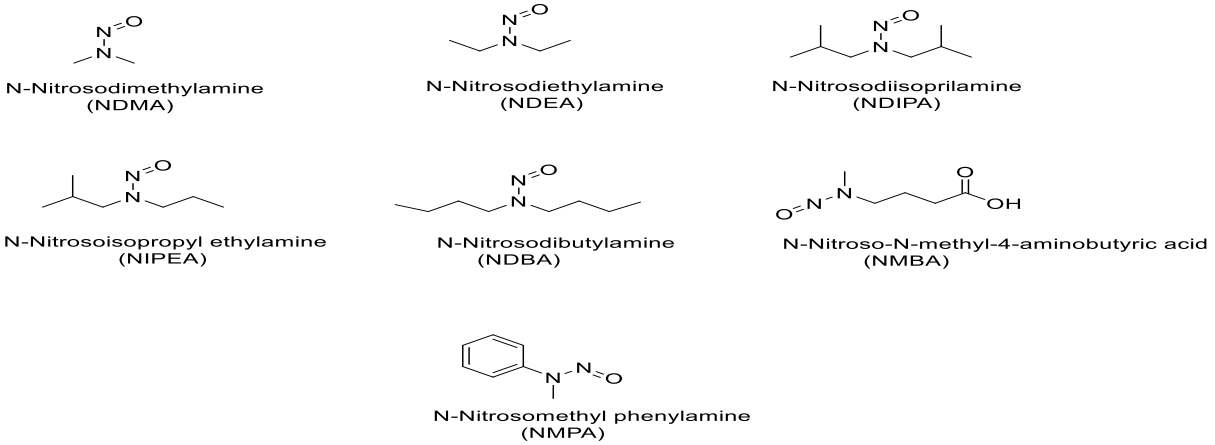


Figure 3: - Chemical Structure of seven potential Nitrosamine impurities in Drug Substance and Drug products.

Below are the acceptable Intake of above Nitrosamine impurities published by USFDA.
The acceptable intake (AI) defined as an intake level that poses a negligible health risk, is a daily exposure to a compound. Acceptable intake concentration in the material varies by product and can be calculated in ppm, based on drug product maximum daily dose (MDD) using following formula.
Limit = AI (ng)/MDD (mg)

Nitrosamine Impurity	**AI Limit (ng/day)**
N-Nitrosodimethylamine (NDMA)	96
N-Nitrosodiethylamine (NDEA)	26.5
N-Nitrosodibutylamine (NDBA)	26.5
N-Nitroso isopropyl ethylamine (NIPEA)	26.5
N-Nitrosomethyl phenylamine (NMPA)	26.5
N-Nitrosodiisopropylamine (NDIPA)	26.5
N-Nitroso-N-methyl 4-aminobutyric acid (NMBA)	96

Mechanism for Nitrosamine reaction with DNA
The N-nitrosamines undergo oxidative metabolism (bio-activation) by cytochrome P450 (CYP) enzymes. The CYP-mediated hydroxylation at the carbon atom α-to the N-nitroso moiety results in the formation of a Nitrosocarbinolamine species, which spontaneously decomposes to corresponding aldehyde and diazohydroxide intermediate. Further, decomposition of N-diazohydroxide yields an electrophile N-alkyl or N-aryl diazonium species capable of covalently adducting to DNA, it is also noted that without α-hydrogen cannot for the alkyl diazonium ion and no adduct formation with DNA.

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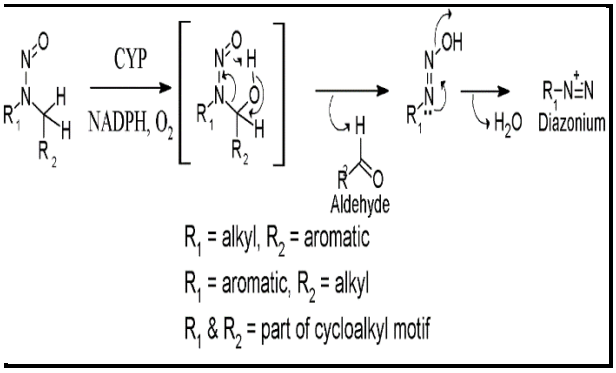


Carcinogenicity
NDMA and NDEA are carcinogenic due to their biotransformation by microsomal liver enzymes, particularly CYP2E1, into their respective alkyl diazonium ions. These ions react with DNA to generate DNA adducts, which are responsible for endogenous DNA damage.

Endogenous or exogenous nitrosamine exposure is a possibility. Exogenous exposure can occur through the consumption of food or drinks (e.g., beer), the inhalation of cigarette smoke, or the use of rubber products and cosmetics. In the stomach,

nitrosation of nitrosamine precursors causes endogenous exposure. Measurement of endogenous N-alkyl nitrosamine creation and its contribution to overall N-nitrosamine exposure has been intensively researched for many years, however there is no contemporary study on the intragastric synthesis of NDMA/NDEA from complicated mixes of precursors and inhibitors in humans.

The target organs of NDMA/NDEA toxicity and malignancies may differ between animals. Despite the fact that nitrosamine metabolism occurs in the liver, it is not concentrated in the liver, and according to research conducted on rats, only around 1% is eliminated intact in the urine. The target organs of NDMA and NDEA in humans are yet unknown, however multiple human investigations have demonstrated a risk of developing cancer due to nitrosamines, particularly stomach cancer.

Risk Evaluation Process:

The following approach should be followed during risk evaluation in pharmaceutical

Step -I: - Perform risk assessment to identify risk of presence of nitrosamines

Step-II: If a risk is identified, proceed with confirmatory testing in order to confirm or refute the presence of nitrosamines.

Step-III: If the presence of nitrosamine is confirmed, then risk mitigation measures to be ensured.

General Root Cause for the Presence of Nitrosamine impurities:

1. General condition that leads to Nitrosamine Impurities formation

i) Formation of Nitrosamine is possible in the presence of secondary, tertiary or quaternary amines and nitrite salts under acidic condition.

ii) Nitrite use in one step can carry over to next steps

2. Sources of Secondary / Tertiary and Quaternary amines that can form Nitrosamines

I) The active pharmaceutical ingredient (or API degradation products), intermediaries, or starting materials may include secondary or tertiary amine functional groups.

ii) Tertiary or quaternary amines may also be purposefully introduced as reagents or catalysts.

iii) Amide solvents that are vulnerable to breakdown under certain reaction circumstances are an additional source of secondary amine.

3. Degradation of raw materials sourced from vendors

i) Nitrosamine contamination occurred when suppliers sent contaminated fresh solvents (ortho-xylene, toluene, and methylene chloride) (e.g., during transfer between storage vessels).

ii) Sodium nitrite is a recognised contaminant in some starting materials (such as sodium azide) and may react with amines under acidic circumstances to produce nitrosamines. Raw materials containing nitrate, such as potassium nitrate, may have nitrite impurities. The tolerable level of nitrite contamination is process-dependent and must be established by each API producer.

iii) Secondary or tertiary amines have been identified as contaminants in some raw materials and in fresh solvents like toluene.

iv) Beginning materials or outsourced intermediates may be at risk of cross-contamination if they are generated at facilities where nitrosamine impurities are created in other processes.

3. Recovered Solvents, Catalysts, and Reagents as Sources of Contamination

Recovered materials such as solvents, reagents, and catalysts may pose a risk of nitrosamine impurities due to the presence of residual amines (such as trimethylamine or diisopropylethylamine). If the recovery process involves a quenching step (i.e., nitrous acid used to decompose residual azide), nitrosamines could form during solvent recovery. These nitrosamines may be entrained if they have boiling points or solubility properties similar to the recovered materials, depending on how recovery and subsequent purification takes place (e.g., aqueous washes or distillation). This further increases the risk of contamination in material recovery. For these reasons, some drug products using APIs manufactured by certain “low” risk processes were found to be contaminated. The Agency has observed the following contaminations due to this root cause:

i) A manufacturing site may produce the same API by more than one synthetic process that uses common solvents. If any of those synthetic processes produces nitrosamines or contains precursor amines, the solvents sent for recovery are at risk. The use of recovered solvents that are comingled from different processes or across manufacturing lines without control and monitoring can introduce nitrosamine impurities. If a recovered solvent is contaminated in this way and then used to manufacture an API, the API will be

contaminated even if the synthetic route is not normally susceptible to nitrosamine formation.

ii) Recovery of raw materials (e.g., solvents, reagents, and catalysts) is often outsourced to third-party contractors. Process outsourcing can pose a risk if the third-party recovery facility does not receive enough specific information on the contents of the materials they are processing and relies solely on routine recovery processes.

iii) Raw materials can be contaminated if adequate cleaning of equipment between customers, or between different materials, is not carried out or is not validated as capable of removing each impurity of concern. It was reported that ortho-xylene and toluene were contaminated during recovery due to inadequate cleaning and to use of shared storage equipment between different customers. Inadequate and unvalidated cleaning procedures can also lead to cross contamination if precautions to avoid nitrosamine contamination are not in place before materials from different customers are combined for recovery. For example, the catalyst tri-N-butyl tin chloride (used as a source of tri-N-butyltin azide) was contaminated at a third-party contractor facility due to the combination of this catalyst from different customers.

4. Quenching Process as a Source of Nitrosamine Contamination

When a quenching phase is carried out directly in the primary reaction mixture, nitrosamine production is possible (i.e., when nitrous acid is added to the reaction mixture to decompose residual azide). This enables for direct interaction between nitrous acid and leftover amines in the production process's raw ingredients. If there are not appropriate removal or purification processes in place, or if the activities are not tailored to remove specific impurities of concern, nitrosamine pollutants may be transported to following phases. If injected, this can contaminate the whole downstream process. Even if the quenching procedure is undertaken outside of the primary reaction mixture, it is still necessary.

5. Lack of Process Optimization and Control

Lack of optimization of the production process for APIs when reaction parameters such as temperature, pH, or the sequence of adding reagents, intermediates, or solvents are incorrect or poorly managed is another possible source of nitrosamine impurities. FDA has seen situations in which reaction conditions for the same API changed significantly across batches and even between processing equipment within the same facility.

Control Strategy

Nitrosamine impurities occur at microgram levels. Hence, sensitive and specific analytical techniques are required for their detection. The quantity of impurities detected in drug products can vary widely depending on who performed the test, the drug manufacturer and the specific batch tested.

Gas chromatography (GC) coupled with thermal energy analysis or mass spectrometry (MS) can be used to detect heat-sensitive nitrosamine impurities. GC coupled with MS is reported to have high selectivity and sensitivity.

Another option is the use of liquid chromatography (LC) coupled with thermal energy analysis, MS or ultraviolet light (UV). LC can be used for both volatile and non-volatile nitrosamines. High-performance liquid chromatography (HPLC) and UV can help analyse low-dose drugs.

Mitigation Plan:

1. During route of synthesis (ROS) development, API producers should optimise the design of the API production process to limit or prevent the generation of nitrosamine impurities.

i) Whenever feasible, avoiding reaction circumstances that may yield nitrosamines; failing that, proving that the process is appropriately regulated and capable of reliably eliminating nitrosamine impurities via fate and purge experiments.

ii) Utilizing bases other than secondary, tertiary, or quaternary amines (where possible) if ROS circumstances have the potential to generate nitrosamines.

iii) Taking care when amide solvents are involved in the ROS (e.g., N, N-dimethylformamide, N, N-dimethylacetamide, and N-methyl pyrrolidone).

iv) Substituting different quenching agents for nitrites in azide breakdown processes.

v) Optimizing and regulating the sequences of reactions, processes, and reaction conditions (such as pH, temperature, and reaction time).

vi) Developing a production procedure that aids the elimination of nitrosamine impurities in following processing processes.

2. API makers should verify their supply chains and monitor them for any raw materials, starting materials, or intermediates that pose a risk.

3. To minimise cross-contamination when recovered materials such as solvents, reagents, and catalysts are employed in the manufacturing process, recovered material should only be used in the same step or in an earlier phase (if

adequate purification exists) of the same process from which it was recovered.

The presence of nitrites in processing water has the potential to contaminate API production with nitrosamines. To avoid unacceptable levels of nitrosamine impurities in APIs, API makers should examine the levels of nitrite and nitrosamine in water and utilise water that has been processed to remove unwanted impurities.

Analytical Method:

The AI (acceptable intake) associated with nitrosamine requires the application of sensitive analytical procedures. In many cases, the most reliable procedures take advantage of the sensitivity and selectivity of chromatographic separation technique coupled with quantitation by mass spectrometry e.g., LCMS, GCMS. Use of accurate mass techniques are required MS/MS or HRMS in order to overcome interference in the identification of the specific peaks of a certain nitrosamine.

Discussion: -Are All Nitrosamines Concerning for Carcinogenicity?

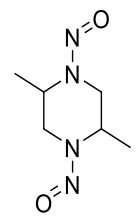
Controlling potentially mutagenic contaminants in pharmaceutical goods is crucial for determining human carcinogenic risk. The recent finding of nitrosamine impurities in several commercially available medications has spurred attention in their potential to cause mutations and cancer. This chemical class is categorised as a "cohort of concern," meaning that typical control techniques, such as the application of a threshold of toxicological concern (TTC), cannot be implemented. While it is known that certain nitrosamines are especially strong carcinogens, it is unclear if this is true of all members of the class. To study the mutagenic and carcinogenic potential of nitrosamines, data were gathered from the published literature and added to the existing information in the Vitic and Lhasa Carcinogenicity Databases. This information was analysed to evaluate the applicability of the ICH M7 guideline to nitrosamine impurity in terms of the Ames test's ability to predict carcinogenic potential and the distribution of carcinogenic potential. Eighteen percent of nitrosamines were shown to be non-carcinogenic. Compared to non-nitrosamine substances, the link between mutagenicity and carcinogenicity was stronger for nitrosamines.

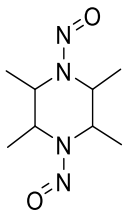
Following factors affect the carcinogenicity potential of Nitrosamine

i) The extent of α-carbon substitution and steric bulk can significantly reduce or eliminate carcinogenicity.
ii) Lower the number of α-hydrogens and substitution of the α-hydrogen may reduce mutagenic potential.
iii) B-carbon strong electron withdrawing group with reduction in carcinogenicity potency.
iv) Branched, bulky or un-metabolizable groups at or near the α-carbon preventing metabolic
v) Activation may reduce or even eliminate the mutagenic and carcinogenic potential of Nitrosamines.
vi) Stability of the diazonium ion effects mutagenic potential stabilizing effect of the substituent is expected to decrease in the order isopropyl > carboxypropyl > ethyl > methyl
vii) Steric hindrance plays a role in DNA reactivity Alkylation of DNA by alkyldiazonium ion follows an SN2 reaction mechanism.

Example 1: -Piperazines

Metabolism Possible Metabolism Partially Blocked





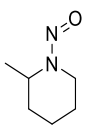
Mutagenic
Carcinogenic

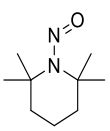
Non-Mutagenic
Non- Carcinogenic

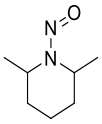
The above di-nitroso piperazine contain both substituted and unsubstituted nitrosamine, structure activity relationship study demonstrates that the unsubstituted dinitroso piperazines is more reactive and is the probable source of mutagenesis and carcinogenesis, whereas the substituted dinitroso piperazine having isopropyl like substitution does not metabolism and it is non-mutagenic or carcinogen, this may be due to presence of steric hindrance in the molecule.

Example-2: Piperidines

Metabolism Possible Metabolism Partially Blocked







Mutagenic
Carcinogenic

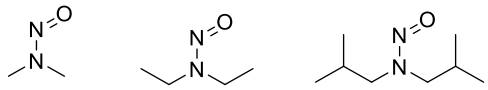
Non-Mutagenic
Non- Carcinogenic

The unsubstituted piperadines may be carcinogen in human since it has shown to cause stomach, esophagus, liver and lungs cancer in animals

whereas Ames study of substituted nitrosopiperadines shows non-mutagenic or non-carcinogenic in nature, which may be due to steric hinderance.

Example-3: Dialkyl Nitrosamines

Metabolism Possible



Mutagenic, Carcinogenic

However lower molecular weight Nitrosamines such as N-Nitroso dimethylamine (NDMA) and N-nitroso diethylamine (NDEA) have been extensively studied foe both experimentally and mechanistically. The potencies of these compound have been used for references for establishing the regulatory limits for many other nitrosamines.

Above scientific rational indicates that all the Nitrosamines compounds are not necessarily carcinogenic in nature. As per ICH M7, all the nitrosamines are categories as Cohort of Concern because of known carcinogenicity of lower molecular weight Nitrosamines. Therefore, regulatory agencies recommended to control these nitrosamines below their acceptable daily intakes. There may Nitrosamine impurities, whose carcinogenic potential is unknown and expected to control as low as 18ng/day by the regulatory agencies. These nitrosamines may include but not limited to starting material, intermediates, raw materials, drug substance and their impurities having secondary amines i.e., all the compounds having secondary amines present in the manufacturing processes are concern for nitrosamine formation. Recently many drug products recalled due to presence of N-nitroso drug substance related impurities (NDSRI) because of unacceptable level of product related nitrosamine impurities such as Varenicline, N-Nitroso quinapril, N-Nitroso Propranolol etc.

Nearly, 40% to drug substances in the market having secondary amines group in their structure and which are susceptible for the formation of corresponding nitrosamine impurities, even though there is no direct source of nitrosating agent is present in the manufacturing processes and impurity may formed from exogenous sources such as water or nitrous oxide from the air. Hence, formation of corresponding NDSRIs, cannot ruled out. These drugs include beta blockers, angiotensin-converting enzyme (ACE) inhibitors, and selective serotonin reuptake inhibitors (SSRIs), with beta blockers being of particular concern. "If a patient needs them, there are no good substitutes, so therefore it has become such an issue for industry, branded and generic alike. Further, control these impurities as below as 18ng /day not currently technically possible. Also, control of these impurities is also challenging where exact source of Nitrosating agent is not available. There are not many publications available for the control or de-nitrosation of N-nitroso impurities except theoretical one. To prevent short and long-term drug shortages, limits for NDSRIs should be set based on scientific grounds and read-across for NDSRIs is possible but sometimes challenging. But regulatory agencies are not currently accepting the justification for higher acceptable intakes (AI) based on scientific grounds. Industry is grappling with how to set daily acceptable limits for these novel nitrosamines, as the toxicological data is not yet available. Further, negative Ames study not accepted by regulatory agencies for claiming higher limit for NDSRI, they expect higher study such as Transgenic Rodent gene mutation assay (TGR assay) for NDSRI to be perform, which is very costly and time taking study.

If such drug recall will keep happening because of presence of nitrosamines impurity, then many patients will be away from lifesaving drugs and their treatment will get delayed. To avoids product contamination, manufacturers have the option of reformulating products or deviating from old formulations, which "is an enormous problem" affecting both brand name and generic companies. Hence, Regulatory agencies and pharmaceutical companies should jointly resolve the challenges and provide solutions to avoid drug shortage.

Industry Next Level Challenges:

Industry is facing following few challenges to control Nitrosamine impurities in their drug products

- Control of 'Nitrite' in Water / Excipients
- Nitrosamine impurities' content in cleaning samples
- Unavailability (either unstable and / or unable to synthesis) of few possible Nitrosamine impurities
- Lack of 'Sufficient Testing infrastructure (in-house and / or CRO labs)'
- High' testing cost
- Lack of skilled manpower for testing
- Nitrosamine impurities assessment (from Packing materials) and control strategy
- Inappropriate support from Raw materials, KSM, API, excipients and Packing materials vendors

Conclusion:

The present review describes the details regarding Nitrosamine impurities in the pharmaceutical Nitrosamines are genotoxic impurities, and due to their carcinogenic behaviour, they pose an alarm to the pharmaceutical industry and patients as well. Nitrosamine impurity formation can be avoided by selecting proper reagent, catalyst and solvents in the manufacturing of drug substances. To alleviate this global issue, regulatory agencies such as CDSCO, US-FDA, and the European Medicines Agency (EMA) should be continuously communicating to pharmaceutical industry and patients through publishing guidelines, new article for awareness. This review is also discussed in details on Carcinogenicity of nitrosamines impurity and challenges in front of pharmaceutical industry.[i]

Abbreviations:

ICH :The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
API : Active pharmaceutical ingredients
DNA : Deoxyribonucleic acid
USFDA : United states Food and Drug Administration
LCMS : Liquid chromatography –mass spectrometry
GCMS : Gas chromatography–mass spectrometry
CDSCO: Central Drugs Standards Control Organization
CRO : Contract Research Organization

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