Nitrosamine Impurities in Pharmaceuticals – An Awareness to enhance its Sustainability

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Abstract

The *N*-Nitrosoamines are the concerning class of genotoxic impurities with higher mutagenic potency that leads to carcinogenesis. These impurities are found in different types of Active pharmaceutical ingredients (APIs) and formulations were reminiscing from the market as soon as they were assessed. The present review represents the formation of nitrosamines by nitrosation reaction. Nitrosamines were classified into three groups according to their carcinogenic potential. The bioactivation of nitrosamines were undergone by two mechanisms i.e., by α- hydroxylation and by Cytochrome P450 Monooxygenase pathway. The methyldiazonium ion and formaldehyde, two reactive intermediates of nitrosamines alkylate the DNA to mutate it. The Acceptable intake (AI) was established with the help of the International Council for Harmonization (ICH) M7 (R1) guideline, using Tumorigenic dose 50 (TD_{50}) data with substance specific animal carcinogenicity data and using Carcinogenic potency categorization approach (CPCA) with no substance specific data. The CPCA approach establishes the five different categories and related specified AI limits for Nitrosamine Drug Substance Related Impurities (NDSRI's). To make drug and drug products sustainable, there is a need to bypass the threat of API scarcities or absolute loss of medicinal choices due to impurities like *N*-nitrosamines, which requires cooperation of Regulatory Authorities with Pharmaceutical industries is of greater importance to prevent economical as well as resources deprivation. Green synthesis of APIs by replacing the use of nitrating reagents with other most suitable green reagents might prevent the formation of nitrosamine impurities.

Keywords

N-Nitrosamines, Genotoxic Impurity, Risk assessment, CPCA approach.

Introduction

The *N*-nitrosamines, also mentioned as nitrosamines, which belong to the category which have nitroso group in its chemical structure. The present nitrosamine compounds have higher mutagenic potential and leads to cancer. Nitrosamines were previously detected in drinking water supplies, tobacco smoke, cosmetic products, beverages, grilled and cured meats, dairy products, and vegetables[1, 2, 3].

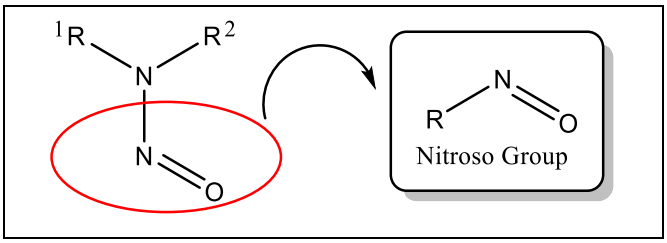

Figure 1: Structure of *N*-nitrosamine

Otto N. Witt the German chemist in 1874, conducted reaction between nitrous acid with secondary and tertiary amines the product formed is called “Nitrosamine”. Since 1954, nitrosamines didn’t get significant attention from the science communities.

In 1954, Peter Magee and John Barnes specified the *N*-nitrosodimethylamine (NDMA) as hepatoxic agent (The chemical which may damage the liver) in a study they found, two out of three men got liver cirrhosis working in laboratories in which the NDMA was used as solvent. They also reported carcinogenicity of NDMA in liver of rats in 1956. Due to this invention the rapid rise in interest of toxicology’s of *N*-nitroso substituents were developed.

In 1960, many farm animals fed with herring preserved in high amounts of sodium nitrite were suffering from liver disorders. The substance responsible for disorders were identified as NDMA. The present contaminant NDMA which might form due to reaction between dimethylamine from fish and nitrosating agent generated due to sodium nitrite.

12 Japanese patients found with hepatic injury because of intake of herbal Chinese dietary supplements in 2002. A hepatotoxic *N*-Nitrosofenfluramine was found in dietary supplement after analytical investigation [4, 5].

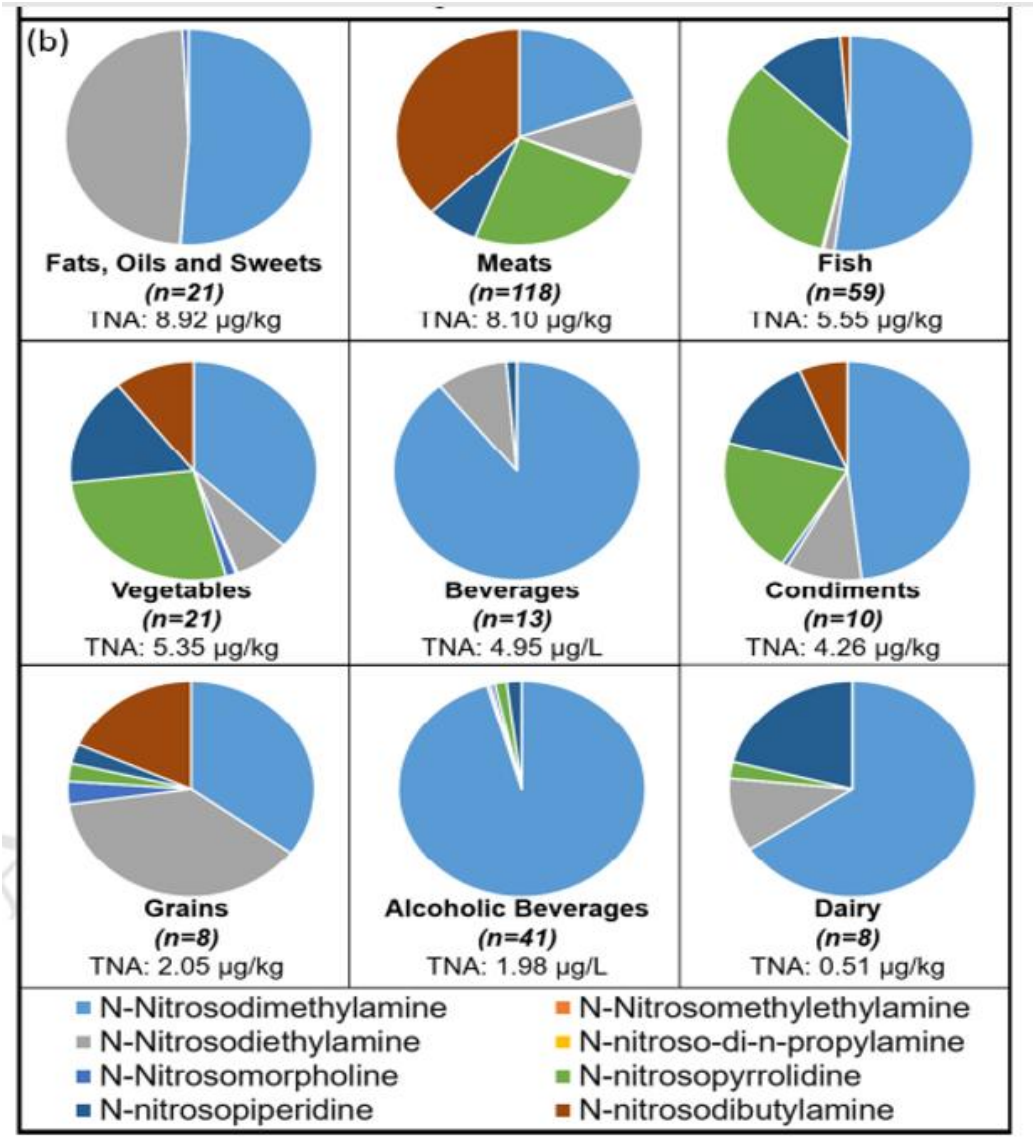

Figure 2: *N*-nitrosamine levels in different food categories.

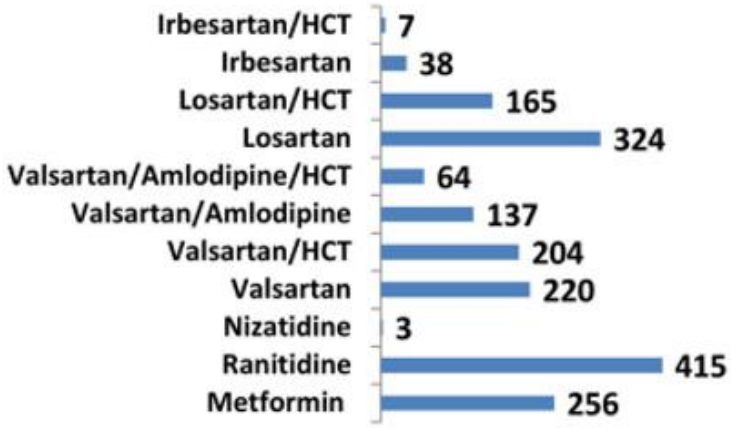

Figure 3: Batches recalled due to the presence of nitrosamines.

Since last 5 years starting from June 2018 the pharmaceuticals have been perceiving the crisis due to Nitrosamines. The Chinese manufacturer reported occurrence of NDMA in Valsartan API. The NDMA, *N*-nitroso-*N*-methyl-4-aminobutyric acid (NMBA), and/or *N*-nitrosodiethylamine (NDEA), as an impurity was established above acceptable limits within API and drug formulations with 22% as well as 18% respectively containing valsartan, losartan and irbesartan. About 1800 formulation batches containing antihistamines, sartans, antidiabetic drugs, and antibiotics were withdrawn from US market because of the presence of Nitrosamine impurities. This makes a compulsion of qualitative evaluation of nitrosamines in API's and medicinal products. The scenario has been changed after recollection of varenicline batches in various countries like Canada, USA and European union in 2021 because of existence of nitrosovarenicline as NDSRIs. The present case is further extended by recollection of various other APIs like quinapril, propranolol and orphenadrine due to their respective NDSRIs. The studies further reported that approximately 40% of common drug substances and 30% of their impurities are the potent precursors for nitrosamine synthesis because of amino functional groups in their structures out of which if we only consider most active secondary amines still 13-15% of drug substances are at potential risk. Therefore, the NDSRIs requires more attention in regulatory as well as industrial approach [6, 7].

1. Formation of Nitrosamines

The basic needs for synthesis of nitrosamines are acid, amine and inorganic nitrite. These requirements are necessary because the nitrite ion (NO_2^-) itself is not effective, therefore it should first react with the acid proton to form nitrous acid (HNO_2). Still HNO_2 is an intermediate for the generation of nitrosating agent dinitrogen trioxide (N_2O_3). The fact taken in consideration that other nitrosating agents were also formed like; nitrosonium ion and nitrous acidium $(H_2NO_2)^+$ ion [8].

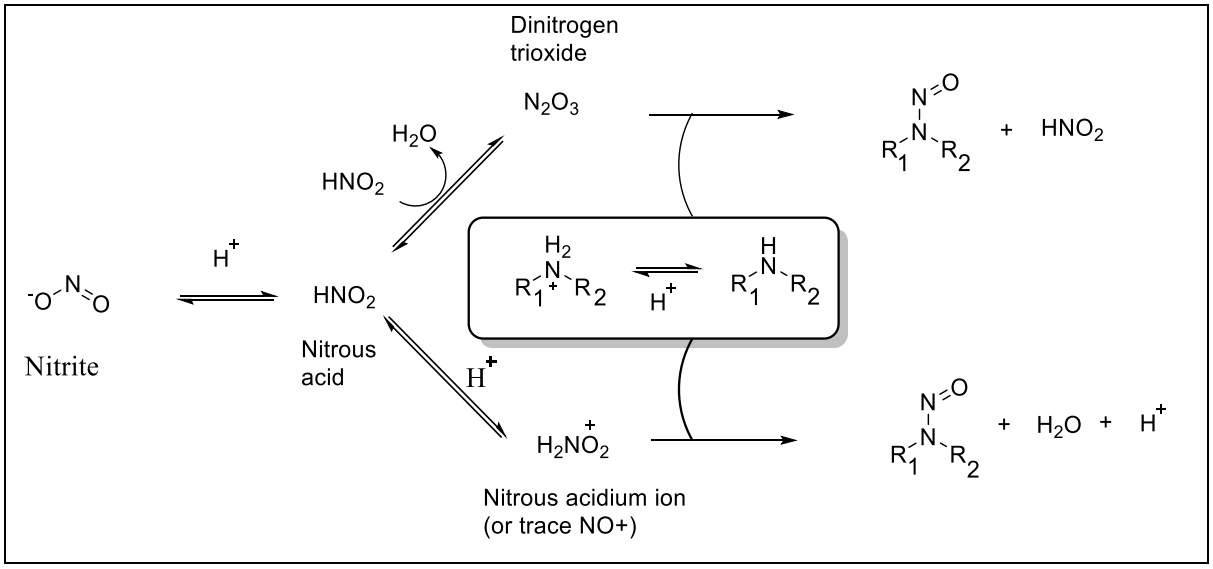

Figure 4: Formation of Nitrosamines

In strong acidic condition the reactivity of amines was reduced due to which nitrosamines formation gets minimized despite that at acidic pH the nitrosation process is enhanced and less reactive in low pH because of amines protonation. The most favored step of nitrosation is to balance pH of the amine basic reagent. The kinetics of nitrosation process rely on formation, reaction conditions as well as nature of [NO^+] carrier. Commonly, the nitrosation is effectively induced by the more electrophilic the [NO^+] carrier. The Secondary amines are easily nitrosated under milder reaction conditions, while tertiary amines demand extreme conditions or use of most active [NO^+] carriers[9].

Nitrosation of different types of Amines

The nitrosating agents can easily nitrosate the primary amines, which generate the nitrosamine products that spontaneously change into diazonium salts. Further, this releases nitrogen to generate carbonium ions that react with water to form compound containing hydroxyl compound. In contrast, secondary amine is more active towards nitrosating agents and forms more stable nitrosamines. Thus, compounds with secondary amines are more prone to generate nitrosamines.

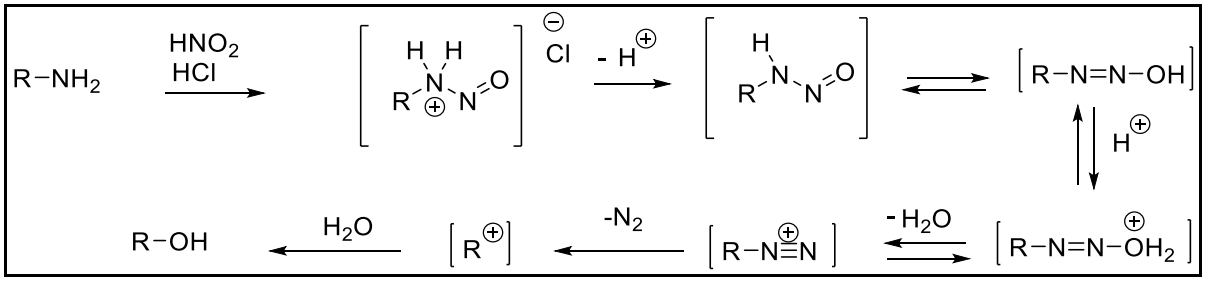

Figure 5: Nitrosation of primary amine

The nitrosation was also possible with tertiary amines and it is known as nitrosative dealkylation or nitrosative cleavage. For generation of nitrosamines the tertiary amines have to release one alkyl group to form secondary amine which further after nitrosation forms its corresponding nitrosamine. The tertiary amines are less reactive to the extent of <1000-fold as compared to secondary amines because of additional dealkylation process. Tertiary amines generally need reaction conditions (higher molar ratios of nitrosating agent, high temperature and more reaction time) and stronger nitrosating agents compared to secondary amines. Concerning the quaternary ammonium compounds, nitrosamines are formed in rather low yield because they undergo indirect non-nitrosative dealkylation to form tertiary amines which further undergo nitrosation to form nitrosamines.

The rate of nitrosation reaction depends on the nucleophilicity of nitrogen, nitrosonium ion reactivity and conditions such as temperature, pH and solvents used. The basicity of the secondary amines contributes to its reactivity. The nitrosation in simple aliphatic amines, includes nucleophilic attack by the amine on the electrophilic nitrosating agent, which forms the amines free base nature which allows nitrosation process to occur, the present scenario is not possible if the lone pair is unavailable due to protonation of secondary amine. As compared to more basic amines, less basic amines are lesser protonated in acidic or neutral pH which tends to easier nitrosation. Secondary aromatic amines give *N*-Nitrosamine derivatives, while tertiary aromatic amines might give ortho or para nitrosamines derivatives due to nitrosation in aromatic ring[10, 11].

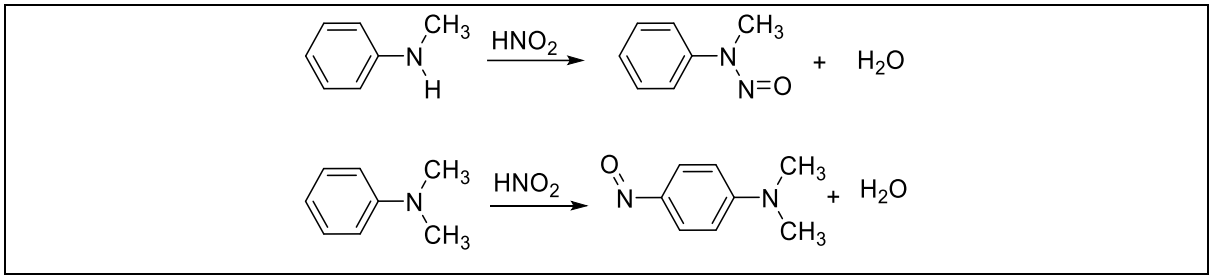

Figure 6: Nitrosation of secondary and tertiary aromatic amines.

The nitrosation process is majorly relied on pH. The rate of nitrosation reaction also depends on the other parameters such as unprotonated amine concentration which reduces with lowering pH. At lower pH the yield of nitrosamines is effectively dependent on the basicity of amino functional group. Therefore, the probability of nitrosation increases with the lower basicity. The free electron pair easily accepts the proton in higher basicity which prevents the

nitrosation. The lower pKa, the stronger acid and the weaker conjugate amine base, i.e., the higher susceptibility to nitrosation.

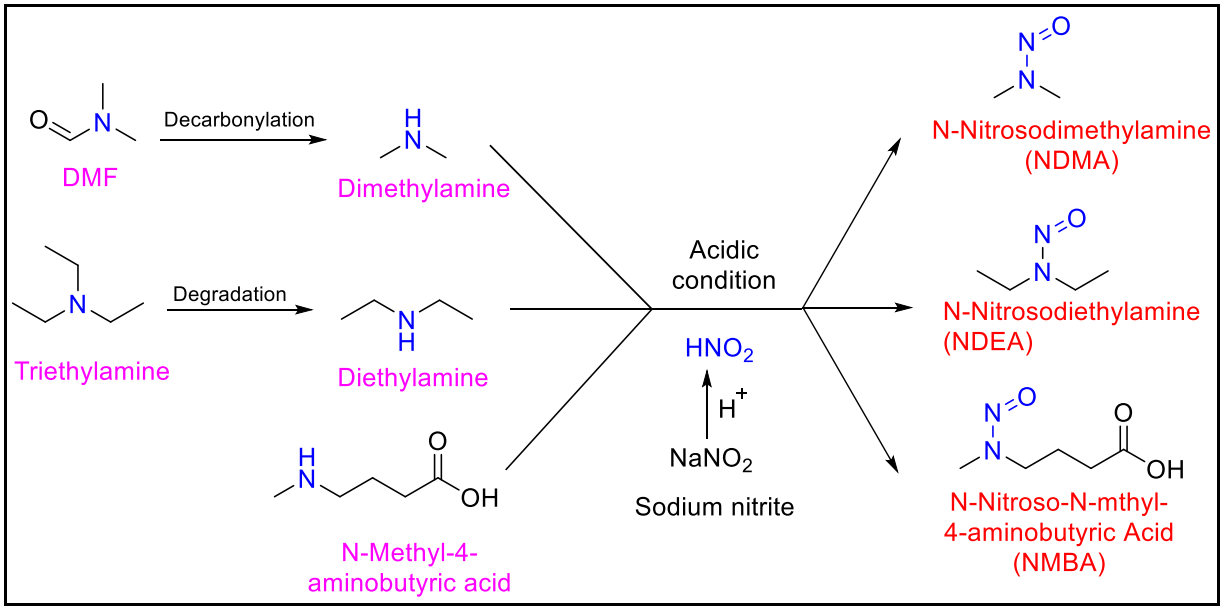

Figure 7: Principal sources for generation of *N*-Nitrosamines

Nitrosamine	**Amine and Amide Precursors**
N-nitroso-dimethylamine (NDMA)	*N*-*N*-dimethyl formamide (DMF) Dimethyl amine (DMA)
N-nitroso-diethylamine (NDEA)	*N*-*N*-dimethyl amine (DEA) Triethylamine (TEA)
N-nitroso-*N*-methyl-4-aminobutyric acid (NMBA)	*N*-methylamino-*N*-butyric acid (MBA) *N*-methyl pyrrolidinone (NMP)
N-nitroso-diisopropylamine (NDIPA)	*N, N*-diisopropylethylamine (DIPEA)
N-ethyl-*N*-nitroso-2-propanamine (NEIPA)	*N, N*-diisopropylethylamine (DIPEA)
N-nitroso-di-n-butylamine (NDBA)	*N,N*-dibutylamine (DBA)
1-methyl-4-nitrosopiperazine (MNP)	1-methyl piperazine
1-Cyclopentyl-4- nitrosopiperazine (CPNP)	1-Cyclopentyl piperazine

Table 1: Amines and amides progenitor of Nitrosamines in API

2. Classification of Nitrosamines

Based on their mutagenic potency the International Agency for Research on Cancer (IARC) categorized the nitrosamines in four different groups. The agents with sufficient evidence to prove it as cancerous to humans are categorized in Group 1. The agents having restricted proofs in humans but adequate proofs in animal study and apparently cancerous to humans are classified in Group 2A. The agents having restricted proofs in humans and animals too with possible carcinogenicity in humans are categorized in Group 2B. The agents with inadequate evidence of carcinogenicity data in humans as well as animals with non-classifiable carcinogenicity in humans are categorized in Group 3[12, 13].

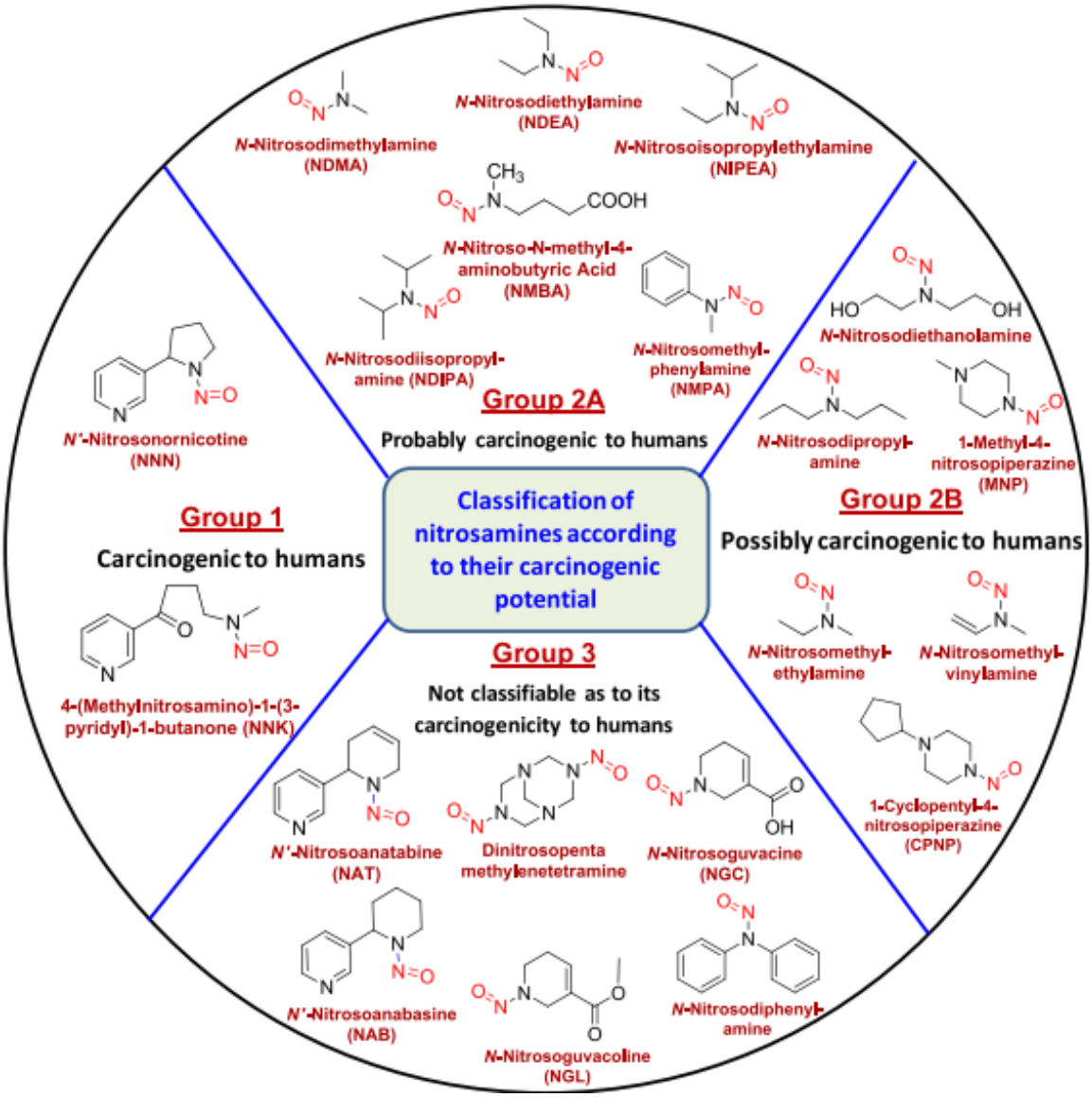

Figure 8: Classification of Nitrosamines

3. Sources of Nitrosamines

1. Sources of Nitrosamines in formulations[14]:

Important Sources of Nitrosamines	**Risks observed**
Carriers (Solvents)	➢ Existence of leftover Tri-substituted amines or dialkyl amines, nitrites, nitrosating agents and acids. ➢ Inferior quality of solvents
Pharmaceutical aids	➢ Existence of nitrosating agents or nitrites or nitrosamine impurities. (if applicable)
Water	➢ Existence of dialkyl amine or the substituents which after degradation forms dialkyl amines. ➢ Existence of nitrosating agents and acids.
Formulations (inclusive of stability)	➢ Presence of quaternary, tertiary or secondary amine group in the API molecule. ➢ Existence of nitrite as impurity in nitrate counter ion. ➢ Implicit reactions in formulation during storage.
Manufacturing process	➢ Presence of contaminants ➢ Utilization of bad quality or reprocessed solvents which might have nitrosamines or their precursors ➢ Contamination of drug substance or product by nitrous oxide present in drying air. ➢ Relative reactive substance carry forwarded to subsequent processes.
Container-closures	➢ Presence of nitrosating agents in packaging material reacts with the amines. Like; amines from ink reacts with nitrocellulose.

Table 2: Sources of Nitrosamines in drug products

2. Sources of Nitrosamines in drug substances[15]

Potential Source of Nitrosamines	**Observed Risk**

API	➢ In acidic conditions existence of quaternary, tertiary or secondary amines as well as nitrites. ➢ Presence of secondary and tertiary amines in API, their intermediates, and/or reactants. The Tertiary and quaternary amine reagents were added into process voluntarily. ➢ Utilization of di- or tri-alkylamines and amides (e.g., dimethylformamide [DMF], dimethylamine [DMA], triethylamine [TEA], *N*-methylpyrrolidone [NMP]) in the acid media containing nitrites. ➢ The raw materials purchased from vendor has been contaminated. ➢ Recycled material like reagents, catalysts and solvents might have nitrosamine impurities. ➢ Inappropriate cleansing of instruments ➢ The Quenching process was directly performed on principal reaction mixture. E.g. Nitrous acid added to mixture to degrade residual azide. ➢ Poor manufacturing process optimization of APIs ➢ Application of disinfected water ➢ Deterioration of API having functional groups which further engage in nitrosation process

Table 3: Sources of Nitrosamines in drug substances

4. Bioactivation of *N*-Nitrosamines

1. Bioactivation of *N*-Nitrosamines via α- hydroxylation mechanism:

Nitrosamines require the α-carbon hydroxylation step for its metabolic activation which leads to carcinogenicity. Cytochrome P450 primarily catalyzes the bioactivation of NDMA to reactive intermediates in human liver microsomes. The oxidation of the methyl group (α-methyl hydroxylation) leads to α-hydroxy NDMA, an unstable and mutagenic intermediate that spontaneously decomposes, generating two reactive species—formaldehyde and methyl diazohydroxide. Methyl diazohydroxide will spontaneously form the highly electrophilic methyl diazonium ion, which alkylates DNA.

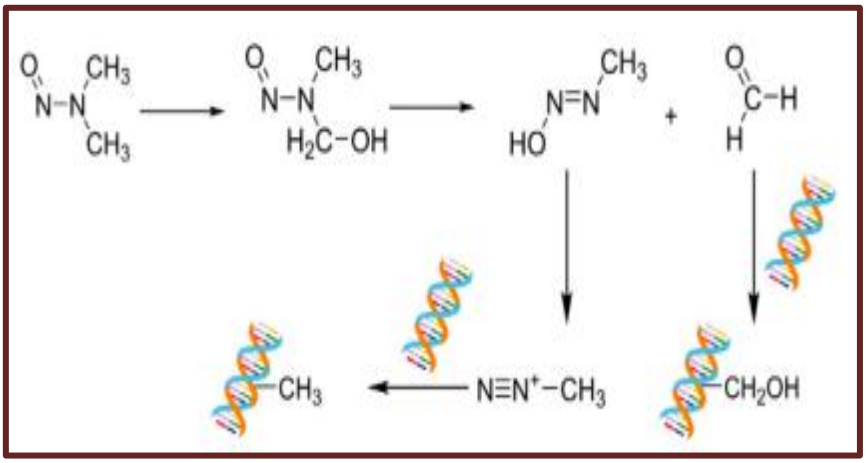

Figure 9: α- hydroxylation mechanism

2. Bioactivation of *N*-Nitrosamines via Cytochrome P450 Monooxygenase mechanism

Cytochrome P450 commonly catalyses the monooxygenase reaction. e.g., one oxygen atom added in aliphatic side chain of organic compound (RH), and reduction of another oxygen atom to water[16].

$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

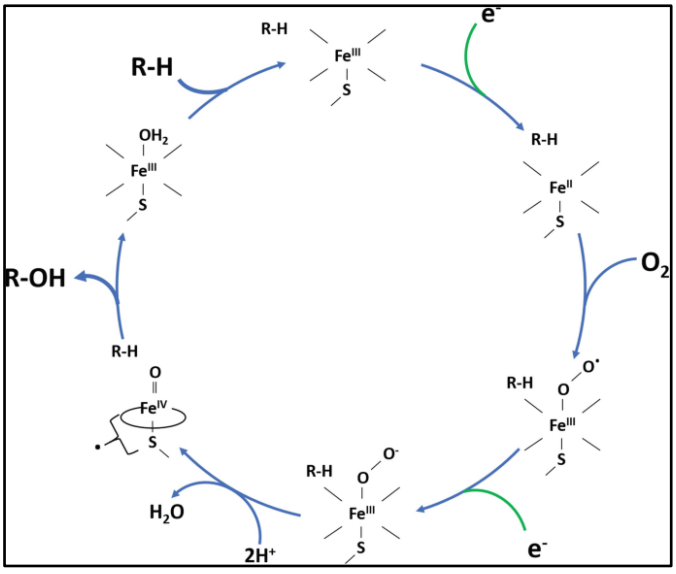

Figure 10: Cytochrome P450 Monooxygenase mechanism

5. DNA Damage

The mutagenicity and genotoxicity of Nitrosamines are well-established. Formaldehyde and methyldiazonium ion are the two reactive intermediates alkylates the DNA to form methyl DNA derivative which play a crucial role in carcinogenesis induced by NDMA.

The alkylating agents are mutagenic and toxic, which is commonly known as human carcinogens. Various positions in the DNA can be substituted via nucleophilic substitution depending on the given alkylating agent. Alkylating agents specifically target oxygen and nitrogen atoms of purines and pyrimidines. Alkylation is highly mutagenic and cytotoxic. N^7-alkylguanine and O^6-alkylguanine represent the more sensitive kind of DNA O-alkylation and *N*-alkylation damage. Alkylation induced specific mispairing. Alkylation of the O^6 position of Guanine as well as the O^4 spot of Thymine, tends to mispairing with the Thymine and Guanine respectively[17].

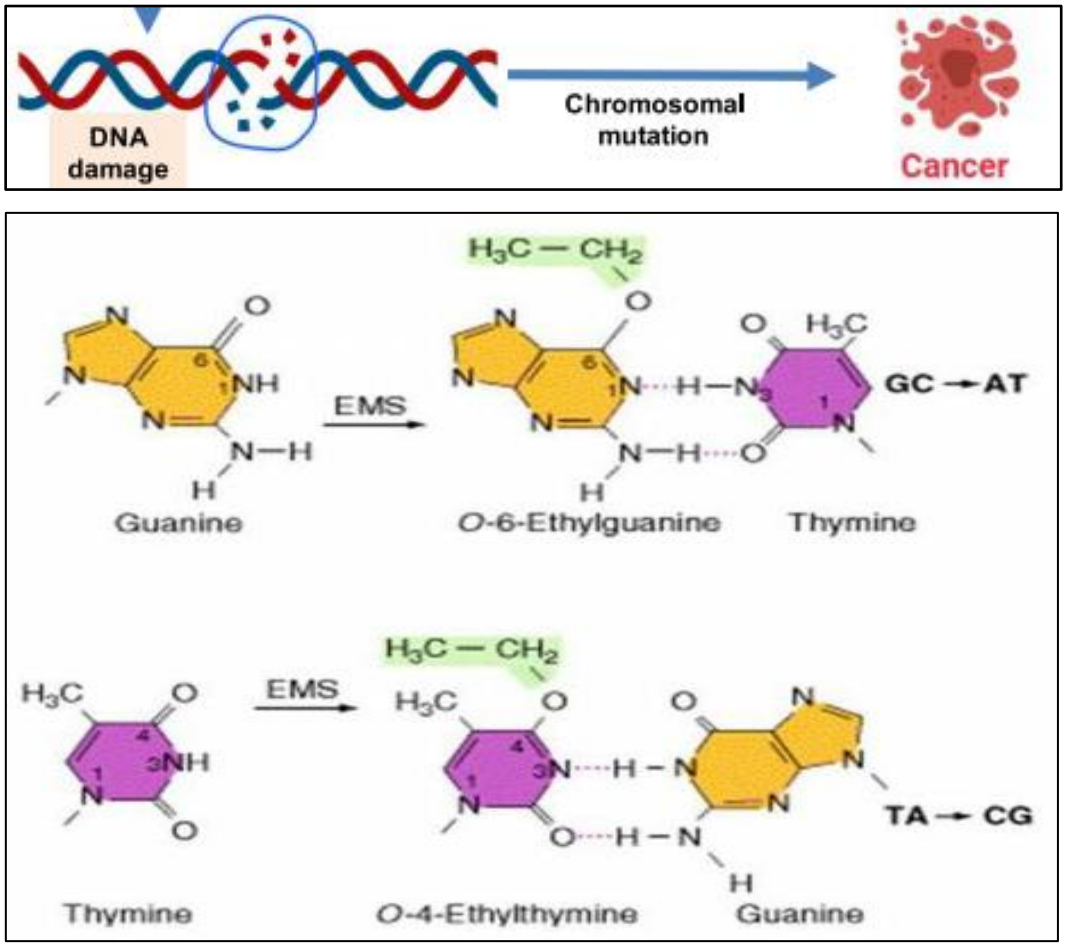

Figure 11: DNA Mutation and carcinogenesis

6. Risk Assessment Approach

The risk assessment is necessary for determination of potency of nitrosamine compounds. In risk assessment approach if the assessment lacks the potential of nitrosamines, then no action is required but if any risk related to nitrosamines is observed in drug substances or products then further confirmation tests were carried out using validated and sensitive analytical methods. Further root cause investigation is required if there is existence of nitrosamine impurities in samples tested. According to the root causes the change in process of manufacturing were made to prevent the contamination of samples with nitrosamine impurities[18].

7. Mitigation of Nitrosamines

As stated above, the optimization of design of manufacturing process for drug substances since development of route of synthesis (ROS) to reduce the generation of nitrosamine impurities. Avoiding reaction conditions (use of nitrosable substances in acidic environment) which leads to generation of nitrosamines whenever possible. Use of purge studies proves the process is controllable and can vanish the nitrosamines. Use of other bases than amines to prevent formation of nitrosamines. Caution is required while using amide solvents.

For decomposition of azide other extinguishing agents were employed in place of nitrites. Optimization of reaction sequence, processes and their conditions like; temperature, pH and time of reaction. The manufacturing sites of API should frequently audit and monitor their supply chains for risk assessment of intermediate and raw materials. Recovered solvents, catalysts and reagents were used to prevent cross-contamination during manufacturing procedure. API manufacturers require analysis of nitrite and nitrosamine levels in water[19].

8. Setting Limits for Nitrosamine Impurities

Nitrosamine impurities have potential and established toxicity with no therapeutic value. As nitrosamines comes under the structurally high-potency mutagenic carcinogen category therefore threshold of toxicological concern (TTC) was not applied, instead it is “cohort of concern”.

The acceptable intake (AI) was established using safety data available which is material specific. The intake level of any drug substance with negligible health risk is known as AI i.e. after 70 years of exposure approximately there is 1:100,000 cancer risk.

Toxicologists have employed various methodologies in determining acceptable intakes (AI). To determine the acceptable risk level, linear extrapolation of median tumorigenic dose (TD_{50}) of NDEA, NDMA and other nitrosamines data were established.

As nitrosamines exposure is relatable to Maximum Daily Dose (MDD) of drug substance therefore various aliquots of nitrosamine (ng/g) would be reliable for individual substituent assessed. The adequate concentration of nitrosamines in the substances changes with product and can be determined in terms of ppm, depending on MDD of drug using following equation

$$\text{Concentration limit (ppm)} = \text{AI (ng/day)} / \text{MDD (mg/day)} \quad (1)$$

Nitrosamine	FDA limit (ng/day)	EMA limit (ng/day)
NDIPA	26.5	26.5
NDMA	96.0	96.0
NEIPA	26.5	26.5
NDEA	26.5	26.5
NMBA	96.0	96.0
NMPA	26.5	34.3
NDBA	26.5	26.5

Table 4: Nitrosamine AI Limits

A TTC of class-specific nitrosamine according to Food and Drug administration (FDA) and European Medicine Agency (EMA) is 26.5 ng/day and 18 ng/day respectively used as default when the impurity recognized with no complete substance specific data to determine substance specific limit. If API has single nitrosamine, then only the limits are applicable. The total amount of nitrosamines should not be greater than 26.5 or 18 ng/day based on MDD if more than one of the nitrosamine impurities are detected. The formulations with less MDD than 880mg/day the specified limit is not greater than 26.5 ng/day and with greater MDD than 880 mg/day, the former should be modified in a way that it is not greater than 26.5 ng/day. Nitrosamines should be controlled within API/drug product specification. Skip testing: consistently ≤ 30% of the limit. Omission from the specification: consistently ≤ 10% of the limit defined.

Limit Calculation for drug product when more than one nitrosamine is identified (EMA)

- Option-1: For all the identified nitrosamines, the total daily intake is not greater than AI of the potent nitrosamine identified.
- Option-2: For all the identified nitrosamines the total risk level does not exceed 1 in 100,000 [20, 21, 22].

Nitrosamines	Option 1	Option 2-Fixed in 20:80 ratio	Option 2 - not Fixed
NDMA	Not needed	0.064 ppm (0.32 ppm X 20 %)	NMT 0.32 ppm
NDEA	Not needed	0.070 ppm (0.088 ppm X 80 %)	NMT 0.088 ppm
Total	NMT 0.088 ppm	Not needed	NMT 100 %

Table 5: Specification possibilities for different control options

9. Establishment of Acceptable Intake (AI)

Two schemes are expected for the identification of newer nitrosamines:

- Nitrosamines recognized with substance specific sufficient animal carcinogenicity data were used to determine TD_{50} and further to determine substance specific limit as described in ICH M7(R1) guideline.
- Nitrosamines recognized with no substance specific animal carcinogenicity data, ICH M7(R1) guideline specifically carcinogenic potency categorization approach (CPCA) were used.

Group AI for complex *N*-Nitrosamines

Primarily the determination of single AIs for greater number of impurities employing a read across approach was carried out and allocated the configurationally complex nitrosamines into 13 groups presumed on the structural resemblance of different nitrosamine substituents. On the basis of previous studies about structure activity relationships for mutagenic/carcinogenic potencies the categories were classified. Certain impurities were inspected for individual category and structural category AIs was pronounced as the highest potency cancerous *N*-nitrosamine in individual categories[23].

10. Structural Features Affecting Carcinogenicity

The critical rate-limiting step in identification, metabolic activation and quantitation of nitrosamines is α-carbon hydroxylation, the effect of different configurational attributes on the present step can furnish beneficial discriminations into their carcinogenic capabilities[17, 24].

Potency diminishing substituents	**Potency enhancing substituents**
Tertiary butyl group	Small alkyl group
Isopropyl group	Benzylic group
Carboxylic acid at any position in the molecule	β-carbonyl group
Aromatic group	Allylic or Propargylic group
Strong β-electron withdrawing group	
β-hydroxyl group	

Table 6: Substituents having considerable impact on carcinogenic potency

11. Carcinogenic Potency Categorization Approach (CPCA) for NDSRIs

The NDSRIs is a category of nitrosamines that are investigated in numerous drug substances and products. The generation of NDSRIs in formulations happens due to nitrosation of API which contains tertiary or secondary amines leads to formation of remnant nitrite within pharmaceutical aids employed to articulate the formulation. Because nitrosamine impurities are investigated in many formulations, disturbances in supply and passage have enhanced, occasionally emerging in drug scarcities.

Deserving AI limit for NDSRIs is frequently too critical compared to deriving limits for smaller molecules, because each API has unique NDSRIs therefore, limited or no safety data are available. NDSRI's have similar structural features like API or API fragment. The CPCA approach is a scientific apocalyptic method to suggest AI limit for different NDSRIs.

The activating or deactivating features are responsible for enhancing or reducing the carcinogenic potency respectively, was specified in this approach. The present perspective also includes SAR studies for nitrosamine substances which describe that the α-hydroxylation reaction for metabolic activation is indebted to its mutagenic potency. Configurational characteristics state that contiguously enhance or reduce the favorable activation or enhance the clearance from another biological processes will have direct impact on its potency. The NDSRIs with two *N*-nitroso groups, the corresponding AI limit is determined on the basis of high predicted carcinogenic potency group for whole molecule. The present approach is

conservative, which explains the better availability of scientific features and is further expected to be more developed as newer insights are available.

- The CPCA is applicable to NDSRIs having carbon atoms on both sides of *N*-Nitroso group, where heteroatom is not directly double bonded with the carbon. (i.e., *N*-nitrosoureas, *N*-nitrosamides, *N*-nitrosoguanidines, and another associated structure are eliminated).
- Moreover, this is not applicable to NDSRIs having *N*-nitroso group in an aromatic ring. (e.g., nitrosated indoles)[25, 26, 27, 28].

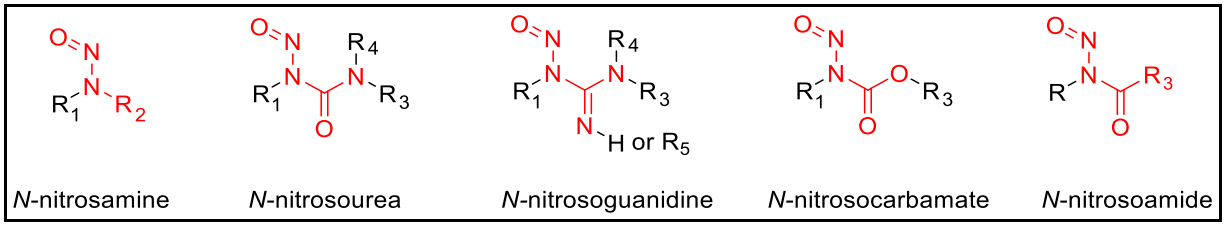

Figure 12: Double bonded heteroatom on an aliphatic chain

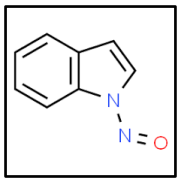

Figure 13: Bonded heteroatom in the aromatic ring

Potency Category	Recommended AI (ng/day)	Comments
1	26.5 or 18	AI limit specified on the basis of most potent nitrosamine (NDEA)
2	100	AI limit specified on the basis of two most potent nitrosamines NDMA and 4-(methylnitrosamino)- 1-(3-pyridyl)-1-(butanone) (NNK)
3	400	Four times lesser carcinogenic strength contemplated to category 2 due to deactivating structural characteristics
4	1500	Lower potency due to disfavored alpha-hydroxylation because of steric and electronic influences.
5	1500	Lower potency due to disfavored alpha-hydroxylation because of steric hindrance or deficiency of α-hydrogens or generate unstable compounds which is unreactive to DNA.

Table 7: Potency categories and their specified AI Limits

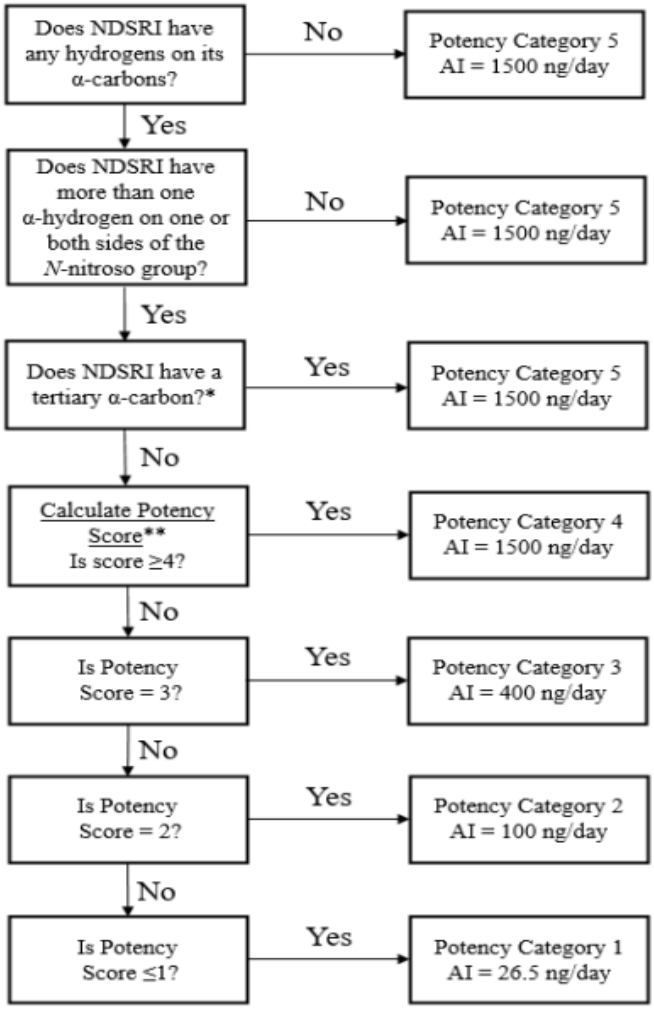

Figure 14: Potency category prediction flowchart

Conclusion

Genotoxic impurities like nitrosamines have carcinogenic potential shows the warning sign for the living things on earth. Moreover, the crucial task for researchers and industrialists is to identify and quantify these impurities from various drug substances precisely. By using genuine catalysts, reagents and solvents for the production of drug substances the formation of nitrosamines can be avoided. The *in-vivo* generation of nitrosamines in humans is a crucial feature to contemplate the threat, this probably happens in GI tract where nitrite and nitrate were ingested from food and acidic pH plays a vital role in formation of nitrosamines.

The Amine groups have higher *in-vivo* volume of distribution (V_d) prone to greater pharmacokinetic characteristics like; extended half-life, leads to good dose frequencies and impressive medicinal compliance. Medicinal products are impossible to make nitrosamine-free

because of the presence of nitrite traces in raw materials and excipients which further generate nitrosamines, the API is also susceptible to nitrosation process.

To make drug and drug products sustainable there is need to bypass the threat of API scarcities or absolute lack of medicinal choices due to impurities like *N*-nitrosamines, which requires cooperation of Regulatory Authorities with Pharmaceutical industries is of greater importance.

References

1. European medicines agency (2020). *Nitrosamine impurities in human medicinal products.* In EMA/369136/2020 (pp. 1–90).
2. U.S. Department of Health and Human Services Food and Drug Administration. (2020). *Control of Nitrosamine Impurities in Human Drugs Guidance for Industry*.
3. Ministry, India. (2022). Pharmacopoeia of India (the Indian pharmacopoeia). *Nitrosamine impurities, chapter*. 5.11
4. Chapter G. 1469 "*Nitrosamine Impurities*". United States Pharmacopoeia.
5. Indian Pharmaceutical Alliance (IPA), *Guidance on nitrosamine impurities*, Oct 2022, (pp. 2-94)
6. Bharate, S. S. (2021). Critical analysis of drug product recalls due to nitrosamine impurities. *Journal of Medicinal Chemistry*, *64*(6), 2923-2936.
7. Nudelman, R., Kocks, G., Mouton, B., Ponting, D. J., Joerg Schlingemann, Simon, S., ... & Werner, A. L. (2023). The nitrosamine "Saga": lessons learned from five years of scrutiny. *Organic Process Research & Development*, *27*(10), 1719-1735.
8. Wichitnithad, W., Nantaphol, S., Noppakhunsomboon, K., & Rojsitthisak, P. (2023). An update on the current status and prospects of nitrosation pathways and possible root causes of nitrosamine formation in various pharmaceuticals. *Saudi Pharmaceutical Journal*, *31*(2), 295-311.
9. Mirvish, S. S. (1975). Formation of *N*-nitroso compounds: chemistry, kinetics, and in vivo occurrence. *Toxicology and applied pharmacology*, *31*(3), 325-351
10. López-Rodríguez, R., McManus, J. A., Murphy, N. S., Ott, M. A., & Burns, M. J. (2020). Pathways for *N*-nitroso compound formation: secondary amines and beyond. *Organic Process Research & Development*, *24*(9), 1558-1585.
11. MAK Value Documentation, *Nitrosation of volatile Amines at the Workplace*, *Occupational Toxicants*, Vol. 1(11), 1985.

12. Guideline, I. H. T. (2006, October). Impurities in new drug substances Q3A (R2). In *Proceedings of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, Switzerland* (Vol. 25). ICH guideline, Impurities: Guideline for residual solvents, Q3C(R8)
13. Guideline, I. H. (2019). Guideline for Elemental Impurities Q3D (R1). *European Medicines Agency: Amsterdam, The Netherlands*.
14. Tuesuwan, B., & Vongsutilers, V. (2021). Nitrosamine contamination in pharmaceuticals: threat, impact, and control. *Journal of Pharmaceutical Sciences*, *110*(9), 3118-3128.
15. Chourasiya, S. S., & Ranbhan, K. J. *Nitrosamine impurities in APIs: A Comprehensive Review,* 2022, Vol. 12(1), (pp. 145-157)
16. Cross, K. P., & Ponting, D. J. (2021). Developing structure-activity relationships for *N*-nitrosamine activity. *Computational toxicology*, *20*, 100186.
17. Fahrer, J., & Christmann, M. (2023). DNA alkylation damage by nitrosamines and relevant DNA repair pathways. *International journal of molecular sciences*, *24*(5), 4684.
18. Schmidtsdorff, S., Neumann, J., Schmidt, A. H., & Parr, M. K. (2022). Risk assessment for nitrosated pharmaceuticals: A future perspective in drug development. *Archiv der Pharmazie*, *355*(4), 2100435
19. Lapo, M. I. F. (2021). *Risk assessment and mitigation of the presence of Nitrosamine impurities in medicines [Dissertation Risk assessment and mitigation of the presence of Nitrosamine impurities in medicines]*.
20. U.S. Department of Health and Human Services Food and Drug Administration. (2023). *Recommended Acceptable Intake Limits for Nitrosamine Drug Substance Related Impurities (NDSRIs) Guidance for Industry*. (pp. 1-24)
21. National Health Surveillance Agency (2023), *Guide on the Control of Nitrosamines in Active Pharmaceutical Ingredients and Medicines*. (pp. 4-31)
22. Elder, D. P., Johnson, G. E., & Snodin, D. J. (2021). Tolerability of risk: A commentary on the nitrosamine contamination issue. *Journal of Pharmaceutical Sciences*, *110*(6), 2311-2328.
23. Ponting, D. J., Dobo, K. L., Kenyon, M. O., & Kalgutkar, A. S. (2022). Strategies for assessing acceptable intakes for novel *N*-nitrosamines derived from active pharmaceutical ingredients. *Journal of Medicinal Chemistry*, *65*(23), 15584-15607.
24. European medicines agency (2024). *Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No*

726/2004 referral on nitrosamine impurities in human medicinal products (pp. 3–28). (2024)

25. Schlingemann, J., Burns, M. J., Ponting, D. J., Avila, C. M., Romero, N. E., Jaywant, M. A., & Wilk, A. (2023). The landscape of potential small and drug substance related nitrosamines in pharmaceuticals. *Journal of Pharmaceutical Sciences*, *112*(5), 1287-1304.
26. Thomas, R., Tennant, R. E., Oliveira, A. A. F., & Ponting, D. J. (2022). What makes a potent nitrosamine? Statistical validation of expert-derived structure–activity relationships. *Chemical research in toxicology*, *35*(11), 1997-2013.
27. Burns, M. J., Ponting, D. J., Foster, R. S., Thornton, B. P., Romero, N. E., Smith, G. F., & Schlingemann, J. (2023). Revisiting the landscape of potential small and drug substance related nitrosamines in pharmaceuticals. *Journal of Pharmaceutical Sciences*, *112*(12), 3005-3011.
28. Kruhlak, N. L., Schmidt, M., Froetschl, R., Graber, S., Haas, B., Horne, I., ... & Whomsley, R. (2024). Determining recommended acceptable intake limits for *N*-nitrosamine impurities in pharmaceuticals: Development and application of the Carcinogenic Potency Categorization Approach (CPCA). *Regulatory Toxicology and Pharmacology*, *150*, 105640.

Glossary

***N*-Nitrosoamines-** A class of organic compounds that are likely carcinogens to human.

Genotoxic- Substances can be chemicals or agents that can directly or indirectly harm an organism's DNA.

Mutagenic- Having ability to cause permanent changes in sequence of an organism’s DNA.

API- It's the active ingredient in a drug that's responsible for its beneficial effects.

Nitrosation reaction- Nitrosation is a chemical reaction where a nitroso group (-NO) is introduced in organic compound, typically an amine.

Cytochrome P450- CYP450 is a family of enzymes that play crucial role in the metabolism of various substances, including drug, toxins, and harmones.

ICH M7 (R1)- This guideline emphasizes considerations of both safety and quality risk management in establishing levels of mutagenic impurities that are expected to pose negligible carcinogenic risk.

Tumorigenic dose 50 (TD_{50})- TD_{50} is the dose of carcinogenic substance required to produce tumors in 50% of the test subjects.

CPCA- CPCA is a model that predicts the carcinogenic potency of a nitrosamine compound. It's used to determine recommended acceptable intake (AI) limits for nitrosamine impurities in pharmaceuticals.

NDSRIs- NDSRIs are a class of nitrosamine impurities in drugs that are structurally similar to the API.

Hepatoxic agent- The chemical which may damage the liver.

Nitrosating agents- Substances that can cause nitrosation, a chemical reaction that produces N-nitroso compounds.

Pharmaceutical aids- Substances which are of little or no therapeutic value, but are necessary in the manufacturing, compounding, storage, etc., of pharmaceutical preparations or drug dosage forms. They include solvents, diluting agents, and suspending agents, and emulsifying agents.

Bioactivation- The process by which enzymes or other biologically active molecules are able to perform their function.

Threshold of Toxicological Concern TTC- TTC is a concept used in toxicology to establish a threshold below which a chemical substance is unlikely to pose a risk to human health.

Cohort of concern- a group of compounds that are highly potent carcinogens and mutagens.

Acceptable intake (AI)- The amount of a substance that can be consumed daily without posing a significant health risk.

***In-vivo*-** That refers to experiments performed on living organisms or cells.