POISON CONTROL

VOLUME 2

Nitrosamine Impurities In Medicines, Acceptable Intake Limits, Hazardous Effects And Control

The unexpected discovery of the presence of nitrosamine impurities in drugs such as Angiotensin-II Receptor Blockers (ARBs), Ranitidine, Nizatidine and Metformin, has triggered a major safety concern on the use of the drugs. This volume aims to briefly highlight on Nitrosamines presence in food, its discovery in drugs, allowable limit and toxicity, hazardous effects, and possible control.

Introduction

The detection of nitrosamines as impurities in drugs was first reported by Medicine Regulatory Authorities in July 2018, following the identification as N-nitrosodimethylamine (NDMA) in Valsartan (an Angiotensin-II Receptor Blocker (ARBs) and belongs to a family of analogue compounds commonly referred to as the sartans). In 2018, this led to a global recall of valsartan-containing preparations in more than 22 countries, including Canada, Europe, and the United States. The impurity was linked to the synthetic process employed by Zhejiang Huahai Pharmaceuticals in China, a major producer of the active pharmaceutical ingredient (API) in valsartan.

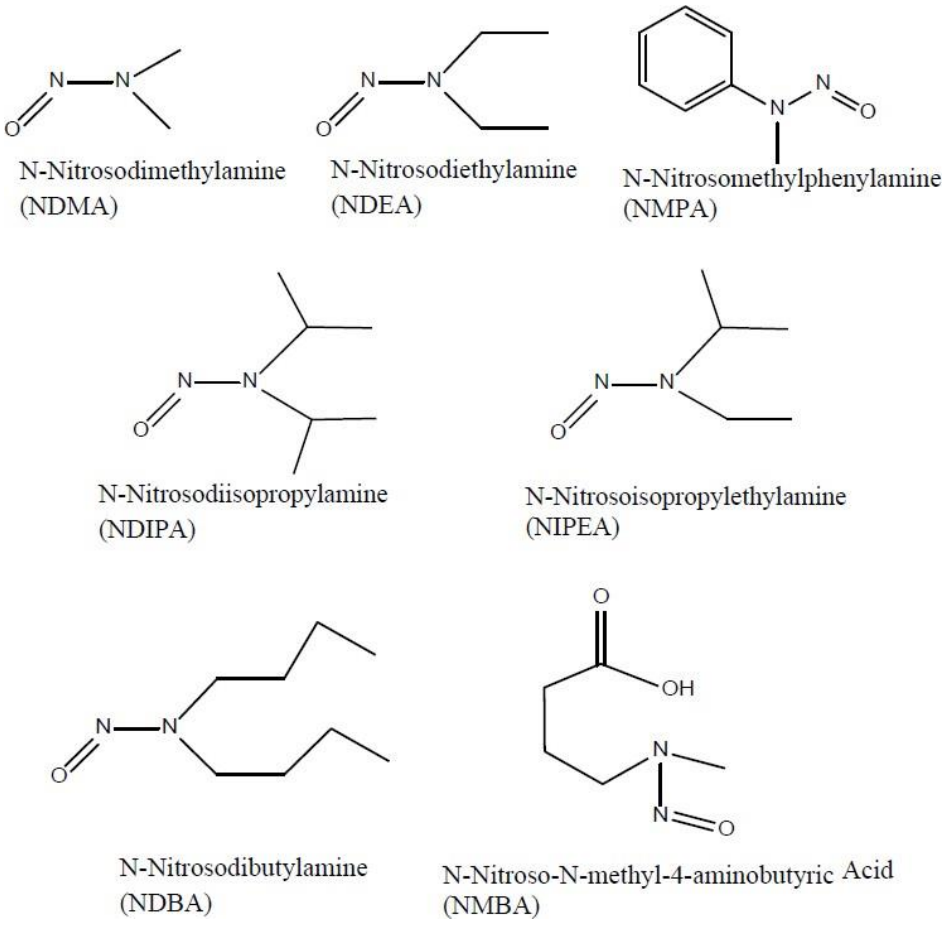
Additional nitrosamine impurities were subsequently detected in other medicines belonging to the sartan family, including N-nitrosodiethylamine (NDEA), N -nitrosodiisopropylamine (NDIPA), N -nitrosoethylisopropylamine (NEIPA) and N -nitroso-N-methyl-4-aminobutyric acid (NMBA).

What are Nitrosamines?

Nitrosamines or more correctly N-nitrosoamines are molecules containing nitroso functional group (N–N=O). These molecules are of concern because they are classified as probable carcinogens by International Agency for Research on Cancer [IARC] based on animal studies. Nitrosamines are common in water and foods, cured and grilled meats, dairy products and vegetables, their presence in medicines is nonetheless considered unacceptable.

There are seven nitrosamine impurities that could be present in drug products theoretically and five of them (NDMA, NDEA, NMBA, NIPEA, and NMPA) have been detected in drug substances or drug products.

Figure 1. Structure of Different Nitrosamine impurities



Common name and chemical name	Acronym	CAS#	Chemical Formula	Molecular Weight
Nitrosodimethylamine/ N-Methyl-N-nitrosomethanamine	NDMA	62-75-9	$C_2H_6N_2O$	74.08
N-Nitrosodiethylamine/ *N*-Ethyl-*N*-nitrosoethanamine	NDEA	55-18-5	$C_4H_{10}N_2O$	102.13
N-Nitrosodiisopropylamine/ N-Isopropyl-N	NDIPA	601-77-4	$C_6H_{14}N_2O$	130.19

nitrosoisopropylamine				
N-nitrosoethyisopropylamine/ N-Ethyl-N-nitroso-2-propanamine	NEIPA	16339-04-1	$C_5H_{12}N_2O$	116.16
N-nitrosodibutylamine/ N-Butyl-N-nitroso- 1-butanamine	NDBA	924-16-3	$C_8H_{18}N_2O$	158.24
N-Nitrosomethylphenylamine/ N-Methyl-N-nitrosophenylamine	NMPA	614-00-6	$C_7H_8N_2O$	136.15
N-Nitrosomethylaminobutyric acid /4-[Methyl(nitroso)amino] butanoic acid	NMBA	61445-55-4	$C_5H_{10}N_2O_3$	146.14

Potential sources of Nitrosamines in Pharmaceuticals

Importantly, formation of Nitrosamine in Medicinal products is process driven and not related to any molecule.

The most common pathway for the formation of Nitrosamine is Nitrosating reaction between amines (secondary, tertiary, or quaternary amines) and nitrous acid (nitrite salts under acidic conditions). Amines and nitrous acid can be present in the reaction process by various reasons.

Secondary, Tertiary and Quaternary Amines, present as a functional group in the active pharmaceutical ingredients (API), intermediate and starting materials or added intentionally as a reagents or catalysts, can react with nitrous acid or other nitrosating agents to form nitrosamines.

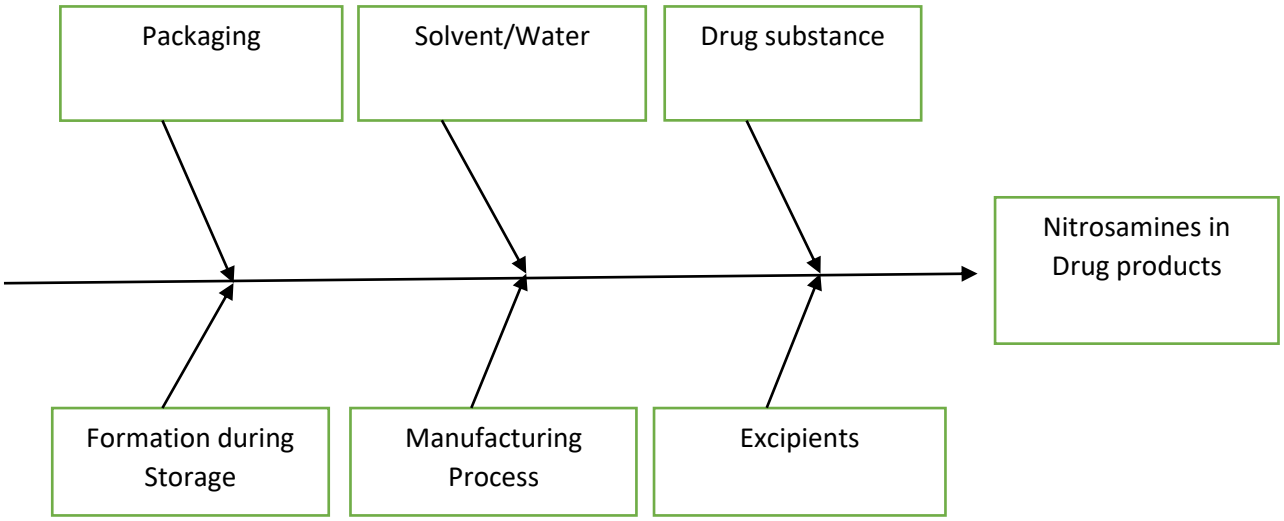
Nitrosamine impurities can be introduced when vendor-sourced materials, including starting materials and raw materials, are contaminated. Evidence suggests the following as potential reasons for formation of nitrosamine impurities in pharmaceuticals;

- Presence of nitrites or amines as unintentional contaminants of starting materials, raw materials, reagents, and solvents (such as dimethylamine in the common solvent dimethyl formamide (DMF)).
- Cross-contamination of out-sourced starting materials and intermediates manufactured at sites where nitrosamine impurities are produced in other processes and equipment used are not adequately cleaned.
- Recovered materials such as solvents, reagents, and catalysts may pose a risk of nitrosamine impurities due to the presence of residual amines.

- Lack of optimization of the manufacturing process for APIs, where reaction conditions such as temperature, pH, or the sequence of adding reagents, intermediates, or solvents are inappropriate or poorly controlled.
- Degradation processes of starting materials, intermediates, and drug substances, including those induced by inherent reactivity in combination with carry-over of nitrosating agents. This could potentially occur also during finished product formulation or storage.
- Use of certain packaging materials. Some finished drug products packaged in blisters may undergo degradation pathways to form nitrosamine impurities during storage.

Nitrosamines are not expected to be formed during the manufacture of most APIs. However, it is now known that these impurities can form during production under certain conditions and when certain solvents, reagents and other raw materials are used. In addition, impurities can be carried over during the manufacturing process when using already-contaminated equipment or reagents.

Figure 2. Summary of Potential sources of nitrosamine impurities in drug products



Toxicity of Nitrosamines

The primary toxicity concern associated with nitrosamines found in the environment, human diet, tobacco products, cosmetics, and as an impurity in marketed drug products has been that these structures are genotoxic chemical carcinogens.

Nitrosamines are described in the International Conference for Harmonisation (ICH) M7(R1) Guideline as Class 1 impurities which are high-potency, mutagenic carcinogens. In addition, nitrosamines along with aflatoxin-like structures and alkyl-

azoxy compounds belong to the infamous “cohort of concern” which is a group of highly potent mutagenic carcinogens that have been classified by the WHO's International Agency for Research on Cancer as probably human carcinogens.

Evidence of carcinogenicity associated with nitrosamines was identified in a variety of nonclinical species and a small number of case-control studies with humans.

In studies, N-nitrosodimethylamine (NDMA) produced tumors in a variety of nonclinical species ranging from fish, amphibians, rodents, and other mammals after exposure via several routes. Benign and malignant tumors after exposure to NDMA were identified in the respiratory tract, kidney, digestive tract, liver and bile duct, hematopoietic system, and female reproductive tract. Similarly, tumors were identified in the liver, kidney, respiratory tract, and the digestive tract of some nonclinical species such as dogs and pigs after exposure to N-nitrosodiethylamine (NDEA).

Although no epidemiological studies evaluating the relationship of exposure to NDEA and human cancer have been conducted, several population-based case-control studies and ecological studies were conducted to assess the relationship between dietary sources of NDMA and cancer. Dose-related associations of colorectal, stomach, esophageal, and oropharyngeal cancers with estimated NDMA exposure were identified in several case-control studies.

Furthermore, an increased risk associated with lung cancer was identified with dose-related increases in estimated dietary exposure to NDMA.

Based on all these evidence from studies, regulatory authorities suspect that NDMA, NDEA, and other structurally related nitrosamines can act as carcinogens in humans. Cancers that are most commonly linked with nitrosamines are bladder, esophageal, nasopharynx, prostate cancers, and non-Hodgkins lymphoma. It may increase the risk of colorectal and stomach cancers. In addition, nitrates in the environment and in food were associated with Alzheimer’s and Parkinson’s diseases and type-2 diabetes.

Nitrosamines can also cause fatty liver disease, obesity, DNA damage, and cell death. In addition, they can cause chromosomal changes, birth defects, and pregnancy loss, based on animal studies.

Allowable limit of nitrosamines in pharmaceutical products

Dietary exposure to nitrosamines is well recognized for many years with their presence in cured meats, tobacco smoke, and beer. For example, NDMA exposure from dietary consumption levels range from 0.0004 to 0.23 μg/day in cured meat, 0.0004 to 1.02 μg/day in smoked meat, and 0.0006 to 0.13 μg/day in grilled meat.

Likewise exposure via drinking water is also recognized, for example the U.S. Environmental Protection Agency (EPA) has set health reference levels for NDEA at 0.8 ng/day and NDMA at 0.6 ng/day.

In addition to environmental exposure, is the recent detection of NDMA as a process-related contaminant in the pharmaceutical product valsartan.

Interim acceptable daily intakes for these specific impurities have been recommended and adopted by most major regulators, as indicated in the table below. It is further recommended that manufacturers use these AIs when determining limits for nitrosamine impurities in APIs and drug products.

Table 1: Interim allowable daily intake limits for selection N-Nitrosamine impurities

Impurity name Abbreviation	Chemical name	Allowable Daily Intake (AI)
NDMA	N-nitrosodimethylamine	96.0 ng/day
NDEA	N-Nitrosodiethylamine	26.5 ng/day
NMBA	N-Nitroso-N-methyl-4-aminobutyric acid	96.0 ng/day
NDIPA	N-nitrosodiisopropylamine	26.5 ng/day
NEIPA	N-nitrosoethylisopropylamine	26.5 ng/day
NMPA	N-Nitrosomethylphenylamine	26.5 ng/day
NDBA	N-Nitrosodibutylamine	26.5 ng/day

The term *acceptable intake (AI)* is used in ICH M7(R1) to indicate the threshold of toxicological concern (TTC) considered for the impurity to be associated with negligible risk of carcinogenicity or other toxic effects.

The AI limit is a daily exposure to a compound such as NDMA, NDEA, NMBA, NMPA, NEIPA, or NDIPA etc. that approximates a 1:100,000 cancer risk after 70 years of exposure.

The limits published were based on the TD50 values for NDMA and NDEA with a 1:100,000 safety factor applied (decreasing the potential cancer risk to 1 in 100,000).

- TD50- dose level showing 50% tumor incidence in animal study.
- Accepted lifetime cancer risk level: 1 in 100,000 patients
- Dividing TD50 by 50,000
- Calculated acceptable NDMA lifetime limit
    - TD50 of 0.0959mg/kg /50,000= 0.000001918mg/kg

- To derive a total human daily dose: 0.000001918mg/kg x 50kg=0.0000959mg/day (=96 ng/day)

How to calculate Acceptable nitrosamine content in a drug product

The conversion of AI limit into ppm varies by product and is calculated based on a drug's maximum daily dose (MDD) as reflected in the drug label (ppm = AI (ng)/MDD (mg))

The acceptable concentration in the product material can be calculated as;

Acceptable nitrosamine content = AI / MDD

Where, AI = Acceptable daily intake of the nitrosamines, ng/day

MDD = maximum daily dose of the API, mg/day

Example: Using AI of 96ng/day for target nitrosamine

Acceptable nitrosamine content= 96ng/day /50 mg x 1000= 1920 ng/g

OR

96ng/day /0.050 g =1920 ng/g

Nitrosamine	**Acceptable concentration, ng/g (ppb) or ng/mg (ppm)**			
	0.050 g (50 mg dose)	0.100 g (100 mg dose)	0.250 g (250 mg dose)	1.000 g (1000 mg dose)
Nitrosamine 1	1920 ng/g	960 ng/g	384 ng/g	96 ng/g

Clinical Management of Nitrosamine intoxication

Nitrosamine exposure is not an acute hazard. Health hazards associated with nitrosamine exposure are limited to cancer, and liver and kidney damage associated with chronic exposure. No specific treatment exists for nitrosamine intoxication. Supportive and symptomatic treatment should be provided.

Since nitrosamines and their precursors are present in food such as cured meats, sausages, salted fish and most dietary/diary products, exposure to nitrosamines cannot be avoided. However, recent studies have shown that ingestion of adequate quantities of vitamin E and selenium may reduce the risk of cancer. It is known that carcinogenic nitrosamines are formed from the reaction of some amines with nitrites and nitrates present in the diet. Vitamin E and selenium have been found to minimize or prevent the reaction of nitrites/nitrates with amines and hence prevent or reduce the formation of carcinogenic nitrosamines. Vitamin C (ascorbic acid) is also known to inhibit nitrosamine formation.

Consumption of nitrosamine prone foods should be done in moderation. Eating vegetables and fruits which are high in antioxidants, vitamin C and phytochemicals (which have shown significant protection against cancer by inhibiting nitrosamine formation) should be encouraged.

Nitrosamine Control Strategies in pharmaceutical products and Regulatory Recommendation

The ICH M7(R1): Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk. This scientific guideline emphasizes considerations of both safety and quality risk management in establishing levels of mutagenic impurities that are expected to pose negligible carcinogenic risk. It outlines recommendations for assessment and control of mutagenic impurities that reside or are reasonably expected to reside in final drug substance or product, taking into consideration the intended conditions of human use.

Companies are required to have appropriate control strategies to prevent or limit the presence of these impurities and, where necessary, to improve their manufacturing processes.

Regulatory agencies, including USFDA, EMA and EDQM, recommends that manufacturers consider the potential causes of nitrosamine formation and evaluate the risk for formation and contamination of nitrosamine impurities in their APIs and drug products based on factors such as maximum daily dose, duration of treatment, therapeutic indication, and number of patients treated. Manufacturers of APIs and drug products should take appropriate measures to prevent unacceptable levels of nitrosamine impurities in their products.

In cases where nitrosamines can form or are carried over during production, the impurities should normally be controlled and removed during the manufacturing process.

Recommendations: API Manufacturers

- Optimize the manufacturing process to minimize or prevent the formation of nitrosamine impurities
- Consider removing quenching steps to avoid nitrosamine formation
- Audit supply chains and monitor for at-risk raw materials, starting materials, and intermediates
- Avoid cross-contamination of solvents, reagents, and catalysts
- Reprocess or rework API batches to control the level of nitrosamine impurities as provided in ICH Q7 (Good Manufacturing Practice Guidance for Active

Pharmaceutical Ingredients Sept. 2016, rev. 1) for amending and controlling such operations

- Develop a strategy to ensure that the nitrosamine level remains within the AI limit

Recommendations: Drug Product Manufacturers

- Conduct risk assessments to determine the potential for nitrosamine impurities in drug products
- Test representative samples of all incoming components, including lots of at-risk API, prior to use, as required under 21 CFR 211.84
- Evaluate whether nitrites could be present during manufacturing

processes where at-risk APIs are used

- Evaluate whether nitrosamines could form in a finished drug product over the drug product's shelf life

Conclusion

Since the recent detection of nitrosamines in widely prescribed pharmaceutical drugs, concerns have been raised about patient safety and has prompted health authorities to mandate risk assessments and confirmatory testing in marketed products. This testing will be aided by sensitive analytical methods like gas chromatography-tandem mass spectrometry (GC-MS/MS) and ultra-high-performance liquid-chromatography-tandem mass spectrometry (UPLC-MS/MS) which are used to test nitrosamine concentrations and determine if products are safe for consumption. These methods have already been established for some of the most commonly occurring nitrosamines. While the pharmaceutical industry and health authorities manage the risk to patients, regulations are expected to rapidly evolve, while the principles of GMP are strictly supposed to be adhered to, to ensure the non-conformities in the manufacturing processes are minimized and by implication mitigate inadvertent contaminations.

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