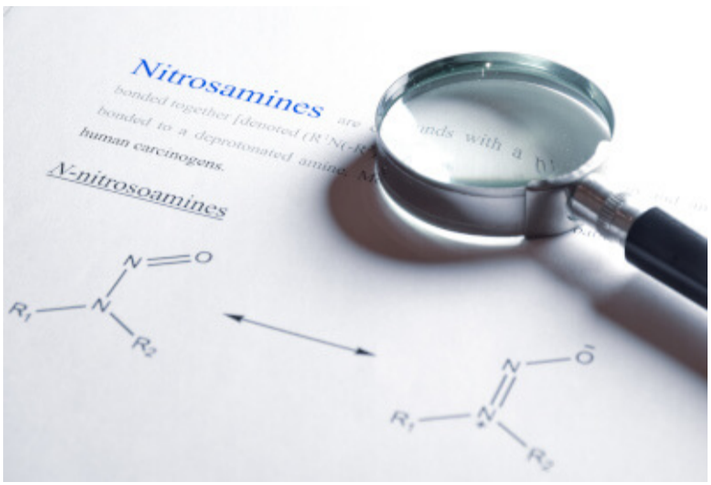
Unveiling the hidden threat: nitrosamine impurities in medicines



Cancer-causing nitrosamines - also known as N-nitrosamines, N-nitroso compounds, or NOCs – first hit the headlines in 2018, when they were detected in batches of bestselling blood pressure drugs. In this article we recount the short but troubled history of pharmaceutical nitrosamine impurities, and global attempts to set acceptable intake limits for both small molecule nitrosamines and NDSRIs in drug products. We also discuss key challenges and advances in pharmaceutical nitrosamine testing, as well as the latest regulatory developments.

Becoming Public Enemy #1

Prior to **threatening the supply of four in ten of the world's medicines**, carcinogenic nitrosamines had received comparatively little scientific attention. They were first detected in the 1870s, but it took until the mid-1950s for Magee and Barnes to **link N-Nitrosodimethylamine (NDMA) with the development of malignant tumours in rats** - while subsequent studies associated it with the deaths of farm animals fed on nitrite-treated herring meal.

Beginning in the 1970s, more rigorous research identified **the presence of N-Nitrosamines in beverages, preserved food, personal care products, tobacco, and chlorinated or contaminated water** – and also implicated them in **the development of bladder, lung, liver, stomach and many other cancers**.

However, it took the discovery of nitrosamines in pharmaceutical drugs to make regulators really sit up and take action - beginning in June 2018, when **the US Food and Drug Administration (FDA) discovered NDMA in batches of the major Angiotensin Receptor Blocker valsartan**. A month later, the European Medicines Agency (EMA) said **NDMA and N-Nitrosodiethylamine (NDEA) had been detected in more sartans blood pressure drugs**, while further testing later revealed **unacceptable levels of NDMA in both the heartburn medicine ranitidine and the anti-diabetic metformin**.

With numerous **batch recalls beginning to threaten the global supply of these essential drugs**, regulators on both sides of the Atlantic demanded that manufacturers carry out **a comprehensive review of all human medicines for the possible presence of nitrosamines**, and submit changes to their manufacturing processes where nitrosamines were detected above permitted levels. "After about three years or so, **we thought we were pretty close to solving the whole problem**," said Astra Zeneca's senior principal scientist in impurity management, Andrew Teasdale. However, the picture changed significantly with the emergence of a new class of nitrosamine impurities called nitrosamine drug-substance-related impurities (NDSRIs), which are intrinsically linked to the active pharmaceutical ingredients (APIs) of many drug products.

We seemed to be heading towards **a catastrophic loss of critical medicines**.

The multiple pathways to nitrosamine formation

Nitrosamine impurities can be formed in many different ways - the most common of which is **an N-N bond formation process** involving **nitrites and vulnerable functional groups such as secondary or tertiary amines**. Nitrite ions can readily transform into nitrosating agents such as N_2O_3 or NO^+, especially in acidic conditions, and then react with amines to form nitrosamines.

Contamination can happen at virtually any stage of the life of a drug compound - from the purchase of ingredients to synthesis, and right up to storage of finished products. Nitrosating agents can be introduced throughout the production process via raw materials and the use of recycled solvents, reagents and catalysts. Chemical processes - such as **quenching with a nitrosating agent in the presence of DMF (dimethylformamide)** and **the use of buffers containing tertiary or quaternary amines to stabilise APIs** - have also been identified as key pathways for nitrosamine formation. Degradation can still occur once a drug product is finished and packed, through the use of **unsuitable primary packaging, such as nitrocellulose blisters**, or printing inks. Furthermore, **nitrite impurities are found in most common drug excipients**, at least in traces, and are therefore another potential contributor to the formation of nitrosamines.

But most in **danger of nitrosamine formation** are the wide range of **drug products whose API structures are comprised of secondary or tertiary amines, or tertiary ammonium salts**. As Teasdale explains, "If the drug itself was a secondary amine, particularly if you're using processes like wet granulation where you've got a fair amount of water present, you can dissolve the trace nitrite that's commonly present in [other ingredients, and] you've got the **chemistry conditions to form a nitrosamine**." These nitrosamine impurities – known as NDSRIs – are unique to individual APIs.

Alternative excipients, and other mitigation strategies

Because N-N=O bonds can occur due to many different precursors and conditions, preventing their formation has been described as "**a multifaceted problem with no single solution**." However, a number of proven mitigation steps have emerged as our understanding of nitrosamines has grown since 2018. These include **careful auditing of supply chains and testing of raw materials**, as well as thorough consideration of where secondary and tertiary aliphatic and aromatic amines may occur during the production process – "including those present as part of the starting material, intermediate or final structure as well as those introduced as **reagents, catalysts, solvents or as impurities**." Excipients containing the **nitrosamine inhibitors ascorbic acid and alpha-tocopherol** are another regulator-sanctioned response - since ascorbic acid in particular reacts with a number of nitrosating agents, and both compounds "bring the added benefit of acting as stabilizers in the finished drug product formulation without safety concerns." Another potential approach is to use **excipients with lower nitrite levels** than those used previously. **A study last year by Boetzel *et al.*** found that crospovidone and magnesium stearate excipients contained significantly higher levels of nitrites than corn starch, lactose monohydrate and microcrystalline cellulose versions.

Challenges and changes in nitrosamine analysis

Since the panicked days of mid-2018, considerable progress has been made on understanding and limiting the threat of small molecule nitrosamine impurities in drug products. **According to the EMA**, the global response rate to regulators' demands for risk evaluations of active substances and finished drugs stood at over 96% in September this year - with just 2.5% of total products assessed so far containing greater than permitted levels of nitrosamines. These Acceptable Intake levels (AIs, see *Table 1*) are necessarily strict, in line with International Council for Harmonisation guidance "that any known mutagenic carcinogen, such as **nitroso compounds, should be controlled at or below levels such that there is a negligible human cancer risk**." However, demanding limits

impurities necessitates careful handling in order to avoid recovery issues, while **NDMA analysis presents "specific challenges due to the small size and high polarity** of both the drug and the contaminant."

Nitrosamine	AI Limit (ng/day)
NDMA	96
NDEA	26.5
NMBA	96
NMPA	26.5
NIPEA	26.5
NDIPA	26.5

Table 1: Acceptable Intake Limits of Small Molecule Nitrosamines

Care is also needed when employing one of the most important analytical techniques for NDSRIs – namely **electrospray ionisation mass spectrometry (ESI-MS)**. The presence of the nitroso group means that **NDSRIs are particularly prone to in-source fragmentation** within the ESI source - "namely the **loss of 30 Da (which)** corresponds to the detachment of the NO radical from the protonated nitrosamine compound." This in turn significantly affects the observed mass spectra, making the correct identification and quantification of target analytes more difficult. There are, however, two main ways of minimising the likelihood of this type of fragmentation – decreasing the decluster potential voltage, and optimising the temperature of the ion source to preserve analyte integrity.

Getting to grips with NDSRIs

When the hazards posed by NDSRIs first became apparent, there was "**little to no safety data available" on these more complex nitrosamines – which in turn made it difficult to set definitively safe limits based on acceptable daily intake**. However, in July this year, the EMA and FDA updated their guidance on nitrosamine impurities with the introduction of the **Carcinogenic Potency Categorisation Approach (CPCA)** – "a science-based predictive solution to recommending AI limits for NDSRIs" for which no direct mutagenicity data exists.

According to the FDA, CPCA "assumes that the **α-hydroxylation mechanism of metabolic activation is responsible for the mutagenic and highly potent carcinogenic response observed for many nitrosamines**. Structural features that directly increase or decrease the favorability of the activation mechanism, or that increase the clearance of the nitrosamine by other biological pathways, will have a corresponding effect on carcinogenic potency. Therefore, **a prediction of the mutagenic potential and carcinogenic potency of an NDSRI can be generated based on its structural features**."

The guidance adds that, once these structural studies have been carried out, **NDSRIs may be placed in one of five potency categories based on AI limits** ranging from 26.5 – 1500 ng/day – enabling manufacturers "to identify the appropriate potency category and associated recommended AI limits for NDSRIs in APIs and drug products, and to facilitate development of methods for confirmatory testing of impurity levels in drug batches." With most drugs expected to fall into the least restricted Category 5, CPCA seems likely to remove many of the nitrosamine-related pressures on drug supply. "For a lot of our existing drugs, **it makes a huge difference**," said Teasdale, welcoming the new approach.
The FDA recently issued another important **update to CPCA** by publishing a list of >260 NDSRIs and their potency categories, together with details of a new analytical requirement – an enhanced bacterial reverse mutagenicity assay, known as the Ames Test, which can be used to assess whether an NDSRI poses a mutagenic risk. Laboratories performing the enhanced assay – which requires **two nitrosamine positive controls that are**

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Small molecule nitrosamines

Part Code	CAS Number	Part Description
MM1204.03	1116-54-7	2,2'-(Nitrosoimino)diethanol (N-Nitrosodiethanolamine)
MM0267.03*	59-89-2	4-Nitrosomorpholine
MM0877.79-0025*	601-77-4	N-Nitrosodiisopropylamine (NdiPA)
MM0487.15-0100*	924-16-3	N-Nitroso-N-di-n-butylamine
MM0487.20-0100	621-64-7	N-Nitroso-N-di-n-propylamine
MM0487.18-0100	54897-63-1	N-Nitroso-N-ethyl-4-aminobutyric Acid
MM0487.17-0100*	16339-04-1	N-Nitroso-N-ethyl-N-isopropylamine
MM0487.19-0025	2624122-60-5	N-Nitroso-N-isopropyl-4-aminobutyric Acid
MM0487.13-0100	30533-08-5	N-Nitroso-N-isopropyl-N-methylamine
MM0487.11-0025	61445-55-4	N-Nitroso-N-methyl-4-aminobutyric Acid
MM0487.16-0100	2680532-87-8	N-Nitroso-N-n-butyl-N-isopropylamine
MM0487.14-0100*	7068-83-9	N-Nitroso-N-n-butyl-N-methylamine
MM3987.06-0025	140-79-4	N,N'-Dinitrosopiperazine (DMP)
MM3987.08-0100	48121-20-6	4-Nitroso-1-piperazineethanol
MM0487.09AME-00.20	62-75-9	N-Nitrosodimethylamine 0.2 mg/ml in Methanol
MM0487.10AME-00.10	55-18-5	N-Nitrosodiethylamine 0.1 mg/ml in Methanol

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MM0477.01	85440-79-5	1H-indole	Indapamide
MM0380.27-0025	2005-04-1	1-(4-Chlorobenzhydryl)-4-nitrosopiperazine	Cetirizine Dihydrochloride
MM0325.02	54786-86-6	2-Nitroso-octahydrocyclopenta[c]pyrrole	Gliclazide
MM0011.12-0025	63779-86-2	4-Nitrosohydrochlorothiazide	Hydrochlorothiazide
MM0257.25-0025	1246819-22-6	8-Chloro-6,11-dihydro-11-(1-nitroso-4-piperidinylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridine	Desloratadine; Loratadine
MM0846.15-0025	1152314-62-9	N-Desmethyl-N-nitrosolevofloxacine	Levofloxacin Hemihydrate
MM3987.13-0025	5336-53-8	N-Nitrosodibenzylamine	Dibenzylamine
MM0935.13-0025	n/a	N-Nitrosoezetimibe	Ezetimibe
MM0608.02	19023-40-6	N-Nitrosofenfluramine	Fenfluramine Hydrochloride
MM0256.22-0025	150494-06-7	N-Nitrosofluoxetine	Fluoxetine Hydrochloride
MM0439.08-0025	134720-06-2	N-Nitrosonadolol	Nadolol
MM2588.22-0100	13256-22-9	N-Nitrososarcosine	Catabolic Amino Acids
MM3380.20-0025	2755871-02-2	N-Nitrosovarenicline	Varenicline Tartrate
MM0420.14-0025	55855-44-2	N-Nitrosodesmethylchlorpromazine	Chlorpromazine Hydrochloride
MM0517.27-0025	16543-55-8	N'-Nitrosonornicotine	Nicotine
MM0938.23-0025	n/a	N-Nitrosotamsulosin	Tamsulosin Hydrochloride
MM1631.02-0025	57830-36-1	N-Nitrosodesmethyltripelennamine	Tripelennamine Hydrochloride
MM0013.20-0025	134720-04-0	N-Nitrosoatenolol	Atenolol
MM0291.24-0025	2248746-67-8	N-Nitrosocarvedilol	Carvedilol
MM0545.15-0025	n/a	N-Nitroso-3-chlorodibenzazepine	Clomipramine Hydrochloride
MM3062.09-0025	2792161-95-4	N,N'-Dinitrosoethambutol	Ethambutol Hydrochloride
MM3264.08-0025	n/a	N-Nitrosovortioxetine	Vortioxetine
MM3564.20-0025	n/a	N-Nitrosovildagliptin	Vildagliptin