Drug Substance Workflow for Quality Risk Management of Nitrosamine Risks in Medicines

Version 3.0

2024

Drug Substance Manufacturing Process Risk Assessment

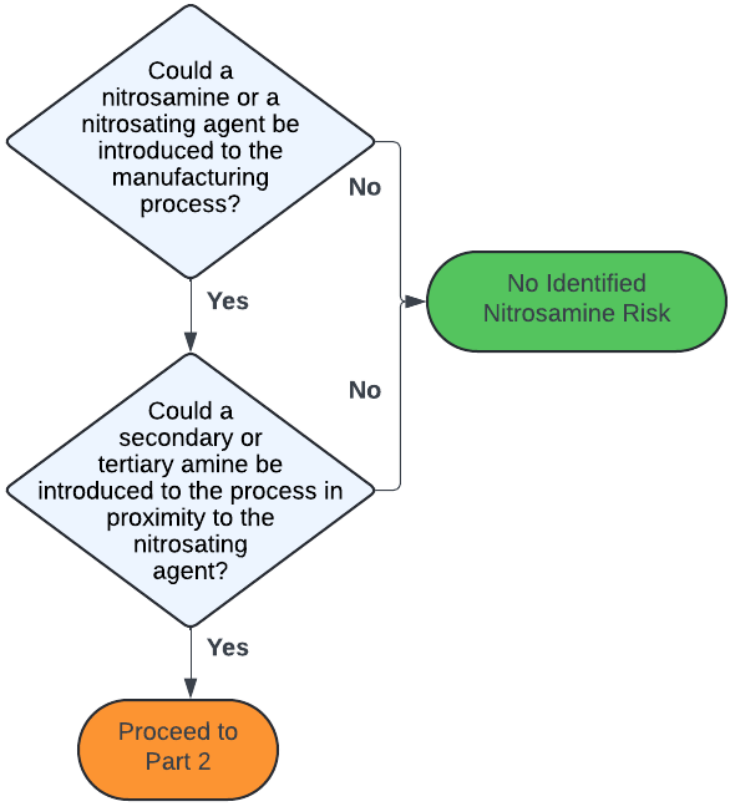
Part 1 - Risk Identification

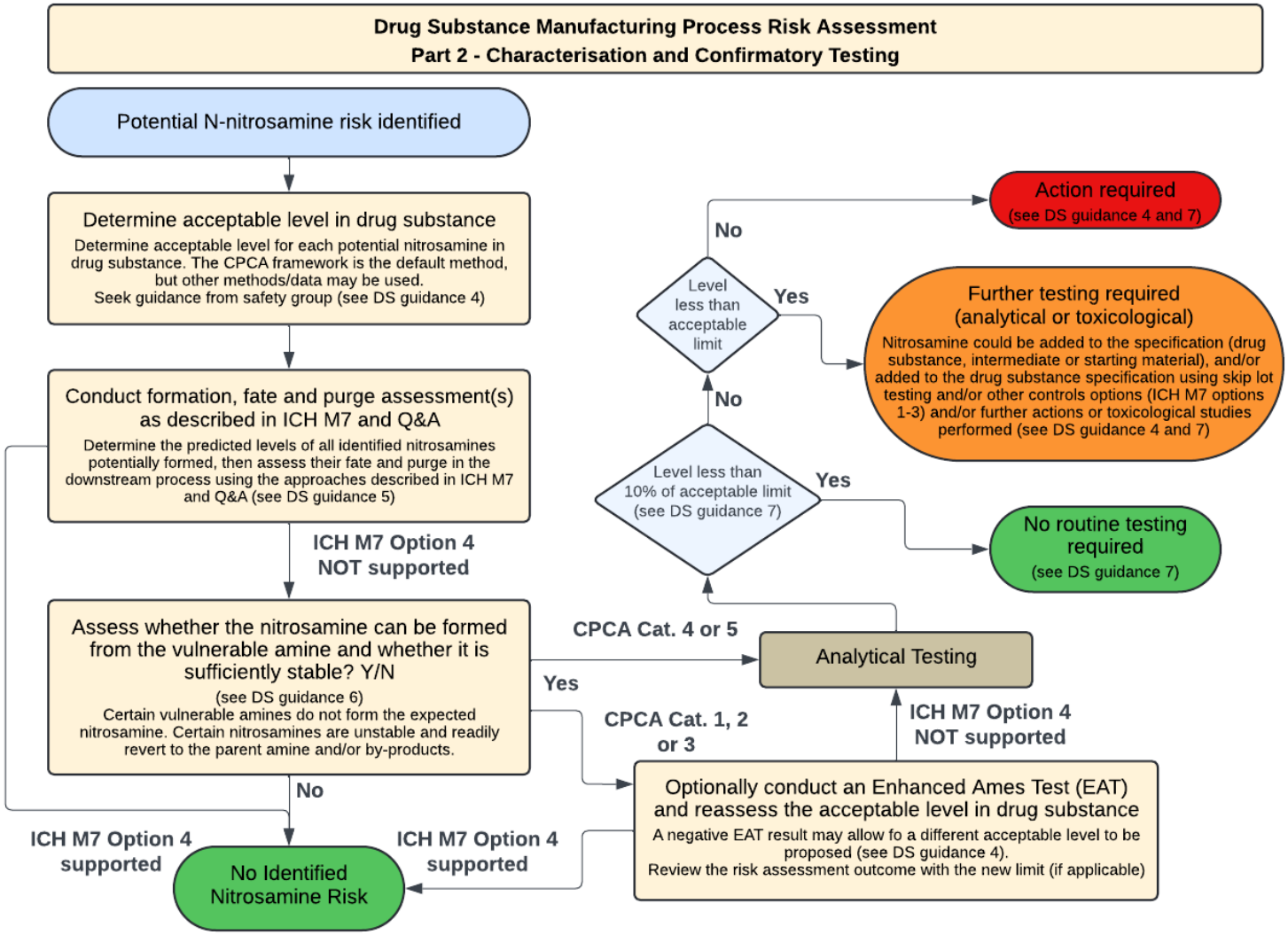
Assess all stages of the drug substance manufacturing route after the registered starting materials. The synthesis routes for registered starting materials also need to be assessed, particularly when they contain amine, nitro functionalities, use nitrosating agents or are introduced late in the synthesis.

Risks associated with the drug substance and associated impurities / degradants containing vulnerable amines in the drug product are addressed in the Drug Product Workflow

Nitrosating agents can be used in the process during a reaction or work-up, introduced as impurities in input materials or processing environment or generated during the process (see DS Guidance 1). Other root causes included in regulatory guidance should also be considered (see DS References 1, 2, 16 and 17).

Certain secondary and tertiary amines are vulnerable towards reaction with nitrosating agents to form nitrosamines. They can be part of the core structures, used in the process as reagents or solvents, introduced as impurities in input materials or generated during the process as impurities. In rare instances, other functional groups may directly lead to a introsamine (see DS guidance 3). Other root causes included in regulatory guidance should also be considered (see DS DS References 1, 2, 16 and 17).




Drug Substance Manufacturing Process Risk Assessment
Part 2 - Characterisation and Confirmatory Testing
Potential N-nitrosamine risk identified
Determine acceptable level in drug substance
Determine acceptable level for each potential nitrosamine in drug substance. The CPCA framework is the default method, but other methods/data may be used.
Seek guidance from safety group (see DS guidance 4)
Conduct formation, fate and purge assessment(s) as described in ICH M7 and Q&A
Determine the predicted levels of all identified nitrosamines potentially formed, then assess their fate and purge in the downstream process using the approaches described in ICH M7 and Q&A (see DS guidance 5)
ICH M7 Option 4 NOT supported
Assess whether the nitrosamine can be formed from the vulnerable amine and whether it is sufficiently stable? Y/N
(see DS guidance 6)
Certain vulnerable amines do not form the expected nitrosamine. Certain nitrosamines are unstable and readily revert to the parent amine and/or by-products.
No
ICH M7 Option 4 supported
No Identified Nitrosamine Risk
Yes
CPCA Cat. 1, 2 or 3
CPCA Cat. 4 or 5
Analytical Testing
Level less than 10% of acceptable limit (see DS guidance 7)
Yes
No routine testing required
(see DS guidance 7)
No
Level less than acceptable limit
Yes
Further testing required (analytical or toxicological)
Nitrosamine could be added to the specification (drug substance, intermediate or starting material), and/or added to the drug substance specification using skip lot testing and/or other controls options (ICH M7 options 1-3) and/or further actions or toxicological studies performed (see DS guidance 4 and 7)
No
Action required
(see DS guidance 4 and 7)
ICH M7 Option 4 NOT supported
Optionally conduct an Enhanced Ames Test (EAT) and reassess the acceptable level in drug substance
A negative EAT result may allow fo a different acceptable level to be proposed (see DS guidance 4).
Review the risk assessment outcome with the new limit (if applicable)
ICH M7 Option 4 supported

"N-Nitrosamine" and "nitrosamine" are synonymous and will be referred to as "nitrosamine" hereafter.

The term "risk" refers to the likelihood of presence or formation of a nitrosamine at a level of concern.

The steps outlined within this guidance can be conducted in different orders if preferred.

Guidance 1 (Sources of nitrosating agents)

Nitrosating agents, and their precursors, to be considered include:

- Nitrites (e.g., sodium nitrite, $NaNO_2$) and nitrous acid (HNO_2), nitric oxide (NO), nitrosyl halides (e.g. ClNO, BrNO), dinitrogen trioxide (N_2O_3), dinitrogen tetroxide (N_2O_4) and organic nitrites (e.g. t-BuONO).
    - It should be noted that nitrite itself is not a nitrosating agent, but it can lead to nitrosating agents (e.g., HNO_2, NO, ClNO, BrNO, N_2O_3, etc..) under certain conditions (e.g. aqueous acidic).
- Nitrosating agents used in the manufacturing process should be considered.
- Impurities acting as nitrosating agents (e.g. from the input materials or water) should be considered if these input materials or water are used in proximity of a vulnerable amine. It should be noted that the risk from water is very low as described below.
- The use of raw materials that contain low levels of nitrosating agents (or vulnerable amines) has been reported as a root cause by Horne et al. in 2023[1] and Bream et al. in 2023.[2]

Additional considerations for potential nitrosation risks:

- Certain input solid materials used during synthesis (e.g. NaCl, NaOH, K_2CO_3 and charcoal) can contain low levels (ppm) of nitrosating agents). Trace analytical methods for nitrite analysis have been reported[3] and can be used to establish nitrite levels in input materials. It should be noted that the grade of the materials may lead to different nitrite contents. Liquid reagents, organic solvents and aqueous solutions at low pH are generally considered to not contain nitrite.

- Analysis has shown that nitrite levels in process water are typically very low (less than 3 ppb for potable water and less than 0.1 ppb for purified water)[4] therefore, an understanding of the nitrite content of the water used has the potential to mitigate water as a risk factor.

- Side reaction in nitration reactions. Nitric acid typically contains nitrogen dioxide and therefore dinitrogen tetroxide as an impurity, additional nitrous acid may also be produced, leading to nitrosation, if any reducing agents are present.[1,5]

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[1] Horne, S. et al. Regulatory Experiences with Root Causes and Risk Factors for Nitrosamine Impurities in Pharmaceuticals *J. Pharm. Sci* **2023**, *112*, 1166-1182. https://doi.org/10.1016/j.xphs.2022.12.022

[2] Bream, R. et al. Formation of N-Nitrosamine Drug Substance Related Impurities in Medicines: A Regulatory Perspective on Risk Factors and Mitigation Strategies *Org. Process Res. Dev.* **2023**, *27*, 1736-1750. https://doi.org/10.1021/acs.oprd.3c00153

[3] Boetzel, R et al. A Nitrite Excipient Database: A useful Tool to Support N-Nitrosamine Risk Assessments for Drug Products, *J. Pharm. Sci.* **2022**, *112*, 1615-1624. https://doi.org/10.1016/j.xphs.2022.04.016

[4] Pfizer internal data shared on 25th Oct 2021 at Global Workshop on Nitrosamine Impurities: Global Workshop on Nitrosamine Impurities (cvent.com)

[5] López-Rodríguez, R et al. Pathways for N-Nitroso Compound Formation: Secondary Amines and Beyond. *Org. Process Res. Dev.* **2020**, *24*, 1558-1585. https://doi.org/10.1021/acs.oprd.0c00323

- Nitroalkanes, halogenated nitro alkanes, Fremy's salt, nitroso sulfonamides and nitroaromatics can all under some circumstances give rise to nitrosating agents.[5]
- Hydroxylamines, hydrazines[6], hydrazides and hydrazones can under oxidative conditions (air, hypochlorite, oxygen, ozone and peroxides) give rise to nitrosating agents.[5, 7, 8]
- Chloramines are known to generate nitrosamines under certain conditions and so should also be considered.[5, 7] Ozone and other strong oxidants may lead to the formation of nitrosamines.[5, 7, 8]
- NOx present in air could lead to the formation of nitrosamines and/or introduce nitrosating agents in materials. Processing operations under inert atmosphere do not present this potential risk. Certain operations performed under air should be assessed (e.g., certain drying and milling operations). In this context, it has been observed that mechanical stress can favour the formation of nitrosamines.[9]

A review of nitrosating agents has been provided by Bream et al.[2]

Guidance 2 (Potential indirect risks)

Consider all potential sources (nitrosamines, nitrosating agents and vulnerable amines) in input materials.

The potential presence of nitrosamines in input materials should be considered, especially if secondary amines are used.[10]

Use of recovered materials (solvents, reagents, catalysts) and associated controls should be assessed. Third party recycling of solvents from a different process should be a particular focus. Recovered materials risks are significantly lower if the recycling is dedicated to the same manufacturing process and/or when performed for early steps. When recycling materials, the following considerations can be useful:

1. Is the pre-recovered stream likely to contain any vulnerable amines, nitrosating agents or nitrosamines.
2. Is the recovery process likely to introduce and / or purge any of the above.
3. Is the recovered material reasonably expected to contain any new or increased levels of vulnerable amines, nitrosating agents or nitrosamines. If so, what is the impact (consider fate and purge).

Carry-over from other processes using shared equipment should be considered. Steps performed under GMP (using solvents/reagents with appropriate controls, and controls on their recovery and reuse, as well as use of appropriate cleaning protocols) are considered to be a lower carry-over risk.

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[6] Lunn, G. et al. Aerial oxidation of hydrazines to nitrosamines. *Environ. Mol. Mutagen*. **1991**, *17*, 0893–6692. https://doi.org/10.1002/em.2850170109

[7] Nawrocki, J et al. Nitrosamines and Water, *J. Hazard. Mater.* **2011**, *189*, 1-18. https://doi.org/10.1016/j.jhazmat.2011.02.005

[8] Jires et al. N-Nitrosation in the absence of nitrosating agents in pharmaceuticals? *J. Pharm. Biomed. Anal.* **2022**, *218*, 114872. https://doi.org/10.1016/j.jpba.2022.114872

[9] Basoccu, F et al. Mechanochemistry for healthcare: revealing the nitroso derivatives genesis in the solid state. *ChemSusChem*, **2023**, e202301034. https://doi.org/10.1002/cssc.202301034

[10] Spiegelhalder et al. Contamination of Amines with *N*-Nitrosamines. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 367– 368, https://doi.org/10.1002/anie.197803672

Guidance 3 (Sources of secondary and tertiary amines)

A "vulnerable" amine is an amine that is capable of reacting with a nitrosating agent to form a stable nitrosamine.

Only secondary and tertiary amines (and salts thereof) are able to form nitrosamines, as primary amines will react with nitrosating agents to produce unstable diazonium species, and tetra substituted quaternary ammonium salts, being coordinatively saturated (and positively charged) cannot directly undergo nitrosation. Note that some quaternary ammonium salts, principally those containing methyl or benzyl substituents, are known to de-alkylate under certain conditions, generating the corresponding tertiary amines which can go on to be nitrosated.[11] Secondary amines are of most concern as they can react with nitrosating agents significantly faster than most tertiary amines. Besides nitrite concentration and pH, the secondary amine pKa impacts the nitrosation rate, with low pKa amines generally being more readily nitrosated even at low nitrite concentrations. Simple tertiary alkylamines react approximately 1000 slower than the corresponding secondary amines while tertiary amines that contain stereo-electronic features (e.g. gramine) or tertiary alkyl aniline derivatives can in some instances form nitrosamines through a multitude of mechanistic pathways.[12,13,14]

Therefore, all secondary and tertiary aliphatic and aromatic amines (amine functionality not being part of the aromatic ring system) should be considered including those:

- present as part of the starting materials, intermediates or drug substance structure
- introduced as reagents, catalysts, solvents
- present as impurities in the input materials or generated in the process (e.g. by hydrolysis of tertiary amides).

Specifically, amines may be introduced as impurities or degradants of:

- Common amide-containing solvents such as *N*,*N*-dimethylformamide (DMF), *N*,*N*-dimethyacetamide (DMAC) and *N*-methylpyrrolidinone (NMP). These solvents can contain secondary amine impurities or generate secondary amines via hydrolysis under various reaction conditions.
- Common tertiary amine bases such as triethylamine, diisopropylethylamine and *N*-methylmorpholine.
- Quaternary ammonium salts such as tetrabutylammonium bromide (TBAB)
- Primary amines such as monoethylamine
- Starting materials, intermediates or the drug substance itself

[11] a) W. A. Mitch et al. Quaternary Amines As Nitrosamine Precursors: A Role for Consumer Products? *Environ. Sci. Technol.* **2010**, *44*, 1224–1231. https://doi.org/10.1021/es902840h b) T.-L. Ho Dealkylation of Quaternary Ammonium Salts with 1,4-Diazabicyclo[2.2.2]octane. *Synthesis* **1972**, 702 DOI: 10.1055/s-1972-21977 and related references.

[12] Curran, T. A et al. Consideration of the Extent That Tertiary Amines Can Form N-Nitroso Dialkylamines in Pharmaceutical Products, *Org. Process Res. Dev.***2023**, *27*, 1714–1718, https://doi.org/10.1021/acs.oprd.3c00073

[13] Ashworth, I. W. et al. Formation of Dialkyl-N-nitrosamines in Aqueous Solution: An Experimental Validation of a Conservative Predictive Model and a Comparison of the Rates of Dialkyl and Trialkylamine Nitrosation *Org. Process Res. Dev.* **2023** *27*, 1759–1766, https://doi.org/10.1021/acs.oprd.2c00366

[14] S. Diab, et al. Formation of N-Nitrosamines by Reaction of Secondary Dialkylamines with Trace Levels of Nitrite in Aqueous Solution: An Automated Experimental and Kinetic Modeling Study Using Di-n-butylamine, *Org. Process Res. Dev.* **2024** *28*, 293-304, https://doi.org/10.1021/acs.oprd.3c00404

Other amine-containing functional groups can also indirectly lead to the formation of nitrosamines under certain conditions, such as 1,1-dialkyl hydrazines which have been reported to oxidize to form nitrosamines.[15]

This evaluation should include the use of all chemicals within a process, including those used during the work-up and isolation as well as during reactive chemistry.

Any secondary and/or tertiary amines which might be reasonably expected to reside in the drug substance should be flagged with approximate levels for inclusion within the assessment of the drug product.

Guidance 4 (Acceptable level determination)

A key basis for a risk assessment is to understand at what levels nitrosamines may be present within a drug product and whether these levels potentially exceed an acceptable intake (AI). Where identified, safety experts may be consulted to determine if an AI is available or whether an AI needs to be calculated using the Carcinogenic Potency Categorisation Approach (CPCA).[16,17] Additional existing compound-specific carcinogenicity data or structural analogues (i.e., read-across, SAR) as recommended by ICH M7[18] and/or categorical based "read across" approaches described by Dobo et al.,[19] Cross and Ponting[20] and FDA[21] have been used. EMA,[22] FDA[17] and other Health Authorities have published product-specific acceptable intakes for a range of marketed assets.

EMA and other Health Authorities (e.g. Health Canada and Swiss Medic) have confirmed that control of CPCA Class 1, 2 or 3 nitrosamines to 1.5 mcg/day is appropriate with a negative result from an enhanced Ames test (EAT). In addition, limits according to ICH Q3A(R2) and ICH Q3B(R2) apply to impurities demonstrated to be non-mutagenic impurities (NMI) based on negative results from an in-vivo mutagenicity study.[16]

FDA has stated that alternative approaches using safety data, such as obtaining compound-specific data or using read-across assessment to a suitable surrogate, could be used to support a higher AI limit. It is likely this proposed new limit would need to be agreed with FDA.

Safety experts may also advise whether the AI may be adjusted based on less than lifetime (LTL) clinical administration in alignment with ICH M7. The use of LTL adjustments has not been applied universally by regulatory authorities. Its use is currently considered a temporary measure by several

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[15] G. Lunn et al. Aerial oxidation of hydrazines to nitrosamines *Environ. Mol. Mutagen*. **1991**, *17*, 0893-6692. https://doi.org/10.1002/em.2850170109

[16] EMA, 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, 15 Jan 2024, EMA Q&A

[17] FDA, Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs) Guidance for Industry, Aug 2023, FDA Guidance

[18] a) ICH Harmonised Guideline "Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk" ICH M7(R2) 4 April 2023; b) ICH M7(R2) Q&A

[19] K. Dobo et al. Practical and Science-Based Strategy for Establishing Acceptable Intakes for Drug Product N-Nitrosamine Impurities *Chemical Research in Toxicology* **2022**, *35* (3), 475-489. https://doi.org/10.1021/acs.chemrestox.1c00369

[20] Cross, K and Ponting, D, *Computational Toxicology*, **2021**, 100186. https://doi.org/10.1016/j.comtox.2021.100186

[21] FDA, AAM/CHPA/PhRMA Questions for May 4th FDA-Industry Meeting to Discuss Nitrosamine Impurities in Pharmaceuticals, 4th May 2021, Document

[22] EMA/CHMP/517258/2023 Rev. 1

Health Authorities for marketed products. Use of LTL beyond this scope may have to be agreed upon with Health Authorities.

For products intended for advanced cancer within the scope of ICH S9,[23] nitrosamines can be controlled to ICH Q3A/B qualification thresholds (as recommended by EMA[16] and FDA[17]).

Guidance 5 (Conducting formation, fate and purge assessments)[24, 25]

The principles of ICH M7[18a] and Q&A[18b] can be followed to determine whether the potential nitrosamine is reasonably expected to be present in the drug substance and if so, estimate its levels.

Where a nitrosating agent and amine have the potential to be concurrently present, an assessment of the process conditions should be conducted to determine if a nitrosamine could potentially be formed.[5]

If the process conditions could potentially promote nitrosamine formation, the maximum amount that could be formed can be calculated by assuming complete consumption of the limiting reagent (nitrosating agent or amine). Alternatively, formation of nitrosamines from amines and nitrosating agents is well understood in solution phase, allowing the use of conservative kinetic models to predict the amount that could form.[26] The following information on solution phase nitrosation may aid such assessments.

- Nitrosation with inorganic nitrite in aqueous or mixed organic/aqueous systems occurs more rapidly under acidic conditions, whereas nitrosation with organic nitrites does not require the presence of an acid.
- Nitrosation can be catalysed by certain anions and aldehydes (notably thiocyanate, halides and formaldehyde), and when catalysed by aldehydes, efficient reaction may also be observed at high pH.[27,28]
- The rate of nitrosation for any given system is principally affected by the system pH and concentrations of both amine and nitrosating agent.
- The relative rates of nitrosation of different amines by nitrite under acidic conditions is principally driven by the basicity of the amines (pKa) with less basic amines being nitrosated much more rapidly.[5,25]
- In general, tertiary amines (and salts thereof) are significantly less reactive than secondary amines as they require an additional de-alkylation step to produce a nitrosamine. Refer to Guidance 3 for exceptions.

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[23] ICH S9, Nonclinical Evaluation for Anticancer Pharmaceuticals, 18 November 2009

[24] Barber, C et al. A consortium-driven framework to guide the implementation of ICH M7 Option 4 control strategies. *Regul. Toxicol. Pharmacol.* **2017**, *90*, 22-28. https://doi.org/10.1016/j.yrtph.2017.08.008

[25] Burns, M et al. Controlling a Cohort: Use of Mirabilis-Based Purge Calculations to Understand Nitrosamine-Related Risk and Control Strategy Options. *Org. Process Res. Dev.* **2020**, *24*, 1531-1535. https://doi.org/10.1021/acs.oprd.0c00264

[26] Ashworth, I et al. Potential for the Formation of N-Nitrosamines during the Manufacture of Active Pharmaceutical Ingredients: An Assessment of the Risk Posed by Trace Nitrite in Water. *Org. Process Res. Dev*. **2020**, *24*, 1629–1646. https://doi.org/10.1021/acs.oprd.0c00224

[27] Diab, S. et al. Investigation of the Formaldehyde-Catalyzed N-Nitrosation of Dialkyl Amines: An Automated Experimental and Kinetic Modelling Study Using Dibutylamine. *J. Pharm. Sci.* **2024**, In Press. https://doi.org/10.1016/j.xphs.2024.01.017

[28] Williams, D. L. H. Nitrosation reactions and the chemistry of nitric oxide. **2004**, Amsterdam, Elsevier. https://doi.org/10.1021/ja041042g

Once the levels of nitrosamines potentially formed have been estimated, a fate and purge assessment can be conducted for the downstream process to predict what levels could remain in the drug substance. Any purge calculations should consider the likely physicochemical characteristics of the nitrosamine which may be formed. For instance, NDMA has a boiling point of 153 °C and will partition in both aqueous and organic layers (NDMA is highly soluble in water and organic solvents). Other, higher molecular weight nitrosamines will likely behave differently. Separation efficiency of nitrosamines can be effectively modelled to more accurately predict the purging through phase separations.[29]

Nitrosamines are relatively stable compounds. A range of conditions, including the following, may result in transformation to a non-nitrosamine but these should be evaluated on a case-by-case basis.[30]

- Strongly acidic condition with a nucleophile trap (e.g. HCl with MeOH)
- Metal reducing conditions (e.g. Zn AcOH; Fe NH_4Cl; Ni/Al KOH)
- Metal catalysed hydrogenation (Ni, Pd and Ru have been shown to be effective catalysts but reactivity is system dependant)
- Grignard or organolithium reagents (RMgX; RLi)
- Strong oxidants (H_2O_2; $KMnO_4$)
- Strong oxophilic electrophiles (e.g. $POCl_3$)
- Photolysis

Purge calculations can be performed on the nitrosating agent or the vulnerable amine in cases when these two components are not introduced in the same step. In those instances, the amount of one or both of the reactant(s) can be estimated using a purge calculation and this amount will guide the estimation of the nitrosamine formed. A second purge calculation on the nitrosamine in the downstream process can then be conducted.

Whilst a strong purge rationale should justify absence of a nitrosamine below a level of concern, this is not always accepted by regulators and additional information / detail may be requested. Refer to ICH M7 Options 3 and 4.[18]

The potential for a nitrosamine to form in the drug product should also be considered (i.e cumulative growth), refer to the EFPIA drug product workflow.

Guidance 6 (1) (Can the *N*-nitrosamine be formed?)[31]

If the nitrosamine has been previously reported, the formation tests described below will typically not inform the assessment further.

In order to evaluate the nitrosamine in toxicity assays (e.g. Enhanced Ames Test or in-vivo tests) or generate analytical data, a physical sample of the nitrosamine is required. Preparative methods for synthesising the nitrosamine typically involve reacting the vulnerable amine with either inorganic nitrite (e.g. sodium nitrite) under aqueous acidic conditions, or organic nitrite (e.g. *tert*-butyl nitrite)

[29] Ashworth, I et al. Prediction of N-Nitrosamine Partition Coefficients for Derisking Drug Substance Manufacturing Processes. *Org. Process Res. Dev.* **2021**, *25*, 871–883. https://doi.org/10.1021/acs.oprd.0c00535

[30] Borths, C. et al. Nitrosamine Reactivity: A Survey of Reactions and Purge Processes. *Org. Process Res. Dev.* **2021,** *25*, 1788-1801. https://doi.org/10.1021/acs.oprd.1c00162

[31] I. W. Ashworth, A. Blanazs et al. Approaches and Considerations for the Investigation and Synthesis of N-Nitrosamine Drug Substance-Related Impurities (NDSRIs) *Org. Process Res. Dev.* **2023**, *27*, 1784–1791. https://doi.org/10.1021/acs.oprd.3c00084

in an organic solvent.[5] Some nitrosamines cannot be directly synthesised from the corresponding amine due to a lack of reactivity of the amine towards nitrosating agents, reaction elsewhere on the substrate, or instability of the transiently formed nitrosamine. In those instances, the lack of formation of the nitrosamine under forcing synthetic conditions (i.e. stoichiometric amounts of nitrosating agents) may be potentially used to justify the absence of risk in a drug substance and/or drug product risk assessment. In cases where the drug substance manufacturing process uses a nitrosating agent, the specific conditions used in the manufacturing process should be assessed for the potential formation of a nitrosamine.

Published conditions[31] that represent optimum preparative conditions for nitrosamine formation (i.e. using typically 10^6 more nitrite than typical levels within a formulation or via presence as impurities in raw materials) should be examined. These conditions are more representative of worst-case scenarios for drug substance and drug product manufacturing with nitrites than the WHO NAP test, [32] which was designed to investigate potential *in-vivo* nitrosamine formation using a large excess of nitrite, elevated temperatures (leading to nitrite decomposition) and only used inorganic nitrite. These three conditions use an excess of nitrosating agent (1.5 eq used compared with ppm amounts potentially present); are orthogonal (e.g. use inorganic and organic nitrite); and are performed at room temperature to avoid depletion of active nitrite through nitrous acid decomposition which is known to occur at elevated temperature. Additionally, this evaluation is performed in the solution-phase (thereby ensuring ideal mixing of reactants) for a period of up to 24 h, and any potential nitrosamine formation is assessed down to 0.5% a/a using an appropriately linear detector (e.g., UV), with peak identification supported by MS (see detailed conditions below). In line with EMA guidance,[16] and given the comprehensiveness of the three conditions, absence of formation of a nitrosamine using these three conditions supports the conclusion that the drug substance and/or drug product is devoid of risk from this nitrosamine.

Certain nitrosamines can form in solution as transient intermediates and are not able to be isolated (e.g. unstable and degrade during the reaction or the work-up/isolation/storage). Such instability can be used as part of a rationale to justify the absence of nitrosamine risk in the drug substance and/or product if considered relevant to the processing/storage conditions.

In cases where a molecule contains more than one amine as part of its scaffold, sub-stoichiometric conditions (i.e. 0.1 or 0.5 eq nitrite added) should be used to determine the nitrosamine product distribution. If certain amines do not undergo nitrosation under sub-stoichiometric conditions, these potential nitrosamines are unlikely to form in the drug substance/drug product. Sub-stoichiometric conditions can also be useful where over-reaction occurs when using excess nitrite.

Conditions 1:
Amine, AcOH (~30% of overall reaction volume), $NaNO_2$ solution in water (1.5 eq), overall reaction media concentration 0.1 M (add water if required to reach this concentration), 20–25 °C temperature with a minimized and static headspace to avoid NOx depletion. No co-solvents to be added — reaction could be a slurry, and care should be taken to ensure the contents are adequately agitated.

Conditions 2:
Amine, dilute HCl* so that pH is between 3 and 4, $NaNO_2$ solution in water (1.5 eq), overall reaction media concentration 0.1 M (add water if required to reach this concentration), 20–25 °C temperature with a minimized and static headspace to avoid NOx depletion. Monitor pH at each

[32] IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Some pharmaceutical Drugs, Volume 24, IARC, Lyon, France, General Considerations on N-Nitrosatable Drugs **1980**, 297 - 314

time points and adjust if necessary**. No co-solvents to be added — reaction could be a slurry, and care should be taken to ensure the contents are adequately agitated.

* If the drug substance is a salt of a strong acid, use the drug substance salt in water instead of dilute HCl and do not adjust the pH. Add 1 eq. of sodium chloride to ensure presence of chloride ion, which can have an accelerating effect on nitrosation via nitrosyl chloride.
** In cases where fluctuation of reaction pH renders this procedure practically challenging, it is suggested that an additional experiment may be of benefit, in which Conditions 1 have been modified by the addition of 1 eq. of sodium chloride.

Conditions 3:
Amine free base, organic solvent that solubilises the amine (e.g. acetonitrile, tetrahydrofuran, dichloromethane), *tert*-butyl nitrite (1.5 eq), overall reaction media concentration 0.1 M, 20–25 °C temperature with a minimized and static headspace to avoid NOx depletion. Reaction could be performed with deuterated solvent in an NMR tube if desired. Note that this anhydrous method can lead to the formation of nitrosamines that are unstable under aqueous conditions. Such instability can be used as part of a rationale to justify the absence of nitrosamine risk in the drug substance and/or product if considered relevant to the processing/storage conditions.

For all conditions:
Samples to be taken prior to nitrite addition and at 1 h, 4 h and 24 h. All peaks that exceed 0.5% by the standard chromatographic purity method (typically UV detection) should be assessed by mass spectrometry (or alternate identification technique). An appropriate analytical detection method for monitoring the potential for formation of a nitrosamine should be used dependent on the structure of the amine. Where MS confirms the potential presence of a nitrosamine and further action is likely to be required, it is recommended to manufacture or isolate an authentic sample for structural confirmation to rule out potential false positives (e.g. *C*-nitroso, *O*-nitroso, oxime, etc.). If significant alternative products (i.e. not the expected nitrosamine) are formed from these screening conditions, then identification of the structures may be considered to understand any potential mutagenicity risk (these impurities can be considered as "reasonably expected" from an ICH M7 perspective).

Note: nitrosamines are classified as potential mutagens and should also be regarded as potentially high energy intermediates. Appropriate safety measures should be taken during their manufacture and handling.

If an ICH M7 control option 4 is not supported, and if it has been shown that the nitrosamine can be formed, the nitrosamine risk assessment should conclude that there is a potential risk of nitrosamine formation.

Guidance 7 (Post Confirmatory Testing)

In cases where confirmatory testing on a suitable number of representative batches has indicated that the level of the nitrosamine is consistently less than 10% of the acceptable intake limit of the nitrosamine being tested, no further testing is required (e.g. ICH M7 Q&A on control option 4). In general, if a nitrosamine is detected, it is helpful to have an understanding of the root cause(s).

In cases where confirmatory testing on a suitable number of representative batches has indicated that the level of the nitrosamine is below 10% of the acceptable limit, this would constitute a negligible toxicological risk.

In cases where confirmatory testing on a suitable number of representative batches has indicated that the level of the nitrosamine is between 10% and 100% of the acceptable limit, further testing or controls may be assessed:

- If the level is between 10% and 30%, the nitrosamine impurity could be included on the specification (e.g. ICH M7 option 1) with skip testing, or no testing can be proposed to the health authorities
- If the level is between 30% and the acceptable limit, the nitrosamine may need to be included on the specification, depending on discussions with the relevant health authority (e.g. ICH M7 option 1)
- If more than one nitrosamine is included on the specification, the total risk level calculated for all identified nitrosamines should not exceed 1 in 100,000. This could be achieved for example by the following specifications: each individual nitrosamine should be below its own acceptable limit and the following equation also should be satisfied: [(Imp 1 level / Imp 1 limit) + (Imp 2 level / Imp 2 limit) + etc…] x 100% ≤ 100%
- Alternatively, a suitable control strategy can be proposed. Such control strategy may include:
    - Upstream control of the nitrosamine at the acceptable limit (e.g. ICH M7 option 2)
    - Upstream control of the nitrosamine at levels higher than the acceptable limit and a demonstration of downstream purge (e.g. ICH M7 option 3)
    - Controls on the levels of the amine or the nitrosating agent at a suitable point (e.g. upstream of the nitrosamine formation point)

In cases where nitrosamine formation has been demonstrated to also occur within the drug product, nitrosamine controls in the drug substance may not be required. In such cases, if control limits in the drug substance are needed or desired (e.g. for products with CEPs (Certificate of suitability to the monographs of the European Pharmacopoeia)), the limit should take into account the downstream formation within the drug product over the shelf life.

In cases where confirmatory testing on a suitable number of representative batches has established that the levels of the nitrosamine is higher than the acceptable limit, an investigation of the root cause of nitrosamine formation is useful to define appropriate corrective actions for mitigation and to implement effective control strategies.

Depending on the root cause, risk mitigation strategies may include but are not limited to:

- Increase the knowledge of the toxicity of the nitrosamine which can lead to a higher AI (see Guidance note 4)
- Propose a temporary acceptable intake during remediation activities
- Changing sources of raw materials shown to contain nitrites or other nitrosating agents
- Changing conditions of process steps critical for nitrosamine formation
- Addition of scavenger agents to critical process steps
- Limiting air exposure
- Reducing levels of vulnerable amines

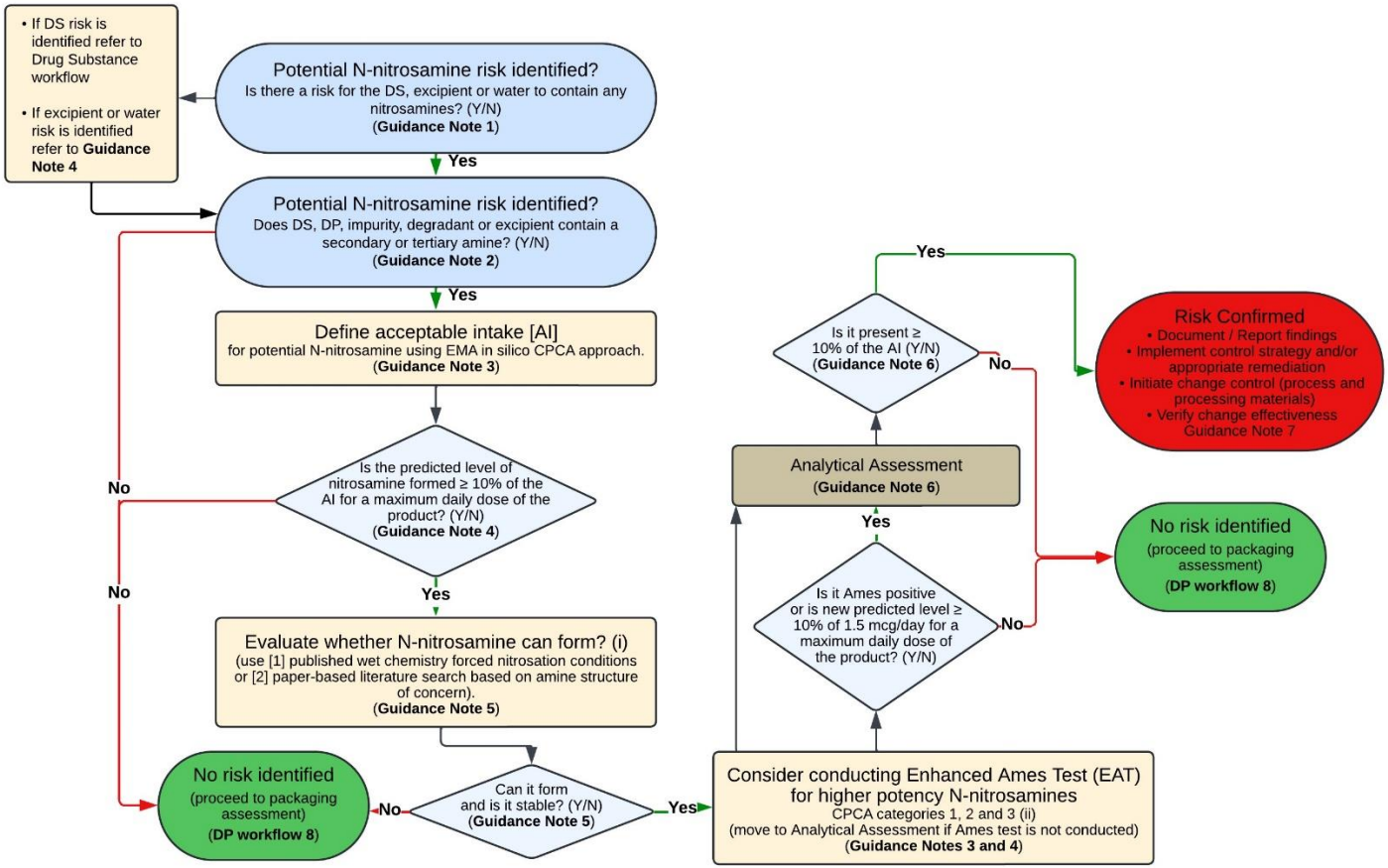
Region specific guidance may also need to be considered.

Drug Product Workflow for Quality Risk Management of Nitrosamine Risks in Medicines

Version 3.0

2024

IQ / EFPIA Drug Product (DP)‡ Workflow 1



Footnotes:
i) Sourcing commercially or manufacture of an N-Nitrosamine (NNA)
ii) EMA – negative Ames test justifies an increased AI for CPCA category 1, 2 or 3 NNAs

The term "Drug product" is equivalent to finished Dosage Forms

"N-Nitrosamine" and "nitrosamine" are synonymous and will be referred to as "nitrosamine" hereafter.

The term "risk" refers to the likelihood of presence or formation of a nitrosamine at a level of concern.

The steps outlined within this guidance can be conducted in different orders if preferred.

Guidance Note 1 (Risk of *N*-nitrosamines in DS, excipients and/or water)

Output from the Drug Substance (DS) risk assessment / excipient evaluation may have identified a risk for the potential presence of nitrosamines. These nitrosamines could be low molecular weight simple nitrosamines or complex nitrosamines, called nitrosamine drug substance related impurities (NDSRIs). Excipients can be evaluated by reference to available supplier questionnaires, consideration of their chemical structure and publicly available information. It is considered unlikely for there to be a nitrosamine present within an excipient. Where excipients contain secondary and/or tertiary amines functional groups further assessment is required (e.g. the EP monograph suggests triethanolamine may contain trace levels of *N*-nitrosodiethanolamine and commercial secondary amines may contain trace levels of N-nitrosamines).[33]

It has been reported that the use of anion exchange resins to purify water could lead to very low levels of nitrosamine and/or amine leachate from the resin.[34] It is also important to consider how the input water may have been purified e.g. by use of chloramines or ozone to disinfect the water. However, it is considered that the risk associated with the water purification process is very low. This is because i) trace levels of secondary amine leachates are unlikely to effectively nitrosate from trace nitrite which may be present within the water and ii) actual levels of nitrosamine, when observed, have been in the ng/L range. Whilst this potential risk needs to be considered as part of the risk assessment, the likelihood for *N*-nitrosamine formation or presence in the product is low. Further assessment is warranted where very large

[33] B. Spiegelhalder, G. Eisenbrand and R. Preussmann; "Contamination of Amines with Nitrosamines"; *Angew. Chem. lnt. Ed. Engl.*; **1978**, *17* (5), 367 to 368.

[34] R. C. Flowers and P. C. Singer, "Anion Exchange Resins as a Source of Nitrosamines and Nitrosamine Precursors"; *Environ. Sci. Technol*. **2013**, *47 (13)*, 7365 to 7372.

volumes (> 1 L) are part of the final product (e.g. plasma derived or continuous infusion products).

Guidance Note 2 (Does DS, DP or excipient contain a secondary/tertiary amine?)

Output from the DS risk assessment, DP degradation and excipient review will have identified amines present either as a structural motif within the DS / excipient(s), or as anticipated impurities or degradants, which could react with a nitrosating agent. Particular consideration should be given to nitrosamines in CPCA categories 1 and 2,[35] especially where the dose is high because these have the lowest Acceptable Intakes (AI) by CPCA and analysis to these AI levels may be difficult to achieve.

Where no source of secondary or tertiary amine is identified, the assessment may conclude no risk for nitrosamine presence or formation.

Excipient related considerations: There are relatively few amine excipients or excipients at risk of containing, or degrading to form, amines. Examples of such excipients include EDTA and its salts, Hunig's base, triethylamine (TEA), triethanolamine, methyl N-methylanthranilate, tetra substituted alkonium salts, certain polymethacrylates functionalized with ammonium/amino groups and fatty acid amides (e.g. coconut diethanolamide). As these excipients may contain vulnerable amines, further assessment is required.

Note: Whilst EDTA, a tertiary alkylamine, is a common excipient in formulations, experimental evidence indicates that there is no reaction with a nitrosating agent under acidic conditions.[36]

Impurity related considerations

Two general cases can be distinguished.

1. If the drug substance and an impurity both contain a vulnerable amine moiety it is unlikely that there is any risk emerging from the impurity, as any nitrosating agent present will predominantly react with the drug substance (that is present at much

[35] Updated requirements for dealing with potential nitrosamine impurities in medicinal products (swissmedic.ch)
[36] Unpublished results from GSK / Haleon.

higher levels). The reactivity and physical properties of the amine impurity versus the drug substance amines should be considered.

2. However, if the drug substance does not contain a vulnerable amine and only the impurity can form a nitrosamine, then the risk emerging from the impurity should be assessed, as nitrosating agents could react with the impurity in the absence of a competing vulnerable amine. At the same time, it is anticipated that low levels of amine impurities[37,38] will constitute a lower risk than parent amine drug substances (low concentration of amine and only trace amounts of nitrite present).

The likelihood for degradation of functionality that could release a secondary amine in the DS or DP (e.g. tertiary amides, sulfonamides, ureas, carbamates etc.) should be assessed for potential nitrosamine formation. During development phases this can be accomplished through evaluation of the forced degradation profile for the parent molecule, to confirm whether hydrolysis to release the secondary amine can occur. If this possibility is identified, then evaluation of accelerated stability samples for the presence of amine impurities is advised.

Reactivity considerations: It is important to note that not all secondary and tertiary amines are reactive towards nitrosation (e.g. flufenamic acid and diphenhydramine) and reference to available literature is appropriate in order to assess the risk. Reference is made to e.g. "Susceptibilities of Drugs to Nitrosation Under Standardised Chemical Conditions".[39]

Given the complexity of DS molecules, often containing multiple reactive functionalities, it is informative to evaluate their reactivity experimentally if they contain a secondary or tertiary amine moiety (See Guidance Note 5). The following may be considered:

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[37] Experimental data from model studies suggests that larger molecular weight amine impurities at ICH Q3A and Q3B identification limits are not considered a risk for nitrosation with trace nitrite: J. Moser; I. A. Ashworth; L. Harris; M. C. Hillier; K. K. Nanda; G. Scrivens "*N*-Nitrosamine Formation in Pharmaceutical Solid Drug Products: Experimental Observations, " *J. Pharm. Sci.* **2023**, *112*, 5, 1255-1267.

[38] The potential for formation/presence of more mobile lower molecular weight amines within the DP may need to be assessed at lower levels than ICH Q3.

[39] P. N. Gillatt, R. J. Hart and C. L. Walter; *Fd Chem. Toxic*. **1984**, *22 (4)*, 269 to 274.

a. Tertiary alkyl amines are significantly less reactive than secondary alkylamines (reports of > 1000-fold lower reactivity[56]) and require an additional de-alkylation step[40], making their nitrosation in the solid state unlikely. Hence, whilst tertiary alkylamines should also be assessed, they would generally be considered negligible risk of forming an *N*-nitrosamine.
b. Certain di-alkyl aromatic amines have been reported in the literature to be significantly more reactive than tri-alkylamines when exposed to nitrosating agents in solution.[41] In certain instances, tertiary amines, where the reactivity towards nitrite is enhanced by particular structural features,[42] could lead to an increased propensity towards nitrosation.

Guidance Note 3 (Acceptable level determination)

A key basis for a risk assessment is to understand at what levels nitrosamines may be present within a DP and whether these levels potentially exceed an acceptable intake (AI). Where identified, safety experts may be consulted to determine if an AI is available or whether an AI needs to be calculated using the Carcinogenic Potency Categorisation Approach (CPCA).[43,44] Additional existing compound specific carcinogenicity data or structural analogues (i.e., read-

[40] https://www.ema.europa.eu/documents/presentation/presentation-nitrosamine-implementation-oversight-group-niog-third-meeting-pharmaceutical-industry_en.pdf
[41] R. N. Loeppky et al. "The mechanistic origin of regiochemical changes in the nitrosative N-dealkylation of N,N-dialkyl aromatic amines" *Org. Biomol. Chem.* **2005**, *3*, 1097 1108. https://doi.org/10.1039/B418457B:
[42] R. N. Loeppky et al. "Rapid Nitrosamine Formation from Tertiary Nitrogen Compounds: An Overview. The significance of N-nitrosation of drugs", Ed. Eisenbrand, G. and Nicolai, H. Gustav Fisher Verlag, **1990**
[43] EMA, 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, 20 May 2022, EMA Q&A
[44] FDA, Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs) Guidance for Industry, Aug 2023, FDA Guidance

across, SAR) as recommended by ICH M7[45] and/or categorical based "read across" approaches as described by Dobo et al.,[46] Cross and Ponting[47] and FDA[48] have been used.

The EMA,[49] FDA[44] and other Health Authorities have published product specific acceptable intakes for a range of marketed assets.

The EMA and other Health Authorities (e.g. Health Canada and Swissmedic) have confirmed that control of CPCA Class 1, 2 or 3 *N*-nitrosamines to 1.5 mcg/day is appropriate with a negative result from an enhanced Ames test (EAT). In addition, limits according to ICH Q3A(R2) and ICH Q3B(R2) apply to impurities demonstrated to be non-mutagenic impurities (NMI) based on negative results from an *in vivo* mutagenicity study.

The FDA has stated that alternative approaches using safety data, such as obtaining compound-specific data or using read-across assessment to a suitable surrogate, could be used to support a higher AI limit. It is likely this proposed new limit would need to be agreed with the FDA.

Safety experts may also advise whether the AI may be adjusted based on less than lifetime (LTL) clinical administration in alignment with ICH M7. The use of LTL adjustments has not been applied universally by regulatory authorities. For commercial products, its use is subject to agreement with regulatory authorities and is currently considered a temporary measure by several Health Authorities. Use of LTL beyond this scope may have to be agreed upon with Health Authorities.

For products intended for advanced cancer within the scope of ICH S9,[50] nitrosamines can be controlled to ICH Q3A/B qualification thresholds (as recommended by EMA[43] and FDA[44]).

Guidance Note 4 (Is predicted level ≥ 10% of the acceptable intake?)

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[45] ICH Harmonised Guideline "Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk" M7(R1) 31 March 2017

[46] K. Dobo et al. Practical and Science-Based Strategy for Establishing Acceptable Intakes for Drug Product N-Nitrosamine Impurities *Chemical Research in Toxicology* 2022, *35* (3), 475-489. https://doi.org/10.1021/acs.chemrestox.1c00369

[47] Cross, K and Ponting, D, *Computational Toxicology*, 2021, 100186. https://doi.org/10.1016/j.comtox.2021.100186

[48] FDA, AAM/CHPA/PhRMA Questions for May 4th FDA-Industry Meeting to Discuss Nitrosamine Impurities in Pharmaceuticals, 4th May 2021, Document

[49] EMA/CHMP/517258/2023 Rev. 1

[50] ICH S9, Nonclinical Evaluation for Anticancer Pharmaceuticals, 29 October 2009

The greatest potential source of nitrosating agent within a drug product has been identified as coming from excipients. Other sources of nitrosating agents are discussed below.

Excipients may contain low levels of potential precursors to nitrosating agents (e.g., nitrite). Typical measured nitrite levels in excipients can be obtained from the Lhasa Vitic Nitrite database. In the absence of data on nitrite content for excipients, a content of 1 ppm[51] nitrite could be used to assess the potential levels of nitrosamine formation. Alternatively, in-house data can be generated using relevant analytical methods, or supplier data can be leveraged.

Calculations using levels of nitrite in excipient are a useful guide for assessing potential levels of *N*-nitrosamine within the drug product. A conservative, potentially maximum, amount of nitrosamine which could be formed from a daily dose of drug product can be calculated by the total amount of nitrite in a daily dose divided by its molecular weight (46 Da) and multiplied by the molecular weight of the nitrosamine.

In case there are multiple non-dose proportional dosage strengths (e.g., 2 mg and 10 mg), a conservative approach may be to perform the calculation for nitrite for each dosage strength individually against the maximum daily dose, even if in practice combinations of the dose strengths are used to achieve the daily doses.

It is considered that the presence of one or more *N*-nitrosamines at <10% of their respective AI constitutes a negligible toxicological risk.[11]

In the absence of a significant body of prior knowledge, it is a conservative approach to assume a 100% conversion of the nitrosating agent present to form the relevant nitrosamine(s) from the vulnerable amine(s) present. At the same time, it can also be appropriate to consider whether a more molecule-specific conversion could be applied for the associated product.

[51] A review of ongoing excipient nitrite testing shows the highest density of maximum values at ~ 1 ppm, with >90% of values being NMT 2 ppm. Boetzel, Ret al.. "A Nitrite Excipient Database: A useful Tool to Support N-Nitrosamine Risk Assessments for Drug Products"; *J. Pharm. Sci*., **2022**, ISSN 0022-3549, https://doi.org/10.1016/j.xphs.2022.04.016: Therefore 2 ppm could be considered as a conservative default value for excipients that do not have data available yet.

To refine the above conservative prediction calculation, a risk-based consideration of all risk factors known to increase or decrease nitrosamine formation can be used to derive an overall formation risk for a specific process.

Multiple factors in the drug manufacturing process may either promote or reduce nitrosamine formation.

Under certain conditions the conversion of nitrite to nitrosating agent and reaction with an amine can be optimal e.g., low pH, low amine pKa, highly aqueous soluble DS, presence of water (e.g., during wet granulation), higher temperature and protracted time (over a shelf life), potentially leading to conversion of available nitrite to nitrosamine. However, there are examples where only a fraction of the available nitrite converts to the nitrosamine.[52]

Appropriate kinetic modelling can complement the risk-based considerations.[53]

Factors that have been shown to impact nitrosamine formation, include the use of nitrosating agent scavenging molecules, use of absorbent (e.g. desiccant), different packaging configurations and temperature/humidity should also be considered for determining the overall formation risk.

If the risk assessment results in potential presence or formation of a nitrosamine at levels below 10% of the AI, this would constitute negligible toxicological risk. If levels are predicted to be at or above 10% of the AI, follow the next step in the DP risk assessment workflow (Guidance Note 5).

<u>Other sources of nitrosating agents</u>

Other sources of nitrosating agents can be derived from the DS, water used in manufacturing processes and NOx in air. Whilst these sources are likely to be a minor contributor to the formation of *N*-nitrosamines in DP, consider their evaluation / assessment.

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[52] Moser, J. *et al.* (2023) 'N-Nitrosamine Formation in Pharmaceutical Solid Drug Products: Experimental Observations', *Journal of Pharmaceutical Sciences*, 112(5), pp. 1255–1267. Available at: https://doi.org/10.1016/j.xphs.2023.01.027.

[53] Carloni, L.-E. *et al.* (2023) 'Solid State Kinetics of Nitrosation Using Native Sources of Nitrite', *Journal of Pharmaceutical Sciences*, 112(5), pp. 1324–1332. Available at: https://doi.org/10.1016/j.xphs.2023.02.014

The DS is assessed for its potential to contain nitrosating agents as part of the DS risk assessment, including potential residual nitrite which may be converted within the DP. Likewise, precursors to nitrosating agents should also be assessed for *in situ* formation during DP processing or over the shelf life.[54] A summary of nitrosating agents and their precursors is captured within Guidance Note 1 of the EFPIA DS Workflow for Quality risk management of nitrosamine risks in medicine.

Water used for formulations is generally purified or water for injection (WFI), where levels of nitrite have been measured as extremely low (0.1 ppb)[55] and as such nitrite in water is considered negligible risk.[56] If purified water, or WFI, has been purified by distillation, is not considered to have the potential for the presence of nitrite.

Exposure of nitrosatable products to air containing higher levels of NOx, e.g. during fluid bed drying or extended storage in open exposed conditions, may also have to be assessed as an additional source of nitrosating agent.

Guidance Note 5 (Evaluate whether the *N*-nitrosamine can form)

If the potential for the *N*-nitrosamine being at or above 10% of the AI (for the maximum daily dose) has been confirmed, it is important to understand whether it can actually form under nitrosation conditions, given that not all structurally complex secondary and reactive tertiary amines can lead to the formation of *N*-nitrosamines under nitrosating conditions. Understanding whether a secondary or tertiary amine can be nitrosated to form an *N*-nitrosamine can be achieved either through [1] conducting preparative chemistry screening or [2] an appropriate literature review of closely related structures.

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[54] R. López-Rodríguez, J. A. McManus, N. S. Murphy, M. A. Ott and M. J Burns "Pathways for N-nitroso compound formation: secondary amines and beyond" *Organic Process Research and Development*; 2020, doi.org/10.1021/acs.oprd.0c00323

[55] Global Workshop on Nitrosamine Impurities (cvent.com)

[56] Ashworth, I.; Dirat, O.; Teasdale, A.; Whiting, M. "Potential for the Formation of *N*-Nitrosamines During the Manufacture of Active Pharmaceutical Ingredients: An Assessment of the Risk Posed by Trace Nitrite in Water", *Org. Process Res. Dev*. **2020**, *24*, 1629-1646.

1. Preparative methods for synthesising the *N*-nitrosamine typically involve reacting the secondary or tertiary amine with either inorganic nitrite (e.g. sodium nitrite) under aqueous acidic conditions, or organic nitrite (e.g. *tert*-butyl nitrite) in an organic solvent.[57] Some *N*-nitrosamines cannot be directly synthesised from the corresponding amine. In those instances, the lack of formation of the *N*-nitrosamine under synthetic conditions using stoichiometric amounts of nitrosating agents may be potentially used as justification that the *N*-nitrosamine would not form in the DS and/or DP.

   The IQ consortium has established a set of three conditions that represent optimum conditions for the formation of *N*-nitrosamines for drug substance and would be considered forcing conditions for DP manufacturing scenarios with trace nitrites.[58] These three conditions use an excess of nitrosating agent (1.5 eq. used compared with ppm amounts potentially present within a formulation), are orthogonal (e.g. use inorganic and organic nitrite), are performed at room temperature to avoid the known nitrite decomposition at elevated temperature, and are in solution phase (thereby ensuring contact of the reactants) for a period of 24 h. Any potential *N*-nitrosamine formation is assessed by analysis down to 0.5% a/a using an appropriately linear detector (e.g.,UV), with peak identification supported by MS. Given the comprehensiveness of the three conditions, absence of formation of an *N*-nitrosamine from the corresponding amine using these three conditions at a level of not greater than 0.5% a/a, leads to the conclusion that the drug substance and/or drug product is absent of risk from this *N*-nitrosamine.

   A summary of the 3 conditions is included in the earlier DS nitrosamine risk assessment workflow (See Drug Substance Guidance Note 6 of that workflow).

   If significant alternative products (i.e. not the expected *N*-nitrosamine) are formed from these screening conditions, then identification of the structures may be considered to understand any potential mutagenicity risk the formation of these impurities may bring.

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[57] R. López-Rodríguez et al. Pathways for N-Nitroso Compound Formation: Secondary Amines and Beyond. *Org. Process Res. Dev.* **2020,** *24* (9), 1558-1585. https://doi.org/10.1021/acs.oprd.0c00323

[58] I. W. Ashworth, A. Blanazs *et al.* "Approaches and Considerations for the Investigation and Synthesis of *N*-Nitrosamine Drug Substance-Related Impurities (NDSRIs)", *Org. Process Res. Dev.* **2023**, *27*, 1784–1791. https://doi.org/10.1021/acs.oprd.3c00084

2. An appropriate literature review can be used to understand potential for formation of *N*-nitrosamines from an amine substrate if the reported structure has sufficient structural similarity to the amine that is being assessed. If literature is to be used as a justification that an *N*-nitrosamine is unlikely to form, it is important that the reported examples are robust i.e. there is close structural similarity and nitrosation conditions have led to high yields of a non-*N*-nitrosamine product.

If the nitrosation screening experiments or the literature search show that an *N*-nitrosamine can be formed then the next step will be a preparative synthesis and isolation of the compound followed by spectroscopic structural confirmation. This exercise will enable determination if the *N*-nitrosamine is a stable compound and can be isolated/stored successfully. The prepared marker can then be used in analytical method development and/or in an Enhanced Ames Test (EAT).

If the hypothetical *N*-nitrosamine cannot be manufactured directly from nitrosation of the secondary or reactive tertiary amine precursor, or is found to be unstable, then this knowledge can be used as justification that formation / persistence of the potential nitrosamine is not reasonably expected and no further action is required.

Guidance Note 6 (Analytical assessment in DP)

Analytical testing the DP formulation (and/or dosage strength) with the associated highest predicted levels of nitrosamine levels relative to their respective AIs should be the initial focus. The number of unit doses for low strength forms which could be used to deliver the maximum daily dose may also need to be considered. A matrixing / bracketing approach for testing multiple strengths of the same dosage form may be considered. Generally, it is recommended to test the DP at, or towards, the end of shelf life as a minimum, but testing an appropriate spread of samples (i.e. after manufacture and during shelf life) can provide invaluable information as to whether a nitrosamine might be increasing during shelf life. If such samples are not available, samples stored at elevated temperature can be considered instead. Such samples however may overpredict real time conditions. Output from this testing can be used to inform the risk associated with other formulations and future product assessments (from a

prior knowledge perspective and/or can inform remediation strategies based on the outcome from root cause evaluation).

Guidance Note 7 (Implementation of a control strategy)

In cases where confirmatory testing on a suitable number of representative batches has indicated that the level of the nitrosamine is between 10% and 100% of the acceptable limit, further analytical testing or controls may be assessed:

- If the level is between 10% and 30%, the nitrosamine impurity could be included on the specification (e.g. ICH M7 option 1) with skip testing, or no testing can be proposed to the health authorities with sufficient justification.
- If the level is between 30% and the acceptable limit, the nitrosamine may need to be included on the specification, depending on discussions with the relevant health authority (e.g. ICH M7 option 1)
- If more than one nitrosamine is included on the specification, the total risk level calculated for all identified nitrosamines should not exceed 1 in 100,000. This could be achieved for example by the following specifications: each individual nitrosamine should be below its own acceptable limit and the following equation also should be satisfied: [(Imp 1 level / Imp 1 limit) + (Imp 2 level / Imp 2 limit) + etc…] x 100% ≤ 100%
- Alternatively, a suitable control strategy can be proposed. Such control strategies may include:
    - Upstream control of the nitrosamine at the acceptable limit (e.g. in the DS if the nitrosamine does not also form in the DP)
    - Controls on the levels of the amine at a suitable point
    - Controls on the levels of the nitrosating agents at a suitable point

In cases where confirmatory testing on a suitable number of representative batches has established that the level of the nitrosamine is higher than the acceptable limit, an investigation of the root cause of nitrosamine formation is necessary to define appropriate corrective actions for mitigation and to implement effective control strategies. Examples of mitigations to reduce / eliminate the nitrosamine risk include:

- Increase the knowledge of the toxicity of the nitrosamine which can lead to a higher AI (see Guidance note 3).
- Propose a temporary acceptable intake during remediation activities.
- Reducing levels for the vulnerable amine if appropriate.
- Consider sourcing/using excipients with lower levels of residual nitrite, if available. It should be acknowledged that the performance of the DP (e.g., dissolution behaviour) could be altered when changing the excipient quality.
- Changing the order of addition of formulation components or doing engineering modifications to remove conditions from the system that could promote nitrosamine formation.
- Cold storage of the bulk drug substance and/or product
- Consider impact of alternative packaging approaches e.g. use of desiccants
- Change of storage and temperature of the finished product
- Management of potential for interaction from NOx within air during critical process steps or intermediate storage

Where mitigation activities are unable to sufficiently reduce levels of the nitrosamine, then reformulation may be required. If the development of a new formulation is needed, then options that can be considered are:

- Consider using a more appropriate formulation approach based on the insights into drivers of nitrosamine presence e.g. powder in a capsule rather than tablet.
- Modify pH for the formation – addition of carbonate to the excipients.
- Change technology of given unit operation (e.g., dry granulation versus wet granulation).
- Modification of particle size excipient / DS etc.

- Inclusion of scavengers e.g. glycine, lysine, histidine, cysteine or antioxidants e.g. ascorbic acid, a-tocopherol, propyl gallate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) within the formulation.[59,60]
    - Consideration should be given to reasonably expected by-products of any scavengers or antioxidants added to the formulation.[61]
    - Consideration should be given that not all scavengers or anti-oxidants are effective for every DS.

Any new formulation would need to be assessed to confirm that the original nitrosamine risk has been effectively reduced below a level of concern or removed. It would also need to be assessed to confirm that there are no new nitrosamine risks with the product.

It is recommended for earlier development assets, where potential for *N*-nitrosamine formation has been identified but a formulation is yet to be developed, to commence *N*-nitrosamine mitigation measures as part of the formulation development activities.

Guidance Note 8 (Managing *N*-nitrosamine risk from packaging)

Packaging materials and processes may contribute to the net nitrosamine risk of a finished product and therefore should be considered. Presently it is known that low concentrations of vulnerable amines (e.g., dimethylamine and diethylamine) may be present in inks that were printed on, and/or covered with nitrocellulose (NC) containing films. The NC or other nitrosating agent present may facilitate the nitrosation of these amines. The risk is that during heat sealing operations (e.g., blister lidding) the nitrosamines may be vaporized from the ink by

[59] K. K. Nanda et al. in Inhibition of N-Nitrosamine Formation in Drug Products: A Model Study"; *J. Pharm. Sci*.; **2021**, *110*, 3773 to 3775

[60] Homšak, M. *et al.* (2022) 'Assessment of a Diverse Array of Nitrite Scavengers in Solution and Solid State: A Study of Inhibitory Effect on the Formation of Alkyl-Aryl and Dialkyl N-Nitrosamine Derivatives', *Processes*, 10(11), p. 2428. Available at: https://doi.org/10.3390/pr10112428.

[61] Cioc, R.C. *et al.* (2023) 'Formation of N-Nitrosamine Drug Substance Related Impurities in Medicines: A Regulatory Perspective on Risk Factors and Mitigation Strategies', *Organic Process Research & Development*, 27(10), pp. 1736–1750. Available at: https://doi.org/10.1021/acs.oprd.3c00153

the 180 – 260°C sealing surface and deposited onto the exposed DP upstream from the sealing operation.[62,63]

It is important to note that generally, the risk is considered very low as observed levels, when formed, have been very low and significantly below the appropriate acceptable daily intakes.[30] Therefore, where a potential risk is identified, testing of product may not be required. However, where multiple daily dosing is required for the respective product, and/or where the printed surface is potentially in contact with the drug product, or where other nitrosamine risks may have been identified within the product assessment as per DP workflow, testing might be appropriate.

Moving to nitrocellulose-free materials or doing relevant modifications to the process or equipment would mitigate this potential packaging risk. This change is not considered a requirement but should be considered if there is a multiple dosing regimen that leads to this potential risk being more significant.

Packaging materials can be potential sources of nitrosamines and low levels of amines from an extractable and leachable perspective. With respect to leachable nitrosamines, this is generally a well understood and managed phenomenon and as such is not considered an additional cause for concern.[64] Likewise, whilst very low levels of amines could leach into the product from packaging materials, it is anticipated that such cross contamination would be at a very low level and such levels of amines potentially reacting with trace nitrite contained within the formulation is not considered a risk.[58] The risk of nitrosamines and amines being derived from an extractable and leachable perspective is therefore considered very low. The related

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[62] N. Golob, R. Grahek, M. Ross, R. Roškar, "Nitrocellulose blister material as a source of N-nitrosamine contamination of pharmaceutical drug products", *International Journal of Pharmaceutics,* **2022**, *618*, 121687.
[63] J. Zheng, A. Brookes, J. Moser, H. Pfeffe, A. Smith. "On the Risk of Nitrosamine Contamination During Drug Product Blister Packaging." *J Pharm Sci.;* **2023***, 112(9), 2321-2325. doi: 10.1016/j.xphs.2023.07.014. Epub 2023 Jul 20. PMID: 37478970 (link)*
[64] General risk assessment for nitrosamines is captured within USP ⟨1664⟩ "Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery systems". Assessment of Elastomeric Component Used in Injectable Pharmaceutical Product Packaging/Delivery Systems are further discussed in USP ⟨1381⟩ and "Orally Inhaled and Nasal Drug Products" within USP ⟨1664.1⟩

risk can be further assessed by using statements of the packaging component suppliers. The risk can be higher for liquid formulations compared to solid oral dosage forms (reference to Borths et al paper, https://doi.org/10.1021/acs.oprd.2c00330).