Workflows for Quality risk management of nitrosamine risks in medicines

Version 2.0

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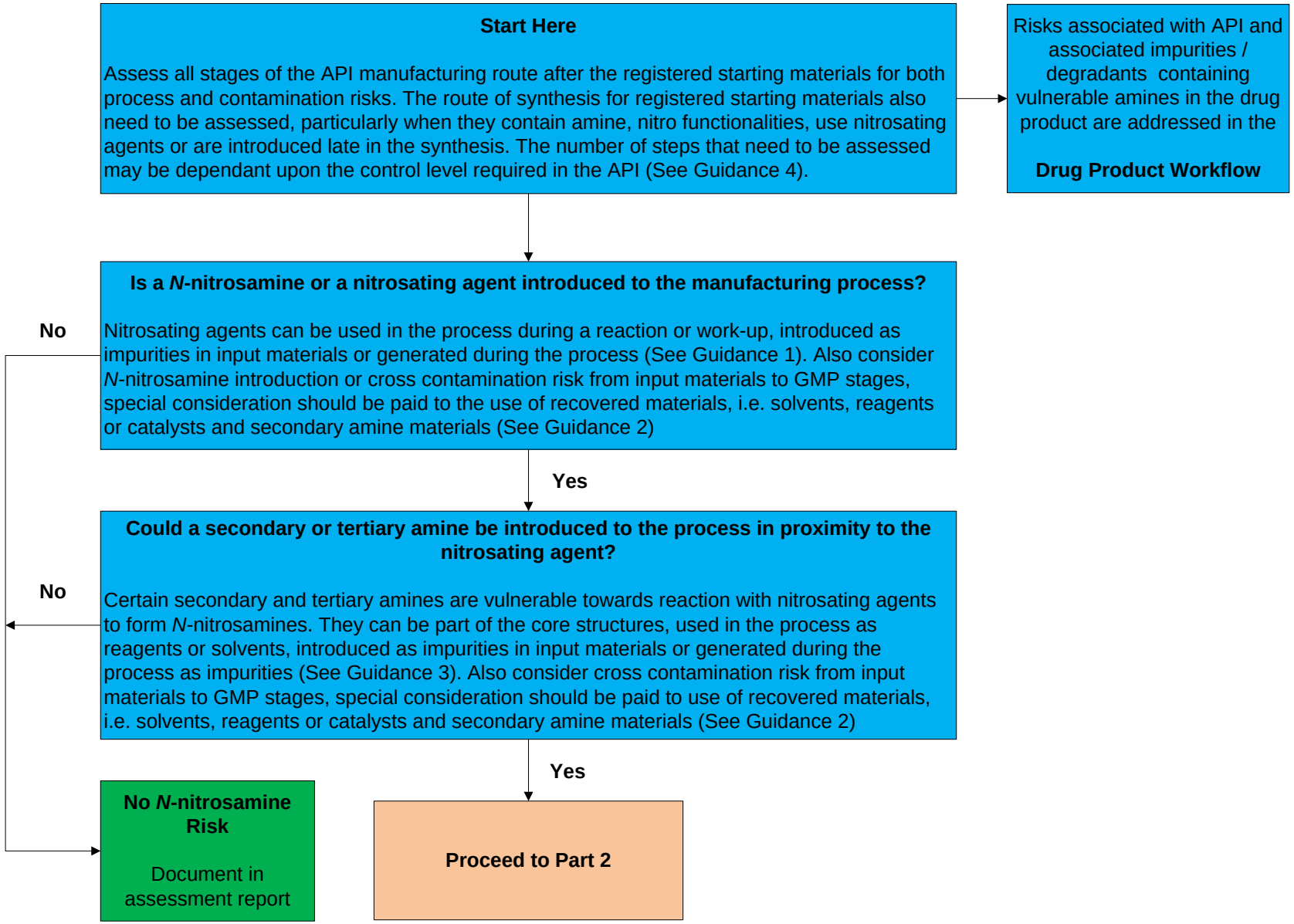
Introduction

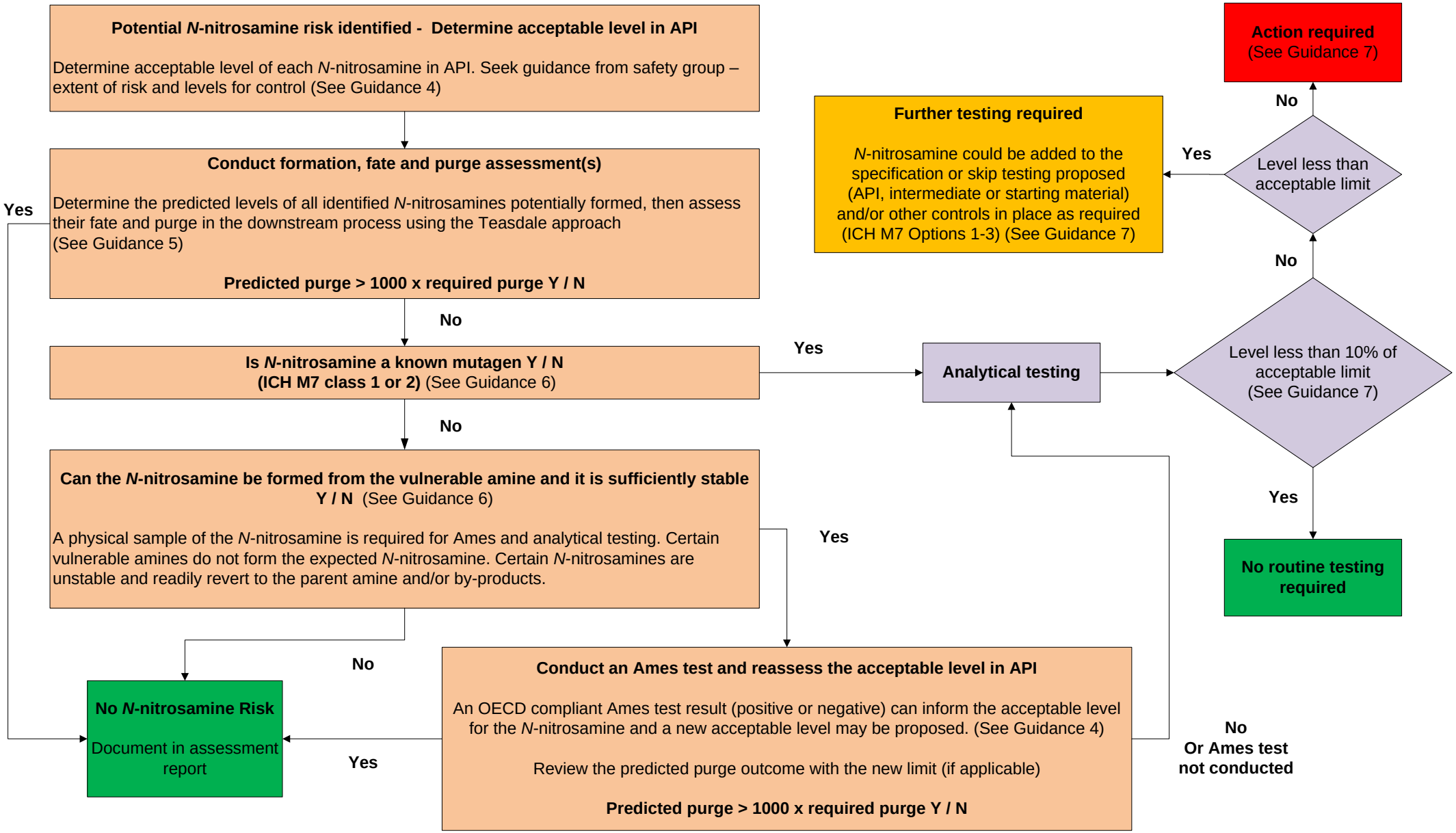
- This document describes workflows for the quality risk management of nitrosamine risks in medicines, developed by experts from EFPIA's manufacturing and Quality Expert Group.
- The workflows and risk assessment principles described here are intended to support the accountabilities of medicine application holders and drug substance manufacturers to identify, assess and mitigate risks from N-nitrosamine impurities.
- Guidance and principles are provided for identification of potential nitrosamine impurities, assessing their risks, and identifying appropriate control strategies, in line with principles and considerations of ICHM7.
- This document contains the following workflows:
    1. Chemical drug substance risk assessment
    2. Drug Product risk assessment
    3. Risk Assessment for nitrocellulose packaging materials
    4. Risk assessment for biological drugs

1. Chemical Drug Substance Risk Assessment

Drug Substance Manufacturing Process Risk Assessment for Presence of *N*-Nitrosamines - Part 1 – Risk Identification



Drug Substance Manufacturing Process Risk Assessment for Presence of *N*-Nitrosamines
Part 2 – Risk Characterisation and Confirmatory Testing



Guidance 1 (Sources of nitrosating agents)

Nitrosating agents 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). Nitrosating agents purposely used in the manufacturing process and/or introduced as impurities (e.g. from the input materials or water) should be considered. This evaluation must include the use of all chemicals within a process, including those used during the quench and work-up as well as during reactive chemistry.

Other potential nitrosation risks:

- Inorganic materials used during synthesis such as NaCl, NaOH and Na_2CO_3 can contain low levels (ppm) of nitrite and trace analytical methods for nitrite analysis have been reported.[1] It should be noted that the grade of the materials could lead to different the nitrite contents.
- 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)[2] meaning an understanding of the nitrite content of the water used has the potential to remove this risk
- 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
- Nitroalkanes, halogenated nitro alkanes, Fremy's salt, nitroso sulfonamides and nitroaromatics (e.g. by fluoro de-nitration) can all under some circumstances give rise to nitrosating agents
- Hydroxylamine, hydrazines, hydrazides and hydrazones can under oxidative conditions (air, hypochlorite, oxygen, ozone and peroxides) give rise to nitrosating agents[3,3a,14]
- Chloramines are known to generate *N*-nitrosamines under certain conditions and so should also be considered[3,14]
- Ozone may lead to the formation of *N*-nitrosamines by initial oxidation of amines to nitrite[3,3a,14]

Guidance 2 (Potential contamination risks)

Consider all potential sources of contamination (*N*-nitrosamines, nitrosating agents and vulnerable amines) in input materials.

The potential presence of *N*-nitrosamines in input materials should be considered, especially if secondary amines are used.

Use of recovered materials (solvents, reagents, catalysts) is of particular concern if appropriate controls are not put in place.

Cross contamination 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) are considered to be a lower cross contamination risk.

Guidance 3 (Sources of secondary and tertiary amines)[4]

A “vulnerable” amine is an amine that is capable of reacting with a nitrosating agent to form a stable *N*-nitrosamine.

Only secondary and tertiary amines (and salts thereof) are able to form *N*-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 substituents, are known to de-alkylate under certain conditions, generating the corresponding tertiary amines which can go on to be nitrosated. Secondary amines are of most concern as they can react with nitrosating agents significantly faster than tertiary amines.

All secondary and tertiary aliphatic and aromatic amines should be considered including those present as part of the starting materials, intermediates or API structure as well as those introduced as reagents, catalysts, solvents or as impurities present in the input materials or generated in the process (e.g. by hydrolysis of amides).

Tertiary amine bases (e.g. triethylamine, diisopropylethylamine and *N*-methylmorpholine) are known to degrade to secondary amines and have been implicated in *N*-nitrosamine formation.

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)
- Quaternary ammonium salts such as tetrabutylammonium bromide (TBAB)
- Primary amines such as monoethylamine
- Starting materials, intermediates or the API itself

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

Guidance 4 (Acceptable level determination)

A key basis for a risk assessment is to understand what limits within a drug substance present a significant risk. Generally, where identified, *N*-nitrosamines containing an alkyl carbon alpha to the nitrogen that bears at least one hydrogen, should be assessed by safety experts.[5a] Safety experts should determine if an acceptable intake (AI) is available (i.e., those with an AI published established by regulatory authorities)[5,6] or can be calculated for any novel *N*-nitrosamines using analogue based "read-across" approaches using existing compound specific carcinogenicity data on closely related structures as recommended by ICH M7[7] and or categorical based "read-across" approaches as described by Dobo et al.[8], Cross and Ponting[9], and FDA[10]). If an AI cannot be calculated in this manner, until a limit is defined by ICH M7, conservative low limits (18 ng/day or 26.5 ng/day) of control have been recommended by regulatory agencies.[5,6] For those nitrosamines which do not possess at least one alpha carbon containing hydrogens then these should be assessed by the representative company for establishing an appropriate AI.

The safety group can also advise whether the AI can be adjusted based on less than lifetime (LTL) clinical administration in alignment with ICH M7. The use of LTL adjustments is subject to agreement with regulatory authorities and could be a temporary measure.

N-Nitrosamines testing negative in an OECD guideline 471 aligned in vitro bacterial reverse mutation assay (Ames test) can be proposed to be controlled to ICH M7 limits (1.5 mcg per day or LTL equivalent). Data from such an Ames test can be used as part of the additional weight of evidence in limit setting. Note that there is an expectation that any non-GLP Ames test are confirmed by a GLP protocol. The understanding of metabolism and metabolic activation pathways could also support the weight of evidence. Additional supporting evidence may also be required (e.g. in-vitro and in-vivo mammalian genotoxicity assays as outlined in ICH M7 note 3)[7] to support levels in excess of ICH M7 limits.

Control to ICH M7 limits (1.5 mcg per day or LTL equivalent) may be considered for those *N*-nitrosamines which prove to be non-mutagenic within the Ames test with metabolic activation (e.g. S9) (required for a *N*-nitrosamine from the cohort of concern), but mutagenic without the metabolic activation.

For products intended for advanced cancer within the scope of ICH S9[11] then *N*-nitrosamines can be controlled to ICH Q3A/B levels (as recommended by EMA[5] and FDA[10]).

If more than one *N*-nitrosamine is included on the specification, the contribution of each *N*-nitrosamine relative to its individual acceptable limit is taken into account and summed up to a total % of expected *N*-nitrosamine control limits.[5]

Guidance 5 (Conducting formation, fate and purge assessments)[12,13]

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 *N*-nitrosamine could potentially be formed,[14] what the maximum realistic level could be formed and what levels would remain in drug substance. Formation of nitrosamines from amines and nitrite is well understood and worst case levels can normally be accurately predicted.[15] Nitrosation with inorganic nitrite occurs more rapidly under acidic conditions, whereas reactions 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.[4, 16] 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.[14,15] The rate of nitrosation for any given system is principally affected by the system pH and concentrations of both amine and nitrosating agent. In general, tertiary amines (and salts thereof) are significantly less reactive (typically > 1000 fold less reactive for simple trialkylamines) than secondary amines as they require an additional de-alkylation step to produce a nitrosamine. Some tertiary amines have higher reactivity towards nitrosation, related to the presence of particular (well understood) structural / electronic features (e.g. di-alkyl aromatic amines).[17,18]

Once the levels of *N*-nitrosamines potentially formed has been estimated, a fate and purge assessment can be conducted for the downstream process. When conducting purge calculations consider the likely physicochemical characteristics of the *N*-nitrosamine which may be formed. For instance, NDMA has a b.p. 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, *N*-nitrosamines will behave differently. Separation efficiency of nitrosamines can be effectively modelled to more accurately predict the purging through phase separations.[19]

N-nitrosamines are relatively stable compounds though a range of conditions are known to result in de-nitrosation[20] these include:

- 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 *N*-nitrosamine formed. A second purge calculation on the *N*-nitrosamine in the downstream process can then be conducted.

Whilst a strong purge rationale should justify absence of a *N*-nitrosamine below a level of concern this is not always accepted by regulators and additional information / detail may be requested.

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

N-Nitrosamines that are know mutagens (ICH M7 class 1 or 2) typically have a simple structure and are known to be able to form from the corresponding amine. In those instances the formation test described below will typically not inform the assessment further.

In order to evaluate the *N*-nitrosamine in toxicity assays (e.g. Ames test) or generate analytical data, a physical sample of the *N*-nitrosamine is required. Preparative methods for synthesising the *N*-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) in an organic solvent.[14] 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 results in the absence of a *N*-nitrosamine risk in the drug substance and/or drug product. In cases where the drug substance manufacturing process uses an intentionally added nitrosating agent, the specific conditions used in the manufacturing process should be assessed for the potential formation of a *N*-nitrosamine.

The IQ consortium has established a set of three conditions (see next slide) that represent a worst case for drug substance and drug product manufacturing scenarios with trace nitrite contamination. 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), performed at room temperature to avoid the known nitrite decomposition at elevated temperature (such a decomposition can occur during the WHO NAP test)[21], are in solution phase (thereby ensuring contact of the reactants) for a period of 24 h to 48 h and any potential *N*-nitrosamine formation assessed down to 0.5% a/a using MS detection (see next slide for detailed conditions). These conditions are more representative of a worst case for drug substance and drug product manufacturing scenarios with trace nitrite contamination than the WHO NAP test which was designed to investigate potential *in-vivo N*-nitrosamine formation using a large excess of nitrite, elevated temperatures (leading to nitrite decomposition) and only used inorganic nitrite. In line with EMA guidance[5] and given the comprehensiveness of the three conditions, absence of formation of a *N*-nitrosamine from the corresponding amine using these three conditions leads to the conclusion that the drug substance and/or drug product is absent of risk from this *N*-nitrosamine.

Certain *N*-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 *N*-nitrosamine risk in the drug substance and/or product.

Guidance 6 (2) (Can the *N*-nitrosamine be formed?)

IQ consortium set of three conditions to investigate the potential formation of a *N*-nitrosamine from an vulnerable amine.

Conditions 1:
Amine, AcOH (~ a third 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), 20-25ºC temperature with a cap on to avoid NOx depletion. No co-solvents to be added - reaction could be a slurry.
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), 20-25ºC temperature with a cap on to avoid NOx depletion. Monitor pH at each time points and adjust if necessary. No co-solvents to be added - reaction could be a slurry.
* if the API is a salt of a strong acid, use the API salt in water instead of dilute HCl and do not adjust the pH
Conditions 3:
Amine free base, organic solvent that solubilises the amine (e.g. acetonitrile, tetrahydrofuran, or other aprotic solvent), *tert*-butyl nitrite (1.5 eq), overall reaction media concentration 0.1 M, 20-25ºC temperature with a cap on 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 *N*-nitrosamine risk in the drug substance and/or product.

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 interrogated by mass spectrometry (or alternate identification techniques). Appropriate monitoring for any weakly responding *N*-nitrosamine fragments should also be ensured. If a *N*-nitrosamine is detected below 10% at 24 h, continue the reaction to 48 h to further inform the formation risk. The amount formed and the kinetic profile will inform the risk assessment. If a *N*-nitrosamine is detected at levels above 10% at 24 h, the formation risk in the drug substance/product should be assessed and a 48 h time point is unnecessary. If more than half of the starting amine has degraded after 24 h and no *N*-nitrosamine is detected above 0.5%, a 48 h time point is also unnecessary. Where MS confirms the potential presence of an *N*-nitrosamine and further action is likely to be required, it is recommended to manufacture / isolate an authentic sample for structural confirmation to rule out potential false positives (e.g. *C*-nitroso, *O*-nitroso, oxime, etc..).

In case 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 *N*-nitrosamine product distribution. If certain amines do not undergo nitrosation under sub-stoichiometric conditions, these potential *N*-nitrosamines are unlikely to form in the drug substance/drug product.

Guidance 7 (Post Confirmatory Testing)

In cases where confirmatory testing on a suitable number of representative batches has established that the levels of the *N*-nitrosamine is consistently less than 10% of the acceptable limit, no further testing is required (e.g. ICH M7 option 4).

In cases where confirmatory testing on a suitable number of representative batches has established that the levels of the *N*-nitrosamine is between 10% and 100% of the acceptable limit, further testing or controls are required:

- If the level is between 10% and 30%, the *N*-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 *N*-nitrosamine impurity should be included on the specification (e.g. ICH M7 option 1)
- If more than one *N*-nitrosamine is included on the specification, the total risk level calculated for all identified *N*-nitrosamines should not exceed 1 in 100,000. This could be achieved for example by the following specifications: each individual *N*-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 could include:
    - Upstream control of the *N*-nitrosamine at the acceptable limit (e.g. ICH M7 option 2)
    - Upstream control of the *N*-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 *N*-nitrosamine formation point)

In cases where confirmatory testing on a suitable number of representative batches has established that the levels of the *N*-nitrosamine higher than the acceptable limit, further actions to be agreed with health authorities and could include remediation and/or establishment of temporary limits and/or further toxicity evaluation.

References

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3a) 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
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5a) CHMP, Nitrosamine impurities in human medicinal products Assessment Report, 25 June 2020, EMA/369136/2020, CHMP Assessment Report
6) FDA, Control of Nitrosamine Impurities in Human Drugs Guidance for Industry, Feb 2021, FDA Guidance
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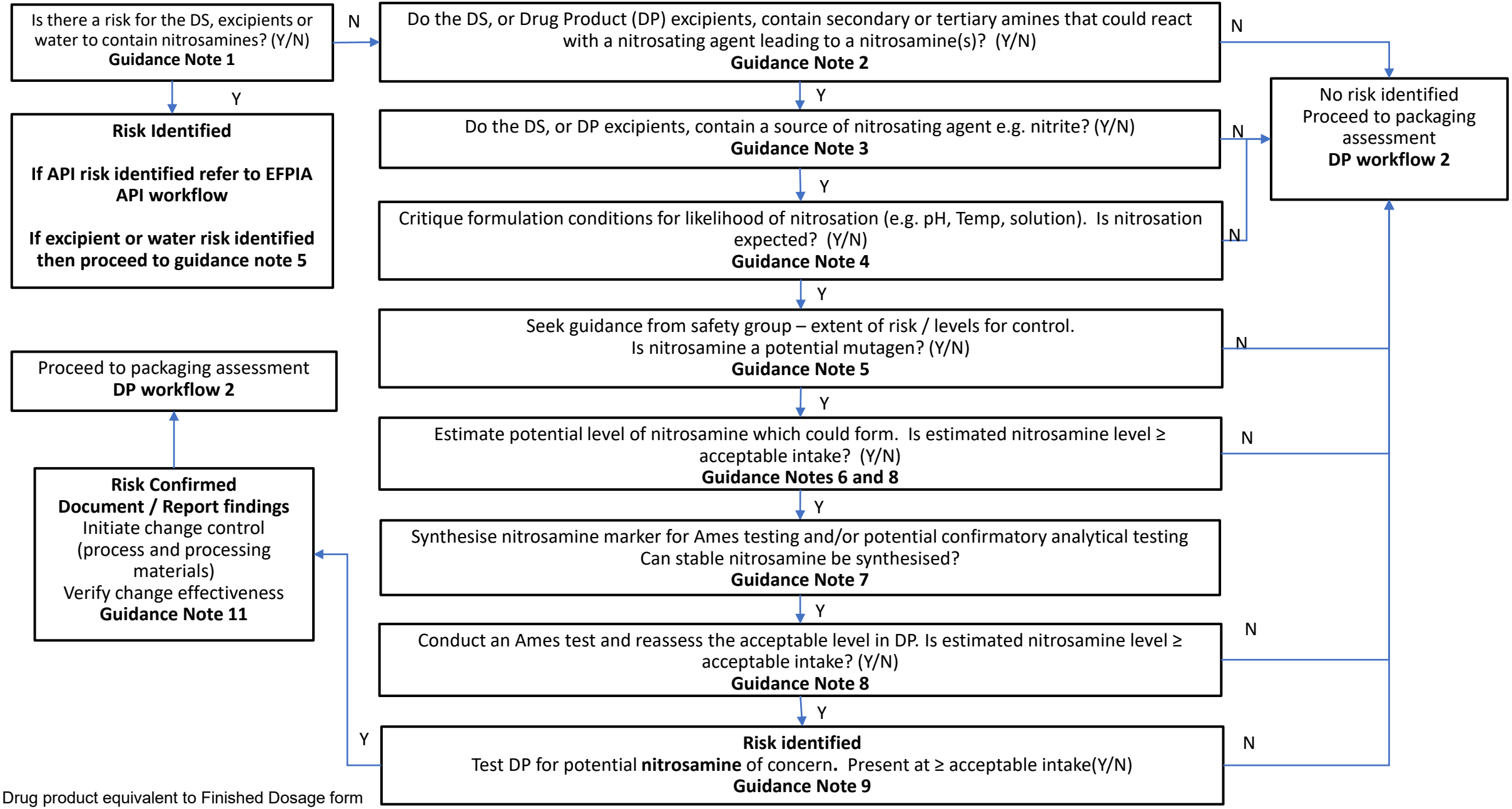
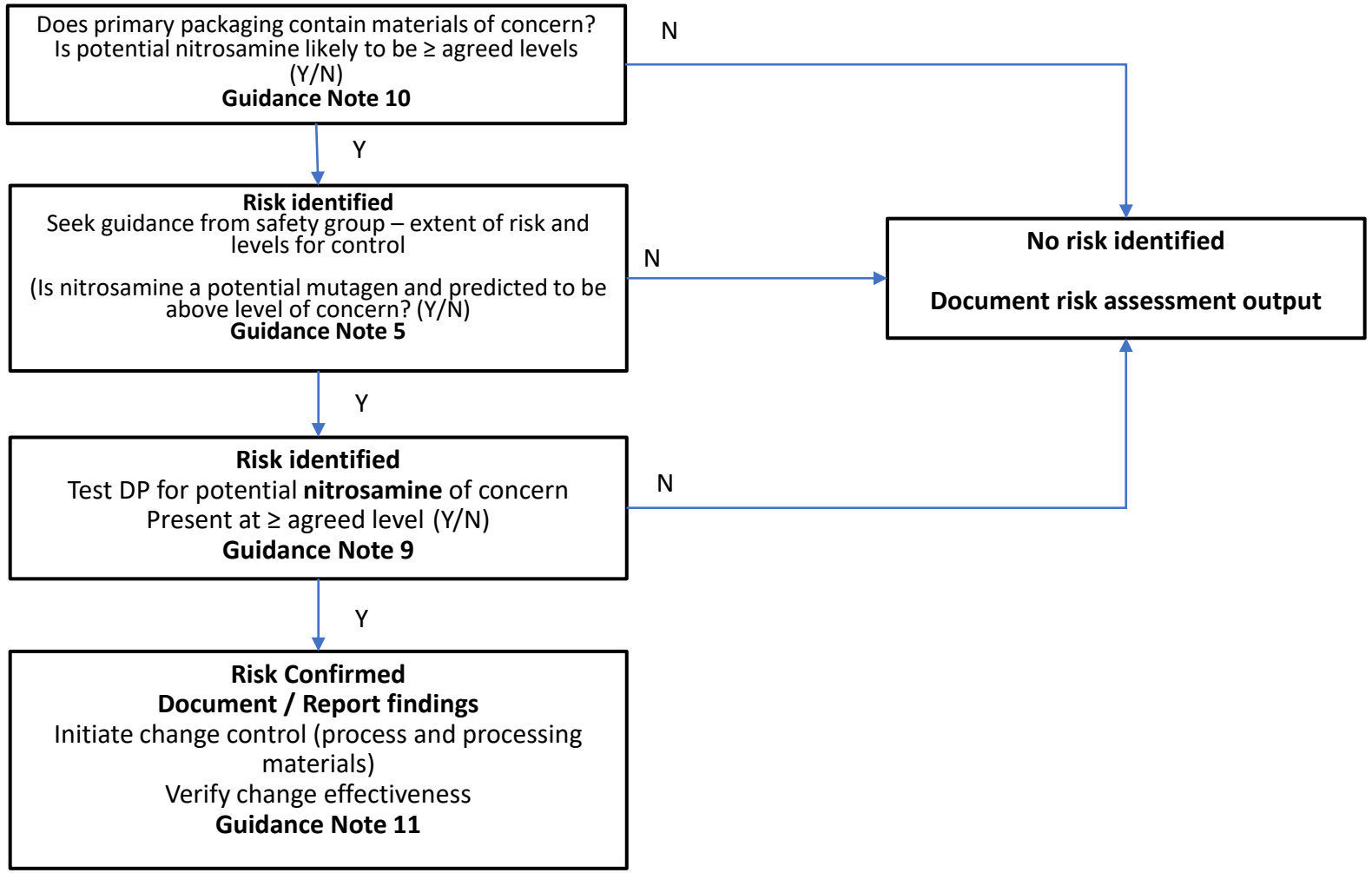
2. Drug Product Risk Assessment

Proposed IQ / EFPIA Drug Product (DP)† Workflow 1



† Drug product equivalent to Finished Dosage form

Proposed EFPIA / IQ Drug product (DP) Workflow 2



Guidance Notes 1 and 2 for EFPIA DP Workflow

Guidance Note 1

Output from the DS risk assessment / excipient evaluation may have identified a risk for the potential presence of nitrosamine impurities. Excipients are 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 (i.e. the EP monograph suggests triethanolamine may contain trace levels of the nitrosamine N-nitrosodiethanolamine) although where secondary amines are concerned this risk may need to be considered.[1] Any identified risk would lead to testing of the DS, excipient or water to confirm presence and at a level advised by the relevant safety group. It is still necessary to continue with a DP risk assessment to identify whether there might be additional nitrosamine risks.

It is considered that the risk associated with contamination during water purification process is very low and only likely to be of concern where very large volumes (> 1 L) are administered to the patient. It has been reported, [2] however, 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. Whilst this potential needs to be assessed, it is considered low potential for product contamination. 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 only been in the ng per litre range thus only significant quantities of water (>1 L) within the formulation could constitute a concern. It is also important to consider how the input water may have been purified e.g. use of chloramines or ozone to disinfect the water (Please refer to Annex 1 for Biologics position paper).

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Guidance Note 2

Output from the DS risk assessment / excipient review will have identified any amines present either as a structural motif within the DS / excipient(s), or as anticipated impurities or degradants, that could react with a nitrosating agent. Amines that could form a nitrosamine containing an alkyl carbon alpha to the nitrogen that contains at least one hydrogen are of particular concern as these may be metabolically activated and potentially mutagenic / carcinogenic to humans. The main focus should be on the most predominant types of nitrosamines with the dimethyl- and diethyl- groups being considered the more important in terms of mutagenic/carcinogenic potency compared to longer chain and cyclic compounds.[12]

Initial evaluation would suggest that there are relatively few amine excipients or excipients at risk of containing amines. Examples of such excipients include EDTA and its salts, triethanolamine, methyl N-methylanthranilate, tetra substituted alkonium salts, certain polymethacrylates functionalized with ammonium/amino groups, fatty acid amides e.g. coconut diethanolamide as these may contain levels of free amine which require assessment.

Guidance Note 2 for EFPIA DP Workflow

Guidance Note 2 (Cont.)

Generally it is anticipated that low level amine impurities[3] should constitute lower risk versus parent amine drug substances given this requires a trace amine to react with trace nitrite. Likewise, nitrogen containing functional group e.g. amides[4] are of lower reactivity because of the electron withdrawing properties of the carbonyl group and as such are considered out of scope of this assessment. The likelihood for degradation of tertiary amides and sulfonamides to form the corresponding secondary amine in the API or DP should be assessed for potential of nitrosamine formation.

Focus should be primarily on those drug substances containing a reactive secondary amine which, if nitrosated, would lead to (ICH M7 Class 1 or 2) nitrosamines. The mutagenicity risk for a nitrosamine derivative of a drug substance which is ICH M7 Class 3 is currently unclear and, whilst they should also be assessed, such materials are the subject of current SAR investigation and development, by industry experts including those from Leadscope and Lhasa. It is important that not all secondary and tertiary amines are particularly reactive to nitrosation (e.g. flufenamic acid and diphenhydramine) and referring to available literature is appropriate to inform likely risk e.g. Susceptibilities of Drugs to Nitrosation Under Standardised Chemical Conditions.[5]

Tertiary amines are significantly less reactive than secondary amines (reports of > 1000 fold lower reactivity[9]) and require an additional de-alkylation step, making their nitrosation in solid state very unlikely. Certain di-alkyl aromatic amines have been reported in the literature to be significantly more reactive than tri-alkyl amines when exposed to nitrosating agents in solution. Certain tertiary amines where nitrosation could lead to class 1 low MW nitrosamines could, in certain instances (e.g. where the reactivity towards nitrite is enhanced by particular structural features[6]), lead to an increased propensity towards nitrosation and should be considered as higher risk. Tertiary alkylamines would generally be considered negligible risk given the mild conditions processing conditions would not be expected to lead to a nitrosamine.

3. 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. 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.
4. Opinion on Nitrosamines and Secondary Amines in Cosmetic Products, Scientific Committee on Consumer Safety (SCCS/1458/11)
5. P. N. Gillatt, R. J. Hart and C. L. Walter; *Fd Chem. Toxic.*; **1984**, *22 (4)*, 269 to 274.
6. 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**: R. N. Loeppky, R. N. 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: https://www.ema.europa.eu/documents/presentation/presentation-nitrosamine-implementation-oversight-group-niog-third-meeting-pharmaceutical-industry_en.pdf

Guidance Notes 3 and 4 for EFPIA DP Workflow

Guidance Note 3

As part of the drug substance risk assessment, the drug substance has been assessed for its potential to contain nitrosating agents.
It is also possible that some excipients may contain low levels of potential precursors to nitrosating agents (e.g. nitrite). In the absence of specific data for excipients, a worst case figure of up to 2 ppm[12] of nitrite for these excipients could be used to assess the risk of nitrosamine formation. Potential precursors to nitrosating agents should be assessed in case there is the opportunity for *in situ* formation during processing or over the shelf life.[7] A summary of nitrosating agents and their precursors is also captured within Guidance Note 1 of the EFPIA Chemical Drug Substance Risk Assessment.

Water used for formulations is generally purified or water for injection where levels of nitrite have been measured as extremely low (0.1 ppb nitrite)[8] and as such nitrite in water is considered to be of negligible risk[9] than potential levels within excipients. If purified or WFI water has been purified by distillation, there is no risk of nitrite presence. Whilst nitrite levels in a limited number of excipients have been reported,[10] recent cross-industry assessment of common excipients suggests levels of nitrite is generally lower than previously published.[11] Further data can be made available within publications and/or a future database, similar to the ICHQ3D database of elemental impurities.[12]

7. 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
8. Global Workshop on Nitrosamine Impurities (cvent.com)
9. 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." *Organic Process Research and Development*; https://doi.org/10.1021/acs.oprd.0c00224
10. Yongmei Wu, Jaquan Levons, Ajit S. Narang, Krishnaswamy Raghavan, and Venkatramana M. Rao; AAPS PharmSciTech, 2011, 12 (4), 1248 to 1263
11. https://www.sciencedirect.com/science/article/pii/S0022354918302120?via%3Dihub
12. 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, R.; Schlingemann, J.; Hickert, S.; Korn, C.; Kocks, G.; Luck, B.; Blom, G.; Harrison, M.; François, M.; Allain, L; Wu, Y. and Bousraf, Y. "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.

Guidance Note 4

When a risk from nitrite and a reactive amine is highlighted, it is important to evaluate the conditions for the formulation manufacturing process and the resulting product to understand the likelihood of nitrosamine formation. Modelling of reaction kinetics may be used to de-risk the potential formation of nitrosamines, i.e. by calculating worst case reaction speed and comparing to conditions the product is exposed to (pH, temperature, reaction time) under the conditions of DP manufacture and under stability. For aqueous solution formulations, the formulation pH is considered the most critical but heat, order of addition and concentration within the formulation are also important.

Guidance Notes 3 and 4 for EFPIA DP Workflow

Guidance Note 4 (Cont.)

It is acknowledged that pH 3 to 4 is considered optimal for nitrosation with nitrous acid[13] and at higher pH the nitrosation reaction becomes much less likely. If pH > 7 for the process and product then the risk for nitrosation of amines with trace nitrite is considered negligible. There is some evidence that pH 5 to 7 can also be low risk for nitrosation but needs to be considered on a case by case basis. The measured pH of the drug substance can be very informative in this respect as can pH of the drug product formulation. For impact of heat, the risk may be higher for terminally sterilised products versus other solution products as nitrosation kinetics may increase. Solid based formulations may also constitute a risk particularly where a vulnerable amine is present in significant quantity and the above risk factors for nitrosation are met. The presence of a vulnerable amine within a solid based formulation should be evaluated as this could be a potential risk.

13. S.S Mirvish; “Kinetics of dimethylamine nitrosation in relation to nitrosamine carcinogenesis” J. Nat. Cancer Inst.; 1970, 44 (3), 633 to 639

Tablet coating operations have no identified risk for nitrosamine formation, either in the coating mixture itself or from interaction of trace components within the coating with the tablet core. This is due to a). Lack of reactive amine source in coatings, b). Dilute [nitrite] in coating suspensions/solutions, c). Low surface to volume ratios leading to minimal interaction between the coating and the core tablet, which is supported by evidence that a well-designed coating process produces little interaction between coating mixture and the tablet core.[14] Capsule shells are also considered to be no identified risk for nitrosamine contamination through either colours used within the capsule coating, neutrality of the plasticisers used, or the external printing ink that may be used. Nitrosation is unlikely to occur from nitrite within the gelatin because pH is likely to be neutral, gelatin contains primary amine scavengers, the low surface to volume ratio (as described above) and the printing process is conducted at room temperature.

14. Ruotsalainen, M. Studies on Aqueous Film Coating of Tablets Performed in a Side-Vented Pan Coater. Academic Dissertation. Pharmaceutical Technology Division, Dept. of Pharmacy. University of Helsinki, Finland. 2003

The use of flavours and fragrances within a drug product also has no identified risk for nitrosamine formation due to them being: a). Used in very small quantities, b). Multicomponent mixtures where a benign carrier/ solvent makes up the bulk of the mixture, c). Food grade therefore used and approved for use, in foods where their safety is covered by food[15] and/or cosmetic[4] standards.

15. EMEA Guideline On Excipients In The Dossier For Application For Marketing Authorisation Of A Medicinal Product ; EU list of flavourings for foodstuffs

Guidance Note 5 for EFPIA DP Workflow (New Guidance Note 5)

Guidance Note 5

A key basis for a risk assessment is to understand what limits within a drug substance present a significant risk. Generally, where identified, N-nitrosamines containing an alkyl carbon alpha to the nitrogen that bears at least one hydrogen, [16] should be assessed by safety experts. Safety experts should determine if an acceptable intake (AI) is available (i.e., those with an AI published established by regulatory authorities)[17,18] or can be calculated for any novel nitrosamines using existing compound specific carcinogenicity data or structural analogues (i.e., read-across) as recommended by ICH M7[19] and or categorical based "read across" approaches described by Dobo et al. ,[20] Cross and Ponting[21] and FDA[22]. If an AI cannot be calculated in this manner, until a limit is defined by ICH M7, conservative low limits (18 ng/day or 26.5 ng/day)[17,18] of control have been recommended by regulatory agencies.[17,18] For those nitrosamines which do not possess at least one alpha carbon containing hydrogens then these should be assessed by the representative company for establishing an appropriate AI.

The safety group can also advise whether the AI can be adjusted based on less than lifetime (LTL) clinical administration in alignment with ICH M7. The use of LTL adjustments is subject to agreement with regulatory authorities and could be a temporary measure.

Nitrosamines testing negative in an OECD guideline 471 aligned in vitro bacterial reverse mutation assay (Ames test) can be proposed to be controlled to ICH M7 limits (1.5 mcg per day or LTL equivalent). Data from such an Ames test can be used as part of the additional weight of evidence in limit setting. Note that there is an expectation that any non-GLP Ames test are confirmed by a GLP protocol. The understanding of metabolism and metabolic activation pathways could also support the weight of evidence. Additional supporting evidence may also be required (e.g. in-vitro and in-vivo mammalian genotoxicity assays as outlined in ICH M7 note 3)[19] to support levels in excess of ICH M7 limits.

Control to ICH M7 limits (1.5 mcg per day or LTL equivalent) may be considered for those nitrosamines which prove to be non-mutagenic within the Ames test with metabolic activation (e.g. S9) (required for a nitrosamine from the cohort of concern), but mutagenic without the metabolic activation.

For products intended for advanced cancer within the scope of ICH S9[23] then nitrosamines can be controlled to ICH Q3A/B levels (as recommended by EMA[17] and FDA[18]).
If more than one nitrosamine is included on the specification, the contribution of each nitrosamine relative to its individual acceptable limit is taken into account and summed up to a total % of expected nitrosamine control limits.[17]

16. https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-assessment-report_en.pdf
17. 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
18. FDA, Control of Nitrosamine Impurities in Human Drugs Guidance for Industry, Feb 2021, FDA Guidance
19. ICH Harmonised Guideline "Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk" M7(R1) 31 March 2017
20. 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
21. Cross, K and Ponting, D, *Computational Toxicology*, 2021, 100186. https://doi.org/10.1016/j.comtox.2021.100186
22. FDA, AAM/CHPA/PhRMA Questions for May 4th FDA-Industry Meeting to Discuss Nitrosamine Impurities in Pharmaceuticals, 4th May 2021, Document
23. ICH S9, Nonclinical Evaluation for Anticancer Pharmaceuticals, 29 October 2009.

Guidance Note 6 for EFPIA DP Workflow

Guidance Note 6

It is known that trace levels of a potential nitrosating agent e.g. nitrite may constitute a risk to a DP. Likely risk can be estimated from calculating the level of nitrite that may be available within the formulation and considering how much nitrosamine would be produced should it **all** react to produce a nitrosating agent. The level of nitrite within the formulation can either be derived from specific excipient testing data, referencing reported data within the Lhasa Vitic Nitrite in Excipient database or by assuming a generic average level of 2 ppm.[12]

If the estimated level of nitrosamine is lower than 10% of the acceptable daily intake,[17] it can be concluded there is no specific risk from the DP and the assessment should move to consider any packaging risks (Guidance Note 9)

If the estimated level of nitrosamine exceeds the allowable daily intake, it is appropriate to consider what a more realistic conversion could be for the associated product. In this respect, risk is assumed proportional to the potential for greatest interaction between the reactive amine and nitrosation agent.
The order of risk is considered to be:

Lower risk < dry blends < direct compression < wet granulation < freeze dried / amorphous < suspension < creams / syrups / solution < Higher risk

The actual conversion will be highly dependent of the formulation process and components e.g. amine reactivity, amine / excipient ratio, pH, particle size, water activity, crystallinity etc. It is useful to refer to the published calculation,[9] which is based on amine pKa, solution pH, amine and nitrite concentrations, total volume and shelf life, in order to estimate conversion for a solution based formulation.

If the estimated level is still in excess of 10% of the allowable daily intake then a potential risk is identified.

If the estimated level of nitrosamine is lower than 10% of the acceptable daily intake, it can be concluded there is no specific risk from the DP and the assessment should move to consider any packaging risks (Guidance Note 10)

Guidance Note 6 for EFPIA DP Workflow (Cont.)

Guidance Note 6 (Cont.)

The formation of N-nitrosamines (NNOs) requires contact between the nitrosating species and the vulnerable amine (VA). The theoretical maximum amount of NNO formed in a drug product is the complete (100%) conversion of the limiting reagent, typically the nitrosating agent. The formation of NNO in solid-phase products will be limited as a significant proportion of the reactants are inaccessible to each other. In a solid matrix there is only very localized mobility of the reactants, so the reaction can only occur where the reactants are in close proximity. In addition, the API in solid drug product is typically present as particles (with impurities contained in the bulk), where only the surface of the particle is available for reaction. Therefore a reduction in accessible VA levels is expected to reduce the plateau level of NNO generated, even in cases where the VA is not the limiting reagent, since the proportion of nitrosating species in proximity to VA will be reduced as the concentration of VA is reduced. Preliminary investigations into the rate and extent of NNO formation in solid drug products containing a model secondary amine have shown that significantly less NNO was formed when the level of VA was lowered, even when the limiting reagent, nitrite, was unchanged.

Therefore, a 'factor' is applied to estimate the extent of NNO formation in cases where the VA is present in solid state at impurity levels in the API. This is based on the fact that as the amount of VA is reduced, the proportion of nitrosating species with potential to come into contact with VA will be reduced, i.e. it is based primarily on a consideration of the 'accessibility of reactants' affecting the maximum extent of reaction. Additionally, a reduction in the VA will also have a kinetic effect (i.e. reduce the rate of formation); therefore, the application of this factor is expected to be a conservative approach for estimating the amount of NNO formed.

This 'factor' is calculated as follows: an impurity at a level of e.g. 0.25% would use a 'factor' of (1/0.0025) = 400. The 'factor' of 400 reflects that the impurity at 0.25% is 400 times less prevalent that an API VA in the solid matrix.

Guidance Note 7 for EFPIA DP Workflow

Guidance Note 7

In progressing to testing, an authentic sample of nitrosated product needs to be obtained to develop a suitable test method and/or perform toxicity assays. Samples of the ICH M7 Class 1 small molecular weight nitrosamines are likely to be readily available but nitrosated versions of DS may require specific manufacture. If the identified nitrosamine cannot be manufactured directly from nitrosation of the VA, or is found to be unstable, then this can be used as justification to take no further action (aligns with Guidance Note 2). A process for the evaluation of feasibility for forming nitrosamines is provided on the next slides.

Manufacture of an identified nitrosamine of unknown mutagenic potential is likely to require appropriate safety input to inform the required occupational handling and containment requirement. Additional safety precautions are likely to be required over and above general laboratory safety working practices as some nitrosamines have been reported to be energetic and sensitive to handle.[24]

Testing the DP formulation with the associated highest risk of nitrosation (Guidance Note 6) should be the focus.

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

N-Nitrosamines that are know mutagens (ICH M7 class 1 or 2) typically have a simple structure and are known to be able to form from the corresponding amine. In those instances the formation test described below will typically not inform the assessment further.

In order to evaluate the N-nitrosamine in toxicity assays (e.g. Ames test) or generate analytical data, a physical sample of the N-nitrosamine is required. Preparative methods for synthesising the N-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) in an organic solvent.[25] 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 results in the absence of a N-nitrosamine risk in the drug substance and/or drug product. In cases where the drug substance manufacturing process uses an intentionally added nitrosating agent, the specific conditions used in the manufacturing process should be assessed for the potential formation of a N-nitrosamine.

The IQ consortium has established a set of three conditions (see next slide) that represent a worst case for drug substance and drug product manufacturing scenarios with trace nitrite contamination. 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), performed at room temperature to avoid the known nitrite decomposition at elevated temperature (such a decomposition can occur during the WHO NAP test)[26], are in solution phase (thereby ensuring contact of the reactants) for a period of 24 h to 48 h and any potential *N*-nitrosamine formation assessed down to 0.5% a/a using MS detection (see next slide for detailed conditions). These conditions are more representative of a worst case for drug substance and drug product manufacturing scenarios with trace nitrite contamination than the WHO NAP test which was designed to investigate potential *in-vivo N*-nitrosamine formation using a large excess of nitrite, elevated temperatures (leading to nitrite decomposition) and only used inorganic nitrite. In line with EMA guidance[17] and given the comprehensiveness of the three conditions, absence of formation of a *N*-nitrosamine from the corresponding amine using these three conditions leads to the conclusion that the drug substance and/or drug product is absent of risk from this *N*-nitrosamine.

25. 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

26. 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

Guidance 7 (Can the *N*-nitrosamine be formed?)

Guidance Note 7 (Cont.)

Certain *N*-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 *N*-nitrosamine risk in the drug substance and/or product.

IQ consortium set of three conditions to investigate the potential formation of a *N*-nitrosamine from an vulnerable amine.

Conditions 1:
Amine, AcOH (~ a third 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^{o}C temperature with a cap on to avoid NOx depletion. No co-solvents to be added - reaction could be a slurry.
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^{o}C temperature with a cap on to avoid NOx depletion. Monitor pH at each time points and adjust if necessary. No co-solvents to be added - reaction could be a slurry.
* if the API is a salt of a strong acid, use the API salt in water instead of dilute HCl and do not adjust the pH
Conditions 3:
Amine free base, organic solvent that solubilises the amine (e.g. acetonitrile, tetrahydrofuran, or other aprotic solvent), *tert*-butyl nitrite (1.5 eq), overall reaction media concentration 0.1 M, 20-25^{o}C temperature with a cap on 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 *N*-nitrosamine risk in the drug substance and/or product.

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 interrogated by mass spectrometry (or alternate identification techniques). Appropriate monitoring for any weakly responding *N*-nitrosamine fragments should also be ensured. If a *N*-nitrosamine is detected below 10% at 24 h, continue the reaction to 48 h to further inform the formation risk. The amount formed and the kinetic profile will inform the risk assessment. If a *N*-nitrosamine is detected at levels above 10% at 24 h, the formation risk in the drug substance/product should be assessed and a 48 h time point is unnecessary. If more than half of the starting amine has degraded after 24 h and no *N*-nitrosamine is detected above 0.5%, a 48 h time point is also unnecessary.

In case a molecule contains more than one amine, sub-stoichiometric conditions (i.e. 0.1 or 0.5 eq nitrite added) should be used to determine the *N*-nitrosamine product distribution. If certain amines do not undergo nitrosation under sub-stoichiometric conditions, these potential *N*-nitrosamines are unlikely to form in the drug substance/drug product.

Guidance Notes 8 and 9 for EFPIA DP Workflow

Guidance Note 8

An OECD compliant Ames test result (positive or negative) can inform the acceptable level for the N-nitrosamine and a new acceptable level may be proposed (Guidance Note 5). Review the predicted nitrosamine formation outcome with the new limit (if applicable – Guidance Note 6).

Guidance Note 9

Testing the DP formulation with the associated highest risk of nitrosation (Guidance Note 6) should be the focus. 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. Output from this testing can be used to inform risk associated with other formulations and future product assessments from a prior knowledge perspective.

Guidance Note 10 for EFPIA DP Workflow

Guidance Note 10

Packaging risks are considered independent of the formulation process and there needs to be an assessment. Packaging and printing materials that are currently considered potentially at risk for the formation of low levels of nitrosamines are nitrocellulose which may react with amines in printing ink to generate nitrosamines which could be transferred to the product under certain packaging operations (e.g. during heat sealing blistering processes *via* vaporisation and deposition onto the drug product).[27]

Generally the risk is considered very low as observed levels, when formed, have been very low and significantly below an acceptable daily intake for the patient. Therefore where a potential risk is identified, testing of product may not be required particularly where there are low numbers of daily doses. Where multiple daily dosing is required for the respective product, or where other nitrosamine risks may have been identified within the product assessment as per workflow 1 might be appropriate.

Moving to nitrocellulose free materials would mitigate this potential risk but 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 leachable nitrosamines, this is generally a well understood and managed phenomenon and as such is not considered an additional cause for concern.[28] 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.[9] The risk of nitrosamines and amines being derived from an extractable and leachable perspective is therefore considered very low and not in scope of this workflow.

27. 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.
28. 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⟩

Guidance Note 11 for EFPIA DP Workflow?

Guidance Note 11

Where a risk for nitrosamine(s) has been confirmed within the drug product, marketing authorisation holders should inform the competent authorities of the outcome of tests. The immediate risk to patients should be assessed based on the appropriate safety limit and action proposed to avoid or minimise the exposure of patients to nitrosamines.[17,18]
With respect to future mitigations to reduce / eliminate the nitrosamine risk, the following can be considered:

- Perform additional toxicological studies to further understand the toxicity potential
- Reducing levels for the vulnerable / reactive amine if appropriate
- Consider sourcing nitrite low / free alternatives if available
- Reformulation may be required

If the development of a new formulation is required, then there are potentially numerous options that can be considered and these include:

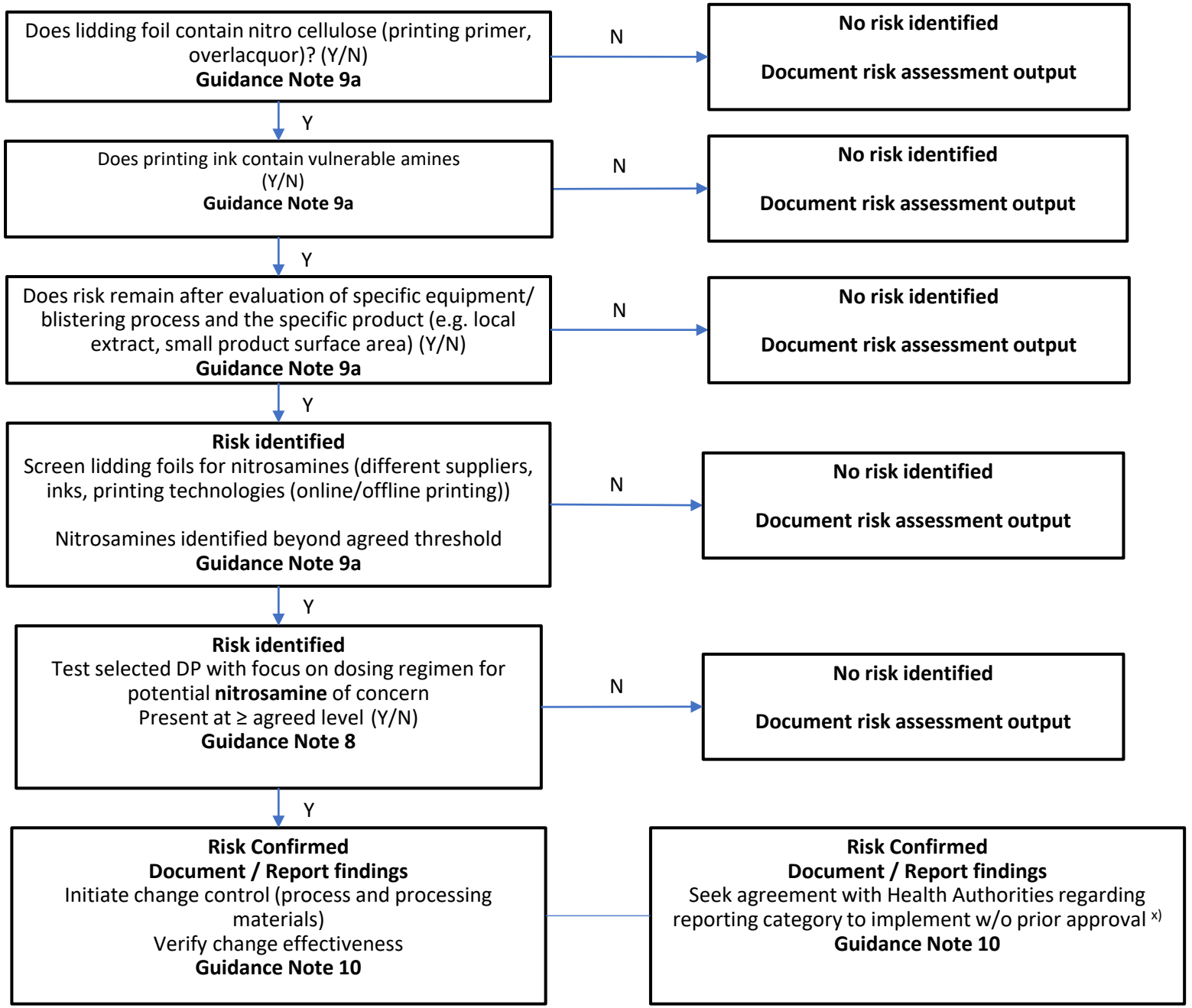
- Use of a simpler formulation i.e. powder in a capsule rather than tablet
- Modify pH for the formation – addition of carbonate to the excipients
- Use of dry granulation versus wet granulation
- Changing order of addition or engineering modifications to remove heat from the system if these aspects were considered potential root causes
- Modification of particle size excipient / DS etc.
- Inclusion of scavengers e.g. glycine, lysine, histidine, or antioxidants e.g. ascorbic acid, a-tocopherol, ferulic or caffeic acids, within the formulation to act as nitrous acid scavengers[29]

Any new formulation would need to 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.

29. 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

3. Risk Assessment for Nitrocellulose Packaging Materials

Proposed EFPIA / IQ Drug product (DP) Workflow 2a - Nitrocellulose containing lidding foil



x): Rationale: lidding foil not in contact with the product and change not expected to affect Quality & Stability (regular FUST proposed) in case aluminum layer & heat seal lacquor are unchanged (water vapor transmission rate, oxygene transmission rate; tightness unchanged)

Guidance Note 9a for EFPIA DP Workflow – Nitro cellulose containing lidding foil

Packaging risks are considered independent of the formulation process and need to be specifically assessed. In general the risk is considered very low as observed levels of Nitrosamines, when formed, have been very low and significantly below an acceptable daily intake for the patient.

Packaging materials that are currently considered potentially at risk for the formation of low levels of nitrosamines are blister lidding foils containing nitrocellulose as printing primer or over-lacquer, which may react with amines in printing ink to generate nitrosamines, which could be transferred to the product under certain packaging operations (e.g. during heat sealing blistering processes *via* vaporization and condensation onto the drug product).

Nitrocellulose is commonly used in blister lidding foils as a print primer and print over-lacquer.

Amines in ink may be part of color pigments but are mainly non functional constituents in the ink and are hence considered to be "Non Intentionally Added Substances" (NIAS). Key factors to consider are extent of ink coverage, colors (e.g. reds, yellows are believed to be higher risk) and location (inner or outer surface).

An evaluation of the blistering process, particular risks and risk mitigation factors (e.g. ventilation) should be considered. The typical blistering operation prevents risk of contamination in upstream blisters from exceeding a few nanograms per blister well. This is due to the following reasons:

1. Very low volatilization rates of NDMA and NDEA from common blister lidding and short time of applied heat during sealing
2. Tortuous pathway for vapors to get upstream into open blister wells
3. Depletion of any volatilized nitrosamines from vapor cloud due to room air changeovers.

Screening of different nitrocellulose containing lidding foil types and inks (potentially containing residual amount of amines as non intentionally added substances) is regarded to be a valuable indicator to assess if tablets could be exposed to Nitrosamines during blistering, and hence a risk in conjunction with the dosing regimen may exist.

In summary, even where a potential theoretical risk is identified e.g. the lidding foil contains nitrocellulose, testing of product may not be required when considered in conjunction with other factors such as foils screening, blister equipment, blistering process, dosing regimen and product surface area. This risk would need to be examined on a case by case basis particularly where multiple daily dosing may be required for the respective product or where other nitrosamine risks may have been identified within the product.

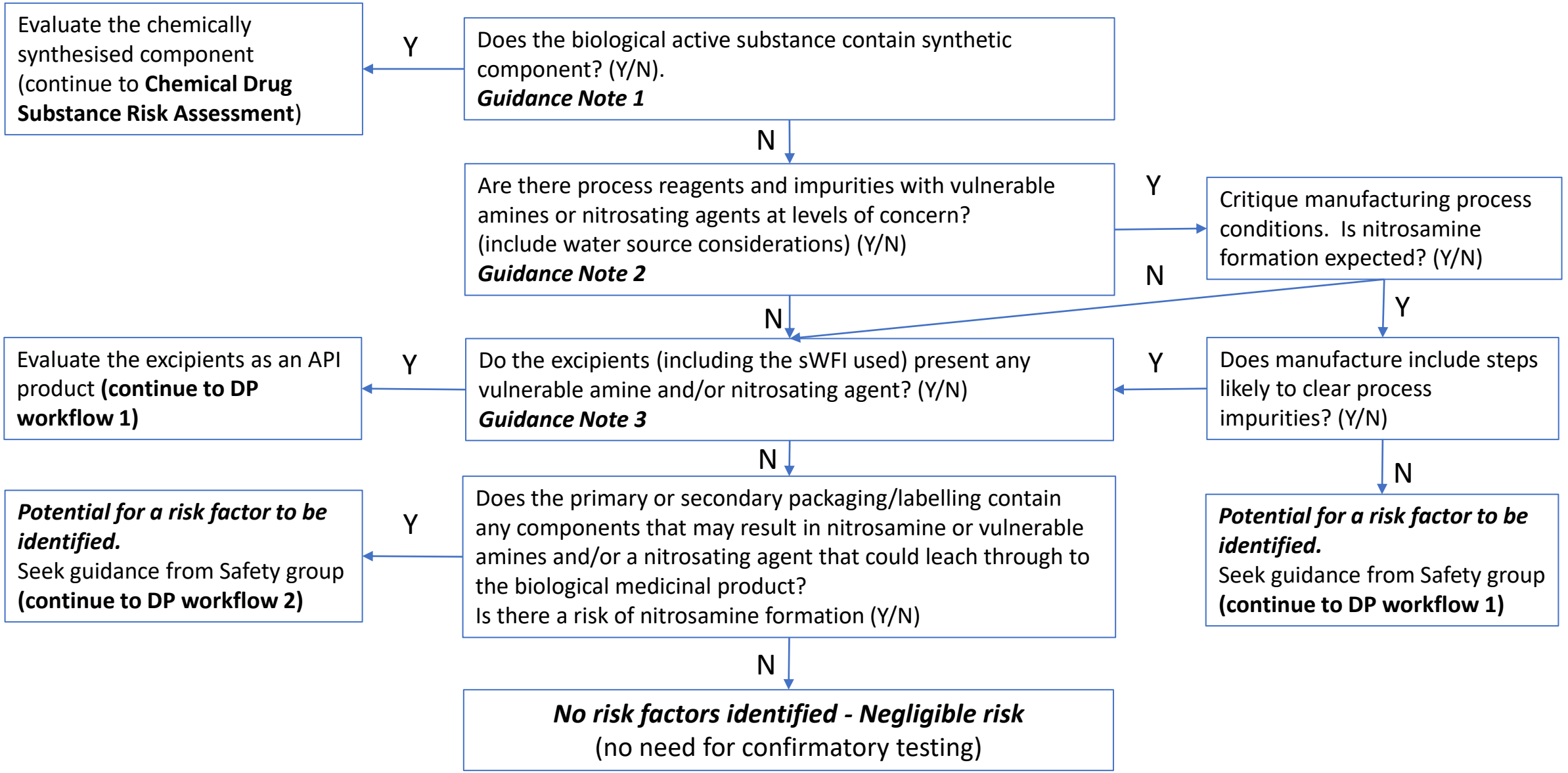
Potential risk removal options are available e.g. moving to nitrocellulose free materials. This change is not considered a requirement but should be considered if there is a multiple dosing regimen that leads to this potential risk being more significant. This change should be considered to be implemented without prior regulatory approval ("do & tell"), as the lidding foil outer surface (Nitrocellulose) is not in contact with the product and the change is not expected to affect Quality & Stability (regular follow up stability is proposed) in case aluminum layer & heat seal lacquer are unchanged (water vapor transmission rate, oxygen transmission rate; tightness unchanged).

Alternative mitigation solutions, such as local extract during blistering operation, would also remove the risk.

The other theoretical risk mitigation possibility is to move to printing ink free of vulnerable amines. However, as secondary amines are NIAS, it is difficult to consistently avoid this risk without testing or robust certification.

4. Risk Assessment for Biological Drugs

ANNEX 1: Biological Medicinal Product Process for Nitrosamine Impurities Risk Evaluation



Guidance Notes for the Biological Medicinal Product Process for Nitrosamine Impurities Risk Evaluation.

Guidance Note 1

When the active substance contains a chemically synthesised component, for example Antigen-Drug Conjugates (ADCs) or PEGylated protein, then the synthesised API component(s) could be assessed as described in the Drug Product Workflow 1. This API assessment should also include the bioconjugation step(s). The risk presented by the biological component of a bioconjugate product could be assessed according to this Biological Medicinal Products Workflow by a 'No' response.

Guidance Note 2

Generally, it would be expected that small molecule process reagents and impurities in raw materials or starting materials would be cleared in biological manufacturing processes that typically employ several steps of bind/elute chromatography and ultrafiltration/diafiltration. Such steps would be expected to clear process reagents and impurities with amines vulnerable to nitrosation or any nitrosating agent below any level of concern. However, it cannot be precluded that a manufacturing process step may require the deliberate addition of a nitrosating agent or precursor[1]. An example would be use of nitrates in a microbial cell culture medium that may be metabolised to form nitrite. Any process introducing a nitrosating agent (or indeed a process reagent vulnerable amine – excluding the biological starting material) should assess if the level presents any concern and proceed with 'Y or N', accordingly.

[1] 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 (EMA/409815/2020), Section 2.

Guidance Note 3

Parenteral biological medicinal products are required to be formulated in Water for Injection (WFI). The WFI is manufactured by distillation (or combined reverse osmosis/ultrafiltration and demonstrated of equivalent or superior quality to distillation[2]). Since WFI is the medium for column chromatography and ultrafiltration/diafiltration, water quality is a recommended consideration, especially should anion-exchange (AEX) chromatography of disinfected source water be used to manufacture Purified Water. Note that Purified Water generation would typically include other steps to deplete disinfectant and nitrosamine impurities. Processing of Purified Water for WFI by distillation may not remove trace levels of nitrosamine. Overall, the processing from Purified Water is expected to result in negligible levels of nitrate/nitrite, disinfectants and nitrosamine impurities in WFI, thereby removing risk of nitrosamine formation and the answer with respect to the WFI would generally be 'N' – an exception may be for very large dose volume products using WFI purified by AEX chromatography from disinfected source water.

If an excipient is used that presents additional risk through nitrosation (Y) then the excipient should be further evaluated. Addition of excipients with vulnerable amines alone is not likely to be of concern for biological medicinal products, so long as the levels of nitrosating agent are assessed as negligible and can respond as 'N'.

[2] EMA Guideline on the quality of water for pharmaceutical use (EMA/CHMP/CVMP/QWP/496873/2018) – Adopted 18 June 2020 and in force from 01 February 2021.