Workflows for Quality risk management of nitrosamine risks in medicines

Version 1.0

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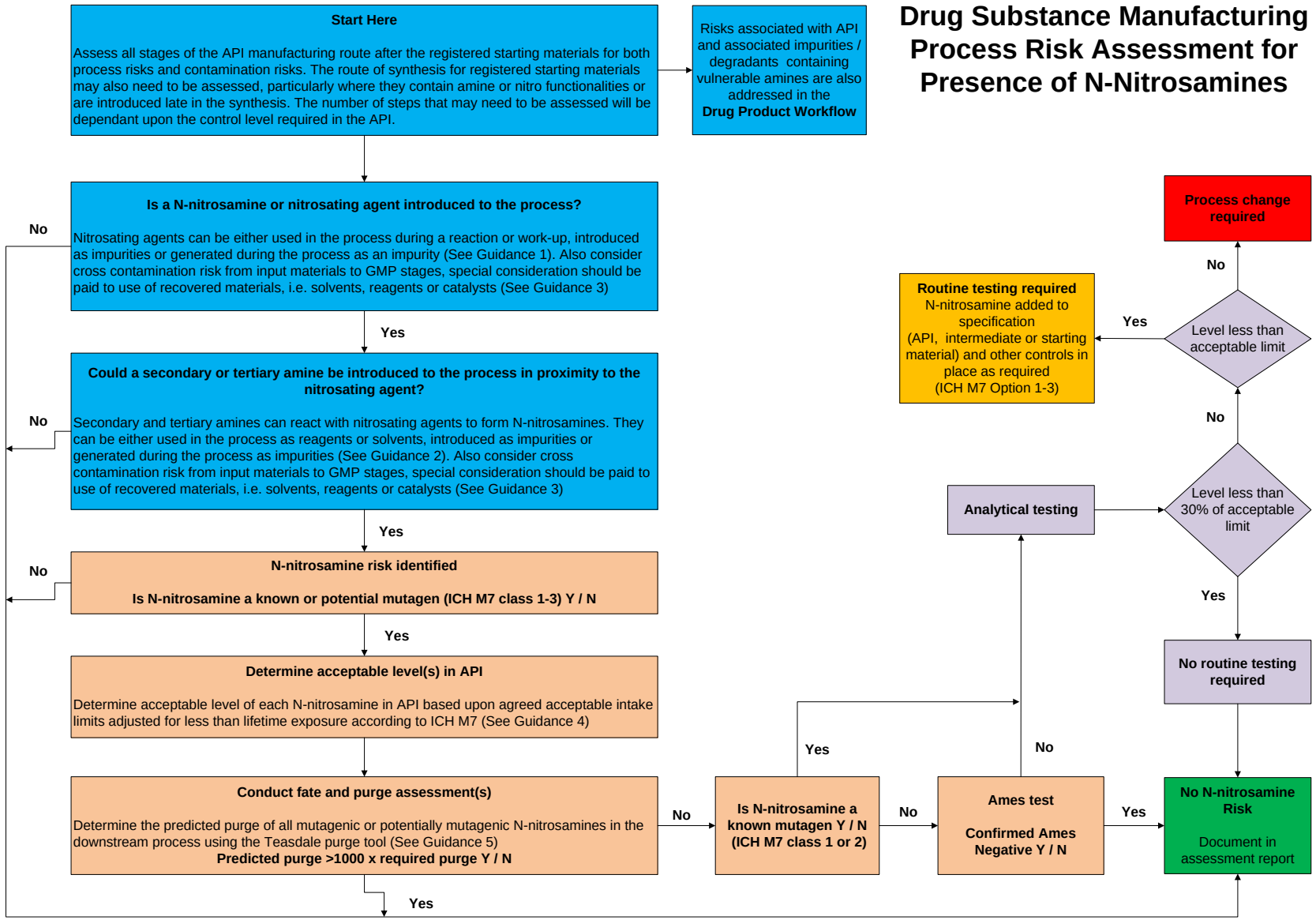
Introduction

- 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



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).

Other potential nitrosation risks:

- Side reaction in nitration reactions. Nitric acid typically contains nitric oxide as an impurity, additional nitrous acid may also be produced, leading to nitrosation, if any reducing agents are present.
- Hydroxylamine under oxidative conditions
- Chloramines are known to generate N-nitrosamines under certain conditions and so should also be considered[1]
- Ozone may lead to the formation of N-nitrosamines by initial oxidation of amines to nitrite[1]

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 2 (Sources of secondary and tertiary amines)[2]

Secondary amines are of greatest concern, however tertiary amines can also undergo nitrosation via more complex pathways. All secondary and tertiary aliphatic and aromatic amines should therefore be considered including those present as part of the starting material, intermediate or API structure as well as those introduced as reagents, catalysts, solvents or as impurities.

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

Amines may also 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)
- Of quaternary ammonium salts such as tetrabutylammonium bromide (TBAB)
- Of primary amines such as monoethylamine
- Of 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 3 (Potential contamination risks)

Consider all potential sources of contamination in input materials

Use of recovered materials (solvents, reagents, catalysts) is of particular concern if appropriate controls are not put in place. The materials DMF, ortho-xylene and tributyltin chloride were highlighted by the EMA as materials at risk of cross contamination by N-nitrosamines. Sodium azide was highlighted by Health Canada for risk of cross contamination with nitrite.

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 4 (Determining an acceptable level)

Interim acceptable daily intakes for chronic exposure to several common N-nitrosamines have been defined, see table 1.

Processes to determine acceptable intakes for all other N-nitrosamines should be in alignment with the EFPIA paper.[3]

These levels should be adjusted for less than lifetime exposures as described in ICH M7.[4]

Calculate acceptable limits in ppm relative to API using the maximum daily dose.

Higher limits may be justified for ICH S9 indications.[5]

Table 1, EMA interim acceptable daily intake for chronic exposure to common N-nitrosamines[6]

Nitrosamine	Abbreviation	EMA acceptable intake for chronic exposure ng/day
N-nitrosodimethylamine	NDMA	96
N-Nitroso-N-methyl-4-aminobutyric Acid	NMBA	96
N-nitrosodiethylamine	NDEA	26.5
N-nitrosodiisopropylamine	DIPNA	26.5
N-nitrosoethylisopropylamine	EIPNA	26.5

Guidance 5 (Conducting purge assessments)[7]

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 and what the maximum realistic level could be. Nitrosation occurs more rapidly under acidic conditions (apart from organic nitrites) and may also be catalysed by certain anions and aldehydes (notably thiocyanate and formaldehyde).[2, 8]

During purge calculations consider the likely physicochemical characteristics of the N-nitrosamine which may be formed. For instance, NDMA has a BP of 153^{o}C and will partition in both aqueous and organic layers. It is highly soluble in water and organic solvents. Other, higher molecular weight, N-nitrosamines will behave differently.

N-nitrosamines are relatively stable compounds though the following conditions are known to result in de-nitrosation:

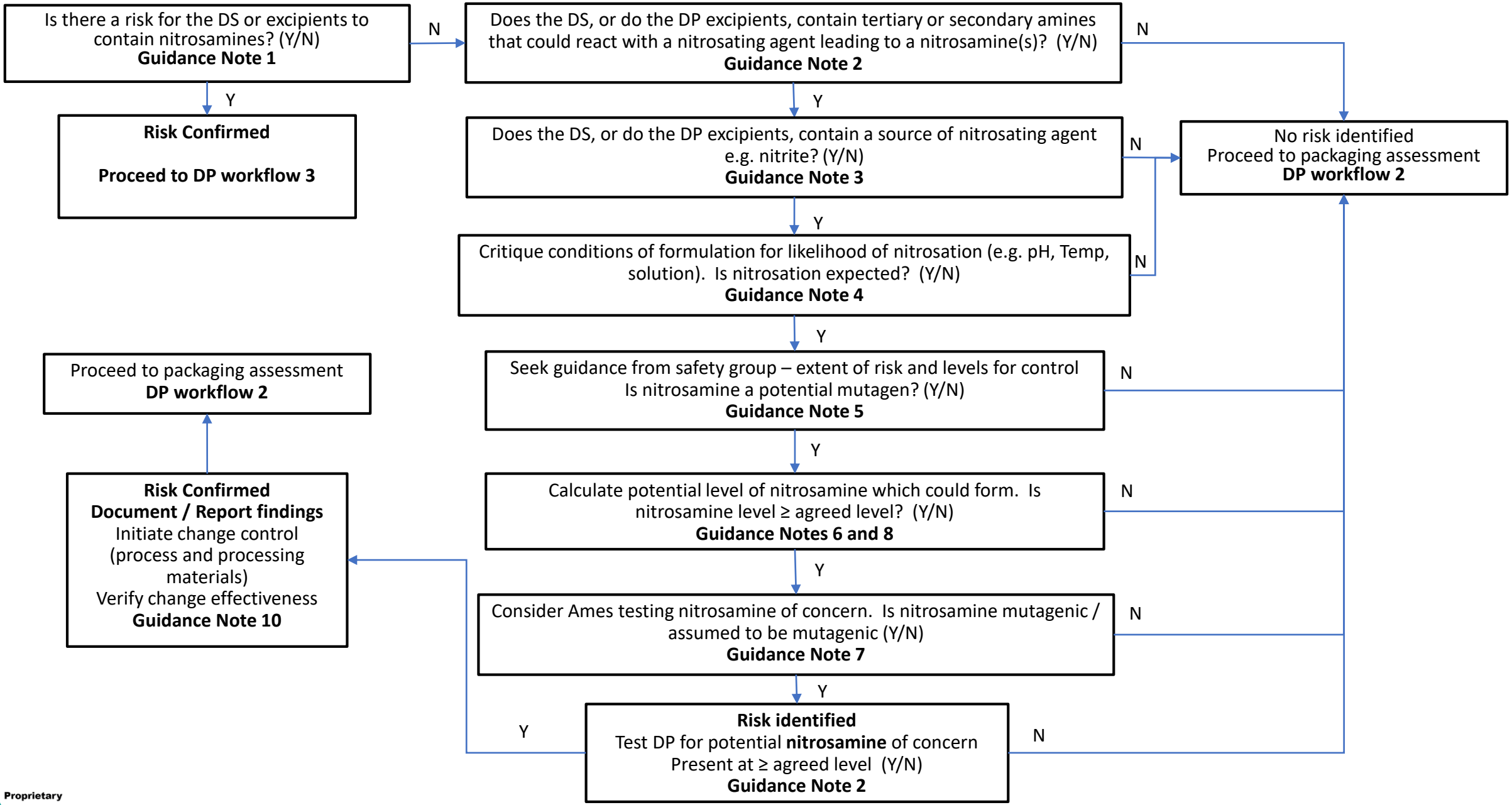
- Strongly acidic condition with a nucleophile trap (e.g. HCl with MeOH)
- Metal reducing conditions (e.g. Zn AcOH; Ni/Al KOH)
- Pd/C Hydrogenation
- Grignards
- Strong oxidants (H_2O_2; $KMNO_4$)

References

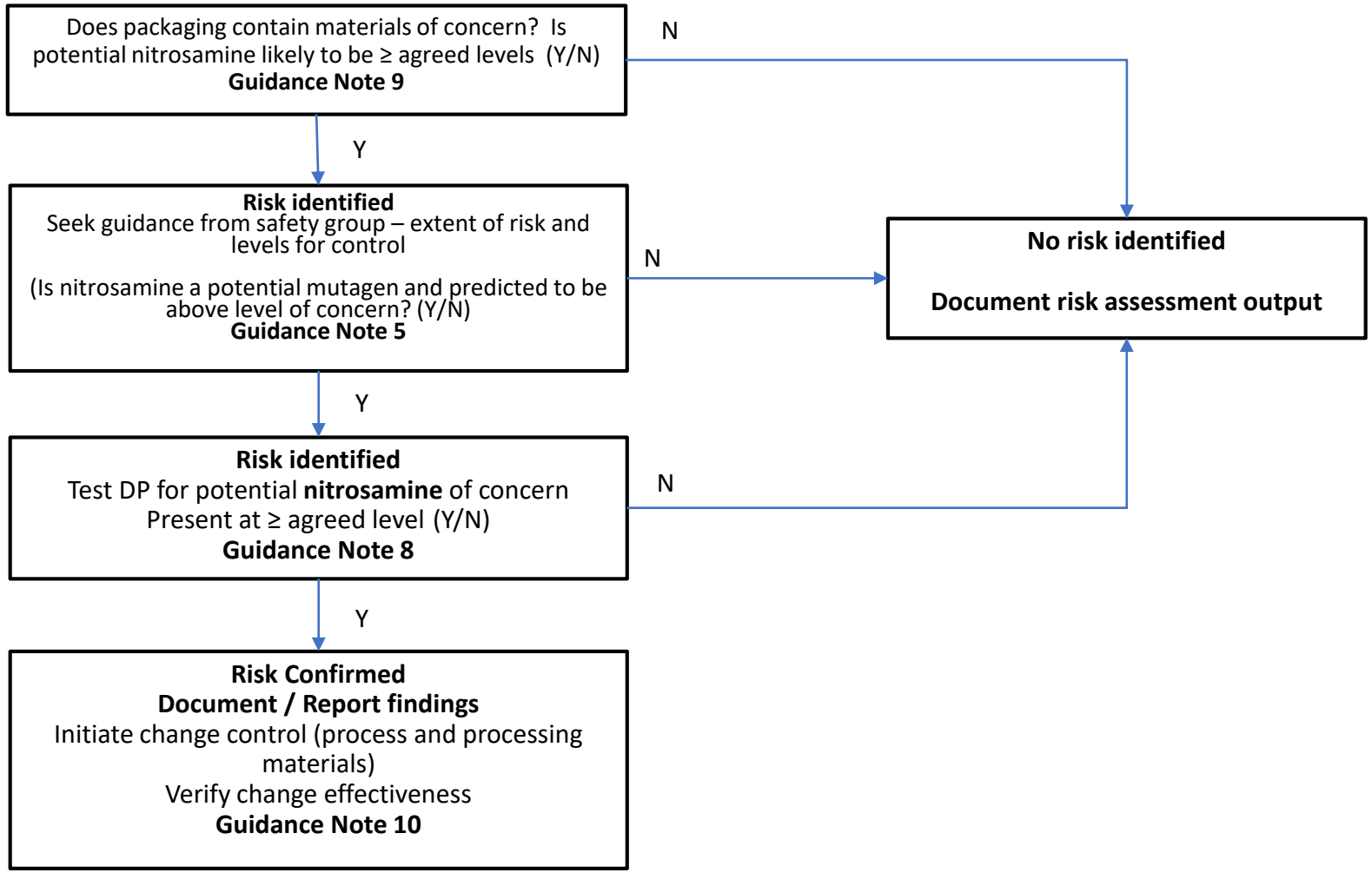
1) Nawrocki, J et al. Nitrosamines and Water, J. Hazard. Mater. 2011, 189, 1-18.
2) SCCS (Scientific Committee on Consumer Safety), Opinion on Nitrosamines and Secondary Amines in Cosmetic Products, 27 March 2012.
3) EFPIA position with respect to safety related aspects of EMA and Health Canada requests for N-nitrosamine evaluations, 2019.
4) ICH M7, Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, 31 March 2017.
5) ICH S9, Nonclinical Evaluation for Anticancer Pharmaceuticals, 29 October 2009.
6) EMA, Temporary interim limits for NMBA, DIPNA and EIPNA impurities in sartan blood pressure medicines, 20 August 2019.
7) 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.
8) Williams, D. L. H. Nitrosation reactions and the chemistry of nitric oxide. 2004, Amsterdam, Elsevier.

2. Drug Product Risk Assessment

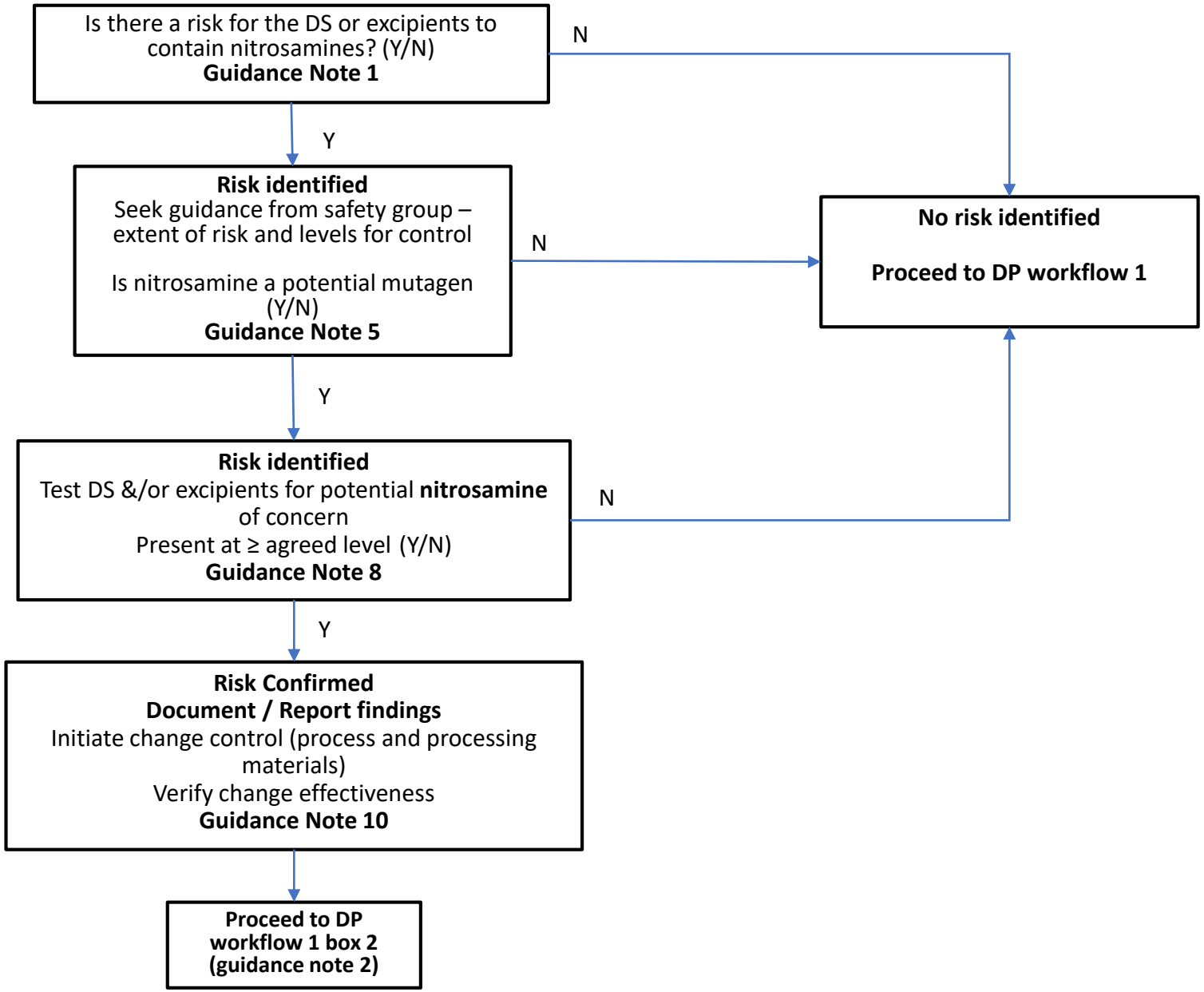
Proposed IQ / EFPIA Drug product (DP) Workflow 1



Proposed EFPIA / IQ Drug product (DP) Workflow 2



Proposed IQ / EFPIA Drug product (DP) Workflow 3



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 very rare for there to be a nitrosamine present within an excipient although where secondary amines are concerned this risk may need to be considered.[1] Any identified risk would lead to testing of the DS or excipient 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.

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

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 Notes 2 for EFPIA DP Workflow

Guidance Note 2 (Cont.)

Generally it is anticipated that low level amine impurities[2] should constitute lower risk versus stoichiometric amines given this requires a trace amine to react with trace nitrite. Likewise, nitrogen containing functional group e.g. amides[3] 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.

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.[4]

Tertiary amines are significantly less reactive than secondary amines (reports of > 1000 fold lower reactivity[8]) and require an additional de-alkylation step, making their nitrosation in solid state very unlikely. 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), lead to an increased propensity towards nitrosation and should be considered as higher risk. Tertiary amines would generally be considered negligible risk given the mild conditions processing conditions would not be expected to lead to a nitrosamine.

2. Experimental data from model studies suggests that amine impurities at ICH Q3A and Q3B identification limits are not considered a risk for nitrosation with trace nitrite
3. Opinion on Nitrosamines and Secondary Amines in Cosmetic Products, Scientific Committee on Consumer Safety (SCCS/1458/11)
4. P. N. Gillatt, R. J. Hart and C. L. Walter; *Fd Chem. Toxic.*; **1984**, *22 (4)*, 269 to 274.
5. S.S Mirvish; “Kinetics of dimethylamine nitrosation in relation to nitrosamine carcinogenesis” *J. Nat. Cancer Inst.*; **1970**, *44 (3)*, 633 to 639

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 5 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.[6]
Whilst potable water can contain low levels of nitrite (<0.1 to 3 ppm as per WHO guidelines[7]), water for formulations is generally further purified (<<0.01 ppm nitrite) and as such nitrite in water is considered to be of lower risk[8] than potential levels within excipients. Whilst nitrite levels in a limited number of excipients have been reported,[9] recent cross-industry assessment of common excipients suggests levels of nitrite is generally lower than previously published.[9]
Further data can be made available within publications and/or a future database, similar to the ICHQ3D database of elemental impurities.[10]

6. 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
7. WHO "Guidelines for Drinking-water Quality", 2008, third edition,
8. 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
9. Yongmei Wu, Jaquan Levons, Ajit S. Narang, Krishnaswamy Raghavan, and Venkatramana M. Rao; AAPS PharmSciTech, 2011, 12 (4), 1248 to 1263
10. https://www.sciencedirect.com/science/article/pii/S0022354918302120?via%3Dihub

Guidance Notes 3 and 4 for EFPIA DP Workflow

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. It is acknowledged that pH 3 to 4 is considered optimal for nitrosation with nitrous acid[5] 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 centrifuged wet granulation mixture. 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 are considered less of a risk, as the availability of the amine within a solid matrix can be substantially lower, lack of availability of nitrite as it may be trapped within a solid matrix and general heterogeneity, and whilst the above factors should be considered, especially pH, the behaviour toward levels of heat and water may be less pronounced.

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.[11]

11. 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). Food grade therefore used, and approved for use, in foods where their safety is covered by food[12] and/or cosmetic[3] standards, b). used in very small quantities, c). multicomponent mixtures where a benign carrier/ solvent makes up the bulk of the mixture.

12. 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

Guidance Note 5

A key basis for a risk assessment is to understand what limits within a drug product present a risk.
Generally, where Identified, structures of concern specifically nitrosamines containing an alkyl carbon *alpha* to the nitrogen that contains at least one hydrogen,[13] should be assessed by safety experts. This group should confirm that the structure of concern is potentially mutagenic and if so determine if an acceptable intake (AI) can be calculated for any novel nitrosamines (i.e., those lacking an AI published established by regulatory authorities) using existing compound specific carcinogenicity data or structural analogues (i.e., read-across) as recommended by ICH M7. If an AI cannot be calculated in this manner, an interim acceptable limit of 44 ng/day until a limit is defined by ICH M7 could be applied but a lower limit of control may be expected.[14] The safety group can also advise whether the AI can be adjusted based on less than lifetime clinical administration in alignment with ICH M7. Control to ICH M7 limits (1.5 mcg per day of LTL equivalent) may be considered for those nitrosamines which prove to be mutagenic within the Ames test without the metabolic activation required for a nitrosamine from the cohort of concern. The assigned level can be used then to understand likely risk in the following steps. For products intended for advanced cancer within the scope of ICH S9 then nitrosamines can be controlled to ICH Q3A/B levels.[14]
Where multiple nitrosamine risks, these would need to be summed appropriately and control to the most potent nitrosamine safety level.[15]

13. https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-assessment-report_en.pdf
14. Committee for Medicinal Products for Human Use (CHMP). Assessment report: Nitrosamine impurities in human medicinal products. EMA/369136/2020. 25 June 2020. Procedure under Article 5(3) of Regulation EC (No) 726/2004
15. https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-questions-answers-marketing-authorisation-holders/applicants-chmp-opinion-article-53-regulation-ec-no-726/2004-referral-nitrosamine-impurities-human-medicinal-products_en.pdf
16. Review of ongoing excipient nitrite testing suggests an average figure of 1 to 2 ppm could be applied. In order to retain some conservatism the figure of 5 ppm has been selected for excipients where nitrite content is not known.

Guidance Note 6

It is currently not well understood whether trace levels of a potential nitrosating agent e.g. nitrite will constitute a risk to a DP. In general, for similar formulations, the greatest risk will be those with the highest level of available nitrite in relation to available amine.

An estimation of 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 or by assuming a generic average level of 5 ppm.[16]

If the estimated level of nitrosamine is lower than the acceptable daily intake,[16] 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)

Guidance Note 6 for EFPIA DP Workflow

Guidance Note 6 (Cont.)

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 risk for an oral solid dose product is therefore much less than for solution phase as any nitrite is likely to be less reactive/available.

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

and more realistic conversion is considered to be that described below from discussions across industry from very limited experimental work:

<1% (dry blends), <10% (direct compression), <15% (wet granulation), <20% (freeze dried/amorphous), <50% (suspensions) and 0-100% (creams/syrup/solution)

The actual conversion will be highly dependent of the formulation process and components e.g. pH, particle size, water activity, crystallinity etc.
It is useful to refer to the published calculation,[8] 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 the allowable daily intake then a risk is identified.

If the estimated level of nitrosamine is lower than 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 9)

Guidance Notes 7, 8 and 9 for EFPIA DP Workflow

Guidance Note 7

Once a potential risk has been confirmed for an ICH M7 Class 3 nitrosamine, suspected mutagen with unknown carcinogenic potential, there are two general options i) proceed to confirmatory product testing to quantify the impurity level and/or ii) Ames test to better understand mutagenicity. Whilst a negative Ames test will be a very useful means to de-risk a potential mutagen, confirming mutagenicity is likely to lead to additional studies in order to better understand the risk to the product. Where a risk has been highlighted with the nitrosation of a secondary amine DS, controls for the DP need to be considered in order to minimise any risk. It is also considered likely that available non-clinical safety data and data from pharmacovigilance would be useful in order to contextualise the level of risk that may be involved for the DP.

Guidance Note 8

In progressing to testing, an authentic sample of nitrosated product may need to be obtained to develop a suitable test method. 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 for scientific reasons then this can be used as justification to take no further action (aligns with Guidance Note 2). 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.

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 9 for EFPIA DP Workflow

Guidance Note 9

Packaging risks are considered independent of the formulation process and there needs to be an assessment. Packaging 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 condensation onto the drug product). **See also the separate EFPIA risk assessment principles for consideration for nitrocellulose packing materials**

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 2 might be appropriate.

Moving to nitrocellulose free materials could 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.[17] 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.[8] 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.

17. 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 10 for EFPIA DP Workflow

Guidance Note 10

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.[12] A risk / benefit product analysis, and shortage management, should be conducted prior to assuming a product is unacceptable or recommending an alternative product to patients. Less than lifetime adjustments are likely to be applied to non-chronic therapy and the resulting acceptable daily level(s) will need to be agreed with the appropriate regulatory authority.

With respect to future mitigations to reduce / eliminate the nitrosamine risk, the following can be considered:

- Reducing levels for the vulnerable / reactive amine if appropriate
- Consider sourcing nitrite low / free alternatives if available
- Reformulation may be required

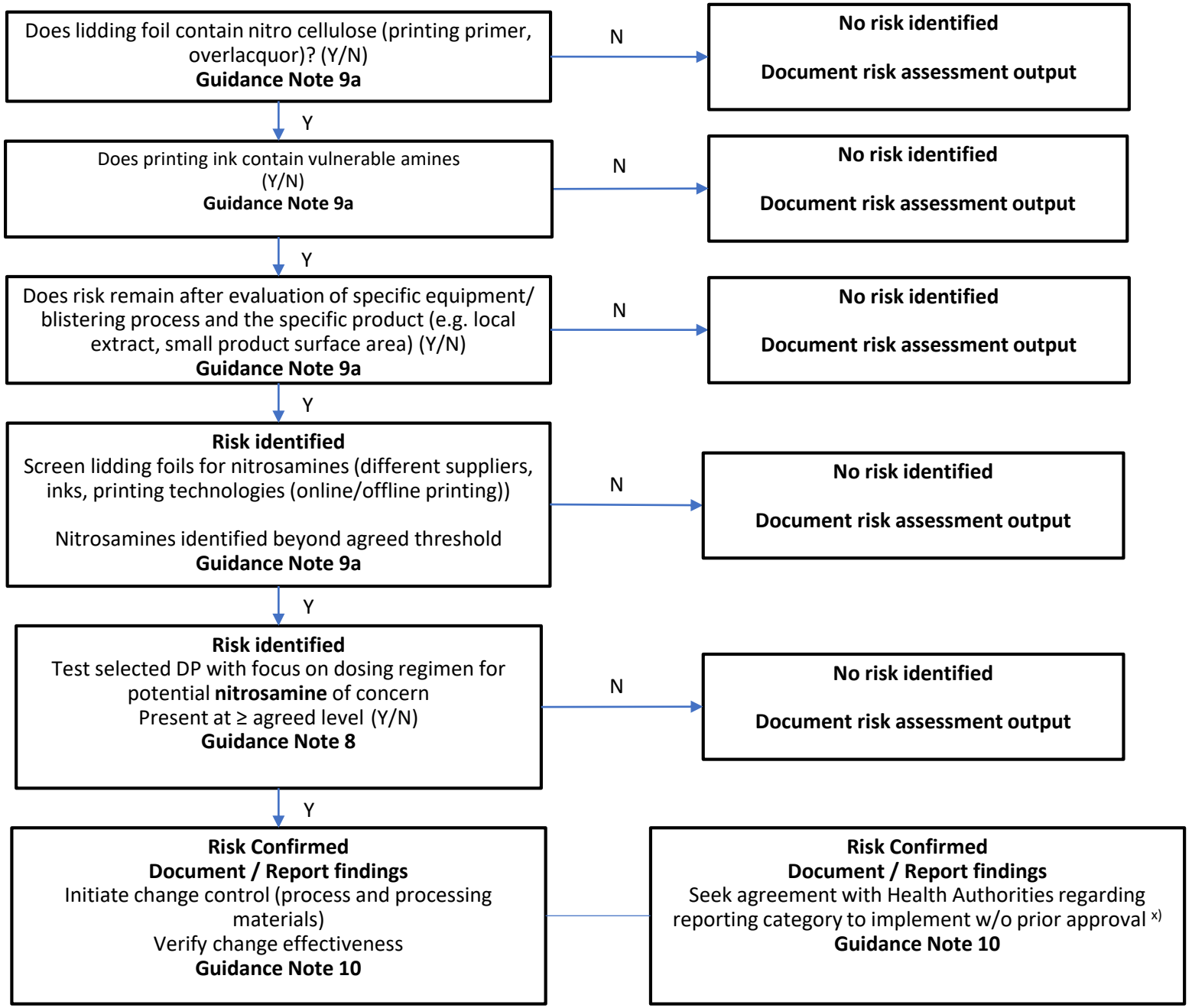
If the development of a new formulation is required, then there are potentially numerous options that can be considered and these include:

- 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 lysine / ascorbic acid within the formulation to act as nitrous acid scavengers

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.

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.

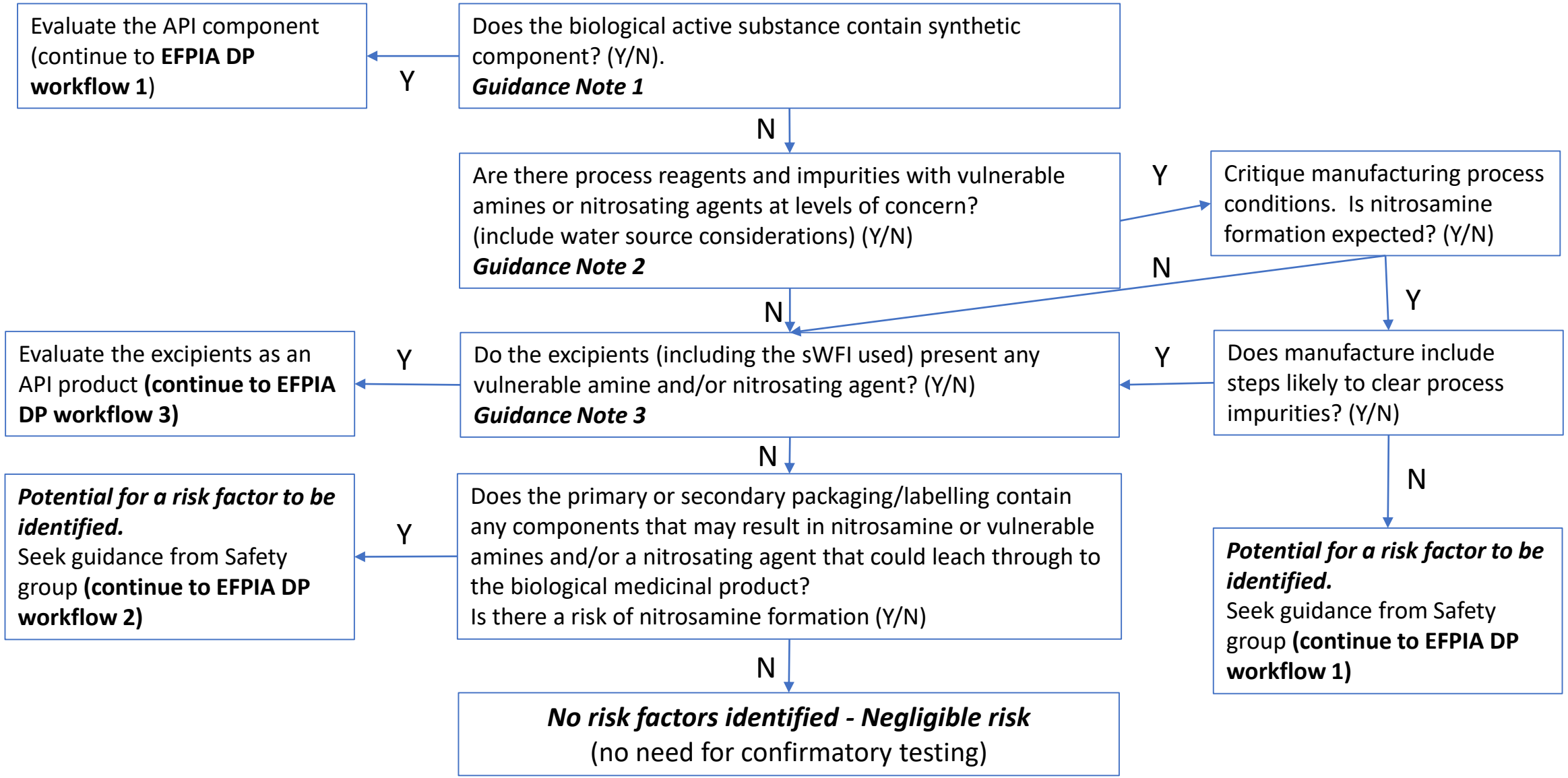
Potential risk removal options are available e.g. moving to nitrocellulose free materials. This change is not considered a requirement but should be considered if there is a multiple dosing regimen that leads to this potential risk being more significant. This change should be considered to be implemented 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 sterile Water for Injection (sWFI). The manufacture of sWFI by distillation (or combined reverse osmosis/ultrafiltration and demonstrated of equivalent or superior quality to distillation[2]). This manufacture is expected to result in negligible levels of nitrate/nitrite in sWFI, thereby removing risk of nitrosamine formation and the answer with respect to the sWFI would be 'N'. However, 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.