

**N-Nitrosamine Impurities in Biological Medicinal Products**

**Executive Summary**

Taking the biological active substance, manufacture, excipients and packaging into consideration, it can be concluded that although vulnerable amines are present in biological medicinal products (from the active substance and certain excipients), there is generally expected to be no meaningful exposure to nitrosating agents or adventitious introduction of N-nitrosamines into biologically-derived medicinal products. It is the position of the EFPIA member companies that it is appropriate to apply a science-based, holistic approach to evaluating the risk nitrosamine impurities present to biological medicinal products. This approach should include consideration of risk and control elements. Risk of incorporating nitrosamines or nitrosating elements into biologics are considered largely hypothetical, and in cases of theoretical occurrence, are expected to be controlled via the application of purification chromatography steps and ultrafiltration/diafiltration during manufacture. There is also expected to be a scavenging effect of any adventitious nitrosating agent, by reaction with the abundant primary amine functionality present within biological products, leading to non-nitrosamine by-products.

 In summary, for the majority of biological products, including cell and gene therapies and vaccines, there is negligible risk from nitrosamine impurities, and confirmatory testing under EMA Article 5(3) should not be required. Further assessment may be needed for those biologics which are conjugated with a chemically synthesised entity (bioconjugate) or those administered with high volumes of water.

**Introduction**

Using the procedure of Article 5(3), the CHMP has finalised its report on the evaluation of the risk presented by N-nitrosamine impurities in human medicines (EMA/369136/2020) and the implementation plan (EMA/425645/2020), which includes all biological medicinal products within scope [1], [2], [3]. This EFPIA position considers the risk of nitrosamine impurities, as well as the impact of Article 5(3), on biological medicinal products being manufactured and developed in the biopharmaceutical industry. The risk of N‑nitrosamine impurities being introduced into biological products is discussed in four parts:

1. active substance
2. chemically modified active biological substances
3. excipients (including water)
4. primary and secondary packaging/labelling

The term ‘biological medicinal products’ is understood in accordance with Directive 2001/83/EC as a product containing a biological substance as the active component, where (with noted exceptions e.g. certain antibiotics) a biological substance is extracted from a biological source. Therefore, the scope of the biologic medicinal products discussed in this position includes vaccines, Advanced Therapeutic Medicinal Products (ATMPs) and recombinant therapeutic proteins. The EFPIA member companies understand that a Step 1 risk assessment is required to be performed on all classes of biological medicinal products, with Step 2 (confirmatory testing) and any subsequent Step 3 (Updating of Marketing Authorisations) activities completed where a risk is identified. This document outlines a holistic, science-based approach to the considerations for a risk assessment of biological products. The discussion should be understood as applicable to all classes of biological medicinal product within the scope of the nitrosamines Article 5(3), unless otherwise described.

The overall conclusion, which aligns with that of the EMA‑BWP, is that most biological medicinal products present no risk of nitrosamine impurities.

A workflow is provided (Annex 1) to assist Marketing Authorisation holders in completing a risk assessment for their biological medicinal products according to the key principles described in the following sections.

***1. Active Substance***

The active substances of biological medicinal products typically consist of proteins, virus particles or cells. Generally, when not subject to chemical derivatisation, such active substances do not contribute nitrosamine risk factors to the finished product and can be considered of negligible risk due to the following general considerations:

1. Biological active substances are manufactured and formulated into (including the cleaning of primary packaging components) and stored in Water for Injection (WFI, PhEur 0169),
2. Most manufacturing steps for biological active substances (and products) are sub-optimal for nitrosation reactions (time, temperature, pH, nitrosating agent concentration),
3. Liquid biological medicinal products are long-term stored under low temperature conditions that would reduce the likelihood of nitrosation which is temperature dependent,
4. Low mass, small molecule impurities are inherently cleared in the manufacturing process by standard unit operations such as bind/elute chromatography, and ultrafiltration/diafiltration that all purge low molecular mass impurities.
5. Biological active substances are structurally complex and highly likely to contain ‘N-nitrosating agent scavenging’ reactive groups (e.g. lysyl, cysteinyl), leading to non-nitrosamine by-products
6. Biological active substances are typically too large for cellular metabolism/activation via cytochrome P450 dependent enzymes to generate a potent mutagenic species from any nitrosamine formed from the active substance,

The following provides additional discussion on aspects of the risks of N‑nitrosamine impurities, as applied to biological medicinal products:

*a) Biological Active Substances are Manufactured and Formulated into and Stored in Water for Injection.*

Overall, the risk posed to the majority of biological products from the use of purified water or WFI is negligible. However, some consideration of potential risk factors should be made.

The EMA Article 5(3) Assessment Report for ‘Nitrosamine impurities in human medicinal products’, lists reaction of residual disinfectant in water with vulnerable amines as a potential source of nitrosamine contamination when there are also residual levels of nitrosating agent in the water [1]. Vulnerable amines may be present as a leachate from specific anion exchange resins used during water treatment. Disinfected water may be used as a source to manufacture the Purified Water and WFI used in the manufacture of biological products [4]. Together water quality could constitute a potential risk factor that should be considered in the risk assessment.

1. *Manufacture of Purified Water*

The content of nitrosamine in Potable Water used to produce Purified Water (PhEur 0008) is typically very low [1], [5] and not considered to present a significant risk to biological medicinal products. Concerning nitrosating agents, Purified Water is depleted in nitrite and nitrate, compared to the allowed and recorded levels in Potable Water [6]. No risk is presented by the nitrite content in Purified Water due to the clearance expected to be provided by the downstream purification steps to manufacture Water for Injections (WFI) used in the manufacture of biological medicinal products.

The potable water used as the source material to manufacture Purified Water may have been disinfected, by e.g. addition of chlorinating agents such as chloramine or ozone, which can potentially result in nitrosamine formation [7]. Disinfectants and other oxidising agents would be removed in the preparation of Bulk Purified Water by distillation, ion exchange, reverse osmosis or by any other suitable method. However, procedures to manufacture Purified Water can influence the risk of further nitrosamine being introduced when the potable water used as the source material has been disinfected, and anion-exchange (AEX) chromatography is used to manufacture the Purified Water*[[1]](#footnote-1)*. It is therefore recommended that medicinal product manufacturers understand how their source water, Purified Water or WFI have been treated; with attention to any amine sources such as anion exchange resins combined with source water that has been treated with an oxidising agent such as chloramine.

1. *Water for Injections Manufactured from Purified Water*

Manufacturing processes for Water for Injections (WFI, Ph Eur 0169 [ref]), are expected to deplete any nitrosating agents in the Purified Water*[[2]](#footnote-2)*. However, depletion of trace volatile nitrosamines will depend on the operating conditions for distillation and the formation of any azeotrope of the nitrosamine and water [8 and references therein].

It is considered that the water used in manufacture, and the final WFI storage or reconstitution solvent, present no meaningful risk of N‑nitrosamine presence to most biological medicinal products given the ng/L levels of nitrosamine detected in water manufactured using AEX chromatography. Only in the case of products with a high maximum volume dose, WFI manufacture may be evaluated as a potential risk with particular attention given to AEX chromatography if it is part of the Purified Water manufacturing process.[[3]](#footnote-3)

*b) Biological medicinal products are manufactured at sub-optimal conditions for N-nitrosamine formation.*

The manufacturing process is unlikely to favour nitrosation by any nitrite in water since for most process steps, the conditions are typically far from optimal for N-nitrosamine formation, with nitrosating agents only at trace impurity levels, if at all present.

The rate of nitrosation of vulnerable amines in protein or amino acids will depend upon the concentrations of the nitrite and the amine in addition to the pH, as described in the literature [6]. This reaction is optimal at or below pH 3.25 for low nitrite concentrations (< 1×10-5 M)[[4]](#footnote-4) and the rate of reaction increases with increasing temperature. Notwithstanding that the presence of nitrosating agent is anticipated to be very low in the purified water medium, virus inactivation in biological product manufacture is typically performed at low pH (typically pH 3 to 4), the concentration of active substance is low , the duration is short (typically 1 to 3 hours) and temperature is low (typically ambient temperature less than 25 ⁰C), compared to optimal reaction conditions. We conclude that there is no overall additional risk presented by viral inactivation due to the mitigating considerations discussed in this document: negligible levels of nitrosating agent, the presence of scavenging reactive groups, clearance of small molecular mass impurities and the negligible risk presented by large nitrosated protein.

It is noted that chromatography and ultrafiltration/ diafiltration steps introduce new process reagents or raw materials as well as product contacting surfaces. Indeed, anion exchange (AEX) or mixed mode anion (MMA) resins may have vulnerable amine groups with the potential for trace quantities to leach as the amine or, after any nitrosation reaction, the corresponding nitrosamine derivative. Therefore, the risk presented by these materials and for leachates also needs to be considered for nitrosamine formation, such as any downstream clearance/purge steps.

Resins used to purify biological medicinal products are stable under operating, cleaning and regeneration operating conditions and unlikely to be exposed to oxidising or dealkylating conditions. The resins would only be exposed to very low levels of nitrosating agent in the aqueous media, with transient exposure and unfavourable reaction conditions. In conclusion, the risk of nitrosamine formation and leaching from AEX or MMA resins is expected to be extremely low.

In summary, it is considered of negligible risk that N-nitrosamine or a nitrosating agent impurity would be introduced from process or raw materials and as discussed above (a), the concentration of nitrosating agent in the water used is negligible. Nevertheless, these considerations should be assessed in the nitrosamine risk evaluation.

*c) Biological medicinal products are stored under conditions unfavourable for temperature-dependent nitrosation reactions*

Biological medicinal products are stored under conditions chosen to maintain product integrity through shelf-life to the point of administration. Therefore, most biological drug substances and products are stored frozen (drug substance) or refrigerated (drug product, 2 - 8⁰C), with certain ATMP’s stored as low as at -70⁰C. The frozen storage condition greatly reduces diffusion rates for chemical reaction, when below the glass transition point for the formulated active substance or product. Such storage conditions would not be conducive to N-nitrosamine formation with any adventitious nitrosating agent that may be present. Any short-term, end-user ambient temperature storage or extended in-use storage such as a product pre-prepared in intravenous bags, should also be included in the evaluation and considering time, temperature, nitrosating agent content etc. For transient storage (e.g. manufacturing intermediates, or final user preparations) no significant increase in risk is expected.

*d) Low mass small molecule impurities (from reagents, raw materials and by-products, etc) are cleared during the manufacturing process*

The manufacture of biological medicinal products involves standard processes that are proven efficient in clearing small molecules from the active substance such as orthogonal, bind/elute and size-based chromatography steps and a final ultrafiltration/diafiltration step that are designed and controlled to effectively deplete small molecular mass impurities to well below any level of concern [10]. These physical purification processes are known to be very effective. As an example, recombinant proteins expressed in biological systems undergo extensive purification involving multiple chromatography and filtration steps designed for clearance of impurities (product or process related) and are validated to clear, or routinely monitored for depletion of, process impurities of potential product safety concern. Due to the vast molecular mass difference between a biological active substance and the small molecular mass of potential process residual impurities, these clearance steps are typically several fold more effective at depleting small molecular mass entities than the crystallisation steps often used in API chemical syntheses to purge impurities. Similarly, large mass process impurities such as Protein A leachate or host cell protein or nucleic acid are not of concern for nitrosamine impurities since there are also effectively cleared and controlled in the manufacture of recombinant protein by the chromatography steps and ultrafiltration/diafiltration. Protein or peptide process impurities would also contain primary amine or thiol groups to act as scavengers of nitrosating agents.

Given that many biological medicinal products use several orthogonal chromatography steps with a final ultrafiltration/diafiltration step, clearance factors of small molecular mass impurities in the order of thousands are typical.

As discussed in Gong (2018) [10] and in the EFPIA position [11], the mass difference between a small molecule impurity and the biologic active substance means that the impurities are at a much smaller proportion of administered product than would be the case for a chemically synthesised API medicinal product. This mass difference should be a consideration in assessing risk associated with dose and posology.

*e) Primary amine (and thiol) groups in biological products are likely to ‘mop up’ nitrosating agents*

Biological products contain reactive groups which can behave as scavengers of nitrosating agents, e.g. primary amines, primary alpha-amides and thiols in the active substance structure or on excipients. Nitrosation of these scavenger groups would not lead to N‑nitrosamine formation [12], [13], [14].

Polypeptides or proteins, provide a source of secondary amines potentially susceptible (e.g. tryptophanyl, histidyl, prolyl residues in polypeptide) to reaction with nitrosating agents (for example nitrites in water), though only the molecule’s outer, solvent accessible amino-acid side chains would be expected to be available for any nitrosating reaction. Although it is secondary or tertiary amines that have potential to form mutagenic N-nitrosamine derivatives, primary amines and thiols may also react with nitrosating agents. However, amino acids with primary amines (lysine), or thiols (cysteine) and the protein N‑terminal primary amine group (an exception being any N-terminal proline) form unstable primary nitrosamine intermediates that rapidly decay to the hydroxyl form and nitrogen [14]. Therefore, primary amine (and free thiol) groups on protein or polypeptide may be considered as a scavenger of nitrosating agents [12] and react with similar rate constants as secondary amines [13].

*f) Activation of nitroso-protein to form a potent mutagen is highly unlikely.*

Large molecules with any trace N-nitrosamine, from vulnerable secondary amine substituents in certain amino acid side groups (e.g. tryptophanyl, prolyl groups), cannot be activated to generate a potent mutagenic entity by the cellular mechanisms that activate small molecules. To form a potent mutagen – due to their stability at physiological pH - nitrosamines require metabolic activation by hydroxylation/oxidation to form an α-hydroxynitrosamine that rapidly rearranges to a diazohydroxide form that can alkylate DNA [15], and references 1 and 3, therein]. In cellular systems, this oxidation mostly occurs enzymatically by cytochrome P450 (CYP) isoenzymes. Large protein molecules, with a nitrosamine group, would be sterically unfavourable substrates for CYP binding and activation in which the N‑N bond needs to be in proximity to the haem group of CYP 16, 17]. The haem group is buried within P450 and access is only through structural channels which restricts the size of substrates. Furthermore, mutagenicity of most nitrosamines has been shown to decrease significantly as the size exceeds 12 to 14 carbons [18].

Additionally, cell compartmentalisation is a consideration since large, biologically active substances are typically, even if internalised by cells, physically separate from the genome in the cell nucleus, making any direct mutagenic activity unlikely.

Note that since current biological medicinal products are parenterally administered and not via the oral route there is no additional risk of nitrosation through gut flora nitrate/nitrite metabolism.

The totality of knowledge outlined above concludes that the risk evaluation for biological medicinal product active substances, that have not been subjected to chemical treatments such as conjugation with a synthetic entity or those administered with high volumes of water, supports a ‘negligible risk’. It is proposed that all such active substances may generally be categorised as ‘no risk’ of significant N-nitrosamine presence. Nevertheless, the MAH should comprehensively evaluate the potential sources of nitrosating agent and their risk to form or introduce nitrosamines. A holistic approach to the risk assessment is considered appropriate for biological active substances.

***2. Bio-conjugated or Chemically Modified Products***

Human medicinal products that contain a synthetically conjugated API component such as Antibody-Drug Conjugate (ADC) products and PEGylated bioconjugates, were already within the scope of Article 5(3). However, until recently, companies have focused their risk assessments on the synthesis of the API component relative to the dose of that entity and any possible risk presented by the conjugation reaction. Expansion of the scope for Article 5(3) requires that the biological component should also be considered, including entities of biological origin that are subsequently chemically modified and may then be used for bioconjugation. For bioconjugates, including ADC products, the nitrosamine risk evaluation may be performed for the drug-linker synthesis (drug intermediate), the recombinant protein production (drug intermediate) in addition to drug substance manufacture (conjugation of the drug intermediates followed by ultrafiltration/diafiltration to finally formulate the drug substance) and drug product manufacture (filling and closure into the primary container closure system) [11]. As outlined in Gong et al., (2018) [10], the active substance purification following bioconjugation reaction, including ultrafiltration, greatly reduces any impurity from the drug-linker synthesis expressed as a (mass ratio) percentage. The final, conjugated bulk drug product would be protected from nitrosation forming N‑nitrosamines as described in Part 1 for the active substance.

As discussed above the risk presented from a typical, chemically modified protein, protein bioconjugate or ADC is likely to be concluded as negligible unless there is a particular risk presented by the chemical nature of the synthetic component and its manufacture.

***3. Excipients***

In general, excipients used to formulate biological medicinal products should not be assessed any differently to excipients used for products containing chemically synthesised APIs, including assessment of risk arising from the manufacture of the excipients [19]. The risks associated with impurities in sourced excipients is adequately treated in the EFPIA position on N-nitrosamines in products containing chemically synthesised APIs and is within the scope of this position for biological medicinal products.

As discussed above, in the context of biological active substances, biological products are usually stored as refrigerated liquid (in aqueous solution or suspension), ‘frozen liquid’ or lyophilised, formulated to give a physiologically compatible pH of the product – under conditions unfavourable for nitrosamine formation. In general, the sole potential sources of nitrosating agent to consider are from any introduced impurity within the raw materials including the excipients. Furthermore, solid phase pharmaceutical forms, e.g. frozen or lyophilised are expected to have a greatly reduced rate of nitrosation reaction especially given the negligible levels of nitrosating agent that could conceivably be present given the clearance/purge steps in the manufacturing purification steps.

*Excipients used to Formulate Biological Medicinal Products*

The excipients used for biological medicinal products can be different to those used to formulate chemical API products and can be required for very different purposes due to the additional complexity of biological structures and their sensitivity to the matrix of excipients and storage conditions [20]. The integrity and activity of protein and cell-based products in aqueous solution, through shelf-life, would be expected to be sensitive to many factors including pH and temperature and therefore, buffering agents are typically employed along with osmolality regulators such as sucrose and stabilising excipients such as polysorbate to minimise product aggregation and effects of surface interactions. Lyophilised biologics also contain bulking agents such as mannitol.

Cell and gene therapy products may be stored in aqueous media containing human serum albumin (HAS) or cryopreservatives such as dimethylsulphoxide. Any HAS, would provide an ample source of primary amine and thiol groups to scavenge any nitrosating agents.

As discussed in the API DP workflow, Guidance Note 5; Excipients at risk of forming structures of concern, specifically N-nitrosamines containing an alkyl carbon alpha to the nitrogen that contains at least one hydrogen[14, guidance note 5], should be assessed by the appropriate company safety group with an acceptable intake (AI) for novel N‑nitrosamines (i.e., those lacking toxicology data to calculate an AI) of 18 ng/day .

The excipients used for biologicals are worthy of some further reflection since certain amino acids used as excipients for biological medicinal products have amine bonds vulnerable to nitrosation e.g. histidine [21], proline [22], arginine [23]. It is also noted that a few biological medicinal products include EDTA (ethylenediaminetetraacetic acid) as a heavy metal chelator which has a reactive tertiary amine group. However, tertiary amines display slow 2‑step kinetics to form nitrosamines, requiring an initial oxidative dealkylation step prior to any nitrosation. Any risk of nitrosamine presence through the use of EDTA should be assessed as per chemically synthesised API products but is not expected to present additional risk to biological products due to their relatively very low reactivity compared to secondary amines.

*Amino Acid Excipients*

Most current, biological medicinal products are formulated using excipients that are not susceptible to nitrosation (e.g. acetate, citrate, phosphate-buffered saline) and present no risk of N-nitrosamine impurity formation *per se*. Other excipients (or adjuvants) have primary amine groups (e.g. L-glutamate, L‑arginine) which can act as nitrosating agent scavengers.

Excipients or adjuvants that do contain vulnerable amine groups (e.g. L-histidine, L-proline, L-arginine) could have the potential to form N-nitrosamine impurities and, therefore, have been considered further:

*L-histidine:* L-histidine is a fairly common excipient in the formulation of biological medicinal products, used in low concentrations (e.g. 10 mM) as a buffering agent.  While nitrosation of L-histidine is possible only one derivative, (1‑nitroso-1H-imidazol-4-yl) acetohydroxamic acid (NIAH), has been shown to be mutagenic. NIAH is not in the so-called ‘cohort of concern’ [24] since it cannot follow the same mechanism of action with respect to mutagenicity [21] as the highly mutagenic N-nitrosamine impurities that require CYP activation and progress via a diazonium ion [25]. Furthermore, NIAH is formed by the action of multiple equivalents (x 4) of nitrosating agent which under conditions of negligible nitrosating agent content (from WFI) is considered highly unlikely[[5]](#footnote-5).

*L-proline*: While nitrosation of the secondary amine of L-proline is possible, any nitroso-proline has been shown not to be carcinogenic as demonstrated in animal studies when L-proline and nitrite are co-ingested [26,27, 28,29].

*L-arginine:* L-arginine is a common excipient in the formulation of biological medicinal products to reduce protein aggregation and enhance thermal stability [20].  While L-arginine has no secondary or tertiary amine, nitrosation of the guanidino group of L-arginine is possible and occurs as part of endogenous cell metabolism to generate NO. However, the resulting derivative is not a nitrosamine but a nitrosourea form [31]. Studies indicate little to no carcinogenicity in animal co-fed arginine and nitrite compared to nitrite alone [32] and weakly mutagenic in an Ames test using one strain of salmonella [23]. Furthermore, the L-arginine primary amine group may also be nitrosated and hence act as a scavenger of low levels of nitrosating agent. It is concluded that the nitrosation products from L-arginine are not within the Cohort of Concern [24] and should be considered in terms of ICH M7.

***4. Packaging***

*Primary packaging*

Biological medicinal products are typically stored in impermeable glass or low permeability resin containers. These containers typically use elastomer stoppers and the risk of any ingress from the external environment is controlled through qualification of the container closure system in Container Closure Integrity studies. The contribution of any vulnerable amines from elastomer leachates to N‑nitrosamine formation is also considered to be negligible due to the absence of any significant level of nitrosating agent. Current manufacture of commonly used elastomer stoppers is not known to create any nitrosating agent nor are any nitrosamines expected to leach into product, as would be detected in extractables and leachate studies. However, since certain older elastomer formulations are cured using processes that result in nitrosamines, it is advisable that the MAH obtain documentation from the supplier that assures the absence of nitrosamines.

Blisters or sachets of proteinaceous powders would require similar consideration as small molecule tablets and capsules.

The mechanism of sterilisation for packaging components should also be a consideration with particular attention to any use of nitrogen dioxide (may form N2O3 and N2O4 that can rapidly react with secondary amines to form N-nitrosamine [33] to sterilise packaging components prior to filling and assembly or as a terminal sterilisation procedure after filling and stoppering of the primary container with the medicinal product [34].

As for active substance, intermediates and drug product manufacture, the risks presented by the immediate (primary) packaging components should be evaluated and the risk assessment documented.

*Secondary packaging*

Biological medicinal products are packaged into primary containers such as vials, syringes or cartridges and are thereby isolated from the external environment. Although some biological medicinal products are then packaged into a blister tray and lid, secondary packaging does not present any added risk of nitrosamines reaching the product.

**Conclusions**

A holistic approach to the evaluation of the risk of N-nitrosamine presence in biological medicinal products is presented in this consensus position for the EFPIA member companies. Key considerations are discussed that may be employed in the risk assessments required by EMA Article 5(3) Assessment Report [1]. In summary, it is expected that, for the vast majority of biological medicinal products with no synthetically-derived component and that are not chemically-modified, there should be no risk from the active substance or its manufacturing process, and no further risks from the formulation and packing materials. Nevertheless, we cannot exclude exceptions and the conclusion of ‘no risk’ should be confirmed for each medicinal product on a case-by-case basis.

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**Abbreviations**

|  |  |
| --- | --- |
| ADC | Antibody Drug Conjugate |
| AEX | Anion Exchange  |
| AI | Acceptable Intake |
| CHMP | Committee for Medicinal Products for Human Use |
| ATMP | Advanced Therapy Medicinal Product |
| API | Active Pharmaceutical Ingredient |
| CYP | Cytochrome P450 enzyme family of genes |
| DP | Drug Product |
| EDTA | Ethylenediaminetetraacetic acid |
| EFPIA | European Federation of Pharmaceutical Industries and Associations |
| EMA | European Medicines Agency |
| BWP | Biologics Working Party |
| MMA | Mixed-Mode Anion |
| NIAH | (1‑nitroso-1H-imidazol-4-yl) acetohydroxamic acid  |
| WFI | Water For Injection |

**Contributions**

The contribution of the following EFPIA MQEG Biomanufacturing and MQEG CMC sub-team members to the development of this paper is acknowledged:

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1. Quaternary ammonium anion-exchange resins can form nitrosamine impurity only when exposed to a dealkylating/oxidising agent followed by nitrosation, of which a low proportion of the nitrosamine may leach into the Purified Water. The degree of leached nitrosamine depends on the specific amine structure as well as source water quality for nitrosating and oxidising agent content and the operating conditions for the AEX chromatography (prewashing the column can substantially reduce the amount of leachate in the Purified Water) [9]. [↑](#footnote-ref-1)
2. Throughout this paper, reference is made to the negligible level of nitrite or nitrosamine in the water for injection (WFI) used to manufacture and store biological medicinal products. WFI manufactured from Purified Water is expected to be essentially free of nitrosamine, nitrite or other known nitrosating agent present. However, the capability of the WFI generation process should be evaluated for depletion of nitrosamines and their precursors. [↑](#footnote-ref-2)
3. A risk assessment approach is proposed since the volume limit which would define a high dose volume of water for a product, is not formally defined and the impact of a theoretical ng/L level of nitrosamine depends on the quality of the Purified Water and WFI. [↑](#footnote-ref-3)
4. The concentration of nitrite in Purified Water of WFI is expected to be far below 2×10-7 M (approximately 0.01 mg/L) as this level is typically seen in potable water. [↑](#footnote-ref-4)
5. Chemical kinetic considerations mean that it is highly unlikely that trace levels of nitrosating agent and molar excess histidine would react in multiple sequential chemical reactions to form NIAH. [↑](#footnote-ref-5)