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Perspective

The Nitrosamine "Saga": Lessons Learned from Five Years of Scrutiny

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ABSTRACT: The onset of the *N*-nitrosamine (NA) saga in 2018 was chiefly related to certain small dialkyl *N*-nitrosamines originating from the synthesis of the active pharmaceutical ingredient (API). However, the subsequent comprehensive assessments performed on APIs, formulated drug products, and packaging put a different type of NAs in the limelight: a diverse range of complex so-called nitrosamine drug-substance-related impurities (NDSRIs). They may form due to the presence of potentially nitrosatable secondary or tertiary amine moieties in APIs or API impurities and nitrosating agents formed from low levels of nitrite present as impurities. The unique properties of the amine functional group make it irreplaceable in the synthesis of APIs. While nitrite levels may be reduced, the formation of NAs in drug products cannot be completely prevented, and the class default acceptable intake (AI) of 18 ng/day currently poses significant challenges in terms of both viable control and analysis at such low levels. Even so, NA exposure through pharmaceuticals is expected to be orders of magnitude lower than the exposure via food or endogenous formation. While robust carcinogenicity data are available for many of the small, simple NAs, there is a distinct absence of such data for most NDSRIs. Many working groups have therefore been established to share data and rapidly improve knowledge (whether in terms of toxicity data, structure−activity relationships, or analytical techniques), to define best practices to assess the genotoxic potential of NDSRIs, and to advance methods to calculate AIs based on solid scientific rationales. Ultimately, to protect patients from true cancer risk and secure access to important medicines, it is crucial for manufacturers and health authorities to pursue efforts to implement NA control strategies that are equally effective and realistic. As patient safety is paramount, the pharmaceutical industry is committed to ensuring that the medicines it supplies are safe and effective. Where legitimate safety concerns exist, it is undisputed that appropriate actions must be taken, which could include withdrawal of products from the market.

KEYWORDS: *nitrosamines, nitrites, amines, NDSRIs, risk assessment, acceptable intake, read-across, data sharing, Ames, carcinogenicity*

1. INTRODUCTION

We are already in the fifth year of the *N*-nitrosamine (NA) saga, which has been a critical concern for pharmaceutical manufacturers and regulators alike since the first detection of *N*-nitrosodimethylamine (NDMA) in valsartan back in June 2018. NDMA and other small and potent NAs stayed in focus, as they were soon after also found in other sartan active pharmaceutical ingredients (APIs),[1,2] in piaglitazone and ranitidine,[3,4] and in metformin drug products.[5,6]

The picture, however, changed dramatically with the recall of Chantix (varenicline) batches in the USA, Canada, and the EU in mid-2021 due to the presence of the nitrosamine drug-substance-related impurity (NDSRI) nitrosovarenicline.[7,8] This was rapidly followed by recalls of propranolol,[9] quinapril,[10] and orphenadrine[11] medicines due to the presence of their respective NDSRIs (Figures 1 and 2). It is to be noted that these recalls were done with the backdrop that there was (and still is) no agreement on how to establish acceptable intakes (AIs) for NDSRIs , based on read-across or biological tests.

While there is comprehensive literature about the formation of NAs from nitrite in solution (e.g., NAP test), the formation of NDSRIs even in solid drug products (DPs) from parts per million levels of nitrite was surprising. It was recently shown that up to 40% of common APIs and 30% of API impurities are potential NA precursors, as they contain vulnerable amine moieties. If only the more reactive secondary amines are considered, still 13−15% of APIs are potentially at risk.[12] Not surprisingly, NDSRIs have become the focus from both an industry and regulatory perspective.

Industry and regulators are now in the challenging situation that NDSRIs may be present in hundreds if not thousands of medicinal products, some of them affecting whole essential drug classes such as β-blockers.[12] A survey conducted by Medicines for Europe among pharmaceutical manufacturers and presented at the fourth meeting of the EMA Nitrosamine Implementation Oversight Group (NIOG) with Industry Associations on November 30, 2022, revealed that so far

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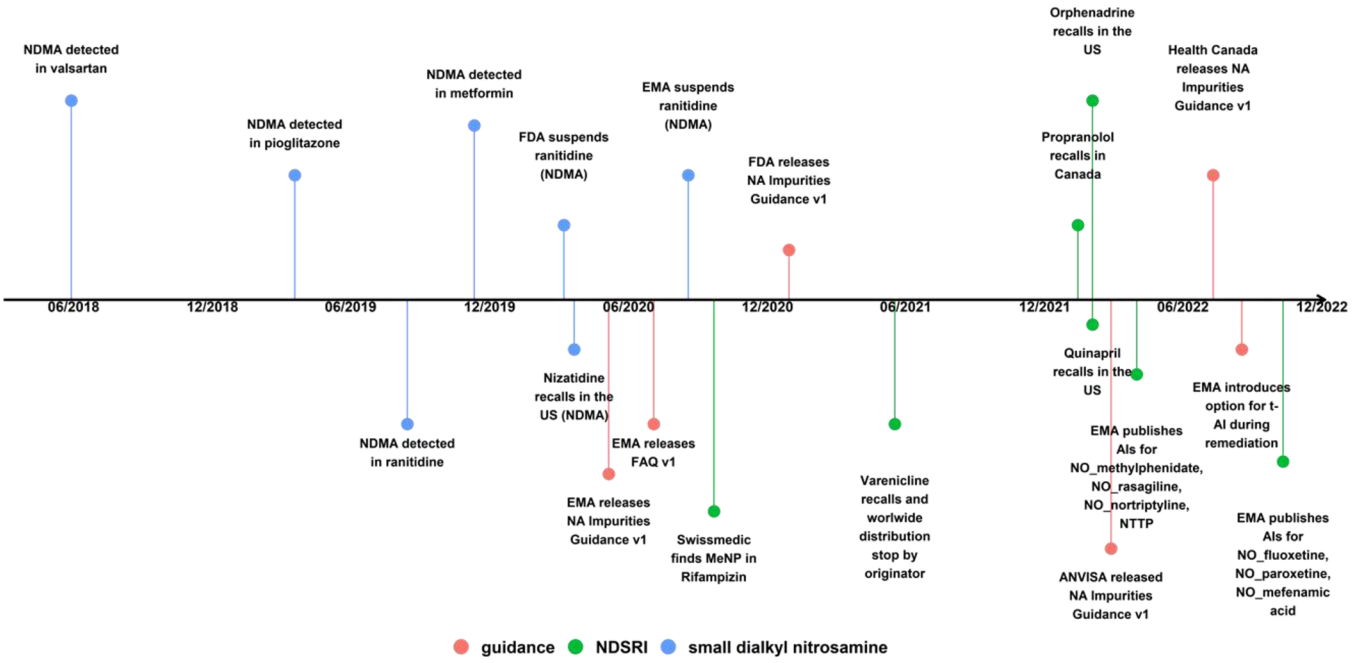

Figure 1. Important occurrences related to NAs in pharmaceuticals in terms of initial detection of small potent NAs and NDSRIs, industry and regulatory responses (recalls, suspensions), and publication of regulatory guidance.

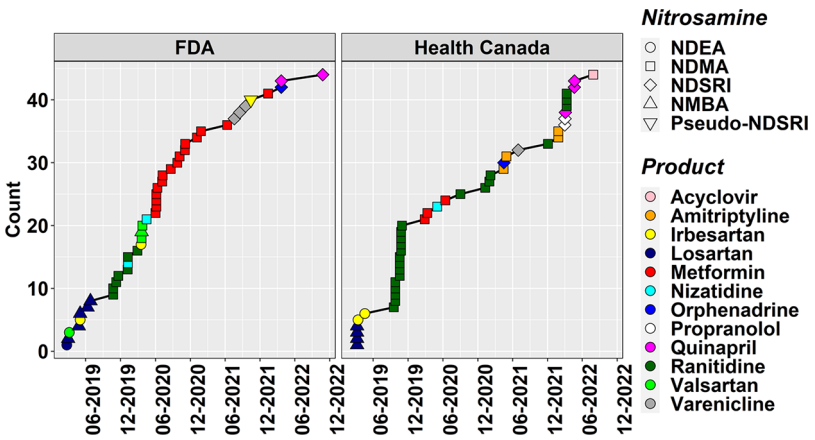

Figure 2. NA-related batch recalls in the USA and Canada since the beginning of 2019. Each point represents the recall of one or several drug product batches from individual marketing authorization holders. The point color indicates the recalled product, whereas the point shape denotes the nitrosamine that triggered the recall. "Pseudo-NDSRI" is Nitrosoirbesartan, which is not a nitrosamine because the nitroso group is attached to the aromatic tetrazole ring.

90% of potential NDSRIs identified in NA risk assessments were later confirmed by analytical testing.

This Perspective aims to put the potential risk from NA-containing pharmaceuticals into perspective by reminding us of what is known about NAs from food as well as endogenous NA formation. NAs are formed from vulnerable amines, so we also discuss why these cannot be avoided and, consequently, why pharmaceuticals cannot be made completely NA-free. In addition, we highlight analytical sensitivity challenges arising from the low default AI that is currently applied for some NAs and discuss how more scientifically justified AIs could be derived using read-across. This also examines the potential for the use of AIs in combination with correction for less-than-lifetime exposure and molecular weight. In light of this, the value of *in vitro* mutagenicity testing (Ames test) and data sharing initiatives remains high. Finally, we summarize major achievements of the past 5 years and give an outlook on the developments we foresee for the nearer future.

2. NITROSAMINES FROM FOOD

It has been known for many years that there is substantial human exposure to NAs from foods. A recent review[13] highlighted the publication of 122 NA studies covering a range of both NAs and their sources, a significant proportion of which were related to food. The presence of NAs in food was also highlighted in a recent review of NAs by the EMA.[14]

Focused on peer-reviewed literature published prior to 2017, the review reported that the food classes with the highest total NA content (TNA) were:

1. Fats, oils, and sweets (average TNA 8.9 ± 3.2 ng/g)
2. Meats (average TNA 8.1 ± 1.4 ng/g)
3. Fish (average TNA 5.6 ± 1.0 ng/g)

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4. Vegetables (average TNA 5.4 ± 1.9 ng/g)

Of the NAs, observed the most prominent was found to be NDMA (2.2 ± 0.3 ng/g), suggesting a daily burden in excess of 2 μg/day (based on a 2000 calorie/day diet composed of 500 g/day vegetables, 170 g/day fats, oils and sweets, and 170 g/day meat). A further 1 μg/day exposure may arise from typical consumption of beer or other malt beverages.

As highlighted, the EFSA Panel on Contaminants in the Food Chain (CONTAM) launched a public consultation on the draft scientific opinion on the risks for animal health related to the presence of NAs in food.[14] This document presented an evaluation of the toxicity of NAs, the estimated dietary exposure of European citizens to the carcinogenic NAs present in food, and, based on these, an assessment of the health risks to the EU population. EFSA evaluated 32 NAs and investigated their presence in food. Quantifiable amounts had only been measured for a certain number of the NAs. The risk characterization was therefore limited to the 10 carcinogenic NAs (TCNAs) occurring in food (i.e., NDMA, *N*-nitrosomethylethylamine (NMEA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodipropylamine (NDPA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosomethylaniline (NMA), *N*-nitrososarcosine (NSAR), *N*-nitrosomorpholine (NMOR), *N*-nitrosopiperidine (NPIP), and *N*-nitrosopyrrolidine (NPYR)).

In total, 2817 results for food samples analyzed from four European countries between 2003 and 2021 were assessed, and in addition, the CONTAM panel also examined results from EU countries (n = 3976) and non-EU countries (n = 27) extracted from articles published between 1990 and 2021, selected based on quality criteria. In comparison to ref 13, the EFSA review adopted a slightly different approach to classification of food categories, defining the five food categories "Alcoholic beverages", "Coffee, cocoa, tea, and infusions", "Fish, seafood, amphibians, reptiles, and invertebrates", "Meat and meat products", and "Seasoning, sauces, and condiments". Their evaluation highlighted that in terms of dietary exposure assessment, "Meat and meat products" was the only food category for which data were available for all of the individual TCNAs.

Direct correlation between the two studies is not straightforward. Results of the EFSA review showed TCNAs exposure ranged from 0 to 208.9 ng/kg bw/day across surveys, age groups, and scenarios, with "Meat and meat products" being the main food category contributing to TCNA exposure.

It is interesting to compare exposure levels of nitrosamines from the diet and pharmaceuticals. Focusing specifically on NDMA, the limit for this is defined as 96 ng/day, based on its TD_{50} value. This level is 10-fold lower than the level of the potent mutagen NDMA typically consumed in food; this is without even considering the benefit associated with pharmaceuticals.

It is important to note that other sources of NAs contribute still further to exogenous exposure, including occupational exposure.[15,16] By far the most significant factor is the use of tobacco products, which truly dwarfs all other exposure sources considering that levels above 20 μg/day have been reported.[13]

3. ENDOGENOUS NITROSAMINE FORMATION

The endogenous formation of NAs in the human body is another important aspect to consider when contextualizing the risk posed by potentially NA-containing pharmaceuticals. The presence of measurable levels of NAs has been reported in the blood and urine. The origin of these NAs is somewhat unclear, although what evidence there is indicates significant levels of endogenous formation, which is most likely to occur in the GI tract,[17] where nitrate and nitrite ingestion from food and acidic pH especially in the stomach create favorable reaction conditions. Inhalation of nitrogen oxide species has also been suggested as a further source.[18] NDSRIs from drug−nitrite interactions[19,20] can contribute to the overall endogenous formation, but the more continual exposure stems from the reaction of amines in food. As an example, the NDMA precursor dimethylamine can be formed by spoilage bacteria from trimethylamine oxide serving as an osmolyte in mollusks, crustaceans, and all marine fishes[21] and is therefore commonly found in seafoods.[22] Furthermore, biogenic amines such as spermidine and putrescine have been identified as precursors of NPYR, NPIP, and other volatile nitrosamines.[23,24] What is most remarkable is the possible scale of the endogenous exposure. It has even been suggested that exposure to NAs is dominated by endogenous exposure,[25] this dwarfing exogenous exposure (discounting tobacco) and certainly the burden associated with consumption of NAs through use of pharmaceuticals. During a U.S. FDA workshop, data were reported relating to endogenous NDMA exposure. Levels of endogenous exposure have been estimated based on measured human blood levels.[26−28] Assuming steady state and a clearance rate in humans of 3.45 L/min, levels of NDMA were observed to range between 100 and nearly 2500 μg/day = 1.4 to 35 μg/kg bw/day. NDMA levels were also estimated based on levels of O6-MeG in leukocytes,[17,29] which correlated well with direct measurement of NDMA levels with a mean value of ca. 1360 μg/day (18 μg/kg/day). At present, there has been no definitive comparison made between exogenous exposure and endogenous formation, but given relative levels and the lower levels still associated with exposure through pharmaceutical use, it is clear that the latter may actually be a minor overall contribution to the total human exposure.

4. WHY SECONDARY AMINES IN APIS CANNOT BE AVOIDED

The development of a novel drug is a complicated endeavor which balances several hundreds of properties to obtain a unique and novel balance of pharmacology, pharmacokinetics, safety, and pharmaceutical properties in a single molecule.[30] A medicinal chemist uses various functional groups to affect and optimize these interactions, and one of the most common is the amine. Recently it was shown that ∼40% of APIs contained tertiary or secondary amines which are at risk of NA formation.[12] Aliphatic and aromatic amines are important for the optimization of drug-like properties in several ways and have proven over time to be safe and readily accessible building blocks for many essential drugs. In fact, whole classes of essential medicines rely on the presence of secondary amines for efficacy.

Several essential properties distinguish amines from any other simple organic functional group: Secondary amines have both hydrogen-bond donor and acceptor properties, which is often essential for potently binding to biological targets made of proteins (consisting, of course, of amino acids with many donor and acceptor groups themselves). Amines typically have a high volume of distribution *in vivo*, leading to better pharmacokinetic properties such as longer half-life, leading to better dosing schedules for patients and better medicine compliance. The pK_a of aliphatic amines, which are typically

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Table 1. Important Organic Reactions Involving Vulnerable Amines as an Educt and/or Product[a]

Reaction	Reactant 1	Reactant 2	Reactant 3	Product
Reductive amination	1°/2° amine	aldehyde or ketone	n/a	2°/3° amine
Eschweiler–Clarke reaction	1°/2° amine	formaldehyde	formic acid	3° amine
Mannich reaction	1°/2° amine	formaldehyde	ketone	1°/2° amine (Mannich base)
Petasis reaction	2° amine	electrophile with carbonyl	vinyl-/aryl-boronic acid	substituted 3° amine
Amine acylation	1°/2° amine	acid chlorides, anhydrides, or esters	n/a	1°/2° amide
Nucleophilic aromatic substitution	1°/2° amine	aryl halide	n/a	2°/3° Arylalkyl amine

[a]Secondary amines are marked in red and tertiary amines in blue due to the reduced reactivity of tertiary amines compared with secondary amines.

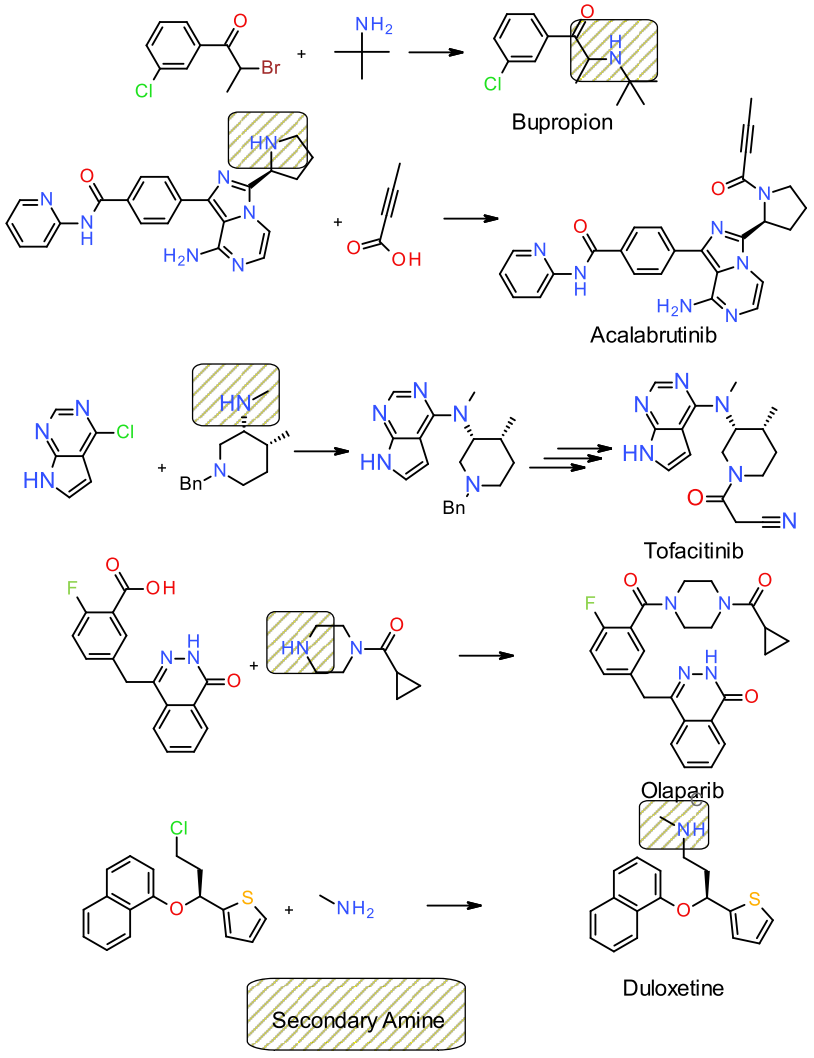

Figure 3. Examples of APIs that are secondary amines or use secondary amine reagents late in their synthesis.

protonated in aqueous solution, typically brings an aqueous solubility enhancement of ∼100-fold versus a neutral molecule, which has the benefit of improving many pharmacokinetic parameters.[31] Aliphatic amines exhibit a 3D structure at nitrogen, which is often required for pharmacological interactions without the synthetic complexity of a chiral carbon center. The availability of a large pool of diverse building blocks as precursors to or containing amines enables access to a vast amount of chemical space for medicinal chemistry structure−activity relationship (SAR) exploration.

The amine functional group also participates in several of the most robust and efficient reactions that are frequently used to make APIs, such as acylation, reductive amination, Buchwald−Hartwig amination, and nucleophilic aromatic substitution (Table 1). Examples of API syntheses that involve secondary amines as a starting material, intermediate, or final

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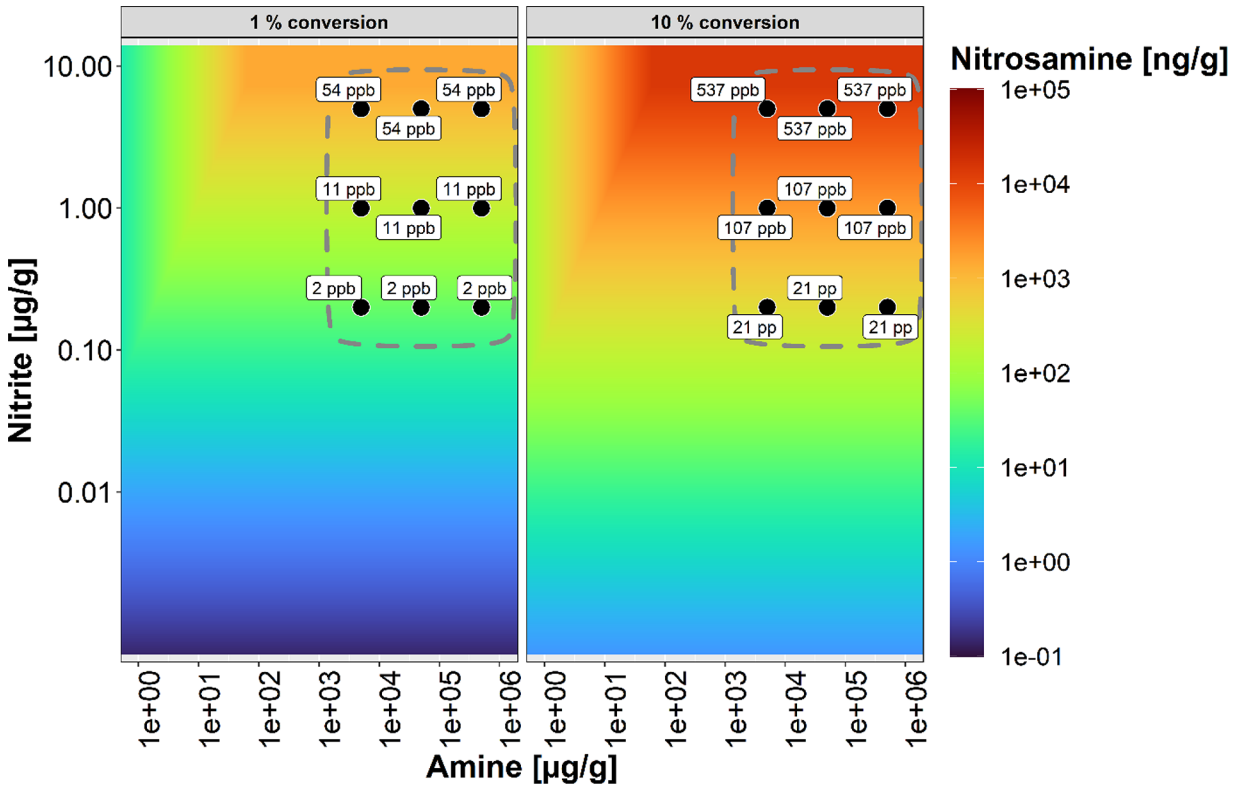

Figure 4. Potential NA content of pharmaceuticals that contain a vulnerable amine. The figures display the content of NA that can be present in a pharmaceutical product assuming that either 1% or 10% of the limiting species is consumed. In most cases, nitrite is the limiting species. Black dots represent the NA contents based on 0.2, 1, and 5 ppm nitrite in combination with 5000, 50000, and 500000 ppm amine, which correspond to drug loadings of 0.5%, 5%, and 50% for the case of NDSRI formation in a formulated drug. The encircled areas represent combinations of nitrite and amine content that can be expected to be most prevalent considering the typical nitrite content of excipients and realistic drug loading.

product are provided in Figure 3. The ability to form salts with acids enables both increased solubility in aqueous environments and the ability to purify by crystallization in large-scale API manufacture. Aliphatic amines are not themselves structural alerts for mutagenicity and in general have safe mechanisms of metabolism and elimination. There is no other functional group which can satisfy such an important and broad range of functions in the discovery and development of an effective medicine as shown above. Aromatic amines are frequently used to connect aliphatic groups to aromatic groups. Thus, many drug-like molecules contain an aryl alkyl secondary or tertiary amine as a linking group.

5. WHY PHARMACEUTICALS CANNOT BE MADE "NITROSAMINE-FREE"

It has been estimated that a typical solid oral dosage form excipient contains on average 1 μg of nitrite/g.[32] Furthermore, it was recently demonstrated that a large fraction of the known APIs and API impurities are potential NA precursors, as they contain vulnerable secondary and/or tertiary amine moieties,[12] for the reasons listed in the previous section. This implies that many pharmaceuticals are at risk of containing NAs formed from those vulnerable amines and nitrosating agents from nitrite in their formulations.

In most cases, the availability of a nitrosating agent will be the limiting factor for NA formation. To illustrate, consider the case of an API with a molecular weight of 1000 g/mol that carries a vulnerable secondary amine moiety. The content of the amine species in the formulated tablet matrix will be in the milligram per gram range, whereas nitrite will be present in the microgram per gram range. Figure 4 visualizes this correlation for 1% and 10% consumption of the limiting species. Depending on the content of vulnerable amine and nitrite, NA amounts exceeding the regulatory default AI of 18 ng or even the 12-month default interim AI of 178 ng can be present per gram of product even if just a fraction of the available nitrite is consumed. In other words, even trace amounts of nitrite from excipients or noncontributary raw materials (NCRMs) can cause relevant amounts of NAs where the API is vulnerable to nitrosation. It is to be noted that the speed at which this conversion occurs will likely depend on the content of reactants, formulation pH, pK_a of the vulnerable amine, water content, temperature, and likely other factors impacting the reactivity, such as particle size (surface area) and degree of crystallinity. Furthermore, nitrosation may be catalyzed by trace aldehydes, which are frequently present at low levels in pharmaceutical excipients,[33] and NO_x species from the air may serve as an additional source of nitrosating agents.

It is to be noted that relevant levels of nitrosation are more likely to be obtained from secondary amines than from tertiary amines, unless the latter contain high levels of the respective secondary amines.[34−37]

6. ANALYTICAL SENSITIVITY REQUIREMENTS VERSUS TECHNICAL FEASIBILITY

When a risk of NA formation has been identified, it must be demonstrated that the content in their product does not exceed a level that corresponds to 10% of the applicable AI, if they want to avoid routine testing.[38] The respective analytical

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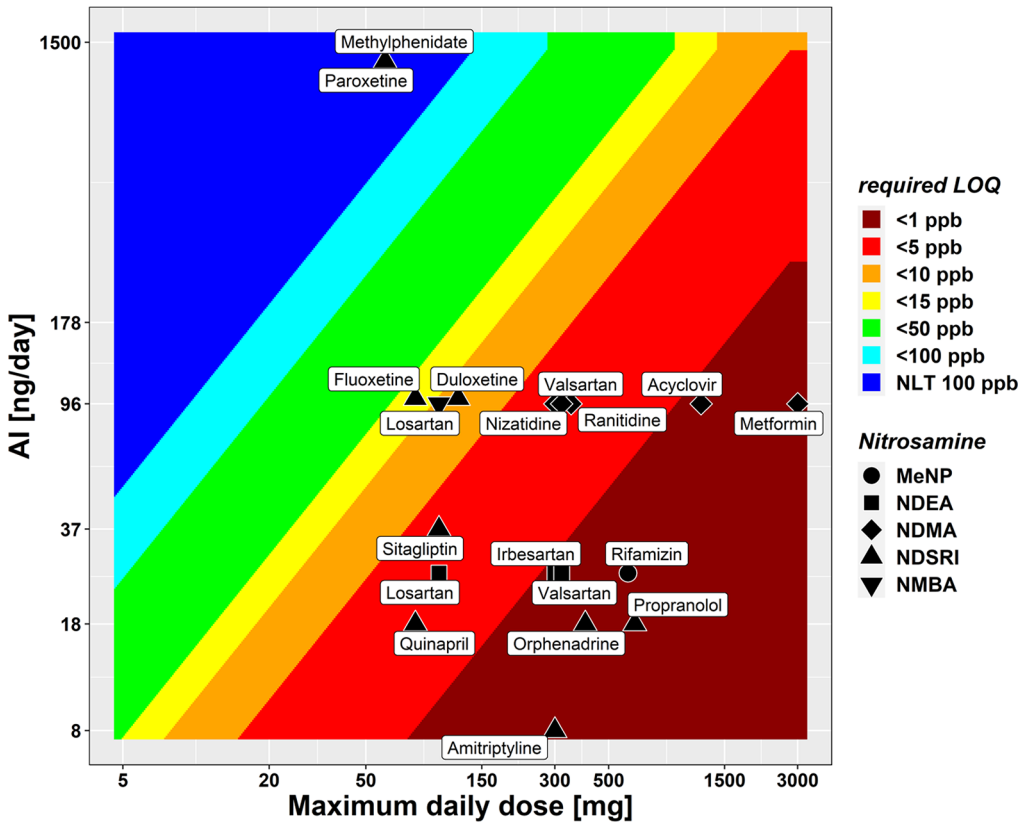

Figure 5. Interrelation of NA AI, API MDD, and required method LOQ, expressed as nanograms per gram of product and assuming a drug loading of 10%. For NDSRIs, calculations were done based on a worst-case AI of 18 ng/day, unless a compound-specific AI is listed in the EMA Q&A, as for nitrosoamitriptyline.

method must have an limit of quantitation (LOQ) that does not exceed this 10% AI level as per regulatory requirement,[38] although technically a limit of detection (LOD) at this level should suffice. The required LOQ (in ng/g of product) can be calculated as follows:

$$\mathrm{LOQ} = \frac{\mathrm{AI} \times 1000 \times \mathrm{DML} \times 0.1}{\mathrm{MDD}}$$

where AI is the acceptable intake per day or lifetime daily exposure (in ng), MDD is the maximum daily dose (in mg), and DML is the drug/mass load of highest dose strength (DML = [dose strength of API in mg]/[dose mass in mg]).

Figure 5 shows the necessary method sensitivity in relationship to the NA AI, the maximum daily dose of API, and the drug load. Plausibly, smaller AIs and larger MDDs require a higher analytical sensitivity, and some unfavorable combinations result in LOQ requirements that exceed what is technically possible. Realistic LOQs are in the range of 5−50 ng/g of product due to the following factors: Sensitivity may be impaired by a reduced ionization efficiency or interfering matrix components. In the case of NDSRIs the API itself can cause major interference, as it is present at much higher concentrations than that of the analyte, and its physicochemical properties will be similar, which complicates effective separation. Another issue of NDSRIs is that their higher molecular weight compared with the small potent dialkyl NAs implies a lower molarity of the analyte and hence potentially reduced method sensitivity.

7. BACTERIAL REVERSE MUTATION (AMES) TEST TO PREDICT CARCINOGENICITY OF NITROSAMINES

NAs are generally considered to be mutagenic impurities and must therefore be controlled according to the ICH M7 guidelines.[39]

As per ICH M7, the first step in the investigation of new potentially mutagenic impurities is to do an *in silico* assessment. Based on their *N*-nitroso moiety, NDSRIs and other NAs will typically trigger an alert for mutagenicity and thus be classified as class 3 impurities (structural alert but no mutagenicity data). Class 3 impurities can, in general, be controlled either at or below the threshold of toxicological concern (TTC) or AI, or an Ames test may be conducted to confirm or disprove potential mutagenicity. In case the Ames test result is negative, the impurity is considered a class 5 nonmutagenic impurity and can be controlled according to ICH Q3A/B limits, being outside the scope of ICH M7. For NAs however, due to their status as a cohort of concern-worthy compounds, they would likely need to be controlled at levels significantly lower than the general TTC of 1500 ng/day. Furthermore, the derisking pathway using the Ames test alone is not yet accepted by health authorities (HAs).

Currently, a negative Ames test conducted according to the OECD 471 test guideline[40] is only accepted for nitrosamines as part of a weight-of-evidence approach but considered not sufficient as sole evidence for absence of mutagenicity.[38] The main concerns of regulators are that there are Ames negatives which have been shown to be *in vivo* carcinogens and, in addition, that the experimental conditions with regard to the type and concentration of solvent or the source of the

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metabolic activation system might not be optimal to detect NA mutagenicity appropriately (EMA Q&A).[38] To address these concerns, many activities are ongoing within industry and also within regulatory bodies (e.g., the U.S. FDA). A significant effort to confirm the conditions for maximal accuracy of the Ames test is being led by the HESI Genetic Toxicology Technical Committee (GTTC). In particular, this committee is working to demonstrate that a negative Ames test can be used as the sole experimental datum to conclude that a nitrosamine is not a mutagenic carcinogen, in alignment with guideline ICH M7. Specifically, an optimized and aligned Ames test protocol is used by all participating companies to test a selection of approximately 30 structurally diverse nitrosamines with mutagenicity and carcinogenicity data to generate a comprehensive data set addressing the regulators' concerns.

Thresher et al.[41] analyzed a large data set extracted from literature, Vitic,[42] and the Lhasa Carcinogenicity Database[43] with respect to the predictivity of the Ames test for the carcinogenic potential of NAs. They found that 18% of NAs were noncarcinogenic and that NAs actually showed a greater correlation between mutagenicity and carcinogenicity compared to non-NAs, indicating that the Ames test is capable of properly predicting carcinogenic potential.[41] Recent publications have shown Ames tests conducted to best current practice, using OECD 471-compliant protocols and strains, can reliably detect the mutagenicity of NAs.[44,45] The authors also addressed the question of whether the choice of solvent impacts the sensitivity of the test. DMSO is one of the most commonly used solvents but was described as an inhibitor of Cyp2E1,[46,47] which is the enzyme predominantly responsible for the metabolic activation of low-molecular-weight NAs.[48] Thus, water has been suggested as the preferred solvent of choice—although the widespread use of this is prevented by issues of solubility when NDSRIs are being tested. Both publications showed that there are no substantial differences in the sensitivities observed when using either DMSO or water.[44,45,45] In addition, for larger NAs, as NDSRIs generally are, other cytochrome P450 isoforms (e.g., 2C9, 2A6, or 3A4) become more important for the α-hydroxylation.[48] This leads to the question of whether the source of the metabolic activation system (S9 liver homogenate fraction) impacts the predictivity of the Ames test. The levels of different isoforms vary between species, which may lead to different experimental results in *in vitro* and *in vivo* studies.[48] The most commonly used S9 is derived from rats pretreated with Aroclor 1254 or phenobarbital/β-naphthoflavone at concentrations of 10−30% v/v used in the Ames test.

Decades ago, it was reported that hamster S9 may be more appropriate for metabolic activation of NAs.[49−51] Based on this literature, it has been reported that some HAs have rejected the negative Ames test on NAs conducted with rat S9 as being not appropriate. Recent investigations on large sets of Ames and carcinogenicity data available in the Lhasa Vitic[52] and Leadscope[53] databases did not confirm a general superiority of hamster S9 compared with rat S9. On the contrary, it could be shown that the specificity of hamster-S9-mediated Ames tests when used to predict carcinogenicity drops significantly compared with rat, while the sensitivity is very similar.[45]

Another variable that is under discussion is the application of a preincubation step, which was initially suggested for low-molecular-weight aliphatic NAs.[54,55] A recent publication comparing plate incorporation versus preincubation protocols showed that only NDMA required the preincubation step to display its mutagenic potential, while all other small alkyl NAs were also reliably detected with the plate incorporation protocol.[44] Trejo-Martin et al. compared the sensitivity and specificity of plate incorporation versus preincubation and did not detect relevant differences between both protocols.[45]

Many of the concerns, uncertainties, and reservations with respect to the Ames test as a robust and reliable method to detect the mutagenic potential of NAs are based on the literature published decades ago, long before the test was standardized and the OECD 471 guideline became available. A considerable fraction of Ames data from those days were derived with protocols that were less standardized and used not all or only the currently recommended strains or other test conditions. Thus, the robustness of these early Ames tests is in some cases questionable. To refine the protocol, there are several initiatives ongoing within HAs (e.g., FDA), international working groups (e.g., HESI GTTC, EMA-MutaMind), and pharmaceutical companies aiming to optimize the Ames protocol.

8. DEFINING A COMMONLY ACCEPTED STRATEGY FOR READ-ACROSS

As certain of the well-characterized low-molecular-weight NAs are exceptionally potent carcinogens (e.g., NDEA and NDMA), with AI levels far below the TTC of 1.5 μg/day, NAs belong to the ICH M7 cohort of concern (CoC), for which the TTC is generally not applicable and individual AI limits should be established. EMA has published AI limits for some already known NAs in their Q&A document for marketing authorization holders (MAHs).[38] This list is growing rapidly, and the proportion of NDSRIs is increasing. For NAs not yet included in this list, the EMA Q&A provides several options:

(1) If sufficient animal carcinogenicity data are available, the TD_{50} derived from a lifetime rodent bioassay can be used to calculate a substance-specific AI by linear extrapolation. This will generally not be the case because hardly any carcinogenicity data for NDSRIs are currently available, and the generation of such data takes several years; even if one were to choose to conduct a carcinogenicity study, it would not be a short-term option, and it is unlikely that further rodent carcinogenicity studies on impurities, even nitrosamines, will be performed when the financial and ethical cost of such is compared to *in silico* predictions or at worst the *in vitro* Ames test and 3R considerations are taken into account.

(2) Without sufficient carcinogenicity data, a class-specific AI of 18 ng/day can be used as the default option, based on the fifth percentile of *N*-nitroso TD_{50} data from the Lhasa carcinogenic potency database. This means that only 5% of NAs are expected to be more potent than this, or in other words, 95% of NAs can be expected to be less potent. Under specific conditions, a 12-month temporary limit based on 178 ng/day (based on the 33rd percentile, rather than the fifth, of *N*-nitroso TD_{50} data from the Lhasa carcinogenic potency database) can be considered (EMA Q&A).[38]

(3) Read-across to a suitable surrogate compound has been suggested by regulatory guidance[14,38,56,57] as an alternative approach in cases where insufficient carcino-

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genicity data exist for the NA in question. However, the selection of this surrogate compound is a challenging process, particularly for NDSRIs. The ideal read-across analogue is one that (a) is structurally similar around the nitrosamine substructure with a similar substitution pattern, (b) has robust carcinogenicity data, (c) can be considered similar in terms of potential metabolic activation and potential DNA reactivity of the diazonium ion that is formed, and (d) can be expected to have a similar DMPK profile to the query compound, such that it can be expected to distribute to similar organs and avoid alternate fates *in vivo* (whether excretion or other metabolic pathways).

In practice, therefore, the selection of a suitable read-across analogue may take into account the parameters listed in Table 2, searching through all available NAs, and then comparison of the different values will allow the selection of the most suitable analogue. The "Tanimoto" similarity is a term which includes a variety of potential similarity metrics; the Tanimoto comparison is a specific method for the similarity comparison of any pair of structural fingerprints rather than a universal number, and there are also other similarity measures available. Comparison of whole-molecule fingerprints, by Tanimoto or other similarity metrics mentioned in Table 2, is often used as a measure of similarity; however, it may not be as relevant for NAs (or perhaps other classes, as reported by Lester et al.[58]). This is due to the high dependence on local reactivity rather than pharmacophoric similarity for the potency of NAs; the two carbons adjacent to the nitrosamine are, respectively, the sites of metabolic activation and formed diazonium. For example, two NAs that are structurally diverse in the pharmacophoric sense may have similar local environments and pharmacokinetic properties and thus may be suitable analogues for each other.

A framework for the assessment of read-across methods for the general chemical use case, as opposed to the specific pharmaceutical use case, the read-across assessment framework (RAAF), has been published by ECHA.[67] This gives two principal hypotheses on which to base read-across that are relevant to NAs: first, biotransformation to common compounds (i.e., the formation of the same diazonium ion from NAs that differ in the aldehyde released, such as methyl-, ethyl-, and isopropyl-substituted isopropyl NAs all forming the isopropyl diazonium ion (Figure 6)), and second, that two similar compounds may have comparable mechanisms, such as comparing 4-methyl nitrosopiperidine to nitrosopiperidine itself.

It is important to note that the RAAF permits the use both of single-compound analogues and category-based read-across. In the case of the category, if the property of concern varies through the category according to a regular and predictive trend (such as the increasing potency of the cycloalkyl rings[47]) with ring size, this can be used to extrapolate; otherwise, a worst-case scenario is recommended. The full details of the RAAF[67] are beyond the scope of this Perspective, but the consideration of routes of metabolism—both for bioactivation and alternative deactivating metabolic pathways—and metabolite profiling are a valuable lesson that has application in the current nitrosamine situation.

It will be noted that typical values of many of the descriptors in Table 2 differ significantly between the small molecules for which most carcinogenicity data are available and the larger

Table 2. Critical Parameters to Consider When Selecting an NA Read-Across Analogue

factor	suggested descriptor	rationale
carcinogenicity data	robust study as described in refs 39, 48, and 59	Read-across to a target with robust data is most easily accepted. Use of lower confidence interval or alternative tools may allow use of less reliable data.
electronic environment of α- and β-carbons	either expert features,[48,59] local similarity as used in refs 60 and 61, or reactivity descriptors[62,63]	There is a need to understand the critical metabolic activation step and ensure that the read-across analogue is of similar reactivity. Both sides of NA are relevant since the diazonium reactivity is also affected by the environment.
steric environment of α- and β-carbons		
accessibility of α-carbon	relative through-bond paths	There is a need to understand more about the availability of the α-carbon than the immediate steric environment can determine (e.g., nitrosamines buried deeply in a molecule are less exposed for metabolism even if unhindered at the α-carbon).
global similarity	Tanimoto/fingerprint comparison	This is normally considered a significant measure of similarity but is less critical for reactivity-driven toxicity such as that of NAs.
	matched molecular pairs[58,64]	An alternative way to understand and interrogate similarity
DMPK	logP	These factors affect distribution and exposure *in vivo*, e.g., polar, ionizable NAs may avoid phase I metabolism since they are already soluble. Furthermore, larger, more complex molecules such as biologics are not metabolized by CYPs, and thus, NA is not activated.
	ionizability[63]	
	molecular weight	
	solubility	
	[predicted] metabolic profile[52,65,66]	If metabolism occurs elsewhere and the molecule is cleared without activation of the α-carbon, diazonium formation is much less likely

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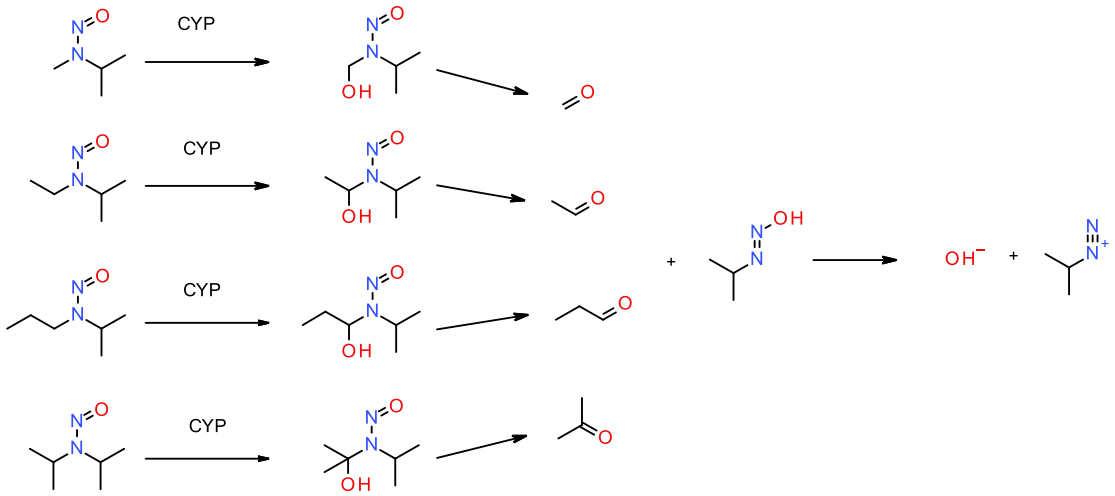

Figure 6. Metabolic activation of different isopropyl dialkyl nitrosamines leads to the formation of the same isopropyl diazonium ion.

NDSRIs for which AI limits are necessary. Selection of an analogue, given the properties listed above, becomes a multiparameter optimization problem, where the most suitable analogue in one category may not necessarily be the most similar in another. A framework for automating, by quantifying, this for the general read-across case has recently been published.[58] The selection of a specific analogue for a given NA may not follow the exact same metrics described by Lester et al., but the general principle may well be useful.

An important point to make is that the difference in molecular weight can and should be used to scale weight-based limits (e.g., the 26.5 ng/day limit applied to NDEA (MW = 102.14) and those compounds read across to it) based on the number of molecules per mole present *in vivo* as a worst-case scenario, simply because NA mutagenicity is mechanistically linked to the molar amount and not to the mass. In other words, if a typical NDSRI of 500 Da is read across to NDEA, the worst-case limit should be 500/102.14 × 26.5 = 129.7 ng/day. Scientifically, this approach is well-justified, but it is currently not accepted by HAs. Further examples for molecular weight correction are provided in Table 3 for the NDSRI AIs from the EMA Q&A. This table also includes correction for less than lifetime (LTL) exposure, which is currently only accepted as a temporary measure during CAPA implementation, although Bercu et al.[68] have clearly shown that LTL-corrected AIs are protective for potential carcinogenic risk to patients. Furthermore, it should be noted that NDEA was a key compound in the original development of the LTL.[69]

Should insufficient data be available for any single compound or the analogues be considered insufficiently close, the class-based approach permitted by the RAAF should be able to be considered, as proposed by Dobo et al.[70] In this case, the compounds of the class should be selected based on relevant structural features, following for example the various classification schema recently proposed,[47,48,59,70] and from the carcinogenicity information across the class a limit can thus be proposed.

EMA and Health Canada have used read-across to determine AI limits for several NDSRIs and published these limits in their Q&A document for marketing authorization holders.[38,71] These surrogate-selection operations vary in the degree of extrapolation required. For instance, EMA used 4-(methylnitrosoamino)-1-(3-pyridinyl)-1-butanone (NNK) as the surrogate to read across from and set an AI of 100 ng/day for nitrosoduloxetine and nitrosofluoxetine. The selection of NNK as the surrogate is easily rationalized by examining the surrounding substituents around the NA. NNK as well as nitrosoduloxetine and nitrosofluoxetine have one *N*-methyl substitution and an *N*-propyl group that is further substituted by an aromatic system. The electronic and steric features surrounding the nitrosamine substructures of these three compounds are very similar. On the other hand, nitrosoduloxetine and nitrosofluoxetine have additional bulky aryl substitutions at the γ-position, adding significant structural hindrance that is not present in NNK, and thus, they are expected to be much less potent carcinogens—indicating that this read-across may result in a lower AI than the nonexistent compound specific data would permit. Other examples of good surrogate selection are nitrosomethylphenidate and nitrosoparoxetine that are read-across from nitrosopiperidine (NPIP) because they all contain a nitrosopiperidine moiety. However, these again are probably very conservative measures of read-across because both nitrosomethylphenidate and nitrosoparoxetine have significant steric bulk on the piperidine ring, adding considerable steric hindrance in comparison to NPIP. Nitrosomefenamic acid is read across from nitrosodiphenylamine (NDPh), as they both have phenyl groups at the two α-positions. A degree of conservatism in these read-across analogue suggestions is to be expected, since (a) if there are two potential analogues of comparable relevance and no mechanistic reason can be found to differentiate between them, the more potent will be chosen, and (b) in almost all cases for nitrosamines, the read-across is from a small molecule with carcinogenicity data to a larger NDSRI that contains either the small molecule as a substructure or something similar to it. The NDSRI in these cases will almost always contain additional steric bulk and of course be a heavier molecule, so the molar dose is lower.

On the other hand, alternative read-across analogues can be and indeed have been proposed[47] for several surrogates that were selected by EMA and Health Canada to be read across to other NDSRIs (Table 4). For example, the surrogate selected to be read across to nitrosovarenicline (NNV) was nitroso-1,2,3,6-tetrahydropyridine (NTHP). NTHP has an olefin at the β-position to the NA that activates the α-position and makes it more prone to hydroxylation—the C−H bond

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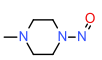
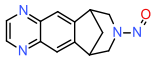
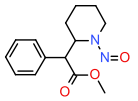
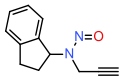
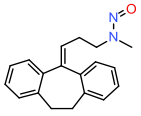
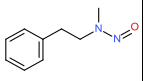
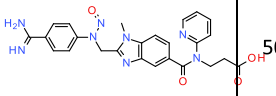
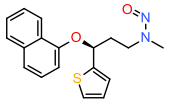
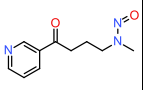
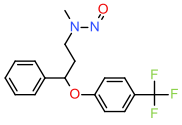
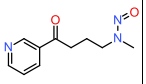
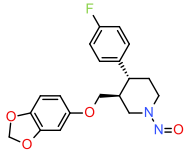
Table 3. Alternative AIs for NDSRIs Based on the LTL and MW Corrections[a]

NDSRI	Structure	MW	Source	EMA AI	RA point of departure	POD MW	MW factor	Treatment duration	LTL factor	Realistic AI[b]	Fold difference
MeNP		129.2	Rifampicin	26.5		73.1	1.8	< 1 m	80	3744	141
NNV		240.3	Varenicline	37		112.1	2.1	1 y	13.3	1054	28
NMPH		262.3	Methyl-phenidate	1300		114.2	2.3	10 y	6.7	20015	15
Nitroso-rasagiline		200.3	Rasagiline	18		73.1	2.7	10 y	6.7	330	18
Nitroso-nortriptyline (NNORT)		292.4	Amitriptyline	8		164.2	1.8	1 y	13.3	189	24
Nitroso-dabigatran		500.5	Dabigatran	18		73.1	6.8	life	1	123	6.8
Nitroso-duloxetine		326.4	Duloxetine	100		207.2	1.6	life	1	158	1.6
Nitroso-fluoxetine		338.3	Fluoxetine	100		207.2	1.6	life	1	163	1.6
Nitroso-paroxetine		358.4	Paroxetine	1300		114.2	3.1	life	1	4081	3.1

[a]In cases where the limit is based on the class-specific threshold of toxicological concern (TTC) of 18 ng/day, NDEA was used as a surrogate point of departure. [b]Realistic AI is calculated as EMA AI × MW factor × LTL factor.

dissociation energy of allylic carbons is significantly reduced, and conjugation to the olefin can stabilize the relevant transition states. NNV does not have a double bond at that position, and other surrogates, such as nitrosohexamethyleneimine (NHEX) with a TD_{50} of 313 μg/kg/day or NPIP with a regulator-accepted limit of 1300 ng/day from a TD_{50} of 1300 μg/kg/day, could be proposed as alternatives. Data for NPIP are perhaps more robust, but as discussed above, where two analogues cannot be differentiated mechanistically, the more potent should be chosen. It should be stressed that while the hexamethyleneimine ring of NNV is unsaturated like that in NTHP, which may explain the choice thereof as an analogue, it is impossible for tautomerization of that ring to occur due to the bridged nature of NNV, and thus, the potent allylic/benzylic α-carbon of NTHP cannot be formed in NNV. By choosing NHEX (as was proposed by the MAH at the time) as the surrogate to be read across to NNV, the AI would be 313 ng/day rather than 37 ng/day as derived from the use of NTHP.

Another example where an alternative surrogate to that requested by regulators may be available is nitrosonortriptyline (NNORT), which was read across from *N*-methyl-*N*-nitrosophenethylamine (NMPEA) with an AI of 8 ng/day. The carcinogenicity data of NMPEA were recently reassessed, and a modified AI of 40.1 ng/day was recommended for NMPEA[72] (see Table 4 footnote *b* for details). The reason for this analogue selection may be that the olefin at the γ-position of NNORT is considered comparable to the phenyl group in

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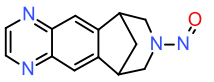
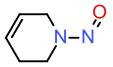
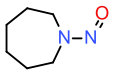
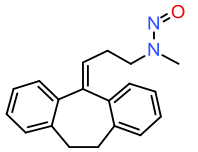
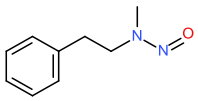
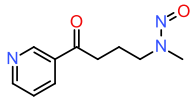
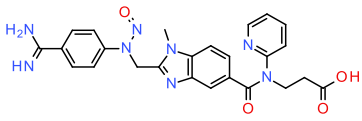
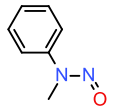
Table 4. Examples of NDSRIs from the EMA Q&A Document for Which Alternative Read-Across Points of Departure Should Be Considered[a]

NDSRI	Structure	RA POD	AI	Alt. RA POD	Alt. AI	Fold difference
NNV			37		313	8.5
Nitroso-nortriptyline (NNORT)			8[b]		100	12.5
Nitroso-dabigatran		n/a	18		34.3	1.9

[a]The use of an alternative read-across would lead to a considerably higher acceptable intake, as shown in the column "Alt. AI". These alternative AIs would even further increase upon application of LTL and MW corrections, as exemplified in Table 3. [b]The use of this value for this compound has been challenged based on the use of a multiorgan (upper GI tract) value rather than an organ-specific value for deriving the TD_{50}; the corresponding single-organ (esophagus) study would indicate a value of 40.1 ng/day.[72]

NMPEA. However, it is not likely that NNORT, with its bulky tricyclic aromatic system, would be of higher carcinogenic potency than NDMA or NDEA. The use of NNK as the surrogate for NNORT could be proposed as an alternative, as these two molecules have similar structural features around the NA functionality—the γ-carbonyl group with its double bond and then aromatic substitution further away—making NNK a potentially closer match to NNORT than the phenylethyl substituent of NMPEA.

A final example is nitrosodabigatran, which was assigned the default AI of 18 ng/day. This default AI may have been assigned because a surrogate from which to read across was not identified. However, to assign the default AI of 18 ng/day is quite inconceivable for a molecule that not only has an aromatic system on one side of the nitrosamine and an α-carbon on the other side, resulting in considerable steric hindrance that limits accessibility, but also has the free carboxylate, which further reduces carcinogenic potency, as well as a high molecular weight. Even NMPA, as the simplest aryl alkyl nitrosamine, could have been selected as a surrogate, although it would have been a very conservative selection because of the lack of steric hindrance (a methyl group at the α-position), low molecular weight, and no free carboxylate. Furthermore, nitrosamines with α-hydrogens but significant steric hindrance were recommended by Ponting and Foster to be potentially excluded from the CoC.[73]

These examples show how critical it is to make the proper selection of surrogates when performing read-across to set AIs for NDSRIs.

9. THE VALUE OF DATA SHARING INITIATIVES

Precompetitive data sharing initiatives have been established as an efficient mechanism for the anonymized distribution of high-quality testing data, both analytical and toxicological.[74,75] The resulting databases have two principal effects: reduction of testing burden and expansion of studied chemical space for SAR. The reduction of testing burden occurs since the members of a data-sharing consortium can access the results that other organizations have generated and shared and, following anonymization, can present them to HAs in the context of a regulatory submission. Various publications report the benefits of *in silico* Ames predictions and read-across in terms of cost and time savings,[76−78] and a similar rationale is very true for precompetitive sharing of Ames data. Both these cost and time considerations compare very unfavorably with a database lookup to determine the same information. In addition, the shared data will constitute the source of knowledge at the heart of the read-across approaches and SAR model refinements. Furthermore, while the Ames test is an *in vitro* assay, it does require rodent liver S9 fraction,[40,79] and thus, there is a potential reduction in the use of laboratory animals and a strong 3R's case for minimizing the use even of this routine *in vitro* test. The expansion of labeled chemical space for SAR is completed by scientists at Lhasa Limited, who have an expert eye that can extract SAR trends from a set of structures and distill these into a structural alert[52,65] without revealing proprietary aspects of the structure. This practice allows both the donor organizations and the wider scientific community to benefit from better predictions for chemical space that would otherwise be poorly covered due to a lack of public data. Two NA-related data-sharing initiatives have been established through Lhasa Limited: Vitic Complex Nitrosamines[76,80,81] and Nitrites.[32,82]

Complex Nitrosamines. An increasing proportion of the NAs that have been detected in marketed drugs are structurally complex and sometimes called NDSRIs—with up to 40% of marketed APIs and 26% of registered impurities containing secondary or tertiary amines[12] that are potentially vulnerable to nitrosation in the presence of nitrite sources (*vide infra*). The vast majority of these were previously unknown

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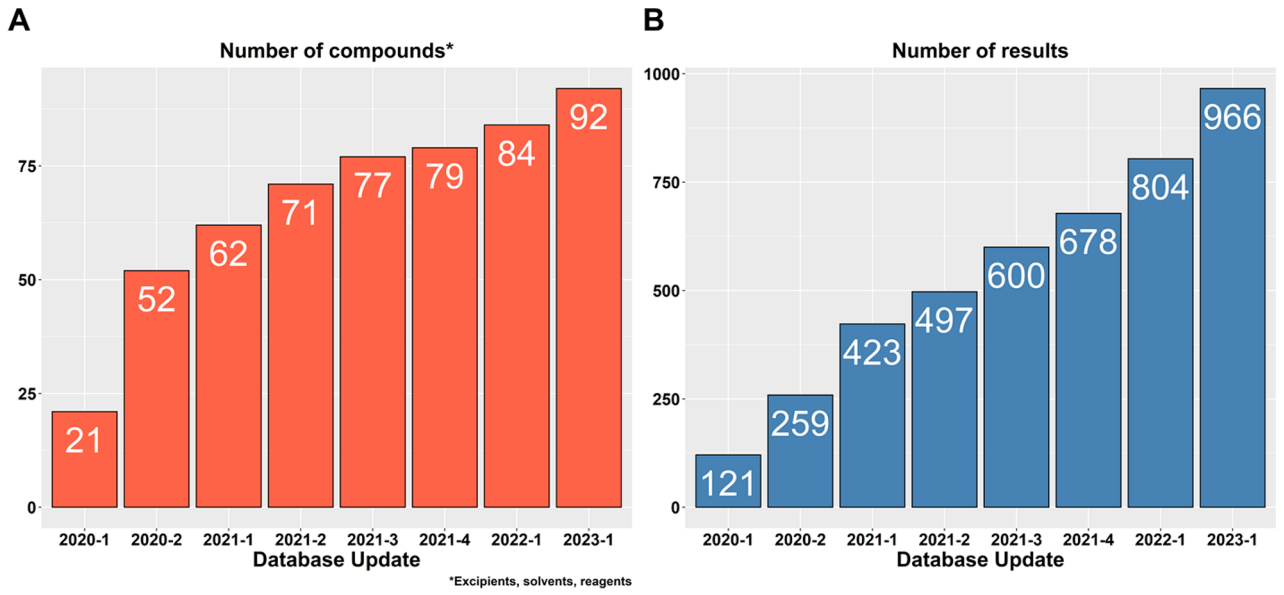

Figure 7. Evolution of the nitrite database since its initial installation in 2020. As of February 2023, it contains 966 results divided over 92 common excipients, reagents, and solvents.

structures; as a result, literature toxicity data are rare. Ames tests, and potentially *in vivo* studies, are being carried out on these to evaluate the overall weight of evidence regarding the mutagenic potential of these NDSRIs. Since many drugs are generic and manufactured by multiple organizations, the harmonization of Ames (and other genotoxicity) testing on these compounds is desirable for the reasons discussed. The global capacity of hamster S9-activated Ames testing is relatively low, and the capacity for follow-up *in vivo* assays is even lower, reinforcing the need for collaborative data-sharing efforts. With respect to the expansion of SAR, this is one of the key drivers to forming a consortium and working collaboratively.[76,80] Most NAs for which toxicity data are known are small NAs, and thus, the majority of SAR work[47,48,59,70,83,84] has been performed on small NAs. It has been hypothesized that while many relevant lessons can be learned from the structural features of small molecules, there are nevertheless significant differences in both potency and prevalence of positive results between the "complex" and small-molecule NAs.[80] The established Complex Nitrosamines consortium has therefore been tasked with curating a sufficiently large data set of high-quality Ames data to test the hypotheses of difference and confirm the utility of read-across from small molecules.[76,80,81] Established in 2022, the second Complex Nitrosamines database update in December 2022 (version 2022.2.0) contains Ames results for 54 NAs and a HPRT assay (hypoxanthine phosphoribosyl transferase, a mammalian cell *in vitro* gene mutation assay[85,86]) for one NDSRI. Most of the Ames results are for NDSRIs, and also a few relevant small-molecule NAs have been donated. *In vivo* mutagenicity studies for NDSRIs are also being conducted across industry, primarily through the transgenic mutation assay (TGR). These studies are expensive and long, require large amounts of test article, and worst of all, their availability in contract research organizations is extremely limited. To avoid redundancy and in the spirit of 3R, the Complex Nitrosamines consortium is also compiling a list of *in vivo* mutagenicity studies that companies are planning to perform, with the intention of eventually sharing the results of these studies within the data-sharing initiative.

Nitrites. Since 2018, it has become increasingly apparent that a major source of nitrite for the nitrosation of vulnerable amines (*vide supra*) is the excipients with which the drug substance has been formulated to create the drug product. Nitrite levels in excipients can be a problem even at parts per million levels, as shown in Figure 4, although it is not the only root cause to consider. The rationale behind and development of a database for the collection of nitrite results on batches of these excipients have recently been described.[32] Since the submission of that article, four further updates of data have been made, and the 2023.1.0 version of the database now contains 966 results for 92 different compounds (Figure 7). In addition to excipients, the database has evolved to meet the additional requirements of the consortium and contains data on solvents and reagent NCRMs, where nitrite levels are equally critical.

Beyond the time- and cost-saving opportunities for reduction of duplicate testing, the database has already had two significant impacts on the wider community: First, the presence of cross-industry blinded testing data and the shared experience and knowledge on testing for nitrite allowed a challenge to be made when unfeasibly high nitrite levels were reported in the literature.[87] Second, the analysis of this data for which excipients routinely contain significant levels of nitrite allows software for the prediction of forced degradation, such as Zeneth,[88] to predict NA formation where these excipients are used in conjunction with a vulnerable amine. The sharing of nitrite levels has increased understanding of the levels and possible variation observed across excipient batches and has shown that relevant levels of nitrite were detected in the majority of tested excipients. Excipient manufacturers supplying the pharmaceutical industry may not be able to solve this challenge, partly as pharmaceuticals are a small part of their overall market. However, collaboration to advance our understanding of the root cause of nitrite in excipients could help to reduce levels of nitrite in specific excipients that are most frequently used in pharmaceuticals in larger quantities

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and therefore could impact the levels of NA formation the most. In this respect, the reduction of nitrite levels in a handful of key excipients would already be a big step forward.

10. OUTLOOK

Currently, the regulatory scene is challenging for both industry and HAs, primarily around acceptable methods to set limits for NDSRIs. The Ames test alone is currently not accepted by HAs to derisk NAs, even when using modified protocols. Using small dialkyl NAs as surrogates for the purpose of read-across to set AIs for NDSRIs is highly challenging due to the structural complexity of NDRSIs. *In vivo* mutagenicity data are slowly being generated for NDSRIs, primarily by TGR and duplex sequencing, but this process takes a long time, primarily due to the low availability of laboratories that can conduct these studies. When the *in vitro* and *in vivo* studies clearly indicate that an NDSRI is nonmutagenic, there is a strong chance of acceptance by the HAs for this compound to be considered an ICH M7 Class 5 impurity. However, when the *in vivo* data show the compound to be positive, these data cannot be used to set compound-specific limits (ICH M7 Q&A 7.2). Therefore, it is necessary to devise an acceptable methodology for setting limits for NDSRIs, particularly with the evolving evidence that most NDSRIs are of weaker mutagenic potency than the small potent dialkyl NAs. Various collaborative expert working groups from industry and regulatory agencies are evaluating the relevance of many of the genotoxicity assays for NAs (e.g., HESI, MutAmind). In the interim, industry is required to report NA levels to HAs and provide justification for AI limits. The problem is that to set a limit for detection and for justification, it is necessary to set an AI for each new NA, including of course NDSRIs. Various methods have been proposed by industry, using a weight of evidence approach, to set AIs or at least interim AIs. For instance, several position papers for NAs of classes of medications have been generated by the Nitrosamine Working Group of the European Federation of Pharmaceutical Industries and Associations (EFPIA) (https://www.efpia.eu/) and have been shared with HAs and circulated within the pharmaceutical industry. These include position papers for nitroso-HCTZ[89] and NAs of calcium channel blockers,[90] β-blockers and β-agonists,[91] and ACE inhibitors.[92] Many of the conclusions from these position papers indicate that full classes of NDSRIs can be considered at the least as non-CoC, but until additional *in vivo* data become available, a pragmatic approach is recommended by the authors, and a temporary limit of the "standard" TTC (1.5 μg/day) is proposed.

Another method for setting limits for NDSRIs, based on positive *in vivo* mutagenicity data, is to perform a benchmark dose (BMD) analysis on the data (for this, it is critical to design the study in advance with a sufficient number of doses) and then to compare the BMD results to the BMDs generated from the same type of study for NAs that also have robust carcinogenicity data. By doing such a comparison, it is feasible to rank the potency of an NDSRI without deriving a compound-specific AI from the *in vivo* mutagenicity data. Several examples of such an analysis have shown that NDSRIs that have been assigned extremely low limits in the EMA Article 5(3) Q&A have much lower mutagenic potency than the surrogates currently used for read-across.

To avoid unjustified shortages of essential medicines, the industry, as well as HAs, must devise acceptable methods to set limits for NDSRIs. Scientifically sound evidence is slowly emerging, indicating that most, if not all, NDSRIs are markedly less potent than the small dialkyl NAs and that blanket inclusion of NDSRIs within the CoC may not be warranted. Although many consortia and mutual (industry and HAs) working groups are collaborating rigorously to investigate the potential risk of exposure to NDSRIs, a much higher risk may be posed to patients if essential medicines are not available.

Medicines may be withdrawn from the market not only where the applicable AI limits are very low but also, more concerningly, if the sales volumes do not justify investigation/mitigation actions for the MAH—a particular concern for long-established, generic drugs, where profit margins per dose can be miniscule—in which case the MAH may simply withdraw the product without intention to replace it. Smaller suppliers of generic drugs may feel this pressure particularly strong since they do not have the same financial strength and technical resources as large multinational pharmaceutical manufacturers. This may result in diminishing supply options and an increase in respective drug prices.

On the positive side of things, we can expect rapidly growing knowledge on the relative risk when results from a multitude of *in vivo* mutagenicity studies with NDSRIs (especially TGR) that have already been started will become available. The TGR data may be complemented with results from highest-resolution next-generation sequencing (NGS)/duplex sequencing,[93] which has the potential to replace TGR studies and bring a significant acceleration of mutagenicity data generation in the future. For those NDSRIs that do not test negative/nonmutagenic, it will be essential to develop an accepted methodology that can derive scientifically justified AIs from the *in vivo* mutagenicity data, as read-across options may be unavailable and carcinogenicity studies are not feasible from a timing, capacity, nor animal welfare perspective.

New products will benefit from having NA control strategies implemented when the drug manufacturing process is being established. NA risk assessments will ultimately become an integral part of product life cycle management (LCM), much like it is the case today for elemental impurities, as per the ICH Q3D guideline. Eventually, this will allow the industry to return to normal operations, where attention to NA risks is part of the new normal, perhaps under a revised ICH M7 framework. This framework will need to allow the differentiation of those nitrosamines that rightfully belong in the cohort of concern from those that are not CoC and those that are not mutagenic altogether.

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Notes

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NONSTANDARD ABBREVIATIONS

AI	acceptable intake
API	active pharmaceutical ingredient
DMPK	drug metabolism and pharmacokinetics
DMSO	dimethyl sulfoxide
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EMA	European Medicines Agency
GTTC	Genetic Toxicology Technical Committee
HA	health authority
HESI	Health and Environmental Sciences Institute
ICH	International Council for Harmonisation
ITEM	Institute for Toxicology and Experimental Medicine
LOQ	limit of quantitation
LOD	limit of detection
LTL	less than lifetime
MAH	marketing authorization holder
MDD	maximum daily dose
NA	*N*-nitrosamine
NCRM	noncontributory raw materials
NDEA	*N*-nitrosodiethylamine
NDMA	*N*-nitrosodimethylamine
NDSRI	nitrosamine drug-substance-related impurity
RAAF	read-across assessment framework
SAR	structure−activity relationship
TGR	transgenic rodent
TNA	total nitrosamine content

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This Perspective published ASAP on July 26, 2023, with errors in Table 3. The structures for MeNP and NNV have been replaced and the corrected version was reposted on July 28, 2023.

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