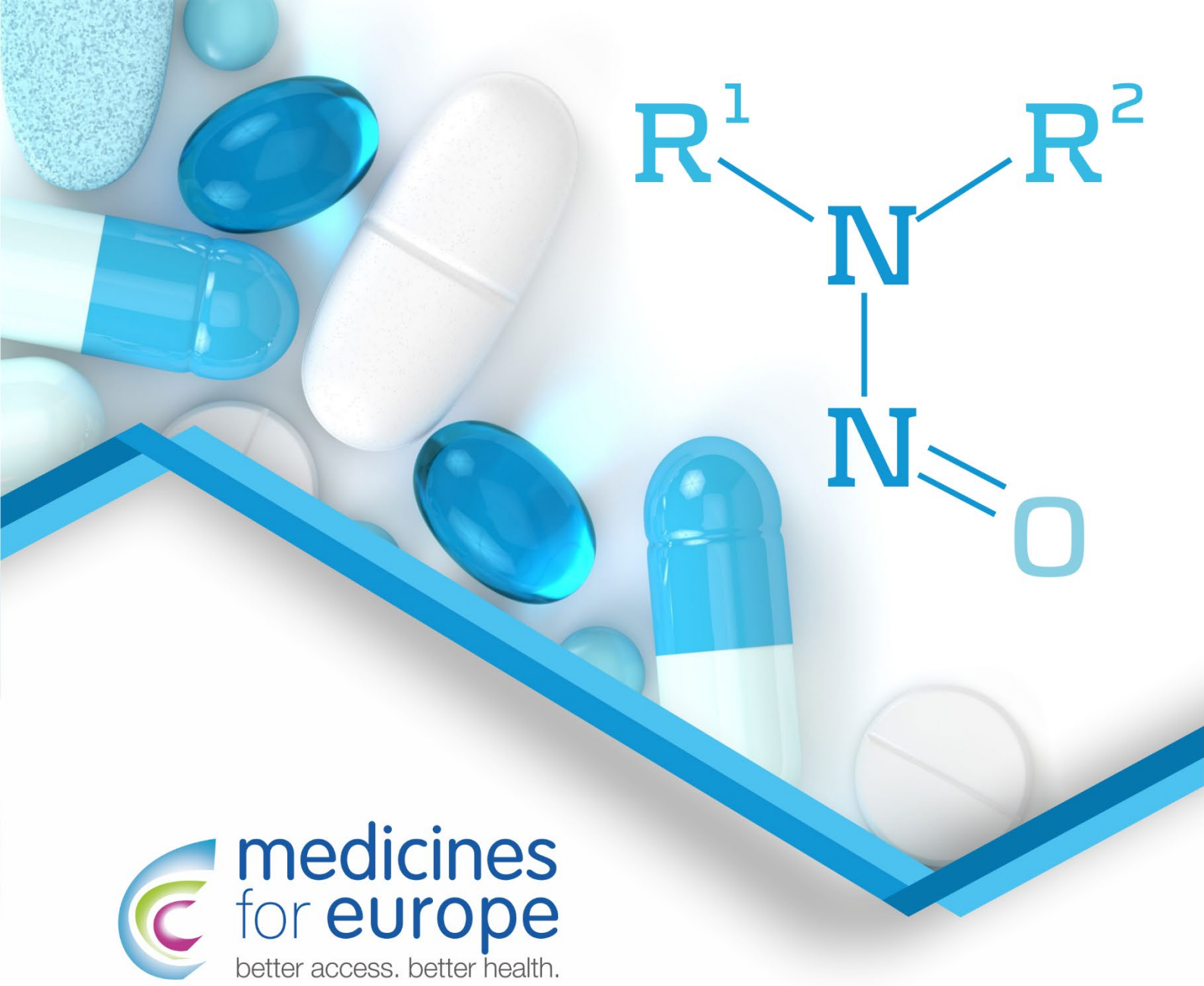

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Review of Nitrosamine Drug Substance Related Impurities (NDSRI) in Pharmaceutical Drugs:

Risk Assessments, Acceptable Intakes, and QSAR Tools

Dr George Johnson

Introduction:

Nitrosamines are organic compounds with a chemical structure R_2N-N=O (Figure 1), where R is usually an alkyl group. These substances are commonly found in food and the environment. Low levels of nitrosamines have been found in a wide array of pharmaceuticals. The acceptable intake (AI) values for many nitrosamines have been published by the regulatory agencies. Many of these are unworkable and have led to recalls of some products (Health-Canada 2023b). Furthermore, some of these products are essential medicines, and recalling these based on the AI's is leading to potential safety issues in patients who no longer have available treatments for their conditions. It is important that the risk assessment and AI levels continue to develop in a science driven manner.

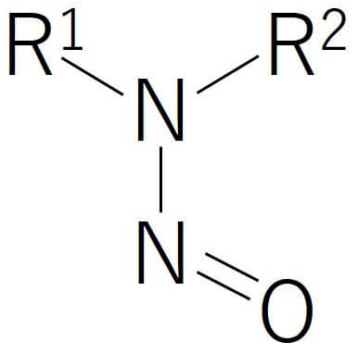
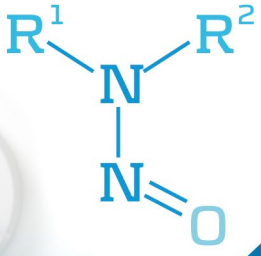


Figure 1: N-Nitrosamine structure ((R7) 2018).

The acceptable intakes (AI) are based on harmonised tumour dose 50 (TD50) values, from which a straight line is drawn back to extrapolate from the dose at which 50% of the animals are estimated to have cancer, to the dose at which 1 in 100,000 animals are assumed to have cancer. The cancer bioassay studies often have 50 rodents per dose or less, and the extrapolation is also from the single data point (TD50), and due to the lack of a variability measure, it doesn't consider the precision of the TD50 or the quality of the dose response data. Extrapolating back from populations of 1 in 50 rodents to 1 in 100,000 humans is also very imprecise. The risk estimate is hypothetical, and only accounts for the animal risk with no extrapolation to humans. This is accepted as the AI is often a very low number, and due to this overly conservative calculation, protects the human population. However, it has no measure of precision, and is a theoretical risk. Moreover, there is often a misunderstanding that any dose above the AI leads to cancer in humans, and this is not the case. We therefore accept that the AI approach has continued application and has been useful to date in protecting the human population. However, it must be accepted that it is overly cautious, very imprecise and that this theoretical assessment is causing major issues with the assessment of products containing low level nitrosamine impurities.



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Risk assessment for nitrosamines is a continuously evolving area in pharmaceutical regulation, and numerous approaches are being considered and often implemented. Recent work in this area has shown (1) a read-across approach to categorize *N*-nitrosamines into three categories with set AIs for each group; (Bercu et al. 2023a) (2) structural activity relationship (SAR) grouping into five categories with set limits (carcinogenic potency categorisation approach, CPCA); (EMA 2023) (3) current resistance to using the permitted daily exposure (PDE) approach for nitrosamines with a "threshold mechanism" of DNA repair; (Johnson et al. 2021) and (4) considerations about using *in vivo* mutation dose−response data for potency ranking and extrapolation from substances of higher potency with defined AIs through the use of a potency factor approach or at least to support a different CPCA category (1−5) as prescribed by the EMA through SAR alone (Teasdale and Johnson 2023).

Risk Benefit

The risk benefit calculation from the European Medicines Agency (EMA) is based on balancing the desired effects of 'benefits' of a medicine against its undesired effects of 'risks'. The EMA can recommend authorisation of a medicine whose benefits are judged to be greater than its risks ((EMA 2012). These considerations and calculations are applied to the parent drug, however, to date they have not been applied to low level impurities. Although there is not a direct benefit to the impurity itself, if the impurity cannot be removed below a certain level, then the risk-benefit is still relevant. This is further highlighted for the essential medicines, where the benefit of having the drug with this low-level impurity, outweighs not having the drug or having a less efficacious replacement drug. In addition, there are also other medicines that could be considered 'essential' beyond the WHO list, and those for chronic disease as well as antibiotics could also be considered to have a higher benefit even with inclusion of the low-level nitrosamine, that outweighs the risk of not having the drug.

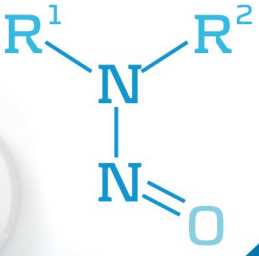
Although the EMA has detailed the risk benefit calculation for use in certain situations, it is not always implemented in local health authorities. There could be different reasons for this, but the result is a non-harmonised approach, and this should be addressed noting that EMA is the thought and guidance leader in this area in Europe.

Using *in vivo* mutation data for human health risk assessment

Risk assessments of nitrosamines are carried out using cancer bioassay data, however *in vivo* genetic toxicity data can also be used to assess human risk. MacGregor et al., 2015 (MacGregor et al. 2015a; MacGregor et al. 2015b) was a landmark paper in this area, the group who wrote this consensus paper, is composed of regulatory experts, along with academics and industrial experts. "Recommendations include the selection of appropriate genetic endpoints and target tissues, uncertainty factors and extrapolation methods to be considered, the importance and use of information on mode of action, toxicokinetics, metabolism, and exposure biomarkers when using quantitative exposure-response data to determine acceptable exposure levels in human populations or to assess the risk associated with known or anticipated exposures. The empirical relationship

between genetic damage (mutation and chromosomal aberration) and cancer in animal models was also examined. It was concluded that there is a general correlation between cancer induction and mutagenic and/or clastogenic damage for agents thought to act via a genotoxic mechanism, but that the correlation is limited due to an inadequate number of cases in which mutation and cancer can be compared at a sufficient number of doses in the same target tissues of the same species and strain exposed under directly comparable routes and experimental protocols" (MacGregor et al. 2015a). Heflich et al (2019) recommend continuing the development of these approaches with the objective of establishing consensus regarding the value of including the quantitative analysis of mutation per se as a required endpoint for comprehensive assessments of toxicological risk (Heflich et al. 2019). The Health and Environmental Science Institute - Genetic Toxicology Technical Committee (HESI-GTTC) has also shown this to be the case, and the groups' paper Johnson et al., (Johnson et al. 2021) showed that nitrosamines can be assessed using *in vivo* gene mutation data, in a protective way for humans. Johnson et al., (2021) showed that the nitrosamines for which dose response and DNA repair information is available, mechanism of action can be used to support the application of the ICH M7 (ICHM7 2017) procedure called the permitted daily exposure (PDE). ICH M7 states in Section 7.2.2: *"The existence of mechanisms leading to a dose response that is non-linear or has a practical threshold is increasingly recognized, not only for compounds that interact with non-DNA (Deoxyribose Nucleic Acid) targets but also for DNA-reactive compounds, whose effects may be modulated by, for example, rapid detoxification before coming into contact with DNA, or by effective repair of induced damage. The regulatory approach to such compounds can be based on the identification of a No-Observed Effect Level (NOEL) and use of uncertainty factors (see ICH Q3C(R5)…) to calculate a Permissible Daily Exposure (PDE) when data are available."* The PDE remains the best approach for assessing risk of nitrosamines when the data is there, but regulatory bodies currently prefer the AI approach. This is likely to change once there is a body of evidence to support the PDE approach for a wide array of nitrosamines. However, even without using the PDE approach, there are still major advantages to using *in vivo* mutation data under the current nitrosamine risk assessment framework. For example, *in vivo* gene mutation data sets for N-Nitrosodiethylamine (NDEA) and N-Nitrosodimethylamine (NDMA) have been repeated, and are now very comprehensive, but still support the same hypotheses presented in Johnson et al., (2021). It is therefore suitable to assess nitrosamines using *in vivo* gene mutation data.

The EMEA/H/A-5(3)/1490 (EMA 2020) document shows some support for using *in vivo* mutation data. "Non-clinical studies are only meaningful when adding to the weight of evidence for quantitative risk assessment. Further lifetime cancer bioassays in rodents should be avoided due to the long time needed (3 years including evaluation) and high costs. In addition, such studies probably would not add any further scientific value due to the high amount of already available carcinogenicity studies for many *N*-nitrosamines. *In vivo* studies such as the transgenic rodent bioassays (TGR) are considered the best choice to determine robust points of departure (PoD) for mutations which are the most important pre-cancerous insult. However, low dose exposure and extensive studies would be needed to enable a robust calculation of benchmark doses (BMD) as the point of departure (PoD) for risk calculation. Studies would also be needed for all *N*-nitrosamines considered relevant. Whether this is ethically acceptable especially with regards to the 3R principle to reduce, refine, replace animal testing." Following this logical advice from EMA and the expert



panel, numerous companies have implemented *in vivo* mutation studies. There is yet to be clear guidance on how to use these data for nitrosamine impurities, but there are numerous options.

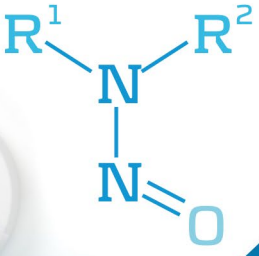
Next Generation Sequencing

The standard *in vivo* mutation test for testing for mutagenicity is the transgenic gene mutation test (TGR). This relies on genetic modified rodents, and their availability is often low, and their price is very high. A new approach for mutagenicity testing is to sequence parts of the genome of exposed rodents, define a mutation frequency, and to carry out the risk assessment on these data. Next generation sequencing (NGS) is an advanced high content methodology, and recent approaches lend themselves well to use in mutagenicity testing.

Error corrected NGS (ecNGS) is being used by an increasing number of industrial, academic and government groups (Marchetti et al. 2023), and there is a building wealth of data on nitrosamines, which is showing the approach to be excellent. NDEA and NDMA have been analysed recently using ecNGS (Bercu et al. 2023b) and there have been some presentations of additional data which are yet to be published. These *in vivo* mutation data on nitrosamines have shown there to be very comparable dose response and resulting benchmark dose (BMD) values between ecNGS and the *in vivo* TGR mutation assay. Furthermore, the ecNGS results confirmed no change in mutation spectra at the lowest doses compared to the vehicle control. Further information on mechanism of action through mutation spectra and the responsible DNA adduct, in addition to the DNA repair pathways were also discussed, and this was excellent and informative. Some early users of the approach consider the ecNGS to provide comparable but more precise BMD CI than those from the *in vivo* TGR mutation assay (Bercu et al. 2023b).

The *in vivo* TGR is the current advised mutation test system, having its own OECD guideline, as well as being supported in the ICH guidelines. NGS is yet to have an OECD guideline, but it is being used readily for nitrosamines due to its price, its availability compared, and the scientific understanding that sequencing will soon be the default test system for assessing *in vivo* mutation. To overcome some potential uncertainties, the users have been using a high level of coverage of the genome. This high level of precision is reflected in the tight variance throughout the dose response, and the tight BMD CI (Bercu et al. 2023b).

Before further guidance is provided, the recommendation is to follow the Bercu et al (2013b) protocol for ecNGS. The Pfizer laboratory and Twinstrand's ecNGS approach currently appear to be the most advanced in assessing nitrosamines for *in vivo* mutagenicity, and numerous groups have NDSRI and model (exemplar) nitrosamine experiments that should start appearing in scientific publications in 2024. Other NGS approaches are also being investigated for *in vivo* mutation dose response analysis. *In vivo* mutation testing using NGS is also being investigated through a HESI GTTC project, and the topic is already being discussed with OECD. Note that the nitrosamine issue and the importance of *in vivo* mutation data, along with the increased precision that ecNGS is offering, means that many stakeholders have committed to the technology prior to an OECD guideline. This is



meaning that a huge amount of data is already being produced, which will greatly advance its integration into hazard and risk assessment as a suitable assay.

DNA repair and Nitrosamines

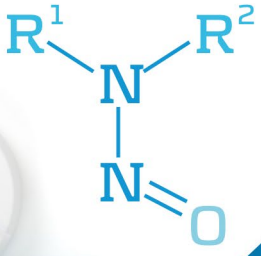
It is well established that NDMA and NDEA generate pro-mutagenic 0^6-alkylguanine (O^6-alkyl-G) adducts (i.e., the modification of guanine through the addition of small alkyl-groups such as e.g. -CH3, -C2H5) which are commonly repaired via dealkylation by DNA alkyl transferases (AGT, also known as methyl-guanine-methyltransferase, MGMT). Other nitrosamines such as NDBA, NDELA, NMEA, NNK and NNN are also known to generate 0^6-alkylguanosine adducts (Dennehy and Loeppky 2005; Coulter et al. 2007).

The efficiency of MGMT-mediated repair of O^6-alkyl-dG adducts decreases with increasing size of the alkyl groups (Du et al. 2019), and larger and more complex O^6-alkyl-dG lesions strongly block replicative polymerases, which often leads to cell death. The latter adducts can be overcome by translesion synthesis (TLS). Depending on the type of DNA lesion and error-prone TLS-polymerase, mismatched nucleotides can be inserted, and if not repaired by mismatch repair (MMR), mutations may arise (Fahrer and Christmann 2023). Apart from direct lesion reversal and TLS, nucleotide excision repair (NER) is thought to be the predominant pathway for repairing bulky O^6-alkyl-dG adducts (Du et al. 2019).

As a result of these different DNA adducts and DNA adduct spectra (and other factors, such as efficiency of metabolic activation impacted by steric hindrance and electron configuration), there is a wide range of mutagenic and cytotoxic potencies of N-Nitrosamines, which can be estimated via structural activity relationship (SAR) (Cross and Ponting 2021; Thomas et al. 2022). The larger nitrosamines which induce larger DNA adducts are predicted to be more cytotoxic than smaller nitrosamines which induce smaller DNA adducts. Smaller DNA adducts are widely accepted as having higher mutagenic potency than larger DNA adducts for this reason (Fine et al. 2023).

Nitrosamines are different and rare.

One misconception is that nitrosamines are somewhat special. This comes from the categorisation that many nitrosamines were in the cohort of concern (CoC), where the AI were below 1.5ug/day so the whole category has been placed within the CoC. All this means is that some nitrosamines are potent within the rodent cancer bioassay, where potency is measured by the TD50. It is also worth noting that the TD50 is not a precise measure of potency. The cancer bioassay is also fraught with issues, and many data sets even within the Gold/Lhasa cancer potency database (CPDB) are not suitable for use in calculating health-based guidance values for a precise risk assessment based on statistical power, number of doses tested, many data sets not having study designs that are OECD compliant and other complexities.



Mutagenic carcinogens are not rare. Mutagenic carcinogens are present in our food (ACSH 2004), the environment, produced endogenously and it is impossible to have zero exposure to them. Nitrosamines are just one group of such compounds, and even these are present in many foods at levels relatively high when compared to those within pharmaceuticals as impurities (EFSA 2023).

We accept that human exposure to nitrosamines should be assessed and controlled, but we do not accept that they are rare substances and that all of them should be controlled to extremely low levels.

Acceptable Intakes (AI)

The underlying assumptions used to calculate acceptable intake (AI) values are often flawed.

1.) The harmonic mean is used in some instances, and this can include data from studies that are not OECD compliant, have poor study designs and lack suitable statistical power.
2.) No measure of precision as there are no confidence intervals or measure of variability on the TD50 or AI.
3.) Protecting the human population to an increased risk of cancer to 1 in 100,000, when the actual incidence of cancer is 1 in 2 for all cancer (NHS 2023), or 1 in 58 for UK males and 1 in 122 for UK females for liver cancer (CRUK 2023) with comparable incidence globally.
    a. This has been accepted in the scientific and regulatory community, as the AI is very low, but this highlights how unprecise the AI is.
4.) Assessment of 1 in 100,000 risk in rodents, as the AI does not have an adjustment factor for humans.
5.) The linear extrapolation from the TD50 to 1 in 100,000 is based on a linear assumption that is not supported by statistical modelling of cancer bioassay data.

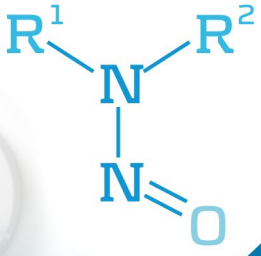
There are also misconceptions of what AI means regarding risk, particularly from a layperson. Experts in this area understand that it is a pragmatic value used as a conservative cutoff, at which dose there is absolute confidence of no increased risk at that dose and below. However, this does not mean that any dose above this AI causes increased risk and is unsafe. An order of magnitude above the AI is still unlikely to cause increased risk of cancer, particularly when there is consideration for the actual risk of cancer.

This issue is not conceptual, the overly conservative nature of risk assessment of nitrosamines in pharmaceuticals, leads to a series of issues; drug availability, misplaced patient safety concerns, ignoring the benefit of the whole drug compared to the risk of the drug plus its low-level impurity, issues of trust in the pharmaceutical industry leading to increased use of untested alternative medicines and reduced use of tested medicines and a general anti-industrialisation movement. One major contributor is the 18ng/day level which is unachievable and usually leads to the end of the

affected drug product. There has been some criticism of this default and very low AI (Ponting et al. 2022 Schlingemann, 2023; Bercu et al. 2023a; Schlingemann et al. 2023).

Structural Activity Relationship (SAR) Approach

The EMA Q&A document presents a structural activity relationship (SAR) based approach, to place compounds into 5 categories based on structural and activity features (EMA 2023). This is called the carcinogenic potency categorisation approach (CPCA). This is a very useful approach in most cases and has enabled AI's to be implemented across the different compounds. Within the appendix, there is a list of example substances that have AIs calculated using cancer bioassay data by EMA, Health Canada and the Food and Drug Administration. There are some substances included in the assessments that have been categorised as having similar potencies to NDMA and NDEA, although the *in vivo* mutation benchmark dose confidence intervals (BMD CI) are a lot higher than those of NDMA and NDEA. It is therefore important to investigate the use of *in vivo* mutation BMD CI to see if this information can be used to calculate AI's directly, or to at least extend the EMA potency categorisation approach. Once data become available for the comparison of *in vivo* mutagenic potency compared to *in vivo* carcinogenic potency for a series of exemplar nitrosamines, numerous opportunities emerge.



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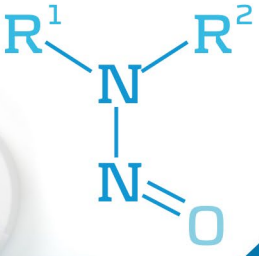
Options for using *in vivo* mutation data to assess nitrosamines.

There is limited cancer bioassay data for most nitrosamines, and there are no cancer bioassay data for the NDSRIs. Therefore, there are a lot of *in vivo* mutation dose-response data being generated to use for risk assessment in place of cancer data. There are different options for how to use positive *in vivo* mutation data, with these options below.

1. AI can be calculated from cancer BMD CI, there is also a potential to calculate AI using *in vivo* mutation BMD CI (EMA 2020).
2. Relative potency analysis based on *in vivo* mutation BMD CI could be used to support definition of categories in a modified CPCA approach (Table 1).
3. AI using relative potency comparisons outside of the CPCA approach.
    a. Use BMD CI to show mutation equipotency to a surrogate that has a defined AI.
    b. Use BMD CI to calculate fold change in potency, to multiply the AI from NDEA/NDEA based on this potency value.
4. If the nitrosamine is positive in the *in vivo* mutation assay or cancer bioassay and there is supportive DNA repair information, a PDE could potentially be calculated based on ICH M7 assumptions (Johnson et al. 2021). Regulatory bodies are currently not supporting the PDE approach for nitrosamines, with a major argument being that these compounds are within the cohort of concern (CoC). However, the CoC is a grouped potency estimation and does not consider mechanism or dose response. Furthermore, the CoC considers all nitrosamines together as equipotent substances when it is becoming clear that most NDRSIs are less potent and non-CoC.
5. Negative *in vivo* mutation data are now accepted by some but not all regulatory bodies, as evidence of non-mutagenicity. A suitable approach to embed this into the CPCA, would be that if the nitrosamine is defined as non-mutagenic using these data, then it should be considered potency category class 5, and limited according to ICH Q3A (R2) and Q3B (R2) 2006 guidelines (ICH 2006a; ICH 2006b).



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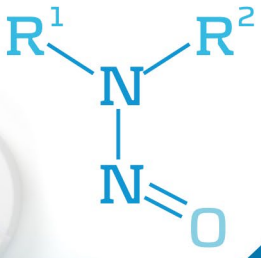
Current European Medicines Agency (EMA) CPCA approach

Table 1: (EMA 2023). The Five Predicted Potency Categories and Associated AI Limits for *N*-Nitrosamines within the CPCA.

Potency Category	Recommended AI Limit (ng/day)	Comments
1	18	The recommended AI limit of 18 ng/day is equal to the class-specific TTC for *N*-nitrosamine impurities.* *N*-nitrosamines assigned to Category 1 are predicted to have high carcinogenic potency; however, the class-specific TTC for *N*-nitrosamine impurities is considered sufficiently protective to patients.
2	100	The recommended AI limit of 100 ng/day is representative of two potent, robustly tested *N*-nitrosamines, *N*-nitrosodimethylamine (NDMA) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-(butanone) (NNK), which have recommended AI limits of 96 ng/day and 100 ng/day, respectively. *N*-nitrosamines assigned to Category 2 are predicted to have carcinogenic potency no higher than NDMA and NNK.
3	400	Compared to Potency Category 2, *N*-nitrosamines in this category have lower carcinogenic potency due to, for example, the presence of a weakly deactivating structural feature. The recommended AI limit was set to reflect a 4-fold decrease in carcinogenic potency from Category 2.
4	1500	*N*-Nitrosamines assigned to Category 4 may be metabolically activated through an α-hydroxylation pathway but are predicted to be of low carcinogenic potency, for example, because the pathway is disfavoured due to steric or electronic influences, or because clearance pathways are favoured. The recommended AI limit of 1500 ng/day is set at the TTC per ICH M7.**
5	1500	*N*-Nitrosamines assigned to Category 5 are not predicted to be metabolically activated via an α-hydroxylation pathway due to steric hindrance or the absence of α-hydrogens or are predicted to form unstable species that will not react with DNA. The recommended AI limit of 1500 ng/day is set at the TTC per ICH M7.**

This EMA CPCA approach (Table 1) is an excellent first step and has helped a lot of drugs to remain available to patients while ensuring their safety. However, many of the AI values are overly conservative. There are growing experimental data showing that NDSRIs have low or no mutagenic potency (Glowienke et al. 2022), and therefore the CoC concept within the TTC should not apply to them. There is also a lot of emerging data from *in vivo* transgenic and ecNGS gene mutation test systems to show that many NDSRIs have considerably lower potency than the smaller nitrosamines. The regulatory experts require data to assess the relationship between potency of nitrosamines in the *in vivo* TGR mutation assay and the cancer bioassay, and this can be provided in the form of dose response data and suitable analysis for model (exemplar) nitrosamines across different potencies.

One major issue in advancing in this area is the need for data sharing. This data sharing comes in all forms, with regulatory bodies, with different regulatory body departments, between regulatory bodies, between companies in some instances with the potential to publish the data, with or without coding or proprietary information included. A starting point is to obtain data from exemplar



nitrosamines that have cancer bioassay data and accepted AI's, in addition to *in vivo* TGR mutation dose response data.

NDMA, NDEA, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK, Cas: 64091-91-4), AI = 100), N-nitrosomorpholine (NMOR, Cas: AI = 127), NPIP (Cas: 100-75-4; AI=1,300), NDELA (Cas: 1116-54-7, AI=1,900) and Nitrosopyrrolidine (NPYR, Cas: 930-55-2; AI=1,700) would be suitable model substances. There are emerging data for some of these substances as well as many NDSRIs.

Bioavailability

The exposed dose is not always the same as the internal dose within the different tissues. For example, a nitrosamine impurity within an external cream would reach different internal organs at a much lower level than if the exposure was through oral ingestion. Even within oral exposure routes, phase 1 and phase 2 metabolism, along with the toxicokinetics means that the percentage of the active compound or metabolite reaching the target organ on which the risk assessment is based, is rarely 100%. This is accepted with the ICH M7 framework, but this decision of ignoring bioavailability highlights that at each decision-making step for setting AIs, the assumption is normally hypothetical and overly conservative.

Less Than Lifetime (LTL)

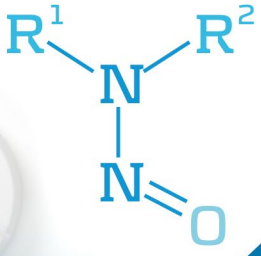
The standard AI approach assumes a lifetime exposure to the substance. However, for pharmaceuticals and other environmental compounds, lifetime exposure is not achieved, and the exact exposure duration is often known. This has led to the less than lifetime (LTL) approach being developed, and the recent update from EMA shows that the LTL can be used for nitrosamine impurities when the data are available. Controlling *N*-nitrosamines to an LTL AI based on the ICH M7 framework is thus demonstrated to be protective for potential carcinogenic risk to patients over the exposure durations typical of clinical trials and many prescribed medicines (Bercu et al. 2021).

There are different uses for the LTL approach within the new EMA Q&A guidance (EMA 2023). For compounds defined as category 4 using the CPCA approach, the substances are limited to 1.5µg/person/day and therefore the LTL is not used to define a higher limit.

LTL with Generic vs. Innovator companies

There is some disparity between the nitrosamine regulatory guidance for generic compared to innovator products.

The LTL approach is applicable to all authorised products that have a duration of treatment not exceeding 10 years (EMA 2023). Q22 within the EMA Q&A 2023 guidance means that a generic



version of that authorised product does not come under this guidance, and therefore the LTL approach is not applied in those scenarios. This is also the case when entering new countries.

Human Exposure to Drugs with Nitrosamine Impurities

Nitrosamine impurities are likely to have been in many drugs, with many of the recalled drugs being on the market for 20-40 years. To date, there has been no direct link to an increased cancer risk in the exposed population.

Some aspects of the recalls can cause major issues. For example, it is often not easy for the clinician and patient to switch from one drug to another due to issues including efficacy, market availability, safety, and potentially other issues. Therefore, a recall can result in a patient being without any suitable medication for their condition.

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The purpose of the report is to consider the 5 following topics.

1. **Exemplar nitrosamine compounds to test relative potency between *in vivo* mutation and cancer.**
2. **Investigate whether NDSRIs have low or no potency and could be considered as non-cohort of concern (non-CoC).**
3. **Investigate using *in vivo* mutation BMD CI for relative potency assessment to further inform on potency categorisation (EMA #1-5).**
4. **Comparisons with levels in food.**
5. **Considering the AI from EMA, USFDA and Health Canada calculated using the CPCA approach.**

1. Exemplar nitrosamine compounds to test relative potency between *in vivo* mutation and cancer.

The HESI GTTC are central to the exemplar nitrosamine work, with its members generously carrying out *in vivo* mutation testing on the range of compounds detailed above. Prior to completion of the project, it will be important to consider how best to use the data. This current report is written independently of the HESI GTTC, but with acknowledgement and support for their work. Note that this report for Medicines for Europe, stemmed from discussions between Dr George Johnson and others at the FDA/ HESI GTTC workshop in Spring 2023 in Washington DC.

The relative potency analysis between mutation and cancer will benefit from both the quantitative potency analysis, as well as the biological understanding of the link between mutation and cancer for each compound in turn. Adverse outcome pathways (AOPs) support the use of *in vivo* mutation as a surrogate for potency estimate of cancer (https://aopwiki.org/aops/139). Furthermore, a recent publication from Chepelev et al (Chepelev et al. 2023) shows that there is a direct relationship between potency of mutation compared to cancer. Additional assessment of the BMD CI values from the *in vivo* TGR mutation and cancer bioassay (supplementary files; Chepelev et al (2023)), shows that in most cases, the BMDL values for cancer and *in vivo* mutation were similar, or mutation BMDL were lower. In the cases where mutation BMDL was higher than cancer BMDL, the maximum difference was only 11xfold. Moreover, in each of these cases, the study design for *in vivo* mutation was not optimised for BMD analysis, and the BMD CI showed a lack of precision through a high BMDL:BMDU ratio and a more tailored study would likely reduce this difference considerably.

There is some apprehension in using *in vivo* gene mutation to assess human cancer risk for nitrosamines. During the HESI FDA workshop (FDA.HESI 2023), there was support for testing the hypothesis that there is relative potency of nitrosamines when assessed using the *in vivo* mutation

assay or the *in vivo* cancer bioassay data. If this hypothesis is shown to hold true, then the acceptable intake (AI) could be calculated using the *in vivo* mutation TGR or even ecNGS data if those data become available.

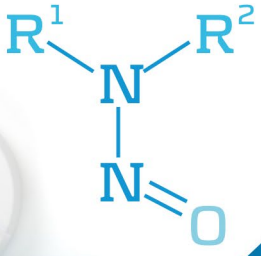
AIs have been calculated for NDEA and NDMA using data from a large number of *in vivo* cancer bioassay studies (EMA 2023). Furthermore, the *in vivo* mutation data for both substances is excellent with Bercu et al (Bercu et al. 2023a) recently generating a new and extensive data set for NDEA and Lynch *et al* (2023) for NDMA (Lynch et al. 2023). Precise and reliable BMD CI are presented from these studies. *In vivo* mutation BMD CI are compared in a relative potency approach. This could be used to define AIs for each NDSRI in turn. A different application is a pragmatic addition to the CPCA approach where potency values can be added to the SAR defined category score, based on the potency comparisons to the exemplar *in vivo* mutation BMD CI.

To use *in vivo* mutation data in place of cancer bioassay data for the calculation of acceptable intakes (AI), there is a request from some regulatory experts to produce data for exemplar (model) nitrosamines. These compounds should have robust cancer bioassay data and accepted AI's and cover a range of potencies. From discussions in the Health and Environmental Sciences Institute Genetic Toxicology Technical Committee (HESI-GTTC), we are aware that the following substances are being or have been studied using *in vivo* TGR mutation assays with optimal study design for BMD analysis.

- NDEA – BigBlue™ Rats – August 2023 publication. BMD CI are available.
- NDMA – MutaMouse™ – August 2023 submission to journal. BMD CI are available.
- NMOR, NNK, NDELA, NPIP and NPYR.

ecNGS mutation analysis will also be carried out for some of these exemplars.

Cancer bioassay defined AI will be calculated and compared to the mutation BMD CI.



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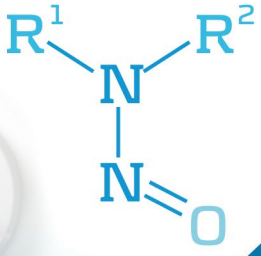
2. **Investigate whether NDSRIs have low or no potency and could be considered as non-cohort of concern (CoC).**

Thresher et al (2000) showed that not all nitrosamines should be considered as cohort of concern (CoC). This paragraph quotes that excellent paper. *"Although carcinogenic nitrosamines, as a class, are typically more potent than other carcinogens, they exhibit a wide distribution of log TD50 values, from NDEA at -2.585mg/kg/day to 1-nitrosopiperazine at -0.781 mg/kg/day. This distribution overlaps with that of the non-nitrosamine carcinogens, including some not present in the 'cohort of concern'. The mean log Lhasa TD50 value of -0.433 suggests NDEA may not be an exemplar of the carcinogenic potency of this chemical class. It was found that 18% of nitrosamines were considered non-carcinogenic. Nitrosamines showed a greater correlation between mutagenicity and carcinogenicity compared to non-nitrosamine compounds. Whilst nitrosamines, in general, are more potent carcinogens than non-nitrosamines, there is a significant overlap between the two distributions of TD50s for each class"* (Thresher et al. 2020).

In addition to numerous nitrosamines being non-CoC, there is a developing body of evidence to show that many NDSRI's are not mutagenic. A lot of this evidence has been presented at scientific conferences with the chemicals name withheld. There are numerous reasons why these newly developed data are yet to be published, but the most suitable example for inclusion within this report is one that is published. (S)-2-(((2'-(1H-tetrazol-5-yl)-[1,1' -biphenyl]-4-yl)methyl)(nitroso)amino)- 3-methylbutanoic acid (named 181-14) was shown by Glowienke *et al* (Glowienke et al. 2022) to be negative in both the Ames test and MutaMice™. There is a level of assurance from some regulatory bodies that a negative *in vivo* TGR mutation test can be accepted as a true result, and the modified *in vitro* Ames mutation test is also providing that same assurance in the absence of *in vivo* data as well. According to ICH M7 (R1) (2018), impurities that are not mutagenic in the modified Ames test would be considered Class 5 impurities and limited according to ICH Q3A (R2) and B (R2) (2006) guidelines (ICH 2006a; ICH 2006b). This shows that not all nitrosamines are equal, and that many could be regulated in an overly conservative manner, particularly the NDSRIs.

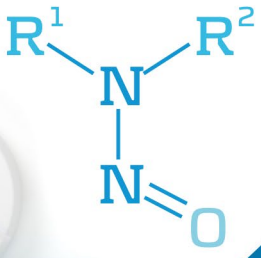
Seven structural groups had at least one *N*-nitrosamine with a TD_{50} similar to or higher than 1.5mg/kg/day, which translates to an AI similar to or higher than the default lifetime TTC (1.5µg/day) defined in ICH M7(R1), and five structural groups had at least one *N*-nitrosamine that was reported as noncarcinogenic, supporting the observation that not all *N*-nitrosamines are CoC carcinogens (Dobo et al. 2022). Higher molecular weight compounds are less likely to be metabolised into reactive substance and are often considered less potent. This means that higher TD50 and AIs are predicted for NDSRIs, noting many NDSRIs are negative as well. Furthermore, TD50 and AI are expressed on a mass basis, whereas Fine et al (Fine et al. 2023) show that they should be corrected for a substance's molecular weight, and when this is carried out, the corrected AI's are higher than the standard mass based AIs.



The EMA Q&A 2023 shows a potency categorisation approach based on structural activity relationship (SAR) principles (EMA 2023). As there are emerging *in vivo* mutation potency data, there is a proposal to use these data to support the EMA potency focussed CPCA.

The relative potency hypothesis is that potency correlates between *in vivo* mutation and *in vivo* cancer bioassay. This is supported by the knowledge that mutagenic carcinogens cause cancer via a mutation adverse outcome pathway (AOP), and protecting at the key event of mutation, will protect for the adverse outcome of cancer (Yauk et al. 2015; Yauk 2023). This is further supported by work such as Chepelev et al 2023 (Chepelev et al. 2023) which shows that protecting for mutation, also protects for cancer by comparing risk assessments based on both data. The risk assessment for ethyl-methansulphonate (EMS) that was acceptable to EMA, also used these principles. Roche used the PDE calculated from *in vivo* TGR mutation data for the alkylating agent EMS in certain batches of Viracept (Muller and Gocke 2009). EMS has a very similar mutation and DNA repair profile to NDMA (Johnson et al. 2021).

Once the relative potency hypothesis is supported for nitrosamines, this should allow decisions to be made based on *in vivo* mutation potency data in the absence of cancer data. One option is that the EMA Q&A CPCA approach could be extended, and *in vivo* mutation BMD CI values could be used to provide potency information which could support adding or subtracting values to the SAR based CPCA potency category value (Table 1). For category 4 or 5, the TTC limit could be used, but there would be merit in using the AI calculated from the potency comparison approach. Another option is to accept that the compound is not CoC and to use the PDE approach (ICHM7 2017). This is a more precise risk assessment that uses a series of adjustment factors and is based on the BMD CI to account for precision in the Point of Departure (PoD). This approach could be first used on certain classes of drugs deemed of very high priority. For example, a high priority drug could be (i) one on the essential medicines list, or (ii) one where the risk-benefit of the parent compound already considers the genotoxic risk of the whole drug, or (iii) one where the risk of the patient being taken off the drug while considering the low level of nitrosamine impurity, is outweighed by the action of the drug.



Examples

NDSRI-1

The following example from Teva Pharmaceuticals, shows an excellent use for the *in vivo* TGR gene mutation data. NDSRI-1-Teva and NDSRI-2-Teva are orders of magnitude less potent than the model nitrosamine NDMA and NDEA (Tables 2 and 3). This information can be used to state that these substances are not as potent as NDMA and NDEA, and that NDRSI-1 and NDSRI-2 are non-CoC (EMA 2023)). Therefore the TTC levels can be used for NDSRI-1 and NDSRI-2.

Positive in Ames test

- Conditions: Pre-incubation, DMSO, +/-rat S9, dose range 313-5000 µg/plate.
- Results: 7-fold increase in revertant numbers in TA1535 with rat S9.

Positive in *in vivo* mutation TGR

- OECD- and GLP-compliant MutaMouse study (reporter gene: *lacZ*)
- BMD modeling of the liver data provided a very precise width of the BMD confidence interval (1.9×)
- Comparison with NDMA and NDEA (Johnson et al. 2021):

Table 2: Table of BMD metrics for the NDEA (Johnson et al. 2021), NDMA (Johnson et al. 2021) and unpublished NDSRI-1 data from Teva.

	Gene Mutation		**Cancer Bioassay**	
Teva	$BMDL_{50}$ (mg/kg/day)	$BMDU_{50}$ (mg/kg/day)	$BMDL_{10}$ (mg/kg/day)	$BMDU_{10}$ (mg/kg/day)
NDMA	0.06	2.34	0.062	0.107
NDEA	0.004	0.028	0.022	0.046
NDSRI-1	28.9	55.7	-	-

- Potency ratio:
  - $BMDL_{50}$(NDSRI-1) ÷ $BMDL_{50}$(NDMA) ≅ 480 ⇨ AI(NDSRI-1) = 96 ng/day × 480 = 46,000 ng/day
  - $BMDL_{50}$(NDSRI-1) ÷ $BMDL_{50}$(NDEA) ≅ 7200 ⇨ AI(NDSRI-1) = 26.5 ng/day × 7200 = 190,800 ng/day
- Conclusion: NDSRI-1 is non-CoC potency.

NDSRI-2

Positive in Ames test

- Conditions: Pre-incubation, DMSO, +/-rat S9, dose range 313-5000 µg/plate.
- Results: 31-fold and 5.4-fold increase in revertant numbers in TA1535 and TA100, respectively, with rat S9.

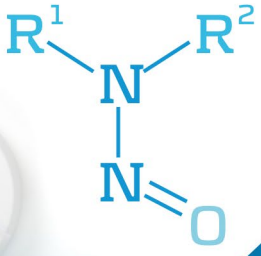
Positive in *in vivo* mutation TGR

- OECD- and GLP-compliant MutaMouse study (reporter gene: *lacZ*).
- BMD modeling of the liver data provided a very precise width of the BMD confidence interval (2.0×).
- Comparison with NDMA and NDEA (Johnson et al. 2021):

Table 3: Table of BMD metrics for the NDEA (Johnson et al. 2021), NDMA (Johnson et al. 2021) and unpublished NDSRI-2 data from Teva.

	Gene Mutation		**Cancer Bioassay**	
Teva	$BMDL_{50}$ (mg/kg/day)	$BMDU_{50}$ (mg/kg/day)	$BMDL_{10}$ (mg/kg/day)	$BMDU_{10}$ (mg/kg/day)
NDMA	0.06	2.34	0.062	0.107
NDEA	0.004	0.028	0.022	0.046
NDSRI-2	8.93	17.9	-	-

- Potency ratio:
  - $BMDL_{50}$(NDSRI-2) ÷ $BMDL_{50}$(NDMA) ≅ 150 ⇨ AI(NDSRI-2) = 96 ng/day × 150 = 14,400 ng/day
  - $BMDL_{50}$(NDSRI-2) ÷ $BMDL_{50}$(NDEA) ≅ 2200 ⇨ AI(NDSRI-2) = 26.5 ng/day × 2200 = 58,300 ng/day
- Conclusion: NDSRI-2 is non-CoC potency.



The level of confidence in using the *in vivo* mutation data in these ways increases based on the number of exemplar nitrosamines that support the relative potency hypothesis *'in vivo* mutation potency (BMD CI) correlates with the cancer-based AI'. There are currently 2 exemplar nitrosamines that support this hypothesis at the low AI levels. If a 3rd exemplar compound supports this hypothesis at the high AI levels, this provides confidence in using *in vivo* mutation BMD CI to support using an AI from an equipotent mutagenic nitrosamine for other nitrosamines. This is particularly relevant for the NDSRIs, that are considered to have low or no potency. Having this 3rd point of comparison will bring sufficient statistical precision and will provide stronger evidence for the significance of trends. This extra data point will act as a redundancy check.

The long-term approach would be to have a full series of exemplar compounds to support the hypothesis of relative potency between *in vivo* mutation and *in vivo* cancer and AI. However, we propose a short-term solution using 3 exemplar compounds for temporary AIs based on relative potency. Time to obtain all exemplar data may lead to unnecessary disruption of the availability of drugs for the patients. An additional long-term proposal is that the BMDL from *in vivo* mutation can be used to define PDE (Johnson et al. 2021).

3. **Investigate using *in vivo* mutation BMD CI for relative potency assessment to further inform on potency categorisation (EMA #1-5).**

The Teva example (Table 2 and Table 3) uses the *in vivo* mutation BMD CI from Johnson et al. 2021, but the studies used to define those BMD CI were not optimised for dose response analysis, and more recent studies are better suited for these comparisons. Bercu et al., (Bercu et al. 2023a) shows an excellent BigBlue Rat *in vivo* mutation dose response for NDEA. The BMD CI are presented in that publication and are used in Table 5 below.

Table 4: Table of BMD metrics for the NDEA (Bercu et al. 2023b) and unpublished NDSRI-1 and NDSRI-2 data from Teva, along with acceptable intakes (AI) for NDEA and 'adjusted AI' calculated using the ratios of the BMD CI for NDSRI-1 and NDSRI-2 to NDEA.

	$BMDL_{50}$	**$BMDU_{50}$**	**AI µg/person/day**
NDEA	0.1	1	0.0265
NDSRI-1	28.9	55.7	
NDSRI-2	8.93	17.9	
			Adjusted AI µg/person/day
NDEA to **NDSRI-1**	289		7.7
NDEA to **NDSRI-2**	89.3		2.4

The potency ratio approach shows that NDSRI1 and NDSRI2 are a lot less potent than NDMA and NDEA. In the approach used in Table 4, the ratio between the BMDL values is then used to calculate the relative potency and transform the NDEA AI value into those for NDSRI1 and NDSRI2. Note that once there are 3 exemplar nitrosamine compounds that have both the *in vivo* transgenic gene mutation BMD CI and cancer derived AI, then this approach is robust enough to use. There are a few other options for how to use relative potency to calculate AI values, and inclusion of upcoming AI values from other exemplar nitrosamines with both *in vivo* mutation and cancer bioassay data should improve the approach.

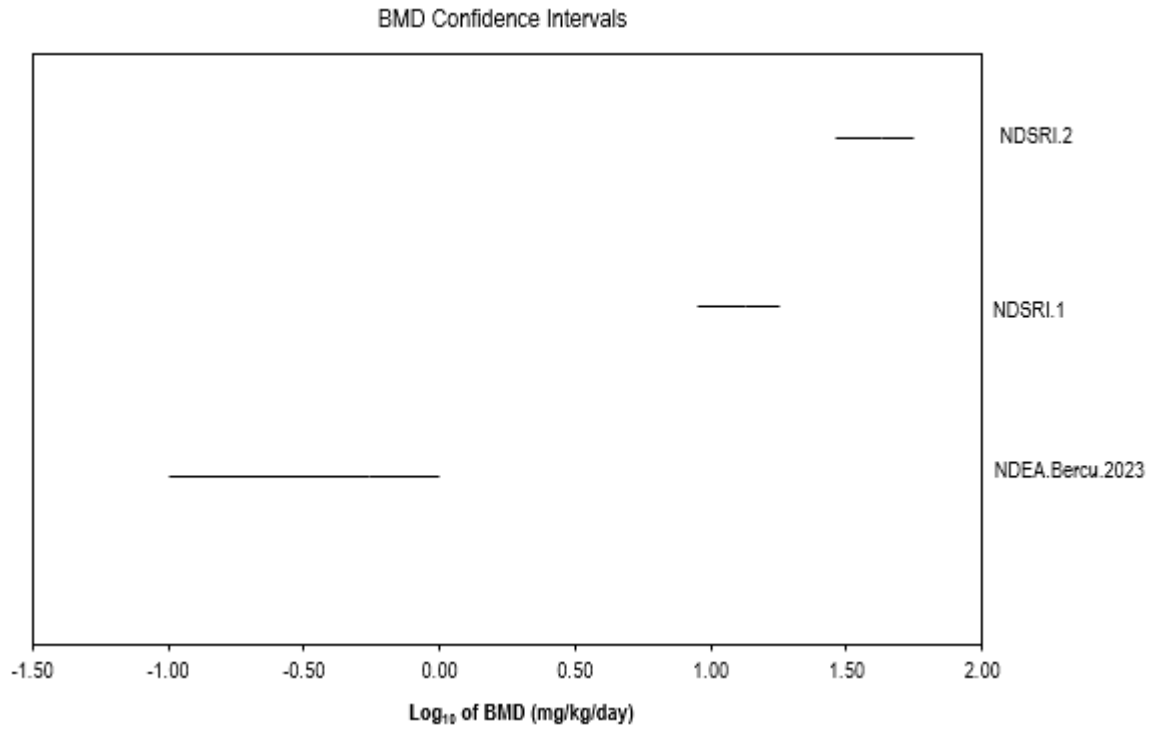


Figure 2: BMD CI potency analysis of *in vivo* gene mutation data from Table 5.

Bercu et al (Bercu et al. 2023b) also presented the BMD CI from ecNGS for NDEA. The BMDL50 was 0.04 and BMDU50 was 0.09mg/kg/day. ecNGS was more sensitive than TGR for mutation analysis. The co-authors are validating the ecNGS approach in-house for the assessment of nitrosamines, in a manner optimised for BMD CI calculation. The most advanced approach in 2023, is ecNGS from TwinStrand Biosciences, and many stakeholders are using this system to assess their nitrosamines of interest, prior to its validation for OECD approval. This is an excellent approach, and stakeholders are recommended to publish their data for hazard and risk assessment of nitrosamines using this ecNGS approach *in vivo*. Publishing these data will provide further confidence in the approach and will enable more advanced use of the data for regulatory decision making.

4 possible options for using relative potency as measured through *in vivo* TGR mutation BMD CI. These approaches could potentially be used with the *in vivo* ecNGS BMD CI as well.

1 - Use the calculated/extrapolated AI values e.g., NDSRI1 has an AI of 7.7µg/person/day (Table 4).

2 – Use the calculated/extrapolated AI values but add in an additional uncertainty factor.

3 – Use the calculated/extrapolated AI to show that the nitrosamine is non-CoC and should be treated according to the ICH M7 for a non-CoC impurity.

4 – Use information on mutagenic potency (Figure 2) to add or subtract an additional value to the SAR defined CPCA category. Cut-off points of BMD CI from the *in vivo* mutation TGR would be used to provide these values but maintain the final goal of defining a CPCA type AI using the category 1-5 approach.

4. Comparisons with levels in food.

Comparing the pharmaceutical AI values of certain nitrosamines to levels of those same nitrosamines in foods, highlights how low and unrealistic many AIs are. Although nitrosamines are found elsewhere in the environment as well as being produced endogenously, they are well researched in foods. The European Food Safety Authority (EFSA) has recently provided an extensive risk assessment of many nitrosamines found naturally in processed and processed food products (EFSA 2023). The nitrosamine levels across a range of food products are presented, and this comparison shows how much of the food would be needed to reach the nitrosamine level at the pharmaceutical AI. This highlights the difference between how the same chemicals are considered when occurring naturally in food, compared to being present within pharmaceutical products. One argument that is regularly used to defend the disparity between risk assessment of foods and pharmaceuticals, is that one does not have a choice whether to eat or not, but they do have a choice whether take pharmaceuticals or not. However, this argument quickly falls apart, as there is a choice in food, but consumers and even producers of food are not aware of the nitrosamine levels in different foods, so actually this choice is a nonsense and is not made by the consumer. Furthermore, many drugs are essential for an individual's health and wellbeing, and some are not replaceable by other medicines. Public perception is also a consideration, but one should not promote bad science and assess risk in an unprecise manner based on public misunderstanding of an industry whose main products are developed to directly improve human lives. There are other options for risk assessment, and although some nitrosamines are very potent, 'safe' levels can still be calculated and adhered to. Note that the term 'safe' is also a problem, as there are few things that are absolutely 'safe', and risk assessment is better defined using terms such as acceptable risk, increased risk, and similar qualified terms. Furthermore, many nitrosamines within pharmaceuticals, mostly termed nitrosamine drug substance-related impurities (NDSRIs), are larger and less potent nitrosamines for which the acceptable intakes (AI) are a lot higher or are even non-mutagenic and non-carcinogenic.

Bercu et al 2023 compared the AI values using the EFSA guidance (EFSA 2023) along with the AI calculated by the co-authors (Bercu et al. 2023a). The differences in AI values arose due to which TD50 was used, and this is well explained and supported.

The analysis below is carried out to compare the levels of a series of noteworthy nitrosamines in foods to the AI's calculated for pharmaceutical impurities. The upper bounds of the levels measured in different foods from EFSA 2023 are included in Table 5, and Figures 3 and 4 compare the upper bound levels of nitrosamines in foods, to the pharmaceutical impurity AI's (EMA 2023).

Table 5: Amount of various nitrosamines in food products (µg/kg), as measured and documented by the European Food Safety Authority (EFSA 2023). TCNA, the 10 carcinogenic N-NAs occurring in food.

N-NA µg/kg	Alcoholic beverages		Fish, seafood, amphibians, reptiles and invertebrates		Meat and meat products	
MaxUB	Beer and beer-like beverage	Unsweetened spirits and liqueurs	Processed fish and seafood	Cooked unprocessed fish	Processed meat	Cooked unprocessed meat
NDMA	0.186	0.692	1.99	1.107	8.284	1.837
NMEA					0.097	0.079
NDEA	0.284		0.5	0.308	3.901	1.874
NDPA					0.559	0.439
NDBA	0.302	4.9	0.35	0.162	0.273	0.28
NMOR	0.319				0.03	0.02
NPIP			2.9	0.908	0.906	1.39
TCNAs	1.09	5.592	9.62	4.264	17.074	17.603
TCNAs with max UB	0.849	1.672	3.692	1.984	9.421	5.15

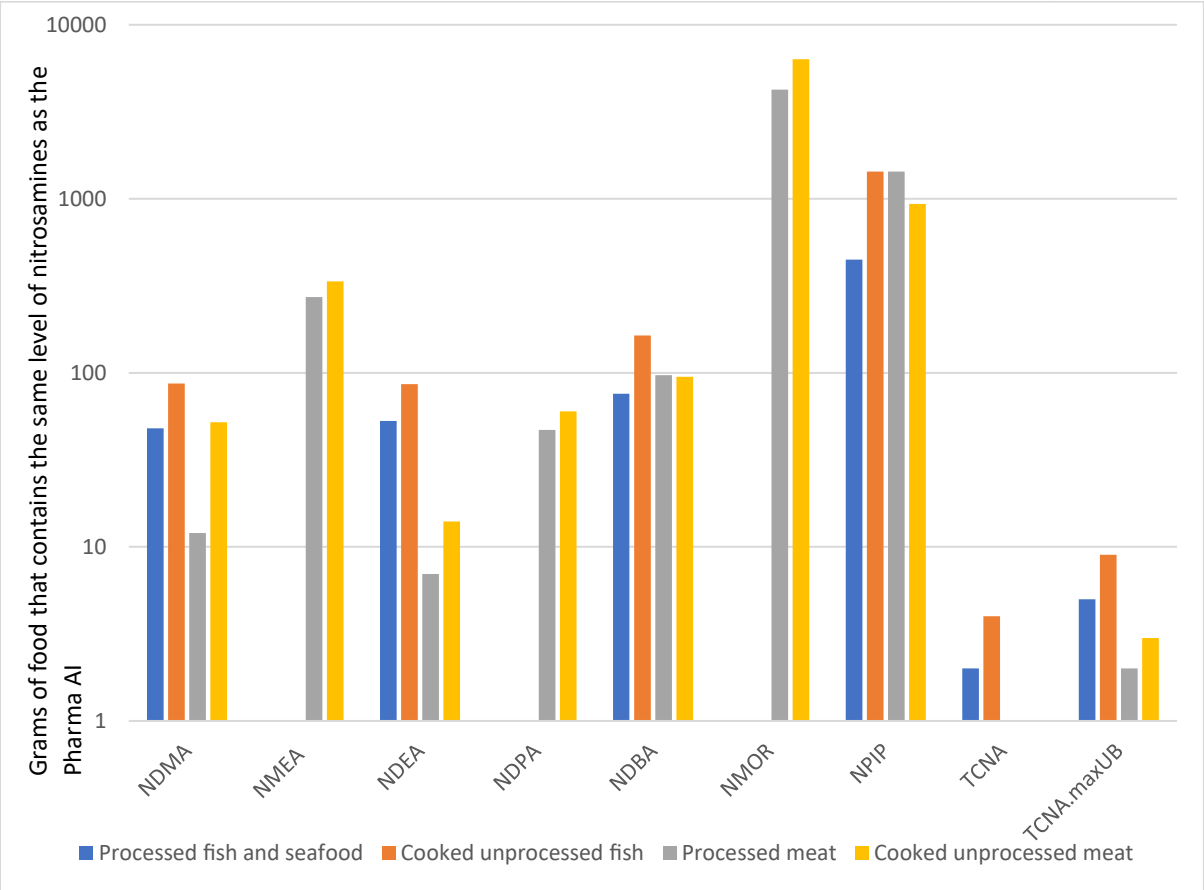


Figure 3: Amount of each food type needed, in order to reach the concentration of nitrosamine at the pharmaceutical acceptable intake (AI), as defined in EMA 2023, with 0.18ng/person/day used for the TCNA categories. 26.5ng/person/day for NMEA, NDEA, NDPA and NDBA, 96ng/person/day for NDMA, 127ng/person/day for NMOR and 1300ng/person/day for NPIP. For TCNA, the value is 1g for both cooked processed meat and processed meat, the other points with no increase in y-axis response do not have data available.

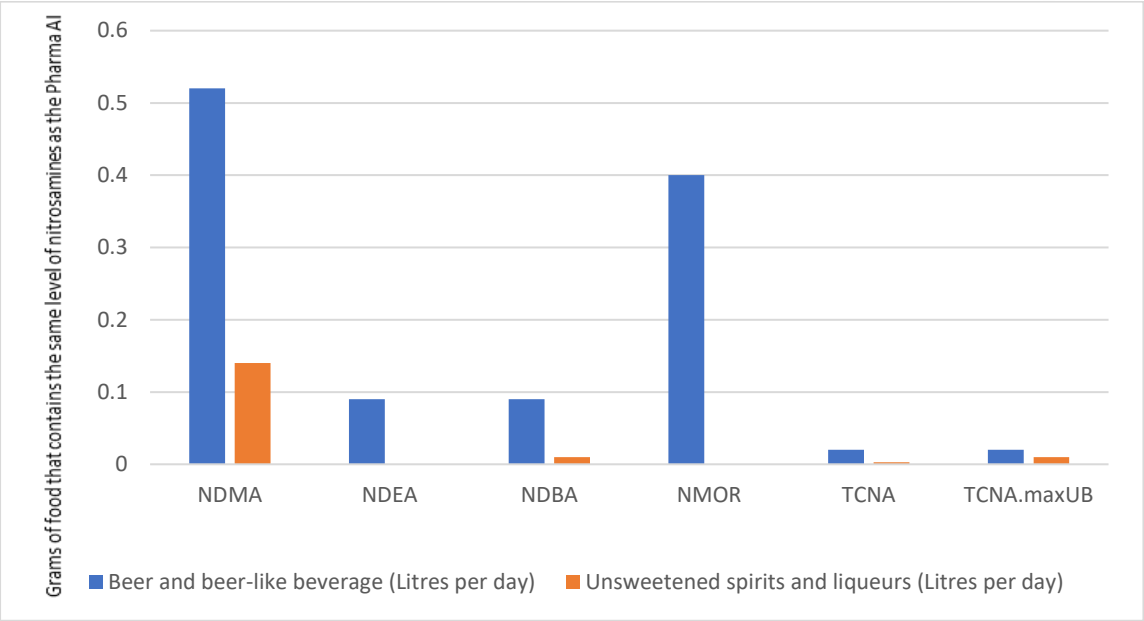
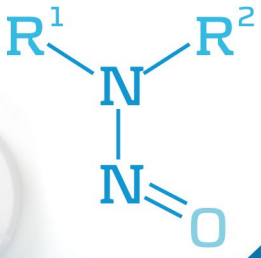


Figure 4: Amount of each drink needed, in order to reach the concentration of nitrosamine at the pharmaceutical acceptable intake (AI) as defined in EMA 2023 (Table 8), with 0.18ng/person/day used for the TCNA categories. 26.5ng/person/day for NMEA, NDEA, NDPA and NDBA, 96ng/person/day for NDMA, 127ng/person/day for NMOR and 1300ng/person/day for NPIP.



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5: Considering the AI from EMA, USFDA and Health Canada calculated using the CPCA approach

EMA (Table 6), USFDA (Table 7) and Health Canada (Table 8) have used the CPCA approach to define AI for an series of nitrosamines. EMA developed and published the approach, which was then adopted and used by the USFDA and Health Canada. This displays a good level of harmonisation between the global regulatory bodies for pharmaceutical impurity assessment.

There are many benefits to this, including a reduced need for follow up *in vivo* studies, as usable AI are now available for a wider range of nitrosamines.

As this guidance and AI have been presented so recently, it is difficult to know all of the intricate details of how to use them. For example, if an AI is yet to be calculated in the EMA guideline (Table 6), the pragmatic approach would be to use one from the USFDA or the Health Canada guideance. If an AI is still not defined, the information from all three could still be used to support an improved surrogate and read across approach.

There are some inconsistencies between the agency defined AI's (Tables, 6-8), and these are shown in Table 9 These are mainly around CPCA category 1 compounds. The base of category 1 is not clear and is more related to an unknown AI where we apply a default limit. One proposal is that if the limit is unknown, in place of applying a limit that is not achievable i.e., category 1, we should wait for weight of evidence produced by the industry to avoid disruption of the market. Achieving the category 1 value of 18ng/day is most often unachievable and placing many nitrosamines into category 1 will lead to the related products disappearing from the market and the clinic. As these category 1 values are defined using the systematic approach, it would be pragmatic to consider making the final call in a data driven manner.

In addition to the CPCA SAR approach (EMA 2023), the AI from Tables 6-8 can be used for NDSRI substances that are not listed as well. This surrogate type of approach is based on similarities between the NDSRI and a substance with a pre-defined AI. At the current time, the surrogate approach is defined by the regulatory body and due to the limited number of substances with well-defined AI values from cancer bioassay, the surrogates can often be those derived using the CPCA approach. An improvement to this on a data driven science-based approach is welcomed. A major improvement will come when the *in vivo* mutation data for exemplar nitrosamines come through and are compared to the *in vivo* cancer bioassay data.

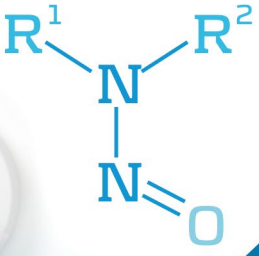
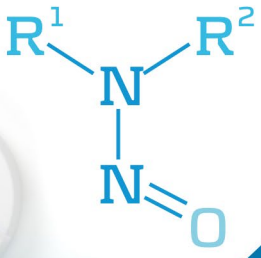
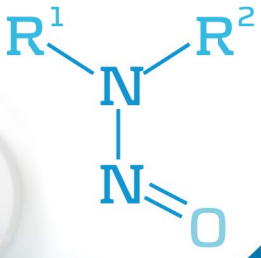


Table 6: Nitrosamine categories and acceptable intakes (AI) (EMA 2023).

Compound	Source	Cas number	CPCA Category	ng/day
N-methyl-N-nitrosophenethylamine, NMPEA		*13256-11-6*		*8*
N-nitroso-nortriptyline	*Amitriptyline, Nortryptyline*			*8*
nitroso-orphenadrine	*Orphenadrine*		*1*	*18*
N-nitroso-betahistine	*Betahistine*		*1*	*18*
N-nitroso-desmethyl-chloropyramine (N-DMCP)	*Chloropyramine*		*1*	*18*
N-nitroso-desmethyl-tripelennamine	*Tripelennamine*		*1*	*18*
N-nitroso-diethylamine, NDEA		*55-18-5*		*26.5*
N-nitroso-diisopropylamine, DIPNA		*601-77-4*		*26.5*
N-nitroso-di-n-butylamine, NDBA		*924-16-3*		*26.5*
N-nitroso-dipropylamine, NDPA		*621-64-7*		*26.5*
N-nitroso-ethylisopropylamine, EIPNA		*16339-04-1*		*26.5*
N-nitroso-N-methylaniline, NMPA		*614-00-6*		*34.3*
7-nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo-[4,3- a]pyrazine	*Sitagliptin*	*2892260-32-9*		*37*
N-nitroso-1,2,3,6-tetrahydropyridine, NTHP		*55556-92-8*		*37*
N-nitroso-varenicline, NNV	*Varenicline*			*37*
N-nitroso-dimethylamine, NDMA		62-75-9		96.0
N-nitroso-N-methyl-4-aminobutyric acid, NMBA		61445-55-4		96.0
4-(methylnitrosoamino)-1-(3-pyridinyl)-1-butanone (NNK)				100
N-nitroso-Atemoxetine	Atomoxetine			100
N-nitroso-duloxetine	Duloxetine			100
N-nitroso-fluoxetine	Fluoxetine			100
N-nitroso-p-chloro-benzylamino-pyridine (N-CBAP)			2	100
N-nitroso-phenylephrine	Phenylephrine		2	100
N-nitroso-rasagiline	Rasagiline		2	100
N-nitroso-sertraline	Sertraline		2	100
N-nitroso-morpholine, NMOR		59-89-2		127
N-nitroso-reboxetine	Reboxetine			127
1-cyclopropylmethyl-4-nitrosopiperazine			*3*	*400*
1-methyl-4-nitrosopiperazine, MeNP	*Rifampicin*	*16339-07-4*	*3*	*400*



nitroso impurity C" [N-(2,6-dimethylphenyl)-2-(4-nitrosopiperazin-1-yl)acetamide]			*3*	*400*
N-nitroso-ambroxol	*Ambroxol*		*3*	*400*
N-nitroso-aryl piperazine / N-nitroso-desalkylquetiapine (NDAQ)	*Quetiapine*		*3*	*400*
N-nitroso-dabigatran	*Dabigatran*		*3*	*400*
N-nitroso-desloratadine	*Desloratadine*		*3*	*400*
N-nitroso-landiolol	*Landiolol*		*3*	*400*
N-nitroso-mirabegron	*Mirabegron*		*3*	*400*
N-nitroso-N-ethyl-valacyclovir	*Valacyclovir*		*3*	*400*
N-nitroso-N-methyl-valacyclovir	*Valacyclovir*		*3*	*400*
N-nitroso-piperazine (NPZ)			*3*	*400*
N-nitroso-pramipexole	*Pramipexole*		*3*	*400*
N-nitroso-trimetazidine (NTMZ)	*Trimetazidine*		*3*	*400*
N-nitroso-vortioxetine	*Vortioxetine*		*3*	*400*
N-nitroso-methylphenidate, NMPH,	Methylphenidate	55557-03-4		1300
N-nitroso-paroxetine	Paroxetine			1300
N-nitroso-piperidine		100-75-4		1300
1-nitroso-pyrrolopiperidine			4	1500
nitroso-praziquanamine [2-nitroso-3,6,7,11b-tetrahydro-1H-pyrazino[2,1- a]isoquinolin-4-one]	Arpraziquantel		4	1500
N-nitroso-2,6-pipecoloxilidide	Ropivacaine		4	1500
N-nitroso-atenolol	Atenolol		4	1500
N-nitroso-benazepril	Benazepril		5	1500
N-nitroso-bisoprolol (NBP)	Bisoprolol		4	1500
N-nitroso-bumetanide (NNB)	Bumetanide		4	1500
N-nitroso-bupropion	Bupropion		5	1500
N-nitroso-cilazapril	Cilazapril		5	1500
N-nitroso-ciprofloxacin	Ciprofloxacin		4	1500
N-nitroso-desmethyl trimebutine	Trimebutine		5	1500
N-nitroso-diclofenac	Diclofenac		5	1500
N-nitroso-enalapril	Enalapril		5	1500
N-nitroso-folic acid (NFA)			4	1500
N-nitroso-ketamine	Ketamine		5	1500
N-nitroso-labetalol	Labetalol		4	1500



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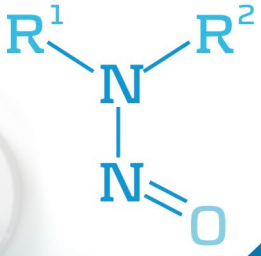
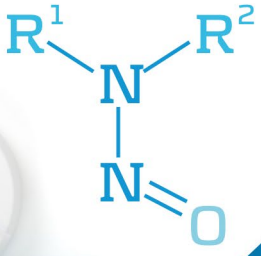
N-nitroso-levofloxacin	Levofloxacin		4	1500
N-nitroso-lisinopril	Lisinopril		5	1500
N-nitroso-metoprolol	Metoprolol		4	1500
N-nitroso-moxifloxacin	Moxifloxacin		4	1500
N-nitroso-nebivolol (NNEB)	Nebivolol		4	1500
N-nitroso-perindopril	Perindopril		5	1500
N-nitroso-propanolol	Propanolol		4	1500
N-nitroso-pseudoephedrine	Pseudoephedrine		4	1500
N-nitroso-ramipril	Ramipril		5	1500
N-nitroso-salbutamol	Salbutamol		5	1500
N-nitroso-sotalol	Sotalol		4	1500
N-nitroso-tamsulosin	Tamsulosin		4	1500
N-nitroso-vildagliptin	Vildagliptin		5	1500
2-nitroso-octahydrocyclopenta(c)pyrrole	Gliclazide			1700
N-nitroso-pyrrolidine NPYR		930-55-2		1700
N-nitroso-diethanolamine NDELA		1116-54-7		1900
N-nitroso-diphenylamine NDPh		86-30-6		78000
N-nitroso-mefenamic acid	Mefenamic acid			78000
N-nitroso-azaerythromycin	Azithromycin			NMI[16]
N-nitroso-desmethylazithromycin	Azithromycin			NMI[16]
N-nitroso-hydrochlorothiazide	Hydrochlorothiazide			NMI[16]
N-nitroso-quinapril	Quinapril			NMI[16]

Table 7: USFDA. 2023 updated acceptable intakes (AI) using the CPCA approach. (FDA 2023).

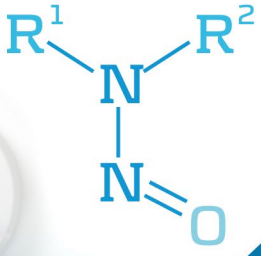
NDSRI Name	Source*	Potency Category	Recommended AI Limit (ng/day)
N-nitroso-atomoxetine	Atomoxetine	1	26.5
N-nitroso-desipramine	Desipramine	1	26.5
N-nitroso-desmethyl-almotriptan	Almotriptan	1	26.5
N-nitroso-desmethyl-amitriptyline	Amitriptyline	1	26.5
N-nitroso-desmethyl-bedaquiline	Bedaquiline	1	26.5
N-nitroso-desmethyl-brompheniramine	Brompheniramine	1	26.5
N-nitroso-desmethyl-cabergoline	Cabergoline	1	26.5
N-nitroso-desmethyl-carbinoxamine	Carbinoxamine	1	26.5
N-nitroso-desmethyl-chlophedianol	Chlophedianol	1	26.5
N-nitroso-desmethyl-chlorpheniramine	Chlorpheniramine	1	26.5
N-nitroso-desmethyl-chlorpromazine	Chlorpromazine	1	26.5
N-nitroso-desmethyl-cidoxepin	Doxepin	1	26.5
N-nitroso-desmethyl-citalopram	Citalopram	1	26.5
N-nitroso-desmethyl-clomipramine	Clomipramine	1	26.5
N-nitroso-desmethyl-cyclobenzaprine	Cyclobenzaprine	1	26.5
N-nitroso-desmethyl-desvenlafaxine	Desvenlafaxine	1	26.5
N-nitroso-desmethyl-dexbrompheniramine	Dexbrompheniramine	1	26.5
N-nitroso-desmethyl-dexchlorpheniramine	Dexchlorpheniramine	1	26.5
N-nitroso-desmethyl-diltiazem	Diltiazem	1	26.5
N-nitroso-desmethyl-diphenhydramine	Diphenhydramine	1	26.5
N-nitroso-desmethyl-doxepin, (e)-	Doxepin	1	26.5
N-nitroso-desmethyl-doxylamine	Doxylamine	1	26.5
N-nitroso-desmethyl-escitalopram	Escitalopram	1	26.5
N-nitroso-desmethyl-nizatidine	Nizatidine	1	26.5
N-nitroso-desmethyl-orphenadrine	Orphenadrine	1	26.5
N-nitroso-desmethyl-pheniramine	Pheniramine	1	26.5
N-nitroso-desmethyl-phenyltoloxamine	Phenyltoloxamine	1	26.5
N-nitroso-desmethyl-propoxyphene	Propoxyphene	1	26.5
N-nitroso-desmethyl-pyrilamine	Pyrilamine	1	26.5
N-nitroso-desmethyl-ranitidine	Ranitidine	1	26.5
N-nitroso-desmethyl-rizatriptan	Rizatriptan	1	26.5
N-nitroso-desmethyl-sumatriptan	Sumatriptan	1	26.5
N-nitroso-desmethyl-tamoxifen	Tamoxifen	1	26.5



N-nitroso-desmethyl-tapentadol	Tapentadol	1	26.5
N-nitroso-desmethyl-tetracaine	Tetracaine	1	26.5
N-nitroso-desmethyl-thonzylamine	Thonzylamine	1	26.5
N-nitroso-desmethyl-tramadol	Tramadol	1	26.5
N-nitroso-desmethyl-trimethobenzamide	Trimethobenzamide	1	26.5
N-nitroso-desmethyl-trimipramine	Trimipramine	1	26.5
N-nitroso-desmethyl-venlafaxine	Venlafaxine	1	26.5
N-nitroso-desmethyl-zolmitriptan	Zolmitriptan	1	26.5
N-nitroso-lorcaserin	Lorcaserin	1	26.5
N-nitroso-oliceridine	Oliceridine	1	26.5
N-nitroso-omadacycline	Omadacycline	1	26.5
N-nitroso-protriptyline	Protriptyline	1	26.5
N-nitroso-trientine	Trientine	1	26.5
N-nitroso-berotralstat	Berotralstat	2	100
N-nitroso-brinzolamide	Brinzolamide	2	100
N-nitroso-colistin a hydrogen methanesulfonate-1	Colistin	2	100
N-nitroso-colistin a hydrogen methanesulfonate-2	Colistin	2	100
N-nitroso-colistin a hydrogen methanesulfonate-3	Colistin	2	100
N-nitroso-colistin a hydrogen methanesulfonate-4	Colistin	2	100
N-nitroso-colistin a hydrogen methanesulfonate-5	Colistin	2	100
N-nitroso-colistin b hydrogen methanesulfonate-1	Colistin	2	100
N-nitroso-colistin b hydrogen methanesulfonate-2	Colistin	2	100
N-nitroso-colistin b hydrogen methanesulfonate-3	Colistin	2	100
N-nitroso-colistin b hydrogen methanesulfonate-4	Colistin	2	100
N-nitroso-colistin b hydrogen methanesulfonate-5	Colistin	2	100
N-nitroso-desmethyl-methylene blue	Methylene Blue	2	100
N-nitroso-desmethyl-mifepristone	Mifepristone	2	100
N-nitroso-desmethyl-minocycline-1	Minocycline	2	100
N-nitroso-desmethyl-neratinib	Neratinib	2	100
N-nitroso-desmethyl-omadacycline-1	Omadacycline	2	100
N-nitroso-desmethyl-padimate o	Padimate O	2	100
N-nitroso-desmethyl-quinupristin	Quinupristin	2	100
N-nitroso-desmethyl-rivastigmine	Rivastigmine	2	100
N-nitroso-desmethyl-spinosad factor a	Spinosad	2	100
N-nitroso-desmethyl-spinosad factor d	Spinosad	2	100
N-nitroso-desmethyl-tigecycline-2	Tigecycline	2	100
N-nitroso-desmethyl-ulipristal acetate	Ulipristal Acetate	2	100



N-nitroso-dipivefrin	Dipivefrin	2	100
N-nitroso-dorzolamide	Dorzolamide	2	100
N-nitroso-epinephrine	Epinephrine	2	100
N-nitroso-fenoldopam	Fenoldopam	2	100
N-nitroso-florbetaben f-18	Florbetaben F-18	2	100
N-nitroso-florbetapir f-18	Florbetapir F-18	2	100
N-nitroso-flutemetamol f-18	Flutemetamol F-18	2	100
N-nitroso-mitoxantrone-2	Mitoxantrone	2	100
N-nitroso-nizatidine-1	Nizatidine	2	100
N-nitroso-phenylephrine	Phenylephrine	2	100
N-nitroso-plazomicin-2	Plazomicin	2	100
N-nitroso-plerixafor-1	Plerixafor	2	100
N-nitroso-plerixafor-2	Plerixafor	2	100
N-nitroso-plerixafor-3	Plerixafor	2	100
N-nitroso-propafenone	Propafenone	2	100
N-nitroso-racepinephrine	Racepinephrine	2	100
N-nitroso-ranitidine-2	Ranitidine	2	100
N-nitroso-rasagiline	Rasagiline	2	100
N-nitroso-sertraline	Sertraline	2	100
N-nitroso-amoxapine	Amoxapine	3	400
N-nitroso-avanafil	Avanafil	3	400
N-nitroso-cangrelor	Cangrelor	3	400
N-nitroso-carvedilol	Carvedilol	3	400
N-nitroso-cinacalcet	Cinacalcet	3	400
N-nitroso-dabigatran etexilate	Dabigatran Etexilate	3	400
N-nitroso-degarelix	Degarelix	3	400
N-nitroso-desloratadine	Desloratadine	3	400
N-nitroso-desmethyl-demeclocycline	Demeclocycline	3	400
N-nitroso-desmethyl-doxycycline	Doxycycline	3	400
N-nitroso-desmethyl-eravacycline	Eravacycline	3	400
N-nitroso-desmethyl-erythromycin ethylsuccinate	Erythromycin Ethylsuccinate	3	400
N-nitroso-desmethyl-methadone	Methadone	3	400
N-nitroso-desmethyl-minocycline-2	Minocycline	3	400
N-nitroso-desmethyl-omadacycline-2	Omadacycline	3	400
N-nitroso-desmethyl-promethazine	Promethazine	3	400
N-nitroso-desmethyl-sarecycline	Sarecycline	3	400



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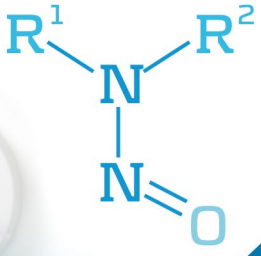
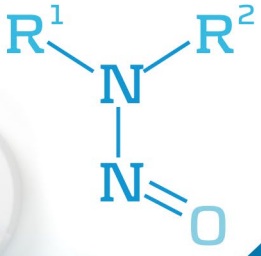
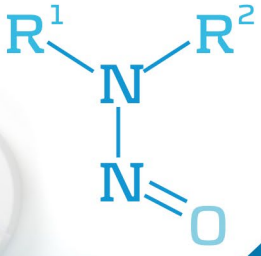
N-nitroso-desmethyl-tetracycline	Tetracycline	3	400
N-nitroso-desmethyl-tigecycline-1	Tigecycline	3	400
N-nitroso-fenfluramine	Fenfluramine	3	400
N-nitroso-frovatriptan	Frovatriptan	3	400
N-nitroso-levmetamfetamine	Levmetamfetamine	3	400
N-nitroso-methamphetamine	Methamphetamine	3	400
N-nitroso-mirabegron	Mirabegron	3	400
N-nitroso-nizatidine-2	Nizatidine	3	400
N-nitroso-ozanimod	Ozanimod	3	400
N-nitroso-pramipexole	Pramipexole	3	400
N-nitroso-propylhexedrine	Propylhexedrine	3	400
N-nitroso-ranitidine-1	Ranitidine	3	400
N-nitroso-relebactam	Relebactam	3	400
N-nitroso-safinamide	Safinamide	3	400
N-nitroso-salmeterol	Salmeterol	3	400
N-nitroso-telavancin-1	Telavancin	3	400
N-nitroso-tetracaine	Tetracaine	3	400
N-nitroso-vilanterol	Vilanterol	3	400
N-nitroso-vortioxetine	Vortioxetine	3	400
N-nitroso-acebutolol	Acebutolol	4	1500
N-nitroso-argatroban	Argatroban	4	1500
N-nitroso-articaine	Articaine	4	1500
N-nitroso-atenolol	Atenolol	4	1500
N-nitroso-betaxolol	Betaxolol	4	1500
N-nitroso-bicisate	Bicisate	4	1500
N-nitroso-bisoprolol	Bisoprolol	4	1500
N-nitroso-bumetanide	Bumetanide	4	1500
N-nitroso-caspofungin	Caspofungin	4	1500
N-nitroso-desmethyl-clarithromycin	Clarithromycin	4	1500
N-nitroso-desmethyl-erythromycin	Erythromycin	4	1500
N-nitroso-desmethyl-olopatadine	Olopatadine	4	1500
N-nitroso-desmethyl-telithromycin	Telithromycin	4	1500
N-nitroso-dobutamine	Dobutamine	4	1500
N-nitroso-elagolix	Elagolix	4	1500
N-nitroso-ephedrine	Ephedrine	4	1500
N-nitroso-ertapenem	Ertapenem	4	1500
N-nitroso-esmolol	Esmolol	4	1500



N-nitroso-ethambutol	Ethambutol	4	1500
N-nitroso-flecainide	Flecainide	4	1500
N-nitroso-folic acid	Folic Acid	4	1500
N-nitroso-formoterol	Formoterol	4	1500
N-nitroso-furosemide	Furosemide	4	1500
N-nitroso-gatifloxacin	Gatifloxacin	4	1500
N-nitroso-isoproterenol	Isoproterenol	4	1500
N-nitroso-labetalol	Labetalol	4	1500
N-nitroso-leucovorin-1	Leucovorin	4	1500
N-nitroso-leucovorin-2	Leucovorin	4	1500
N-nitroso-levoleucovorin-1	Levoleucovorin	4	1500
N-nitroso-levoleucovorin-2	Levoleucovorin	4	1500
N-nitroso-levomefolic acid-1	Levomefolic Acid	4	1500
N-nitroso-levomefolic acid-2	Levomefolic Acid	4	1500
N-nitroso-mefloquine	Mefloquine	4	1500
N-nitroso-meropenem	Meropenem	4	1500
N-nitroso-metoprolol	Metoprolol	4	1500
N-nitroso-migalastat	Migalastat	4	1500
N-nitroso-mitoxantrone-1	Mitoxantrone	4	1500
N-nitroso-moxifloxacin	Moxifloxacin	4	1500
N-nitroso-nebivolol	Nebivolol	4	1500
N-nitroso-hydrochlorothiazide	Hydrochlorothiazide	4	1500
N-nitroso-oritavancin-1	Oritavancin	4	1500
N-nitroso-ozenoxacin	Ozenoxacin	4	1500
N-nitroso-pindolol	Pindolol	4	1500
N-nitroso-plazomicin-1	Plazomicin	4	1500
N-nitroso-prilocaine	Prilocaine	4	1500
N-nitroso-proline	Proline	4	1500
N-nitroso-propranolol	Propranolol	4	1500
N-nitroso-pseudoephedrine	Pseudoephedrine	4	1500
N-nitroso-sapropterin-1	Sapropterin	4	1500
N-nitroso-silodosin	Silodosin	4	1500
N-nitroso-sotalol	Sotalol	4	1500
N-nitroso-streptomycin	Streptomycin	4	1500
N-nitroso-tamsulosin	Tamsulosin	4	1500
N-nitroso-telavancin-2	Telavancin	4	1500
N-nitroso-telavancin-3	Telavancin	4	1500



N-nitroso-tirofiban	Tirofiban	4	1500
N-nitroso-vancomycin	Vancomycin	4	1500
N-nitroso-abacavir	Abacavir	5	1500
N-nitroso-acarbose	Acarbose	5	1500
N-nitroso-albuterol	Albuterol	5	1500
N-nitroso-amlodipine	Amlodipine	5	1500
N-nitroso-benazepril	Benazepril	5	1500
N-nitroso-bendroflumethiazide	Bendroflumethiazide	5	1500
N-nitroso-brilliant blue g	Brilliant Blue G	5	1500
N-nitroso-bupropion	Bupropion	5	1500
N-nitroso-carteolol	Carteolol	5	1500
N-nitroso-chloroquine	Chloroquine	5	1500
N-nitroso-clevidipine	Clevidipine	5	1500
N-nitroso-clozapine	Clozapine	5	1500
N-nitroso-diclofenac	Diclofenac	5	1500
N-nitroso-duvelisib	Duvelisib	5	1500
N-nitroso-enalapril	Enalapril	5	1500
N-nitroso-enalaprilat	Enalaprilat	5	1500
N-nitroso-esketamine	Esketamine	5	1500
N-nitroso-etravirine	Etravirine	5	1500
N-nitroso-felodipine	Felodipine	5	1500
N-nitroso-fosdenopterin-1	Fosdenopterin	5	1500
N-nitroso-fosdenopterin-2	Fosdenopterin	5	1500
N-nitroso-fostamatinib-1	Fostamatinib	5	1500
N-nitroso-fostamatinib-2	Fostamatinib	5	1500
N-nitroso-hydroxychloroquine	Hydroxychloroquine	5	1500
N-nitroso-imatinib	Imatinib	5	1500
N-nitroso-isoxsuprine	Isoxsuprine	5	1500
N-nitroso-isradipine	Isradipine	5	1500
N-nitroso-ivacaftor	Ivacaftor	5	1500
N-nitroso-ketamine	Ketamine	5	1500
N-nitroso-levalbuterol	Levalbuterol	5	1500
N-nitroso-levamlodipine	Levamlodipine	5	1500
N-nitroso-levobunolol	Levobunolol	5	1500
N-nitroso-lisinopril	Lisinopril	5	1500
N-nitroso-mecamylamine	Mecamylamine	5	1500
N-nitroso-meclofenamic acid	Meclofenamic Acid	5	1500



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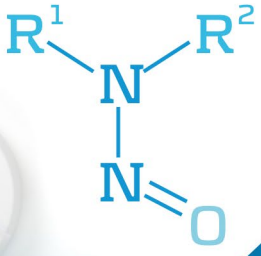
N-nitroso-metolazone	Metolazone	5	1500
N-nitroso-moexipril	Moexipril	5	1500
N-nitroso-nadolol	Nadolol	5	1500
N-nitroso-neratinib	Neratinib	5	1500
N-nitroso-nicardipine	Nicardipine	5	1500
N-nitroso-nifedipine	Nifedipine	5	1500
N-nitroso-nimodipine	Nimodipine	5	1500
N-nitroso-nintedanib	Nintedanib	5	1500
N-nitroso-nisoldipine	Nisoldipine	5	1500
N-nitroso-olanzapine	Olanzapine	5	1500
N-nitroso-olodaterol	Olodaterol	5	1500
N-nitroso-oritavancin-2	Oritavancin	5	1500
N-nitroso-perindopril	Perindopril	5	1500
N-nitroso-polythiazide	Polythiazide	5	1500
N-nitroso-primaquine	Primaquine	5	1500
N-nitroso-quinapril	Quinapril	5	1500
N-nitroso-ramipril	Ramipril	5	1500
N-nitroso-rifabutin	Rifabutin	5	1500
N-nitroso-rilpivirine-1	Rilpivirine	5	1500
N-nitroso-rilpivirine-2	Rilpivirine	5	1500
N-nitroso-risdiplam	Risdiplam	5	1500
N-nitroso-rolapitant	Rolapitant	5	1500
N-nitroso-sapropterin-2	Sapropterin	5	1500
N-nitroso-tafenoquine	Tafenoquine	5	1500
N-nitroso-telavancin-4	Telavancin	5	1500
N-nitroso-terbutaline	Terbutaline	5	1500
N-nitroso-ticagrelor	Ticagrelor	5	1500
N-nitroso-tigecycline	Tigecycline	5	1500
N-nitroso-timolol	Timolol	5	1500
N-nitroso-torsemide	Torsemide	5	1500
N-nitroso-trandolapril	Trandolapril	5	1500
N-nitroso-vibegron	Vibegron	5	1500

Table 8: Health Canada, acceptable intakes (AI) using the CPCA approach. (Health-Canada 2023a).

Related Drug Substance / Drug Product	***N*-Nitrosamine**	**CAS RN (if available)**	**CPCA category**	**AI limit ()***
Betahistine	*N*-nitroso-betahistine	32635-81-7	1	18
Citalopram	*N*-nitroso-desmethyl citalopram	-	1	18
Desvenlafaxine	*N*-nitroso-*N*-desmethyl desvenlafaxine	-	1	18
Diphenhydramine hydrochloride	*N*-(2-(benzhydryloxy)ethyl)-*N*-methylnitrous amide	55855-43-1	1	18
Doxepin	*N*-nitroso-nordoxepin	-	1	18
Doxylamine	*N*-nitroso-*N*-methyl-2-[1-phenyl-1 (2-pyridinyl)ethoxy]ethanamine	-	1	18
Doxylamine	*N*-nitroso-*N*-methyl-2-[1-phenyl-1-(2-pyridinyl)methoxy]ethanamine	-	1	18
Lapatinib	*N*-nitroso-lapatinib: *N*-[3-chloro-4-[(3-fluorophenyl)methoxy]phenyl]-6- [5-[[[2-(methylsulfonyl)ethyl]nitrosoamino]-methyl]-2-furanyl]-4- quinazolinamine	-	1	18
Orphenadrine	*N*-nitroso-*N*-desmethyl orphenadrine (NMOA)	-	1	18
Penicillin G benzathine	*N*-nitroso-*N,N'*-dibenzylethanediamine	-	1	18
Penicillin G benzathine	*N,N'*-dinitroso-*N,N'*-dibenzylethanediamine	-	1	18
Sumatriptan	3-[2-(*N*-nitroso-*N*- methyl)amino)ethyl]-*N*-methyl-1*H*- indole-5-methanesulfonamide	-	1	18
Terbinafine	*N*-nitroso-*N*-methyl-1-naphthylmethylamine	-	1	18
Terbinafine	*N*-nitroso-*N*-desmethyl terbinafine	-	1	18
Terbinafine	*N*-[(2*E*)-6,6-dimethyl-2-hepten-4-yn- 1-yl]-*N*-nitrosomethanamine	-	1	18
Tripelennamine	*N*-nitroso-*N*-desmethyl- tripelennamine	-	1	18
Dorzolamide	*N*-nitroso-dorzolamide	-	2	100
Phenylephrine	*N*-nitroso-phenylephrine	78658-64-7	2	100
Rasagiline	*N*-nitroso-rasagiline	2470278-90-9	2	100
Rivastigmine	*N*-nitroso-*N*-desmethyl rivastigmine	-	2	100
Sertraline	*N*-nitroso-sertraline	-	2	100
-	1-methyl-4-nitrosopiperazine (MNP)	16339-07-4	3	400
-	*N*-nitroso-piperazine	5632-47-3	3	400
Ciprofloxacin	*N,N*'-dinitrosopiperazine	140-79-4	3	400



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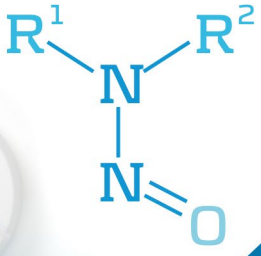
Dabigatran	*N*-nitroso-dabigatran	-	3	400
Desloratadine	*N*-nitroso-desloratadine	1246819-22-6	3	400
Doxycycline	*N*-desmethyl-*N*-nitroso-doxycycline	-	3	400
Frovatriptan	*N*-nitroso-frovatriptan: (3*R*)-2,3,4,9-tetrahydro-3-(*N*-methyl(nitroso)amino)-1*H* carbazole-6-carboxamide	-	3	400
Hydroxyzine	2-(2-(4-nitrosopiperazin-1-yl)ethoxy)ethan-1-ol	73486-81-4	3	400
Mirabegron	*N*-nitroso-mirabegron	-	3	400
Pramipexole	*N*-nitroso-pramipexole	-	3	400
Quetiapine	*N*-nitroso-desalkylquetiapine (NDAQ): 1-(4-dibenzo[*b*,*f*][1,4]thiazepin-11- yl)-4-nitrosopiperazine	-	3	400
Rifapentine	1-cyclopentyl-4-nitrosopiperazine	61379-66-6	3	400
Valacyclovir	*N*-nitroso-*N*-methyl-valacyclovir	-	3	400
Valacyclovir	*N*-nitroso-*N*-ethyl-valacyclovir	-	3	400
Vortioxetine	*N*-nitroso-vortioxetine	-	3	400
Atenolol	*N*-nitroso-atenolol	134720-04-0	4	1500
Bisoprolol	*N*-nitroso-bisoprolol	-	4	1500
Ciprofloxacin	*N*-nitroso-ciprofloxacin	864443-44-7	4	1500
Clarithromycin	*N*-nitroso-*N*-desmethyl clarithromycin	-	4	1500
Dextromethorphan	*N*-nitroso-*N*-desmethyl dextromethorphan	-	4	1500
Flecainide	*N*-nitroso-flecainide	-	4	1500
Folic acid	*N*-nitroso-folic acid	26360-21-4	4	1500
Furosemide	*N*-nitroso-furosemide	2708280-93-5	4	1500
Labetalol	*N*-nitroso-labetalol	2820170-74-7	4	1500
Leucovorin	*N*-nitroso-folinic acid: *N*-[4-[[(2-amino-5-formyl-3,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl)methyl]nitrosoamino]benzoyl]-L-glutamic acid	-	4	1500
Levofloxacin	9-fluoro-2,3-dihydro-3-methyl-10-(*N*-nitroso-2-aminoethyl)-7-oxo-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid	-	4	1500
Levofloxacin	*N*-nitroso-*N*-desmethyl levofloxacin	-	4	1500
Lidocaine	*N*-nitroso-lidocaine EP Impurity E	-	4	1500
Meropenem	*N*-nitroso-meropenem	-	4	1500
Metoprolol	*N*-nitroso-metoprolol	138768-62-4	4	1500
Moxifloxacin	*N*-nitroso-moxifloxacin	-	4	1500



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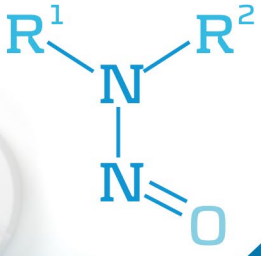
Nebivolol	*N*-nitroso-nebivolol	1391051-68-5	4	1500
Propranolol	*N*-nitroso-propranolol	84418-35-9	4	1500
Pseudoephedrine	*N*-nitroso-pseudoephedrine	1850-88-0	4	1500
Sotalol	*N*-nitroso-sotalol	134720-07-3	4	1500
Tamsulosin	*N*-nitroso-tamsulosin	-	4	1500
Benazepril	*N*-nitroso-benazepril	-	5	1500
Bupropion	*N*-nitroso-bupropion	-	5	1500
Cilazapril	*N*-nitroso-cilazapril	1053740-92-3	5	1500
Clozapine	*N*-nitroso-clozapine	156632-03-0	5	1500
Diclofenac	*N*-nitroso-diclofenac	66505-80-4	5	1500
Enalapril	*N*-nitroso-enalapril	-	5	1500
Ketamine	*N*-nitroso-ketamine	86144-35-6	5	1500
Lisinopril	*N*-nitroso-lisinopril: N^2-[(1*S*)-1- carboxy-3-phenylpropyl]-N^2-nitroso- L-lysyl-L-proline	-	5	1500
Perindopril	*N*-nitroso-perindopril	-	5	1500
Ramipril	*N*-nitroso-ramipril	-	5	1500
Salbutamol	*N*-nitroso-salbutamol	-	5	1500
Tigecycline	*N*-nitroso-tigecycline: (4*S*,4a*S*,5a*R*,12a*S*)-4,7-bis(dimethylamino)-9-[[2-[(1,1-dimethylethyl)nitrosoamino]acetyl]amino]-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11- dioxo-2-naphthacenecarboxamide	-	5	1500
Trimebutine	*N*-nitroso-*N*-desmethyl trimebutine	-	5	1500
-	*N*-methyl-*N*-nitroso-phenethylamine (NMPEA)	13256-11-6	-	8
Amitriptyline	*N*-nitroso-nortriptyline (NNORT)	55855-42-0	-	8
Nortriptyline	*N*-nitroso-nortriptyline (NNORT)	55855-42-0	-	8
-	*N*-nitroso-dibutylamine (NDBA)	924-16-3	-	26.5
-	*N*-nitroso-diethylamine (NDEA)	55-18-5	-	26.5
-	*N*-nitroso-diisopropylamine (NDIPA)	601-77-4	-	26.5
-	*N*-nitroso-dipropylamine (NDPA)	621-64-7	-	26.5
-	*N*-nitroso-ethylisopropylamine (NEIPA)	16339-04-1	-	26.5
-	*N*-nitroso-1,2,3,6-tetrahydropyridine (NTHP)	55556-92-8	-	37
Sitagliptin	7-nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo-[4,3-*a*]pyrazine (NTTP)	762240-92-6	-	37
Varenicline	*N*-nitroso-varenicline (NNV)	535920-98-0	-	37
-	*N*-nitroso-4-(methylamino)butyric acid (NMBA)	61445-55-4	-	96



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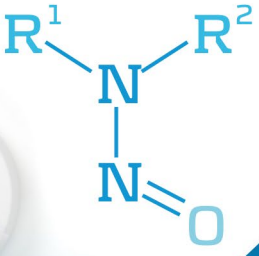
-	*N*-nitroso-dimethylamine (NDMA)	62-75-9	-	96
-	4-(methylnitrosamino)-1-(3-pyridyl)- 1-(butanone) (NNK)	64091-91-4	-	100
Atomoxetine	*N*-nitroso-atomoxetine	-	-	100
Duloxetine	*N*-nitroso-duloxetine (NDLX)	-	-	100
Fluoxetine	*N*-nitroso-fluoxetine	150494-06-7	-	100
-	*N*-nitroso-morpholine (NMOR)	59-89-2	-	127
-	*N*-nitroso-piperidine (NPIP)	100-75-4	-	1300
Methylphenidate	*N*-nitroso-methylphenidate	55557-03-4	-	1300
Paroxetine	*N*-nitroso-paroxetine	2361294-43-9	-	1300
Felodipine	*N*-nitroso-felodipine	-	-	1500
-	*N*-nitroso-pyrrolidine	930-55-2	-	1700
-	*N*-nitroso-diethanolamine (NDELA)	1116-54-7	-	1900
-	*N*-nitroso-diphenylamine	86-30-6	-	78000
Mefenamic acid	*N*-nitroso-mefenamic acid	2114-63-8	-	78000
Azithromycin	*N*-nitroso-azaerythromycin: (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,4*R*)-13-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl- 3,4,10-trihydroxy-3,5,8,10,12,14- hexamethyl-6-nitroso-11-[[3,4,6- trideoxy-3-(dimethylamino)-β-D- *xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one	-	-	**
Azithromycin	*N*-nitroso-*N*-desmethyl azithromycin: (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl- 3,4,10-trihydroxy- 3,5,6,8,10,12,14-heptamethyl-11- [[3,4,6-trideoxy-3-(methyl(nitroso)amino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one	-	-	**
Hydrochlorothiazide	*N*-nitroso-hydrochlorothiazide	63779-86-2	-	**
Valsartan	(*S*)-2-(((2'-(1*H*-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)(nitroso) amino)-3-methylbutanoic acid	2254485-68-0	-	**
Quinapril	*N*-nitroso-quinapril	158522-79-3		**

Table 9: There are some differences in AI defined by the different regulatory agencies, as explained in the table below.

Related Drug Substance / Drug Product	N-Nitrosamine	CAS RN		AI limit ()*		
		(if available)	CPCA category	Health Canada	EMA	FDA
Atomoxetine	N-nitroso-atomoxetine		NA/1	100	100	26.5
Citalopram	N-nitroso-desmethyl citalopram		1	18		26.5
Desvenlafaxine	N-nitroso-N-desmethyl-desvenlafaxine		1	18		26.5
Diphenhydramine hydrochloride	N-(2-(benzhydryloxy)ethyl)-N-methylnitrous amide	55855-43-1	1	18		26.5
Doxepin	N-nitroso-nordoxepin		1	18		26.5
Doxylamine	N-nitroso-N-methyl-2-[1-phenyl-1-(2-pyridinyl)ethoxy]ethanamine		1	18		26.5
Orphenadrine	N-nitroso-N-desmethyl orphenadrine (NMOA)		1	18	18	26.5
Sumatriptan	3-[2-(N-nitroso-N-methyl)amino)ethyl]-N-methyl-1H-indole-5-methanesulfonamide		1	18		26.5



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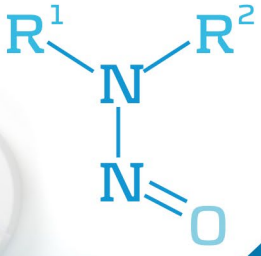

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