The regulatory challenge of determining acceptable intakes for nitrosamine drug substance-related impurities while ensuring medicinal product supply

Masterarbeit

zur Erlangung des Titels

„Master of Drug Regulatory Affairs, M.D.R.A."

der Mathematisch-Naturwissenschaftlichen Fakultät

der Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

Marianna Costa

Bonn 2023

Erstgutachter: Dr. Josef Hofer
Zweitgutachterin: Prof. Dr. Christa Müller

Acknowledgements

I wish to express my sincere gratitude to my supervisor Dr. Josef Hofer for his helpful suggestions during the preparation of this thesis. I would also like to thank Prof. Dr. Christa Müller for her interest in this topic and her time in reviewing this work. My thanks also go to Jörg for the inspiration for the subject of this master thesis. For the continuous organizational advice, I would like to thank Dr. Jasmin Fahnenstich of the DGRA office.

Last but not least, special thanks go to my husband René for his patience and support.

Table of content

Abbreviations

Acc.	According
ADME	Absorption, Distribution, Metabolism, Elimination
ANDA	Abbreviated New Drug Application
AI	Acceptable Intake
API	Active Pharmaceutical Ingredient
Art.	Article
BCS	Biopharmaceutical Classification System
BE	Bioequivalence
CA	Competent Authority
CAP	Centrally Authorized Product
CAPA	Corrective and Preventive Action
CDER	Center for Drug Evaluation and Research
CERSI	Centers of Excellence in Regulatory Science and Innovation
CHMP	Committee for Medicinal Products for Human Use
CMDh	Coordination Group for Mutual Recognition and Decentralized Procedures
CoC	Cohort of Concern
CPCA	Carcinogenic Potency Categorization Approach
CPDB	Carcinogenic Potency Database
CPNP	1-cyclopentyl-4-nitrosopiperazine
CRCG	Center for Research on Complex Generics
CT	Confirmatory Testing
CTD	Common Technical Document
CYP	Cytochrom P-450
DIN	Drug Identification Number
EAT	Enhanced Ames test
EC	European Commission
EDQM	European Directorate for the Quality of Medicines & Health Care
EFPIA	European Federation of Pharmaceutical Industries and Associations
EIPNA	N-nitrosoethylisopropylamine
EMA	European Medicines Agency

EU	European Union
FDA	Food and Drug Administration
FP	Finished Product
GMP	Good Manufacturing Practice
GLP	Good Laboratory Practice
HC	Health Canada
HMA	Heads of Medicines Agency
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ITEM	(Frauenhofer-) Institut für Toxikologie und Experimentelle Medizin
LCBP	Lhasa Carcinogenicity Database
LOD	Limit of Detection
LOQ	Limit of Quantification
LTL	Less Than Lifetime
MAA	Marketing Authorisation Application
MAH	Marketing Authorisation Holder
MDD	Maximum Daily Dose
MeNP	1-Methyl-4-nitrosopiperazine
MNP	1-Methyl-4-nitrosopiperazine
NAP	Nationally Authorized Product
NAP (test)	Nitrosation Assay Procedure
NcWP	Non-clinical Working Party
NDA	New Drug Application
NDBA	*N*-Nitroso-di-*n*-butylamine
NDEA	N-nitrosodiethylamine
NDELA	*N*-nitroso-diethanolamine
NDMA	N-nitrosodimethylamine
NDPh	*N*-nitroso-diphenylamine
NDSRI	Nitrosamine drug substance-related impurity
NEIPA	N-nitrosoethylisopropylamine
NHEX	N-nitrosohexamethyleneimine
NIPEA	N-nitrosoethylisopropylamine

NMOR	*N*-Nitrosomorpholine
NMPA	*N*-Nitroso-*N*-methylaniline
NMT	Not More Than
NNK	4-(Methylnitrosoamino)-1-(3-pyridinyl)-1-butanone)
No.	Number
NPYR	*N*-nitroso-pyrrolidine
NTHP	*N*-Nitroso-1,2,3,6-tetrahydropyridine
NTTP	7-nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo [4,3-a]pyrazine
OECD	Organisation for Economic Cooperation and Development
OMCLs	Official Medicines Control Laboratories
Ph. Eur.	European Pharmacopeia
PKPD	Pharmacokinetic/Pharmacodynamic
Ppb	Parts per billion
Ppm	Parts per million
Q&A	Questions and Answers
QbD	Quality by Design
(Q)SAR	(Quantitative) Structure-Activity Relationship
RA	Risk Assessment
RE	Risk Evaluation
RP	Radiopharmaceutical
SWP	Safety Working Party
TD_{50}	median toxic dose
TTC	Threshold of Toxicological Concern
US	United States
USP	United States Pharmacopeia

List of figures

List of tables

1 Introduction

1.1 Background

The presence of *N*-Nitrosamines (hereafter simply referred to as nitrosamines) in pharmaceuticals have been of growing concern to regulatory authorities and the pharmaceutical industry equally for more than five years now. Nitrosamines are known as mutagenic compounds that may induce carcinogenesis in humans. As early as 1977, analgesic aminophenazone preparations were removed from the market due to contamination with N-nitrosodimethylamine (NDMA) [1]. But it was not until 2018, starting with NDMA and N-nitrosodiethylamine (NDEA) findings in valsartan drug substance [2], that a major regulatory initiative was triggered to prevent and control nitrosamine impurities in medicines. As a consequence of ongoing nitrosamine findings in various therapeutical groups, regulatory authorities all over the world established guidance and obligations for marketing authorization holders (MAHs) aiming to ensure the quality, safety and, last but not least, availability of medicinal products on the market.

To limit the presence of nitrosamine impurities in their authorized products, manufacturers and MAHs have to undertake a three-step mitigation process (named *call for review* by the European Medicines Agency (EMA)) which was one of the outcomes of the Art. 5 (3) procedure of Regulation (EC) No. 726/2004 for nitrosamine impurities in human medicinal products [3]. Accordingly, they have to carry out risk assessments, confirmatory testing and, if necessary, develop mitigation strategies to reduce nitrosamine levels in their products. However, this task as well as the detailed Assessment Report on the Art. 5 (3) procedure, describing risk factors and root causes for nitrosamine impurities and their mitigation strategies, were published in mid-2020 and thus at a time when a certain new class of nitrosamines, the nitrosamine drug substance-related impurities (NDSRIs), had not yet been detected in medicinal products. Consequently, the focus of the first published guidance by the regulatory authorities was on a few simple dialkyl-nitrosamines known so far, such as NDMA and NDEA, resulting predominantly from active pharmaceutical ingredient (API) synthesis conditions and whose carcinogenicity was proven in animal studies on the basis of which compound-

specific acceptable intakes (AIs) for humans in line with the ICH M7 guideline to control mutagenic impurities in pharmaceuticals [4] can be determined.

With increasing discoveries of NDSRIs from mid-2021 onwards, the first assumption that nitrosamine impurities could be almost or completely avoided in medicinal products by eliminating their root causes soon proved to be incorrect, as NDSRIs are directly related to the API structure and therefore cannot be evaded. Furthermore, these nitrosamines "*are typically in a different chemical space than the simple dialkyl nitrosamines"* [5] indicating that an equal regulatory treatment of these two kinds of nitrosamines may not be justified. Where initially only single cases of NDSRI findings were reported, an in-silico analysis published by Schlingemann et al. [6] in late 2022 predicted that about 40% of APIs could be at risk to form NDSRIs. Simultaneously, NDSRIs have been frequently found in the course of the three-step mitigation process. Comprehensive lists of confirmed and potential NDSRIs and their AIs were recently published in updated regulatory guidance for nitrosamine impurities in mid-2023 [7–9], while mutagenicity and carcinogenicity data for NDSRIs are largely missing [10].

Since the presence of nitrosamine impurities in medicinal products has led to numerous drug recalls in the past, there is concern about further drug recalls due to increasing NDSRI findings [11].

1.2 Aim description

Even though regional and national guidelines for nitrosamine impurities in medicinal products have been continuously revised and supplemented in the last years, taking into account new developments, the question arises whether the current recommendations sufficiently address the challenges posed by the presence of nitrosamine impurities, especially NDSRIs, in medicinal products. This is to be doubted due to the original focus of the guidelines on simple dialkyl-nitrosamines as explained above. Furthermore, differing published AIs for nitrosamine impurities by the EMA, the United States (US) Food and Drug Administration (FDA) and Health Canada (HC) [7–9] indicate that there lies a special challenge in determining AIs for nitrosamine impurities.

The aim of this master thesis is therefore to investigate the regulatory challenges in the determination of AIs for nitrosamine impurities, in particular NDSRIs, and their control in

medicinal products. A further objective is to find out whether the present regulatory status quo is sufficient to overcome the observed challenges and finally end the ongoing nitrosamine crisis, currently dominated by NDSRIs. Therefore, a thorough observation of regulatory events regarding nitrosamine impurities as well as a detailed analysis and comparison of the available guidance on nitrosamine impurities provided by the EMA, the FDA and HC will be carried out. Differences in recommendations will be evaluated and considerations for a reasonable regulatory handling of nitrosamine impurities in medicinal products will be developed always with the focus to ensure drug supply.

Drug shortages are currently considered critical for the availability of medicines, while drug recalls do not consistently have a bad reputation. Thus, the FDA states on its website that *"a drug recall is the most effective way to protect the public from a defective or potentially harmful product"* [12]. The nitrosamine guidance from the EMA, the FDA and HC repeatedly emphasize that it is intended to ensure drug supply and protect public health [8–13–14]. However, de Weerdt et al. identified 26 more or less divergent definitions for drug shortages from various stakeholders [15]. In this respect, the question arises as to what is meant by an ensured drug supply and what objective should be aimed at in this context with regard to regulatory guidance for the control of nitrosamine impurities in medicinal products. In the following, this thesis also tries to answer this question.

2 Material and methods

First of all, this master thesis briefly presents the essential basics of nitrosamine impurities in medicinal products and their regulatory environment in order to support the understanding of the results and the discussion of this master thesis.

To track the evolution of the regulatory response to nitrosamine impurities in medicinal products in the past five years, announcements of drug recalls and other information provided primarily by the EMA, the FDA and HC on their websites, and from the European Directorate for the Quality of Medicines & Healthcare (EDDM) and the United States Pharmacopeia (USP) were reviewed. Furthermore, a quantitative evaluation of drug recalls in general and due to nitrosamines impurities between 2018-2022 was performed based on FDA enforcement reports taking the US market as example.

To answer the question what is meant by an ensured drug supply, an analysis of the qualitative impact of drug recalls was done by literature review covering drugs recalls in general and those due to nitrosamine impurities in particular.

To evaluate the current recommendations on nitrosamine impurities in medicinal products, the following main guidance documents were analyzed and compared thoroughly:

- EMA: Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products (July 2023, Revision 17) [13];
- FDA: Control of Nitrosamine Impurities in Human Drugs – Guidance for Industry (February 2021, Revision 1)[14];
- FDA: Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs) – Guidance for Industry (August 2023) [10];
- HC: Guidance on nitrosamine impurities in medications (July 2023, Revision 3) [8].

Previous versions of the HC and EMA guidance documents were analyzed too, as they were revised several times during the preparation of this master thesis, giving insight into earlier regulatory challenges and development of recommendations.

Additionally, although the focus was on the official main guidance documents listed above, other information regarding nitrosamine impurities published on the agencies' websites were also considered.

Differences in recommendations were evaluated and discussed in the light of an ensured short-, mid- and long-term drug supply and by taking relevant scientific literature on nitrosamine topics into account.

The terms *pharmaceutical, medicine*, *medicinal product* and *drug product* are used synonymously in this master thesis to take into account regional linguistic differences and to avoid repetition of words.

As the control of nitrosamine impurities in medicinal products is a highly topical issue, regulatory requirements can change at any time, and therefore the most recent changes incorporated into the examined guidelines may not have been taken into account at the time of the completion of this master thesis.

3 Nitrosamine impurities in medicinal products

3.1 General aspects on structure and formation

Nitrosamines are organic chemical compounds containing a nitroso functional group derived from a secondary amine (see Figure 1) [16–17]. They are counted among a class of compounds because of their common structural element of the nitroso group attached to an amine.

In principle, amine structures from secondary to quaternary amines could lead to the formation of stable nitrosamines. However, secondary amines represent the most relevant nitrosamine precursors as they do not have to go through any previous dealkylation steps and are directly reactive. [18–19] Once they come into contact with a nitrosating agent, i.e. a nitrosonium ion carrier {NO^+}, this electrophilic structure undergoes nucleophilic attack by the amino compound and a nitrosamine can be formed [18].

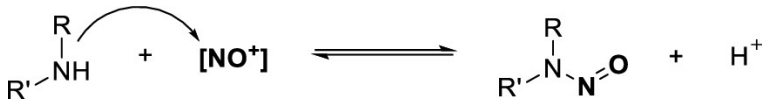


Figure 1. N-Nitrosation of a secondary amine by {NO}+ Carrier

Source: López-Rodríguez et al., 2020 [18]

A typical nitrosating agent is nitrite, present in many pharmaceutical excipients, from which nitrous acid can form in an acid environment, which ultimately provides the nitrosonium ion carrier {NO^+} [18].

Thus, the formation of a nitrosamine requires the presence of two essential structures in the reactive system: a vulnerable amine structure and a nitrosating agent. Decisive for the reactivation of these nitrosamine precursors is the pH of the reaction medium as nitrosation is facilitated under acidic conditions [20]. Furthermore, the risk of nitrosamine formation generally increase with high temperatures [19].

Guidance documents and assessment reports have been published by different authorities summarizing the root causes and risk factors of nitrosamine formation in medicinal products. An overview of available literature on that topic published by the competent authorities (CAs) is given in Annex I. In addition, the paper by Horne et al.[20],

published in late 2022, provides a comprehensive overview of the current scientific knowledge on the numerous possibilities of nitrosamine formation in medicinal products.

3.2 NDSRIs

NDSRIs are also called API-derived nitrosamines and their amine source can be either a vulnerable amin-containing degradant of the API, an API impurity or, as in the most cases, the API itself. Thus, the term NDSRI is used as a general term for all those API-derived nitrosamines which share structural similarity with and are specific to the API [10].

According to current knowledge, the formation of NDSRIs in medicinal products is caused mainly by excipients contaminated with nitrites [10–21]. Even small quantities in the ppm range can lead to NDSRI formation [22], whereby the amounts vary depending on the type of excipient, different suppliers and even on different batches from same supplier of same excipients [23]. Furthermore, water used in the manufacturing process may be contaminated with nitrite or chloramine, also known as nitrogen source [13–20]. Certain manufacturing operations, e.g. wet granulation and fluid bed drying were identified as risk factors for NDSRI formation as they could facilitate contact between nitrosamine precursors, create favorable conditions for the dealkylation of tertiary amines or could promote the reaction of nitrogen oxides in the air with a nitrosatable amine [8–20].

3.3 Carcinogenicity of nitrosamines

At present, N-nitroso compounds are classified according to ICH M7 guideline as mutagens with such high potency that they belong to the cohort of concern (CoC) group due to their nitroso structure [4]. The evidence on the carcinogenicity of nitrosamines is mainly based on rodent studies, which can only be transferred to humans to a limited extent due to species-related differences [1].

Controlled human studies to prove carcinogenicity are unethical, hence human data can only be obtained through post-approval epidemiological studies with which a clinically relevant causality is difficult to verify. Epidemiological studies with valsartan medicines containing NDMA did not show an increased risk for cancer overall, but a slight increase in liver cancer and melanoma [24–25]. The evidence for the carcinogenicity of nitrosamines in humans thus can be considered as in need of further development.

As indicated by the data on rodent carcinogenicity, there is a wide range of carcinogenic potency among nitrosamines, the underlying mechanisms of which are not yet fully understood [26].

However, it has been scientifically verified that the high carcinogenic potency of dialkyl-nitrosamines is due to a well-defined activation process mediated by cytochrome P450 (CYP), in which highly reactive diazonium or carbenium ions are formed that can subsequently bind to DNA and damage it (see Figure 2).

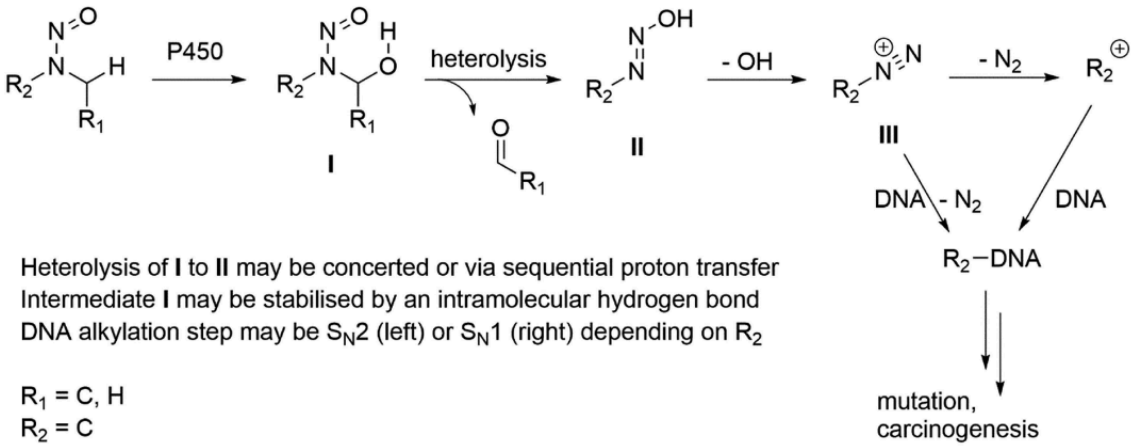


Figure 2. Metabolic activation of dialkyl-nitrosamines by α-carbon hydroxylation

Source: Cross and Ponting, 2021 [27]

3.4 Control of mutagenic impurities acc. to ICH M7

In general, the control of impurities in medicinal products is carried out either for classical impurities as related substances, residual solvents and elemental impurities according to the ICH Q3A-D guidelines [28], or for mutagenic compounds in line with the ICH M7 guideline (hereafter referred to as ICH M7) [4]. Additionally, the ICH Q3E guideline is currently being drafted with the aim of providing internationally harmonized requirements for the assessment and control of extractables and leachables in drug products [29].

The control strategy of mutagenic impurities involves setting acceptable limits in APIs and drug products ensuring that the impurities pose a negligible toxic risk. Since the toxicological profile of many detected impurities is unknown, the threshold of toxicological concern (TTC) concept has been developed which allows the prediction of

the toxicological risk of an unknown chemical substance based on toxicological data of a wide range of chemical substances [30]. Accordingly, a general threshold value of 1.5 µg/day/70 years was established to control mutagenic impurities in drug products. This value is associated with a theoretical cancer risk of less than 1:100000 meaning a negligible increased cancer risk compared to the lifetime cancer incidence of greater than 1 in 3 [4]. However, according to ICH M7, the TTC concept does not apply to the so-called cohort of concern (CoC) mutagenic impurities, which includes aflatoxin-like, N-nitroso and alkyl-azoxy compounds [4].

The TTC concept is in general applicable for substances classified as class 2 or 3 impurities (see Table 1). Class 2 includes known mutagens whose carcinogenic potency is unknown due to lack of rodent studies. In class 3 impurities, an alerting structure unrelated to the structure of the drug substance is present while data on both mutagenicity and carcinogenicity are missing. In case of class 1 impurities where sufficient data demonstrating the carcinogenicity of the impurity are available, a compound-specific AI has to be calculated using linear extrapolation of the TD_{50} derived from rodent cancer studies.[4]

Table 1. AI derivation based on impurity classification acc. to ICH M7

Impurity class	Definition	AI
1	Known mutagenic carcinogens	Compound-specific
2	Known mutagens with unknown carcinogenic potential	TTC-based
3	Alerting structure unrelated to the structure of the drug substance	TTC-based or acc. to ICH Q3A/B with negative Ames test result
4	Altering structure corresponding to alert in drug substance or qualified related substances (non-mutagenic)	Acc. to ICH Q3A/B
5	No structural alerts/altering structure proven to be non-mutagenic or non-carcinogenic	Acc. to ICH Q3A/B

Source: ICH M7 guideline [4], modified

Alerting structures are identified by computer-assisted methods and a prediction for the mutagenic potential can be made by (quantitative) structure-activity relationships ((Q)SAR) (hereafter referred to as SAR). If the result of the SAR analysis predicts mutagenicity for a compound, it is possible to disprove this hypothesis by a standard negative bacterial mutagenicity test, better known as Ames test, and control the impurity as non-mutagenic according to ICH Q3A/B. In the case of a positive Ames test and

impurity levels not controllable at or below the AI, an in vivo gene mutagenicity assay or other genotoxicity assays, if justified, are recommended in order to support the setting of a compound-specific AI. [4]

ICH M7 points out that its principles also apply to the establishment of AIs for CoC compounds. However, for CoC compounds the derivation of the AI should be a case-by-case decision taking carcinogenicity data of closely related structures into account. [4]

4 Evolution of regulatory response to nitrosamine impurities

4.1 Regulatory history

This section looks at the most important regulatory events concerning nitrosamine impurities of the last five years. A detailed chronological list of events from June 2018 to August 2023 can be found in Annex II of this master thesis.

4.1.1 Findings, recalls, referrals

The detection of NDMA in valsartan API in June 2018 was accompanied by subsequent worldwide recalls of affected batches of valsartan-containing medicines [31]. In order to assess the risk of the presence of nitrosamine impurities in these antihypertensive agents, the EMA immediately started a referral under Art. 31 of Directive 2001/83/EC [2]. A few weeks later, NDMA was also found in valsartan API from other sources [2]. Furthermore, NDEA was discovered in valsartan API and in other sartan APIs by different manufacturers which led to the extension of the Art. 31 referral to all sartan medicines [2]. When the corresponding assessment report was finally published in early 2019, new nitrosamines findings occurred, e.g. NDMA was found in pioglitazone [2], followed by the detection of NMDA in ranitidine medicines [2]. Nitrosamine impurities were obviously no longer a problem specific to one structural class of medicines and therefore a broader evaluation became necessary through an Art. 5 (3) procedure of Regulation (EC) No 726/2004 [3]. In parallel, a further Art. 31 referral for the assessment of ranitidine medicines was initiated and voluntary recalls by pharmaceutical companies of ranitidine medicines started worldwide [32–34]. The associated assessment report, which explains in detail why the risk-benefit ratio for all ranitidine medicines is unfavorable, was published in September 2020 [35]. However, a few months earlier, the Committee for Medicinal Products for Human Use (CHMP) had already recommended the withdrawal of all marketing authorizations for ranitidine medicines [32].

In the meantime, the review under Article 5 (3) of Regulation (EC) No. 726/2004 was also completed and the respective assessment report was published shortly afterwards in July 2020, providing MAHs with recommendations on how to avoid the presence of nitrosamine impurities in their medicinal products [36].

In late 2019, NDMA was found also in metformin drug products from the Singapore market leading to few drug recalls [37]. While no EU products with unacceptable intake levels were found, amounts above the AI were also detected in Canadian and US products and therefore the products concerned were recalled [38–39].

A different situation arose when the nitrosamines 1-Methyl-4-nitrosopiperazine (MeNP or MNP, hereafter abbreviated with MNP) and 1-cyclopentyl-4-nitrosopiperazine (CPNP) were discovered in rifampicin and rifapentin medicines in August 2020 [40]. Despite nitrosamine levels of all medicinal products on the market clearly exceeding the AIs, there were no recalls of these lifesaving medicines, but higher AIs were temporarily permitted [41].

Apart from that, in June 2021, after the detection of unacceptable levels of the NDSRI N-nitroso-varenicline in the varenicline-containing drug Champix®, this medicine was recalled worldwide due to its non-criticality [36–42]. This was the first time in the ongoing nitrosamine crisis that the formation of an NDSRI was laboratory confirmed.

Shortly afterwards, a number of other national, and in some cases cross-national NDSRI findings followed, e.g. N-nitroso-irbesartan [43], N-nitroso-quinapril [44], N-nitroso-rasagiline [45], which were also responded to with recalls of the respective drug products.

The AIs listed by the EMA, previously provided under Q&A 10 of the EMA guidance [46], have been expanded several times since June 2021 for new nitrosamine impurities, as can be tracked from the revision history of the EMA Q&A document [13], especially between mid-2022 and December 2022, and lately with a large extension provided as an appendix [7]. However, for the period June 2022 to August 2023, only two confirmed NDSRIs led to drug recalls, as can be tracked from respective notices on the agency's websites and press releases. Thus, in March 2023 dabigatran medicines were recalled in the US and in May 2023 atomoxetine medicines were recalled in Germany (see Annex II) [47–48]. Furthermore, the inclusion of additional NDSRIs and their AIs in the EMA Q&A document was halted although nitrosamines continued to be found regularly and persistently in pharmaceuticals, as can be concluded from the Coordination Group for Mutual Recognition and Decentralized Procedures (CMDh) Minutes from 2022 and 2023 [49] (see Annex III). The missing updates to the AI table of the EMA Q&A document thus already

suggested changes in the AI determination of nitrosamine impurities which were confirmed with latest guidance updates published by the EMA and HC in July 2023, followed by the FDA in August 2023 and will be discussed in detail in section 5.3..

4.1.2 Guidance on nitrosamine impurities

In September 2019, the EMA published first guidance to support the initial call for review through a question and answer (Q&A) document which was revised two times until March 2020 [36–50]; it was finally replaced in August 2020 by the currently valid Q&A document for MAHs, reflecting the main principles adopted in the Art. 5 (3) procedure of Regulation (EC) No. 726/2004 [13]. In the meantime, this guidance was revised 17 times.

HC also published a question and answer document on nitrosamines in November 2019, which was finally replaced by the official *Guidance on nitrosamine impurities in medications* in April 2022 [51]; it has since been revised three times, most recently in July 2023 [8].

The FDA only published corresponding recommendations on nitrosamine impurities in September 2020, after all European referral procedures had been completed. The respective guidance called *Control of Nitrosamine Impurities in Human Drugs* has so far been updated once in order to extend the recommended timeline for steps 2 and 3 of the call for review to October 2023 [52–53]. In August 2023, the FDA published a separate guidance for NDSRIs that focuses on the derivation of AIs [10].

4.1.3 EDQM and Pharmacopeia activities

The EDQM has been actively involved in addressing the nitrosamine crisis from the outset through various activities to detect and control nitrosamine impurities in APIs and medicinal products [54]. It had a coordinative function in the activities of the Official Medicines Control Laboratory (OMCL) network regarding the detection of nitrosamine impurities.

In June 2019 the European Pharmacopeia (Ph. Eur.) monographs for sartan APIs were revised to include specification limits for NDMA and NDEA. Likewise, the general chapter on *N-nitrosamine impurities in active substances (2.5.42)* was published on the EDQM website in December 2020 [54]. In November 2022, the general monographs *Substances for pharmaceutical use (2034)* and *Pharmaceutical preparations (2619)* were revised to

include a paragraph explaining the Ph. Eur. approach to nitrosamines impurities [54]. Currently, the European Pharmacopeia Commission is in the process of defining clear rules on when it is appropriate to include a specification for a nitrosamine impurity or statement in the production section of an individual monograph [55].

Similarly, the USP has responded to the nitrosamine crisis with standards, tools and solutions for the risk assessment (RA) as well as for the detection and quantification of nitrosamines impurities in drug products [56]. In December 2021, the comprehensive new *general chapter <1469> on nitrosamine impurities* in the USP became official, containing information on possible root causes of nitrosamine formation and the development of control strategies, and also providing several analytical methods to test for nitrosamine impurities [56].

Additionally, reference standards for simple nitrosamine impurities were developed and provided by the EDQM and the USP [54–56]. In the meantime, numerous reference standards for NDSRI have also been made available by the USP [57].

4.2 Drug recalls

This section will provide a quantitative and qualitative evaluation of drug recalls in general and of nitrosamine-related drug recalls as well as their impact on the availability of medicines to patients.

4.2.1 Reasons for drug recalls

The ICH Q6A guideline attributes the quality of APIs and medicinal products to their design, development, in-process controls, GMP controls, process validation, and specification parameters applied to them during development and manufacturing [58]. Section 4 (15) of the German Medicines Act similarly defines quality as *the nature of a medicinal product determined by identity, content, purity and other chemical, physical and biological properties or by the manufacturing procedure* [59]. Accordingly, all non-conformities with the specifications of a drug product and also good manufacturing practice (GMP) violations are to be considered quality defects.

As can be confirmed by data extraction from FDA enforcement reports [60] of the past years, quality issues are the most common grounds for drug recalls. Figure 3 gives an

overview of recall reasons between 2018-2022 for the US market. Over 50% of drug recalls were due to purity issues, namely lack of sterility assurance (29%), contaminations (15%) and failed specifications for impurities or newly discovered impurities (10%). In addition, GMP deviations (21%), which generally also lead to potential or actual quality defects in starting materials, active ingredients or the finished product (FP), frequently caused drug recalls. Adding the 5% of drug recalls due to failed assay specifications, it can be stated, taking the US market as an example, that an overwhelming proportion of drug recalls in the last years, in fact about 80%, were triggered due to quality issues. Only 4% of drug recalls were due to incorrect labeling. Furthermore, the 16% of drug recalls included under other reasons in Figure 3 incorporate other quality-related recalls, such as defective bottles, presence of foreign particles (for further examples see Annex IV, Table 2), but individually these represent a comparatively small subset compared to the recall reasons outlined in Figure 3. Consequently, less than 16% of drug recalls were for other non-quality related issues like marked products without an approved NDA/ANDA.

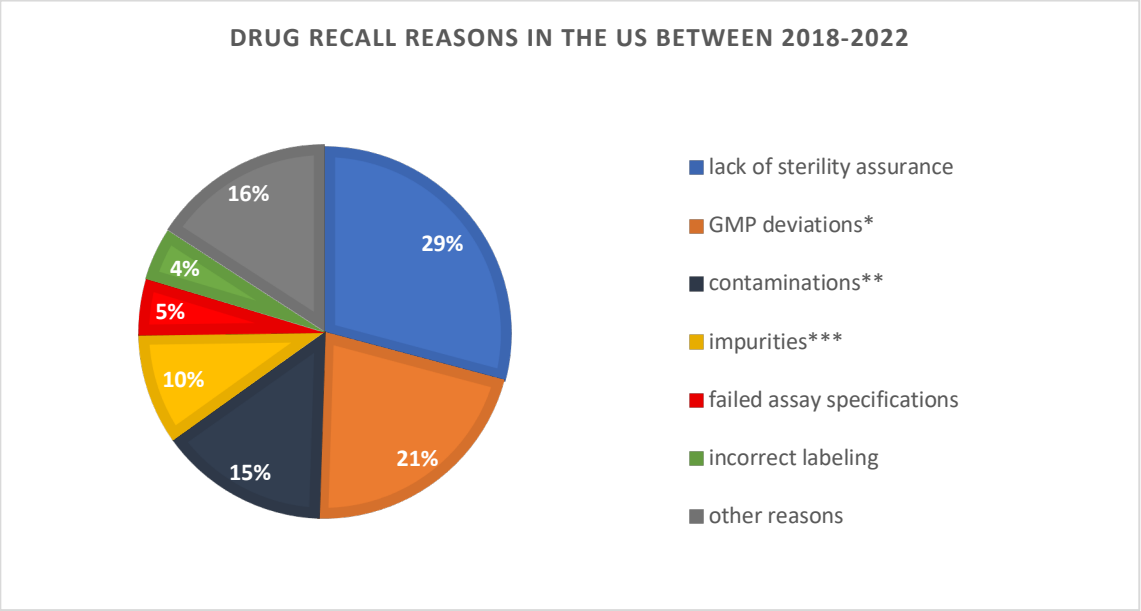


Figure 3. Recall reasons in the US between 2018 - 2022

Source: Own illustration based on FDA enforcement reports [55], for tracking data see Annex IV

*Excluded are GMP deviations with confirmed or possible consequences of contaminations, impurities, lack of sterility assurance, failed assay specifications; these are recorded under the respective recall reason
**Included are cross, chemical and microbiological contaminations
***Included are failed impurities/degradation specifications or presence of new impurities

4.2.2 Drug recalls due to nitrosamine impurities

Examining the number of drug recalls due to nitrosamine impurities since the beginning of the nitrosamine crisis two waves of drug recalls can be observed (see Figure 4). The first started with the detection of NDMA in valsartan and led to 105 drug recalls in 2018, reaching an absolute high of 112 drug recalls in 2019 followed by a significant decline in 2020. A low point since the beginning of the crisis was reached in 2021, where only 15 drug recalls can be counted. A second comparatively minor wave of recalls due to nitrosamine impurities occurred in 2022, where the number of recalls increased again slightly up to 22 drug recalls and then fell from mid-2022 onwards. Until August 2023, only three drug recalls in the US were done due to nitrosamine impurities.

The second wave of drug recalls is attributable to the first-time discoveries of NDSRIs, i.e. N-nitroso-varenicline and N-Nitroso-quinapril (see Annex II and Annex IV, Table 5). Figure 5 shows the course of drug recalls due to NDSRI discoveries compared to other nitrosamines. With an absolute number of 12 in 2021 and 15 drug recalls in 2022, corresponding to 1.6% of all observed drug recalls in these years, they represent a small share of drug recalls compared to the first sartan recall wave resulting in 6.7% of drug recalls from 2018 - 2019.

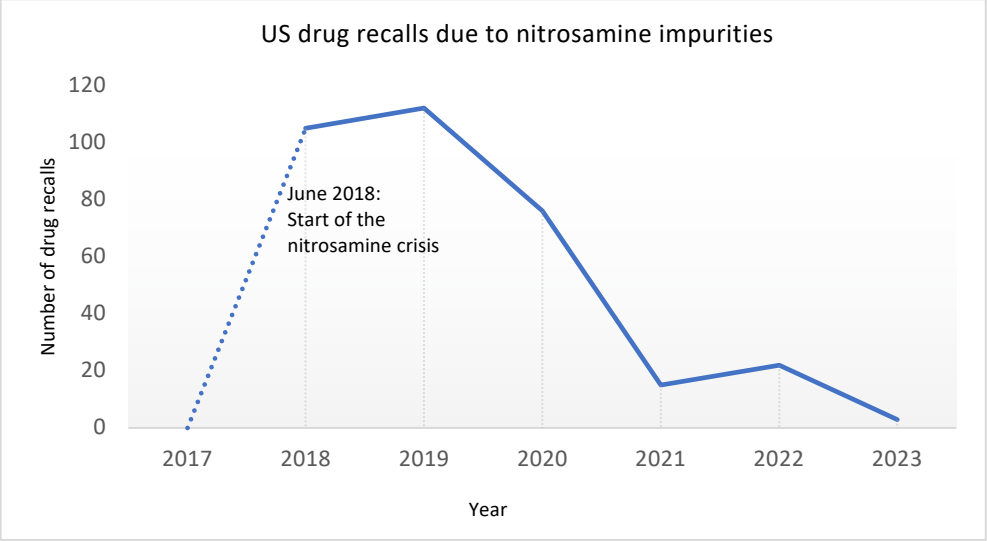


Figure 4. Total number of US drug recalls due to nitrosamine impurities

Source: Own illustration based on FDA enforcement reports [55], for tracking data see Annex IV

However, compared to other nitrosamines, NDSRIs have been predominantly responsible since 2021 for drug recalls due to nitrosamine impurities in medicines (see Figure 5 and Annex II).

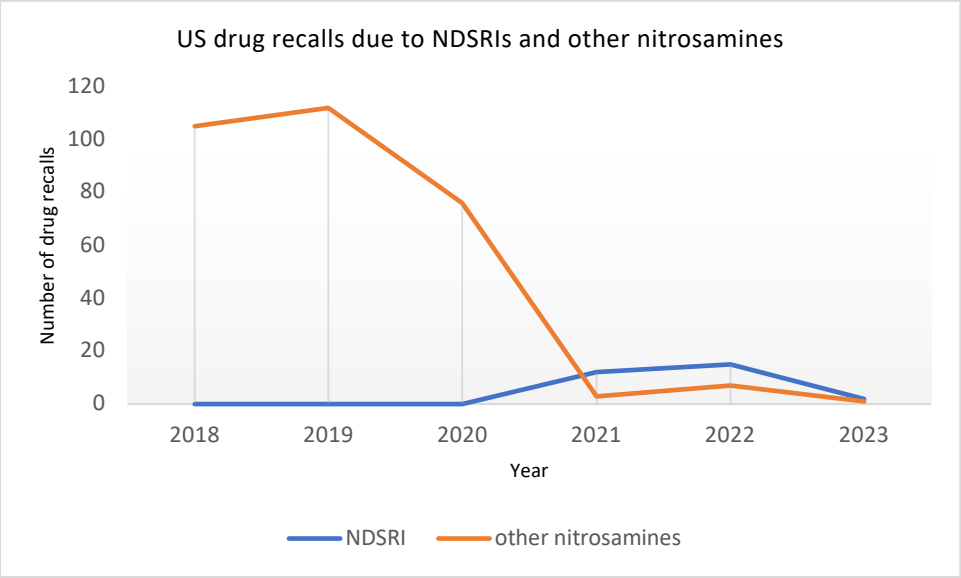


Figure 5. Total number of US drug recalls due to NDSRIs and other nitrosamines

Source: Own illustration based on FDA enforcement reports [55], for tracking data see Annex IV

Consequently, drug recalls in the US due to nitrosamine impurities have decreased significantly over the course of the nitrosamine crisis which corresponds to the decline in public announcements on drug recalls. Nevertheless, as can be observed from the regulatory history described in section 4.1, the decline in drug recalls does not correlate with an ebbing or even end of the nitrosamine crisis, but suggests significantly changed regulatory responses to nitrosamine findings.

4.2.3 The impact of drug recalls/drug shortages

Drug recalls are by definition voluntary actions by MAHs [61], but the authorities can ask them to remove their product from the market. Drug recalls lead to supply disruptions and may result in drug shortages. The reasons for drug shortages given by pharmaceutical companies and authorities correlate with the typical reasons for drug recalls as presented in section 4.2.1: manufacturing problems in the supply chain due to quality/GMP issues [62–63].

Quality issues are not always quick to fix. For failed specifications, the causes may first need to be investigated and eliminated before production can be restarted. Production

deficiencies found during GMP inspections could lead to immediate production stops, and a potentially necessary improvement of manufacturing processes could be lengthy [64].
In the worst case, drug shortages can lead to critical medical care situations if no sufficient alternative therapies exist. However, even if alternative medicines are available, switching drugs usually also has an impact on patients. They may experience delayed treatment or even not receive therapy at all. Furthermore, an alternative medicine could be less effective or less well tolerated leading to patients' non-compliance with a possibly serious health impact. Healthcare professionals need to invest time and resources in the management of supply shortages, including patient education and encouragement. [65]

However, the current jointly agreed definition by the Heads of Medicines Agency (HMA), the EMA and other stakeholders for the purpose of reporting and managing shortages is: "*A shortage of a medicinal product for human or veterinary use occurs when supply does not meet demand at a national level"* [66]. This definition implies that drug shortages can be solved by decreasing demand due to a switch to alternative therapies. But the following examples show that this definition falls short with regard to the quality of treatment or impact at patient level.

In September 2021, the CHMP justified the recall of Champix® due to unacceptable levels of N-nitroso-varenicline on the grounds that it was not a critical medicine for which alternatives were available [67]. In contradiction, less than a month later, the World Health Organisation placed varenicline on the Model List of Essential Medicines [68]. Furthermore, Lang et al. [69] showed that the unavailability of varenicline after the recall did not lead to an increase in prescriptions for alternative medicines used for nicotine dependence in the US. Another finding of the study was that although prescriptions for varenicline-containing medicines increased again after the drug shortage ended in October 2021, these settled substantially below the level before the drug recall [69].

A study conducted by the EMA based on German prescription data demonstrated that most patients were switched successfully from valsartan to another angiotensin-II-receptor blocker (ARB), mostly candesartan, in the course of the valsartan recalls [70]. However, results of other analyses of the consequences of valsartan recalls are less positive. Jackevicius et al. [71] highlighted that three month after the recall of valsartan from the Canadian market, nearly 11% did not receive alternative therapy, as well as

there being a significant short-term increase in emergency department visits after the valsartan recalls. A similar evaluation, also for the Canadian market, was conducted by McAlister and Youngson [72], who identified an increased number of outpatient physician visits for hypertension following valsartan recalls. Additionally, in an analysis of German prescription data, Beck et al. [73] concluded from the valsartan incident that the absence of a generic prescription drug results in long-lasting disruptions and volume shifts where the availability of alternative medicines may also be impaired. Along with this, switching to an alternative medication is a great, unreimbursed additional effort, both in procurement and in advising and informing patients [73]. There may be clinically relevant differences between alternative medications, even with the same mechanism of action, e.g. due to a different potential for drug-drug interactions [74–75]. Medication errors can also be a consequence of drug recalls and may have a negative impact on clinical outcomes [74–76].

Considering the effects of interruptions in the supply of medicines, in the context of this master thesis an ensured drug supply is understood as the continuous availability of their prescribed medicines to patients, avoiding a negative impact on drug therapy. A harmonized definition of drug shortages should be achieved for the purpose of uniform cross-regional criteria for decisions on drug recalls, which takes greater account of the impact on patients, supported by increasing evidence on this topic. The evolution of the regulatory response to nitrosamine findings over the course of the nitrosamine crisis shows that a shift in decision-making on drug recalls has already taken place in this respect.

However, the uninterrupted availability of medicines does not mean that less safe medicines should be tolerated on the market. The aim should be to avoid the necessity of recalling medicines in the first place, which can only be achieved by improving manufacturing processes and product quality. With regard to the control of nitrosamines and other impurities in medicinal products, regulatory requirements should be defined against this background.

5 Comparison of the guidance on nitrosamine impurities in medicinal products

5.1 Instructions on the three-step mitigation process (call for review)

The following section discusses the recommendations given in the different guidance provided by the EMA, the FDA and HC for implementing the three-step mitigation process for nitrosamine impurities in human medicinal products which includes RA, confirmatory testing (CT) and changes to the marketing authorization. Table 2 gives an overview of important aspects of the call for review.

Table 2. Comparison of recommendations for the three-step mitigation process (call for review)

Call for review	FDA	EMA	HC
Scope	Chemically synthesized APIs and drug products, biologics containing synthesized fragments and biologic-led combination products	Chemical and biological medicinal products, radiopharmaceuticals included	Chemical, biological and radiopharmaceutical (RP) drug products
Timelines	Extended for NDSRIs: Step 1: 31.03.2021 01.11.2023 Step 2: 01.10.2023 01.08.2025 Step 3: 01.10.2023 01.08.2025	Chemical: Biological: Step 1: 31.03.2021 21.07.2021 Step 2: 26.09.2022 01.07.2023 Step 3: 01.10.2023 01.07.2023	Chemical: Biological/RP: Step 1: 31.03.2021 30.11.2021 Step 2: 01.10.2022 30.11.2023 Step 3: 01.10.2023 30.11.2023
RE/RA requirements	Collaboration between drug product and API manufacturers; responsibilities for RAs divided among the different manufacturers of the supply chain; RA to be completed during CT	Collaboration between MAHs and API, FP manufacturers and raw material suppliers including provision of all information necessary; RA to be completed during CT	Co-operation between MAHs, API, excipient and drug product manufacturers; robust RA using a holistic approach; conducted by personnel with acceptable qualifications and expertise; statements or declarations by manufacturers and suppliers do not replace overall robust documented RA by the MAH; third-party approach may be applied; RA to be completed before CT
CT requirements*	Number of batches to be tested should be representative of the manufacturing process; batch to batch variability should be considered	6 pilot scale or 3 production scale batches; in case of high risk of nitrosamine presence the number of tested batches should be higher	6 pilot scale or 3 production scale batches; in case of high risk of nitrosamine presence the number of tested batches should be higher; minimum of 6 months accelerated and long-term stability data
Expectations of MAHs if nitrosamines are detected in the course of CT	Levels above LOQ: development of a control strategy and mitigation strategies; Levels above AI: inform FDA; in all cases the agency should be contacted if drug recalls are likely	At any level: inform authority by using dedicated step 2 reporting templates [36]; At levels not exceeding AI: Below AI, but >10% of AI: variation; ≤ 10 % of AI: no action; At levels exceeding AI: testing results, interim investigation plan, risk mitigation plan, benefit/risk assessment, CAPAs	At levels above AI**: inform HC and provide CT results and available details of RA; additional expectations: health risk assessment, assessment to determine medical necessity and possibility for product supply disruptions, detailed investigation report with description of CAPAs included, risk mitigation plan; engage HC prior to taking any market action to minimize impacts on drug supply

Submitting changes as part of step 3	Acc. to FDA regulations (21 CFR 314.60, 314.70, 314.96, 314.97); drug product changes as supplement; reformulation changes as prior approval supplement	Acc. to existing variations Classification Guideline	For chemically synthesized and semisynthetic APIs: Level I - Supplements or Post-Drug Identification Number (DIN) Change submissions; for biological and radiopharmaceutical products: Level I - Supplements or Level II - Notifiable changes or Post-Drug Identification Number (DIN) Change submissions (PDCs))

Information sources: [8–10–13–14–53–77]

* Expectations of analytical procedures see Table 3
**Recommendations changed with third HC guidance revision, dated 24 July 2023, see Annex V, Topic 17

CAPAs Corrective and Preventive Actions
LOQ Limit of Quantification

Status: August 2023

5.1.1 Scope and timelines

In its general nitrosamine guidance the FDA does not indicate to which drug products the call for review applies [14]. The FDA's response to the question of whether biologics, pure fermentation products and semisynthetic products are exempt from RA for nitrosamine impurities, given during the stakeholder meeting between the FDA and industry representatives in May 2021 [53], indicates that these products generally do not need to be evaluated for risk of nitrosamine impurities unless they contain synthesized fragments. This recommendation is also consistent with the FDA's recently published guidance on NDSRIs [10], which specifies biologic-led combination products and biological products containing chemically synthesized fragments as products within the scope of the guidance. The FDA specifies that synthetic conjugated API components included in biologics could present a risk of nitrosamine presence and refers to its general nitrosamine guidance according to which an assessment for chemically synthesized APIs has to be carried out [14–53].

However, the EMA provides two further rationales for the extension of the scope to biologicals. One is that processes can take place during the manufacture of biologicals that lead to the release of nitrosating agents, and secondly, biologicals can be packaged in primary packaging materials containing nitrocellulose [13], known as potential nitrite source [20]. Therefore, according to the EMA and HC as well, apart from chemical products, all biological products are in the scope of the call for review [8–13]. Although the risk of nitrosamine presence in biological medicinal products is lower compared to chemical medicinal products, it cannot be excluded and should be determined individually for each medicinal product [78] making the general inclusion of biologicals reasonable.

HC additionally explicitly lists radiopharmaceuticals as drug products to be assessed for nitrosamine risk [8]. The EMA had to clarify this in the CMDh guidance on the implementation of the call for review, where radiopharmaceuticals are mentioned as being within scope [77]. As radiopharmaceuticals are drug products where a radionucleotide is attached to a chemical or biological entity, the nitrosamine risk may originate either from the radioisotope or the chemical or biological entity [79]. Therefore, they should also be included in the scope of the call for review.

Comparing the timelines given by the authorities (see Table 2), they are similar, especially for medicinal products with chemically active substances. While the FDA provides a common deadline for steps 2 and 3, the EMA and HC say that step 2 should already be completed about one year in advance for chemical medicinal products.

Looking at the deadline for step 1 (31.03.2021 for chemical medicines), it is noticeable that this ended before the start of the NDSRI recall wave in the middle of 2021 (see section 4.2.2). This means that the RA for all chemical medicines should have been completed by that time. The EMA and FDA drew attention to the risk of NDSRI formation on their websites and demanded that the new findings/evidence on NDSRIs should be included in their RA [21–36]. However, these new findings did not result in an official extension of the deadlines at that time, leading to some manufacturers and applicants not having considered NDRSIs in their RAs [10]. With the new NDSRI guidance, the FDA finally clarifies that the original timelines of the three-step mitigation process for nitrosamine impurities do not apply to NDRSIs and defines new timelines (01.11.2023 for Step 1; 01.08.2025 for Step 2 and 3) [10].

5.1.2 Risk assessment

HC clearly indicates in its guidance that the main responsibility for carrying out the RA lies within the MAHs. They should conduct robust RAs by assessing all stages of the products' life cycle and by using a holistic approach. Therefore, MAHs should cooperate with API, excipient and finished product manufacturers [8].

In this context, HC proposes a third-party approach for cases where manufacturers do not provide sufficient information to MAHs, for example due to confidentiality reasons. Then, the MAH can involve a consultant who works directly with the manufacturer to complete the RA. MAHs are also responsible for ensuring that RAs are carried out by sufficiently qualified and trained personnel. To fulfill this request, MAHs may also use the third-party approach. HC points out that statements and declarations by manufacturers and suppliers are no substitute for a comprehensive RA conducted by the MAH. Moreover, HC mentions another general responsibility of MAHs, namely that they should establish an ongoing monitoring program in order to be able to identify trends with regard to quality.

In this way, HC addresses the early detection of quality issues, this being the most common cause for drug recalls, in this specific guidance for nitrosamine impurities. [8]

Similar to HC, the EMA emphasizes the importance of the cooperation between MAHs and drug product manufacturers and their raw material suppliers as well as the obligation to provide the MAHs with all necessary information to conduct a comprehensive risk evaluation [13]. Thus, a primary responsibility on the part of the MAH can also be concluded from the EMA guidance. However, the expectations on responsibilities for RAs are not clearly elucidated, as they are by HC, and upcoming problems such as withholding information are not addressed.

The FDA mentions two actors involved in the RA to identify the potential for nitrosamine impurities. Firstly, the API manufacturer, which should analyze its manufacturing process and perform an RA, and secondly, the drug product manufacturer, which, in collaboration with the API manufacturer, should examine the potential for nitrosamine impurities in the FP [14]. The MAH is not named in the FDA guidance as responsible for the RA and there is also no suggestion by the FDA of a comprehensive process-wide assessment that pools all the information from the manufacturers and suppliers involved [14]. Despite the FDA generally explaining in the FDA-industry meeting [53] that MAHs should have an adequate overview of the quality and safety of their drug products, it sees the responsibilities for RAs divided among the different manufacturers of the supply chain. Therefore, each manufacturer is responsible for the RA regarding its own conducted operation(s). For the previous steps and the quality of the incoming product(s) to be further processed, the manufacturer can refer to the RA/quality testing of the prior manufacturer [53]. This approach is in line with long-standing practice, where there has been little or no cooperation between the various manufacturers and the MAH of a drug product. For a pharmaceutical company, a lack of communication and cooperation within the supply chain can lead to gaps in process and product understanding, making successful quality risk management more difficult. Furthermore, the high proportion of drug recalls due to quality defects as outlined in section 4.2.1 can also be seen as an indication that the status quo does not ensure sufficient product quality in many cases.

HC further addresses the question of whether CT can be carried out without prior RA. Since step 1 serves to identify possible root causes, it is not recommended to skip the RA.

Furthermore, the development and validation of appropriate analytical methods would depend on the precise identification of the possibly present nitrosamine impurities. [8]

On the other hand, the EMA distinguishes between a risk evaluation (RE) in step 1 and the actual RA that follows, if the RE has revealed a potential risk [13]. CT should begin immediately after a potential risk has been identified. Continuing RA simultaneously to CT is also foreseen by the FDA, as the following wording can be found in the guidance: "*If a nitrosamine impurity is detected, manufacturers should investigate the root causes (...).*" [14]. Accordingly, a detailed investigation of the root causes might not begin until nitrosamines have actually been detected. This is contrary to the recommendations of HC, which emphasizes that the RA should be completed before CT begins.

Identifying a general risk, e.g. based on the presence of nitrosamine precursors with a lack of understanding of the exact chemical reaction pathway leading to the formation of a specific nitrosamine, may be predictive of already known nitrosamines for whose identification and quantification analytical methods have already been established by EDQM, USP or in-house, but it could lead to the overlooking of new nitrosamines.

Consequently, the HC recommendation to complete RA before CT seems reasonable in view of the danger of otherwise overlooking potential root causes and thus unknown or multiple present nitrosamines. Likewise, unsuitable analytical methods could lead to false negative CT results which would counteract the entire process of the three-step mitigation policy.

Step 1 thus forms the foundation for a successful three-step mitigation process and for preventing drug recalls due to subsequently discovered nitrosamines or GMP-violations due to lack of thoroughness of RAs revealed through inspections. In this context, the recommendations for HC's RA, such as on how to deal with difficulties in cooperation between MAH and manufacturers and the clear assignment of the responsibility to the MAH, are to be appreciated. Appropriately qualified and experienced staff performing the RA will further ensure the validity of the RA.

While HC provides some important aspects to ensure reliable RAs, the expectations from the EMA and the FDA for the conduct of RAs are more general. All three guidance documents recommend the application of quality risk management principles in

accordance with the ICH Q9 guideline [80]. However, the application of risk management, including RAs, by different stakeholders is usually subjective and thus performed differently [80]. Among other things, this could be attributed to the fact that not all companies have the same financial and personnel resources at their disposal. Therefore, it is important to provide companies with available tools to perform valid RA on nitrosamine impurities. While the workflows for the quality management of nitrosamine impurities provided by the European Federation of Pharmaceutical Industries and Associations (EFPIA) are directly available online [81], the software for carrying out nitrosamine impurity RAs provided by Lhasa Limited is only available to members, meaning sponsors and contributors of this data sharing initiative [82]. Access also for non-contributing companies would be desirable to ensure that not only the companies benefit that already have knowledge, but also those that are most in the need of the knowledge of other parties. USP Nitrosamine Exchange [83] is a free and after one-time registration publicly accessible exchange platform that offers the possibility of knowledge sharing on RA strategies, tools and technologies. In addition, workshops such as the one organized by the FDA and the Center for Research on Complex Generics (CRCG) in June 2023 [84], where RAs on NDSRIs were discussed among other topics, can contribute to the exchange of knowledge and training for both authorities and industry and thus assure thoroughness of RAs.

5.1.3 Confirmatory testing

The requirements for step 2 of the three-step-mitigation process are uniform for all authorities, in that sensitive validated test methods must be used for the confirmation of nitrosamine impurities [8–13–14]. The FDA emphasizes that analytical methods should have excellent chromatographic separation due to the physicochemical properties of nitrosamines (e.g. low molecular weight, volatility) [14]. The analysis of the small dialkyl-nitrosamines like NDMA and NDEA was a major challenge, especially at the beginning of the nitrosamine crisis, that was met by the collaboration of OMCLs, EDQM and USP by providing analytical methods and reference standards as described in section 4.1.3. So far, no specific analytical methods for NDSRIs have been made available by these institutions. But the established liquid chromatography with mass spectrometry (LC-MS) methods can in principle also be used for the confirmation of NDSRIs and seem to have an

improved method performance due to the enhanced detectability of the generally larger NDRSI molecules [85]. However, the available mass spectrometry-based analytical techniques need to be validated for each individual sample matrix [85] which could be challenging. Furthermore, a prerequisite for the quantification of present NDSRI levels is the availability of a suitable primary reference standard. Primary reference standards can either be obtained from an officially recognized body (EDQM/USP) or produced in-house [86], whereby the nitrosamines suspected of being present have to be produced and characterized by the manufacturer [58–86] which could be a considerable effort. Thus, the availability of certified reference standards is important for the acceleration of CT and completion of the three-step mitigation process. Therefore, USP provides an increasing number of NDSRI reference standards and should continue to do so as proactively as possible, taking into account confirmed and hypothetical NDSRIs. The EMA, followed by HC in its latest guidance update, address the issue of potentially not synthesizable nitrosamine impurities, leading to lack of reference standards for CT and simultaneously indicating that the nitrosamines actually could not be formed in practice [8–13]. Accordingly, the synthesis tests carried out on potential NDSRIs can also serve to exclude a nitrosamine risk. The widespread use of a reliable and easy-to-perform nitrosation assay procedure (NAP test) as developed by Sharma et al. [87] could be used for RA and also for the synthesis of reference standards [87]. This would be an option for institutional bodies to further support MAHs in RAs.

The EMA and HC specify an equal minimum number of batches to be tested (see Table 2) during CT by MAHs and manufacturers and that the number should be commensurate with the risk. Furthermore, the number of batches should be representative of sources of components and manufacturing sites of the drug product [8–13]. HC gives as examples of high risk the formation of nitrosamines towards the end of the manufacturing process or during storage or the presence of nitrosamine precursor groups in the API [8]. HC further recommends testing stability batches if there is a risk of nitrosamine levels increasing over time in the API or FP or if it is unclear how levels will develop over the lifetime of the product. From these recommendations it can be concluded that for predictive results of CT for NDSRI presence in drug products, which are typically formed during manufacturing or storage, a large number of batches has to be tested. However, it remains open how

many tested batches are sufficient for reliable results of the second step of the call for review.

The FDA guidance itself does not indicate the number of batches to be tested. In the FDA-industry meeting [53], the FDA clarifies that the batches to be tested should be representative of the manufacturing process, i.e. must take into account all sources used for the marketed product. But how to deal with the risk posed by fluctuating nitrite levels from the same suppliers of the same excipients [23]? This question is currently not addressed in the available guidance documents of the CAs.

According to the EMA guidance, samples should be tested using orthogonal analytical test methods to investigate the influence of technical factors of the different methods, such as interactions with test materials or contamination with nitrosamines during sample preparation [13], thus addressing the challenge of ensuring reproducible and effective sample preparation [85]. HC emphasizes that testing must take place in a GMP-compliant facility [8].

In summary, the guidance on confirmatory testing is largely congruent between the authorities with some additional different inputs from each, providing insight into the challenges of analytical testing for nitrosamine impurities trying and are intended to ensure valid CT results. With regard to the establishment of a control strategy for NDSRIs based on the results of the CT, it is unclear which number of tested batches is considered sufficient by the authorities to justify, for example, the omission of routine testing or testing at all (for more information see 5.2.3).

5.1.4 Expectations of MAHs if nitrosamines are detected

For medicinal products approved in the EU, the CT results have to be submitted to the CAs using the step 2 templates available on the EMA website [36]. Further actions are dependant on the levels detected. Therefore, the EMA specifies a threshold of 10% of the AI above which a variation has to be filed to include a specification for the respective nitrosamine impurity. If levels above the AI are detected, CT results, the interim investigation plan, a risk mitigation plan and a benefit-risk assessment have to be submitted immediately, and the planned corrective and preventive actions (CAPAs) have to be communicated. Then, a multi-stage evaluation process is initiated under the

supervision of a lead authority, which lastly also recommends market actions, if necessary. [13]

Initially, with published version two of the HC guidance document [88] HC wanted to be immediately informed of nitrosamine contamination at any level following the completion of CT (see Annex V, Topics 3 and 15). Furthermore a copy of the RA and CT results should have been submitted in parallel irrespective of the quantities detected [88]. Since the last update of the HC guidance in July 2023, HC only requires reporting when nitrosamines are detected above the AI. Similarly, topic 17 of the guidance has been adjusted to reduce the additional expectations of MAHs, as a health risk assessment including medical necessity of the drug product, a detailed investigation plan with a description of the planned CAPAs, and a risk mitigation plan do not need to be sent to HC in parallel as previously requested, but "*MAHs should have completed or be completing as necessary"* the above listed documentation [8] (see Annex V, Topic 17).

The FDA recommends the development of a control strategy including the implementation of specification limits for findings below the AI to ensure that the nitrosamine levels are permanently at or below the AI [10–14]. In case of nitrosamine levels above AI in drug products on the market, the FDA should be contacted for the decision on regulatory actions to be taken. In summary, informing the FDA in the course of CT is only required in case of nitrosamine levels above AI and/or if a disruption in the drug supply is likely. The FDA guidance does not specify what information manufacturers should submit to the agency in these cases. [10–14]

These requirements clarify that drug recalls should not be initiated by MAHs without prior consultation with the relevant CA. Thus, it can be concluded that all authorities want to be involved in the decision on regulatory actions in critical cases with the potential of causing supply disruptions. An early exchange with the authorities on the further course of action based on all information on the medicine concerned and its nitrosamine risk is desirable in order to counteract supply problems well in advance. The EMA and HC provide requirements for documents making a comprehensive benefit-risk evaluation possible. However, the need to submit this documentation to HC is now no longer reflected in the updated topic 17 of the HC guidance [8]. Thus, HC followed the FDA's

approach of only reviewing cases that are considered most critical for drug supply because of AI exceedances.

Relying primarily on CT results for marketed drug products without checking the thoroughness of the RA and other documentation, i.e. the overall situation, may be a risky strategy to ensure drug supply in the long term. Considering the fact that the nitrite content in excipients of different batches from the same supplier can vary [23], it is possible that significant higher NDSRI levels may be present in future batches of the finished product compared to the levels determined during the CT, especially if there are no clear recommendations on the number of batches to be tested.

Specification limits set on the basis of CT results would prevent the release or initiate the recall of batches with out-of-specification levels and thus would protect the patient from an unsafe medicine, but batch rejections and drug recalls should be thought through further. As illustrated in section 4.2.3, the consequences of drug recalls can also put patients' health at risk due to interruptions and changes in therapy.

Therefore, HC's initially stricter handling of nitrosamine impurities by assessing each drug product affected independently of its nitrosamine levels could have been the more enduring approach to prevent drug shortages caused by nitrosamine impurities. On the other hand, the strict regulatory requirements probably could not have been realized by many affected MAHs given the tight timelines of the call for review. Overly stringent requirements could potentially lead to MAHs withdrawing their products from the market. Furthermore, it can also be assumed that the workload on the regulatory side was too great to check the documentation of the numerous drug products affected by NDSRIs, possibly leading to prolonged regulatory procedures for impacted or other drug products. Lengthy regulatory decisions and non-implementable regulatory requirements could also endanger drug supply.

The latest update of the HC guidance regarding the expectations of MAHs if nitrosamines are detected reflects the challenges posed by the numerous drug products affected by NDSRIs in the light of ensuring drug supply. The prioritization towards high-risk products identified during CT recommended by the authorities ensures short-term drug supply.

Whether this strategy will also have a sustainable effect on supply with products already on the market remains to be seen.

5.1.5 Changes to the marketing authorization

If the risk of nitrosamine impurities is confirmed during CT, the next step for the MAH is to make changes to the marketing authorization. If applicable, tests for the potentially present specific nitrosamines with suitable limits are to be included in the specification of the API and/or FP. However, in order to reduce nitrosamine levels, further changes might be necessary which have to be notified to or approved by the CAs. The regulatory challenges to mitigate NDSRIs in drug products will be discussed in section 5.4.

According to the FDA guidance, the relevant FDA regulations should be followed for changes to the marketing authorization to reduce nitrosamine levels [14]. However, changes to the drug product have to be submitted at least as a supplement [14], meaning they are classified as moderate changes which are considered to possess a moderate risk of having an adverse effect on the drug product [89]. The EMA requires the submission of suitable variations in accordance with the existing guidelines on the details of the various categories of variations [13–90]. HC also refers to its relevant guidelines for post-authorization changes [91–92], but additionally lists the possible application types to be suitable dependent on the drug product type (see Table 2) [8]. Furthermore, HC points out that changes aiming to reduce nitrosamine impurities in APIs and drug products are due to safety concerns and that a critical assessment by HC is required before their implementation by the manufacturer [8]. In addition, the FDA indicates in its NDSRI guidance that the reformulation of an approved drug product is a major change which has to be submitted as a prior approval supplement [10].

Thus, while HC and the FDA provide for a more or less intensive review of the changes before implementation or distribution of the drug product concerned by specifying the application type, the EMA does not comment on the type of variation to be submitted in its Q&A document, indicating that the submission of type IA variations is possible to implement changes as part of step 3 of the three-step mitigation process. Thus, HC and the FDA apply stricter regulatory requirements by clarifying that changes to reduce nitrosamines impurities in drug products should be assessed by the CA. The EMA on the

other hand, relies on the individual responsibility of the MAH by simply referring to the applicable guideline.

Clear provisions on how the changes should be submitted would be helpful in order to prevent regulatory procedures from being prolonged due to validation issues or non-acceptances. Furthermore, an assessment of the changes by the authorities contributes to quality assurance and is therefore generally useful, but should not lead to unnecessary delays in the implementation of the changes and thus jeopardize the availability of the drug products concerned.

5.2 Quality aspects

In the following, general recommendations with regard to quality aspects regarding nitrosamine impurities in drug products from the FDA, the EMA and HC are compared, some of which are also relevant for the call for review.

Table 3. Comparison of recommendations on quality aspects

Quality aspect	FDA	EMA	HC
RA **submission** for new/ongoing MAAs	not required for ongoing MAAs; necessary for new MAAs	RE (using CMDh template) required for ongoing and new MAAs	required for ongoing and new MAAs; detailed expectations of the summary and discussion
RA **submission** for variations/changes	not required*	generally not required	required for relevant quality changes** with summary and discussion
Control options acc. to ICH M7	control options 1 - 3	control options 1 - 3	control options 1 - 3; option 4 for new MAAs possible on a case-by-case basis
Routine testing	usually required; alternative approaches supported by sufficient process understanding and evidence of statistical control possible	<u>required except:</u> (1) amount is consistently*** below 10% of the AI (test omission); (2) level of a single nitrosamine is consistently*** below 30% (skip testing)	<u>required if:</u> API: high risk and/or CT results CT > 30% of the AI; FP: potential for nitrosamine introduction during manufacturing, packaging and storage and/or nitrosamine detected during CT and unknown root cause; test omission if levels are below 10% of the AI****
Analytical procedures	sensitive, validated methods	sensitive, validated methods; use of orthogonal analytical methods recommended	sensitive, fully (for quantification) validated analytical methods conducted at a GMP-compliant facility

Information sources: [8–10–13–14–53]

* Manufacturers should consider manufacturing changes and shifts that may impact the potential for nitrosamine impurities
**Changes in drug substance or drug product manufacturing processes, drug product composition (API, excipients), introduction of a new dosage form, changes to the container closure system are given as examples
***See CT requirements, table 2
****Included with latest, third guidance revision (July 2023)

MAAs = marketing authorization applications

Status: August 2023

5.2.1 New/ongoing marketing authorization applications

According to the FDA, the three-step mitigation process should be completed prior to the submission of a new marketing authorization application (MAA)[14].

The EMA requires a completed RE submitted as attachment to Module 1 with a corresponding reference to Module 3.2 as part of a new MAA [13]. It should be carried out using the *template for nitrosamine risk evaluation in marketing authorisation applications* available on the CMDh nitrosamines website [93] consisting of a checklist that queries the root causes of nitrosamine formation and presence mentioned in the EMA Q&A document. The use of this template is mandatory for nationally authorized products (NAPs), including products authorized through mutual recognition and decentralized procedures, and optional for centrally authorized products. The CMDh emphasizes that the completion of the RE template is not a substitute for a robust RA [77]. However, neither the EMA Q&A document nor the CMDh procedural guidance contain specific requirements for the RA. If a risk has been identified during RE, an RA including benefit-risk considerations and the risk mitigation strategy, as well as CT results or at least plans for CT, have to be included with submission [13]. Like the FDA, the EMA admits that the RA as well as plans for CT can be provided while the assessment process is ongoing [13–14].

HC recommends that all three steps of the call for review should be completed before the submission of a new MAA. Sections concerned of the Common Technical Document (CTD) should include relevant information from the RAs and a summary and discussion of the RA should be submitted in section 3.2.P.2 of the CTD. In this context, HC has added the following passage with the second revision of its guidance in April 2023 (see Annex VI, Topic 19): "*This summary is expected to include sufficient detail to allow Health Canada to assess the adequacy and robustness of the risk assessment. Expectations for the content of the summary and discussion of risk assessments are found under number 20."* [8] HC gives clear provisions in this on what aspects should be addressed in the RA and points out that submission of checklists that lack sufficient detailed information and discussion should be avoided (see Annex VI, Topic 20). Here, HC clearly differs from the EMA guidance which accepts the submission of the CMDh checklist solely in case of no risk is identified during the initial RE.

The requirements for CT by the EMA and HC for new or ongoing MAAs correspond to those for already approved MAAs (see 5.1.3).

It can be stated that all authorities require an RA for nitrosamines for future MAA submissions. The requirements for the documentation of the RA differ between the EMA and HC in that as per HC, a summary and discussion of the RA should be submitted as part of the CTD with prescribed content, whereas the EMA does not provide any detailed requirements for the RA but refers to the available RE template of the CMDh, which, however, should only be used mandatorily for NAPs.

Both the EMA and HC introduce their recommendations for new/ongoing MAAs with the note that the formation of nitrosamines or contamination with nitrosamines should be prevented as early as during the development of a medicinal product [8–13]. If pharmaceutical companies follow this crucial recommendation and consider all possible risk factors and root causes for nitrosamine formation when developing their products, nitrosamine impurities in new drug products could be successfully tackled in the long term. From this point of view, the requirement to submit RAs for nitrosamines with new MAAs should be highly supported. An assessment of the robustness of the RA for each new MAA, as carried out by HC on the basis of the expected RA summary and discussion, could ensure the absence or suitable control of nitrosamine impurities in new drug products coming into the market. Therefore, the CAs should have sight of meaningful documentation of the RA in the drug product dossier. In view of the importance of the RA to mitigate nitrosamine impurities on the one hand and the high number of drug recalls due to GMP issues on the other hand (see section 4.2), only selective checks of the detailed RA documentation during GMP inspections could be insufficient to ensure drug supply in the long-term. In any case, assessors and inspectors should be trained accordingly to assess the content of the RA documentation to ensure the reliability of the RA.

5.2.2 Changes/variations

HC requires an RA for all Supplements, Notifiable Changes and Post-DIN Change submissions after the Step 1 deadline relating to quality changes that may impact nitrosamine presence. With its latest guidance update, HC has added examples of such

changes that may lead to an altered nitrosamine risk. In addition to changes in the manufacturing process, changes in the composition, dosage form and container closure system of the finished product are considered to possibly alter the nitrosamine risk [8]. Otherwise, no RA is generally expected by the EMA for line extension or variation applications, but questions on the presence of nitrosamine in the product could be raised by the authority during the assessment [13].

The FDA indicates that manufacturers should have in mind that the risk of nitrosamine impurities may change during the life cycle of the product in case of manufacturer changes and changes in the manufacturing processes. The risk should be periodically reassessed in line with quality management principles. There is no requirement for the submission of an RA for changes to the marketing authorization. [14]

All authorities indicate in their guidance that the risk of nitrosamine impurities in drug products may have to be reassessed due to quality changes to the marketing authorization. While the EMA and FDA generally do not require RAs to be provided to the authorities [13–14], the submission of an RA, including summary and discussion, and its presentation in the drug product dossier is asked by HC for relevant quality changes [8].

Due to the meanwhile numerous, identified and still growing root causes and risk factors of nitrosamine formation, it can be expected that more than just a few quality changes will alter the risk of nitrosamine formation. The focus on manufacturer changes or changes in the manufacturing process is no longer appropriate in view of the extensive possibilities for the origin of nitrosamine contamination. Furthermore, the evaluation of the relevance of quality changes for the nitrosamine risk cannot be considered trivial and necessarily requires a more or less extensive RA. An RA should therefore be the rule rather than the exception in the case of quality changes. As with new MAAs, inclusion of RAs in the dossier and cross-checking by the authority, as practiced by HC, would be appropriate in order to sustainably manage nitrosamine impurities in drug products.

5.2.3 Control strategy

Regarding the development of a control strategy for nitrosamine impurities, the EMA and HC refer to the control options described in ICH M7. However, it should be noted, that

these four options available are outlined for the control of process-related impurities in APIs [94] (see Annex VII).

For the control of nitrosamine impurities, control options 1–3 may be used, as stated in the EMA and HC guidance [8–13]. HC also admits option 4 as a control strategy for new MAAs, but the authority wants to decide on the acceptability of this approach on a case-by-case basis [8].

According to the FDA guidance, routine testing for nitrosamines in APIs and, if necessary, in FPs is required. This reflects standard control option 1 for nitrosamine impurities in line with ICH M7. However, the FDA does not mention periodic verification testing as an option for the control of nitrosamine impurities and justifies the need for routine testing with existing uncertainties regarding nitrosamine impurities in drugs. Alternative procedures should be based on sufficient process understanding and evidence of statistical control and must be approved prior to implementation by the FDA. [14]

Exemptions from routine testing are permitted by the EMA and HC if the root cause of nitrosamine contamination is known [8–13]. According to the EMA these are dependent on the nitrosamine levels determined during CT and not only for the API but also for the FP. In addition to the application of periodic testing if impurity levels are consistently below 30% of the AI, testing can even be omitted completely if the levels detected are consistently below 10% of the AI. [13]

HC requires a routine test in the API specification whenever the risk of the presence of nitrosamines is high. The following examples are given by HC for a high risk [8]:

- *potential for nitrosamine formation on storage*
- *presence of nitrosamine precursor functional groups in the API*
- *late-stage formation/introduction of a nitrosamine impurity in the manufacturing process*

A routine test is also required if the concentrations found during CT were greater than 30% of the AI, meaning nitrosamine levels below 30% of the AI permit periodic testing, which is in line with the ICH M7 requirements [8].

HC expects routine testing for nitrosamines in the finished product if

- *the potential for nitrosamine introduction during drug product manufacturing, packaging and storage is identified and/or*
- *a nitrosamine impurity is detected in the drug product during confirmatory testing and the root cause is unknown.* [8]

These requirements suggest that regardless of the results of the CT of the FP, HC thus also requires routine testing of the FP solely on the basis of a present risk. However, in July 2023 with the latest guidance update, HC introduced the EMA's recommended test omission for nitrosamine levels <10% of the AI, but it is unclear whether this also applies to APIs and FPs at high risk of NDSRIs [8].

If the API itself represents the nitrosamine precursor structure (amine) for the corresponding NDSRI, the risk of nitrosamine formation and presence in the API can be considered high according to HC criteria. Furthermore, the potential for the formation of an NDSRI during the manufacture and storage of a drug product whose API includes a nitrosamine precursor structure is basically given. Thus, in the case of drug products with amin-containing APIs, the HC criteria for general routine testing for potentially forming nitrosamines in the API as well as in the FP are fulfilled.

These aspects indicate that HC considers periodic testing to be inappropriate as a control option for NDSRIs in drug products, while the same recommendation cannot be derived from the EMA Q&A document, whose recommendations on the control strategy for nitrosamines are based on the levels detected during CT (see Table 3).

The control options according to ICH M7 are primarily based on the assumption that the fate of the impurity on the way to the final API is known and that removal or reduction of the impurity during the manufacturing process is possible. This makes these control options suitable for nitrosamine impurities whose formation or origin are clearly identified and which are formed from nitrosamine precursor structures that are not part of the API or can be formed from it, but are formed, for example, from reagents, solvents or other substances used in the synthesis of the API or from API intermediates. If the API itself contains an amine function or a nitrogen component, or both as was the case with

ranitidine [35], these risk factors cannot be eliminated during API synthesis and consequently also remain during the manufacturing process and storage of the FP.

The above considerations support the requirement for routine testing for NDSRIs. However, it is questionable whether the presence of an amine-containing API alone is sufficient to justify the need for routine testing, since the formation of NDSRIs is only possible if correspondingly favorable reaction conditions and at least one nitrosating agent are present. The exclusion of routine testing should therefore be possible, where appropriate, by means of a valid risk assessment. Nevertheless, a distinction between process-related nitrosamines and NDSRIs in the regulatory guidance, reflecting the different risk of their formation once their actual presence was confirmed, seems necessary to provide clarity on the control options for NDSRIs. As already mentioned, demonstrating consistent NDRSI levels over time may be complicated, for example, by variable nitrite levels in batches from the same excipient supplier.

5.3 Safety aspects

The following table gives an overview of safety-relevant aspects for nitrosamine impurities outlined in the guidance of the FDA, EMA and HC which will be discussed in this section.

Table 4. Comparison of recommendations on safety aspects

Safety aspect	FDA	EMA	HC
Number of established AIs*	259 (of which 250 NDSRIs)	83 (of which 61 NDSRIs)	100 (of which 82 NDSRIs)
Lifetime AIs for new nitrosamines	Approaches outlined in ICH M7; recommendation for the application of the **CPCA for NDSRIs**	<u>Sufficient carcinogenicity data:</u> substance specific AI acc. to ICH M7; <u>no/insufficient carcinogenicity data:</u> 1. use **CPCA for nitrosamines** 2. control at 1.5 µg/day with neg. EAT result 3. use TD_{50} from a suitable surrogate (SAR/read-across) 4. control acc. to ICH Q3A/B limits with neg. in vivo mutagenicity study result	<u>Reliable compound-specific data:</u> 1. linear extrapolation from most relevant TD_{50} from robust carcinogenicity study (Ref. to ICH M7); 2. control at 1.5 µg/day with neg. EAT result; 3. control acc. to ICH Q3A/B limits with neg. in vivo mutagenicity data <u>Insufficient reliable compound-specific data:</u> 1. conduct SAR/read-across: use TD_{50} from a suitable surrogate; 2. use **CPCA for nitrosamines**
LTL application/interim limits	Permission of exposures above lifetime AI on a case-by-case basis; LTL approach officially not applied	LTL application after consultation with CA as a temporary measure to set interim limit during CAPA implementation ≤ 3 years; not applicable for new/ongoing applications	Intermin higher limits on a case-by-case basis in exceptional circumstances (to avoid a drug shortage for a drug product that is considered medical necessary or medically important)
AI in case of multiple nitrosamines	Apply most potent nitrosamine (corresponds to EMA option 1); alternative approaches possible after FDA consultation	Two options provided (with detailed examples): option 1 or option 2, fixed or flexible approach; nitrosamines present at ≤ 10% of their AI do not need to be calculated in the total nitrosamine level	Apply most conservative AI (corresponds to EMA option 1) or limit for each nitrosamine set at a percentage of its AI limit such that the sum of the % AI limits for each specified nitrosamine does not exceed 100% (corresponds to EMA option 2, fixed approach); alternative approaches will be assessed by HC on a case-by-case basis; nitrosamines present at ≤ 10% of their AI do not need to be calculated in the total nitrosamine level

Information sources: [8–10–13–14–36–53]

For a complete overview of all recommended nitrosamine impurities and their AIs see Annex VIII

CPCA = Carcinogenic Potency Categorization Approach
EAT = Enhanced Ames test
LTL = Less than lifetime

Status: August 2023

5.3.1 Lifetime AIs for nitrosamine impurities

In their guidance, the authorities specify AIs for nitrosamine impurities that should be applied as specification limits for APIs and FPs to limit carcinogenic risks for lifetime exposure to not more than 1:100000. Until the publication of the latest guidance updates by the EMA, the FDA and HC in July and August 2023, only few AIs for nitrosamines, including NDSRIs, were listed in them (see Annex IX). In the meantime, however, more than 300 different nitrosamines with corresponding AIs are provided by the CAs. A complete list of currently published AIs is given in Annex VIII of this master thesis. The majority of the listed nitrosamines are NDSRIs and not only those actually detected in medicinal products, but also for potentially formable NDSRIs. These hypothetical NDSRIs are included in Table 1 provided on the FDA website where 247 published AIs are listed [9]. It is not clear, even for the significant lower number of NDSRIs and their AIs published by the EMA and HC, for which APIs NDSRIs had been actually confirmed, meaning that the current extent of affected medicines is only known by the authorities.

With the latest updates of their guidelines, the authorities have harmonized the AIs of several nitrosamines. Table 5 shows AIs published by the FDA, the EMA and HC before the updates and afterwards. The harmonization, highlighted through the color switch from red to yellow or green and yellow to green, was achieved through the application of the Carcinogenic Potency Categorization Approach (CPCA) by all CAs, which will be further introduced in section 5.3.1.1. But also the acceptance of 4(Methylnitrosoamino)-1-(3-pyridinyl)-1-butanone (NNK) as a surrogate for N-nitroso-duloxetine and N-Nitroso-1,2,3,6-tetrahydropyridine (NTHP) as a surrogate for 7-nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyrazine (NTTP), related to sitagliptin, and N-nitroso-varenicline for the application of read-across by the FDA contributes to the harmonization of the respective AIs. Furthermore, N-Nitrosopiperidine (NPIP), N-Nitrosomorpholine (NMOR) and N-Nitrosopyrrolidine (NPYR) are listed as suitable surrogate compounds for NDSRIs in the FDA NDSRI guidance [10], but without corresponding AIs and NDSRIs for which read-across could be applied. Therefore it is not clear, despite the FDA having assigned them robust mutagenicity and carcinogenicity data [10], that the TD_{50} values applied by the EMA and HC are accepted by the FDA for AI determination (see Table 5).

The application of the CPCA made it possible to establish significantly higher AIs for the NDSRIs that were already listed in previous guidance versions where the class-specific AI of 18 ng/day was applied in several cases (see N-nitroso-rasagiline, N-nitroso-dabigatran and N-nitroso-tamsulosin before and after the latest guidance update, Table 5).

Table 5. Comparison of differences in lifetime AIs (ng/day) before and after July/August 2023

Nitrosamine	AI before July/August 23				AI after July/August 23			
	EMA	HC	FDA	AI derivation	EMA	HC	FDA	AI derivation
MeNP/MNP (1-Methyl-4-nitrosopiperazine)	26.5	96	96	EMA: SAR/NDEA FDA/HC: SAR/NDMA	400	400	96	FDA: SAR/NDMA EMA/HC: CPCA 3
NMOR (N-Nitrosomorpholine)	127	127	/	Gold TD_{50} 0.127 mg/kg/day[1]	127	127	?[2]	Gold TD_{50} 0.127 mg/kg/day[1]
NMPA (*N*-Nitroso-*N*-methylaniline)	34.3	/	26.5	FDA: SAR/NDEA; EMA: Gold TD_{50} 0.0343 mg/kg/day[1]	34.3	/	26.5	FDA: SAR/NDEA; EMA: Gold TD_{50} 0.0343 mg/kg/day[1]
NNK (4(Methylnitrosoamino)-1-(3-pyridinyl)-1-butanone)	100	100	/	Gold TD_{50} 0.0999 mg/kg/day[3]	100	100	100[4]	Gold TD_{50} 0.0999 mg/kg/day[3]
NPIP (N-Nitrosopiperidine)	1300	1300	/	Gold TD_{50} 1.3 mg/kg/day[3]	1300	1300	?[2]	Gold TD_{50} 1.3 mg/kg/day[3]
NPYR (*N*-nitroso-pyrrolidine)	1700	/	/	Gold TD_{50} 1.7 mg/kg/day[1]	1700	1700	?[2]	Gold TD_{50} 1.7 mg/kg/day[1]
NTHP (*N*-Nitroso-1,2,3,6-tetrahydropyridine)	37	37	/	Gold TD_{50} 0.0374 mg/kg/day[1]	37	37	37[4]	Gold TD_{50} 0.0374 mg/kg/day[1]
NTTP (7-Nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3- a]pyrazine)[5]	37	37	/	SAR/NTHP	37	37	37	SAR/NTHP
N-nitroso-vareniclin	37	37	/	SAR/NTHP	37	37	37	SAR/NTHP
N-nitroso-duloxetine	100	100	/	SAR/NNK	100	100	100	SAR/NNK
N-nitroso-rasagiline	18	18	/	Class specific TTC	100	100	100	CPCA 2
N-nitroso-dabigatran	18	18	/	Class specific TTC	400	400	400	CPCA 3
N-nitroso-tamsulosin	/	18	/	Class specific TTC	1500	1500	1500	CPCA 4

Information sources: [7–9–40]

List is limited to selected nitrosamines that were not harmonized before the latest guidance updates; complete lists of nitrosamines and their AIs before and after the latest guidance updates are provided in Annexes VIII and IX.
Columns in red are not aligned, columns in yellow are partly aligned, columns in green are fully aligned.
CPCA 2, 3, 4 = CPCA potency category 2, 3, 4.
AI = TD_{50} (mg/kg/day)/50 000 (linear extrapolation from cancer risk probability of 1:2 to 1:100 000) x 50 kg (human body weight).
[1] Most sensitive TD_{50} from most robust dataset
[2] No AI provided, only recommended as suitable surrogate compound for NDSRIs [10]
[3] Harmonic mean TD_{50}; most sensitive rodent species
[4] AI can be transferred from the AI for the structurally similar NDSRI published on the FDA website, which was derived by read-across from this nitrosamine [9]
[5] NTTP is also known as nitroso-STG-19 sourced from sitagliptin

Status: August 2023

Still not fully aligned AIs, highlighted in yellow, can be observed for the nitrosamines N-Nitroso-N-methylaniline (NMPA), MNP, NPIP, NMOR, N-nitroso-diphenylamine (NDPh) and NPYR.

Furthermore, the newly added AIs for NDSRIs do not match in many cases either, as Table 6 shows for selected nitrosamines. Most of the 290 NDSRIs are listed by only one or two CAs, while only 30 NDSRIs are simultaneously listed by the EMA, HC and FDA with matching AIs (see Annex VIII, green table rows). For example, HC and the FDA list AIs for the NDSRIs related to citalopram, diphenhydramine and doxepin, while these are absent from the EMA list. Others, such as N-nitroso-fluoxetine, N-nitroso-hydrochlorothiazide and N-nitroso-quinapril are listed by the EMA and HC, while the FDA has not yet published AIs for these NDSRIs. A discussion of the possible reasons for the differences in AIs provided by the CAs follows for some examples in section 5.3.1.5. First, an overview of the different approaches to derive AIs and their challenges will be given in the next section.

Table 6. Differences in newly added AIs (ng/day) for NDSRIs between EMA, HC and FDA

Related drug substance	NDSRI	EMA	HC	FDA	AI derivation
Amitrptyline, Nortriptyline	N-nitroso-nortriptyline	8	8	26.5	EMA/HC: SAR/NMPEA; FDA: CPCA 1
Atomoxetin	N-nitroso-atomoxetine	100	100	26.5	EMA/HC: SAR/NNK; FDA: CPCA 1
Ciprofloxacin	N-nitroso-ciprofloxacin	1500	1500	pending	CPCA 4; FDA: pending surrogate for SAR/read-across
Citalopram	N-nitroso-desmethyl citalopram	/	18	26.5	CPCA 1
Diphenhydramine	N-nitroso-desmethyl-diphenhydramine*	/	18	26.5	CPCA 1
Doxepin	N-nitroso-desmethyl-doxepin	/	18	26.5	CPCA 1
Fluoxetine	N-nitroso-fluoxetine	100	100	/	EMA/HC: SAR/NNK
Hydrochlorothiazide	N-nitroso-hydrochlorothiazide	NMI	NMI	/	negative in vivo mutagenicity test
Quinapril	N-nitroso-quinapril	NMI	NMI	/	negative in vivo mutagenicity test

Information sources: [7–9]

List is not exhaustive; for a complete overview of recommended AIs see Annex VIII.
Columns in red are not aligned, columns in yellow are partly aligned.
CPCA 1 = CPCA potency category 1;
NMI = non-mutagenic impurity.

*listed as N-(2-(benzhydryoxy)ethyl)-N-methylnitrous amide by HC [8]

Status: August 2023

5.3.1.1 Approaches to derive AIs for nitrosamines in line with ICH M7

If sufficiently robust carcinogenicity data are available for a nitrosamine, a compound-specific AI can be calculated using the TD_{50} value based on available rodent carcinogenicity data for the respective nitrosamine as it was done for example for NDMA and NDEA (see Annex IX). However, for newly identified nitrosamines, it is very unlikely that carcinogenicity data are available and thus compound-specific AIs can be determined. In this case, MAHs can either apply a class-specific AI or carry out structure-activity relationship (SAR) considerations with read-across to derive an AI for the respective nitrosamine.

SAR is an approach to estimate the potential adverse effects of a chemical on the basis of its chemical structure [95]. Existing SAR models are used for the qualitative prediction of mutagenicity to classify mutagenic impurities as recommended in ICH M7 [4–95]. SAR models could further be applied to identify a surrogate compound with sufficient carcinogenicity data for read-across. To establish an AI for the target compound, the TD_{50} of the identified structurally similar substance (surrogate compound) can be used to make a quantitative estimation of carcinogenicity by read-across (see Figure 6).

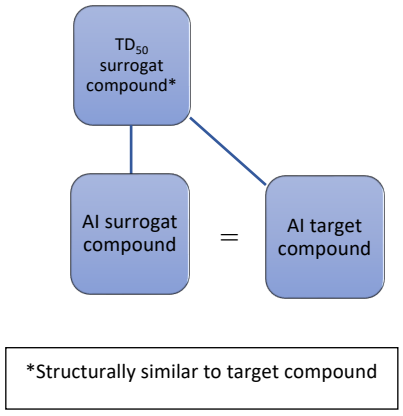


Figure 6. AI derivation based on SAR/read-across

Class-specific AIs can be derived based on structural similarity to a chemically defined class of known carcinogens, collecting all available TD_{50} values of the related compounds and using the lowest value as the class-specific AI [4].

The foundation of the above-described approaches to establish AIs for nitrosamines is TD_{50} values derived from carcinogenicity data in each case. The TD_{50} corresponds to the daily dose for a lifetime that causes tumors in half of the test animals that would have

remained tumor-free at a dose of zero [96]. This established risk can be linear extrapolated to the acceptable human cancer risk of 1: 100000.

An essential resource for selecting a TD_{50} value is the Carcinogenicity Potency Database (CPDB) [96] or Lhasa Carcinogenicity Database (LCDB) [97], which contain results of relevant long-term animal carcinogenicity tests. The LCBD is an extension and further development of the CPDB, which is no longer updated. All data from the CPDB were imported into the LCBD and in addition an improved calculation of TD_{50} values was created to account for the weaknesses of the CPBD calculation which are described by Thresher et al. [98]. If the data are poor, consisting of studies where less than 2 dose levels were tested, Lhasa in contrast to Gold, director of the carcinogenic potency project [96], did not calculate a TD_{50} value [99]. For studies with 2 dose levels, a TD_{50} value was calculated if no other quality-reducing conditions, like for example studies where the lifetable method was used, were present [100]. Therefore, the absence of a Lhasa TD_{50} value for a specific compound in the LCBD indicates a poor study situation for this compound. On the other hand, an existing Lhasa TD_{50} value does not necessarily mean that the data are robust as defined in ICH M7 (for more information see section 5.3.1.3).

5.3.1.2 Carcinogenic Potency Categorization Approach (CPCA)

The CPCA is a methodology developed by the FDA in collaboration with other international CAs enabling the carcinogenic potency of a nitrosamine to be determined quickly and easily on the basis of its molecular structure. It is based on the assumption that the α-hydroxylation mechanism of metabolic activation is responsible for the high carcinogenic potency of nitrosamines. Several scientific publications published in the last two years have been able to demonstrate that certain structural features around the nitrosamine motif and above either increase or decrease the decisive activation mechanism [27–101–102]. Taking into account this evidence, nitrosamines can be classified into five potency categories (see Table 7) mostly based on the structural elements at the α- and β-carbon (see Figure 7). [8–10–13]

The potency score, reflecting the carcinogenic potency category, is the sum of the α-hydrogen score, the deactivation feature score and the activation feature score. The relevant tables, a flow chart and examples are provided with the updated guidelines to

assign nitrosamines to a respective potency category. The relevant instructions can be found in Annex X and some examples for the CPCA application are given in the next sections as well as in Annex XI of this master thesis.

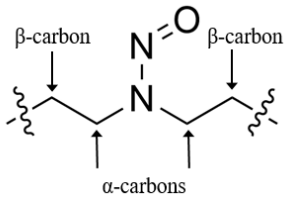


Figure 7. Structural representation of α- and β-carbons on an N-nitrosamine

Source: EMA Q&A document, Annex 2 [8]

Table 7. Carcinogenic Potency Categories according to CPCA

Potency Category	AI (ng/day)	Justification
1	18; 26.5*	Class-specific limit
2	100	Potency predicted to be no higher than NDMA (AI 96 ng/day) and NNK (100 ng/day)**
3	400	4-fold decrease in potency compared to category 2 due to the presence of a weakly deactivating feature
4	1500	TTC acc. to ICH M7; Metabolic activation*** possible, but disfavored due to steric or electronic influences or favored clearance pathways
5	1500	TTC acc. to ICH M7; Metabolic activation not possible due to steric hindrance or absence of α-hydrogens or formation of unstable, not reactive species

Source: modified table included in FDA, HC and EMA guidance [8–10–13]

*Different limits applied by EMA, HC (18ng /day) and FDA (26.5 ng/day)
**Two robustly tested nitrosamines
***α-hydoxylation pathway

5.3.1.3 The challenge of determining AIs for NDSRIs

Many of the available TD_{50} values for nitrosamines are attributed to non-robust carcinogenicity studies and thus often may not provide sufficient evidence to predict carcinogenic potency. The decision on the suitability of a TD_{50} value therefore represents a major obstacle in the reliable estimation of the carcinogenic potency of a substance as the criteria for selecting an appropriate TD_{50} value to derive a compound-specific AI for a nitrosamine impurity are not standardized. The general approach used to derive AIs for the mutagenic chemicals outlined in the addendum of ICH M7 is based on pre-calculated TD_{50} values from the CPDB or on a TD_{50} calculation from robust literature data using

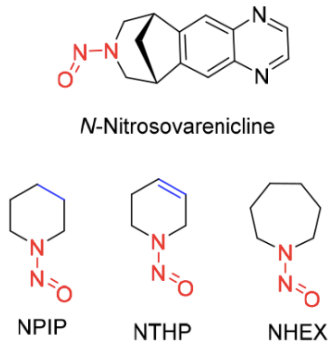
methods described in the CPBD [103]. Furthermore, the use of harmonic mean TD_{50} values provided in the CPDB, pooling the results of existing studies, is not described in the Addendum of ICH M7. Instead, the lowest TD_{50} values were selected by the ICH to give a worst-case estimate of carcinogenic potency [103]. In contrast, for some nitrosamines, the harmonic mean TD_{50} was used by the CAs to determine compound-specific AIs and for N-Methyl-N-nitrosophenethylamine (NMPEA) the Lhasa TD_{50} was used instead of the Gold TD_{50} [13]. An overview of the TD_{50} values used for the derivation of AIs published by the EMA and the study situation on which the TD_{50} values are based on can be found in Annex IX.

ICH M7 provides a framework for defining sufficiently robust carcinogenicity data that can be used to calculate a TD_{50} value (see Annex XII), but also acknowledges that in some cases less robust data can be used [4]. Some TD_{50} values selected by the EMA to determine AIs for nitrosamines do not meet all criteria for robust data as they are based on studies with less than three tested dose levels (see AI derivation for NMPA and NDPh, Annex IX). The FDA has obviously assessed these data situations as insufficient and set an AI of 26.5 ng/day for NMPA [14], while no AI has yet been published for NDPh. As ICH M7 does not define criteria where less robust data can be accepted, it can be concluded that the selection of suitable TD_{50} values remains subjective to a certain extent.

Another challenge lies in the selection of a similar chemical structure for read-across as there is no commonly agreed validated method for the identification of structural similarity between the target and the surrogate compound. According to the FDA, the "*structural environment surrounding the N-nitroso group"* [10] should be considered for the selection of a surrogate, while HC clarified in its latest guidance update that local and overall structural similarity should be taken into consideration to justify an appropriate surrogate for read-across [8]. The EMA does not make any recommendations on this topic in its Q&A document, but some advice for conducting SAR/read-across is given by the CHMP in the assessment report of the Art. 5 (3) procedure for nitrosamine impurities [1]. The rationale for the chosen surrogate compound should not only be based on structural similarity, but also take into account structural differences including their influence on absorption, distribution, metabolism and excretion (ADME) [1]. Although all these notes provide some orientation, in the absence of a standardized method for selecting

surrogate compounds, the derivation of AIs based on SAR/read-across remains a great challenge and subjective to some degree as illustrated by Ponting et al. [102] for N-nitroso-varenicline.

Ponting et al. [102] identified N-nitrosohexamethylenimine (NHEX), resulting in an AI of 313 ng/day, or N-nitrosopiperidine (NPIP), resulting in an AI of 963 ng/day, both based on lowest reported TD_{50}, instead of NTHP as used by the CAs (currently giving an AI of 37 ng/day based on most sensitive TD_{50} from most robust dataset), as suitable surrogate to establish an AI for N-nitroso-varenicline (see Figure 8).



Theoretical CPCA potency score for N-nitroso-varenicline:

Count of hydrogen atoms on each α-Carbon = 2,2 corresponds to an α-hydrogen-score of 1; N-nitroso group in a 7-membered ring results in a deactivating feature score of 1 and lack of activating features finally leads to a potency score of 2 (α-hydrogen score (1) + deactivating score (1)).

For full traceability apply tables and instructions in Annex X.

Figure 8. Read-across surrogates considered for N-nitroso-varenicline

Source: Ponting et al., 2022 [102]

The authors consider NTHP to be an unsuitable surrogate compound due to the olefin structure present, which leads to increased acidity at the α-hydrogen atom and thus to an increased likelihood of CYP oxidation at this site and ultimately overestimation of carcinogenic potency. However, allylic and propargylic side chains are currently not considered as activating properties in the CPCA either. On the other hand, the existing studies on the more structurally similar compound NHEX are not robust [102]. The study situation for NPIP, however, is robust, as the FDA also states in its NDSRI guidance [10]. Thus, it remains open why the authorities nevertheless chose NTHP as a surrogate compound for N-nitroso-varenicline. Applying the CPCA to N-nitroso-varenicline, considering the 7-membered saturated ring, gives an AI of 100 ng/day (potency category 2), which is much closer to the current AI of 37 ng/day than the AIs of 963 ng/day based on lowest reported TD_{50} [102] or 1300 ng/day based on harmonic mean Gold TD_{50} selected by the EMA for NPIP as a surrogate [7]. These observations illustrate the magnitude of the challenges in deriving AIs. For the selection of a surrogate compound,

the degree of structural similarity may have to be weighed against the robustness of the respective carcinogenicity data leading to possible deviating selections of surrogate compounds. The application of the CPCA to NDSRIs for which surrogate compounds are available may create additional uncertainty. As long as there is no standardized procedure for SAR/read-across, it remains difficult to achieve harmonized AIs for nitrosamine impurities based on this method.

Furthermore, for the majority of NDSRIs, surrogate compounds are missing as most of the tested nitrosamines providing carcinogenicity data do not show sufficient structural similarity. This led to the setting of the class-specific AI of 18 ng/day for some NDSRIs in the past, established by the Safety Working Party (SWP) based on the most potent nitrosamines according to the LCBD [1]. This class-specific AI was also applied by HC but not by the FDA, which always referred to the AI of NDEA or NDMA as default option if no suitable surrogate was available [53]. The application of these conservative limits for NDSRIs, not reflecting that many nitrosamines, especially NDSRIs, may have only a weak carcinogenic potency compared to NDEA and NDMA, posed a great threat to the drug supply for quite a while. Increasing evidence suggests the exclusion of some NDSRIs from the CoC [5–104–105]. In the EFPIA supported position papers, Nudelman et al. proposed the use of a weight of evidence approach mainly based on SAR considerations for the establishment of AIs for NDSRIs from ß-blocker and ß-agonists as well as ACE-inhibitors [5–105]. SAR considerations for N-nitroso-ACE derivatives [5] are summarized in Annex XIII exemplified by N-nitroso-ramipril and may have been an important driver for the development of new approaches to predict carcinogenic potency for NDSRIs. Finally, with the introduction of the CPCA, the broad application of the class-specific AI for NDSRIs has become obsolete.

An additional newly introduced option to de-risk NDSRIs is the enhanced Ames test (EAT), which is intended to represent the metabolism in humans more precisely than the standard Ames Test [8–10–13]. NDSRIs and other nitrosamines for which neither mutagenicity nor carcinogenicity data are available would fit into class 3 impurities according to ICH M7 (see 3.4) and thus could in principle be de-risked to class 5 impurities with a negative bacterial mutagenicity test and controlled as non-mutagenic impurities according to ICH Q3A/Q3B [4]. However as nitrosamines also belong to the CoC,

carcinogenicity data from structurally similar substances should be included in the hazard assessment as stated in ICH M7, indicating that treating nitrosamines as non-mutagenic impurities solely based on a negative Ames test may not be appropriate [4].

Indeed, the CAs still do not accept a negative Ames test as sole evidence for lacking mutagenicity at present [8–10–13–53]. However, according to the EMA and HC guidance, a negative EAT may justify a limit of 1.5 µg/day, corresponding to the TTC for mutagenic impurities not included in the CoC [8–13]. The FDA also lists the EAT for assessing mutagenic risk and cites a negative test result as an option to justify a higher NDSRI limit [9]. However, the FDA does not specify the limit of 1.5 µg/day and reserves the right to request further safety data in these cases [9]. This means that there still remains uncertainty for MAHs about the usefulness of the Ames test, which is now also more elaborate to perform. A comparison between the standard OECD Ames test and the EAT is provided in Annex XIV.

The introduction of the option to control an NDSRI at 1.5 µg/day by a negative EAT result goes some way to solving the dilemma that the interpretation of ICH M7 requirements has created for regulators and industry to control NDSRIs. As many NDSRIs have a much lower carcinogenic potency compared to simple dialkyl-nitrosamines, the blanket inclusion of NDSRIs in the CoC because of being N-nitroso-compounds, and thus denial of the possibility to classify them as Class 5 impurities based on a negative Ames test (see 3.4) seems questionable. Now, although a negative EAT does still not allow assignment of nitrosamines to Class 5 impurities, it at least makes it possible to exclude these from the CoC. This prospect for a clear control strategy creates an incentive for pharmaceutical companies to carry out Ames testing with NDSRIs, which in turn provides useful mutagenicity data on NDSRIs.

Furthermore, the EMA and HC recommend in vivo mutagenicity testing to control a nitrosamine as non-mutagenic impurity under ICH Q3A/Q3B [8–13]. With the Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays according to OECD No. 488, HC indicates suitable in vivo tests in its guideline [8]. In contrast, the FDA does not specify any requirements for follow-up testing in the latest guidance update, but as outlined in the FDA industry meeting [53], in vitro testing is also possible, besides in vivo testing, to confirm the negative Ames test result.

There is currently a lot of research going on in the field of in vitro genotoxicity testing for nitrosamines as the typically used model cell lines lack effective metabolic capability and thus are less specific for compounds that need metabolic activation to induce genotoxicity, as is the case for nitrosamines [106]. Investigations like those by Li et al., supported by the Center for Drug Evaluation and Research (CDER), where TK6 cell lines expressing human cytochrome P450s were developed [106–107], and joint research projects like the EMA-funded project led by the Fraunhofer Institut für Toxikologie und Experimentelle Medizin to evaluate other novel in vitro genotoxicity tests as comet assays in liver cell models [108] are promising efforts for the establishment of improved in vitro testing systems to better mimic human metabolism and thus reliably predict mutagenicity for nitrosamine, including NDSRIs.

5.3.1.4 Application of AI determination methods

The newly introduced options for determining AIs provide practical approaches to establish science-based lifetime AIs in a timely manner and thus finally enable MAHs and manufacturers to develop effective mitigation measures for their approved drug products, if necessary. But does the introduction of the CPCA really represent a milestone in the regulatory approach to control nitrosamine impurities in medicinal products, as it is currently communicated in the regulatory community [109]?

Figure 9 shows the proportion of nitrosamines assigned by the EMA, the FDA and HC to the respective CPCA categories. The differences in the percentages of nitrosamines by CPCA category between the CAs are based on the differences in the numbers and kind of listed nitrosamines. They may be due to deviating market statuses, different CT results, or potential uncertainties regarding the method or AIs to be adopted. Further, the FDA applied the CPCA only for NDSRIs, while this method was also followed by the EMA and HC for a few other nitrosamines (e.g. N-nitroso-piperazine, MNP).

However, it can be stated that for around 50% of the NDSRIs whose AI was determined by the CPCA, an AI of 1500 ng/day can be applied in all regions because they were assigned to either potency category 4 or 5. It can be expected that this high AI will ensure the availability of many approved drugs affected by NDSRIs and, dependent on the levels detected, even without the need to implement reducing strategies. On the other hand, it

is notable that HC with 23% and the FDA with 18%, have assigned a relatively high proportion of NDSRIs to category 1 compared to the EMA, where only 8% are included in category 1. Together with the 7% (HC) and 17% (FDA) of CPCA potency category 2, approximately one-third of the NDSRIs are still assigned a similarly high carcinogenic potency as NDEA or NDMA. The sum of the potency categories 1 and 2 applied by the EMA is only 16%. However, many of those CPCA category 1 NDSRIs listed by the FDA and HC are missing on the EMA list (see Annex VIII) although these are also potential NDSRIs of APIs approved in the EU.

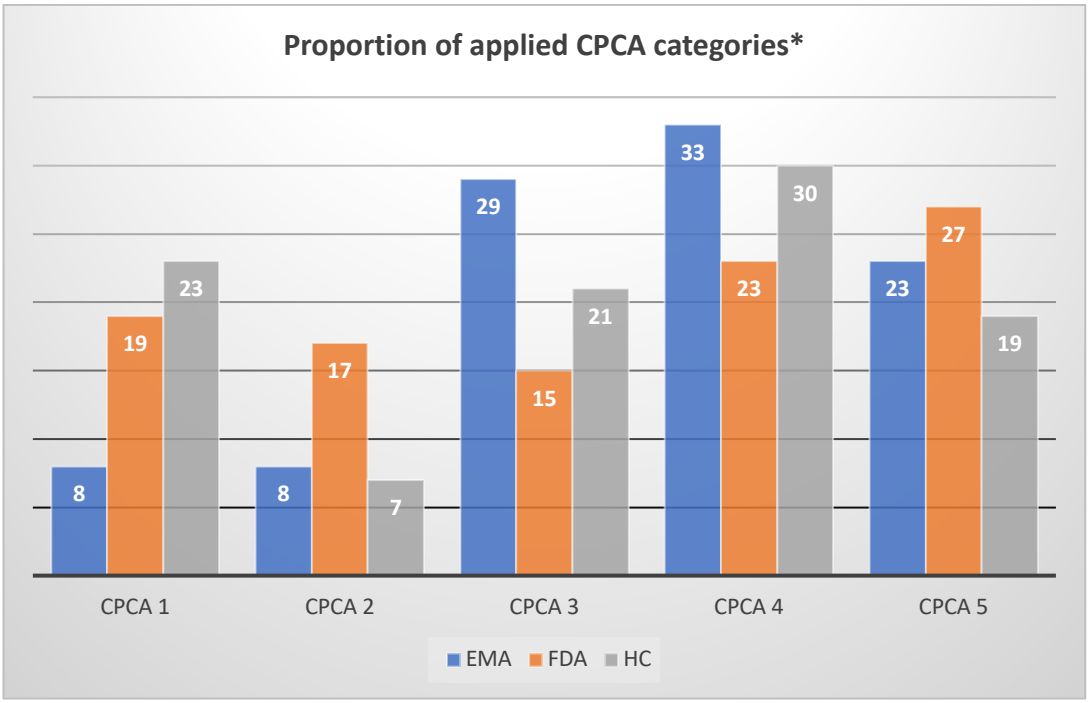


Figure 9. Proportion of applied CPCA categories

Source: Own illustration based on EMA website (Appendix 1) [7], HC guidance (Appendix 2) [8], FDA website (FDA Recommended AI Limits for Certain Hypothetical NDSRIs) [9]

*Shown is the percentage of nitrosamines assigned to a CPCA category out of all nitrosamines to which the CPCA was applied.

The current potency categorization means that a more or less large proportion of NDSRIs still need to be controlled as highly potent carcinogens.

The FDA published AIs for three NDSRIs (NTTP, N-nitroso-varenicline and N-nitroso-duloxetine) based on SAR/read-across on its website in August 2023 [9]. While a further SAR/read-across-based AI is pending for N-nitroso-ciprofloxacin, all other AIs for NDSRIs are based on the CPCA [9].

Table 6 introduced above shows some examples of newly listed NDSRIs that are not fully harmonized between the FDA, EMA and HC, either because they are not listed by all CAs or because there is no agreement on the method used. As can be seen in the overview of Table 6, the EMA and HC have already determined an AI of 1500 µg/day for N-nitroso-ciprofloxacin based on the CPCA category 4 while the FDA will publish an AI based on SAR/read-across. Conversely, for N-nitroso-nortriptyline, the FDA has published an AI of 26.5 ng/day according to CPCA potency category 1, while the EMA and HC preserve the SAR/read-across-based AI of 8 ng/day. For other NDSRIs, for which a SAR/read-across-based AI has been published by the EMA and HC, except for those outlined in Table 5, the FDA has not yet published an AI. These include, for example, N-nitroso-paroxetine and N-nitroso-fluoxetine. The same applies to NDSRIs that have been classified as non-mutagenic by the EMA and HC on the basis of negative in vivo mutagenicity tests (e.g. N-nitroso-hydrochlorothiazide, N-nitroso-quinapril) which are missing on the FDA list.

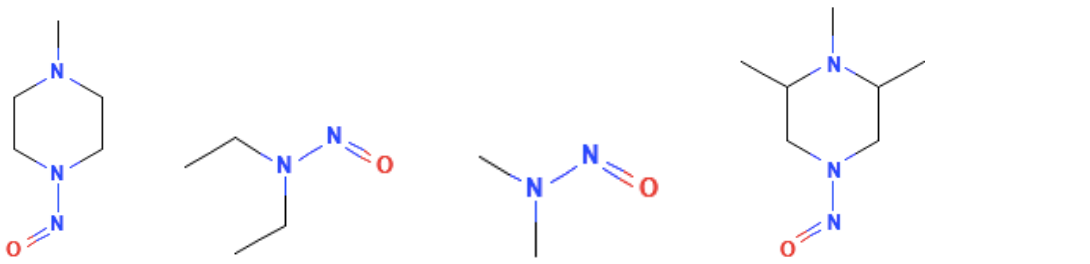
CPCA potency category 1 differs between the FDA and the EMA as well as HC, as the FDA assigns an AI of 26.5 ng/day to it based on NDEA, while the EMA and HC have set the class-specific AI of 18 ng/day established by the SWP (see Table 6 and Table 7). It is surprising that it was not possible to agree on a uniform AI here. Even if the values differ only slightly, this difference also demonstrates a certain degree of inconsistency.

While the EMA and the FDA explicitly request the use of the CPCA as the method of choice to derive AIs, such a clear recommendation cannot be read from the HC guideline [8–10–13]. However, the EMA recommends the CPCA *"unless other robust data are available that would override this AI"* [13]. But which data can be considered more robust apart from a negative EAT or negative in vivo mutagenicity test? Could a more reliable mapping of the carcinogenic potency be achieved by SAR/read-across if there is sufficient structural similarity and robust carcinogenicity data? As the criteria for these prerequisites are currently not standardized, it remains unclear when an AI based on SAR/read-across more reliably reflects the carcinogenic potency of a nitrosamine than one determined by the CPCA. This uncertainty is also reflected in the still diverging AIs for nitrosamines published by the CAs, based on different methods of derivation.

5.3.1.5 Analysis of AIs and their derivation approaches

AIs for nitrosamines based on SAR/read-across established by the EMA and HC before the introduction of the CPCA are still applied with one exception. The previous AI of 26.5 and 96 ng/day for MNP derived by SAR/read-across from NDEA and NDMA, respectively, is aligned to 400 ng/day by the EMA and HC based on CPCA potency category 3 (see Table 5). In MNP the N-Nitroso-group is embedded in a 6-membered ring, which according to the CPCA is considered as deactivating feature for metabolic activation via α-hydroxylation mechanism (see Figure 10 and Annex X, Table 2). As these deactivating features are not present in NDEA or NDMA, insufficient structural similarity for SAR/read-across for MNP is evident, but the FDA still has not changed its recommended AI of 96 ng/day [40]. Structural similarity is given between MNP and 1,2,6-trimethyl-N-nitrosopiperazine (see Figure 10), which is proposed by Dobo et al. [110] as surrogate for read-across resulting in an AI of 153 ng/day. However, the used Lhasa TD_{50} (0.153 mg/kg/day) is based on only one study in which tumors were detected at two dose levels, which could not be considered sufficiently robust carcinogenicity data, at least not according to ICH M7 criteria. Consequently, the suitability of 1,2,6-trimethyl-N-nitrosopiperazine as surrogate compound for MNP is also questionable.

The FDA's non-application of the CPCA for MNP is consistent with its exclusive use of this AI determination method for NDSRIs as presented in the FDA guidance [10]. Since MNP could not be considered structurally related and unique to rifampicin, but is formed from the nitrosation of 1-amino-4-methylpiperazine [111], which is essentially required for the final synthesis step to rifampicin, it is not considered an NDSRI under the current definition [10]. However, the high MNP levels found in rifampicin medicines are due to their late introduction in API synthesis and difficult to avoid as the MNP precursor structure is simultaneously the API precursor structure. Whether this aspect played a role in the selection of the CPCA as the AI determination method for MNP, allowing the application of a relative high AI, and if this approach most reliably reflects the carcinogenic potency of MNP remains open. Nevertheless, it seems to be a pragmatic approach to ensure the continuous availability of rifampicin medicines, which furthermore are not taken for lifetime.



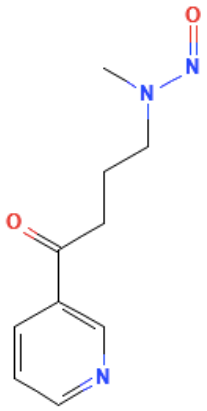
MNP NDEA NDMA 1,2,6-trimethyl-N-Nitrosopiperazine

Figure 10. Comparison of applied surrogate compounds for MNP

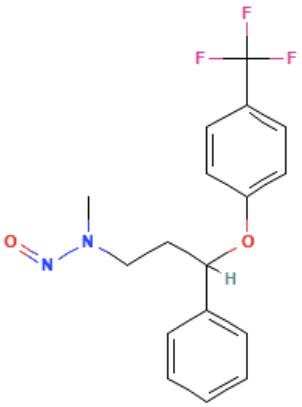
Sources of chemical structures:
MNP: https://pubchem.ncbi.nlm.nih.gov/compound/1-Methyl-4-nitrosopiperazine, accessed 04.06.2023;
NDEA: https://pubchem.ncbi.nlm.nih.gov/compound/5921, accessed 04.06.2023;
NDMA: https://pubchem.ncbi.nlm.nih.gov/compound/6124, accessed 04.06.2023;
1,2,6-trimethyl-N-Nitrosopiperazine = 1,2,6-trimethyl-4-Nitrosopiperazine: https://pubchem.ncbi.nlm.nih.gov/compound/150692 ; accessed 04.06.2023

The SAR/read-across-based AI of 37 ng/day for N-nitroso-varenicline and NTTP was kept by the EMA and HC and recently also accepted by the FDA. However, this AI derivation is discussed controversial by Ponting et al. as already explained in section 5.3.1.3. Equally, the AI of 100 ng/day for N-nitroso-duloxetine derived by read-across from NNK, is now fully aligned between the EMA, the FDA and HC (see Table 5).

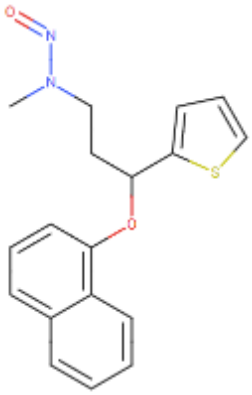
As can be seen in Figure 11, structural similarity to NNK further exists for N-nitroso-fluoxetine and N-nitroso-atomoxetine, but in contrast to HC and the EMA, the FDA did not apply the SAR/read-across-based approach for these NDSRIs. Instead, CPCA potency category 1 was determined for N-nitroso-atomoxetine, and for N-nitroso-fluoxetine no AI was yet published by the FDA (see Table 6). What could be the reasons behind this?



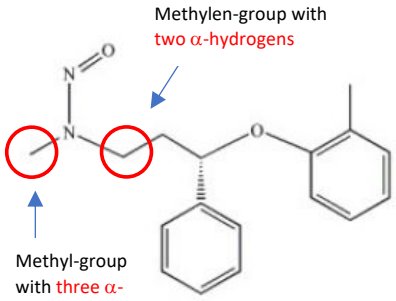
NNK (MW: 207.23 g/mol)



N-nitroso-fluoxetine (MW: 338.32 g/mol)



N- nitroso-duloxetine (MW: 326.4 g/mol)



N-nitroso-atomoxetine (MW: 284.35 g/mol)

CPCA for N-nitroso-atomoxetine and related NDSRIs:

Count of hydrogen atoms on each α-carbon = 2,3 corresponds to an α-hydrogen-score of 1; no deactivating and activating features lead to a potency score of 1 (corresponding to the α-hydrogen-score).

For full traceability apply tables and instructions in Annex X.

Figure 11. Comparison between NNK and structurally similar NDSRIs

MW= molecular weight;
Sources of chemical structures and MW:
NNK: https://pubchem.ncbi.nlm.nih.gov/compound/47289, accessed 04.06.2023
N-nitroso-duloxetine: https://nitrosamines.usp.org/t/ai-for-nitroso-duloxetine/3573 ; accessed 04.06.2023;
N-Nitroso-fluoxetine: https://pubchem.ncbi.nlm.nih.gov/compound/9840784, accessed 04.06.2023;
N-nitroso-atomoxetine: https://www.hpc-standards.com/shop/ReferenceMaterials/PharmaceuticalsVeterinaryProducts/NNitrosoAtomoxetine.htm, accessed 13.08.2023.

While local similarity is evident between NNK and N-nitroso-duloxetine, N-nitroso-fluoxetine and N-nitroso-atomoxetine, it is questionable, whether overall structural similarity, as required by the latest HC guidance update, is also sufficiently met to get a reliable picture of the carcinogenic potency of these NDSRIs by SAR/read-across. Differences in the elements distant from the nitrosamine-motif between these NDSRIs and NNK, like, for example, the bulky naphtyl substituent in N-nitroso-duloxetine, may influence the metabolism as discussed by Ponting et al. [102] and thus the carcinogenic potency of NDSRIs.

Another aspect that would take account of the overall structure for carcinogenicity prediction of a nitrosamine is the molecular weight, as high molecular weight nitrosamines are usually more bulky and less accessible to metabolic enzymes. In

addition, such nitrosamines have fewer nitroso groups available per defined mass than nitrosamines with a lower molecular weight. The mutagenic potential consequently decreases with the increase in molecular weight. [6] However, at present this factor is officially not taken into account in the AI derivation.

Applying the CPCA on N-nitroso-duloxetine, N-nitroso-fluoxetine and N-nitroso-atomoxetine, the low α-hydrogen score of 1 and the lack of both deactivating and activating properties results in CPCA potency category 1 as illustrated by way of example for N-nitroso-atomoxetine in Figure 11. So far, science has sufficient data for some functional groups to classify them as deactivating or activating functional elements and these features are predominantly taken into account for α- and β-carbons in the CPCA. Thus, the influence of the oxy-groups in γ-position and the aryl-groups of N-nitroso-duloxetine, N-nitroso-fluoxetine, N-nitroso-atomoxetine remains unconsidered in the CPCA at this time. The CPCA considers carboxyl- and hydroxyl groups as deactivating factors that increase polarity and solubility and thus significantly alter stability and metabolism [27]. However, predicted metabolic fates of NDSRIs like favored hydroxylation reactions distant from the nitrosamine motif as considered by Nudelman and Czich [5] for N-nitroso-ramipril (see Annex XIII), leading to none or negligible α-hydroxylation, still need to be confirmed for most NDSRIs [102] and thus are currently not taken into account.

Atomoxetine is an API used to treat attention deficit hyperactivity disorder in children aged 6 years and older [112], whereas duloxetine is used only in adults [113]. This could be a reason for the FDA's conservative AI derivation for N-nitroso-atomoxetine based on the CPCA. In addition, the lower molecular weight of N-nitroso-atomoxetine compared to the other NDSRIs may play a role. It is interesting at this point to see which AI the FDA will publish for N-nitroso-fluoxetine, which is the NDSRI with the highest molecular weight of the NNK-like NDSRIs (see Figure 11) and its corresponding drug substance is approved from the age of 8 [114].

The general difference in the application of the CPCA, where the FDA explicitly applies this method only to NDSRIs while the EMA and HC also allow for it for other nitrosamines, could lead to further confusion as well as the inconsistent application of this method by

the EMA and HC. For example, the application of the CPCA to N-nitrosoethylisopropylamine (EIPNA/NEIPA/NIPEA, hereafter abbreviated with NEIPA) which is currently assigned an AI of 26.5 ng/day based on read-across from NDEA (see Annex VIII), would result in an AI of 400 ng/day according to CPCA potency category 3 (see Annex XI, Example 2). The question arises why the AI of NEIPA remains unchanged although its structure is even explicitly listed as an example in the table for calculating the α-hydrogen score and why the authorities do not adjust this AI as it was done for MNP.

The above considerations show that there are obviously several limitations of the CPCA for the prediction of carcinogenic potency for nitrosamines. Accordingly, the guidelines indicate that the CPCA is a conservative approach that takes into account the current state of science and will be further adapted in the future [8–10–13]. The continuing differences in the published AIs and applied methods of the authorities indicate that a uniform regulatory approach to determine AIs for nitrosamines remains a great challenge at present.

More knowledge about the metabolic fate of NDSRIs and extended SAR considerations, for example the inclusion of molecular weight, could be important steps towards a more reliable prediction of carcinogenic potency for NDSRIs and other nitrosamines whose structural features are currently insufficiently included in the calculation. In addition, since a particular NDSRI is unique to the drug product concerned, consideration should be given to whether the user group should be taken into account in the AI derivation for NDSRIs for as long as the prediction of carcinogenic potency is based on a certain degree of uncertainty. In addition, the nitrosamine impurities to which the CPCA applies should be clearly and uniformly defined. This would increase the acceptance of the CPCA among the different CAs and promote a uniform application.

5.3.1.6 AIs for multiple nitrosamine impurities

The large number of possible root causes and risk factors for nitrosamine impurities in drug products leads to situations where several nitrosamines can be present in a drug product.

The EMA gives two principal options for the control of multiple nitrosamines and presents by way of example how the respective AIs could be reflected in the specification. Applying

option 1, the total daily intake must not exceed the AI of the most potent nitrosamine. In this case, the individual limits for the nitrosamines present do not have to be specified. Option 2 considers the total risk level, which should not be greater than 1:100000 for all nitrosamines. This could be realized using the fixed or the flexible approach. In the fixed approach, fixed limits as percentages of the respective AIs are set for the individual nitrosamines such that the sum of the percentage AI limits gives 100%. A total content of nitrosamines does not have to be specified in this case. In the flexible approach, the individual nitrosamines are specified with their AI limit. In addition, the total content of nitrosamines must be controlled with NMT 100%. [13]

HC´s recommendations for the control of multiple nitrosamines correspond to option 1 and option 2, fixed approach, while the FDA mentions only option 1 according to the EMA in its guidance. However, the possibility of alternative approaches is offered by both, HC and the FDA [8–10–14].

Table 8. FP specification possibilities as control options for multiple nitrosamines (here for NDEA and N-nitroso-dabigatran)

Nitrosamine	Option 1		Option 2 - fixed Example 20-80 ratio*		Option 2 - flexible	
	Limit (ppb)	Results (ppb)	Limit (ppb)	Results (ppb)	Limit	Results
NDEA (AI 26.5 ng/day)	Not needed	(6)	NMT 9 (44x 0.2)	6	NMT 44**	6 (14% of AI)
N-nitroso-dabigatran (AI 400 ng/day)	Not needed	(390)	NMT 534 (667 x 0.8)	390	NMT 667**	390 (58% of AI)
Total NA	NMT 44**	396	Not needed	-	NMT 100%	72%

Source: fictive results based on EMA Q&A document, Q&A 10 [15]

*Different ratios could be used in different situations dependent on relative amounts present, sum of the % AI limits should be ≤ 100%
**AI limit of nitrosamine expressed in ppb calculated by dividing the AI by the MDD (600 mg/day) for dabigatran [115]:
(NDEA: 26.5 ng/day : 600 mg/day = 44 ppb; N-nitroso-dabigatran: 400 ng/day : 600 mg/day = 667 ppb))

Table 8 shows the different control options for the constellation that a nitrosamine of high carcinogenic potency (NDEA) is present in low quantity together with a nitrosamine of lower carcinogenic potency (N-nitroso-dabigatran) in high quantity in a drug product. For example, if NDEA levels of 6 ppb and N-nitroso-dabigatran levels of 390 ppb were present in one batch, the acceptable total specification limit of 44 ppb – applying option 1 – would be clearly exceeded and the respective batch rejected. Option 1 would not take into account the quantitative ratio of the two nitrosamines as well as the different

carcinogenic potencies and represents a very stringent option, especially in those cases where the carcinogenic potency differs considerably among the nitrosamines. Since NDSRI impurities are derived from the API and are therefore more difficult to remove or reduce from the drug product than process-related nitrosamines, thus are typically present at much higher levels [10], a constellation as in the example is likely to be present in a drug product.

With control option 2, the MAH has the possibility to control the carcinogenic risk for the above-mentioned situation or similar constellations reflecting the individual carcinogenic potency of existing nitrosamines while the overall carcinogenic risk of the drug product not exceeding 1:100000 is preserved. The flexible approach would even allow compliance with the specification and batch release in the case of fluctuating batch results with regard to the individual nitrosamine levels (see Table 9). A higher NDEA level could thus be compensated by a lower N-nitroso-dabigatran level or the other way around.

Table 9. Comparison of batch analysis results for fixed and flexible approach for multiple nitrosamines (here for NDEA and N-nitroso-dabigatran)

Nitrosamine	Limits and batch results (ppb)					
	Option 2 - fixed Example 20-80 ratio*			Option 2 - flexible		
	limit	batch 1	batch 2	limit	batch 1	batch 2
NDEA (AI 26.5 ng/day)	NMT 9 (44 x 0.2)	6	18	NMT 44**	6 (14% of AI)	18 (41% of AI)
N-nitroso-dabigatran (AI 400 ng/day)	NMT 534 (667 x 0.8)	390	365	NMT 667**	390 (58% of AI)	365 (55% of AI)
Total NA	Not needed	(396)	(383)	NMT 100%	72%	96 %

Source: fictive results based on EMA Q&A document, Q&A 10, option 2 [15]

*Different ratios could be used in different situations dependent on relative amounts present, sum of the % AI limits should be ≤ 100%
**AI limit of nitrosamine expressed in ppb calculated by dividing the AI by the MDD (600 mg/day) for dabigatran [115]:
(NDEA: 26.5 ng/day : 600 mg/day = 44 ppb; N-nitroso-dabigatran: 400 ng/day : 600 mg/day = 667 ppb))

If the MAH opted for the fixed approach to control the two present nitrosamines, it would have to reject batch 2 even with a lower total nitrosamine content (383 ppb) than acceptable batch 1 (396 ppb) as the individual limit of NDEA is exceeded at 18 ppb. With the flexible approach, on the other hand, it would not have to reject batch 2, as the total content remains below 100%. Thus, the flexible approach represents the least stringent

approach to control multiple nitrosamines and is therefore likely to be the preferred control strategy adopted by MAHs.

According to the EMA, followed by HC with its latest guidance update, the presence of one or more nitrosamines at levels always below 10% is considered as negligible toxicological risk and therefore such nitrosamines do not have to be calculated into the total nitrosamine level [8–13]. HC states as a prerequisite for disregarding nitrosamine levels <10% of the AI that the root causes have to be sufficiently understood and that appropriate controls must ensure that the nitrosamine level is always below 10% of the AI. However, if multiple nitrosamines are present in a drug product, disregarding them even if their individual levels are consistently <10% of the respective AI could lead to a significant nitrosamine level in total (e.g. 3 x 9 % = 27 %; 4 x 9% = 36%). Therefore, this approach contradicts the EMA request for single nitrosamines where routine testing for levels above 30% and at least periodic testing for levels between 10 and 30% of the AI has to be conducted. It is also in conflict with the routine testing required by HC for high-risk APIs and drug products that form nitrosamines during manufacture or storage (see section 5.2.3). Disregarding nitrosamine levels below 10% of the AI could be a risky strategy as omission of the control of lowest AI levels of one or more nitrosamines in a drug product for batch release and during shelf-life could lead to an underestimation of the carcinogenic risk of the product. On the other hand, ICH M7 points out that the translation of the numerical risk level of 1 in 100000 into corresponding AIs is a very hypothetical and conservative concept that should not be interpreted as realistically reflecting the actual risk [4]. Thus, exceeding the calculated AI is not necessarily associated with an increased carcinogenic risk und therefore could be considered appropriate from a safety perspective to ensure drug supply in the short term. But thinking in the long term, it might fail to motivate companies to invest in improving the quality of their products and processes. Although the EMA encourages manufacturers to do so, even in case of levels <10% of the AI of individual nitrosamines, MAHs may welcome the disregard limit since the improvement of manufacturing processes and/or the development of new formulations require significant efforts. Therefore, it is questionable whether the disregard limit provides the right incentives for a quality improvement that ensures the availability of safe drug products in the long term.

The EMA, with option 2 and its sub-options, provides pragmatic recommendations for the control of multiple nitrosamines in one drug product and thus offers reasonable control strategies for the combination of simple nitrosamines and NDSRIs. The flexible approach will ensure short-term availability of already marketed drug products. However, acceptance of this control strategy for new MAAs should be viewed critically, as new drug products should be developed in such a way that they show as little variation in nitrosamine levels as possible confirming sufficient product and process understanding.

5.3.2 Interim AIs

According to ICH M7, less-than-lifetime (LTL) limits are applicable for mutagenic impurities based on the principle that the cancer risk from exposure to a low dose over a lifetime would be equivalent to the cancer risk from equal cumulative exposure over a shorter duration [4]. Therefore, higher AIs for mutagenic impurities for drug products with a shorter treatment duration, including those with compound or class specific AIs [94] are generally in line with ICH M7. Accordingly, the LTL concept could in principle also be applied to nitrosamines.

Nevertheless, according to the EMA, HC and the FDA, no general LTL adjustments should be made to the AI for nitrosamines [8–13–53]. However, the CAs allow higher limits on a case-by-case basis as a temporary solution to avoid supply disruptions until measures are implemented that reduce the level of nitrosamines to the AI of lifetime exposure [8–13–14]. While the FDA and HC do not provide guidance on the level of interim AIs, the EMA specifies LTL adjustments as the rationale for its calculation.

While the temporary AI limit of 178 ng/day introduced by the EMA in October 2022, which could have been applied during the period of outstanding AIs for new nitrosamines, has now become obsolete due to the introduction of the CPCA, interim limits based on LTL adjustments are still applicable [13]. According to step 3 of the call for review, variations to control the nitrosamines to an acceptable level have to be submitted by 1 October 2023. However, as the final AI is essential for the development of mitigation measures and many outstanding AIs were only published in mid-2023, compliance with the above-mentioned deadline is hardly achievable. Necessary changes to the formulation actually discussed with regard to NDSRIs are complex and possibly time-

consuming (see section 5.4.) and require a more or less lengthy assessment by the authority on the regulatory side. Consequently, in December 2022, the EMA recommended a period of 3 years to implement CAPAs from the publication of the final AI and in which higher limits may be acceptable. However, an official extension of the timeline by almost two years to 1 August 2025 has so far only been communicated by the FDA in its recently published NDSRI guidance [10].

Initially, applying interim AIs during CAPA implementation was only possible if the duration of treatment with the drug product concerned did not exceed 10 years following the principle of LTL application according to ICH M7 [46]. However, among the drug products affected by NDSRIs are many of those typically used for chronic diseases which require a treatment duration for lifetime. Consequently, the EMA extended the application of the LTL approach to drug products with a treatment period beyond 10 years [13]. In order to ensure drug supply during the period of CAPA implementation, this was an important change in the latest EMA guidance update. At the same time, the acceptance of LTL-adjusted nitrosamine limits for three years for medicinal products that are used for longer than 10 years leads to an increased cancer risk compared to medicinal products used for a shorter period of time, given the subsequent potentially lifetime continued intake of the medicinal product. However, this seems to be balanced by the high benefit of many chronically used medicines and the harm that could result from their recalls.

Table 10 shows possible interim AIs for some NDSRIs using LTL adjustment factors according to ICH M7. An interim AI of 1500 ng/day corresponding to the TTC for non-CoC compounds can be applied to NDSRIs of CPCA potency category 3 and 4 and even beyond in the case of category 5 NDSRIs (e.g. N-nitroso-ramipril). Furthermore, for NDEA and NDMA as well as for NDSRIs of the CPCA categories 1-2 or whose lifetime AIs were determined by SAR/read-across (e.g. N-nitroso-varenicline), significantly higher nitrosamine values can temporarily be accepted in the affected products.

Table 10. Interim limits for selected nitrosamines based on LTL adjustments permitted by EMA

Nitrosamine (AI in ng/day)	Interim AIs (ng/day)	
	13.3 x AI* (< 12 months)	6.7 x AI* (> 12 months up to 10 years)
NDEA (26.5)	352	178
NDMA (96)	1277	643
N-nitroso-betahistine (18)[1]**	239	121
N-nitroso-duloxetine (100)[2]	1330	670
N-nitroso-dabigatran (400)[3]	1500*	1500*
N-nitroso-bisoprolol (1500)[4]	1500*	1500*
N-nitroso-ramipril (1500)[5]	19950*	10050*
N-nitroso-varenicline (37)	492	248

Source: EMA Q&A document, Q&A 22 [15]

* In any case the limit should not exceed 1.5 µg/day unless the listed AI is >1.5 µg/day, the nitrosamine belongs to CPCA category 5 or is shown to be negative in an EAT
**CPCA category 1 acc. to EMA and HC
[1-5]CPCA categories

Status: August 2023

The application of interim AIs based on LTL adjustments provides a rational solution to the current regulatory dilemma of guaranteeing the supply of medicines on the one hand, but also ensuring that they are sufficiently safe on the other. Proactive discussions should be held on what happens if the CAPA implementation to reduce NDSRIs in medicinal products takes significantly longer than the envisaged timeframe.

5.4 Mitigation strategies for NDSRIs

The prevention of NDSRIs in drug products cannot usually be achieved solely by modifying manufacturing processes or conditions, as is possible in many cases for simple nitrosamines resulting from the API manufacturing process or from API contamination.

A comprehensive RA lays the foundation for an appropriate mitigation strategy. Amine-containing APIs or API impurities, nitrite presence in excipients as well as acidic conditions during manufacturing and/or storage are listed as important risk factors for the formation of nitrosamines in drug products by the EMA, the FDA and HC. Furthermore, certain drug manufacturing operations like wet granulation and fluid bed drying can promote nitrosamine formation [8–10]. Horne et al. [20], citied by HC as peer-reviewed literature to be considered in RA, further identified functional groups in APIs (amides, hydrazones

and hydrazides) that can be degraded (e.g. by hydrolysis) to amines. However, as APIs are an integral part of the drug product, their presence cannot be avoided or reduced.

Based on the preventable risk factors for the presence of NDSRIs in drug products known so far, measures to reduce NDSRI levels are discussed in the following.

5.4.1 Supplier qualification and nitrite control strategy

Nitrite impurities in various excipients at ppm levels are a common root cause of the presence of NDSRIs in drug products [21]. The variation in nitrite levels was explained by Wu et al. [22] by different process conditions (used water, acid titration, drying conditions) in the production of the excipients. Changing the supplier as a consequence of a supplier qualification, which is also mentioned in the FDA guidance as mitigation strategy, could therefore be an effective approach to limit the NDSRI level in a drug product. But how to conduct a supplier qualification to mitigate NDSRI formation? As explicitly stated in the HC guidance, excipient suppliers should provide MAHs with the necessary information for the RA of their drug products [8]. Since the nitrite content in excipients is crucial for the formation of NDSRIs, ideally excipient suppliers should know the nitrite and nitrate (reduced form of nitrite) content of their excipients, lower it if necessary, and control it. However, at present, excipient suppliers usually only give an estimate of nitrite content by providing an RA [116]. The certificates of analysis often do not contain nitrite or nitrate levels, and if they do, they were probably carried out using test methods with limits of quantification above the actual trace but relevant nitrite levels [117].

For a reliable and effective control strategy based on supplier qualification, testing for nitrites at both the supplier and the finished product manufacturer is advisable. A close exchange with the respective supplier to determine acceptable levels depending on the identified risks is essential for this purpose. The inclusion of nitrite as a specification parameter with a general limit in pharmacopeial monographs of certain excipients might be an option. However, since there is no general risk from nitrites in excipients, but rather it depends on the individual drug formulation (presence of a vulnerable amine, pH, manufacturing process), the inclusion of nitrite in the specification as an additional in-house test parameter seems more reasonable. However, the EDQM and USP could

establish sensitive analytical methods to reliably determine very low levels of nitrite in order to support manufacturers to mitigate NDSRI formation in their high-risk drug products.

5.4.2 Reformulation and manufacturing changes

Based on a study by Nanda et al. [118], the FDA recommends the addition of antioxidants (ascorbic acid or alpha-tocopherol) as they could significantly reduce the formation of NDSRIs [21]. This approach is supported by a recent publication by Homšak et al. [119] who identified further compounds besides the known antioxidants with high nitrite scavenging activity. However, they also concluded that the chemical compatibility of the scavenger with the API and excipients, the pharmaceutical form and the route of administration should be considered when selecting the nitrite scavenger. This means that the selection has to be product-specific and there can be no general recommendation at present for all products at NDSRI risk. The manufacturer has to carry out more or less extensive testing to find the suitable nitrite scavenger in a proper concentration. In addition, auxiliary substances such as sodium carbonate to change or stabilize the pH value could be included in the formulation to prevent an acid environment favoring nitrosamine formation [21].

The introduction of nitrite scavengers as well as pH stabilizers require reformulation of the drug product. Similarly, changing a filler or disintegrant in a tablet to reduce the nitrite content would require reformulation. Heat and moisture during production can be avoided by changing the manufacturing process, e.g. by switching from wet granulation to direct tableting. However, these are generally major changes requiring thorough assessments by manufacturers and authorities. According to the EMA *Guideline on the investigation of bioequivalence* [120], generally in vivo bioequivalence (BE) studies are required to demonstrate comparable bioavailability for reformulations or manufacturing changes that may impact bioavailability. In vivo BE studies are clinical trials that involve costs and time. This is particularly challenging for generic companies, especially if many of their products are affected by NDSRIs.

The addition of antioxidants, for instance, may affect intestinal transport molecules important for drug absorption and thus change the bioavailability of a drug. To

investigate the effects of antioxidants in drug products, a collaborative project between the FDA, the CDER and Centers of Excellence in Regulatory Science and Innovation (CERSI) has been ongoing since May 2022 where 31 antioxidants are screened for their potential to inhibit three important intestinal drug transporters [121]. The results will help to decide on the need for in vivo BE studies for drug products that are reformulated to reduce nitrosamine formation. Further research, such as that presented by Fang Wu [122], on the use of pharmacokinetic/pharmacodynamic (PKPD) absorption modelling to justify biowaivers for regulatory submissions, including Biopharmaceutical Classification System (BCS) class 3 generic drug applications with different quantitative and qualitative excipient composition compared to the reference product, could also be supportive in reformulating drug products contaminated with NDSRIs or other nitrosamine impurities. As long CAPA implementation could jeopardize drug supply due to lengthy development of mitigation strategies and regulatory procedures, alternative approaches to in vivo BE studies to predict bioequivalence could be an important step forward in securing drug supply in the medium term.

In any case, due to the narrow time frame for implementation combined with existing uncertainties with regard to the BE demonstration, an early exchange with the authorities on the development of a new formulation and/or modification of the manufacturing process to reduce NDSRIs in approved drug products, e.g. in the form of scientific advice, is reasonable. This is also explicitly recommended by the FDA [21].

5.4.3 Mitigation strategies for API-impurity-related nitrosamines

If the amine precursor is not the API itself but an API impurity, lowering the specification limit for the relevant amin-containing impurity in the API could reduce the risk of relevant nitrosamine levels. If a mitigation strategy is necessary, the API manufacturer could be consulted in order to assess whether it is possible to reduce the impurity levels, for example by changing the API manufacturing process. If cases of NDSRIs resulting from related substances of the API are confirmed, a change in the corresponding pharmacopeial monographs might be appropriate. However, Moser et al. [123] observed that trace impurity levels specified at Q3A/B limits pose a signficantly lower risk compared to amin API precursors which are present typically at mg concentrations. Even though significant levels of NDSRI could theoretically form from ppm levels of an amine

impurity and nitrite, Moser et al. determined that the reactants are not fully available for reaction in solid dosage forms [123]. The same considerations apply equally to API impurities/degradants with amine-containing functional groups which could further react to actual amine precursors. As nitrosamine formation needs to be preceded by another necessary reaction step in these cases, the formation of relevant levels of NDSRIs is even less likely.

More research is needed in this area to understand the mechanism of nitrosamine formation in drug products and thus to define more precisely what high risk APIs mean in terms of selecting a suitable control and mitigation strategy.

5.4.4 Overall discussion on mitigation strategies for NDSRIs

Changes in the manufacturing technique for tablets and reformulations for solid dosage forms usually have a major impact on the drug product dossier, up to and including the results of clinical studies to prove BE, while the change of an excipient supplier represents generally a minor change from a regulatory perspective. The effort to reduce impurity levels in the API in case they are amine-precursors depends on the API manufacturers available, their ability to change the manufacturing process or introduce further purification steps, and their willingness to do so. For high-risk drug products, several measures may be necessary to lower the NDSRI levels to the long-term AI.

The implementation of the above measures may pose several challenges. The development of the manufacturing process and consequently the final drug formulation usually takes place in early clinical development. The choice of an optimal qualitative and quantitative combination of APIs and excipients is complex and requires thorough testing, the results of which have to be presented in the drug product dossier and approved by the CAs [124]. These aspects support a new, defined deadline for Step 3 of the call for review for NDSRI-affected medicinal products, as already published by the FDA, providing clarity and planning security for MAHs.

Further research on the effect and interaction potential of nitrite scavengers and on in vitro BE detection as well as the development of suitable analytical methods for the detection of trace nitrite levels is necessary to support pharmaceutical companies in a rapid CAPA implementation, but also in the development of new drug products.

6 ICH quality guideline for nitrosamine impurities in medicinal products

The control of nitrosamine impurities is currently based on the ICH M7 guideline, according to which nitrosamines are to be treated not only as mutagenic but also as CoC compounds. The non-reflection of the different carcinogenic potency of N-nitroso-compounds in ICH M7 has led to partly different interpretations of ICH M7 requirements to control nitrosamine impurities. Even though many recommendations of regional guidelines have already been harmonized among the CAs as presented in this thesis, there are still important differences in the regulatory treatment of medicines affected by nitrosamine impurities. These differences lead to uncertainties for regulators and industry and thus may prolong regulatory procedures, which, in the worst case, could endanger drug availability.

Therefore, the establishment of an internationally harmonized guideline by the ICH to control nitrosamine impurities in drug products is proposed following the series of the impurity guidelines ICH Q3A-E. This section presents considerations for uniform recommendations based on the previous results and discussion of the analysis of the EMA, HC and the FDA guidance carried out in this master thesis. The proposals are oriented towards the structure and content of the existing ICH guidelines Q3C [125] and Q3D [126].

6.1 Scope

The guideline should be applied to new finished drug products as well as to new drug products containing already known APIs. A uniform scope of the guideline should be defined, specifying exactly which drug products require an RA for nitrosamine impurities and which do not. For a possible differentiation of drug products, e.g. different biologics, the results of the three-step mitigation process gained and to be gained in future could be helpful. Based on the scope of the three-step mitigation process for approved drug products, respective drug properties and the currently known risk factors and root causes for nitrosamine presence, all chemically defined, biological and radiopharmaceutical drug products should be included in the scope as discussed in section 5.1.1.

6.2 Risk assessment

The importance of a comprehensive and robust RA for sustainable safe and available drug products was elaborated in this master thesis. In order to ensure an objective RA, the ICH should therefore lay down precise recommendations for the performance of the RA for nitrosamine impurities. This should include detailed recommendations on RA by the provision of the requirements for qualified personnel, the proposal for a third-party approach as well as the detailed requirements for the summary and discussion of the RA to be included in the drug product dossier enabling the CAs to routinely evaluate the validity of the RA. The risk factors and root causes that are known for nitrosamine presence in drug products should be listed in the guideline and/or reference should be made to scientific publications to be consulted to ensure that all the available evidence is taken into account in the RA. In addition, specially developed and validated RA tools for determining nitrosamine risk could be recommended in the guideline. For APIs including a vulnerable amine structure or amine precursor structure, the performance of a standardized NAP test [87] could be recommended to confirm or exclude the risk for the formation of NDSRIs. Special aspects to be considered for biological and radiopharmaceutical drug products regarding their nitrosamine risk could be included in addition to those already established for chemical drugs.

The guideline should point out that the risk of nitrosamine impurities should already be taken into account during the early development of a drug product following quality by design (QbD) concepts leading to robust formulations and manufacturing processes which consistently deliver the desired product quality [127]. In this way, this recommendation could also encourage a general forced implementation of QbD concepts in product development leading to a quality improvement which goes beyond the control of nitrosamine impurities and could ultimately contribute to the reduction of drug recalls due to quality deficiencies.

6.3 Acceptable intakes

Harmonized AIs form the basis for a harmonized control strategy for drug products affected by nitrosamine impurities. They should reflect the carcinogenic potency to the best possible extent following a weight-of-evidence approach. Therefore, harmonized AIs should be defined by ICH for each known nitrosamine impurity based on carcinogenicity

and/or mutagenicity data, if available, as well as on SAR considerations. Additionally, the respective approach applied to determining the AI should be made transparent.

The guideline should further specify which methods are applicable to derive AIs for new nitrosamine findings in order to create certainty for MAHs and regulators and thus accelerate the mitigation process for the respective nitrosamine impurity if necessary. With regard to the CPCA or any other method, it should be clearly defined to which type of nitrosamine impurities it can be applied. Defining sufficiently robust carcinogenicity data beyond the requirements outlined in ICH M7 would be important in order to standardize the selection of TD_{50} values to derive compound-specific AIs and improve the derivation and acceptability of SAR/read-across based AIs.

The establishment of internationally agreed suitable mutagenicity tests and their harmonized evaluation would be crucial to exclude nitrosamines from the CoC or to prove non-mutagenicity. Increasing results of enhanced genotoxicity tests for nitrosamines will contribute to the refinement of SAR tools, which in the future will hopefully be able to predict the mutagenicity and carcinogenicity of a nitrosamine on the basis of its chemical structure alone, making biological test systems dispensable [95–108]. In the meanwhile, ICH should recommend internationally accepted in vivo or in vitro follow-up tests to avoid or reduce in vivo testing whenever possible in accordance with Art. 4 of Directive 2010/63/EU to protect animals used for scientific purposes [128].

Risk-benefit consideration regarding the target group to be treated (children versus older patients) and duration of use (temporary versus long-term) should be taken into account in the AI determination for NDSRIs as NDSRIs are unique to the respective drug product. For example, a conservative approach should be chosen for drug products used for children, at least if the available evidence for carcinogenicity prediction for the respective NDSRI is ambiguous. On the other hand, LTL adjustments in line with ICH M7 could be used to determine AIs if the affected medicine is one, that is only used for a short time, such as an anti-infective.

6.4 Control options

Clear guidance on the application of control options for nitrosamine impurities should be developed by ICH for API and finished product specifications. Thereby a differentiation

between process-related nitrosamine impurities and NDSRIs should be established, reflecting the different risk for nitrosamine presence. In the case of NDSRI control in the drug product, test omission below a defined threshold as well as periodic testing could be tied to the application of QbD principles that would ensure a thorough RA combined with a sufficient statistical control of NDSRI levels. Since the risk of NDSRI formation differs depending on the amine precursor structure (API, API impurity, API degradant), a further differentiation between NDSRIs according to the corresponding amine source may be useful from a regulatory perspective. Improvements in RAs for NDSRIs as well as comprehensive CT results based on suitable validated analytical methods will certainly contribute to the establishment of reasonable control strategies for NDSRIs in the future.

Control options for multiple nitrosamine impurities, taking into account the potentially widely varying carcinogenicity potencies of nitrosamine impurities in a single drug product, should be defined in the guideline. The flexible control option 2, as currently applicable according to the EMA Q&A document, should be critically considered as control strategy for new drug products, as it contradicts consistent product quality by accepting batch-to-batch variability. The application of the fixed control option 2, on the other hand, meets the standard of high product and process understanding that ensures the supply of medicines in the long term while taking differing carcinogenic potencies of nitrosamines into account.

6.5 Lifecycle Management

In the event of post-authorization changes that may lead to altered nitrosamine levels in the drug product, the RA should be re-evaluated and control strategies adapted if necessary. For this purpose, typical quality changes with possible impact on nitrosamine levels should be listed as examples in the proposed guidance to make clear that an RA is necessary in the case of a large number of changes and therefore represents the rule rather than the exception in lifecyle management of drug products. The importance of intensive cooperation between MAHs and suppliers and manufacturers in the supply chain during lifecycle management should be emphasized as well as the overall responsibility of the MAH for the RA.

Furthermore, in terms of simplified life cycle management and quality improvement, the ICH should encourage MAHs to apply QbD concepts, such as the establishment of a design space ensuring the quality of the drug product via a predefined and tested variation of critical quality attributes or process parameters (e.g. nitrite content in excipients, temperature and moisture in the manufacturing process) and within which post-approval changes can be made without regulatory submissions [129]. This would also obviate the need to reassess the risk of nitrosamines for relevant quality changes as it could be considered equal within the approved design space. However, a continuous monitoring programme should ensure that the quality attributes of the medicinal product are as predicted by the design space [129].

7 Conclusion and outlook

While the first nitrosamine findings in valsartan and other medicines led to immediate and multiple drug recalls, the response to the increasing NDSRI findings was different than expected. It was shown that the numerous NDSRI findings from 2022 onwards did not lead to an increase in drug recalls. Instead, they were averted by the application of temporary higher AIs and an exchange between MAHs and regulatory authorities regarding market actions. The importance of avoiding drug recalls to ensure drug supply, taking into account patient's view and drug therapy safety, has been highlighted in this thesis. Thus, short-term drug supply for approved drug products could be ensured with the help of the present guidance despite the numerous medicinal products affected by NDSRIs.

In this master thesis, the challenges for MAHs regarding the implementation of the three-step mitigation process for nitrosamine impurities in medicinal products were elaborated. It was found that the timelines initially published by the authorities were too tight to fulfil the tasks for drug products affected by NDSRIs satisfactorily as it was not until mid-2023, with the publication of long-term AIs and the introduction of the CPCA, that the foundation was laid for an effective mitigation process for NDSRIs. New timelines are expected globally in order to ensure thorough assessment of approved medicinal products regarding NDSRIs and implementation of mitigation strategies to reduce NDSRI levels if necessary.

Finally, with the introduction of the CPCA and the exclusion of NDSRIs from the CoC by means of negative enhanced Ames testing, it can be predicted that major regulatory challenges regarding NDSRIs could be solved. The AI determination is now no longer dependent on read-across from a more or less suitable surrogate compound, but can be done simply and reproducibly on the basis of established SAR concepts reflecting the current scientific knowledge on nitrosamine impurities and NDSRIs as well as their carcinogenic potency. Approximately half of the NDSRIs found or hypothesized so far can thus be controlled according to the TTC of 1.5 µg/day for mutagenic impurities, which simplifies mitigation strategies or probably even makes them entirely dispensable. This may be a key factor in ensuring supply of many NDSRI-contaminated medically important drug products currently on the market.

However, as numerous NDSRIs included in CPCA category 1 and 2 are still assigned a high carcinogenic potency comparable with NDEA or NDMA, the regulatory challenges for medicinal products affected by those NDSRIs remain. The exclusion of NDSRIs from the CoC by a negative EAT, as recommended by the EMA and HC, is likely to de-risk further NDSRIs, but the testing results need to be awaited first. In this light, it is still necessary to support MAHs in the CAPA implementation for their affected products. Ongoing close cooperation between industry, regulators, institutions and research will be crucial to promote successful mitigation strategies and their regulatory approvals and thus ensure mid-term drug supply of the drug products concerned. The provision of suitable analytical methods for the detection of nitrite facilitating supplier qualification, as well as acceptable in vitro test systems that offer the possibility of waiving BE studies for reformulations and manufacturing changes, could support pharmaceutical companies in implementing CAPAs for NDSRIs more quickly. Scientific advice for and accelerated assessment of respective changes to the marketing authorization by the CAs for the implementation of CAPAs can also contribute to assurance that these medicines will be available in future without supply disruptions.

On the one hand, recent updates of the guidelines have achieved harmonization of AIs among regulatory authorities for various nitrosamines, but on the other hand, there remain relevant differences in the published AIs and applied methods which lead to the conclusion that it still remains a challenge to determine AIs for nitrosamine impurities. This could be attributed to the fact that all methods to determine AIs for nitrosamine impurities without carcinogenicity data are not yet fully developed and are thus based on more or less solid evidence. With the CPCA, at least one standardized method is now available which would allow a harmonized AI determination. However, this approach does not seem to reflect the carcinogenic potency for all NDSRIs with sufficient accuracy either, especially of those that have to be assigned to CPCA categories 1 and 2. Many more developments are to be expected in the coming years, in which SAR concepts will improve steadily and test results from in vivo and in vitro investigations will contribute to increasing evidence on the mutagenicity and carcinogenicity of nitrosamine impurities, including NDSRIs. This will be crucial to establish internationally harmonized AIs and methods for AI determination for all nitrosamine impurities.

In addition to the partly not aligned AIs, the detailed analysis and comparison of the guidelines further revealed some divergent or inconclusive requirements regarding RAs and control strategies for nitrosamine impurities. In particular, the differences between NDSRIs and simple dialkyl-nitrosamines regarding their risk for presence and formation are not equally taken into account. Even though the FDA has published its own guidance on NDSRIs, it does not contain recommendations on quality aspects.

As drug products are affected by nitrosamine impurities all over the world, they should be limited or avoided equally. Deviating or unclear regulatory provisions lead to uncertainties for MAHs and regulators, which may prolong drug development and regulatory procedures. Although the total number of medicines affected by nitrosamine impurities is currently undisclosed due to the decrease in drug recalls and regulatory discretion about nitrosamine findings, a huge extent can be concluded from the recent updated guidelines by the EMA, HC and the FDA. Moreover, due to their intrinsic nature and risk factors known so far, NDSRI formation will not be preventable in many cases but can only be reduced to an acceptable level. This means that their presence in drug products has to be permanently managed. Nitrosamine impurities in medicines can therefore no longer be considered only a temporary crisis that will be overcome after completion of the three-step mitigation process, but should be regulated as an additional known impurity class that has to be taken into account in product development and continuously in the life cycle of medicinal products.

Based on the above considerations, the current regulatory provisions for the control of nitrosamine impurities in medicinal products are considered not sufficient to ensure the purity of medicinal products and thus drug product supply in the long term. Consequently, the development of harmonized regulatory requirements by the ICH for new MAAs to control nitrosamine impurities in drug products seems justified.

The considerations for a harmonized control of nitrosamine impurities in medicinal products as presented in this master thesis are based on current scientific insights and the recommendations published to date by the EMA, the FDA and HC. However, there are still many challenges to solve regarding nitrosamine impurities which make final recommendations difficult at the present time. Further national guidelines from other

authorities have not been included, as this would have exceeded the scope of this master thesis. However, their review could possibly provide additional useful inputs.

Overall, the discovery of NDSRIs in medicines can be seen as an opportunity to avoid similar crises in the future. Avoiding impurity issues and other quality defects in drugs represents the most effective way to continuously provide safe drug products to patients. Thus, the focus of pharmaceutical companies should be on the prevention of the reasons for drug recalls. Drug recalls should not be considered as a standard regulatory tool to protect public health. Instead, they should only be used in a few cases in close consultation with the CAs. Of course, improving quality systems cannot be implemented in the short term and drug recalls will remain unavoidable in some cases. However, preventing regulatory challenges of similar magnitude as those posed by nitrosamine impurities should trigger a fundamental rethink of quality management in the pharmaceutical industry.

8 Summary

Approximately two years after the discovery of the probable carcinogens N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) in Valsartan, which led to global recalls of affected medicinal products, manufacturers and marketing authorization holders (MAHs) were requested to undertake a three-step mitigation process in which they were expected to review their products for the presence of nitrosamine impurities and, if necessary, initiate measures to remove or reduce them.

In addition to the simple dialkyl-nitrosamines like NDMA and NDEA, which were detected in various active pharmaceuticals ingredients (APIs) and drug products between 2018 and 2020, others, and predominantly nitrosamine drug substance-related impurities (NDSRIs), were subsequently found which typically differ significantly from the simple nitrosamines in their overall chemical structure and thus in their carcinogenic potency. As NDSRIs are related to the API structure, the risk of their presence and formation in drug products is high and the reduction of NDSRI levels is usually difficult to achieve. These peculiarities lead to various regulatory challenges, first and foremost the determination of acceptable intakes (AIs) which form the basis for the control strategy and the necessity of corrective and preventive actions. Lastly, the high number of drug products or even whole therapeutical drug classes actually or potentially affected by NDSRIs poses a threat to the drug supply.

To support MAHs in implementing the three-step mitigation process, guidelines for the control of nitrosamine impurities have been published by various competent authorities and were revised more or less frequently over the past five years. This master thesis deals with the question of whether the currently existing regulatory framework can be considered sufficient to provide safe and continuously available drug products to patients. A thorough assessment and comparison of the guidelines provided by the European Medicines Agency (EMA), Health Canada (HC) and the United States (US) Food and Drug Administration (FDA), as well as an analysis of historical and recent regulatory events regarding nitrosamine impurities, show that the short-term supply of drug products affected by nitrosamine impurities could be ensured with the help of adjusted regulatory recommendations, whereby the application of temporary higher AIs based on less-than-lifetime adjustments is decisive. Assurance of long-term supply is now also likely

for many approved drug products affected by NDSRIs with the recently introduced Carcinogenic Potency Categorization Approach (CPCA), which assigns significantly higher lifetime AIs up to the threshold of toxicological concern of 1.5 µg/day for mutagenic impurities to a large number of NDSRIs. However, a considerable proportion of NDSRIs will still need to be controlled in the medium-term with an AI of 18 ng/day or 100 ng/day and thus are assigned a carcinogenic potency about as high as NDEA and NDMA. Therefore, ongoing intensive cooperation between industry, authorities, research and other institutions to support MAHs with suitable analytical methods, innovative in vitro testing systems and regulatory advice and flexibility appears to be crucial to avoid drug recalls of approved drug products in the medium-term, given the challenges associated with the implementation of mitigation strategies for NDSRIs.

Despite many consistent recommendations, the detailed comparison of the EMA, FDA and HC guidelines also reveals some differences, discussed and evaluated in this master thesis in the light of ensuring long-term drug supply. Examples of the effects of drug recalls are used to illustrate that an ensured drug supply in the interest of public health is linked to an uninterrupted availability of drug products.

Deviations in the observed guidelines are found in the recommended lifetime AIs, control options and provisions for risk assessments which may lead to uncertainties for industry and regulators potentially resulting in prolonged pharmaceutical development and regulatory procedures. The focus in the existing guidelines lies on the mitigation of nitrosamine impurities in approved drug products, but given the various risk factors and root causes for the presence of nitrosamine impurities and their widespread occurrence in medicinal products, they need to be continuously considered and prevented during product development and also throughout the life cycle of medicines to ensure drug supply in the long-term. While the differing carcinogenic potency of NDSRIs and simple dialkyl-nitrosamines is now better reflected in the guidelines by the application of the CPCA, there are confounding regional differences in the application of AI derivation methods for NDSRIs and other nitrosamines, highlighting the challenge of adequately quantifying carcinogenic risk of nitrosamine impurities and the need for a harmonized approach to determine AIs.

Taking into account the continuously evolving science regarding nitrosamines, an international guideline should be envisaged with harmonized recommendations for the control of nitrosamine impurities in drug products to equally ensure a sustainable supply of safe medicines worldwide.

9 References

1. European Medicines Agency, *Assessment report: Procedure under Article 5(3) of Regulation EC (No) 726/2004: Nitrosamine impurities in human medicinal products. Procedure number: EMEA/H/A-5(3)/1490.* (EMA/369136/2020 Committee for Medicinal Products for Human Use (CHMP), 2020).

2. Heads of Medicines Agency & European Medicines Agency, *Lessons learnt from presence of N-nitrosamine impurities in sartan medicines. Overview and recommendations.* (EMA/526934/2019, 2020).

3. European Medicines Agency, Heads of Medicines Agency, & Co-ordination Group for Mutual Recognition and Decentralised Procedures-human, *European Medicines Regulatory Network approach for the implementation of the CHMP Opinion pursuant to Article 5(3) of Regulation (EC) No 726/2004 for nitrosamine impurities in human medicines* (EMA/425645/2020, 2021).

4. European Medicines Agency, *ICH guideline M7(R2) on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk.* (EMA/CHMP/ICH/83812/2013 Committee for Human Medicinal Products, 2023).

5. R. Nudelman & A. Czich, Nitroso-Derivates of ACE Inhibitors. (2023). https://www.efpia.eu/media/677168/position-paper-for-nitroso-ace-inhibitors-20feb2023.pdf.

6. J. Schlingemann, M. J. Burns, D. J. Ponting, C. Martins Avila, N. E. Romero, M. A. Jaywant, G. F. Smith, I. W. Ashworth, S. Simon, C. Saal, & A. Wilk, The Landscape of Potential Small and Drug Substance Related Nitrosamines in Pharmaceuticals. *Journal of Pharmaceutical Sciences*, (2022). https://doi.org/10.1016/j.xphs.2022.11.013.

7. European Medicines Agency, Appendix 1: Acceptable intakes established for N-nitrosamines. (2023). https://www.ema.europa.eu/en/documents/other/appendix-1-acceptable-intakes-established-n-nitrosamines_en.pdf (accessed August 27, 2023).

8. Health Canada, *Guidance on nitrosamine impurities in medications. Effective Date: July 24, 2023* (2023).

9. U.S. Food and Drug Administration, Updated Information | Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs). (2023). https://www.fda.gov/regulatory-information/search-fda-guidance-documents/updated-information-recommended-acceptable-intake-limits-nitrosamine-drug-substance-related (accessed August 5, 2023).

10. U.S. Food and Drug Administration, *Recommended Acceptable Intake Limits for Nitrosamine Drug Substance- Related Impurities (NDSRIs). Guidance for Industry* (2023).

11. S. Schmidtsdorff, J. Neumann, A. H. Schmidt, & M. K. Parr, Prevalence of

nitrosamine contaminants in drug samples: Has the crisis been overcome? *Archiv der Pharmazie*, **356** (n.d.) e2200484. https://doi.org/10.1002/ardp.202200484.

12. U.S. Food and Drug Administration, Drug Recalls. (2023). https://www.fda.gov/drugs/drug-safety-and-availability/drug-recalls (accessed April 14, 2023).

13. European Medicines Agency, *Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products.* (EMA/409815/2020 Rev. 17, 2023).

14. U.S. Food and Drug Administration, *Control of Nitrosamine Impurities in Human Drugs - Guidance for Industry* (2021).

15. E. De Weerdt, S. Simoens, M. Casteels, & I. Huys, Toward a European definition for a drug shortage: a qualitative study. *Frontiers in Pharmacology*, **6** (2015) 253. https://doi.org/10.3389/fphar.2015.00253.

16. World Health Organization, Update on nitrosamine Impurities. *Information Note Nitrosamine impurities*, (2019). https://www.who.int/news/item/20-11-2019-information-note-nitrosamine-impurities (accessed March 3, 2023).

17. J. C. Beard & T. M. Swager, An Organic Chemist's Guide to N-Nitrosamines: Their Structure, Reactivity, and Role as Contaminants. *The Journal of Organic Chemistry*, **86** (2021) 2037–2057. https://doi.org/10.1021/acs.joc.0c02774.

18. R. López-Rodríguez, J. A. McManus, N. S. Murphy, M. A. Ott, & M. J. Burns, Pathways for N-Nitroso Compound Formation: Secondary Amines and Beyond. *Organic Process Research & Development*, **24** (2020) 1558–1585. https://doi.org/10.1021/acs.oprd.0c00323.

19. I. W. Ashworth, O. Dirat, A. Teasdale, & M. Whiting, Potential for the Formation of N-Nitrosamines during the Manufacture of Active Pharmaceutical Ingredients: An Assessment of the Risk Posed by Trace Nitrite in Water. *Organic Process Research & Development*, **24** (2020) 1629–1646. https://doi.org/10.1021/acs.oprd.0c00224.

20. S. Horne, M. D. Vera, L. R. Nagavelli, V. A. Sayeed, L. Heckman, D. Johnson, D. Berger, Y. Yean Yip, C. Lopes Krahn, L. Oyamada Sizukusa, N. Flavio Moura Rocha, R. N. Bream, J. Ludwig, D. A. Keire, & G. Condran, Regulatory Experiences with Root Causes and Risk Factors for Nitrosamine Impurities in Pharmaceuticals. *Journal of Pharmaceutical Sciences*, (2023) S0022354922006141. https://doi.org/10.1016/j.xphs.2022.12.022.

21. U.S. Food and Drug Administration, Updates on possible mitigation strategies to reduce the risk of nitrosamine drug substance-related impurities in drug products. (2021). https://www.fda.gov/drugs/drug-safety-and-availability/updates-possible-mitigation-strategies-reduce-risk-nitrosamine-drug-substance-related-impurities (accessed June 4, 2023).

22. Y. Wu et al., Reactive Impurities in Excipients: Profiling, Identification and

Mitigation of Drug–Excipient Incompatibility. *AAPS PharmSciTech*, **12** (2011) 1248–1263. https://doi.org/10.1208/s12249-011-9677-z.

23. R. Boetzel, J. Schlingemann, S. Hickert, C. Korn, G. Kocks, B. Luck, G. Blom, M. Harrison, M. Francois, A. Leonardo, Y. Wu, & Y. Bousraf, A Nitrite Excipient Database: A useful Tool to Support N-Nitrosamine Risk Assessments for Drug Products. *Journal of Pharmaceutical Sciences*, (2022) S0022-3549(22)00168-X. https://doi.org/10.1016/j.xphs.2022.04.016.

24. W. Gomm, C. Röthlein, K. Schüssel, G. Brückner, H. Schröder, S. Heß, R. Fröschtl, K. Broich, & B. Haenisch, N-Nitrosodimethylamin-kontaminiertes Valsartan und Krebsrisiko. Eine longitudinale Kohortenstudie mit deutschen Krankenkassendaten. *Deutsches Ärzteblatt*, (2021). https://www.aerzteblatt.de/archiv/219177/N-Nitrosodimethylamin-kontaminiertes-Valsartan-und-Krebsrisiko (accessed April 2, 2023).

25. I. Mansouri, B. Jeremie, L. Semenzato, N. Haddy, & M. Zureik, N-nitrosodimethylamine-Contaminated Valsartan and Risk of Cancer: A Nationwide Study of 1.4 Million Valsartan Users. *Journal of the American Heart Association*, **11** (2022) e8067. https://doi.org/10.1161/JAHA.122.026739.

26. A. Thresher, R. Foster, D. J. Ponting, S. A. Stahlford, R. E. Tennant, & R. Thomas, Are all nitrosamines concerning? A review of mutagenicity and carcinogenicity data. *Regulatory Toxicology and Pharmacology*, **116** (2020) 104749. https://doi.org/10.1016/j.yrtph.2020.104749.

27. K. P. Cross & D. J. Ponting, Developing structure-activity relationships for N-nitrosamine activity. *Computational Toxicology*, **20** (2021) 100186. https://doi.org/10.1016/j.comtox.2021.100186.

28. European Medicines Agency, ICH: quality. *Human Regulatory. Scientific Guidelines*, (n.d.). https://www.ema.europa.eu/en/human-regulatory/research-development/scientific-guidelines/ich/ich-quality (accessed April 4, 2023).

29. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, Final Concept Paper. ICH Q3E: Guideline for Extractables and Leachables (E&L). Endorsed by the Management Committee on 10 July 2020. (2020). https://database.ich.org/sites/default/files/ICH_Q3E_ConceptPaper_2020_0710.pdf (accessed April 4, 2023).

30. R. Kroes, J. Kleiner, & A. Renwick, The Threshold of Toxicological Concern Concept in Risk Assessment. *Toxicological Sciences*, **86** (2005) 226–230. https://doi.org/10.1093/toxsci/kfi169.

31. M. J. Farrukh et al., Valsartan recall: global regulatory overview and future challenges. *Therapeutic Advances in Drug Safety*, **10** (2019) 2042098618823458. https://doi.org/10.1177/2042098618823458.

32. European Medicines Agency, Suspension of ranitidine medicines in the EU. (2020). https://www.ema.europa.eu/en/news/suspension-ranitidine-medicines-eu

(accessed March 11, 2023).

33. Government of Canada, Nitrosamine impurities in medications: recalls. (2021). https://www.canada.ca/en/health-canada/services/drugs-health-products/compliance-enforcement/information-health-product/drugs/nitrosamine-impurities/recalls.html (accessed March 12, 2023).

34. U.S. Food and Drug Administration, FDA Requests Removal of All Ranitidine Products (Zantac) from the Market. (2020). https://www.fda.gov/news-events/press-announcements/fda-requests-removal-all-ranitidine-products-zantac-market (accessed March 10, 2023).

35. European Medicines Agency, *Assessment report. Referral under Article 31 of Directive 2001/83/EC. INN: ranitidine.* (EMA/599846/2020 Corr. 1 Committee for Medicinal Products for Human Use (CHMP), 2020).

36. European Medicines Agency, Nitrosamine impurities. *Human Regulatory*, (2019). https://www.ema.europa.eu/en/human-regulatory/post-authorisation/referral-procedures/nitrosamine-impurities (accessed March 10, 2023).

37. Health Science Authority, HSA Recalls Three out of 46 Metformin Medicines. (2019). https://www.hsa.gov.sg/announcements/news/hsa-recalls-three-out-of-46-metformin-medicines (accessed March 11, 2023).

38. U.S. Food and Drug Administration, FDA Alerts Patients and Health Care Professionals to Nitrosamine Impurity Findings in Certain Metformin Extended-Release Products. (2020). https://www.fda.gov/news-events/press-announcements/fda-alerts-patients-and-health-care-professionals-nitrosamine-impurity-findings-certain-metformin (accessed March 10, 2023).

39. Government of Canada, Certain Metformin diabetes drugs recalled due to the presence or possible presence of NDMA - Recalls, advisories and safety alerts – Canada.ca. *Recalls and safety alerts*, (2021). https://recalls-rappels.canada.ca/en/alert-recall/certain-metformin-diabetes-drugs-recalled-due-presence-or-possible-presence-ndma (accessed March 11, 2023).

40. U.S. Food and Drug Administration, FDA Updates and Press Announcements on Nitrosamines in Rifampin and Rifapentine. *FDA*, (2021). https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-nitrosamines-rifampin-and-rifapentine (accessed March 10, 2023).

41. U.S. Food and Drug Administration, Laboratory analysis of rifampin/rifapentine products. (2021). https://www.fda.gov/drugs/drug-safety-and-availability/laboratory-analysis-rifampinrifapentine-products (accessed March 11, 2023).

42. U.S. Food and Drug Administration, FDA Updates and Press Announcements on Nitrosamine in Varenicline (Chantix). (2022). https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-nitrosamine-varenicline-chantix (accessed February 11, 2023).

43. U.S. Food and Drug Administration, Lupin Pharmaceuticals, Inc. Issues Voluntarily Nationwide Recall of All Irbesartan Tablets and Irbesartan and Hydrochlorothiazide Tablets Due to Potential Presence of N-nitrosoirbesartan Impurity. (2023). https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/lupin-pharmaceuticals-inc-issues-voluntarily-nationwide-recall-all-irbesartan-tablets-and-irbesartan (accessed March 10, 2023).

44. U.S. Food and Drug Administration, Pfizer Voluntary Nationwide Recall of Lots of ACCUPRIL® (Quinapril HCl) Due to N-Nitroso-Quinapril Content. (2022). https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/pfizer-voluntary-nationwide-recall-lots-accuprilr-quinapril-hcl-due-n-nitroso-quinapril-content (accessed March 10, 2023).

45. D. Moll, Rückruf wegen Nitroso-Rasagilin. *DAZ.online*, (2022). https://www.deutsche-apotheker-zeitung.de/news/artikel/2022/05/16/rueckruf-wegen-nitroso-rasagilin (accessed March 10, 2023).

46. European Medicines Agency, *Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products.* (EMA/409815/2020 Rev. 15, 2023).

47. U.S. Food and Drug Administration, Company Announcement. Ascend Laboratories LLC. Issues Voluntary Nationwide Recall of Dabigatran Etexilate Capsules, USP 75 mg and 150 mg, Due to the Detection of N-Nitroso-dabigatran (NDAB) Impurity. (2023). https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/ascend-laboratories-llc-issues-voluntary-nationwide-recall-dabigatran-etexilate-capsules-usp-75-mg (accessed May 5, 2023).

48. Deutsche Apotheker Zeitung, Wegen Nitrosamin-Verunreinigung – Rückruf zweier Atomoxetin-Chargen. *DAZ.online*, (2023). https://www.deutsche-apotheker-zeitung.de/news/artikel/2023/05/30/wegen-nitrosamin-verunreinigung-rueckruf-zweier-atomoxetin-chargen (accessed September 7, 2023).

49. Co-ordination Group for Mutual Recognition and Decentralised Procedures-human, CMDh Agendas and Minutes. (n.d.). https://www.hma.eu/human-medicines/cmdh/agendas-and-minutes.html (accessed May 5, 2023).

50. European Medicines Agency, *Questions and answers on "Information on nitrosamines for marketing authorisation holders"* (EMA/CHMP/428592/2019 Rev. 3, 2020).

51. Government of Canada, New guidance for evaluating and managing the risks of N-nitrosamine impurities in products. (2023). https://www.canada.ca/en/health-canada/services/drugs-health-products/compliance-enforcement/establishment-licences/drug-establishment-licensing-bulletin/guidance-evaluating-managing-risks-nitrosamine-impurities.html (accessed March 17, 2023).

52. U.S. Food and Drug Administration, Information about Nitrosamine Impurities in Medications. (2021). https://www.fda.gov/drugs/drug-safety-and-availability/information-about-nitrosamine-impurities-medications (accessed

March 17, 2023).

53. U.S. Food and Drug Administration, AAM/CHPA/PhRMA Questions for May 4th FDA-Industry Meeting to Discuss Nitrosamines Impurities in Pharmaceuticals. (2021). https://www.fda.gov/media/150864/download (accessed March 19, 2023).

54. European Directorate for the Quality of Medicines & HealthCare, N-nitrosamine contamination in brief - European Directorate for the Quality of Medicines & HealthCare - EDQM. (n.d.). https://www.edqm.eu/en/n-nitrosamine-contamination-in-brief (accessed March 15, 2023).

55. European Directorate for the Quality of Medicines & HealthCare, N-nitrosamine impurities in Ph. Eur. monographs: update on approach - European Directorate for the Quality of Medicines & HealthCare - EDQM. *Newsroom*, (2023). https://www.edqm.eu/en/-/n-nitrosamine-impurities-in-ph.-eur.-monographs-update-on-approach (accessed August 6, 2023).

56. USP, Nitrosamine Impurities. (n.d.). https://www.usp.org/small-molecules/nitrosamine-impurities (accessed March 17, 2023).

57. USP, USP Reference Standards. USP catalog. (n.d.). https://www.usp.org/reference-standards (accessed July 31, 2023).

58. European Medicines Agency, *ICH Topic Q 6 A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances* (CPMP/ICH/367/96, 2000).

59. Federal Ministry of Justice, Medicinal Products Act in the version published on 12 December 2005 (Federal Law Gazette [BGBl.]) Part I p. 3394, last amended by Article 8c of the Act of 20 December 2022 (Federal Law Gazette I p. 2793). *Translation provided by the Language Service of the Federal Ministry of Health*, (n.d.). https://www.gesetze-im-internet.de/englisch_amg/englisch_amg.html#p0104 (accessed June 3, 2023).

60. U.S. Food and Drug Administration, Enforcement Report. U.S. Food and Drug Administration Recall Information Search. (n.d.). https://www.accessdata.fda.gov/scripts/ires/index.cfm#tabNav_advancedSearch (accessed April 17, 2023).

61. U.S. Food and Drug Administration, FDA's Role in Drug Recalls. (2018). https://www.fda.gov/drugs/drug-recalls/fdas-role-drug-recalls (accessed April 14, 2023).

62. Kooperation im Geschäftsbereich des Bundesministerium für Gesundheit, Veröffentlichte Lieferengpassmeldungen. *PharmNet.Bund. Arzneimittleinformation für alle*, (n.d.). https://anwendungen.pharmnet-bund.de/lieferengpassmeldungen/faces/public/meldungen.xhtml (accessed April 12, 2023).

63. U.S. Food and Drug Administration, Frequently Asked Questions about Drug

Shortages. General Questions. Q2. What is the major reason for drug shortages? *FDA*, (2023). https://www.fda.gov/drugs/drug-shortages/frequently-asked-questions-about-drug-shortages (accessed April 12, 2023).

64. The Multi-Stakeholder Steering Committee on drug shortages in Canada, Multi-Stakeholder Toolkit. A Toolkit for Improved Understanding and Transparency of Drug Shortage Response in Canada. (2017). https://www.drugshortagescanada.ca/files/MSSC_Toolkit_2017.pdf.

65. U.S. Food and Drug Administration, Drug Shortages: Root Causes and Potential Solutions. A Report by the Drug Shortages Task Force. Updated on 02/12/2020. (2019). https://www.fda.gov/media/131130/download (accessed April 12, 2022).

66. European Medicines Agency & Heads of Medicines Agency, *Guidance on detection and notification of shortages of medicinal products for Marketing Authorisation Holders (MAHs) in the Union (EEA)* (EMA/674304/2018, 2019).

67. European Medicines Agency, Meeting highlights from the Committee for Medicinal Products Human Use (CHMP) 13-16 September 2021. (2021). https://www.ema.europa.eu/en/news/meeting-highlights-committee-medicinal-products-human-use-chmp-13-16-september-2021 (accessed May 5, 2023).

68. World Health Organization, Two new tobacco cessation medicines added to the WHO essential medicines list. (2023). https://www.who.int/news/item/05-11-2021-two-new-tobacco-cessation-medicines-added-to-the-who-essential-medicines-list (accessed May 5, 2023).

69. A. E. Lang, U. Patel, J. Fitzpatrick, T. Lee, M. McFarland, & C. B. Good, Association of the Chantix Recall With US Prescribing of Varenicline and Other Medications for Nicotine Dependence. *JAMA Network Open*, **6** (2023) e2254655. https://doi.org/10.1001/jamanetworkopen.2022.54655.

70. K. Hedenmalm, C. Quinten, X. Kurz, M. Bradley, H. Lee, & E. Eworuke, A collaborative study of the impact of N-nitrosamines presence and ARB recall on ARB utilization - results from IQVIA™ Disease Analyzer Germany. *European Journal of Clinical Pharmacology*, (2023). https://doi.org/10.1007/s00228-022-03439-3.

71. C. A. Jackevicius, H. M. Krumholz, A. Chong, M. Koh, A. F. Ozaki, P. C. Austin, J. A. Udell, & D. T. Ko, Population Impact of Generic Valsartan Recall. *Circulation*, **141** (2020) 411–413. https://doi.org/10.1161/CIRCULATIONAHA.119.044494.

72. F. A. McAlister & E. Youngson, Impact of the Generic Valsartan Recall in Alberta, Canada. *Journal of the American College of Cardiology*, **75** (2020) 1860–1862. https://doi.org/10.1016/j.jacc.2020.02.034.

73. T. Beck, U. Hüsgen, & J. Meuser, Schadensanalyse: Ein Jahr mit dem Valsartan-Störfall. *Pharmazeutische Zeitung online*, (2019). https://www.pharmazeutische-zeitung.de/ein-jahr-mit-dem-valsartan-stoerfall/seite/5/ (accessed April 12, 2023).

74. J. M. Phuong, J. Penm, B. Chaar, L. D. Oldfield, & R. Moles, The impacts of medication shortages on patient outcomes: A scoping review. *PLoS ONE*, **14** (2019)

e0215837. https://doi.org/10.1371/journal.pone.0215837.

75. A. M. Algabbani, W. A. Alkeridy, M. A. Alessa, & A. A. Alrwisan, The Inadvertent Consequences of Drug Recalls: A Case Study of a Recall of Pantoprazole Generics from the Markets. *Saudi Pharmaceutical Journal*, (2023). https://doi.org/10.1016/j.jsps.2023.04.011.

76. B. N. Reed, E. R. Fox, M. Konig, C. A. Jackevicius, F. A. Masoudi, A. A. Rabinstein, & R. L. Page, The impact of drug shortages on patients with cardiovascular disease: causes, consequences, and a call to action. *American Heart Journal*, **175** (2016) 130–141. https://doi.org/10.1016/j.ahj.2016.02.004.

77. Co-ordination Group for Mutual Recognition and Decentralised Procedures-human, *CMDh practical guidance for Marketing Authorisation Holders of nationally authorised products (incl. MRP/DCP) in relation to the Art. 5(3) Referral on Nitrosamines. Rev.20* (CMDh/412/2019, 2023).

78. EFPIA, N-nitrosamine Impurities in Biological Medicinal Products. (2020). https://www.efpia.eu/media/580595/n-nitrosamine-impurities-in-biological-medicinal-products.pdf (accessed August 20, 2023).

79. Biologic and Radiopharmaceutical Drugs Directorate, Information to Marketing Authorization Holders (MAHs) of Human Pharmaceutical Products Regarding Nitrosamine Impurities Request to evaluate the risk of the presence of nitrosamine impurities in biologics and radiopharmaceuticals. (2020). https://www.cosmeticsalliance.ca/wp-content/uploads/2020/12/MAH-Letter_ENGLISH-Final_BRDDsign.pdf (accessed May 15, 2023).

80. European Medicines Agency, *ICH guideline Q9 on quality risk management* (EMA/CHMP/ICH/24235/2006, 2015).

81. EFPIA, Workflows for Quality risk management of nitrosamine risks in medicines. Version 2.0. (2022). https://www.efpia.eu/media/676632/efpia-nitrosamines-quality-risk-management-workflows-sep-2022.pdf (accessed July 7, 2023).

82. Lhasa Limited, Lhasa Limited | Shared Knowledge, Shared Progress. (2023). https://www.lhasalimited.org/ (accessed July 7, 2023).

83. USP, Nitrosamines Exchange - A knowledge based community for all-things Nitrosamine. (n.d.). https://nitrosamines.usp.org/ (accessed July 7, 2023).

84. Center for research on complex generics, Mitigation Strategies for Nitrosamine Drug Substance Related Impurities: Quality and Bioequivalence Considerations for Generic Products. (2023). https://www.complexgenerics.org/NDSRIs_mitigation_strategies/ (accessed July 7, 2023).

85. J. Yang, Performance Characteristics of Mass Spectrometry- Based Methods for Quantitation of Nitrosamines: (2023). https://www.complexgenerics.org/media/SOP/complexgenerics/pdf/Conference-Slides/NDSRIsmitigationstrategies/1-3_Session1_JingyueYang.pdf (accessed July 3,

2023).

86. European Medicines Agency, *ICH Topic Q7 Good Manufacturing Practice for Active Pharmaceutical Ingredients* (CPMP/ICH/4106/00, 2006).

87. N. Sharma, R. Patel, T. Bothara, S. Jain, & R. P. Shah, Modified NAP Test: A Simple and Responsive Nitrosating Methodology for Risk Evaluation of NDSRIs. *Journal of Pharmaceutical Sciences*, (2023). https://doi.org/10.1016/j.xphs.2023.02.024.

88. Health Canada, *Guidance on nitrosamine impurities in medications. Effective Date: April 17, 2023* (2023).

89. U.S. Food and Drug Administration, *Guidance for Industry. Changes to an Approved NDA or ANDA. Revision 1* (Center for Drug Evaluation and Research (CDER), 2004).

90. European Commission, *Guidelines on the details of the various categories of variations, on the operation of the procedures laid down in Chapters II, IIa, III and IV of Commission Regulation (EC) No 1234/2008 of 24 November 2008 concerning the examination of variations to the terms of marketing authorisations for medicinal products for human use and veterinary medicinal products and on the documentation to be submitted pursuant to those procedures* (2013).

91. Health Canada, *Guidance Document Post-Drug Identification Number (DIN) Changes* (2009).

92. Health Canada, *Guidance Document: Post-Notice of Compliance (NOC) Changes: Quality Document* (2018).

93. Co-ordination Group for Mutual Recognition and Decentralised Procedures-human, Call for review for chemically synthesised and biological medicinal products. (n.d.). https://www.hma.eu/human-medicines/cmdh/nitrosamine-impurities.html (accessed May 22, 2023).

94. European Medicines Agency, *ICH M7(R2) Questions and Answers on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk.* (EMA/CHMP/ICH/321999/2020, 2023).

95. M. Honma, An assessment of mutagenicity of chemical substances by (quantitative) structure–activity relationship. *Genes and Environment*, **42** (2020) 23. https://doi.org/10.1186/s41021-020-00163-1.

96. L. S. Gold, The Carcinogenic Potency Database (CPDB). *The Carcinogenic Potency Project (CPDB)*, (2011). https://files.toxplanet.com/cpdb/index.html (accessed May 30, 2023).

97. Lhasa Limited, Lhasa Carcinogenicity Database. (2022). https://carcdb.lhasalimited.org/ (accessed June 3, 2023).

98. A. Thresher, J. P. Gosling, & R. Williams, Generation of TD50 values for carcinogenicity study data. *Toxicology Research*, **8** (2019) 696–703. https://doi.org/10.1039/c9tx00118b.

99. Lhasa Limited, Lhasa Carcinogenicity Database - Study Information. *N-Nitroso-N-methylaniline*, (2022). https://carcdb.lhasalimited.org/study-information/44605646 (accessed June 3, 2023).

100. Lhasa Limited, Lhasa Carcinogenicity Database - Study Information. *Nitrosodiphenylamine*, (2022). https://carcdb.lhasalimited.org/study-information/44696259 (accessed June 3, 2023).

101. R. Thomas, R. E. Tennant, A. A. F. Oliveira, & D. J. Ponting, What Makes a Potent Nitrosamine? Statistical Validation of Expert-Derived Structure–Activity Relationships. *Chemical Research in Toxicology*, **35** (2022) 1997–2013. https://doi.org/10.1021/acs.chemrestox.2c00199.

102. D. J. Ponting, K. L. Dobo, M. O. Kenyon, & A. S. Kalgutkar, Strategies for Assessing Acceptable Intakes for Novel N-Nitrosamines Derived from Active Pharmaceutical Ingredients. *Journal of Medicinal Chemistry*, **65** (2022) 15584–15607. https://doi.org/10.1021/acs.jmedchem.2c01498.

103. European Medicines Agency, *ICH M7(R2) Addendum on application of the principles of the ICH M7 guideline to calculation of compound-specific acceptable intakes* (EMA/CHMP/ICH/502766/2021, 2023).

104. D. J. Ponting & R. S. Foster, Drawing a Line: Where Might the Cohort of Concern End? *Organic Process Research & Development*, (2023). https://doi.org/10.1021/acs.oprd.3c00008.

105. R. Nudelman, K. L. Dobo, & M. O. Kenyon, Nitroso-Derivatives of β-blocker and β-agonists. (2022). https://www.efpia.eu/media/677182/position-paper-for-beta-blockers-and-beta-agonists-10jan2023.pdf.

106. X. Li, S. Chen, X. Guo, Q. Wu, J.-E. Seo, L. Guo, M. G. Manjanatha, T. Zhou, K. L. Witt, & N. Mei, Development and Application of TK6-derived Cells Expressing Human Cytochrome P450s for Genotoxicity Testing. *Toxicological Sciences*, **175** (2020) 251–265. https://doi.org/10.1093/toxsci/kfaa035.

107. X. Li, X. He, Y. Le, X. Guo, M. S. Bryant, A. H. Atrakchi, T. J. McGovern, K. L. Davis-Bruno, D. A. Keire, R. H. Heflich, & N. Mei, Genotoxicity evaluation of nitrosamine impurities using human TK6 cells transduced with cytochrome P450s. *Archives of Toxicology*, **96** (2022) 3077–3089. https://doi.org/10.1007/s00204-022-03347-6.

108. Better models to shed light on the mutagenicity of N-nitrosamines - Fraunhofer ITEM. *Fraunhofer Institute for Toxicology and Experimental Medicines*, (2022). https://www.item.fraunhofer.de/en/press-and-media/press-releases/mutagenicity-of-nitrosamines.html (accessed June 19, 2023).

109. Lhasa Limited, What Does The EMA Update On Nitrosamine Impurities Mean For You? (2023). https://www.lhasalimited.org/what-does-the-ema-update-on-nitrosamine-impurities-mean-for-you/ (accessed August 28, 2023).

110. K. L. Dobo, M. O. Kenyon, O. Dirat, M. Engel, A. Fleetwood, M. Martin, S. Mattano, A. Musso, J. C. McWilliams, A. Papanikolaou, P. Parris, J. Whritenour, S. Yu, & A. S.

Kalgutkar, Practical and Science-Based Strategy for Establishing Acceptable Intakes for Drug Product N-Nitrosamine Impurities. *Chemical Research in Toxicology*, **35** (n.d.) 475–489. https://doi.org/10.1021/acs.chemrestox.1c00369.

111. S. Paglialunga & A. van Haarst, The Impact of N-nitrosamine Impurities on Clinical Drug Development. *Journal of Pharmaceutical Sciences*, **112** (2023) 1183–1191. https://doi.org/10.1016/j.xphs.2023.01.017.

112. Zentiva Pharma GmbH, Gebrauchsinformation: Information für Anwender Atomoxetin Zentiva 80 mg Hartkapseln. (2022). https://www.zentiva.de/-/media/files/zentivade/produkte/atomoxetin-zentiva/gi_atomoxetin-80mg.pdf (accessed September 16, 2023).

113. Heumann Pharma, Duloxetin Heumann 30 mg/60 mg magensaftresistente Hartkapseln. Fachinformation. (2021). https://www.heumann.de/fileadmin/user_upload/produkte/infos/Duloxetin_Heumann_30_mg__60_mg_magensaftresistente_Hartkapseln_022291_2019-07.pdf (accessed September 16, 2023).

114. Fachinfo-Service, Fachinformation für Fluoxetin AbZ 20mg Hartkapseln. (2020). https://www.fachinfo.de/suche/fi/003564 (accessed June 21, 2023).

115. European Medicines Agency, Annex I Summary of Product Characteristics.Product information. 25/07/2022 Pradaxa - EMEA/H/C/000829 - N/0141. *Pradaxa*, (2022). https://www.ema.europa.eu/en/documents/product-information/pradaxa-epar-product-information_en.pdf (accessed September 8, 2023).

116. S. van Gassel, Reducing Nitrosamines Without the Use of Scavengers: The Critical Role of Excipients. An excipient manufacturer's view. *FDA-CRCG Workshop*, (2023). https://www.complexgenerics.org/NDSRIs_mitigation_strategies/ (accessed July 7, 2023).

117. R. Grahek, Determination of nitrite in pharmaceutical excipients; air as source for higher nitrite levels. *FDA-CRCG Workshop*, (2023). https://www.complexgenerics.org/NDSRIs_mitigation_strategies/.

118. K. K. Nanda, S. Tignor, J. Clancy, M. J. Marota, L. R. Allain, & S. M. D'Addio, Inhibition of N-Nitrosamine Formation in Drug Products: A Model Study. *Journal of Pharmaceutical Sciences*, **110** (2021) 3773–3775. https://doi.org/10.1016/j.xphs.2021.08.010.

119. M. Homšak, M. Trampuž, K. Naveršnik, Z. Kitanovski, M. Žnidarič, M. Kiefer, & Z. Časar, Assessment of a Diverse Array of Nitrite Scavengers in Solution and Solid State: A Study of Inhibitory Effect on the Formation of Alkyl-Aryl and Dialkyl N-Nitrosamine Derivatives. *Processes*, **10** (2022) 2428. https://doi.org/10.3390/pr10112428.

120. European Medicines Agency, *Guideline on the investigation of bioequivalence* (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr, 2010).

121. U.S. Food and Drug Administration, Effects of Antioxidants in Drug Products on

Intestinal Drug Transporters. (2023). https://www.fda.gov/science-research/advancing-regulatory-science/effects-antioxidants-drug-products-intestinal-drug-transporters (accessed June 19, 2023).

122. F. Wu & U.S. Food and Drug Administration, Challenges and Opportunities when Using Oral PBPK to Support Risk Assessment and Biowaiver in Regulatory Submissions. (2022). https://www.fda.gov/media/166598/download (accessed July 7, 2023).

123. J. Moser, I. W. Ashworth, L. Harris, M. C. Hillier, K. K. Nanda, & G. Scrivens, N-Nitrosamine Formation in Pharmaceutical Solid Drug Products: Experimental Observations. *Journal of Pharmaceutical Sciences*, (2023) S0022-3549(23)00028-X. https://doi.org/10.1016/j.xphs.2023.01.027.

124. European Medicines Agency, *Guideline on excipients in the dossier for application for marketing authorisation of a medicinal product* (Doc. Ref. EMEA/CHMP/QWP/396951/2006, 2007).

125. European Medicines Agency, *ICH guideline Q3C (R8) on impurities: guideline for residual solvents* (EMA/CHMP/ICH/82260/2006 Corr.*, 2022).

126. European Medicines Agency, *ICH guideline Q3D (R2) on elemental impurities* (EMA/CHMP/ICH/353369/2013, 2022).

127. L. X. Yu, G. Amidon, M. A. Khan, S. W. Hoag, J. Polli, G. K. Raju, & J. Woodcock, Understanding Pharmaceutical Quality by Design. *The AAPS Journal*, **16** (2014) 771–783. https://doi.org/10.1208/s12248-014-9598-3.

128. Offical Journal of the European Union, *Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Amended by Regulation (EU) 2019/1010 of the European Parliament and of the Council of 5 June 2019.* (2010).

129. European Medicines Agency, *ICH guideline Q8 (R2) on pharmaceutical development* (EMA/CHMP/ICH/167068/2004, 2017).

130. European Medicines Agency, *Assessment report Referral under Article 31 of Directive 2001/83/EC angiotensin-II-receptor antagonists (sartans) containing a tetrazole group* (2019).

131. Nitrosamines risk assessment: update for CEP holders. *European Directorate for the Quality of Medicines & HealthCare*, (n.d.). https://www.edqm.eu/en/w/nitrosamines-risk-assessment-update-for-cep-holders-1 (accessed March 15, 2023).

132. D. A. Keire, R. Bream, U. Wollein, J. Schmaler-Ripcke, A. Burchardt, M. Conti, A. Zmysłowski, P. Keizers, J. Morin, J. Poh, M. George, & M. Wierer, International Regulatory Collaboration on the Analysis of Nitrosamines in Metformin-Containing Medicines. *The AAPS Journal*, **24** (2022) 56. https://doi.org/10.1208/s12248-022-00702-4.

133. GMP Verlag Peither AG, USP: New General Chapter <1469> on Nitrosamine

Impurities. (2021). https://gmp-publishing.com/content/en/gmp-news/news-about-gmp-cgmp/d/usp-new-general-chapter-1469-on-nitrosamine-impurities (accessed March 16, 2023).

134. Health Science Authority, Recall of Ventolin 2mg Tablets. (2021). https://www.hsa.gov.sg/announcements/product-recall/recall-of-ventolin-2mg-tablets (accessed March 17, 2023).

135. U.S. Food and Drug Administration, Sandoz, Inc. Issues Nationwide Recall of 13 Lots of Orphenadrine Citrate 100 mg Extended Release Tablets Due to Presence of a Nitrosamine Impurity. (2022). https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/sandoz-inc-issues-nationwide-recall-13-lots-orphenadrine-citrate-100-mg-extended-release-tablets-due (accessed March 10, 2023).

136. Government of Canada, Pfizer recalls Inderal-LA (propranolol hydrochloride) capsules due to a nitrosamine impurity - Recalls, advisories and safety alerts – Canada.ca. (2022). https://recalls-rappels.canada.ca/en/alert-recall/pfizer-recalls-inderal-propranolol-hydrochloride-capsules-due-nitrosamine-impurity (accessed March 12, 2023).

137. Government of Canada, Apotex recalls Apo-Acyclovir tablets due to a nitrosamine impurity - Recalls, advisories and safety alerts – Canada.ca. (2022). https://recalls-rappels.canada.ca/en/alert-recall/apotex-recalls-apo-acyclovir-tablets-due-nitrosamine-impurity (accessed March 12, 2023).

138. U.S. Food and Drug Administration, FDA works to avoid shortage of sitagliptin following detection of nitrosamine impurity. (2022). https://www.fda.gov/drugs/drug-safety-and-availability/fda-works-avoid-shortage-sitagliptin-following-detection-nitrosamine-impurity (accessed March 12, 2023).

139. Government of Canada, Amitriptyline antidepressant drugs recalled because of a nitrosamine impurity - Recalls, advisories and safety alerts. (2023). https://recalls-rappels.canada.ca/en/alert-recall/amitriptyline-antidepressant-drugs-recalled-because-nitrosamine-impurity (accessed March 12, 2023).

140. Health Canada, *Guidance on nitrosamine impurities in medications. Effective Date: September 01, 2022* (2022).

141. Lhasa Limited, Current Status Of The Ames Test For N-nitrosamines. (2023). https://www.lhasalimited.org/current-status-of-the-ames-test-for-n-nitrosamines/ (accessed August 27, 2023).

142. OECD, Test Guideline No. 471: Bacterial Reverse Mutation Test. (2020). https://doi.org/10.1787/9789264071247-en.

Annex I: Overview of available guidance addressing root causes and risk factors

Guidance	Authority	Publication date
Assessment report Referral under Article 31 of Directive 2001/83/EC angiotensin-II-receptor antagonists (sartans) containing a tetrazole group [130]	EMA	February 2019
Assessment report Procedure under Article 5(3) of Regulation EC (No) 726/2004 Nitrosamine impurities in human medicinal products [1]	EMA	June 2020
Lessons learnt from the presence of *N*-nitrosamine impurities in sartan medicines [2]	HMA, EMA	June 2020
Assessment report Referral under Article 31 of Directive 2001/83/EC INN: ranitidine [35]	EMA	September 2020
Control of Nitrosamine Impurities in Human Drugs – Guidance for industry [14]	FDA	February 2021*
Guidance on nitrosamine impurities in medications [8]	HC	July 2023*
Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products [13]	EMA	July 2023*
Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities [10]	FDA	August 2023

Sources: included in table

*latest revision

Annex II: Regulatory history

- June 2018: NDMA detection in valsartan [2]
- July 2018: Start of worldwide recalls of valsartan medicines [31]
- July 2018: Start of referral under Article 31 of Directive 2001/83/EC for the assessment of valsartan medicines [2]
- August 2018: NDMA detection in valsartan from other sources [2]
- August 2018: NDEA detection in valsartan [2]
- September 2018: NDEA in other sartans, e.g. losartan, irbesartan from different sources [2]
- September 2018: Article 31 extension for the assessment of all sartan medicines [2]
- January 2019: NMBA detection in losartan [2]
- January 2019: NDMA detection in pioglitazone [2]
- February 2019: Publication of final assessment on sartan referral with recommendations for sartan medicines [2]
- June 2019: Publication of revised Ph. Eur. monographs for valsartan, candesartan, irbesartan, losartan and olmesartan [54]
- July 2019: NMPA detection in valsartan [2]
- September 2019: NMDA detection in ranitidine and start of worldwide recalls of ranitidine medicines [32–34]
- September 2019: Start of Article 31 referral for the assessment of ranitidine medicines [2]
- September 2019: Start of Article 5 (3) procedure for medicines containing chemically synthesized active substances [2]
- September 2019: Start of the call for review for human medicines containing chemically synthesized APIs [3]
- September 2019: Publication of EMA *Questions and answers on Information on nitrosamines for marketing authorisation holders*, 3 revisions, last update in March 2020 [36]
- October 2019: Start of EDQM call for review for CEP holders [131]

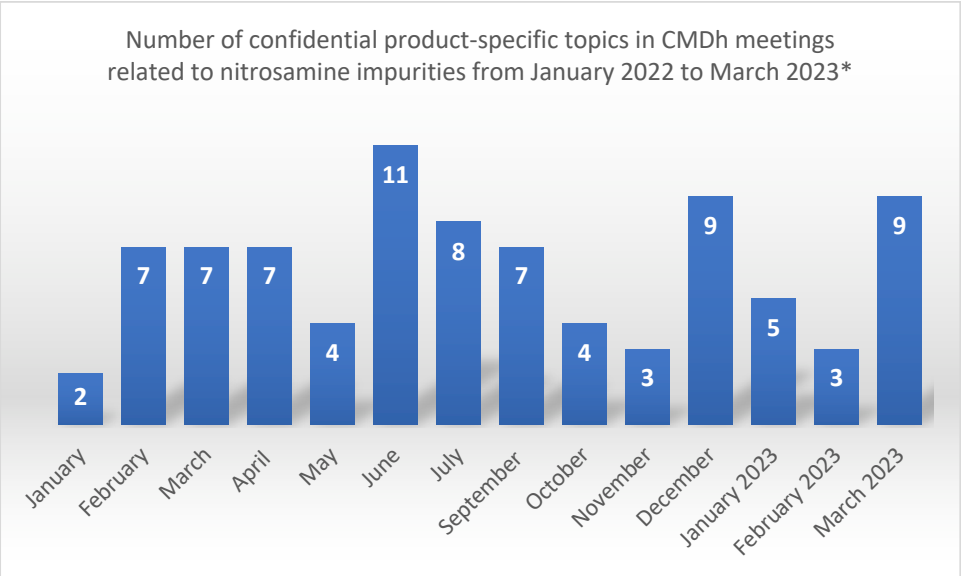
- November 2019: HC issued questions-and-answers document on nitrosamines [51]
- December 2019: NDMA detection in metformin medicines in Singapore following recalls by Health Science Authority [37]
- November 2019 - July 2020: Regulatory laboratory testing of metformin APIs and FPs by OMCLs [132]
- February 2020: Start of recalls of certain metformin extended-release products in HC and later in US [38–39]
- June 2020: Publication of assessment report for Article 5 (3) procedure for nitrosamine impurities in human medicinal products [1]
- June 2020: Publication of *Lessons learnt from presence of N-nitrosamine impurities in sartan medicines* [2]
- June 2020: HC updated questions-and-answers document on nitrosamines [51]
- July 2020: Extension of the call for review by EMA and EDQM to include biological medicines [13–54]
- August 2020: Detection of MNP in rifampicin and CPNP in rifapentine [40]
- August 2020: Publication of EMA *Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products*, 17 revisions, last updated in July 2023 [13]
- September 2020: Publication of assessment report for Art. 31 referral on ranitidine [35]
- September 2020: Publication of FDA Guidance Document *Control of Nitrosamine Impurities in Human Drugs,* 1 Revision in February 2021 [52]
- November 2020: Adoption of new Ph. Eur. general chapter on the analysis of N-nitrosamines in active substances (2.5.42) by Ph. Eur. Commission [54]
- December 2020: EDQM provides seven reference standards for the analysis of nitrosamine impurities [54]
- December 2020: HC updated questions-and-answers document on nitrosamines [51]
- December 2020: Publication of general chapter *N-nitrosamine impurities in active substances (2.5.42)* on the EDQM website [54]

- February 2021: Publication of revised sartan monographs on EDQM website to keep Ph. Eur. requirements in line with latest EMA regulatory decisions [54]
- June 2021: Start of worldwide recalls of Champix due to the presence of N-nitroso-varenicline [33–36–42]
- October 2021: Recall of irbesartan medicines in US due to the presence of N-nitroso-irbesartan [43]
- November 2021: FDA update on possible mitigation strategies to reduce the risk of nitrosamine drug substance-related impurities in drug products [52]
- December 2021: Publication of new *general chapter <1469> nitrosamine impurities* in the USP [133]
- December 2021: Recall of salbutamol medicines due to the presence of N-nitroso-salbutamol in Singapore [134]
- March 2022: Recall of orphenadrine medicines in US due to presence of Nitroso-Orphenadrine [135]
- March 2022: Recall of propranolol medicines in Canada due to presence of N-nitroso-propranolol [136]
- April 2022: Worldwide recalls of quinapril medicines due to presence of N-nitroso-quinapril [44]
- April 2022: Publication of HC *Guidance on nitrosamine impurities in medicinal products*, three revisions, last updated in July 2023 [8]
- May 2022: Recall of rasagiline medicines in Germany due to N-nitroso-rasagiline [45]
- July 2022: Extension of the call for review step 3 deadline by EDQM and EMA for chemical medicines [36]
- July 2022: Recall of acyclovir medicines in Canada due to NDMA [137]
- September 2022: NTTP (= Nitroso-STG-19) in Sitagliptin above AI [138]
- November 2022: Adoption of revised general monographs *Substances for pharmaceutical use (2034)* and *Pharmaceutical preparations (2619)* [54]
- February 2023: Recall of amitriptyline medicines in Canada due NDMA [139]
- March 2023: Recall of dabigatran-containing medicines in US due to N-nitroso-dabigatran [47]

- May 2023: Recall of atomoxetine medicines in Germany due to N-nitroso-atomoxetine [48]
- August 2023: Publication of FDA Guidance *Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities* [10]
- August 2023: Provision of AI limits and further updated information for NDSRIs on FDA website [9]

events are not exhaustive

Annex III: Number of confidential product-specific topics in CMDh meetings related to nitrosamine impurities from January 2022 to March 2023



Source: Own illustration acc. to published CMDh minutes [49]

*Counted are subtopics filled with the sentence *"Information related to the section cannot be released at the present time as it is deemed to contain commercially confidential information"* found under the topic "*Presence of nitrosamine impurities in human medicinal products containing chemically synthesized active pharmaceutical ingredients"*.

Annex IV: Additional information to figures in section 4.2 based on FDA enforcement reports

Table 1. Total numbers of recall reasons per year and search terms for derivation of Figure 3

Recall reason[1]	search term(s)	2018	2019	2020	2021	2022	sum
Lack of sterility assurance	"sterility"	353	815	236	316	311	2031
GMP deviations*	"GMP"	195	178	230	273	611	1487
Non-microbial contamination**	"cross contam", "chemical contam"	77	35	62	68	73	315
Microbial contamination	"micro contam"	99	550	12	18	28	707
Impurities***	"impurit"	163	207	131	56	112	669
Failed assay specifications	"superpotent", "subpotent"	131	35	77	50	44	337
Incorrect labeling	"labelling"	106	56	76	41	37	316
Other reasons	/	281	287	215	216	105	1104

Source: [60]

[1] all classes and not classified

* Excluded are GMP deviations with confirmed or possible consequences of contaminations, impurities, lack of sterility assurance, failed assay specifications; these are recorded under the respective recall reason

** Included are cross and chemical contaminations

***Included are failed impurities/degradation specifications or presence of new impurities

Table 2. Total numbers of recall reasons between 2018-2022 supporting Figure 3.

Recall reason[1]	Total number
Lack of sterility assurance	2031
GMP deviations*	1487
Contaminations**	1022
Impurities***	669
Failed assay specifications	337
Incorrect labeling	316
Other reasons****	1104

Source: [60]

[1]All classes and not classified
*Excluded are GMP deviations with confirmed or possible consequences of contaminations, impurities, lack of sterility assurance, failed assay specifications; these are recorded under the respective recall reason
**Included are cross, chemical and microbiological contaminations
***Included are failed impurities/degradation specifications or presence of new impurities
****Other reasons representing only small fractions of drug recalls:

- Customer complaints
- Marked without an approved NDA/ANDA
- Defective container
- Presence of particulate matter
- Failed content uniformity specifications
- Failed dissolution specifications
- Failed stability specifications
- Discoloration
- Lack of processing controls
- Defective delivery system

Table 3. Total number of drug recalls due to nitrosamine impurities supporting Figure 4

Year	Total number
2018	105
2019	112
2020	76
2021	15
2022	22
2023	3

Source: [60]

*Status: July 2023

Table 4. Total number of drug recalls due to NDSRIs and other nitrosamines supporting Figure 5

	Total number of drug recalls	
Year	NDSRIs	Other nitrosamines
2018	0	105
2019	0	112
2020	0	76
2021	12	3
2022	15	7
2023*	2	1

Source: [60]

*Status: July 2023

Table 5. Total number of drug recalls listed according to affected drugs supporting Figure 4 and Figure 5

API-containing medicines	2018	2019	2020	2021	2022	2023*
Irbesartan	10	5	0	5	0	0
Valsartan	95	68	0	0	0	0
Losartan	0	15	8	0	0	0
Nizatidin	0	0	3	0	0	0
Ranitidin	0	24	25	0	0	0
metformin	0	0	40	3	2	1
vareniclin	0	0	0	7	0	0
quinapril	0	0	0	0	14	0
rifampicin	0	0	0	0	5	0
orphenadrin	0	0	0	0	1	0
dabigatran	0	0	0	0	0	2
All	105	112	76	15	22	3

Source: [60]

*Status: July 2023

Annex V: Comparison HC guidance between revision 2 and 3

Topic	Revision 2, April 17, 2023	Revision 3, July 24, 2023
3. Outcomes of risk assessments (Step 1) and what is provided to Health Canada (updated)	Risk assessment documentation should be retained by the MAH, unless nitrosamine impurities are detected in the API, drug product or both during confirmatory testing. Following the completion of confirmatory testing, if any nitrosamine impurity is detected at any level, Health Canada must be informed immediately. The available details of the risk assessment should be submitted at the same time that Health Canada is informed of the detection. Please note that Health Canada may request to review the MAH's risk assessment for all products and will request this information directly from the MAH, as necessary. (…)	Risk assessment documentation should be retained by the MAH, unless nitrosamine impurities are detected in the API, drug product or both during confirmatory testing. Following the completion of confirmatory testing of the drug product, Health Canada must be informed if the nitrosamine impurity is detected above the established Acceptable Intake (AI) limit for the nitrosamine impurity in question, or above the class-specific threshold of toxicological concern (TTC) of 18 ng/day for N-nitrosamines if an AI limit has not been established by Health Canada. The available details of the risk assessment should be submitted at the same time that Health Canada is informed of the detection. Refer to the information in number 15. Please note that Health Canada may request to review the MAH's risk assessment for all products and will request this information directly from the MAH, as necessary. (…)
15. Contacting Health Canada if nitrosamine impurities are detected following the completion of confirmatory testing (updated)	MAHs must inform Health Canada immediately if nitrosamine impurities are detected at any level in the API, drug product or both following the completion of confirmatory testing. MAHs must provide a copy of the risk assessment and confirmatory testing results. (…) If nitrosamines are not detected during confirmatory testing (for example, less than the appropriate limit of detection of the validated test method), MAHs do not need to communicate to Health Canada. However, they should keep the risk assessment, analytical testing results and analytical method validation documentation on hand in case Health Canada requests them.	MAHs must inform Health Canada if nitrosamine impurities are detected above the established AI limit for the nitrosamine impurity in question, or above the class-specific TTC of 18 ng/day for N-nitrosamines if an AI limit has not been established by Health Canada in the drug product following the completion of confirmatory testing. MAHs must provide the confirmatory testing results. Health Canada recognizes the challenges faced by MAHs to decrease levels of nitrosamine impurities in their drug products while maintaining drug supply to Canadians. To minimize the impacts on drug supply within the Canadian market, MAHs are requested to engage Health Canada prior to taking any market action of a drug product due to a nitrosamine impurity issue including where the nitrosamine impurity is detected above 1500 ng/day. (…) If nitrosamines are not detected during confirmatory testing (for example, less than the appropriate limit of detection of the validated test method) or are detected below the established AI limit for the nitrosamine impurity in question or the class-specific TTC of 18 ng/day for N-nitrosamines, MAHs do not need to communicate this information to Health Canada. However, they should keep the risk assessment, analytical testing results and analytical method validation documentation on hand in case Health Canada requests them. (…)
17. Additional expectations of MAHs if nitrosamine impurities are detected in the API and/or drug product (updated)	Where 1 or more nitrosamine impurities are detected following the completion of confirmatory testing (for multiple nitrosamines, refer to number 27), in addition to notifying Health Canada, MAHs should provide: • a health risk assessment posed by the presence of the nitrosamine(s) along with intentions related to any actions, as necessary, for the batches on the Canadian market o where product recalls are warranted, consult the Drugs and Natural Health Products Recall Guide (GUI-0039) for procedures • indicate if the product is considered to	Where 1 or more nitrosamine impurities are detected (for multiple nitrosamines, refer to number 27), in addition to notifying Health Canada, MAHs should have completed or be completing as necessary: • a health risk assessment posed by the presence of the nitrosamine(s) along with intentions related to any actions, as necessary, for the batches on the Canadian market where product recalls are warranted, consult the Drugs and Natural Health Products Recall Guide (GUI-0039) for procedures • an assessment to determine if the product

	be medically necessary and if any disruption to product supply is expected
	• a detailed investigation report assessing all possible root causes of the detected nitrosamine impurity (or impurities) and describing corrective and preventive actions
	  ○ perform investigations in accordance with written procedures
	  ○ evaluate all potential changes to facilities, materials, equipment and/or process intended to reduce the levels of the nitrosamine impurities through a formal change control system
	• a risk mitigation plan to ensure that, moving forward, nitrosamine impurity levels will be consistently below the Acceptable Intake (AI) limit at the end of the retest period for the API or the shelf-life for the drug product (refer to number 24 for a list of established AIs)
	
	MAHs are reminded to submit changes to the market authorization as per Step 3 of the October 2, 2019 letter. Refer to number 13 on how changes should be submitted.
	
	Health Canada may use such notifications to request additional actions and/or information. For example, the origin of nitrosamine impurities may be attributed to the type of process chemistry used and the risk mitigation plan may necessitate the establishment of a control strategy by manufacturers for each detected nitrosamine impurity according to the ICH M7 guideline.
	
	We may request additional actions by other MAHs of the same products to mitigate any risks identified and protect people's health and safety if necessary.
	
	(…)
		is considered to be medically necessary or medically important and if any disruption to product supply is expected should market action be taken
		• a detailed investigation report assessing all possible root causes of the detected nitrosamine impurity (or impurities) and describing corrective and preventive actions
		  ○ perform investigations in accordance with written procedures
		  ○ evaluate all potential changes to facilities, materials, equipment and/or process intended to reduce the levels of the nitrosamine impurities through a formal change control system
		• a risk mitigation plan to ensure that, moving forward, nitrosamine impurity levels will be consistently below the Acceptable Intake (AI) limit at the end of the retest period for the API or the shelf-life for the drug product (refer to Appendix 2 for a list of established AI limits)
		
		MAHs are reminded to submit changes to the market authorization as per Step 3 of the October 2, 2019 letter. Refer to number 13 on how changes should be submitted.
		
		Health Canada may use such notifications to request additional actions and/or information. For example, the origin of nitrosamine impurities may be attributed to the type of process chemistry used and the risk mitigation plan may necessitate the establishment of a control strategy by manufacturers for each detected nitrosamine impurity according to ICH's M7 guideline.
		
		We may request additional actions by other MAHs of the same products to mitigate any risks identified and protect people's health and safety if necessary.
		
		(…)

Sources: Information extracted from HC guidance revision 2 [88] and revision 3 [8]

Annex VI: Comparison HC guidance between revision 1 and 2

Topic	Revision 1, September 01, 2022	Revision 2, April 17, 2023
19. Approach for drug products that are planned for submission or are already filed with Health Canada (updated)	A summary and discussion of the risk assessment for nitrosamine impurities in the drug product should be placed in section 3.2.P.2 of the CTD. Confirmatory testing results and updated control strategy (where warranted) should also be included in the drug application (for example, under sections 3.2.S.2, 3.2.S.4, 3.2.S.7, 3.2.P.3, 3.2.P.4, 3.2.P.5, 3.2.P.8). (…)	A summary and discussion of the risk assessment for nitrosamine impurities in the drug product should be placed in section 3.2.P.2 of the CTD. This summary is expected to include sufficient detail to allow Health Canada to assess the adequacy and robustness of the risk assessment. Expectations for the content of the summary and discussion of risk assessments are found under number 20. Confirmatory testing results and updated control strategy (where warranted) should also be included in the drug application (for example, under sections 3.2.S.2, 3.2.S.4, 3.2.S.7, 3.2.P.3, 3.2.P.4, 3.2.P.5, 3.2.P.8). (…)
20. Risk assessments for the potential presence of nitrosamine impurities as part of the expected content for new submissions (updated)	Risk assessments for nitrosamine impurities should be conducted routinely during API and drug product development. (…) Failure to include this information could result in requests for additional information, delays in the review process, and potentially the issuance of negative decisions.	Risk assessments for nitrosamine impurities should be conducted routinely during API and drug product development. (…) The summary and discussion of the risk assessment for the drug product is expected to include sufficient detail to allow Health Canada to assess the adequacy and robustness of the risk assessment. It should include a discussion of the risk factors and potential root causes considered in relation to specific knowledge of the drug product and its components (including the API). Checklists lacking sufficient discussion and detail should be avoided. The summary and discussion should include the following: • identification of any third parties (for example, suppliers, manufacturers, consultants) who have been authorized to perform the risk assessment on behalf of the applicant • identification of intrinsic and extrinsic risk factors related to formation or introduction of nitrosamine impurities originating from all drug product components and quality/compliance considerations • identification of those nitrosamines potentially formed and/or introduced • information on the established process and/or analytical controls and how they may mitigate risk • supporting scientific data (for example, confirmatory testing results) and calculations • an overall conclusion on the risk of presence of nitrosamines in the drug product together with an appropriate scientific rationale/justification For Supplements, Notifiable Changes and Post-DIN Change submissions (for quality changes that may impact the potential presence of nitrosamine impurities in the API or drug product), the summary and discussion of the risk assessment should address the impact of the proposed change(s) on nitrosamine impurities relative to the approved drug product. (…) Failure to include this information could result in requests for additional information, delays in the review process, and potentially the issuance of negative decisions.

Sources: Information extracted from HC guidance revision 1 [140] revision 2 [88]

Annex VII: Control of process-related impurities for drug substances acc. to ICH M7

- Option 1: routine testing in the API with acceptance criteria at or below the AI; periodic verification testing possible if levels $<$ 30% AI for $\geq$ 6 consecutive pilot scale or $\geq$ 3 consecutive production scale batches.
- Option 2: control in upstream (raw material, starting material, intermediate) specifications with acceptance criteria at or below the AI.
- Option 3: control in upstream (raw material, starting material, intermediate) specification with acceptance criteria exceeding the AI and demonstration that the level in the drug substance will be $<$ 30% AI.
- Option 4: control based on scientific risk assessment shown as estimated purge factor for clearance of the impurity by the process giving sufficient confidence that the level in the drug substance will be < AI.

Source: ICH M7 guideline [4]

Annex VIII: Recommended AIs for nitrosamine impurities

NDSRIs	Source[1]	FDA	EMA	HC	AI derivation	Potency Category
N-nitroso-desmethyl-almotriptan	Almotriptan	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-amitriptyline	Amitriptyline, Nortryptylin	26.5 ng/day	8 ng/day	8 ng/day	SAR/NMPEA; CPCA	1
N-nitroso-atomoxetine	Atomoxetine	26.5 ng/day	100 ng/day	100 ng/day	SAR/NNK; CPCA	1
N-nitroso-desmethyl-bedaquiline	Bedaquiline	26.5 ng/day	/	/	CPCA	1
N-nitroso-betahistine	Betahistine	/	18 ng/day	18 ng/day	CPCA	1
N-nitroso-desmethyl-brompheniramine	Brompheniramine	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-cabergoline	Cabergoline	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-carbinoxamine	Carbinoxamine	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-chlophedianol	Chlophedianol	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-chlorpheniramine	Chlorpheniramine	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-chloropyramine (N-DMCP)	Chloropyramine	/	18 ng/day	/	CPCA	1
N-nitroso-desmethyl-chlorpromazine	Chlorpromazine	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-citalopram	Citalopram	26.5 ng/day	/	18 ng/day	CPCA	1
N-nitroso-desmethyl-clomipramine	Clomipramine	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-cyclobenzaprine	Cyclobenzaprine	26.5 ng/day	/	/	CPCA	1
N-nitroso-desipramine	Desipramine	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-desvenlafaxine	Desvenlafaxine	26.5 ng/day	/	18 ng/day	CPCA	1
N-nitroso-desmethyl-dexbrompheniramine	Dexbrompheniramine	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-dexchlorpheniramine	Dexchlorpheniramine	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-diltiazem	Diltiazem	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-diphenhydramine[2]	Diphenhydramine	26.5 ng/day	/	18 ng/day	CPCA	1
N-nitroso-desmethyl-cidoxepin	Doxepin	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-doxepin, (e)-	Doxepin	26.5 ng/day	/	18 ng/day	CPCA	1
N-nitroso-desmethyl-doxylamine	Doxylamine	26.5 ng/day	/	18 ng/day	CPCA	1
N-nitroso-N-methyl-2-[1-phenyl-1(2-	Doxylamine	/	/	18 ng/day	CPCA	1

pyridinyl)methoxy]ethanamine						
N-nitroso-desmethyl-escitalopram	Escitalopram	26.5 ng/day	/	/	CPCA	1
N-nitroso-lapatinib	Lapatinib	/	/	18 ng/day	CPCA	1
N-nitroso-lorcaserin	Lorcaserin[3]	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-nizatidine	Nizatidine	26.5 ng/day	/	/	CPCA	1
N-nitroso-oliceridine	Oliceridine	26.5 ng/day	/	/	CPCA	1
N-nitroso-omadacycline	Omadacycline	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-orphenadrine/nitroso-orphenadrine	Orphenadrine	26.5 ng/day	18 ng/day	18 ng/day	CPCA	1
N-nitroso-N,N'-dibenzylethanediamine	Penicillin G benzathine	/	/	18 ng/day	CPCA	1
N,N'-dinitroso-N,N'dibenzylethanediamine	Penicillin G benzathine	/	/	18 ng/day	CPCA	1
N-nitroso-desmethyl-pheniramine	Pheniramine	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-phenyltoloxamine	Phenyltoloxamine	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-propoxyphene	Propoxyphene	26.5 ng/day	/	/	CPCA	1
N-nitroso-protriptyline	Protriptyline	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-pyrilamine	Pyrilamine	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-ranitidine	Ranitidine	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-rizatriptan	Rizatriptan	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-sumatriptan	Sumatriptan	26.5 ng/day	/	18 ng/day	CPCA	1
N-nitroso-desmethyl-tamoxifen	Tamoxifen	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-tapentadol	Tapentadol	26.5 ng/day	/	/	CPCA	1
N-nitroso-N-methyl-1naphthylmethylamine	Terbinafine	/	/	18 ng/day	CPCA	1
N-nitroso-N-desmethyl terbinafine	Terbinafine	/	/	18 ng/day	CPCA	1
N-[(2E)-6,6-dimethyl-2-hepten-4-yn1-yl]-N-nitrosomethanamine	Terbinafine	/	/	18 ng/day	CPCA	1
N-nitroso-desmethyl-tetracaine	Tetracaine	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-thonzylamine	Thonzylamine	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-tramadol	Tramadol	26.5 ng/day	/	/	CPCA	1
N-nitroso-trientine	Trientine	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-trimethobenzamide	Trimethobenzamide	26.5 ng/day	/	/	CPCA	1

N-nitroso-desmethyl-trimipramine	Trimipramine	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-tripelennamine	Tripelennamine	/	18 ng/day	18 ng/day	CPCA	1
N-nitroso-desmethyl-venlafaxine	Venlafaxine	26.5 ng/day	/	/	CPCA	1
N-nitroso-desmethyl-zolmitriptan	Zolmitriptan	26.5 ng/day	/	/	CPCA	1
N-nitroso-berotralstat	Berotralstat	100 ng/day	/	/	CPCA	2
N-nitroso-brinzolamide	Brinzolamide	100 ng/day	/	/	CPCA	2
N-nitroso-colistin a hydrogen methanesulfonate-1	Colistin	100 ng/day	/	/	CPCA	2
N-nitroso-colistin a hydrogen methanesulfonate-2	Colistin	100 ng/day	/	/	CPCA	2
N-nitroso-colistin a hydrogen methanesulfonate-3	Colistin	100 ng/day	/	/	CPCA	2
N-nitroso-colistin a hydrogen methanesulfonate-4	Colistin	100 ng/day	/	/	CPCA	2
N-nitroso-colistin a hydrogen methanesulfonate-5	Colistin	100 ng/day	/	/	CPCA	2
N-nitroso-colistin b hydrogen methanesulfonate-1	Colistin	100 ng/day	/	/	CPCA	2
N-nitroso-colistin b hydrogen methanesulfonate-2	Colistin	100 ng/day	/	/	CPCA	2
N-nitroso-colistin b hydrogen methanesulfonate-3	Colistin	100 ng/day	/	/	CPCA	2
N-nitroso-colistin b hydrogen methanesulfonate-4	Colistin	100 ng/day	/	/	CPCA	2
N-nitroso-colistin b hydrogen methanesulfonate-5	Colistin	100 ng/day	/	/	CPCA	2
N-nitroso-dipivefrin	Dipivefrin	100 ng/day	/	/	CPCA	2
N-nitroso-dorzolamide	Dorzolamide	100 ng/day	/	100 ng/day	CPCA	2
N-nitroso-epinephrine	Epinephrine	100 ng/day	/	/	CPCA	2
N-nitroso-fenoldopam	Fenoldopam	100 ng/day	/	/	CPCA	2
N-nitroso-florbetaben f-18	Florbetaben F-18	100 ng/day	/	/	CPCA	2
N-nitroso-florbetapir f-18	Florbetapir F-18	100 ng/day	/	/	CPCA	2
N-nitroso-flutemetamol f-18	Flutemetamol F-18	100 ng/day	/	/	CPCA	2
N-nitroso-desmethyl-methylene blue	Methylene Blue	100 ng/day	/	/	CPCA	2
N-nitroso-desmethyl-mifepristone	Mifepristone	100 ng/day	/	/	CPCA	2
N-nitroso-desmethyl-minocycline-1	Minocycline	100 ng/day	/	/	CPCA	2
N-nitroso-mitoxantrone-2	Mitoxantrone	100 ng/day	/	/	CPCA	2
N-nitroso-desmethyl-neratinib	Neratinib	100 ng/day	/	/	CPCA	2
N-nitroso-nizatidine-1	Nizatidine	100 ng/day	/	/	CPCA	2
N-nitroso-desmethyl-omadacycline-1	Omadacycline	100 ng/day	/	/	CPCA	2

N-nitroso-desmethyl-padimate o	Padimate O	100 ng/day	/	/	CPCA	2
N-nitroso-phenylephrine	Phenylephrine	100 ng/day	100 ng/day	100 ng/day	CPCA	2
N-nitroso-plazomicin-2	Plazomicin	100 ng/day	/	/	CPCA	2
N-nitroso-plerixafor-1	Plerixafor	100 ng/day	/	/	CPCA	2
N-nitroso-plerixafor-2	Plerixafor	100 ng/day	/	/	CPCA	2
N-nitroso-plerixafor-3	Plerixafor	100 ng/day	/	/	CPCA	2
N-nitroso-propafenone	Propafenone	100 ng/day	/	/	CPCA	2
N-nitroso-desmethyl-quinupristin	Quinupristin	100 ng/day	/	/	CPCA	2
N-nitroso-racepinephrine	Racepinephrine	100 ng/day	/	/	CPCA	2
N-nitroso-ranitidine-2	Ranitidine	100 ng/day	/	/	CPCA	2
N-nitroso-rasagiline	Rasagiline	100 ng/day	100 ng/day	100 ng/day	CPCA	2
N-nitroso-desmethyl-rivastigmine	Rivastigmine	100 ng/day	/	100 ng/day	CPCA	2
N-nitroso-sertraline	Sertraline	100 ng/day	100 ng/day	100 ng/day	CPCA	2
N-nitroso-desmethyl-spinosad factor a	Spinosad	100 ng/day	/	/	CPCA	2
N-nitroso-desmethyl-spinosad factor d	Spinosad	100 ng/day	/	/	CPCA	2
N-nitroso-desmethyl-tigecycline-2	Tigecycline	100 ng/day	/	/	CPCA	2
N-nitroso-desmethyl-ulipristal acetate	Ulipristal Acetate	100 ng/day	/	/	CPCA	2
N-nitroso-ambroxol	Ambroxol	/	400 ng/day	/	CPCA	3
N-nitroso-amoxapine	Amoxapine	400 ng/day	/	/	CPCA	3
N-nitroso-avanafil	Avanafil	400 ng/day	/	/	CPCA	3
N-nitroso-cangrelor	Cangrelor	400 ng/day	/	/	CPCA	3
N-nitroso-carvedilol	Carvedilol	400 ng/day	/	/	CPCA	3
N-nitroso-cinacalcet	Cinacalcet	400 ng/day	/	/	CPCA	3
N-nitroso-dabigatran etexilate	Dabigatran Etexilate	400 ng/day	400 ng/day	400 ng/day	CPCA	3
N-nitroso-degarelix	Degarelix	400 ng/day	/	/	CPCA	3
N-nitroso-desmethyl-demeclocycline	Demeclocycline	400 ng/day	/	/	CPCA	3
N-nitroso-desloratadine	Desloratadine	400 ng/day	400 ng/day	400 ng/day	CPCA	3
N-nitroso-desmethyl-doxycycline	Doxycycline	400 ng/day	/	400 ng/day	CPCA	3
N-nitroso-desmethyl-eravacycline	Eravacycline	400 ng/day	/	/	CPCA	3
N-nitroso-desmethyl-erythromycin ethylsuccinate	Erythromycin Ethylsuccinate	400 ng/day	/	/	CPCA	3

N-nitroso-fenfluramine	Fenfluramine	400 ng/day	/	/	CPCA	3
N-nitroso-frovatriptan	Frovatriptan	400 ng/day	/	400 ng/day	CPCA	3
2-(2-(4-nitrosopiperazin-1yl)ethoxy)ethan-1-ol	Hydroxyzine	/	/	400 ng/day	CPCA	3
N-nitroso-landiolol	Landiolol	/	400 ng/day	/	CPCA	3
N-nitroso-levmetamfetamine	Levmetamfetamine	400 ng/day	/	/	CPCA	3
N-nitroso-desmethyl-methadone	Methadone	400 ng/day	/	/	CPCA	3
N-nitroso-methamphetamine	Methamphetamine	400 ng/day	/	/	CPCA	3
N-nitroso-desmethyl-minocycline-2	Minocycline	400 ng/day	/	/	CPCA	3
N-nitroso-mirabegron	Mirabegron	400 ng/day	400 ng/day	400 ng/day	CPCA	3
N-nitroso-nizatidine-2	Nizatidine	400 ng/day	/	/	CPCA	3
N-nitroso-desmethyl-omadacycline-2	Omadacycline	400 ng/day	/	/	CPCA	3
N-nitroso-ozanimod	Ozanimod	400 ng/day	/	/	CPCA	3
N-nitroso-pramipexole	Pramipexole	400 ng/day	400 ng/day	400 ng/day	CPCA	3
N-nitroso-desmethyl-promethazine	Promethazine	400 ng/day	/	/	CPCA	3
N-nitroso-propylhexedrine	Propylhexedrine	400 ng/day	/	/	CPCA	3
N-nitroso-aryl piperazine / N-nitroso-desalkylquetiapine (NDAQ)	Quetiapine	/	400 ng/day	400 ng/day	CPCA	3
N-nitroso-ranitidine-1	Ranitidine	400 ng/day	/	/	CPCA	3
N-nitroso-relebactam	Relebactam	400 ng/day	/	/	CPCA	3
1-cyclopentyl-4-nitrosopiperazine	Rifapentine	/	/	400 ng/day	CPCA	3
N-nitroso-safinamide	Safinamide	400 ng/day	/	/	CPCA	3
N-nitroso-salmeterol	Salmeterol	400 ng/day	/	/	CPCA	3
N-nitroso-desmethyl-sarecycline	Sarecycline	400 ng/day	/	/	CPCA	3
N-nitroso-telavancin-1	Telavancin	400 ng/day	/	/	CPCA	3
N-nitroso-tetracaine	Tetracaine	400 ng/day	/	/	CPCA	3
N-nitroso-desmethyl-tetracycline	Tetracycline	400 ng/day	/	/	CPCA	3
N-nitroso-desmethyl-tigecycline-1	Tigecycline	400 ng/day	/	/	CPCA	3
N-nitroso-trimetazidine (NTMZ)	Trimetazidine	/	400 ng/day	/	CPCA	3
N-nitroso-N-ethyl-valacyclovir	Valacyclovir	/	400 ng/day	400 ng/day	CPCA	3
N-nitroso-N-methyl-valacyclovir	Valacyclovir	/	400 ng/day	400 ng/day	CPCA	3

N-nitroso-vilanterol	Vilanterol	400 ng/day	/	/	CPCA	3
N-nitroso-vortioxetine	Vortioxetine	400 ng/day	400 ng/day	400 ng/day	CPCA	3
N-nitroso-acebutolol	Acebutolol	1500 ng/day	/	/	CPCA	4
N-nitroso-argatroban	Argatroban	1500 ng/day	/	/	CPCA	4
nitroso-praziquanamine	Arpraziquantel	/	1500 ng/day	/	CPCA	4
N-nitroso-articaine	Articaine	1500 ng/day	/	/	CPCA	4
N-nitroso-atenolol	Atenolol	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	4
N-nitroso-betaxolol	Betaxolol	1500 ng/day	/	/	CPCA	4
N-nitroso-bicisate	Bicisate	1500 ng/day	/	/	CPCA	4
N-nitroso-bisoprolol	Bisoprolol	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	4
N-nitroso-bumetanide	Bumetanide	1500 ng/day	1500 ng/day	/	CPCA	4
N-nitroso-caspofungin	Caspofungin	1500 ng/day	/	/	CPCA	4
N-nitroso-ciprofloxacin	Ciprofloxacin	pending	1500 ng/day	1500 ng/day	SAR/?; CPCA	4
N-nitroso-desmethyl-clarithromycin	Clarithromycin	1500 ng/day	/	1500 ng/day	CPCA	4
N-nitroso-N-desmethyl dextromethorphan	Dextromethorphan	/	/	1500 ng/day	CPCA	4
N-nitroso-dobutamine	Dobutamine	1500 ng/day	/	/	CPCA	4
N-nitroso-elagolix	Elagolix	1500 ng/day	/	/	CPCA	4
N-nitroso-ephedrine	Ephedrine	1500 ng/day	/	/	CPCA	4
N-nitroso-ertapenem	Ertapenem	1500 ng/day	/	/	CPCA	4
N-nitroso-desmethyl-erythromycin	Erythromycin	1500 ng/day	/	/	CPCA	4
N-nitroso-esmolol	Esmolol	1500 ng/day	/	/	CPCA	4
N-nitroso-ethambutol	Ethambutol	1500 ng/day	/	/	CPCA	4
N-nitroso-flecainide	Flecainide	1500 ng/day	/	1500 ng/day	CPCA	4
N-nitroso-folic acid	Folic Acid	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	4
N-nitroso-formoterol	Formoterol	1500 ng/day	/	/	CPCA	4
N-nitroso-furosemide	Furosemide	1500 ng/day	/	1500 ng/day	CPCA	4
N-nitroso-gatifloxacin	Gatifloxacin	1500 ng/day	/	/	CPCA	4
N-nitroso-isoproterenol	Isoproterenol	1500 ng/day	/	/	CPCA	4
N-nitroso-labetalol	Labetalol	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	4
N-nitroso-leucovorin-1	Leucovorin	1500 ng/day	/	1500 ng/day	CPCA	4

N-nitroso-leucovorin-2	Leucovorin	1500 ng/day	/	/	CPCA	4
N-nitroso-levofloxacin	Levofloxacin	/	1500 ng/day	1500 ng/day	CPCA	4
9-fluoro-2,3-dihydro-3-methyl-10-(Nnitroso-2-aminoethyl)-7-oxo-7Hpyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid	Levofloxacin	/	/	1500 ng/day	CPCA	4
N-nitroso-levoleucovorin-1	Levoleucovorin	1500 ng/day	/	/	CPCA	4
N-nitroso-levoleucovorin-2	Levoleucovorin	1500 ng/day	/	/	CPCA	4
N-nitroso-levomefolic acid-1	Levomefolic Acid	1500 ng/day	/	/	CPCA	4
N-nitroso-levomefolic acid-2	Levomefolic Acid	1500 ng/day	/	/	CPCA	4
N-nitroso-lidocaine EP Impurity E	Lidocain	/	/	1500 ng/day	CPCA	4
N-nitroso-mefloquine	Mefloquine	1500 ng/day	/	/	CPCA	4
N-nitroso-meropenem	Meropenem	1500 ng/day	/	1500 ng/day	CPCA	4
N-nitroso-metoprolol	Metoprolol	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	4
N-nitroso-migalastat	Migalastat	1500 ng/day	/	/	CPCA	4
N-nitroso-mitoxantrone-1	Mitoxantrone	1500 ng/day	/	/	CPCA	4
N-nitroso-moxifloxacin	Moxifloxacin	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	4
N-nitroso-nebivolol	Nebivolol	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	4
N-nitroso-desmethyl-olopatadine	Olopatadine	1500 ng/day	/	/	CPCA	4
N-nitroso-oritavancin-1	Oritavancin	1500 ng/day	/	/	CPCA	4
N-nitroso-ozenoxacin	Ozenoxacin	1500 ng/day	/	/	CPCA	4
N-nitroso-pindolol	Pindolol	1500 ng/day	/	/	CPCA	4
N-nitroso-plazomicin-1	Plazomicin	1500 ng/day	/	/	CPCA	4
N-nitroso-prilocaine	Prilocaine	1500 ng/day	/	/	CPCA	4
N-nitroso-proline	Proline	1500 ng/day	/	/	CPCA	4
N-nitroso-propranolol	Propranolol	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	4
N-nitroso-pseudoephedrine	Pseudoephedrine	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	4
N-nitroso-2,6-pipecoloxilidide	Ropivacaine	/	1500 ng/day	/	CPCA	4
N-nitroso-sapropterin-1	Sapropterin	1500 ng/day	/	/	CPCA	4
N-nitroso-silodosin	Silodosin	1500 ng/day	/	/	CPCA	4
N-nitroso-sotalol	Sotalol	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	4

N-nitroso-streptomycin	Streptomycin	1500 ng/day	/	/	CPCA	4
N-nitroso-tamsulosin	Tamsulosin	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	4
N-nitroso-telavancin-2	Telavancin	1500 ng/day	/	/	CPCA	4
N-nitroso-telavancin-3	Telavancin	1500 ng/day	/	/	CPCA	4
N-nitroso-desmethyl-telithromycin	Telithromycin	1500 ng/day	/	/	CPCA	4
N-nitroso-tirofiban	Tirofiban	1500 ng/day	/	/	CPCA	4
N-nitroso-vancomycin	Vancomycin	1500 ng/day	/	/	CPCA	4
N-nitroso-abacavir	Abacavir	1500 ng/day	/	/	CPCA	5
N-nitroso-acarbose	Acarbose	1500 ng/day	/	/	CPCA	5
N-nitroso-albuterol	Albuterol	1500 ng/day	/	/	CPCA	5
N-nitroso-amlodipine	Amlodipine	1500 ng/day	/	/	CPCA	5
N-nitroso-benazepril	Benazepril	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	5
N-nitroso-bendroflumethiazide	Bendroflumethiazide	1500 ng/day	/	/	CPCA	5
N-nitroso-brilliant blue g	Brilliant Blue G	1500 ng/day	/	/	CPCA	5
N-nitroso-bupropion	Bupropion	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	5
N-nitroso-carteolol	Carteolol	1500 ng/day	/	/	CPCA	5
N-nitroso-chloroquine	Chloroquine	1500 ng/day	/	/	CPCA	5
N-nitroso-cilazapril	Cilazapril	/	1500 ng/day	1500 ng/day	CPCA	5
N-nitroso-clevidipine	Clevidipine	1500 ng/day	/	/	CPCA	5
N-nitroso-clozapine	Clozapine	1500 ng/day	/	1500 ng/day	CPCA	5
N-nitroso-diclofenac	Diclofenac	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	5
N-nitroso-duvelisib	Duvelisib	1500 ng/day	/	/	CPCA	5
N-nitroso-enalapril	Enalapril	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	5
N-nitroso-enalaprilat	Enalaprilat	1500 ng/day	/	/	CPCA	5
N-nitroso-esketamine	Esketamine	1500 ng/day	/	/	CPCA	5
N-nitroso-etravirine	Etravirine	1500 ng/day	/	/	CPCA	5
N-nitroso-felodipine	Felodipine	1500 ng/day	/	1500 ng/day[4]	CPCA	5
N-nitroso-fosdenopterin-1	Fosdenopterin	1500 ng/day	/	/	CPCA	5
N-nitroso-fosdenopterin-2	Fosdenopterin	1500 ng/day	/	/	CPCA	5
N-nitroso-fostamatinib-1	Fostamatinib	1500 ng/day	/	/	CPCA	5

N-nitroso-fostamatinib-2	Fostamatinib	1500 ng/day	/	/	CPCA	5
N-nitroso-hydroxychloroquine	Hydroxychloroquine	1500 ng/day	/	/	CPCA	5
N-nitroso-imatinib	Imatinib	1500 ng/day	/	/	CPCA	5
N-nitroso-isoxsuprine	Isoxsuprine	1500 ng/day	/	/	CPCA	5
N-nitroso-isradipine	Isradipine	1500 ng/day	/	/	CPCA	5
N-nitroso-ivacaftor	Ivacaftor	1500 ng/day	/	/	CPCA	5
N-nitroso-ketamine	Ketamine	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	5
N-nitroso-levalbuterol	Levalbuterol	1500 ng/day	/	/	CPCA	5
N-nitroso-levamlodipine	Levamlodipine	1500 ng/day	/	/	CPCA	5
N-nitroso-levobunolol	Levobunolol	1500 ng/day	/	/	CPCA	5
N-nitroso-lisinopril	Lisinopril	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	5
N-nitroso-mecamylamine	Mecamylamine	1500 ng/day	/	/	CPCA	5
N-nitroso-meclofenamic acid	Meclofenamic Acid	1500 ng/day	/	/	CPCA	5
N-nitroso-metolazone	Metolazone	1500 ng/day	/	/	CPCA	5
N-nitroso-moexipril	Moexipril	1500 ng/day	/	/	CPCA	5
N-nitroso-nadolol	Nadolol	1500 ng/day	/	/	CPCA	5
N-nitroso-neratinib	Neratinib	1500 ng/day	/	/	CPCA	5
N-nitroso-nicardipine	Nicardipine	1500 ng/day	/	/	CPCA	5
N-nitroso-nifedipine	Nifedipine	1500 ng/day	/	/	CPCA	5
N-nitroso-nimodipine	Nimodipine	1500 ng/day	/	/	CPCA	5
N-nitroso-nintedanib	Nintedanib	1500 ng/day	/	/	CPCA	5
N-nitroso-nisoldipine	Nisoldipine	1500 ng/day	/	/	CPCA	5
N-nitroso-olanzapine	Olanzapine	1500 ng/day	/	/	CPCA	5
N-nitroso-olodaterol	Olodaterol	1500 ng/day	/	/	CPCA	5
N-nitroso-oritavancin-2	Oritavancin	1500 ng/day	/	/	CPCA	5
N-nitroso-perindopril	Perindopril	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	5
N-nitroso-polythiazide	Polythiazide	1500 ng/day	/	/	CPCA	5
N-nitroso-primaquine	Primaquine	1500 ng/day	/	/	CPCA	5
N-nitroso-ramipril	Ramipril	1500 ng/day	1500 ng/day	1500 ng/day	CPCA	5
N-nitroso-rifabutin	Rifabutin	1500 ng/day	/	/	CPCA	5

N-nitroso-rilpivirine-1	Rilpivirine	1500 ng/day	/	/	CPCA	5
N-nitroso-rilpivirine-2	Rilpivirine	1500 ng/day	/	/	CPCA	5
N-nitroso-risdiplam	Risdiplam	1500 ng/day	/	/	CPCA	5
N-nitroso-rolapitant	Rolapitant	1500 ng/day	/	/	CPCA	5
N-nitroso-salbutamol	Salbutamol	/	1500 ng/day	1500 ng/day	CPCA	5
N-nitroso-sapropterin-2	Sapropterin	1500 ng/day	/	/	CPCA	5
N-nitroso-tafenoquine	Tafenoquine	1500 ng/day	/	/	CPCA	5
N-nitroso-telavancin-4	Telavancin	1500 ng/day	/	/	CPCA	5
N-nitroso-terbutaline	Terbutaline	1500 ng/day	/	/	CPCA	5
N-nitroso-ticagrelor	Ticagrelor	1500 ng/day	/	/	CPCA	5
N-nitroso-tigecycline	Tigecycline	1500 ng/day	/	1500 ng/day	CPCA	5
N-nitroso-timolol	Timolol	1500 ng/day	/	/	CPCA	5
N-nitroso-torsemide	Torsemide	1500 ng/day	/	/	CPCA	5
N-nitroso-trandolapril	Trandolapril	1500 ng/day	/	/	CPCA	5
N-nitroso-desmethyl trimebutine	Trimebutine	/	1500 ng/day	1500 ng/day	CPCA	5
N-nitroso-vibegron	Vibegron	1500 ng/day	/	/	CPCA	5
N-nitroso-vildagliptin	Vildagliptin	/	1500 ng/day	/	CPCA	5
N-nitroso-azaerythromycin	Azithromycin	/	NMI	NMI	neg. in-vivo mutagenicity test	/
N-nitroso-N-desmethyl azithromycin	Azithromycin	/	NMI	NMI	neg. in-vivo mutagenicity test	/
N-nitroso-hydrochlorothiazide	Hydrochlorothiazide	/	NMI	NMI	neg. in-vivo mutagenicity test	/
N-nitroso-quinapril	Quinapril	/	NMI	NMI	neg. in-vivo mutagenicity test	/
(S)-2-(((2'-(1H-tetrazol-5-yl)-[1,1'biphenyl]-4-yl)methyl)(nitroso) amino)-3-methylbutanoic acid	Valsartan	/	/	NMI	neg. in-vivo mutagenicity test	/
N-nitroso-duloxetine	Duloxetine	100 ng/day	100 ng/day	100 ng/day	SAR/NNK	/
N-nitroso-fluoxetine	Fluoxetine	/	100 ng/day	100 ng/day	SAR/NPYR	/
2-nitroso-octahydrocyclopenta(c)pyrrole	Gliclazide	/	1700 ng/day	/	SAR/NDPh	/
N-nitroso-mefenamic acid	Mefenamic acid	/	78000 ng/day	78000 ng/day	SAR/N-nitroso-	/

					piperidine	
N-nitroso-methylphenidate, NMPH	Methylphenidate	/	1300 ng/day	1300 ng/day	SAR/N-nitroso-piperidine	/
N-nitroso-paroxetine	Paroxetine	/	1300 ng/day	1300 ng/day	SAR/NMOR	/
N-nitroso-reboxetine	Reboxetine	/	127 ng/day	/	SAR/NTHP	/
7-nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo-[4,3- a]pyrazine	Sitagliptin	37 ng/day	37 ng/day	37 ng/day	SAR/NTHP	/
N-nitroso-varenicline	Varenicline	37 ng/day	37 ng/day	37 ng/day	SAR/NTHP	/

Other nitrosamines[5]	Source[1]	FDA	EMA	HC	AI derivation	Potency Category
1-cyclopropylmethyl-4-nitrosopiperazine	/	/	400 ng/day	/	CPCA	3
1-methyl-4-nitrosopiperazine, MNP/MeNP	(Rifampicin)[6]	96.0 ng/day[7]	400 ng/day	400 ng/day	SAR/NDMA; CPCA	3
1-nitroso-pyrrolopiperidine	/	/	1500 ng/day	/	CPCA	4
4-(methylnitrosoamino)-1-(3-pyridinyl)-1-butanone (NNK)	/	100 ng/day	100 ng/day	100 ng/day	compound-specific	/
nitroso impurity C" [N-(2,6-dimethylphenyl)-2-(4-nitrosopiperazin-1-yl)acetamide]	/	/	400 ng /day	/	CPCA	3
N-methyl-N-nitrosophenethylamine, NMPEA	/	/	8 ng/day	8ng/day	compound-specific	/
N-nitroso-1,2,3,6-tetrahydropyridine, NTHP	/	37 ng/day	37 ng/day	37 ng/day	compound-specific	/
N-nitroso-diethanolamine NDELA	/	/	1900 ng/day	1900 ng/day	compound-specific	/
N-nitroso-diethylamine, NDEA	/	26.5 ng/day	26.5 ng/day	26.5 ng/day	compound-specific	/
N-nitroso-diisopropylamine, DIPNA	/	26.5 ng/day	26.5 ng/day	26.5 ng/day	SAR/NDEA	/
N-nitroso-dimethylamine, NDMA	/	96.0 ng/day	96.0 ng/day	96.0 ng/day	compound-specific	/
N-nitroso-di-n-butylamine, NDBA	/	/	26.5 ng/day	26.5 ng/day	SAR/NDEA	/
N-nitroso-diphenylamine NDPh	/	/	78000 ng/day	78000 ng/day	compound-specific	/
N-nitroso-dipropylamine, NDPA	/	/	26.5 ng/day	26.5 ng/day	SAR/NDEA	/

N-nitroso-ethylisopropylamine, EIPNA/NEIPA/NIPEA	/	26.5 ng/day	26.5 ng/day	26.5 ng/day	SAR/NDEA	/
N-nitroso-pyrrolidine, NPYR	/	/	1700 ng/day	1700 ng/day	compound-specific	/
N-nitroso-morpholine, NMOR	/	/	127 ng/day	127 ng/day	compound-specific	/
N-nitroso-N-methyl-4-aminobutyric acid, NMBA	/	96.0 ng/day	96.0 ng/day	96.0 ng/day	SAR/NDMA;	/
N-nitroso-N-methylaniline, NMPA	/	26.5 ng/day	34.3 ng/day	/	SAR/NDEA; compound-specific	/
N-nitroso-p-chloro-benzylamino-pyridine (N-CBAP)	/	/	100 ng/day	/	CPCA	2
N-nitroso-piperazine (NPZ)	/	/	400 ng/day	400 ng/day	CPCA	3
N-nitroso-piperidine	/	/	1300 ng/day	1300 ng/day	compound-specific	/
N,N'-dinitrosopiperazine	Ciprofloxacin	/	/	400 ng/day	CPCA	3

Information sources: FDA nitrosamine guidance [14]; Table 1 and 2 on FDA website [9]; Appendix 2 of HC guidance [8] ; Appendix 1 to EMA guidance [7]

Total number of nitrosamine impurities with published AIs: 313 (290 NDSRIs and 23 other nitrosamines);
highlighted in yellow: different AIs based on different methods of AI derivation;
highlighted in green: full harmonized AIs between FDA, EMA and HC

[1] Source is the form of the drug substance in the free base or free acid form
[2] = N-(2-(benzhydryoxy)ethyl)-N-methylnitrous amide listed by HC
[3] Missing from the EMA and HC AI list although presented in EMA and HC guideline as example 8 for the application of the CPCA with corresponding AI of 18 ng/day
[4] CPCA method not specified by HC for AI derivation
[5] Not clearly falling under the current definition of NDSRIs
[6] Source only given by the EMA
[7] Provided in FDA public announcement from 28.01.2021

Status: August 2023

Annex IX: Comparison of AIs (ng/day) and their derivation methods until July/August 2023

N-Nitrosamine	FDA	EMA	HC	Gold TD_{50}[1] (mg/kg/day)	Lhasa TD_{50}[1] (mg/kg/day)	≥ 3 dose levels	AI derivation
NDMA (*N*-Nitrosodimethylamine)	96	96.0	96.0	0.0959	0.177	yes	Gold TD_{50}[1]
NDEA (*N*-Nitrosodiethylamine)	26.5	26.5	26.5	0.0265	0.0177	yes	Gold TD_{50}[1]
NMBA (*N*-Nitroso-*N*-methyl-4-aminobutyric acid)	96	96.0	96.0	0.982	/	no	SAR/NDMA
NMPA (*N*-Nitroso-*N*-methylaniline) = (*N*-Nitrosomethylphenylamine (FDA))	26.5	34.3	/	0.142	0.106	no	FDA: SAR/NDEA; EMA: Gold TD_{50} 0.0343 mg/kg/day[2,3]
NIPEA/EIPNA/NEIPA (*N*-Nitrosoethylisopropylamine) = (*N*-Nitrosoisopropylethlyamine (FDA)	26.5	26.5	26.5	No entry			SAR/NDEA
NDIPA/DIPNA (*N*-Nitrosodiisopropylamine)	26.5	26.5	26.5	No entry			SAR/NDEA
MeNP/MNP (1-Methyl-4-nitrosopiperazine)	/	26.5	96	No entry			EMA: SAR/NDEA HC: SAR/NDMA
NDBA (*N*-Nitroso-di-*n*-butylamine)	/	26.5	26.5	0.691	/	no	SAR/NDEA
NDPA (*N*-Nitrosodipropylamine)	/	26.5	26.5	0.816	/	no	SAR/NDEA
N-Nitrosopiperidine	/	1300	1300	1.3	1.12	yes	Gold TD_{50}[1]
NMOR (*N*-Nitrosomorpholine)	/	127	127.0	0.109	0.135	yes	TD_{50} 0.127 mg/kg/day[3]
NNK (4-(Methylnitrosoamino)-1-(3-pyridinyl)-1-butanone)		100	100.0	0.0999	0.142	yes	Gold TD_{50}
NMPH (*N*-Nitrosomethylphenidate)	/	1300	1300	Only negative results		no	SAR/N-Nitrosopiperidine
NTHP (*N*-Nitroso-1,2,3,6-tetrahydropyridine)	/	37	37.0	0.0601	0.0599	yes	TD_{50} 0.0374 mg/kg/day[3]
NNV (*N*-Nitrosovareniclin)	/	37.0	37.0	No entry			SAR/NTHP
7-Nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3- a]pyrazine	/	37	37.0	No entry			SAR/NTHP
NMPEA (*N*-Methyl-N-nitrosophenethylamine)	/	8	8.0	0.00998	0.00797	yes	Lhasa TD_{50}[1]
N-Nitrosonortriptyline	/	8	8.0	No entry			SAR/NMPEA
N-Nitrosorasagiline	/	18	18.0	No entry			Class specific TTC
N-Nitrosodabigatran	/	18	18.0	No entry			Class specific TTC
N-nitrosoduloxetine	/	100	100.0	No entry			SAR/NNK
N-nitroso-fluoxetine	/	100	100	No entry			SAR/NNK
N-nitroso-tamsulosin	/	/	18.0	No entry			Class specific TTC
N-nitrosoparoxetine	/	1300	1300	No entry			SAR/N-Nitrosopiperidine
NDPh *(N*-nitroso-diphenylamine)	/	78000	78000	167	/	no	TD_{50} 78.4 mg/kg/day[4] (lower boundary of the 99% CI)
N-nitroso-mefenamic acid	/	78000	78000	No entry			SAR/NDPh
NPYR (*N*-nitroso-pyrrolidine)	/	1700	/	0.679	2.02	yes	TD_{50} 1.7 mg/kg/day[3]
NDELA (*N*-nitroso-diethanolamine)	/	1900	1900	3.17	3.38	yes	TD_{50} 1.9 mg/kg/day[3]

Sources:[14–46–88]

AI derivation is based on information from EMA Q&A Document, Rev. 15 [46]; the criterion "≥ 3 dose levels" indicates a robust study situation (see Annex XII).

[1]Harmonic mean TD_{50}; most sensitive rodent species

[2]While the FDA gives a limit of 26.5 ng/day for NMPA derived by SAR/read-across approach using the TD_{50} of NDEA as point of departure [14], the EMA gives as a reference for the establishment of the AI of 34.3 ng/day the harmonic mean Gold TD_{50} [13]. However, in the LCPD, the harmonic mean Gold TD_{50} for NMPA is listed with 0.142 mg/kg/day [99], which would result in an AI of 142 ng/day. In fact, the lowest Gold TD_{50} value for NMPA, i.e. 0.0343 mg/kg/day, taken from the most robust dataset, results in an AI of 34.3 ng/day. The derivation of the harmonic mean TD_{50} value by Lhasa in contrast to the calculation by Gold did not include the studies in which tumors were detected at a single concentration. Only the most robust study in which tumor incidence was detected at two dose concentrations is included in the calculation of Lhasa. Consequently, the TD_{50} value (0.106 mg/kg/day) of Lhasa determined from this study corresponds to the harmonic mean Lhasa TD_{50}, but differs significantly from the value calculated by Gold for this study (0.0343mg/kg/day) [99]. Even though the EMA used the TD_{50} value of the most robust study for the calculation of the AI of NMPA, this study with only two dose concentrations is a less qualitative study according to ICH M7 and probably the reason why the FDA did not use it to determine NMPA's AI but indicates an AI of 26.5 ng/day based on read-across from NDEA.

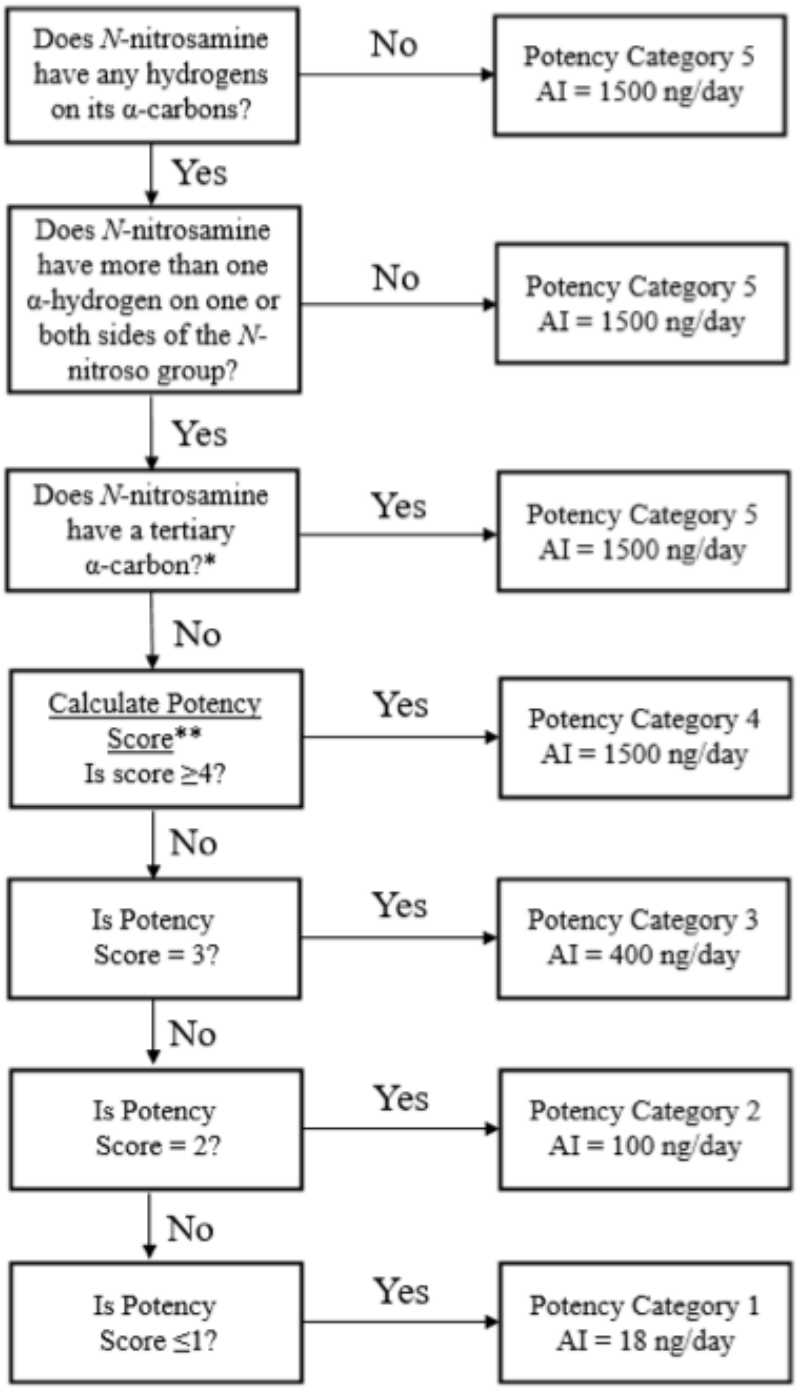
[3]Most sensitive Gold TD_{50} from most robust dataset

[4]The AI for NDPh is even based on the lower bound of the confidence interval (78.4 mg/kg/day) of the most sensitive TD_{50} of the most robust study [13–100]. This would actually result in an AI of 78400 ng/day, which was rounded down to 78000 ng/day. No TD_{50} value from Lhasa was obtained for NDPh due to the use of the lifetable method even though two dose concentrations were investigated in this study. In this respect, it is remarkable that the EMA, despite poorly robust studies, did not apply a SAR approach for NDPh as it was the case for the other nitrosamines with similar poor data. Instead, the EMA applied the worst-case TD_{50} of the most robust study available when deriving the AI for NDPh. This seems justified due to the fact that the EMA used NDPh as a surrogate compound for the derivation of the AI for N-nitroso-mefenamic acid based on a SAR/read-across approach. However, this practice remains controversial because it is not in line with the recommendations of ICH M7, which provides for a SAR approach only for substances with sufficiently robust carcinogenicity data [4]. Nevertheless, HC has included the EMA-derived AIs for NDPh and N-nitroso-mefenamic acid in the table of its guidance revision from April 2023 [88], thus following the EMA's approach.

Status: April 2023

Annex X: Tables and figures to predict the CPCA Potency Category

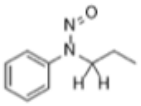
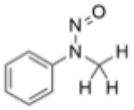
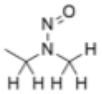
Figure 1. Flowchart to Predict the Carcinogenic Potency Category of an *N*-Nitrosamine



Source: EMA, Annex 2 [13]; the meaning of the two stars marking was adjusted

* A tertiary α-carbon is defined as an α-carbon atom in a sp^3 hybridization state, bonded to three other carbon atoms.
** **Potency Score** = α-**Hydrogen Score** + **Deactivating Feature Score** (sum all scores for features present in the N-nitrosamine) + **Activating Feature Score** (sum all scores for features present in the *N*-nitrosamine)

Table 1. Derivation of α-Hydrogen Score

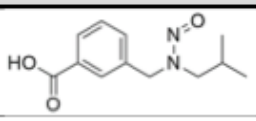
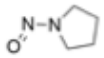
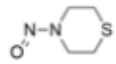
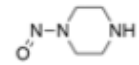
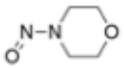
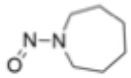
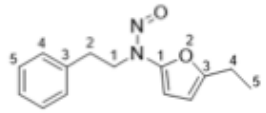
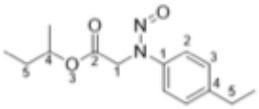
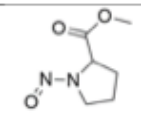
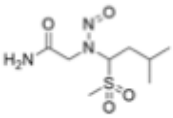
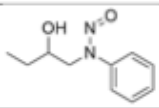
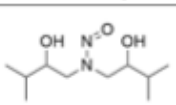
Count of Hydrogen Atoms on Each α-Carbon, Lowest First	Example	α-Hydrogen Score
0,2		3*
0,3		2
1,2		3
1,3		3
2,2		1
2,3		1

Source: EMA 2023, Annex 2 [13]

Count of hydrogen atoms on each α-carbon (lowest count first) and corresponding α-Hydrogen Score. Examples are intended to be illustrative only and are not intended to be exhaustive.

*A score of 3 applies when the methylene α-carbon is not part of an ethyl group. If the methylene α-carbon is part of an ethyl group, a score of 2 should be applied.

Table 2. List of deactivating features and associated scores

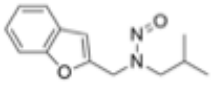
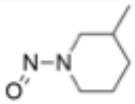
Deactivating Feature	Example	Individual Deactivating Feature Score
Carboxylic acid group anywhere on molecule		+3
N-nitroso group in a pyrrolidine ring		+3
N-nitroso group in a 6-membered ring containing at least one sulfur atom		+3
N-nitroso group in a 5- or 6-membered ring*		+2
N-nitroso group in a morpholine ring		+1
N-nitroso group in a 7-membered ring		+1
Chains of ≥5 consecutive non-hydrogen atoms (cyclic or acyclic) on both side of acyclic *N*-nitroso group. Not more than 4 atoms in each chain may be in the same ring.	 	+1
Electron-withdrawing group** bonded to α-carbon on only one side of *N*-nitroso group (cyclic or acyclic)		+1
Electron-withdrawing groups** bonded to α-carbons on both sides of *N*-nitroso group (cyclic or acyclic)		+2
Hydroxyl group bonded to β-carbon*** on only one side of *N*-nitroso group (cyclic or acyclic)		+1
Hydroxyl group bonded to β-carbon*** on both sides of *N*-nitroso group (cyclic or acyclic)		+2

Source: EMA 2023, Annex 2 [13]

*Excludes examples where N-nitroso group is in a pyrrolidine ring, a 6-membered ring containing at least one sulfur atom or a morpholine ring (all counted separately).
**Excludes carboxylic acid and aryl (counted separately), and ketone (conflicting data). Additional electron withdrawing group examples are limited to those described in Cross KP and Ponting DJ, 2021, Developing StructureActivity Relationships for N-Nitrosamine Activity, Comput Toxicol, 20:100186, where they are referred to as "βcarbon electron withdrawing groups."
***β-Carbon must be in an sp3 hybridization state for this feature to apply.

To calculate Deactivating Feature Score, sum the individual scores for all listed features present in the *N*-nitrosamine structure. Each deactivating feature row in the table may only be counted once. For *N*-nitrosamines where the *N*-nitroso group is within more than one ring, the feature score for only the smallest matching ring should be applied. Examples are intended to be illustrative only and are not intended to be exhaustive.

Table 3. List of activating features and associated scores

Activating Feature	Example	Individual Activating Feature Score
Aryl group bonded to α-carbon (i.e., benzylic or pseudo-benzylic substituent on *N*-nitroso group)		-1
Methyl group bonded to β-carbon (cyclic or acyclic)		-1

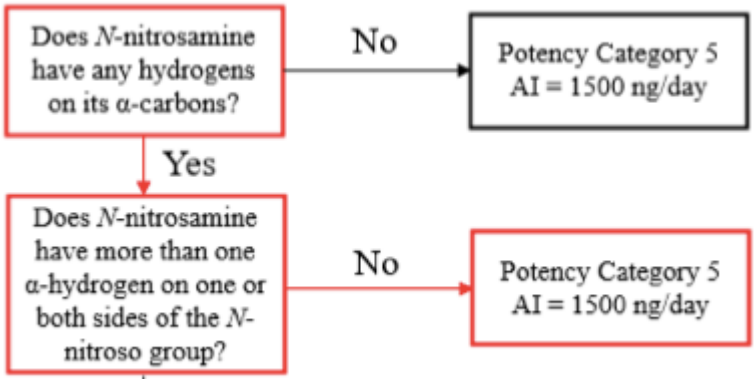
Source: EMA 2023, Annex 2 [13]

To calculate Activating Feature Score, sum the individual scores for all listed features present in the *N*-nitrosamine structure. Each activating feature row in the table may only be counted once.
Examples are intended to be illustrative only and are not intended to be exhaustive.

Annex XI: Examples for CPCA application

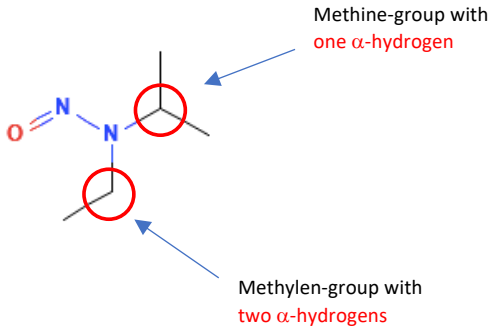
Example 1. CPCA application for *N*-Nitroso-enalapril

Potency Category 5	**AI = 1500 ng/day**

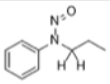
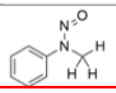


Source: Extracts from EMA Q&A document, Annex 2, Appendix B, Example 2 [8]

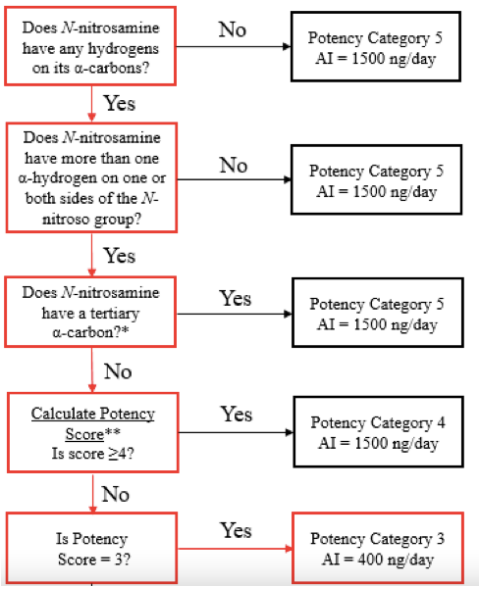
Example 2. Theoretical CPCA application for NEIPA



Own illustration based on chemical structure sourced from: https://pubchem.ncbi.nlm.nih.gov/compound/27824#section=2D-Structure, accessed 17.09.2023

Count of Hydrogen Atoms on Each α-Carbon, Lowest First	Example	α-Hydrogen Score
0,2		3*
0,3		2
1,2		3
1,3		3
2,2		1
2,3		1

Source: Modified extract from EMA Q&A document, Annex 2, Appendix A [13]



As no deactivating or activating features are present in NEIPA, the α-hydrogen score corresponds to the potency score.

Source: Extract from EMA Q&A document, Annex 2, Appendix B, Example 6 [13]

Annex XII: Criteria for carcinogenicity studies of lesser quality acc. to ICH M7

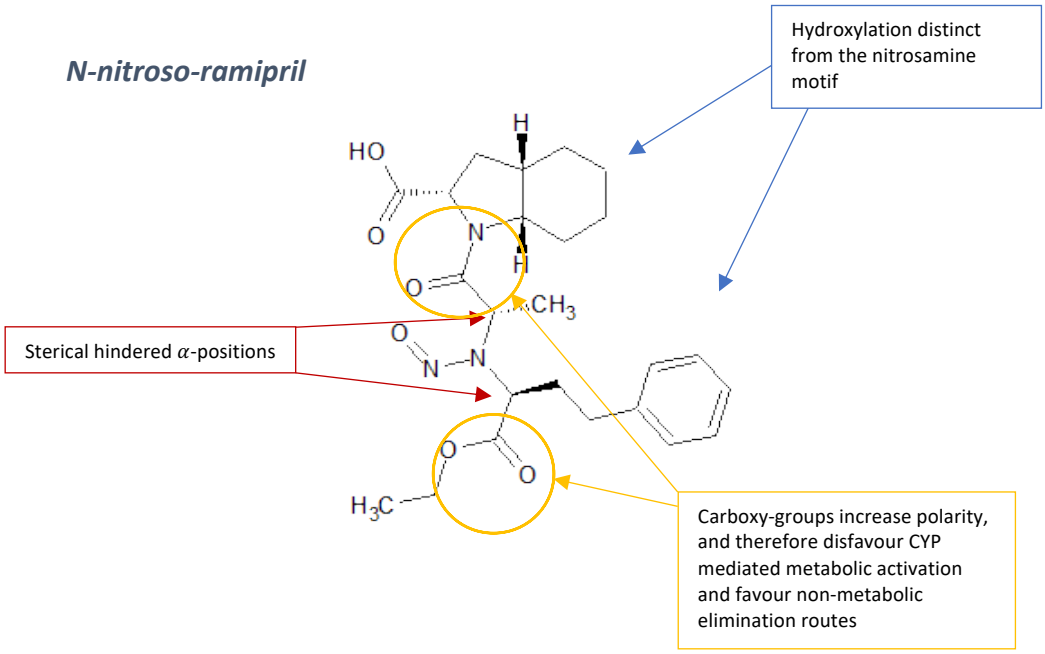
- < 50 animals per dose per sex;
- < 3 dose levels;
- Lack of concurrent controls;
- Intermittent dosing (< 5 days per week);
- Dosing for less than lifetime.

Source: ICH M7 guideline [4]

Annex XIII: SAR considerations for N-nitroso-ACE-inhibitors

Nudelman and Czich [5] first analyzed the typical structural elements in nitroso derivatives of ACE inhibitors and identified steric hindrance at α-positions to the nitrosamine motif and carboxy groups (carboxamide and carboxylate) at β-positions, increasing polarity and thus disfavour CYP-mediated metabolism, as structural features that prevent the key reaction sequence leading to the high carcinogenic potency of small dialkyl-nitrosamines, starting with the CYP 450-mediated hydroxylation of the α-position to the nitrosamine up to the formation of stable DNA alkylating diazonium and carbonium ions which are able to form DNA adducts.

For N-nitroso-enalapril and N-nitroso-ramipril the authors show visually in a 3D model that both α-positions of the nitroso derivatives are strongly sterically hindered [5]. Thus, leading to the conclusion that metabolic α-hydroxylation is highly unlikely. Furthermore, even if a reactive ion were to form, it would also be large and bulky, which would prevent the binding of its reactive centre (ion structure) to the DNA.



Source: own illustration based on EFPIA position paper [5] and https://acanthusresearch.com/products/drug-impurities-reference-standards/ramipril-n-nitroso/, accessed 17.06.2023

In vitro and in silicone analyses of the metabolism of N-nitroso-ramipril combined with quantum chemical calculations have shown that hydroxylations take place at other sites of the molecule, such as the toluene structure or the octahydrocyclopenta-pyrrole, but not at the α- or β-position of the nitroso group [5].

Based on the above considerations, Nudelman and Czich proposed to exclude N-nitroso-ACE-inhibitors from the CoC.

Annex XIV: Comparison between Standard OECD Ames and Enhanced Ames Test conditions

Condition	Standard OECD Ames test*	Enhanced Ames test**
Tester strains	S. typhimurium • TA98 • TA100 • TA1535 • TA1537 or TA97 or T A97a E. coli • WP2 uvrA or WP2 uvrA (pKM101) or S. typhimurium TA102	S. typhimurium • TA98 • TA100 • TA1535 • TA1537 E. coli • WP2 uvrA (pKM101)
Protocol	(20 mins incubation time) or plate incorporation	Preincubation (30 mins incubation time).
Metabolic activation	5-30% S9 prepared from the livers of rodents treated with enzyme-inducing agents such as Aroclor 1254 or a combination of phenobarbitone and ß-naphthoflavone, and in the absence of S9.	30% rat liver S9, 30% hamster liver S9, as well as in the absence of S9. S9 should be prepared from rodents treated with inducers of cytochrome P450 enzymes (e.g., a combination of phenobarbital and β-naphthoflavone).
Solvent/Negative control	Water/organic solvent.	Water/organic solvent (the lowest possible volume should be included in the pre-incubation mixture with justification to indicate that the volume of solvent does not interfere with metabolic activation of the *N*-nitrosamine).
Positive control	Concurrent strain-specific positive controls.	In addition to concurrent strain specific positive controls, two *N*-nitrosamines that are known to be mutagenic in the presence of S9 should be included, the choice of which should be justified based on the anticipated metabolism of the *N*-nitrosamine and the cytochrome P450 enzymes most likely involved.

Source: Lhasa Limited, 2023 [141]

*OECD Guideline No. 471 [142]

** EMA guidance (Annex 3) [13], HC guidance (Annex 3) [8], FDA (Recommended Safety Testing Methods for NDSRIs) [9]

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, die Arbeit selbstständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet zu haben.

_____	_____
Ort, Datum	Unterschrift