Nitrosamine Risk Management for Medicinal Products: WHO's Role and a Case Study of Camzyos®

Masterarbeit

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Abbreviations

Ac	-acetate
ADI	Acceptable daily intake
AI	Acceptable intake
AIBL	Ab Initio Bond Lengths
API	Active pharmaceutical ingredient
APIMF	Active Pharmaceutical Ingredient Master File
CoC	Cohort of concern
CPCA	Carcinogenic Potency Categorization Approach
CPNP	1-Cyclopentyl-4-nitroso-piperazine
CYP	Cytochrome P450
DIPNA	N-nitroso-diisopropylamine
EC	European Commission
ECSPP	WHO Expert Committee on Specifications for Pharmaceutical Preparations
EDQM	European Directorate for the Quality of Medicines & HealthCare
EFPIA	European Federation of Pharmaceutical Industries and Associations
EIPNA	Nitroso-ethylisopropylamine
EMA	European Medicines Agency
EML	WHO Model List of Essential Medicines
EOI	Expression of Interest for Product Evaluation
EPAR	European Public Assessment Report
Et	Ethyl-
FAQ	Frequently Asked Questions
FDA	U.S. Food and Drug Administration

FP	Finished product
FPP	Finished pharmaceutical product
GLP	Good laboratory practice
HCT	Hydrochlorothiazide
HPLC-UV	High-performance liquid chromatography-ultraviolet
IARC	International Agency for Research on Cancer
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IN	Immediate notification
LCDB	Lhasa Carcinogenicity Database
LC-MS	Liquid chromatography–mass spectrometry
LOQ	Limit of quantification
LTL	Less-than-lifetime
MA	Marketing authorization
MAH	Marketing authorization holder
MNP	1-Methyl-4-nitroso-piperazine
NA	Nitrosamine
NAP	Nitrosation assay procedure
NDEA	N-nitroso-diethylamine
NDSRI	Nitrosamine drug substance related impurity
NMBA	N-nitroso-N-methyl-4-aminobutyric acid
NMDA	N-nitroso-dimethylamine
NMI	Non-mutagenic impurity
NMP	1-methyl-4-nitroso-piperazine

NNA	N-Nitrosamine
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNM	N-nitroso-mavacamten
NNN	N-nitroso-nornicotine
NOC	N-nitroso compound
NTTP	7-Nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyrazine
oHCM	Obstructive hypertrophic cardiomyopathy
OMCL	Official Medicines Control Laboratory
Ph. Eur.	European Pharmacopoeia
ppb	Parts per Billion
ppm	Parts per Million
PQP	WHO Prequalification of Medicines Programme
PQT/MED	WHO Prequalification Unit – Medicines Assessment Team
Q&A	Questions and Answers
QCL	Quality control laboratory
QM	Quantum Mechanics
REMPAN	Radiation Emergency Medical Preparedness and Assistance Network
SAR	Structure activity relationship
SmPC	Summary of Product Characteristics
TCM	Traditional Chinese Medicine
TD50	Median toxic dose
TEBAC	Triethylbenzylammonium chloride
TTC	Threshold of Toxicological Concern

USP	United States Pharmacopeia
UV-VIS	Ultraviolet–visible
WFI	Water for Injection
WHO	World Health Organization

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1 Introduction

1.1 Background

N-nitrosamine impurities (hereafter simply referred to as nitrosamines, NAs or NNAs) have been presenting a significant challenge to the pharmaceutical industry and regulatory bodies for six years now, as they are a class of organic compounds characterized by the nitroso functional group (–NO), which are known or suspected carcinogens in humans. Primarily nitrosamines have been associated with environmental pollutants, such as tobacco smoke and certain foodstuffs. However, in mid-2018 the detection of N-nitroso-dimethylamine (NMDA) and N-nitroso-diethylamine (NDEA) in valsartan drug substance prompted intensified scrutiny of the potential presence of nitrosamines in pharmaceuticals from regulatory authorities and industry stakeholders. (1, 2)

The crisis deepened as subsequent recalls and investigations uncovered additional instances of nitrosamine contaminations in various classes of medication, raising significant concerns among healthcare providers, patients, and regulatory authorities due to the potential carcinogenicity of these compounds and the widespread use of the affected medications. Investigations revealed that nitrosamines can form when a nitrosatable amine group and nitrosating agents, frequently derived from inorganic nitrite, are combined under favourable conditions, particularly during the synthesis of the active pharmaceutical ingredient (API). (1)

Regulatory agencies worldwide issued guidelines and recommendations for pharmaceutical manufacturers to undertake a three-step mitigation process in which they were expected to review and test their products for the presence of nitrosamine impurities and, if necessary, initiate measures to remove or reduce them to ensure patient safety. (3)

While initially only simple dialkyl-nitrosamines like NDMA and NDEA were considered, from 2021 attention gradually shifted to the nitrosamine drug substance related impurities (NDSRIs), (4–8) which are directly associated to the chemical structure and synthesis of the active pharmaceutical ingredient itself and are therefore difficult to prevent. An in-silico analysis conducted by Schlingemann et al. in late 2022 projected that approximately 40% of APIs could be susceptible to NDSRI formation. (9) 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 therapeutic diversity.

Recently updated regulatory guidance in May 2024 provides comprehensive lists of confirmed and potential NDSRIs and their associated AIs. However, data on the mutagenicity and carcinogenicity of NDSRIs remain generally unavailable. (10, 11) To support marketing authorization holders (MAHs) in implementing the three-step mitigation process, guidelines for the control of nitrosamine impurities have been published by various competent authorities and were updated several times over the past six years. (1, 2, 10, 12–15, 11)

While the differing carcinogenic potency of NDSRIs and simple dialkyl-nitrosamines is now better reflected in the guidelines by the application of the Carcinogenic Potency Categorization Approach (CPCA), (11) there are still 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.

1.2 Purpose of this thesis

Even though many recommendations of regional guidelines have already been harmonized among the competent authorities there are still important differences in the regulatory treatment of medicines affected by nitrosamine impurities. These differences lead to uncertainties for regulators and the pharmaceutical industry and thus may prolong regulatory procedures, which, in the worst case, could endanger therapeutic diversity. As the World Health Organization (WHO) describes itself as the “United Nations agency that connects nations, partners and people to promote health, keep the world safe and serve the vulnerable – so everyone, everywhere can attain the highest level of health”, (16) one might reasonably expect the WHO to assume the responsibility of harmonising and standardizing the regulatory framework globally concerning nitrosamine impurities, ensuring higher quality and safety in pharmaceutical products and finally maintaining therapeutic diversity.

The purpose of this thesis is therefore to investigate the role of the WHO during the last six years during the nitrosamine crisis and to critically scrutinize the WHO’s activities in

evaluating the associated risks, in offering guidance for the pharmaceutical industry, patients and regulatory bodies and in implementing mitigation measures.

In this context it should also be demonstrated how a thorough nitrosamine risk assessment on the basis of a concrete example could be conducted, taking into account global marketability and emphasizing the need for harmonization of regulatory frameworks to ensure pharmaceutical quality, safety, and therapeutic diversity.

2 Material and methods

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 announcements of nitrosamine detections and resulting drug recalls a review of websites of the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) was conducted.

To evaluate the WHO's activities in response to nitrosamines over the last six years, especially with regard to essential drugs and prequalified medicinal products its press releases, WHO Model Lists of Essential Medicines (hereafter referred to as Essential Medicines Lists or EMLs) and lists of prequalified finished pharmaceutical products (FPPs) and APIs were analysed.

To perform the theoretical risk assessment for the newly approved medicinal product Camzyos® the relevant guidelines of the EMA and FDA were consulted. Furthermore, information was gathered from publicly available documents like the European Public Assessment Report (EPAR) and the Summary of Product Characteristics (SmPC).

Additionally, other information regarding nitrosamine impurities published in scientific literature was also considered by accessing public databases, including Google Scholar, PubMed®, ScienceDirect®, ResearchGate and Bonn University and State Library.

Molecular structures were drawn individually with ChemDraw® Prime v23.

In this master thesis, the terms *pharmaceutical*, *medicine*, *medicinal product*, and *drug product* are employed interchangeably.

Due to the current prominence of addressing nitrosamine impurities in medicinal products, regulatory requirements are subject to potential changes. Consequently, the latest modifications integrated into the examined guidelines may not have been considered during the completion of this master thesis.

3 Nitrosamine impurities: Structure, formation, and carcinogenicity

Nitrosamines, or more formally N-Nitrosamines, are organic substances having the chemical formula $R_1R_2N\text{-}N{=}O$, where $R_{1/2}$ is often an alkyl group. They contain a nitroso group (NO^+) coupled to a deprotonated amine *(Figure 1).* (17)

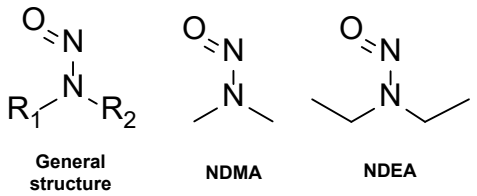


Figure 1-General structure of nitrosamines and two typical examples NDMA and NDEA

N-Nitrosamines are typically formed through the N-nitrosation of NH-containing compounds (e.g., typically secondary amines), involving the formation of an N–N bond via reaction with an nitrosonium ion ([NO^+]), which is electrophilic and undergoes nucleophilic attack by an amino compound (*Figure 2*).

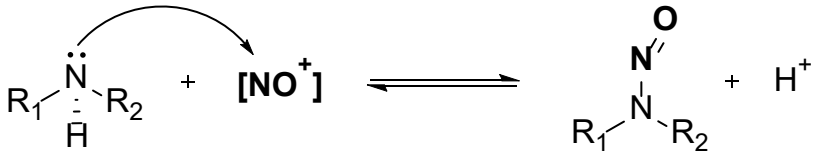


Figure 2-Nitrosation pathway

Any NH-containing compound is potentially vulnerable to electrophilic N-nitrosation, which can occur under various specific conditions. (18) The parade example for a nitrosating agent is nitrite (NO_2^-). Under acidic conditions it forms nitrous acid (HNO_2) which can lead to the nitrosonium ion. In many cases, nitrosation occurs under acidic conditions. However, nitrosation has also been observed under neutral or basic conditions, for example, when catalysed by a carbonyl group. Moreover, the likelihood of nitrosamine formation typically rises with elevated temperatures. (19)

Since the discovery of unacceptable levels of N-nitroso-dimethylamine (NDMA) and N-nitroso-diethylamine (NDEA) (*Figure 1*) in valsartan drug substance from different manufacturers from mid-2018 onwards nitrosamines have drawn increased attention resulting in several drug recalls and new regulatory guidance all over the world. Initially known only for

its high toxicity and used for several industrial applications, NDMA was found to be hepatocarcinogenic in rats by John Barnes and Peter Magee in 1956. (20, 21) Following this observation, the carcinogenicity of several N-nitroso compounds was extensively investigated and reported by Druckrey et al. (1967) and was reviewed by Magee & Barnes (1967). (22, 23) Reviews on their toxicity, carcinogenicity and metabolism, teratogenicity and mutagenicity were also published. (24)

In 1977, aminophenazone (i.e. amidopyrine) preparations were withdrawn from the market (25), because API batches were contaminated with NDMA. NDMA formation from aminopyrine was previously discovered in-vitro and in-vivo by Lijinsky et al. and associated with liver tumours in rats. (26) As a result, the aminophenazone monograph was removed from pharmacopoeias, including the European Pharmacopoeia (Ph. Eur.). Following these findings, investigation into N-nitrosamine impurities in other APIs and finished products (FP) were conducted. (25, 27–30)

Today we know that most nitrosamines are carcinogenic, and several are genotoxic. Several epidemiological studies have detailed the impact of nitrosamines on the risk of developing esophageal, colon, hepatocellular, and other types of cancers. (31–33)

Although not all nitrosamines are mutagenic, understanding their structures and toxicity levels is crucial for effective risk assessment. Based on their chemical structures and origins, nitrosamine impurities can be categorized into three groups:

- Dialkylnitrosamines: These relatively small compounds, such as NDMA and NDEA, often originate from reagents or solvents used in the API manufacturing process, API process impurities, or the degradation of products containing a secondary functional amine group. Consequently, the same nitrosamine can be found in a variety of APIs or drug products.
- Related-Substance-Derived Nitrosamine Impurities (RSNIs): These impurities arise from amine-containing by-products or intermediates formed during drug substance synthesis, or from the degradation products of APIs containing amine groups. Therefore, this group of nitrosamines resembles related substance impurities.
- Drug-Substance-Derived Nitrosamine Impurities (NDSRIs): These impurities are directly generated from the drug substances themselves. (34)

The ICH M7 guideline (hereafter referred to as ICH M7) delineates the evaluation and regulation of mutagenic impurities in pharmaceutical products concerning their carcinogenic potential. Within this framework the Threshold of Toxicological Concern (TTC) defines an acceptable patient intake for any unstudied chemical posing a negligible risk of carcinogenicity or other toxic effects. The TTC is extrapolated linearly from preclinical TD_{50} values, representing the dose at which the probability of remaining tumour-free after chronic administration for the standard lifespan would be halved, derived from experimental tumour incidence data. It is set at 1.5 µg/day for pharmaceuticals. N-nitrosamines are considered to pose a significant carcinogenic risk at intakes below the TTC, which is why they are grouped within the cohort of concern (CoC) of the ICH M7. This CoC contains high potency mutagenic carcinogens, defined by the presence of particular structural alerts just as the N-nitroso moiety in case of nitrosamines. (35) The evidence regarding the carcinogenicity of nitrosamines primarily stems from rodent studies, which have limited applicability to humans owing to species-related differences. Therefore, the evidence for the carcinogenicity of nitrosamines in humans requires further research. (1)

The carcinogenicity of nitrosamines primarily arises from their metabolic activation, which leads to the formation of highly reactive intermediates that can interact with DNA, causing mutations. Empirical and computational evidence indicate that the nitrosamine first undergoes enzymatic α-hydroxylation with cytochrome P450 (CYP) via a radical intermediate and subsequently forms the dealkylated primary nitrosamine. The unstable primary nitrosamine further decomposes to diazonium, a DNA alkylating agent. The resulting DNA damage can lead to cancer.

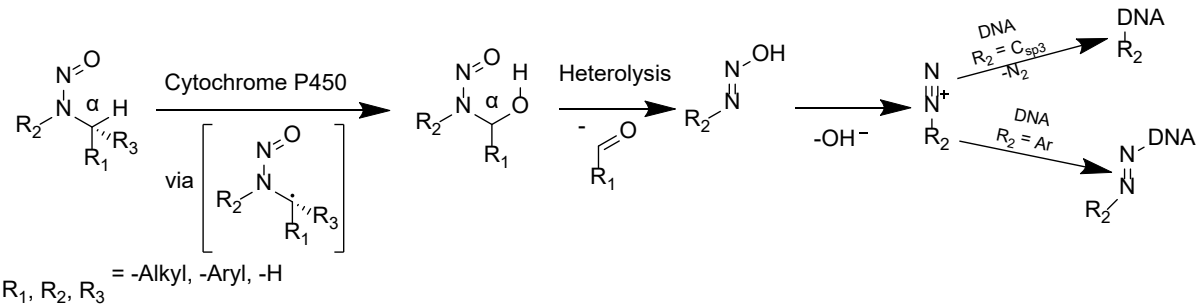


Figure 3-Metabolic activation and DNA adduct formation of nitrosamines

Alkyl diazonium ions can interact with DNA through either an S_N1 reaction involving the loss of nitrogen (N_2) followed by the reaction of the carbocation with DNA, or a S_N2 reaction, where nitrogen is directly displaced by a nucleophile on the DNA. Aryl diazonium ions cannot undergo either S_N1 or S_N2. Instead, they initially form a diazo adduct, which may subsequently rearrange via an S_NAr mechanism. However, the metabolic activation step is consistent no matter the class of nitrosamine. There are competing sites of CYP metabolism (e.g., β-carbon), and other reactive species can form following initial bioactivation, although these alternative pathways tend to decrease rather than enhance carcinogenic potency. The persistence of DNA damage and the failure of repair mechanisms contribute to the mutagenic and carcinogenic potential of nitrosamines. (36, 37, 9)

4 World Health Organization

4.1 Key activities and responsibilities

The World Health Organization (WHO) is a specialised agency of the United Nations established on 7 April 1948. It acts as a coordinating authority on international public health and deals with health, sanitation, and diseases. (38) Its tasks include the worldwide coordination of national and international activities in the fight against communicable diseases such as AIDS, malaria, SARS, tuberculosis and the coronavirus (e.g. it sends medical teams to help combat epidemics), the launch of global vaccination programs, including for the prevention of pandemics, and programs against health risk factors such as smoking or obesity, the regular collection and analysis of global health and disease data. Its responsibilities also include the support for the development of the most effective and affordable health systems in developing countries, supervising the Programme for International Drug Monitoring, (39) the creation of a model list of essential medicines, publishing the World Health Report on global health care and existing disease problems and providing medical assistance in radiological emergencies through the Radiation Emergency Medical Preparedness and Assistance Network (REMPAN). (40)

A key area of responsibility is to develop, harmonize and enforce guidelines, standards, and methods in health-related areas worldwide. The WHO Constitution provides three instruments for this: international treaties, regulations directly based on the WHO Constitution and non-binding recommendations. (41)

4.2 Essential drugs

The WHO's concept of essential drugs developed in 1977 is outlined in the WHO Model List of Essential Medicines (EMLs), which plays a significant role in global healthcare by identifying the medications considered to be most effective and safe to meet the most important needs in a health system. The list, which is regularly updated, is designed to guide countries in establishing their own lists of essential medicines, taking into account their specific health priorities, available resources, and epidemiological profiles. (42) While most medications on the list are available as generic products, being under patent does not preclude inclusion. (43)

The concept of essential drugs, as defined by the WHO, refers to medications that are considered crucial for meeting the basic healthcare needs of a population. These drugs are selected based on their efficacy, safety, and cost-effectiveness, and they are intended to be available in sufficient quantities, in appropriate dosage forms, and at affordable prices.

The key principles and objectives of the WHO's essential drugs concept include:

- Access to Basic Healthcare: Essential drugs are selected to ensure that individuals and communities have access to the most necessary medications for treating common health conditions.
- Promotion of Rational Drug Use: The list aims to promote the rational use of drugs by emphasizing medications with proven efficacy, safety, and cost-effectiveness.
- Affordability: Essential drugs should be affordable to individuals and healthcare systems, contributing to the goal of universal health coverage.
- Public Health Impact: The selection of essential drugs is based on their potential to address major public health issues, such as infectious diseases, maternal and child health, and chronic conditions.
- Sustainability: The list is regularly updated to incorporate new therapeutic options and remove outdated or less effective drugs, ensuring that healthcare systems adapt to evolving medical knowledge.
- Quality and Safety: Essential drugs must meet quality standards to ensure their safety and effectiveness. The WHO provides guidance on quality assurance and regulatory aspects.

By promoting the use of essential drugs, the WHO aims to improve the availability and affordability of key medications, particularly in resource-limited settings, and contribute to the overall enhancement of healthcare systems worldwide. The Model List of Essential Medicines is a valuable tool for countries in prioritizing and planning their pharmaceutical procurement, distribution, and use. (44)

4.3 Prequalification of medicinal products

The WHO Prequalification of Medicines Programme (PQP), established in 2001, aims to ensure the quality, safety, and efficacy of essential health products, particularly in low- and middle-income countries. Health products are either immunization devices, in vitro

diagnostics, medicinal products, vaccines, or vector control products. Within the PQP, the WHO Prequalification Unit – Medicines Assessment Team (PQT/MED) plays a critical role. It is responsible for the prequalification process, which involves the assessment of product dossiers for FPPs or master files for APIs, inspections of manufacturing and clinical sites, and organization of quality control testing. The standards for evaluating FPPs, APIs, and their manufacturing sites are derived from principles and practices established by the world's leading regulatory agencies and endorsed by the WHO Expert Committee on Specifications for Pharmaceutical Preparations (ECSPP). The PQT/MED also prequalifies quality control laboratories (QCLs) and offers training, technical assistance, and accelerated registration procedures. Detailed guidance and prequalification reports are provided to support manufacturers, regulators, and procurement agencies in ensuring the production of quality-assured medicines. This team collaborates closely with national regulatory authorities, manufacturers, and other stakeholders to support capacity building and regulatory harmonization. (30) The current scope covers HIV/AIDS, malaria, tuberculosis, reproductive health, hepatitis, diarrhoeal diseases and selected neglected tropical diseases. It recently initiated prequalification of biotherapeutic products: selected products to treat certain types of cancer, and human insulin for diabetes. (31)

FPP refers to the final medicinal product that is ready for patient use. In the context of the PQP, the Medicines Assessment team evaluates the FPP based on its formulation, manufacturing process, stability, and packaging. This evaluation ensures that the FPP consistently meets required standards throughout its shelf life. Manufacturers submit a comprehensive dossier detailing the FPP's development, production, and quality control processes as part of the prequalification process. (45)

The Active Pharmaceutical Ingredient Master File (APIMF) provides detailed information about the API used in the FPP. It includes data on the synthesis, characterization, and quality control of the API. The PQT/MED assesses the API separately to ensure its quality and consistency before it is used in the FPP. This separate evaluation allows the API manufacturer to submit confidential information directly to the PQP without disclosing proprietary details to the FPP manufacturer. The quality of the API is then integrated into the overall assessment of the FPP, ensuring that both components meet international standards. (46)

Medicinal products are included into the WHO List of Prequalified Medicinal Products after the data submitted for an invited product has been evaluated, and relevant sites have been inspected by WHO and are considered to meet WHO prequalification. (47)

The prequalification process for pharmaceutical products begins with their inclusion in an Invitation to Manufacturers to Submit an Expression of Interest for Product Evaluation (EOI), issued by the WHO. These EOIs are categorized by therapeutic area, such as HIV/AIDS, TB, malaria, and COVID-19, following consultation with WHO disease programs and clinical specialists. The products invited for evaluation are deemed crucial by WHO experts for effective treatment and expansion of treatment programs or ensuring reproductive health. Typically, these products are already listed in the EML or treatment guidelines, though exceptions may occur during public health emergencies when unlisted products are deemed vital for addressing treatment needs. (47)

An evaluation of these activities showed that WHO's prequalification programme led to a greater donor-funded market volume of around US$ 3.5 billion of health products, to production innovations and development like HIV self-testing diagnostics and to higher production standards in low- and middle-income countries. (48)

4.4 Regulatory history and response to nitrosamines

About 25 % (24,4%) of the APIs listed in the 2021 WHO EML are potential nitrosamine precursors. (17) According to own calculations this remains valid for the 2023 WHO EML (25,3%) (for calculation please refer to *Annex IV: Analysis of APIs listed in the 23rd WHO EML (2023) for nitrosamine precursors*)**.** Since medications listed on the EML are widely used and distributed, any recall associated with nitrosamine contamination can have significant implications for healthcare systems globally. Consequently, this raises the question to what extent the WHO has dealt with nitrosamine impurities over the last six years to equally maintain therapeutic diversity worldwide and provide safe medicinal products. However, the WHO's response to nitrosamine contamination started at an earlier stage:

Triggered by substantial evidence that nitrosamines can be formed via the reaction of nitrite and amines under acidic conditions, (49) the International Agency for Research on Cancer (IARC), an intergovernmental agency forming part of the WHO, launched a comprehensive research program on nitrosamines. (50) The IARC proposed a nitrosation assay

procedure (NAP) for assessing the reactivity of nitrogen-containing drugs to nitrosating agents simulating in vivo formation of nitrosamines in the stomach (please refer to *Annex I: Nitrosation assay procedure (NAP test) (WHO, 1978)*). It is designed as an in vitro forced degradation test with fourfold excess nitrite in acidic solution. However, it has not been included in the drug discovery process ever since, as selective and sensitive analytical techniques were not commonly available at that time. (51) Lately, the NAP test has emerged as the first prospective method in drug development for identifying and screening the potential for nitrosation of a target drug substance, using all relevant reagents in synthetic reactions. In a study, thirty-three pharmaceuticals showed suspected signals at the exact masses of their corresponding N-nitroso compounds (NOCs) after interaction with nitrite. (52)

Nitrosamines were categorized as probable or possible carcinogens by the IARC. They have been classified into four groups: (50)

- Group 1 compounds, like N-nitroso-nornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), have sufficient incidences of carcinogenic effects on humans.
- Group 2A compounds, such as NDMA, NDEA are probably carcinogenic to humans, with limited evidence in humans but sufficient evidence in animals.
- Group 2B compounds, including 1-methyl-4-nitroso-piperazine (NMP) and 1-cyclopentyl-4-nitroso-piperazine (CPNP), are possibly carcinogenic to humans, with limited evidence in both humans and animals.
- Compounds in Group 3, like N-nitroso-diphenylamine and N-nitroso-guvacoline, have inadequate data on their carcinogenicity.

Following the detection of NDMA in valsartan API in June 2018, global recalls of affected batches of valsartan-containing medications were initiated. (53) In response to this, the EMA promptly initiated a referral under Article 31 of Directive 2001/83/EC to evaluate the risk of nitrosamine impurities in these Angiotensin II receptor blockers. Shortly thereafter, NDMA was also detected in valsartan API from various other sources. Additionally, NDEA was found in valsartan API and other sartan APIs manufactured by different companies, prompting the extension of the Article 31 referral to include all sartan medications.

In early 2019 new findings emerged. For instance, NDMA was detected in pioglitazone, followed by its discovery in ranitidine and nizatidine medications. (2, 54) This indicated that nitrosamine impurities were not limited to a specific class of medicines, necessitating a broader evaluation through an Article 5(3) procedure of Regulation (EC) No 726/2004. (3) Concurrently, an additional Article 31 referral was initiated to assess ranitidine medicines, prompting pharmaceutical companies worldwide to voluntarily recall these products. (55–57)

In the same month the PQT/MED responded to the international nitrosamine contamination findings on its website for the first time. (58) It was explained that the PQT/MED was actively monitoring announcements from major regulators regarding nitrosamine impurities in sartan-related products, pioglitazone, and ranitidine. The risk of nitrosamine formation in prequalified products was regarded as generally low. Furthermore, manufacturers were recommended to follow the advice from the major regulators. Appropriate actions in response to the nitrosamine issue were being considered by the PQT/MED.

2 weeks later the PQT/MED published an information note on nitrosamine impurities on its webpage, (59) providing some general information about formation and toxicity of nitrosamines and explaining how the EMA, FDA, other regulatory agencies and companies have been responding to the nitrosamine issue. A short reference to the test methods on nitrosamines published by the FDA and the Official Medicines Control Laboratories (OMCLs) Network of the Council of Europe was also given. At the end of the note the WHO made the following recommendations to regulatory agencies: Nitrosamine levels in products like sartans and ranitidine should be actively verified through national testing laboratories or supplier declarations, using appropriate methods. They should require manufacturers to conduct risk assessments to identify sources of contamination and establish limits to control nitrosamine impurities and also take steps to ensure future nitrosamine levels are at a minimum. For this transitional period, it was recommended to be guided by the interim allowable daily intake limits for NDMA, NDEA, N-nitroso-N-methyl-4-aminobutyric acid (NMBA), N-nitroso-diisopropylamine (DIPNA) and -nitroso-ethylisopropylamine (EIPNA), adopted by most major regulators to prevent supply disruptions until measures are in place to reduce nitrosamine levels to the AI for lifetime exposure. (14, 15) The determination of interim allowable daily intake limits for other nitrosamines should be based on the ICH M7. (35)

These interim limits or less-than-lifetime (LTL) limits are grounded in the principle that the cancer risk from a low-dose lifetime exposure is equivalent to the cancer risk from the same cumulative exposure over a shorter duration. Thus, higher AIs for mutagenic impurities in drug products with shorter treatment durations, including those with compound or class-specific AIs, generally align with ICH M7. (60) Manufacturers were requested to consider broader factors contributing to nitrosamine contamination and mitigate risks, accordingly, as outlined in the EMA's advice on steps to take to avoid nitrosamines in human medicines. (61)

Products with nitrosamine levels below acceptable limits were generally considered safe, while those exceeding limits or containing multiple nitrosamines should not be permitted unless alternative options are available. Patients were advised not to discontinue treatment without consulting healthcare professionals.

PQT/MED committed to maintaining surveillance over nitrosamine impurities. Additionally, it was advised to keep up to date with developments on the websites of the FDA and the EMA, as they would publish any new information from ongoing investigations.

In contrast to the ranitidine case, the WHO did not react to the recalls of metformin drug products in late 2019 on the Singapore, Canadian and US markets, (62–64) although it had already been included in the EML before the nitrosamine crisis (please refer to *Table 4* in *Annex III: NA detection and essential drug and prequalification status*). (65)

In April 2020 the PQT/MED announced that it will contact FPP applicants and APIMF holders to request a risk assessment which should cover any prequalified API or FPP, accepted APIMF, or any FPP or APIMF currently undergoing evaluation by December 31, 2020. (66) For submissions before this date, a risk assessment is not required upon submission but must be completed within six months of acceptance. Submissions after December 31, 2020, should include a prior risk assessment. Outcome reports of evaluations should be sent to the WHO Prequalification Unit using designated templates. While the assessments themselves do not need to be submitted, they may be requested by the WHO Prequalification Inspection Team for certain APIs known to be at risk of nitrosamine content. APIMF holders are advised to share risk assessment information with FPP customers, regardless of

identified risks, to fulfil responsibilities promptly. Again, WHO referred to websites of FDA and EMA.

Four weeks later EMA published its press release on suspension of all ranitidine medicines in the EU as a precautionary measure due to the fact that some ranitidine medicines contain unacceptable levels of NDMA. The source of these impurities is, however, unclear. (55)

In July 2020 it was published that the PQT/MED had been in contact with Sanofi about the nitrosamine impurity CPNP in their tuberculosis treatment Priftin® 150 mg (drug substance: rifapentine) which was prequalified by the PQT/MED 2017. (67) Priftin® 300 mg strength has been included in the EML since 2015 (please refer to *Table 4* in *Annex III: NA detection and essential drug and prequalification status*). (68) Sanofi has paused batch release while considering remedial actions and was updating the PQT/MED regularly on this issue. (69)

The FDA reported the detection of CPNP in rifapentine only in August 2020 in parallel with the detection of 1-methyl-4-nitrosopiperazine (MNP) in rifampicin (also known as rifampin) which was included in the EML before the valsartan case (please refer to *Table 4* in *Annex III: NA detection and essential drug and prequalification status*). (70, 71) Although the nitrosamine levels in all available medicinal products exceeded the AI limits of 0.16 ppm for MNP and 0.1 ppm for CPNP, there were no recalls of these critical medications. Instead, temporary allowances for higher intake limits of 5 ppm for MNP and 14 ppm for CPNP ppm were granted. (72, 70) Concerned manufacturers of rifampicin products have not been publicly provided, which makes a correlation to prequalified rifampicin medicines hard to conduct.

Two months later the PQT/MED recognized this decision with regards to rifapentine and informed on its website that is had instructed all API and medicine applicants to test for MNP. Decisions regarding each product will be made individually based on actual nitrosamine levels and assessments of toxicity and risk/benefit balance. The PQT/MED advises against interrupting rifampicin treatment or taking any action until conclusions are reached. The PQT/MED again recommended that all companies should conduct risk assessments to evaluate the potential for the presence of nitrosamine impurities in due time. It was also reassured that the PQT/MED remained committed to evaluating information regarding nitrosamine impurities and their associated risks. (73, 74)

At the end of 2020 the PQT/MED published an Update and FAQs document regarding nitrosamine concerns for rifapentine and rifampicin. (75) According to this note PQT/MED was conducting product-specific risk assessments, evaluating actual reported levels while considering clinical, toxicological, and quality aspects of these medications. Additionally, PQT/MED was also in contact with other international regulatory bodies as well as professional and patient advocacy groups concerning this matter. Regarding rifapentine PQT/MED recognized the FDA's decision to accept the release of new batches meeting the CPNP temporary limit of 20 ppm. Regarding rifampicin PQT/MED anticipated receiving all results, along with risk assessment reports, by the first quarter of 2021. For APIs where results had already been reported by manufacturers, a temporary interim limit for the MNP was being established. PQT/MED assured that it was collaborating closely with these companies to implement mitigation measures promptly to reduce the impurity to acceptable lifetime levels. Based on the initial risk assessment and reported results, PQT/MED had not suspended any rifampicin prequalified APIs or medications. At that time, no alert had been deemed necessary, and the recommendation from PQT/MED not to interrupt any rifampicin treatment remained unchanged. It was again reassured that the PQT/MED continued to closely monitor responses concerning rifampicin products and the ongoing efforts by manufacturers.

Interestingly, rifapentine 300 mg was also added to the EML in 2021 (please refer to *Figure 12* in *Annex II: Timeline of nitrosamine detections and WHO's corresponding activities*). (68) This was justified based on the previous inclusion of 150 mg strength 2015. It was suggested that adverse effects of the 300 mg formulation are not expected to differ from those of the 150 mg formulation, provided the product is quality-assured with proven bioavailability. It was highlighted that shorter treatment regimens facilitated by rifapentine 300 mg can lead to better patient compliance, fewer cases of drug resistance, and overall better public health outcomes. The issues with nitrosamine impurities were also addressed. Monitoring and Sanofi's cooperation would ensure that rifapentine remains a safe option for tuberculosis treatment within the established safety limit of below 20 ppm. (68)

Furthermore, the fixed-dose combination product isocyanid/rifapentine was also added to the EML in 2021 (please refer to *Figure 12* in *Annex II: Timeline of nitrosamine detections*

and WHO's corresponding activities), also justified by particularly through shorter treatment regimens, which enhance patient adherence and reduce duration. (68)

In June 2021, following the discovery of elevated levels of N-nitroso-varenicline in Champix®, a drug containing varenicline, it was globally recalled due to its non-critical nature, for which alternatives such as bupropion were available. (76–78) This marked the first instance during the ongoing nitrosamine crisis where the formation of an NDSRI was confirmed through laboratory testing.

However, in October 2021, the World Health Organization took a contrary stance by adding varenicline to the Model List of Essential Medicines without even mentioning the recent findings in Champix® justified by demonstrating safety and efficacy in aiding tobacco cessation for individuals who find it challenging to quit solely with behavioural counselling or brief advice. It was highlighted that cigarette smoke comprises approximately 7000 distinct chemical compounds, with at least 70 identified or suspected as human carcinogens, including nitrosamines. (79)

Moreover, Lang et al. demonstrated that the absence of varenicline post-recall did not result in an increase in prescriptions for alternative medications for nicotine dependence in the US. Additionally, the study revealed that while prescriptions for varenicline-containing drugs rebounded after the drug shortage concluded in October 2021, they remained significantly lower than pre-recall levels. (80)

In October 2021 the PQT/MED announced that the analyses conducted on all prequalified rifampicin APIs and FPPs had revealed trace levels (≤ 5ppm) of MNP present in every batch tested (81). Beforehand, the FDA released laboratory test findings indicating that all examined batches exhibited levels of MNP surpassing the acceptable threshold of 0.16 ppm but remaining below the interim limit of 5 ppm. None of the tested products had been prequalified by the WHO. (72, 67) PQT/MED had evaluated the nitrosamines risk quality data for all prequalified rifampicin products (both APIs and FPPs), including comprehensive toxicological assessments and weighing the risk-to-benefit balance. Temporary interim limits for MNP had been provisionally established for all prequalified APIs, guided by process capability considerations tailored to individual cases. These interim limits would undergo regular review. Similarly, interim limits for prequalified FPPs were under evaluation at that time.

It is worth mentioning that bupropion was also added to the 22nd EML. The WHO expert committee found that varenicline had significantly higher rates of sustained abstinence at 6 months compared to both placebo and bupropion. Additionally, varenicline outperformed nicotine replacement therapy in achieving abstinence at 24 weeks. (68)

In the following months salbutamol medicines were recalled in Singapore due to the presence of N-nitroso-salbutamol and orphenadrine medicines in USA and Canada due to the presence of N-nitroso-orphenadrine. (82, 83, 56)

In May 2022 MacLeods Pharmaceuticals' isoniazid/rifapentine fixed-dose combination product was prequalified. (67)

In summer 2022 the PQT/MED gave an update on rifapentine products (84): As of then the PQT/MED had prequalified two finished pharmaceutical products based on rifapentine: Sanofi's Priftin® and MacLeods Pharmaceuticals' fixed-dose combination product isoniazid/rifapentine. Due to the medicine's crucial role in public health, the FDA allowed temporary distribution of Priftin® tablets with CPNP impurity content at or below 20 ppm while corrective measures were being pursued by the manufacturer. MacLeods Pharmaceuticals Ltd's product, the first isoniazid/rifapentine generic product, having essential drug status since 2021, (68) had been prequalified with an interim release limit of NMT 20 ppm for CPNP impurity. This limit, determined based on risk-benefit considerations and process capability, would undergo regular review. Additionally, the rifapentine API from MacLeods Pharmaceuticals Ltd, had been accepted with an interim release limit for CPNP based on process capability. Within this update the PQT/MED announced that a field sampling and testing study was also planned.

Soon after, several additional national and, in certain instances, cross-national discoveries of NDSRIs emerged, such as N-nitroso-irbesartan (5), N-nitroso-quinapril (6), N-nitroso-rasagiline, (7) and 7-nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-a]pyrazine (nitroso-STG-19 or NTTP) related to sitagliptin (8) (please refer to *Figure 12* in *Annex II: Timeline of nitrosamine detections and WHO's corresponding activities*). The former three findings prompted recalls of the corresponding drug products as well.

In September 2022 the PQT/MED published a nitrosamine contamination policy for APIs, in which a comprehensive nitrosamine risk assessment report was requested for all new

APIMF procedure applications and new API prequalification applications submitted since 1 January 2021. (85) Furthermore, the PQT/MED also required that manufacturers assess the potential effect of proposed changes on nitrosamine contamination. In line with this, the API Amendment Guidance had been updated to address this concern. (86)

In addition, three months later the PQT/MED published a policy for FPP applications, in which the completion of nitrosamine risk assessment before submission was requested for all new FPP applications submitted since 1 January 2021. (87) They referred to the update of "FAQ: Variations to prequalified pharmaceutical products", (88) published on this very day, regarding variation applications for changes to specifications, processes and controls resulting from step 3 ("Updates to the relevant authority regarding mitigation strategy, if the presence of nitrosamines is confirmed") of the three-step mitigation process. Any change to include control of nitrosamine impurities should be submitted as an immediate notification (IN). In this third revision it was also expected that manufacturers assess how proposed changes to the FPP manufacturing process, sources of raw materials, packaging, and storage might influence the risk of nitrosamine contamination.

From March 2022 to September 2023, several instances of nitrosamine impurities resulted in drug recalls, as evidenced by notices on agency websites and press releases (please refer to *Figure 12* in *Annex II: Timeline of nitrosamine detections and WHO's corresponding activities*). (89–97) Specifically, in March 2023, dabigatran medicines were recalled in the US due to the detection of N-nitroso-dabigatran above the established acceptable daily intake (ADI) level. Dabigatran is an essential drug since 2019. (98) In September 2023 lisinopril medicines were recalled in Germany due to the detection of N-nitroso-lisinopril above the established ADI level. (90) Lisinopril in fixed-dose combination with amlodipine and HCT is also an essential drug since 2019 (please refer to *Figure 12* in *Annex II: Timeline of nitrosamine detections and WHO's corresponding activities*). (98)

Beginning of September 2023 MacLeods Pharmaceuticals' rifapentine mono product was prequalified as well (please refer to *Figure 12* in *Annex II: Timeline of nitrosamine detections and WHO's corresponding activities*). (67)

On 18.09.2023 the PQT/MED released their latest update on nitrosamine contamination in prequalified products up to now (effective 29.05.2024). (99)

For rifampicin it had still accept interim limits for MNP impurity at or below 5 ppm, until manufacturers would have reduced it to lifetime acceptable levels. Rifampicin API and FPP manufacturers had to implement corrective measures to meet AI or lower limits by the end of 2023.

PQT/MED would monitor responses and manufacturer progress towards meeting the revised limit. Applicants were made aware of the fact that the AI for MNP had been significantly increased to 400 ng/ day through the Carcinogenic Potency Categorization Approach (CPCA) (please refer to *6.5 Deduction of AIs*). However, it was omitted by the WHO that this did not apply to the FDA limit of 20 ppm for MNP.

For rifapentine PQT/MED would still accept interim release limits for CPNP at or below 20 ppm, until manufacturers had reduced it to lifetime acceptable levels. The CPCA might be employed to revisit the AI for CPNP.

For other products than rifampicin or rifapentine manufacturers of FPPs containing APIs with secondary or tertiary amine groups were urged to revise the risk assessments conducted for these products to account for the possible presence of N-nitrosamines, including NDSRIs. If necessary, they should conduct additional testing to confirm their presence. In this regard, the CPCA, mentioned earlier can aid in determining the carcinogenic potency of the suspected nitrosamine impurity, thereby guiding prioritization for confirmatory testing. For instance, suspected nitrosamines classified under potency categories 1, 2, or 3 based on the CPCA should be given priority for confirmatory testing, unless the risk assessment demonstrates that the formation of the suspected nitrosamine is unlikely.

In April 2024 the WHO released a new draft guidance on nitrosamine contamination for consultation. (100) Up to now the “WHO good manufacturing practices considerations for the prevention and control of nitrosamine contamination in pharmaceutical products” has been the WHO’s latest contribution to this topic (effective 27.05.2024). Pharmaceutical manufacturers are urged by the WHO and other regulators to conduct a risk assessment regarding potential nitrosamine contamination. The process involves three key steps:

- Step 1: Conduct a risk evaluation to determine if APIs or finished products might be susceptible to nitrosamine presence. Additional nitrosamine sources highlighted in

the WHO guidance include recycled solvents or reused catalysts lacking adequate control and monitoring.

- Step 2: Upon identifying a risk, proceed with confirmatory testing to verify nitrosamine presence. Prompt reporting of outcomes to the appropriate regulator is advised.
- Step 3: If nitrosamines are confirmed, implement effective risk mitigation measures, and notify regulatory authorities of manufacturing variations.

The WHO guidance supplements these steps by considering factors such as daily dosage, duration of medication use, and nitrosamine levels in the finished product. Storage conditions, manufacturing processes, and understanding chemical interactions are also emphasized. A comprehensive understanding of the manufacturing process is crucial, particularly where amines and nitrite salts converge under acidic conditions, leading to potential nitrosamine formation. Special attention is given to cross-contamination risks in facilities with shared equipment, necessitating rigorous cleaning validation procedures. The WHO advises performing risk assessments for each supplier of APIs and excipients to evaluate the potential for nitrosamine presence. Attention is directed towards substances like nitrites, nitrates, and their sources or processing aids. Detailed discussions with vendors supported by audits are recommended. During drug process development, the WHO suggests understanding and avoiding conditions that may lead to nitrosamine formation. This may involve optimizing pH, temperature, and reaction time to mitigate risks.

4.5 Overall discussion on WHO's role during the nitrosamine crisis

On the one hand the WHO has been actively addressing the issue of nitrosamine contamination in prequalified pharmaceutical products, i.e., rifapentine and rifampicin over the last six years. The PQT/MED closely monitored announcements from major regulators regarding nitrosamine impurities in various medications, including sartan-related products, pioglitazone, ranitidine, and others. The WHO provided recommendations to regulatory agencies, urging them to actively verify nitrosamine levels in prequalified products, conduct risk assessments, establish limits to control nitrosamine impurities, and ensure future nitrosamine levels are minimized. Manufacturers were advised to consider broader factors contributing to nitrosamine contamination and take appropriate mitigation measures. They communicated updates and recommendations through their website, information

notes, and updates on nitrosamine impurities in prequalified products. Additionally, they collaborated with international regulatory bodies, professional organizations, and patient advocacy groups to address the issue comprehensively. The WHO implemented policies requiring comprehensive nitrosamine risk assessments for APIs and FPPs submitted for prequalification. Manufacturers were urged to assess potential nitrosamine contamination risks and implement effective risk mitigation measures. They continuously evaluated responses from manufacturers and regulatory agencies regarding nitrosamine contamination. They revised interim limits for nitrosamine impurities based on ongoing assessments and collaborated with manufacturers to ensure compliance with acceptable levels. None of the prequalified products were removed from the list of prequalified products due to nitrosamine findings, which is in line with the decision of the EMA and FDA to not disrupt distribution.

On the other hand, there was no press release or other communication indicating that the WHO had also been dealing with products that are not prequalified. The main updates provided by the WHO were only regarding the prequalified products rifapentine and rifampicin and the API rifampicin. It also would have been useful to show more transparency in providing the manufacturers' names of the rifampicin medicines affected by nitrosamine impurities.

Rifapentine and rifampicin are also essential drugs. Regarding the other essential drugs which are not prequalified from any manufacturer like metformin, varenicline, dabigatran and lisinopril (in fixed-dose combination) the WHO has not reacted or taken any measures so far. As outlined before quality and safety are one of the key objectives of the WHO's essential drugs concept. (1) If an essential drug is found to contain unacceptable levels of nitrosamine impurities, it is expected that the WHO would consider its removal from the list of essential drugs due to the significant implications for public health and safety.

However, for ranitidine the WHO made some recommendations to regulatory authorities but did not support EMA's suspension of all ranitidine medicines by considering its essential drug status.

The WHO also developed a draft guidance on nitrosamine contamination, providing detailed steps for risk assessment, confirmatory testing, and risk mitigation measures. The

guidance emphasized the importance of understanding manufacturing processes and potential sources of nitrosamine contamination.

Conversely, the WHO needed approximately half a year to react for the first time to the discovery of nitrosamines in medicines, which is late compared to the EMA's and FDA's prompt responses. Additionally, the draft guideline was not published until six years after the first detection of nitrosamines in valsartan. The guideline only highlights what has already been communicated by the EMA and FDA and does not provide any further details, e.g., number of batches to be tested during confirmatory testing. Before the release of this guidance, it was sufficient for the WHO to simply refer to the EMA's and FDA's websites, which was not conducive to the harmonization across global regulatory practices and often led to inconsistencies in how different regions handled the issue.

To enhance the safety and quality of pharmaceuticals, the WHO should advocate for the implementation of a standardized NAP test. This test would be applied to starting materials, intermediates, and APIs that include vulnerable amine structures or amine precursors. Conducting this test during the manufacturing process development would help confirm or exclude the risk of forming NDSRIs. Furthermore, the results of this NAP test should be incorporated into the registration dossier of drug substances and products to ensure comprehensive safety assessments and regulatory compliance.

5 Nitrosamine risk management

To comprehensively undertake a nitrosamine risk assessment for a medicinal product, it necessitates a thorough examination encompassing the entire lifecycle, ranging from the origin of the API to the storage of the final product. This entails an exhaustive scrutiny for the presence of nitrosatable molecular structures as well as potential nitrosating agents. While attention is naturally directed towards deliberately incorporated raw materials, equal emphasis must be placed on discerning potential sources of contamination.

The formation of N-nitrosamines in drug products requires the presence of three factors:

1) a nitrosating agent or its precursor, particularly nitrites.
2) a vulnerable secondary or tertiary amine
3) favourable reaction conditions such as elevated temperatures, acidic conditions, or the presence of a liquid phase.

These three factors may not be sufficient for N-nitrosamine formation to occur as the kinetics of N-nitrosamine formation can vary significantly depending on the structure and environment. (101, 18)

5.1 API synthesis

To assess the risk of nitrosamine formation, a comprehensive review of the manufacturing process of the drug substance in question is imperative. This review should encompass not only an examination of the reagents utilized in synthesis but also an exploration of potential contaminations in these reagents that could act as nitrosamine precursors. It is essential to evaluate not only the actual synthesis steps but also the purification procedures and pH adjustments employed throughout the process. By thoroughly scrutinizing each aspect of the manufacturing process, including reagents, synthesis steps, purification methods, and pH adjustments, the potential for nitrosamine formation can be effectively assessed.

5.1.1 Origin and nature of the API

As described by FDA and EMA chemically synthesized API are the most critical ones regarding nitrosamine contamination. (102, 15, 14) According to the FDA, biologics, pure fermentation products, and semisynthetic products typically do not need to be assessed for the risk of nitrosamine impurities unless they contain synthesized fragments. (103) This stance aligns with the FDA's guidance on NDSRIs, which includes biologic-led combination

products and biological products with chemically synthesized fragments. (11) The FDA notes that synthetic conjugated API components in biologics could pose a nitrosamine risk, and thus, an assessment for chemically synthesized APIs should be conducted as per the general nitrosamine guidance. (15, 103) However, the EMA points out that the formation of nitrosamines 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, known as potential nitrite source. (14, 19) 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. (104)

FDA's guidelines and calls for review do not explicitly extend to herbal medicinal products but do not exclude them either. (15). According to a new question and answer concerning medicinal products developed by the EMA and included in the practical guidance for MAHs, new marketing authorisation/registration applications for traditional herbal medicinal products must include a risk assessment. Already authorised/registered herbal medicinal products are not within the scope of the call for review unless a marketing authorisation holder suspects a risk of contamination. A rationale for this approach is not provided. (105)

Traditional Chinese Medicine (TCM) has been used for centuries as an alternative form of treatment for various conditions. However, recent studies have discovered that some TCM products may contain nitrosamine impurities. One such product is Tripterygium wilfordii, a herb commonly used to treat autoimmune and inflammatory disorders. The presence of nitrosamines in this herb has been attributed to the use of nitrate-containing fertilizers or pesticides during cultivation. (106–108)

Pesticides, the most common groundwater contaminants, often leak into groundwater from agricultural fields through rain, snow melting, and irrigation. Many pesticides are nitrosatable due to their nitrogen content. Coexisting contaminants in groundwater, such as metals, catalyse nitrosamine formation. Despite extensive pretreatment and purification, the high solubility and in-situ formation of nitrosamines via polymers and ion-exchange resins exacerbate the issue. Thus, water used for pharmaceutical purposes must be rigorously screened for nitrosamines. Municipal or groundwater used in the industry is purified, but the quality of purified water depends on the quality of the source water. Nitrosamines

have been detected in treated water due to their presence in the source water. For instance, WHO reported that 30% of 2000 surveyed water sources worldwide contained more than 24 mg/L of nitrosamines. Industrial and agricultural activities, especially the use of nitrogenous fertilizers and pesticides, contribute precursors to water bodies that react to form nitrosamines. Studies have found nitrosamines in soil and sewage water samples, indicating a significant environmental presence. (108, 109)

However, nitrosating agents can enter the API not only through contaminated water. Plants used in the production of APIs can absorb nitrosating agents from contaminated soil, water, or air. When these plants are harvested and processed to extract the active ingredients, any nitrosating agents present in the plant material may carry over into the final API product. Animals raised for pharmaceutical purposes may ingest nitrosating agents through contaminated feed, water, or environmental exposure. If the APIs are derived from these animals (such as hormones, antibodies, or enzymes), there is a risk that nitrosating agents absorbed by the animals could be present in the final API product. In contrast the processes involved in manufacturing and storing these substances, including formulation into Water for Injection (WFI) and storage under low-temperature conditions, are generally suboptimal for nitrosation reactions. Furthermore, biological active substances often contain reactive groups, like primary amines and thiols, which can neutralize nitrosating agents, further mitigating the risk. Additionally, the size and structure of biological molecules make them unlikely to be activated to form mutagenic compounds, even if trace amounts of nitrosamines are present. (104)

5.1.2 Structural alerts in APIs

The primary reason behind the presence of nitrosamine impurities in drug substances is their unintended generation during various stages of the API synthesis process.

Minimization of the risk of nitrosamine formation should be a key target of early drug development phase. In order to assess the risk of nitrosamine contamination of a medicinal product the first step would be a thorough analysis of the API structure and identification of any potential precursors of NDSIRs, followed by confirmatory testing to assess the contamination level and establish control strategies.

Primary amines typically do not form stable nitrosamines but instead undergo degradation into arene diazonium salts and deamination products, such as alcohols, ethers, or alkenes for aromatic and aliphatic amines, respectively. Similarly, other compounds containing the NH_2 group usually yield deamination products; for instance, primary amides produce the respective carboxylic acids. (110)

Secondary amines, however, readily form nitrosamines when exposed to nitrosating agents due to their higher reactivity, making them the likely source of contamination, influenced by structural and physicochemical factors such as pK_a, ionization and steric environment near the nitrogen atom. The relationship between pK_a and susceptibility to N-nitrosation has been extensively investigated for different nitrosating agents, notably N_2O_3 generated from $NaNO_2/H^+$(aq). (110) Additionally, other secondary NH-containing systems have been identified as undergoing N-nitrosation, e.g. secondary amides**.** (111) Generally, these systems exhibit significantly lower reactivity compared to secondary amines due to the NH moiety's weaker nucleophilic nature, as nitrosation begins with a nucleophilic attack by the lone pair of electrons on nitrogen, thus preventing quaternary ammonium or protonated forms from undergoing direct nitrosation. However, hydroxylamine- and hydrazine-type compounds are exceptions, possessing nucleophilicity similar to amines. Furthermore, aromatic N–H moieties, such as those present in pyrroles and tetrazoles, have also been observed to undergo N-nitrosation. Generally speaking, stronger bases offer better nucleophilicity but may hinder the reaction in acidic conditions by protonating more. In addition to amide hydrolysis to form precursor amines, direct nitrosation of amides followed by hydrolysis to nitrosamines has been suggested. In order to determine which one of both NH-groups is more prone to nitrosation, pk_a of its conjugate ammonium ions (pk_{aH}) needs to be compared. A higher pK_{aH} indicates a weaker acid and a stronger conjugate base for the amine, resulting in lower susceptibility to nitrosation. pk_{aH} values can be predicted by quantum mechanical calculations (e.g. Augmentation of Sparse Experimental Datasets with Accurate AIBL (QM) Derived Values (112)). A faster and simpler method suitable only for rough comparison is the estimation of pk_{aH} values on the basis of known pk_{aH} values for molecules resembling the electronic adjacency of each of the NH-group.

Tertiary amines are only suitable substrates for N-nitrosation if they bear protons alpha to the nitrogen. Dealkylation of the tertiary amine leading to a secondary amine is followed

by nitrosation, hence the reaction rate of tertiary amine nitrosation is significantly slower than that of secondary amines.

Secondary amines are expected to undergo N-nitrosation under mild conditions with most nitrosating agents. However, tertiary amines, (hetero)amides, and other NH-compounds necessitate harsher conditions and/or the use of more potent [NO+] carriers for successful nitrosation. (18) Tertiary amides cannot undergo direct nitrosation; instead, they require prior hydrolysis, resulting in the formation of a carboxylic acid and a secondary amine. This secondary amine can then be nitrosated. However, amides typically exhibit high resistance to hydrolysis, necessitating prolonged heating under acidic or basic conditions. Hence, tertiary amide moieties shall not be considered in this thesis.

Although quaternary ammonium salts cannot undergo direct nitrosation, they require non-nitrosative dealkylation to produce a nitrosatable tertiary amine, which in turn can form nitrosamines (*Figure 4*). Notably, since quaternary ammonium compounds originate from tertiary amine precursors, these precursors might exist as impurities. (9)

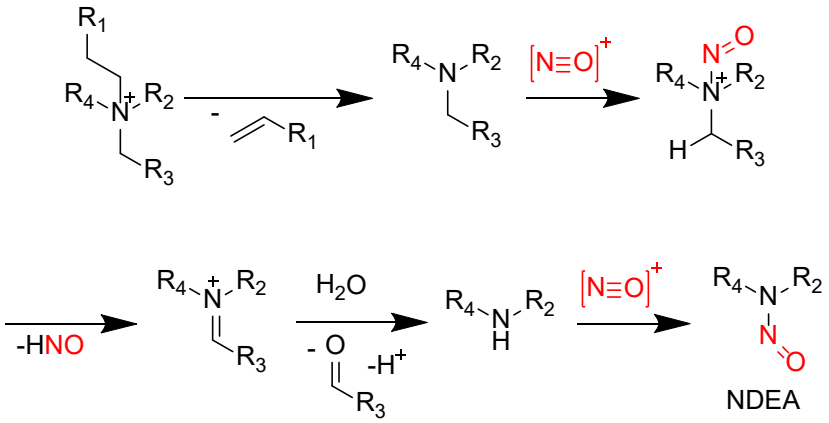


Figure 4-Dealkylation and nitrosation of quaternary ammonium salts

Nitrosation of imides are not known.

Aromatic rings can undergo nitrosation forming N-nitroso-heteroaromatic compounds. However, their mutagenicity is not based on the mechanism of N-nitrosamines (*Figure 3*) as α-hydroxylation and C-N cleavage would require breaking the aromaticity of the ring which is energetically disfavoured. Therefore, any potential toxicity is expected to arise from a mechanism not specific to the CoC, with existing data indicating their behaviour may be similar to that of other C-nitroso aromatic compounds. (113) Nitrosation of basic

aromatic rings, such as pyridine or quinoline necessitates an excess of the potent nitrosating agent, nitrosonium tetrafluoroborate, whereas an excess of the ring results in its cleavage. (9) Hence, basic aromatic moieties shall not be considered in this thesis.

5.2 Drug product

5.2.1 Excipients

The formation of nitrosamines is often determined by the concentration of nitrites within excipients and the conversion rate. While the variation in nitrite concentrations among different excipient types, batches and suppliers is well acknowledged, the influence of excipient choice on nitrosamine formation has not been systematically examined. Nitrites, existing as impurities in excipients, serve as the primary source of nitrosating agents. Typically, the limiting factor in nitrosamine formation is the trace amounts of nitrite rather than the more abundant secondary amine. Such an evaluation needs to take into account the realistic values of both nitrite levels in excipients and the percentage of conversion into nitrosamines. (114–116) The lack of comprehensive validated data on nitrite levels in excipients has hindered scientists' ability to accurately assess the risk of nitrosamine formation in pharmaceutical products. To overcome this obstacle, Lhasa Limited maintains a database that holds validated information provided by pharmaceutical company members on nitrite concentrations in common excipients, collected through validated analytical procedures. The aim of this data sharing initiative is to provide a common framework for contextualizing and estimating the risk posed by the presence of nitrites, which contribute to the formation of nitrosamines in drug products. Creating a comprehensive and reliable dataset is of great relevance because nitrite is generally not included in any pharmacopeial excipient specification. However, for the purpose of this master thesis the database could not be made use of, since only sponsors and contributors of this data sharing initiative have access. Due to the fact that excipients represent the largest component in medicinal products, there is a possibility that high levels of nitrite could be present as a reaction partner. In cases where the product contains vulnerable amines, selecting raw materials or processing methods that reduce nitrite levels by excipient manufacturers could reduce the risk of nitrosamine formation in the finished drug product formulations. (116)

The Lhasa approach does not take into account that there are several more nitrosating agents than solely nitrite salts which can act as an [NO^+] carrier, either in combination or

under specific reaction conditions. (49) In order to assess the risk of an excipient, also the content of nitrosonium salts/nitrosyl halides and derivatives, nitrite esters, organic nitro compounds, nitrogen oxides, peroxynitrites, nitrosodisulfonates, oxyhyponitrites and others should be determined. At least the probability of their presence should be assessed. Furthermore, some impurities (e.g. nitromethane) may not be traceable and therefore a formulation which is believed to be free of nitrites could still form nitrosamines. (101)

The Lhasa risk assessment evaluates the worst-case scenario, incorporating theoretical maximum levels of both nitrite content and conversion extent (i.e., 100%). Ultimately, the risk is assessed by comparing these calculated levels against the ADI for the relevant nitrosamine. Estimating nitrosamine content based on these extreme scenarios of nitrite levels in excipients and complete conversion of 100% likely leads to a significantly exaggerated risk compared to realistic conditions. The percentage of conversion is dependent on the formulation and the manufacturing and storage conditions, which will be discussed below. It was proven by Moser et al. that the most favourable conditions for maximum conversion were large excesses of secondary amine API, API in the salt form, wet granulation manufacturing process and storage in high-humidity conditions. It was also highlighted that amines found in tablets at low levels (e.g., 0.1% w/w), either as impurities or degradation products, undergo minimal conversion into N-nitrosamines, often falling near or below the analytical method's limit of quantification (LOQ). This stands in contrast to amines associated with APIs, which are present in higher concentrations and exhibit a greater propensity for conversion into NDSRIs. Wet granulation enhances the diffusion and mobility of nitrites and amine salts, thereby increasing the likelihood of their interaction and reactivity. Due to the same reason a 100 % conversion of nitrites and nitrosamines in solid state (e.g., in tablets, capsules) is extremely unlikely. In all cases the level of N-nitrosamine formation in solid dosage forms reached a plateau at a level significantly below the maximum theoretical yield calculated on the level of nitrite present. The highest percentage of conversion measured in Moser’s study was 38%. (117)

In order to establish valid and robust values, more methodical and systemic experimental studies like Moser’s varying individual factors are needed. Furthermore, it would be of great advantage if the excipient suppliers knew the content of nitrosating agents (especially nitrite) of their excipients and provided the information to the MAH or MA seeker. In

reality, the suppliers usually only provide documentation for risk of presence of nitrosamines and precursors in their excipients. For a reliable and effective risk assessment the nitrite contents should be tested by both the supplier and the drug product manufacturer. Nitrite contents of excipients can be determined by the following analytical methods:

- Ion chromatography with conductivity detection
- Griess method coupled with HPLC-UV
- Direct analysis by HPLC-UV
- And UV-VIS spectroscopy (118)

The challenge lies in testing hundreds of chemicals at trace level (low ng/g) for nitrite to assess their risk. Interestingly technique sensitivity is not the limiting factor, but rather the omnipresence of nitrite as contaminant in many chemicals, plastics, filters, and glassware etc. Nitrous gases from air can also be the source of nitrite in excipients (e.g. air used for tablet drying after wet granulation and coating). (119)

Although screening for excipients with minimal nitrite content may not be fully adequate as a standalone measure, it can serve as a significant component within a comprehensive approach aimed at minimizing the potential formation of N-nitrosamines.

5.2.2 Container closure system

The container closure system and primary packaging materials can be significant contributors to nitrosamine contamination in drug products. A clear example of nitrosamine generation from primary packaging materials involves nitrocellulose-containing blister packs. (120) Studies have shown that sample tablets in such blister packs exhibit elevated levels of NDMA and NDEA. Nitrocellulose contains nitrate esters, which can undergo hydrolysis to release nitrogen oxide, a nitrosating agent. (121) Simultaneously, the labeling ink used on these blister packs often contains trace amounts of dimethylamine and diethylamine. (120) This combination of nitrosating agents and amines can react to form nitrosamines.

During the blister pack sealing process, the required heating can vaporize NDMA and NDEA. These vapours can migrate and contaminate the drug within the primary packaging. While the levels of nitrosamines generated from the packaging process are usually small compared to the AI levels of each nitrosamine, the quality of materials and process conditions can influence nitrosamine levels.

Additionally, nitrosamine contamination in elastomers used in pharmaceutical packaging is well-documented. (122) Acceptance limits for nitrosamines in rubber materials have been proposed to minimize the risk of leaking into contained drugs.

5.2.3 Water

Apart from excipients which remain in the final drug product, water is often used during the manufacturing process but evaporates and is therefore not part of the final product composition. However, water may be contaminated with nitrite which can lead to nitrosamines under the following conditions: less basic amines, higher processing temperatures, and/or acidic conditions. Nitrite is a controlled impurity in water, with a WHO guideline limit of 3 mg/L. (123). Levels can be significant enough if proper downstream processing measures are not implemented to sufficiently remove them. Please note that the risk factor water was also discussed above (please refer to *5.1.1*)

5.3 Degradation

The degradation of the API, excipients or already formed nitrosamines can occur during the drug manufacturing process, especially during high-energy mixing, wet granulation and compression steps. (104)

As long as NDSIRs are formed during formulation, it should be noticed during the final quality control of the finished product, and thus the batch should be rejected, provided that one has actually searched for the NDSIR.

However, nitrosamine impurities in pharmaceutical products can also arise from the breakdown of APIs under specific storage conditions. Nitrosamines are particularly sensitive to pH, temperature, and light, which can lead to their degradation into other nitrosamines or nitrosamine precursors. Nitrosation can also occur during storage as a solid-state reaction. Since the two molecules, amines, and nitrites, must lie in the correct distance from each other in the solid dosage form, e.g., capsule in order to react with each other, the reaction is not frequent. Nevertheless, we see nitrosamine concentrations above the allowed level. (117, 124)

5.4 Potential contamination of manufacturing equipment

Cross-contamination can occur due to different processes being run successively on the same manufacturing line, leading to the carry-over of impurities between process steps.

This issue may arise from operator-related errors or inadequately detailed batch records, such as insufficient phase separations during work-up procedures. Additionally, cross-contamination by equipment or process and the purging capabilities of the process must be considered. The use of contaminated recovered or recycled materials, such as solvents, reagents, and catalysts, especially when recovery is outsourced to third parties unaware of the content of the materials, they are processing, poses a significant risk. Recovery processes carried out in non-dedicated equipment further worsen this concern. (104, 19)

5.5 Post-approval activities

In general the EMA does not require a risk assessment for line extension or variation applications, although questions regarding the presence of nitrosamines in the product may arise during assessment. (14) The FDA advises manufacturers to periodically reassess the risk of nitrosamine impurities throughout the product's lifecycle, especially following changes in manufacturing processes or manufacturer. This reassessment should align with quality management principles, though there is no requirement to submit a risk assessment for changes to the marketing authorization. (67)

Both regulatory authorities emphasize the need to reassess nitrosamine impurity risks due to quality changes in the marketing authorization, although the EMA and FDA generally do not mandate the submission of risk assessments. Given the numerous and still increasing root causes and risk factors for nitrosamine formation, a risk assessment should be standard practice for quality changes.

5.6 Establishment of acceptable intake limits

When sufficient substance specific animal data are available, the TD_{50} (tumorigenic dose for 50% of the population) is calculated. This TD_{50} value is then used to derive a substance-specific limit for lifetime exposure. The acceptable intake limit is set to correspond to a theoretical excess cancer risk of one in 100,000 patients, consistent with the ICH M7(R2). (19) An essential resource for selecting a TD_{50} value is the Lhasa Carcinogenicity Database (LCDB), (125) which contains results of relevant long-term animal carcinogenicity tests. If a specific compound lacks a TD_{50} value in the LCDB, it suggests a deficiency in studies conducted for that compound. Conversely, the presence of a TD_{50} value in the LCDB does not automatically guarantee the robustness of the data, as defined in ICH M7.

The availability of TD_{50} values for nitrosamines is often linked to non-robust carcinogenicity studies, raising doubts about their reliability in predicting carcinogenic potency. Selecting an appropriate TD_{50} value to derive a compound-specific AI for nitrosamine impurities lacks standardization, posing a significant challenge. While the general approach outlined in ICH M7 addendum relies on pre-calculated TD_{50} values from the LCDB or robust literature data, the use of harmonic mean TD_{50} values, derived from the results of existing studies, commonly provided in the LCDB, is not specified in the ICH M7 Addendum. Instead, the ICH opts for selecting the lowest TD_{50} values to provide a worst-case estimate of carcinogenic potency. (126) However, some regulatory authorities have employed harmonic mean TD_{50} values to determine compound-specific AIs for certain nitrosamines. (14)

For newly identified nitrosamines, however, it is very unlikely that carcinogenicity data are available and thus compound-specific AIs can be determined. In the past the EMA and the FDA responded to the discovery of nitrosamine impurities promptly by establishing conservative AI limits for nitrosamines, often using a class-specific threshold of 18 ng/day. (14, 11) Several proposals have been suggested to reassess these limits, including methods such as leveraging structure-carcinogenicity relationships of N-nitrosamines, (14, 11) surrogate analysis, (127) comprehensive toxicity data analysis, (128) factoring in structural complexity and size of NDSRIs (129) and prediction of α-carbon hydroxylation. (130) Despite these efforts, achieving broad consensus and acceptance of results from these approaches has been challenging.

The pharmaceutical industry's confusion was resolved when the EMA and FDA issued revised guidance on N-nitrosamines in summer 2023, recommending the Carcinogenic Potency Categorization Approach (CPCA). (11, 12)

The CPCA is based on the premise that the α-hydroxylation mechanism of metabolic activation is key to the high carcinogenic potency of nitrosamines. This approach involves determining a potency score based on the structural features of the nitrosamine. The score is calculated by summing the α-hydrogen score (derived from the count of hydrogen atoms on the α-carbon), the activating features score (based on structural features that could enhance carcinogenicity), and the deactivating features score (based on structural features that could reduce carcinogenicity). Nitrosamines are then categorized into different

potency categories, each with specific acceptable intake limits (*Table 1*). The relevant instructions can be found in *Annex V: Carcinogenic potency categorization approach (CPCA)*.

According to EMA's Q&A document the CPCA for N-nitrosamines should be used to establish the AI unless other robust data are available that would override this AI. A negative result in a GLP-compliant enhanced Ames test allows control of the N-nitrosamine at 1.5 µg/day. (13) For substances testing positive, the AI should be established using the CPCA or by considering a surrogate nitrosamine with sufficiently robust carcinogenicity data. The TD_{50} from the surrogate substance can serve as a point of departure for AI derivation by structure activity relationship (SAR) considerations with read across. Alternatively, a negative result in a relevant, well-conducted in vivo mutagenicity study can allow control of the N-nitrosamine as a non-mutagenic impurity (NMI), according to ICH Q3A(R2) and ICH Q3B(R2) limits, irrespective of the limit calculated through the CPCA or surrogate approach. (47)

Table 1-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 disfavoured due to steric or electronic influences or favoured clearance pathways
4	1500	TTC acc. to ICH M7; Metabolic activation not possible due to steric hindrance or absence of a-hydrogens or formation of unstable, not reactive species

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

SAR is an approach used to estimate the potential adverse effects of a chemical based on its chemical structure (131). Existing SAR models are employed for the qualitative prediction of mutagenicity to classify mutagenic impurities, as recommended in ICH M7. (35, 131) Additionally, SAR models can identify a surrogate compound with sufficient carcinogenicity data for read-across purposes. To establish an AI for the target compound, the TD_{50} of the

identified structurally similar substance can be used to make a quantitative estimation of carcinogenicity through read-across.

Since November 2023 EMA permits sponsors to use non-EMA sources to categorize nitrosamines not listed in Appendix 1 of the Q&A document. (14, 10) The ability to utilize CPCA categories published by other regulatory authorities may be advantageous if the EMA updates its requirements more slowly than other regulators. This allowance enables MAHs to perform risk mitigation and meet reporting requirements using values from another reference regulator that may update its requirements more quickly when justified.

Globally, several major regulators have acted promptly and collaboratively to harmonize approaches addressing nitrosamine impurities in drug substances. The recent recognition of the threat posed by NDSRIs and the adoption of new assessment methods and AIs, such as the enhanced Ames test and the CPCA approach, have occurred concurrently. The EMA's permissive approach to relying on other regulators' CPCA determinations is facilitated by this unified stance, alleviating at least one aspect of the challenging situation for sponsors.

The AI limits are mass-based which do not account for the molecular weight of different nitrosamines, leading to inconsistencies in risk assessments for not experimentally derived AIs. Therefore, AI limits should be recalculated on a molar basis to ensure each nitroso group (responsible for DNA mutation) is adequately considered. Molecular weight scaling would result in more relevant and protective AI limits for NDSRIs. (132)

Although the guidelines now better reflect the differing carcinogenic potency of NDSRIs and simple dialkyl-nitrosamines through the application of the CPCA, regional differences in AI derivation methods for NDSRIs and other nitrosamines still pose challenges. These discrepancies underscore the difficulty in accurately quantifying the carcinogenic risk of nitrosamine impurities and highlight the need for a harmonized approach to determine acceptable intakes.

5.7 Confirmatory testing

The guidance on confirmatory testing is largely consistent between EMA and FDA, though specifics may vary, reflecting the challenges of analytical testing for nitrosamine impurities. It remains unclear how many batches are sufficient for reliable confirmatory testing results,

complicating the establishment of a control strategy based on these results (please refer to *5.8*).

For confirmatory testing the use of sensitive validated test methods and excellent chromatographic separation due to the low molecular weight and volatility of nitrosamines are required. (14, 15) The challenges in analysing small dialkylnitrosamines like NDMA and NDEA were addressed through collaboration between OMCLs, European Directorate for the Quality of Medicines & HealthCare (EDQM), and United States Pharmacopeia (USP). So far, specific analytical methods for seven NDSRIs and intermediate-related contaminants have been made available by these institutions. (133) For other NDSIRs LC-MS methods can be used for their confirmation but require validation for each sample matrix. (134) Suitable primary reference standards, either sourced from recognized bodies or produced in-house, are essential for quantifying NDSRI levels. (135, 136) The availability of certified reference standards is crucial for timely mitigation, and USP is actively increasing the provision of these standards. EMA addresses the issue of non-synthesizable nitrosamine impurities and suggests that synthesis tests on potential NDSRIs can help exclude nitrosamine risk. (14)

EMA specifies the minimum number of batches to be tested as six pilot scale or three production scale batches. In case of high risk of nitrosamine presence, the number of tested batches should be higher. If a product comes in various strengths of the same form, each with identical risk factors, it may be rational to test only the highest-risk strength to streamline testing. The decision to adopt this approach should be supported by the MAH with case-specific justifications. (14) The FDA does not specify the number of batches but emphasizes representative sampling of the manufacturing process. (15) The issue of fluctuating nitrite levels in excipients remains unaddressed in current guidance. EMA recommends using orthogonal analytical methods to address technical factors and ensure reproducible results. (14)

5.8 Control strategy

In developing a control strategy for nitrosamine impurities, the EMA reference the control options delineated in the ICH M7. While these options primarily address process-related impurities in APIs, the EMA permit the use of options 1-3 for controlling nitrosamine impurities. (60, 14)

The FDA mandates routine testing for nitrosamines in APIs and, if deemed necessary, in FPs, aligning with standard control option 1 from ICH M7. However, the FDA does not advocate for periodic verification testing for nitrosamine impurities, citing existing uncertainties. Alternative procedures require FDA approval, contingent upon sufficient process understanding and evidence of statistical control. (15)

Exemptions from routine testing are allowed by the EMA if the root cause of nitrosamine contamination is identified, based on nitrosamine levels determined during confirmatory testing. Testing may be omitted if levels consistently remain below certain thresholds. (14)

The control options in ICH M7 assume knowledge of impurity fate and the possibility of removal during manufacturing. For APIs containing nitrosamine precursors like amines, risks persist throughout manufacturing and storage, supporting routine testing requirements. However, the mere presence of amine-containing APIs may not justify routine testing alone, necessitating a valid risk assessment.

It is crucial to differentiate between process-related nitrosamines and NDSRIs in regulatory guidance to provide clarity on control options. Demonstrating consistent NDSRI levels can be complicated by variable nitrite levels from the same excipient supplier.

5.9 Setting the specification

The determination of a specification limit in ppm for a specific medicinal product involves dividing the corresponding AI (ng) by the maximum daily dosage (mg) specified in the SmPC. The calculation of the specification limit does not take into account the molecular weight of the nitrosamine.

If the quantity of nitrosamine consistently remains below 10% of the acceptable limit derived from the AI in the API or in the final product, then testing for nitrosamine may be waived from the specifications. Similarly, if levels of any individual nitrosamine consistently stay below 30% of the acceptable limit derived from the AI in the API or the finished product, skip-testing in accordance with the ICH Q6A definition might be considered acceptable.

When multiple nitrosamines are identified in a product, the acceptable risk level must not exceed 1:100,000, as per the ICH M7(R2). There are two acceptable options to determine the limits, following the EMA's Q&A document: (14)

1. Total Daily Intake option: Ensure the total daily intake of all identified N-nitrosamines does not exceed the AI of the most potent N-nitrosamine identified.
2. Total Risk Level option: Ensure the total risk level calculated for all identified N-nitrosamines does not exceed 1 in 100,000.

The chosen option must be justified by the MAH or applicant. Specifications for individual N-nitrosamines should include an AI limit expressed in ppm or ppb. This limit is calculated by dividing the AI (in ng/day) by the maximum daily dose (in mg) of the product, as indicated in the SmPC.

N-nitrosamines present at less than 10% of their AI are considered to pose negligible toxicological risk and do not need to be specified or factored into the limits for individual or total N-nitrosamines. Despite this, manufacturers should aim to minimize the presence of N-nitrosamines as much as possible in accordance with the overall principle of Article 5(3).

For the first option, the AI limit for total N-nitrosamines should be based on the most potent N-nitrosamine that is present at or above 10% of its AI. The most potent nitrosamine is the one with the lowest AI. Limits for individual N-nitrosamines can be defined but are not required. It should be clearly stated which N-nitrosamines are included in the total calculation.

For option 2, the limits for N-nitrosamines should ensure an overall risk of not more than 1 in 100,000.

Different approaches can be used to achieve this risk requirement:

The fixed approach involves setting fixed AI limits in ppm/ppb for individual nitrosamines, with no need for a limit on the total N-nitrosamines. The limit for each nitrosamine should be set at a percentage of its AI limit, ensuring that the sum of these percentages does not exceed 100%. The absolute limits depend on the relative amounts of the different nitrosamines present in the batch.

The flexible approach, on the other hand, requires specifying each nitrosamine at its AI limit in ppm/ppb and additionally setting a limit for the total N-nitrosamines. A calculation method for determining the total N-nitrosamines is also provided in the Q&A document and will be examined exemplary below (please refer to 6.5)

FDA mentions only the first option according to the EMA in its guidance, but also offers the possibility of alternative approaches. (11, 15)

6 Case study: Nitrosamine risk management using the example of Camzyos®

6.1 Introduction

As a first step of the risk assessment publicly available information on the medicinal product are provided hereafter, which will be referred to later on:

Camzyos® was approved in April 2022 (28.04.2022) by the FDA (137) and one year later by the EMA (26.06.2023) (138) for the treatment of certain classes of obstructive hypertrophic cardiomyopathy (oHCM), a condition where the heart's main pumping chamber muscles thicken or enlarge, potentially obstructing blood flow. It is intended for adults experiencing symptoms of the disease, categorized as class II or class III oHCM, which indicate varying levels of limitation in physical activity.

Camzyos® is an orally active cardiac myosin inhibitor developed by MyoKardia, wholly owned subsidiary of Bristol Myers Squibb. It is expected that the global sales forecast in 2028 will be $1.658 billion. (139) Camzyos® is available as a hard capsule, taken by mouth once daily. The dose depends on the activity of a liver enzyme, CYP2C19, which is involved in the breakdown of the drug substance and the patient's response to treatment. (140) Camzyos® is available in four different presentations: 2.5 mg, 5 mg, 10 mg, and 15 mg. Its maximum daily dose is 15 mg. (141)

The active ingredient in Camzyos® is mavacamten (*Figure 5*). After being absorbed in the body, mavacamten is converted to its active form and binds to myosin, preventing it from attaching to actin, which reduces the excessive connections between these two proteins. This allows the heart muscle to relax more, thereby improving the symptoms of oHCM. (102)

Mavcacamten is manufactured using the synthetic route, based on the patent US9585883B2, depicted in *Figure 6*. (142)

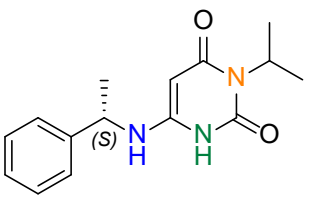


Figure 5-Structure of mavacamten

The drug substance is micronized after its synthesis. (102)

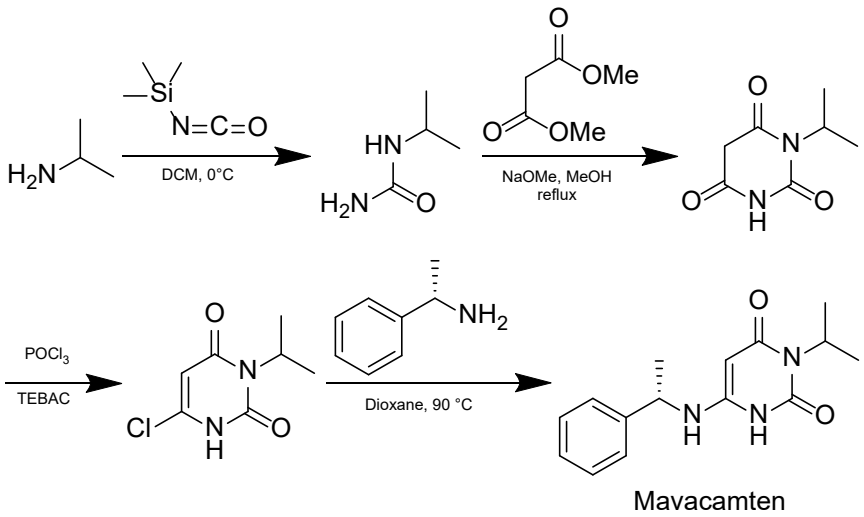


Figure 6-Synthesis of mavacamten as proposed by MyoKardia

According to the EPAR the commercial manufacturing process for Camzyos® is comprised of eight main steps: pre-blending, wet granulation, fluid bed drying, milling, final blending and lubrication, encapsulation and packaging. (102)

6.2 Structure analysis of the API

Mavacamten *(Figure 5*) bears a secondary amine moiety (blue), a secondary amide moiety (green) and a tertiary imide moiety (orange). As explained previously, only the secondary amine will be considered further in this thesis. Nitrosation of mavacamten could theoretically lead to the structure depicted in *Figure 7*, which will be called N-nitroso-mavacamten (NNM).

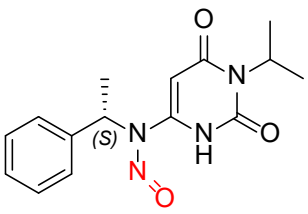


Figure 7-Structure of N-nitroso-mavacamten

6.2.1 Drug substance synthesis

The synthesis of mavacamten as proposed by MyoKardia (*Figure 6*) starts from isopropylmine which upon reaction with trimethylsilyl isocyanate is converted to isopropylurea. The

crude residue of isopropylurea is recrystallized from methanol (CH_3OH)/diethyl ether (Et_2O) (1:20).

The reaction between isopropylurea and dimethyl malonate in the presence of sodium methoxide as base produces 1-isopropyl barbituric acid, adjusting the pH of the reaction mixture to 3 using aqueous concentrated hydrochloric acid (HCl). The residue is taken up in ethanol (EtOH) and filtered. The filtrate is concentrated under reduced pressure and the residue is purified by silica gel column chromatography using dichloromethane (DCM)/CH_3OH (20:1) as eluent.

The subsequent conversion to the chloride derivative is mediated by phosphoryl chloride ($POCl_3$) using triethylbenzylammonium chloride (TEBAC) as phase transfer catalyst. The residue is dissolved in DCM followed by slow addition of water (H_2O). The phases are separated, and the organic layer is washed with water (H_2O), dried with anhydrous sodium sulfate (Na_2SO_4), and concentrated under reduced pressure. The crude residue is purified by silica gel column chromatography using ethyl acetate (EtOAc)/petroleum ether (1:1) as eluent.

The last stage involves the nucleophilic addition of (S)-methylbenzylamine to the chloride derivative to yield mavacamten. The residual is taken up in EtOAc and washed with aqueous 1N HCl and brine. The organic layer is dried with anhydrous Na_2SO_4 and then concentrated under reduced pressure to half the original volume to yield a precipitate. Hexane is added and the mixture is stirred at room temperature. The resulting solid is collected by filtration, washed with hexane, and dried.

The only possible precursor from the synthesis pattern is TEBAC, a quaternary ammonium ion which could yield a nitrosatable tertiary amine, which can yield NDEA (*Figure 8)* as explained before (*Figure 4*).

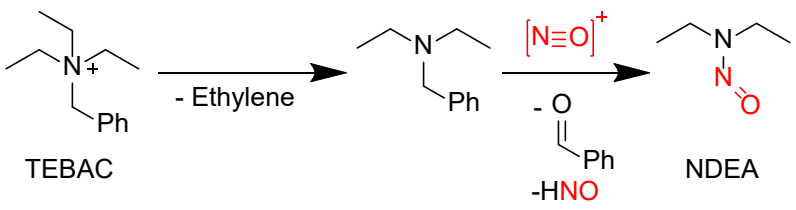


Figure 8-Dealkylation and nitrosation of TEBAC forming NDEA

6.3 Degradation products

Not only the API itself can be nitrosylated. It must be also taken into account that during synthesis, manufacturing, and storage degradation processes can occur. These degradation products can also be prone to nitrosation and should also be included in a holistic risk assessment. Gola et al. investigated mavacamten for its stability behaviour under various stress conditions and found that it is stable under basic and neutral hydrolytic, oxidative, thermal, and photolytic conditions. However, under acidic hydrolytic conditions two degradations impurities were formed: 1-isopropylpyrimidine-2,4,6 (1H,3H,5H)-trione and 1-phenylethanamine (*Figure 9*). (143)

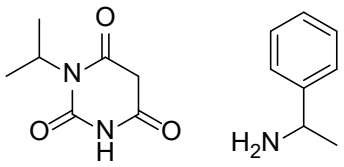


Figure 9-Structures of 1-isopropylpyrimidine-2,4,6 (1H,3H,5H)-trione (left) and 1-phenylethanamine (right)

These molecules do not bear vulnerable amines whereas they are not considered as potential nitrosamine precursors.

6.4 Raw materials

Table 2 lists all excipients contained in the medicinal product Camzyos® according to the product information. (141) Publicly accessible nitrite contents are also included.

In order to assess the risk originating from the excipients, the theoretical amount of nitrosamines per each excipient formed needs to be calculated:

$$\text{Nitrosamine [ppm]} = \frac{\text{nitrite content [ppm]} \cdot \text{MW Nitrosamine} \left[{}^{\text{g}}/_{\text{mol}}\right] \cdot \text{percentage of conversion}}{46 \left[{}^{\text{g}}/_{\text{mol}}\right]}$$

The denominator of 46 represents the molecular weight of nitrite. MW is the molecular weight. (116, 144)

This calculation needs to be performed for the nitrosamine, which is expected to be the most toxic one, as a worst-case estimation. Which one this could be, will be discussed later.

Table 2-Excipients contained in Camzyos®

Excipient	All Strengths	2.5 mg	5 mg	10 mg	15 mg	Mean nitrite content [µg/g] (116, 117)
Capsule Content						
Silica, colloidal hydrated	✓	✓	✓	✓	✓	0.87 (For silicon dioxide)
Mannitol (E421)	✓	✓	✓	✓	✓	0.38
Hypromellose (E464)	✓	✓	✓	✓	✓	0.80
Croscarmellose sodium (E468)	✓	✓	✓	✓	✓	0.42
Magnesium stearate	✓	✓	✓	✓	✓	2.6
Capsule Shell						
Gelatine	✓	✓	✓	✓	✓	N/A
Titanium dioxide (E171)	✓	✓	✓	✓	✓	N/A
Iron oxide black (E172)	✓				✓	N/A
Iron oxide red (E172)	✓			✓		N/A
Iron oxide yellow (E172)			✓			N/A
Printing Ink						
Iron oxide black (E172)	✓	✓	✓	✓	✓	N/A
Shellac (E904)	✓	✓	✓	✓	✓	N/A
Propylene glycol (E1520)	✓	✓	✓	✓	✓	N/A
Ammonia solution, concentrated (E527)	✓	✓	✓	✓	✓	N/A
Potassium hydroxide (E525)	✓	✓	✓	✓	✓	N/A

Due to the found variance of average nitrite contents, nitrites levels should be determined for every excipient for every single batch and for every single supplier. For the purpose of this thesis, nitrate contents originating from excipients cannot be calculated, since the amounts of excipients are not publicly available and the percentage of conversions are not known.

Apart from the nitrite level in the excipients, the possibility that vulnerable amines etc. are present should of course also be taken into consideration. Theoretically, one could think of

the amine functionalities in gelatine. According to the European Federation of Pharmaceutical Industries and Associations (EFPIA) capsule shells are considered to be no risk for nitrosamine contamination through either colourant or printing ink. Nitrosation is unlikely to occur from nitrite within the gelatine because pH is likely to be neutral, gelatine contains primary amine scavengers, the low surface to volume ratio leading to minimal interaction between the capsule shell and the capsule content, and the printing process is conducted at room temperature. (145)

6.5 Deduction of AIs for nitrosamines in Camzyos®

So far two structures, the API and TEBAC, which are prone to nitrosylation, have been identified. We have also observed that none of the known nitrosating agents are purposely added to these vulnerable structures during any step of the product's life cycle. However, we are aware of the fact that nitrites are ubiquitous contaminants and very difficult to avoid during API synthesis or drug product manufacturing. Consequently, it cannot be ruled out that nitrosamines can be formed in Camzyos®. These are N-nitroso-mavacamten and NDEA.

For NDEA robust rodent carcinogenicity data are already available and a substance specific AI has been calculated using the TD_{50} value. This is 26.5 ng/day for EMA and FDA. (10, 11)

However, for newly identified nitrosamines like N-nitroso-mavacamten no carcinogenicity data are available. For a long period, this meant that one had to adhere to the default AI set by the EMA at 18 ng/day and FDA at 26.5 ng/day. (1, 11)

Nowadays the CPCA can be used to determine an AI quickly and easily on the basis of its molecular structure. Recalling the structure of N-nitroso-mavacamten (*Figure 10*) we can clearly see that only one α-hydrogen is present. Following the flow chart from updated Appendix 2 of EMA's Q&A document (*Figure 11*) an AI of 1500 ng/day can be established, meaning that N-nitro-mavacamten belongs to Potency Category 5.

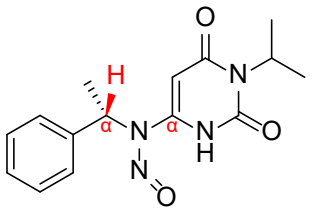


Figure 10-Structure of N-nitroso-mavacamten

According to Appendix 2 to the EMA's Q&A document N-Nitrosamines of this category "are not predicted to be metabolically activated via an α-hydroxylation pathway due to steric hindrance or the absence of α-hydrogens or are predicted to form unstable species that will not react with DNA. Therefore, the recommended AI limit of 1500 ng/day is set at the TTC per ICH M7". (12) (35)

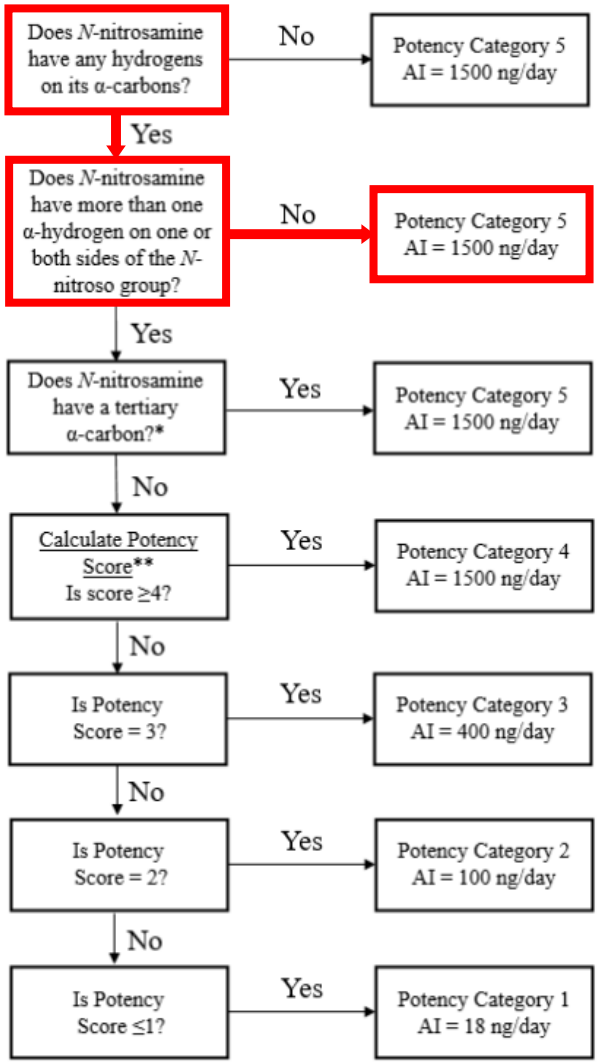


Figure 11-Flowchart for prediction of the Potency Category of nitrosamines using the example of NNM

6.6 Setting the specification for nitrosamines in Camzyos®

Assuming that both theoretically identified nitrosamines have been detected at or above 10% of their respective AI in Camzyos® with a maximum daily dose of 15 mg, (141) two options can be applied for setting respective specifications (please refer to 5.9):

For option 1 only the most potent nitrosamine NDEA needs to be considered, whose AI is 26.5 ng/day. Divided by the maximum daily dose of 15 mg, the specification for total nitrosamines in Camzyos® would be NMT 1.76 ppm.

For option 2 two approaches are available, for which calculations are conducted according to *Annex VI: Determination of specification limits for option 2 of EMA's Q&A*. For the flexible approach, specifications are set for both individual nitrosamines and for total nitrosamines. For N-nitroso-mavacamten it is 500 ng/d divided by 15 mg leading to NMT 100 ppm. For the fixed approach specifications are set for individual nitrosamines NDEA and N-nitroso-mavacamten based on their relative amount present in the batch.

An overview of possible specification settings according to the EMA's Q&A document are provided in *Table 3*. (14) Option 1 would also be the specification according to FDA's guideline on nitrosamines. (15)

Table 3-Overview on possible specification settings

Nitrosamine	**Option 1**	**Option 2 – Fixed** (Depends on relative amount of NDEA and NNM)	**Option 2 – Flexible**
NDEA	N/A	1.76 ppm x relative amount of NDEA	NMT 1.76 ppm
N-nitroso-mavacamten (NNM)	N/A	100 ppm x relative amount of NNM	NMT 100 ppm
Total nitrosamines	NMT 1.76 ppm	N/A	NMT 100%[1]

[1] $\frac{[NDEA]\ ppm}{relative\ amount\ of\ NDEA\ ppm} + \frac{[NNM] ppm}{rel.\ amount\ of\ NNM\ ppm} \cdot 100\% \leq 100\%$

7 Conclusion and outlook

While the main regulatory agencies reacted promptly to the first nitrosamine detections in valsartan 2018, the WHO reacted with a delay. It was demonstrated that over the past six years, the WHO has been actively addressing the issue of nitrosamine contamination in prequalified pharmaceutical products, particularly focusing on rifapentine and rifampicin. The PQT/MED closely monitored announcements from major regulators about nitrosamine impurities in various medications, providing recommendations to regulatory agencies and manufacturers to control and minimize these impurities. The WHO's efforts included continuous communication through various channels and collaboration with international regulatory bodies and other stakeholders.

The WHO implemented policies requiring comprehensive nitrosamine risk assessments for APIs and FPPs submitted for prequalification. They also evaluated responses from manufacturers and regulatory agencies, revising interim limits for nitrosamine impurities as necessary and ensuring compliance with acceptable levels. Importantly, the prequalification status was not withdrawn from any product due to nitrosamine findings, aligning with decisions from the EMA and FDA to maintain distribution without disruption.

However, the WHO's communication and actions were primarily focused on prequalified products. There was a lack of transparency regarding non-prequalified essential drugs and the manufacturers of affected rifampicin medicines. Despite the significance of drugs like metformin, varenicline, dabigatran, and lisinopril, the WHO has not taken similar measures for these non-prequalified essential drugs. The delayed response to the discovery of nitrosamines in medicines and the late publication of a draft guideline, which largely reiterated existing EMA and FDA communications, highlighted gaps in the WHO's proactive stance.

To enhance the safety and quality of pharmaceuticals globally, the WHO should consider adopting more proactive measures. This includes implementing a standardized NAP test, applied to starting materials, intermediates, and APIs with vulnerable amine structures or amine precursors. Conducting this test during the manufacturing process development would help confirm or exclude the risk of forming NDSRIs. Incorporating the results of the NAP test into the registration dossier of drug substances and products would ensure comprehensive safety assessments and regulatory compliance. Furthermore, the WHO should

expand its scope to include non-prequalified and non-essential drugs, ensuring consistent and transparent communication about potential nitrosamine contamination across all medicines. By advocating for harmonized global regulatory practices and timely, detailed guidance, the WHO can better safeguard public health and enhance the overall quality and safety of pharmaceutical products worldwide.

A detailed case study on the nitrosamine risk management of Camzyos® provided a practical illustration of how a meticulous risk assessment and adherence to regulatory guidelines could mitigate potential nitrosamine contamination. The case study highlighted the complexity of root cause analysis and setting AI limits.

It was demonstrated that early identification of vulnerable amines during the drug discovery process are key for a thorough risk assessment. This may enable reformulation with lower nitrite-content excipients or usage of nitrite scavengers. Sufficient time is also required for the relevant toxicity assays to be performed and a protective and practical AI limits determined. Sharing of data on sections of the hazard profile, such as nitrite levels in excipient batches and toxicity data between different manufacturers is necessary to enhance overall industry knowledge and facilitate more effective risk management strategies.

A more proactive approach and broader nitrosamine risk assessments in the pharmaceutical industry could significantly enhance drug safety and quality. In Europe and the US, in-process analysis is a critical component of ensuring drug quality during synthesis. This practice includes real-time monitoring of the production process to detect any deviations or impurities early on. However, the same rigorous standards and practices may not always be enforced consistently in other parts of the world. This discrepancy can lead to variations in the quality and safety of drug substances produced in different regions. Implementing global standards for in-process analysis would help harmonize quality assurance practices and reduce risks.

A reaction matrix could be an invaluable tool in drug substance production. This matrix would outline potential reactions between starting materials, impurities, reagents, catalysts, and solvents. By assessing these interactions proactively, manufacturers can better predict and prevent the formation of harmful nitrosamines. Understanding how impurities

in starting materials or solvents might react under various conditions can help with identifying and mitigating risks early in the production process. This would involve not only regular screening of excipients for nitrites and other nitrosating agents but also systematically assessing their potential interactions with the API and other excipients and consequently determining their influence on nitrosamine formation.

A proactive approach to quality analysis can lead to more consistent product quality and higher safety standards. This involves regular, comprehensive testing and analysis at all stages of production.

Staying ahead of potential issues can also help manufacturers remain compliant with evolving regulatory requirements, which increasingly emphasize the importance of risk management and quality assurance. Ultimately, proactive quality assurance measures can help maintain and enhance patient trust in pharmaceutical products, ensuring that they are safe, effective, and of high quality.

Adopting a more proactive stance with broader risk assessments and consistent quality analysis is essential. This involves implementing global standards for in-process analysis, developing comprehensive reaction matrices, and conducting thorough toxicity assessments. By doing so, the pharmaceutical industry can better predict, prevent, and mitigate potential risks, ensuring safer and more reliable medications for patients worldwide.

NA risk assessments will eventually become a fundamental aspect of product life cycle management, much like the current approach for elemental impurities as outlined by the ICH Q3D guideline. Over time, this integration will enable the industry to resume standard operations, with NA risk considerations becoming a routine part of procedures, possibly under an updated ICH M7 framework. At the ICH Assembly of Members in Fukuoka, from 4th to 5th June 2024, plans were announced to issue a common addendum to the existing ICH M7 addressing the safety assessment and establishment of appropriate controls for nitrosamine impurities utilising the Formal ICH procedure. (146)

The addendum will be developed in a staged approach. The first stage will establish principles for the design and use of in vitro assays, such as the Ames test, to differentiate between mutagenic and non-mutagenic nitrosamines. It will also define AIs based on SAR,

considering the structural features of the nitrosamine molecule. Additionally, it will involve applying LTL adjustments to AIs based on exposure, provided sufficient scientific data is available. The second stage will develop principles for the design and use of in vivo mutation studies as follow-ups to in vitro mutation studies and/or for the derivation of AIs. It will also create a framework for deriving AIs based on read-across methods. After completing Stage 2, a harmonized set of AIs for nitrosamines following the M7 maintenance process shall be developed. Training materials, including examples, will also be created to elaborate on relevant quality principles and the application of the ICH M7 to nitrosamines risk assessment and control. (147) It is desirable that this addendum distinguishs between nitrosamines that are part of the cohort of concern and those that are not. It should describe the detailed methods for setting acceptable intakes for which type of nitrosamine impurities. Additionally, all chemically synthesized, biological, and herbal drug products should be explicitly included in the addendum. The issue of non-synthesizable nitrosamine impurities should be addressed and standardized criteria for accepting the justification for excluding nitrosamine risk should be established. If regulations are finally harmonized, companies with global operations will not have to take different approaches to meet the regulations of each country. A great benefit would be to clearly defining the responsibilities between the drug substance manufacturer and the drug product manufacturer. The excipient suppliers should know the content of nitrosating agents (especially nitrite) of their excipients and should be obliged to provide the information to the MAH or MA seeker.

8 Summary

Nitrosamine impurities have posed a significant challenge to the pharmaceutical industry and regulatory bodies for six years due to their carcinogenic potential. The discovery of nitrosamines in valsartan in 2018 heightened scrutiny of their presence in pharmaceuticals leading to recalls and investigations uncovering more contaminations in various medications, raising safety concerns.

Regulatory agencies worldwide responded by issuing guidelines for manufacturers to review, test, and mitigate nitrosamine impurities in their products. While the initial focus was on simple dialkyl-nitrosamines like NDMA and NDEA, attention shifted in 2020 to nitrosamine drug substance-related impurities (NDSRIs), which are linked to the chemical structure and synthesis of APIs.

A 2022 study predicted that about 40% of APIs could form NDSRIs, complicating regulatory efforts to determine acceptable intakes (AIs) and necessitating corrective actions. Updated guidelines in 2024 listed confirmed and potential NDSRIs and their AIs, though data on their mutagenicity and carcinogenicity remains limited. Despite improved guidelines reflecting the varying carcinogenic potency of NDSRIs and simple nitrosamines, regional differences in AI derivation methods persist, underscoring the need for a harmonized approach.

This thesis investigates the World Health Organization’s (WHO) role during the last six years during the nitrosamine crisis and critically scrutinizes the WHO’s activities in evaluating the associated risks, in offering guidance for the pharmaceutical industry, patients and regulatory bodies and in implementing mitigation measures, especially with regards to their essential medicines and prequalified finished pharmaceutical products (FPP). In this context it also demonstrated how a thorough nitrosamine risk assessment on the basis of a concrete example could be conducted taking into account global marketability and emphasizing the need for harmonization of regulatory frameworks to ensure pharmaceutical quality, safety, and therapeutic diversity.

By evaluating the WHO’s press releases, Model Lists of Essential Medicines (EML) and lists of prequalified FPPs and APIs it is demonstrated that the WHO in general has actively addressed nitrosamine issues, focusing on prequalified products, such as rifapentine and rifampicin. They monitored major regulators' announcements, provided recommendations,

and implemented policies requiring comprehensive risk assessments for APIs and FPPs submitted for prequalification. The WHO revised interim limits for nitrosamines as needed, ensuring compliance with acceptable levels, and no product's prequalification status was withdrawn due to nitrosamine findings. However, the WHO's delayed response to the nitrosamine contamination crisis in pharmaceuticals and the late publication of a draft guideline only reiterating existing EMA and FDA communications highlighted gaps in their proactive stance. Additionally, this thesis reveals that their focus has been primarily on prequalified products, lacking transparency regarding non-prequalified essential drugs and manufacturers of affected rifampicin medicines. Despite the significance of drugs like metformin and lisinopril, similar measures were not taken for non-prequalified drugs.

To improve pharmaceutical safety and quality globally, the WHO should adopt more proactive measures, such as standardized NAP tests during manufacturing to confirm or exclude NDSRI risks. Incorporating these test results into registration dossiers would ensure comprehensive safety assessments. The WHO should also expand its scope to include non-prequalified essential drugs, ensuring consistent communication about potential nitrosamine contamination.

The case study on recently approved Camzyos® demonstrates the complexity of nitrosamine risk management, emphasizing early identification of vulnerable amines, sufficient time for toxicity assays, and sharing data between manufacturers to enhance industry knowledge. Proactive quality analysis, including global standards for in-process analysis and developing reaction matrices, can help predict and prevent harmful nitrosamine formation, ensuring consistent product quality and safety. It is expected that Nitrosamine risk assessments become fundamental in the product life cycle management.

The conclusion of the thesis underscores the critical importance of a harmonized global approach to managing nitrosamine risks in pharmaceuticals. It calls for consistent and coordinated efforts to protect patient safety and maintain the therapeutic diversity. The WHO's role is highlighted as essential in standardizing regulatory frameworks and facilitating international cooperation to effectively mitigate nitrosamine impurities. The ICH's initiative to issue a harmonized addendum to the ICH M7 guideline on nitrosamines represents a promising step forward, though it is still in its initial stage.

9 References

(1) COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE. *Nitrosamines EMEA-H-A5(3)-1490 - Assessment report* [online]. 25 June 2020, 12:00 [viewed 5 March 2024]. Available from: https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-assessment-report_en.pdf.

(2) EUROPEAN MEDICINES AGENCY AND HEADS OF MEDICINES AGENCIES. *Lessons learnt from presence of N-nitrosamine impurities in sartan medicines* [online]. 23 June 2020, 12:00 [viewed 13 March 2024]. Available from: https://www.ema.europa.eu/system/files/documents/report/sartans_lessons_learnt_exercise_report_en.pdf.

(3) EUROPEAN MEDICINES AGENCY, HEADS OF MEDICINES AGENCIES, and 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* [online]. 22 February 2021, 12:00 [viewed 13 March 2024]. Available from: https://www.ema.europa.eu/en/documents/referral/european-medicines-regulatory-network-approach-implementation-chmp-opinion-pursuant-article-53-regulation-ec-no-726-2004-nitrosamine-impurities-human-medicines_en.pdf.

(4) EUROPEAN MEDICINES AGENCY. *Meeting highlights from the Committee for Medicinal Products Human Use (CHMP) 13-16 September 2021* [online] [viewed 6 March 2024]. Available from: https://www.ema.europa.eu/en/news/meeting-highlights-committee-medicinal-products-human-use-chmp-13-16-september-2021.

(5) 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* [online]. 14 October 2021, 12:00 [viewed 25 March 2024]. Available from: https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/lupin-pharmaceuticals-inc-issues-voluntarily-nationwide-recall-all-irbesartan-tablets-and-irbesartan.

(6) U.S. FOOD AND DRUG ADMINISTRATION. *Pfizer Voluntary Nationwide Recall of Lots of ACCUPRIL® (Quinapril HCl) Due to N-Nitroso-Quinapril Content* [online]. 22 April

2022, 12:00 [viewed 25 March 2024]. Available from: https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/pfizer-voluntary-nationwide-recall-lots-accuprilr-quinapril-hcl-due-n-nitroso-quinapril-content.

(7) DEUTSCHE APOTHEKER ZEITUNG. *Rückruf wegen Nitroso-Rasagilin* [online]. 16 May 2022, 12:00 [viewed 25 March 2024]. Available from: https://www.deutsche-apotheker-zeitung.de/news/artikel/2022/05/16/rueckruf-wegen-nitroso-rasagilin.

(8) U.S. FOOD AND DRUG ADMINISTRATION. *FDA works to avoid shortage of sitagliptin following detection of nitrosamine impurity* [online]. 9 August 2022, 12:00 [viewed 2 April 2024]. Available from: https://www.fda.gov/drugs/drug-safety-and-availability/fda-works-avoid-shortage-sitagliptin-following-detection-nitrosamine-impurity.

(9) SCHLINGEMANN, Joerg, Michael J. BURNS, David J. PONTING, Carolina MARTINS AVILA, Naiffer E. ROMERO, Mrunal A. JAYWANT, Graham F. SMITH, Ian W. ASHWORTH, Stephanie SIMON, Christoph SAAL, and Andrzej WILK. The Landscape of Potential Small and Drug Substance Related Nitrosamines in Pharmaceuticals [online]. *Journal of pharmaceutical sciences.* 2023, **112**(5), 1287-1304 [viewed 14 April 2024]. Available from: 10.1016/j.xphs.2022.11.013.

(10) EUROPEAN MEDICINES AGENCY. *Appendix 1: Acceptable intakes established for N-nitrosamines* [online]. 5 June 2024, 12:00 [viewed 5 June 2024]. Available from: https://www.ema.europa.eu/en/documents/other/appendix-1-acceptable-intakes-established-n-nitrosamines_en.xlsx.

(11) U.S. FOOD AND DRUG ADMINISTRATION. *Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs) Guidance for Industry* [online]. 4 August 2023, 12:00 [viewed 1 June 2024]. Available from: https://www.fda.gov/media/170794/download.

(12) EUROPEAN MEDICINES AGENCY. *Appendix 2 to 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* [online]. 12 October 2023, 12:00 [viewed 5 November 2024]. Available from: https://www.ema.europa.eu/en/documents/other/appendix-2-carcinogenic-potency-categorisation-approach-n-nitrosamines_en.pdf.

(13) EUROPEAN MEDICINES AGENCY. *Appendix 3 to 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 [online]. 26 March 2024, 12:00 [viewed 6 May 2024]. Available from: https://www.ema.europa.eu/en/documents/other/appendix-3-enhanced-ames-test-conditions-n-nitrosamines_en.pdf.

(14) EUROPEAN MEDICINES AGENCY AND CO-ORDINATION GROUP FOR MUTUAL RECOGNITION AND DECENTRALISED PROCEDURES-HUMAN. *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* [online]. 15 January 2024, 12:00 [viewed 31 May 2024]. Available from: https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-questions-answers-marketing-authorisation-holders-applicants-chmp-opinion-article-53-regulation-ec-no-726-2004-referral-nitrosamine-impurities-human-medicinal-products_en.pdf.

(15) U.S. FOOD AND DRUG ADMINISTRATION. *Control of Nitrosamine Impurities in Human Drugs* [online]. 24 February 2021, 12:00 [viewed 31 May 2024]. Available from: https://www.fda.gov/media/141720/download.

(16) WORLD HEALTH ORGANIZATION. *About WHO* [online] [viewed 6 June 2024]. Available from: https://www.who.int/about.

(17) BEARD, Jessica C., and Timothy M. SWAGER. An Organic Chemist's Guide to N-Nitrosamines: Their Structure, Reactivity, and Role as Contaminants [online]. *The Journal of Organic Chemistry.* 2021, **86**(3), 2037-2057. Available from: 10.1021/acs.joc.0c02774.

(18) LÓPEZ-RODRÍGUEZ, Rocío, James A. MCMANUS, Natasha S. MURPHY, Martin A. OTT, and Michael J. BURNS. Pathways for N -Nitroso Compound Formation: Secondary Amines and Beyond [online]. *Organic Process Research & Development.* 2020, **24**(9), 1558-1585. Available from: 10.1021/acs.oprd.0c00323.

(19) HORNE, Stephen, Matthew D. VERA, Laxma R. NAGAVELLI, Vilayat A. SAYEED, Laurel HECKMAN, Deborah JOHNSON, Dan BERGER, Yean Yean YIP, Carolina Lopes KRAHN, Leticia Oyamada SIZUKUSA, Nayrton Flavio Moura ROCHA, Robert N. BREAM, Joachim LUDWIG, David A. KEIRE, and Gary CONDRAN. Regulatory Experiences with Root Causes and Risk Factors for Nitrosamine Impurities in Pharmaceuticals

[online]. *Journal of Pharmaceutical Sciences.* 2023, **112**(5), 1166-1182. Available from: 10.1016/j.xphs.2022.12.022.

(20) MAGEE, P. N., and J. M. BARNES. The production of malignant primary hepatic tumours in the rat by feeding dimethylnitrosamine [online]. *British journal of cancer.* 1956, **10**(1), 114-122. Available from: 10.1038/bjc.1956.15.

(21) AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY. *Toxicological Profile for N-Nitrosodimethylamine (NDMA)* [online]. 04.2023 [viewed 22 May 2024]. Available from: https://www.atsdr.cdc.gov/toxprofiles/tp141.pdf.

(22) DRUCKREY, H., R. PREUSSMANN, S. IVANKOVIC, D. SCHMHL, J. AFKHAM, G. BLUM, H. D. MENNEL, M. MLLER, P. PETROPOULOS, and H. SCHNEIDER. Organotrope carcinogene Wirkungen bei 65 verschiedenen N-Nitroso-Verbindungen an BD-Ratten [online]. *Zeitschrift fr Krebsforschung.* 1967, **69**(2), 103-201. Available from: 10.1007/BF00524152.

(23) MAGEE, P. N., and J. M. BARNES. Carcinogenic Nitroso Compounds. In: Alexander Haddow and Sidney Weinhouse, eds. *Advances in Cancer Research:* Academic Press, 1967, pp. 163-246.

(24) MAGEE, P. N., A. E. PEGG, and P. F. SWANN. Molecular Mechanisms of Chemical Carcinogenesis. In: H.-W. Altmann, F. Büchner, H. Cottier, E. Grundmann, G. Holle, E. Letterer, W. Masshoff, H. Meessen, F. Roulet, G. Seifert, G. Siebert, D. Bierwolf, F. Fey, A. Graffi, E. Hecker, K. von der Helm, K. Kammer, P. N. MAGEE, B. Micheel, K. Munk, W. Nakahara, A. E. Pegg, R. Preussmann, T. Schramm, H. Sugano, P. F. Swann, V. Wunderlich, T. H. Yosida, and Ekkehard Grundmann, eds. *Geschwülste · Tumors II. Virale und chemische Carcinogenese/Viral and Chemical Carcinogenesis.* Berlin: Springer Berlin, 2013, pp. 329-419.

(25) EISENBRAND, G., B. SPIEGELHALDER, J. KANN, R. KLEIN, and R. PREUSSMANN. Carcinogenic N-nitrosodimethylamine as a contamination in drugs containing 4-dimethylamino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one (amidopyrine, aminophenazone) [online]. *Arzneimittel-Forschung.* 1979, **29**(6), 867-869. Available from: 10.1002/chin.197938327.

(26) LIJINSKY, W., H. W. TAYLOR, C. SNYDER, and P. NETTESHEIM. Malignant tumours of liver and lung in rats fed aminopyrine or heptamethyleneimine together with nitrite [online]. *Nature.* 1973, **244**(5412), 176-178. Available from: 10.1038/244176a0.

(27) CASTEGNARO, M., B. PIGNATELLI, and E. A. WALKER. Analysis of volatile N-nitrosamines in commercial drugs [online]. *Food and Cosmetics Toxicology.* 1981, **19**(4), 489-491. Available from: 10.1016/0015-6264(81)90455-7.

(28) BELLANDER, T., B. G. OSTERDAHL, and L. HAGMAR. Formation of N-mononitrosopiperazine in the stomach and its excretion in the urine after oral intake of piperazine [online]. *Toxicology and Applied Pharmacology.* 1985, **80**(2), 193-198. Available from: 10.1016/0041-008X(85)90075-4.

(29) TAYLOR, P., P. BRADDOCK, and D. CARTER. The analysis of N-nitrosodimethylamine in antihistamines and cough/cold preparations. *IARC scientific publications.* 1980, (31), 575-587.

(30) DAWSON, B. A., and R. C. LAWRENCE. Analysis of selected drug formulations for volatile nitrosamines [online]. *Journal - Association of Official Analytical Chemists.* 1987, **70**(3), 554-556. Available from: https://pubmed.ncbi.nlm.nih.gov/3610969/.

(31) GHAFFARI, Hamid Reza, Simin NASSERI, Masud YUNESIAN, Ramin NABIZADEH, Farhad POURFARZI, Hossein POUSTCHI, Alireza SADJADI, Mohammad Reza FATTAHI, and Ali Reza SAFARPOUR. Monitoring and exposure assessment of nitrate intake via fruits and vegetables in high and low risk areas for gastric cancer [online]. *Journal of environmental health science & engineering.* 2019, **17**(1), 445-456. Available from: 10.1007/s40201-019-00363-0.

(32) ZHAO, Chao, Jingjing ZHOU, Yun GU, Enchun PAN, Zhongming SUN, Hu ZHANG, Qiang LU, Ying ZHANG, Xiaojin YU, Ran LIU, Yuepu PU, and Lihong YIN. Urinary exposure of N-nitrosamines and associated risk of esophageal cancer in a high incidence area in China [online]. *The Science of the total environment.* 2020, **738**, 139713. Available from: 10.1016/j.scitotenv.2020.139713.

(33) ZHENG, Jiali, Carrie R. DANIEL, Rikita I. HATIA, Janice STUFF, Ahmed A. ABDELHAKEEM, Asif RASHID, Yun Shin CHUN, Prasun K. JALAL, Ahmed O. KASEB, Donghui LI, and Manal M. HASSAN. Dietary N-Nitroso Compounds and Risk of Hepatocellular Carcinoma: A USA-Based Study [online]. *Hepatology (Baltimore, Md.).* 2021, **74**(6), 3161-3173. Available from: 10.1002/hep.32046.

(34) TUESUWAN, Bodin, and Vorasit VONGSUTILERS. Current Threat of Nitrosamines in Pharmaceuticals and Scientific Strategies for Risk Mitigation [online]. *Journal of*

pharmaceutical sciences. 2023, **112**(5), 1192-1209. Available from: 10.1016/j.xphs.2023.01.028.

(35) INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE (ICH) and EUROPEAN MEDICINES AGENCY. *ICH M7(R2) Guideline on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk* [online]. *Step 5.* 18 July 2023, 12:00 [viewed 5 November 2024]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-m7r2-guideline-assessment-and-control-dna-reactive-mutagenic-impurities-pharmaceuticals-limit-potential-carcinogenic-risk-step-5_en.pdf.

(36) SNODIN, David J. Mutagenic impurities in pharmaceuticals: A critical assessment of the cohort of concern with a focus on N-nitrosamines [online]. *Regulatory toxicology and pharmacology: RTP.* 2023, **141**, 105403 [viewed 5 May 2024]. Available from: 10.1016/j.yrtph.2023.105403.

(37) CROSS, Kevin P., and David J. PONTING. Developing Structure-Activity Relationships for N-Nitrosamine Activity [online]. *Computational Toxicology.* 2021, **20**, 100186. Available from: 10.1016/j.comtox.2021.100186.

(38) WORLD HEALTH ORGANIZATION. *What we do* [online] [viewed 6 March 2024]. Available from: https://www.who.int/about/what-we-do.

(39) WORLD HEALTH ORGANIZATION. *The WHO Programme for International Drug Monitoring* [online] [viewed 6 March 2024]. Available from: https://who-umc.org/about-the-who-programme-for-international-drug-monitoring/.

(40) WORLD HEALTH ORGANIZATION. *Radiation Emergency Medical Preparedness and Assistance Network (REMPAN)* [online] [viewed 6 March 2024]. Available from: https://www.who.int/groups/rempan.

(41) BUNDESGESUNDHEITSMINISTERIUM. *World Health Organization (WHO)* [online]. 28 September 2023, 12:00 [viewed 30 May 2024]. Available from: https://www.bundesgesundheitsministerium.de/en/en/international/who-english.

(42) WORLD HEALTH ORGANIZATION. *Strengthening access to essential medicines* [online] [viewed 26 May 2024]. Available from: https://www.who.int/activities/strengthening-access-to-essential-medicines.

(43) REED F BEALL. *The patent landscape of the WHO Model List of Essential Medicines* [online]. Global Challenges Brief [viewed 26 May 2024]. Available from: https://www.wipo.int/edocs/mdocs/mdocs/en/wipo_gc_ip_ge_16/wipo_gc_ip_ge_16_brief.pdf.

(44) KAR, Sitanshu Sekhar, Himanshu Sekhar PRADHAN, and Guru Prasad MOHANTA. Concept of essential medicines and rational use in public health [online]. *Indian journal of community medicine : official publication of Indian Association of Preventive & Social Medicine.* 2010, **35**(1), 10-13. Available from: 10.4103/0970-0218.62546.

(45) WORLD HEALTH ORGANIZATION - PREQUALIFICATION UNIT - MEDICINES. *Finished pharmaceutical products | WHO - Prequalification of Medical Products (IVDs, Medicines, Vaccines and Immunization Devices, Vector Control)* [online]. 11 July 2024, 12:00 [viewed 11 July 2024]. Available from: https://extranet.who.int/prequal/medicines/finished-pharmaceutical-products.

(46) WORLD HEALTH ORGANIZATION - PREQUALIFICATION UNIT - MEDICINES. *Prequalification Procedures and Fees: FPPs, APIs & QCLs | WHO - Prequalification of Medical Products (IVDs, Medicines, Vaccines and Immunization Devices, Vector Control)* [online] [viewed 30 May 2024]. Available from: https://extranet.who.int/prequal/medicines/prequalification-procedures-and-fees-fpps-apis-qcls.

(47) WORLD HEALTH ORGANIZATION - PREQUALIFICATION UNIT - MEDICINES. *General Information – WHO List of Prequalified Medicinal Products | WHO - Prequalification of Medical Products (IVDs, Medicines, Vaccines and Immunization Devices, Vector Control)* [online] [viewed 26 May 2024]. Available from: https://extranet.who.int/prequal/medicines/general-information-who-list-prequalified-medicinal-products.

(48) WORLD HEALTH ORGANIZATION. *History and Mission of WHO Prequalification* [online] [viewed 6 March 2024]. Available from: https://extranet.who.int/prequal/about.

(49) SANDER, J., F. SCHWEINSBERG, and H. P. MENZ. Untersuchungen über die Entstehung cancerogener Nitrosamine im Magen. *Hoppe-Seyler's Zeitschrift fur physiologische Chemie.* 1968, **349**(12), 1691-1697.

(50) INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. *Some N-Nitroso compounds* [online]. 05.1978 [viewed 31 May 2024]. Available from: https://publications.iarc.fr/_publications/media/download/1565/12f27b1bb900b6286a5da21c926a24880fc9399e.pdf.

(51) INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. *Some pharmaceutical drugs* [online]. 09.1980 [viewed 31 May 2024]. Available from: https://publications.iarc.fr/_publications/media/download/1572/280837b296cfecf310c28f0c729a36f2e9016384.pdf.

(52) SCHMIDTSDORFF, Sebastian, Jonas NEUMANN, Alexander H. SCHMIDT, and Maria K. PARR. Risk assessment for nitrosated pharmaceuticals: A future perspective in drug development [online]. *Archiv der Pharmazie.* 2022, **355**(4), 2100435. Available from: 10.1002/ardp.202100435.

(53) FARRUKH, Muhammad Junaid, Muhammad Haseeb TARIQ, Obaidullah MALIK, and Tahir Mehmood KHAN. Valsartan recall: global regulatory overview and future challenges [online]. *Therapeutic Advances in Drug Safety.* 2019, **10**, 2042098618823458. Available from: 10.1177/2042098618823458.

(54) U.S. FOOD AND DRUG ADMINISTRATION. *Laboratory Tests | Ranitidine* [online]. 1 November 2019, 12:00 [viewed 30 March 2024]. Available from: https://www.fda.gov/drugs/drug-safety-and-availability/laboratory-tests-ranitidine.

(55) EUROPEAN MEDICINES AGENCY. *Suspension of ranitidine medicines in the EU* [online]. 30 April 2020, 12:00 [viewed 13 March 2024]. Available from: https://www.ema.europa.eu/en/documents/press-release/suspension-ranitidine-medicines-eu_en.pdf.

(56) HEALTH CANADA. *Nitrosamine impurities in medications: recalls - Canada.ca* [online]. 15 March 2024, 12:00 [viewed 28 May 2024]. Available from: https://www.canada.ca/en/health-canada/services/drugs-health-products/compliance-enforcement/information-health-product/drugs/nitrosamine-impurities/recalls.html.

(57) U.S. FOOD AND DRUG ADMINISTRATION. *FDA Requests Removal of All Ranitidine Products (Zantac) from the Market* [online]. 4 February 2020, 12:00 [viewed 28 May 2024]. Available from: https://www.fda.gov/news-events/press-announcements/fda-requests-removal-all-ranitidine-products-zantac-market.

(58) WORLD HEALTH ORGANIZATION - PREQUALIFICATION UNIT - MEDICINES. *Nitrosamine contamination – Recent international alerts* [online]. 6 November 2019, 12:00 [viewed 11 March 2024]. Available from: https://extranet.who.int/prequal/news/nitrosamine-contamination-recent-international-alerts.

(59) WORLD HEALTH ORGANIZATION. *Update on Nitrosamine impurities* [online]. 20 November 2019, 12:00 [viewed 12 March 2024]. Available from: https://cdn.who.int/media/docs/default-source/essential-medicines/medical-alert-2019/informationnotenitrosamine-impurities-nov2019en.pdf?sfvrsn=d189497f_21.

(60) COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE. *ICH M7(R2) Questions and Answers on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk* [online]. 19 July 2023, 12:00 [viewed 31 May 2024]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-m7r2-questions-and-answers-assessment-and-control-dna-reactive-mutagenic-impurities-pharmaceuticals-limit-potential-carcinogenic-risk-step-5_en.pdf.

(61) EUROPEAN MEDICINES AGENCY. *EMA advises companies on steps to take to avoid nitrosamines in human medicines* [online]. 26 September 2019, 12:00 [viewed 13 March 2024]. Available from: https://www.ema.europa.eu/en/documents/press-release/ema-advises-companies-steps-take-avoid-nitrosamines-human-medicines_en.pdf.

(62) HEALTH SCIENCES AUTHORITY SINGAPORE. *HSA Recalls Three out of 46 Metformin Medicines* [online]. 4 December 2019, 12:00 [viewed 17 March 2024]. Available from: https://www.hsa.gov.sg/announcements/news/hsa-recalls-three-out-of-46-metformin-medicines.

(63) U.S. FOOD AND DRUG ADMINISTRATION. *FDA Alerts Patients and Health Care Professionals to Nitrosamine Impurity Findings in Certain Metformin Extended-Release Products* [online]. 28 May 2020, 12:00 [viewed 17 March 2024]. Available from: https://www.fda.gov/news-events/press-announcements/fda-alerts-patients-and-health-care-professionals-nitrosamine-impurity-findings-certain-metformin.

(64) HEALTH CANADA. *Certain Metformin diabetes drugs recalled due to the presence or possible presence of NDMA* [online]. 13 June 2020, 12:00 [viewed 17 March 2024].

Available from: https://recalls-rappels.canada.ca/en/alert-recall/certain-metformin-diabetes-drugs-recalled-due-presence-or-possible-presence-ndma.

(65) WORLD HEALTH ORGANIZATION. *WHO Model List of Essential Medicines* [online]. 08.2017 [viewed 27 March 2024]. Available from: https://iris.who.int/bitstream/handle/10665/273826/EML-20-eng.pdf?sequence=1.

(66) WORLD HEALTH ORGANIZATION. *WHO Prequalification Unit – Medicines request to manufacturers to conduct risk assessments to evaluate the potential for the presence of nitrosamine impurities* [online]. 2 April 2020, 12:00 [viewed 13 March 2024]. Available from: https://extranet.who.int/prequal/news/who-prequalification-unit-medicines-request-manufacturers-conduct-risk-assessments-evaluate.

(67) WORLD HEALTH ORGANIZATION - PREQUALIFICATION UNIT - MEDICINES. *Medicines (Finished Pharmaceutical Products/Biotherapeutic products) - Prequalification* [online] [viewed 30 May 2024]. Available from: https://extranet.who.int/prequal/medicines/prequalified/finished-pharmaceutical-products.

(68) WORLD HEALTH ORGANIZATION. *The Selection and Use of Essential Medicines* [online]. *Report of the WHO Expert Committee on Selection and Use of Essential Medicines, 2021 (including the 22nd WHO Model List of Essential Medicines and the 8th WHO Model List of Essential Medicines for Children).* 27 January 2021, 12:00 [viewed 3 April 2024]. Available from: https://iris.who.int/bitstream/handle/10665/351172/9789240041134eng.pdf?sequence=1.

(69) WORLD HEALTH ORGANIZATION - PREQUALIFICATION UNIT - MEDICINES. *Nitrosamine concerns in Priftin (Rifapentine)* [online]. 9 July 2020, 12:00 [viewed 18 March 2024]. Available from: https://extranet.who.int/prequal/news/nitrosamine-concerns-priftin-rifapentine.

(70) U.S. FOOD AND DRUG ADMINISTRATION. *FDA Updates and Press Announcements on Nitrosamines in Rifampin and Rifapentine* [online]. 28 February 2021, 12:00 [viewed 18 March 2024]. Available from: https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-nitrosamines-rifampin-and-rifapentine.

(71) WHO EXPERT COMMITTEE ON THE SELECTION AND USE OF ESSENTIAL MEDICINES. *The selection and use of essential medicines* [online]. *Report of the WHO Expert Committee, 2017 (including the 20th WHO Model List of Essential Medicines and the*

6th WHO Model List of Essential Medicines for Children). 2017 [viewed 31 May 2024]. Available from: https://iris.who.int/bitstream/handle/10665/259481/9789241210157-eng.pdf?sequence=1&isAllowed=y.

(72) U.S. FOOD AND DRUG ADMINISTRATION. *Laboratory analysis of rifampin/rifapentine products* [online]. 28 February 2021, 12:00 [viewed 18 March 2024]. Available from: https://www.fda.gov/drugs/drug-safety-and-availability/laboratory-analysis-rifampinrifapentine-products.

(73) WORLD HEALTH ORGANIZATION - PREQUALIFICATION UNIT - MEDICINES. *Nitrosamine concerns for rifapentine and rifampicin* [online]. 23 October 2020, 12:00 [viewed 18 March 2024]. Available from: https://extranet.who.int/prequal/news/nitrosamine-concerns-rifapentine-and-rifampicin.

(74) WORLD HEALTH ORGANIZATION - PREQUALIFICATION UNIT - MEDICINES. *Nitrosamine concerns for Priftin (rifapentine) - Update* [online]. 30 October 2020, 12:00 [viewed 18 March 2024]. Available from: https://extranet.who.int/prequal/news/nitrosamine-concerns-priftin-rifapentine-update.

(75) WORLD HEALTH ORGANIZATION - PREQUALIFICATION UNIT - MEDICINES. *Nitrosamine concerns for rifapentine and rifampicin Update and FAQs* [online]. 18 December 2020, 12:00 [viewed 19 March 2024]. Available from: https://extranet.who.int/prequal/sites/default/files/document_files/FAQ_Nitrosamine_18Dec2020.pdf.

(76) EUROPEAN MEDICINES AGENCY. *Meeting highlights from the Committee for Medicinal Products for Human Use (CHMP) 13-16 September 2021* [online]. 17 September 2021, 12:00 [viewed 25 March 2024]. Available from: https://www.ema.europa.eu/en/news/meeting-highlights-committee-medicinal-products-human-use-chmp-13-16-september-2021.

(77) EUROPEAN MEDICINES AGENCY. *Shortage of Champix (varenicline) Film-coated tablets, 0.5 mg, 1 mg* [online]. 24 September 2021, 12:00 [viewed 25 March 2024]. Available from: https://www.ema.europa.eu/en/documents/shortage/champix-varenicline-supply-shortage_en.pdf.

(78) U.S. FOOD AND DRUG ADMINISTRATION. *https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-nitrosamine-varenicline-chantix* [online]. 5 May 2022, 12:00 [viewed 25 March 2024]. Available from:

https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-nitrosamine-varenicline-chantix.

(79) WORLD HEALTH ORGANIZATION. *Two new tobacco cessation medicines added to the WHO essential medicines list* [online]. 5 November 2021, 12:00 [viewed 6 March 2024]. Available from: https://www.who.int/news/item/05-11-2021-two-new-tobacco-cessation-medicines-added-to-the-who-essential-medicines-list.

(80) LANG, Adam Edward, Urvashi PATEL, Jonathan FITZPATRICK, Tiffany LEE, Margo MCFARLAND, and Chester B. GOOD. Association of the Chantix Recall With US Prescribing of Varenicline and Other Medications for Nicotine Dependence [online]. *JAMA network open.* 2023, **6**(2), e2254655. Available from: 10.1001/jamanetworkopen.2022.54655.

(81) WORLD HEALTH ORGANIZATION - PREQUALIFICATION UNIT - MEDICINES. *Nitrosamine concerns in rifampicin products - Update* [online]. 8 October 2021, 12:00 [viewed 19 March 2024]. Available from: https://extranet.who.int/prequal/news/nitrosamine-concerns-rifampicin-products-update.

(82) HEALTH SCIENCE AUTHORITY. *Recall of Ventolin 2mg Tablets* [online]. 9 December 2021, 12:00 [viewed 29 May 2024]. Available from: https://www.hsa.gov.sg/announcements/product-recall/recall-of-ventolin-2mg-tablets.

(83) 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* [online]. 22 March 2022, 12:00 [viewed 30 March 2024]. Available from: 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.

(84) WORLD HEALTH ORGANIZATION - PREQUALIFICATION UNIT - MEDICINES. *Nitrosamine concerns in rifapentine products - Update* [online]. 12 July 2022, 12:00 [viewed 19 March 2024]. Available from: https://extranet.who.int/prequal/news/nitrosamine-concerns-rifapentine-products-update.

(85) WORLD HEALTH ORGANIZATION - PREQUALIFICATION UNIT - MEDICINES. *Nitrosamine contamination policy for APIs* [online]. 25 September 2022, 12:00 [viewed 21 March 2024]. Available from:

https://extranet.who.int/prequal/sites/default/files/document_files/Guidance_Nitrosamine_Contamination_Policy_APIs_Rev1_0.pdf.

(86) WORLD HEALTH ORGANIZATION - PREQUALIFICATION UNIT - MEDICINES. *Guidance on amendments to an active pharmaceutical ingredient master file (APIMF) submitted in support of a prequalified pharmaceutical product (FPP) or prequalified active pharmaceutical ingredient (API)* [online]. 6 February 2023, 12:00 [viewed 23 March 2024]. Available from: https://extranet.who.int/prequal/sites/default/files/document_files/Amendment-Guidance_20230209.pdf.

(87) WORLD HEALTH ORGANIZATION - PREQUALIFICATION UNIT - MEDICINES. *Nitrosamine contamination policy for FPP applications (an update)* [online]. 15 December 2022, 12:00 [viewed 31 May 2024]. Available from: https://extranet.who.int/prequal/sites/default/files/document_files/NitrosamineContaminationPolicy_FPP-Applications-Dec2022%20.pdf.

(88) WORLD HEALTH ORGANIZATION - PREQUALIFICATION UNIT - MEDICINES. *FAQ: Variations to prequalified pharmaceutical products* [online]. 15 December 2022, 12:00 [viewed 24 March 2024]. Available from: https://extranet.who.int/prequal/sites/default/files/document_files/FAQ_Variations-revision_15Dec2022.pdf.

(89) RP. INSTITUT ZUR SICHERUNG DER ARZNEIMITTELVERORDNUNG. *Wichtige Arzneimittelinformationen* [online]. *Chargenrückruf.* Implicor 25 mg/5 mg und 50 mg/5 mg, „EurimPharm“. 11 July 2023, 12:00 [viewed 27 March 2024]. Available from: https://ami.deutschesarztportal.de/?id=3134&Arzneimittelinformationen%5Bdatum%5D=%3E%3D+2019-01-29&page_no_top=0.

(90) APOTHEKE ADHOC. *Rückruf: Lisinopril mit Nitrosaminen verunreinigt* [online]. 19 September 2023, 12:00 [viewed 27 March 2024]. Available from: https://www.apotheke-adhoc.de/nachrichten/detail/pharmazie/rueckruf-lisinopril-mit-nitrosaminen-verunreinigt/.

(91) HEALTH CANADA. *Amitriptyline antidepressant drugs recalled because of a nitrosamine impurity* [online]. 7 February 2023, 12:00 [viewed 2 April 2024]. Available from: https://recalls-rappels.canada.ca/en/alert-recall/amitriptyline-antidepressant-drugs-recalled-because-nitrosamine-impurity.

(92) HEALTH CANADA. *Apotex recalls Apo-Acyclovir tablets due to a nitrosamine impurity* [online]. 29 July 2022, 12:00 [viewed 2 April 2024]. Available from: https://recalls-

rappels.canada.ca/en/alert-recall/apotex-recalls-apo-acyclovir-tablets-due-nitrosamine-impurity.

(93) SWISSMEDIC. *Chargenrückruf – Fluoxetin Sandoz 20 mg, dispergierbare Tabletten* [online]. 21 July 2023, 12:00 [viewed 2 April 2024]. Available from: https://www.swissmedic.ch/dam/swissmedic/de/dokumente/marktueberwachung/chargenrueckruf/chargenrueckruf-fluoxetin-sandoz.pdf.download.pdf/Kundeninformation_Fluoxetin_SDZ_DT_DE_final.pdf.

(94) HEALTH SCIENCES AUTHORITY SINGAPORE. *Recall of Apo-Amitriptyline 10mg Tablet* [online]. 16 August 2023, 12:00 [viewed 29 May 2024]. Available from: https://www.hsa.gov.sg/announcements/product-recall/recall-of-apo-amitriptyline-10mg-tablet.

(95) U.S. FOOD AND DRUG ADMINISTRATION. *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* [online]. 22 March 2023, 12:00 [viewed 27 March 2024]. Available from: https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/ascend-laboratories-llc-issues-voluntary-nationwide-recall-dabigatran-etexilate-capsules-usp-75-mg.

(96) HEALTH CANADA. *Pfizer recalls Inderal-LA (propranolol hydrochloride) capsules due to a nitrosamine impurity* [online]. 1 March 2022, 12:00 [viewed 2 April 2024]. Available from: https://recalls-rappels.canada.ca/en/alert-recall/pfizer-recalls-inderal-propranolol-hydrochloride-capsules-due-nitrosamine-impurity.
DEUTSCHE APOTHEKER ZEITUNG. *Wegen Nitrosamin-Verunreinigung – Rückruf zweier Atomoxetin-Chargen* [online]. 30 May 2023, 12:00 [viewed 27 March 2024]. Available from: https://www.deutsche-apotheker-zeitung.de/news/artikel/2023/05/30/wegen-nitrosamin-verunreinigung-rueckruf-zweier-atomoxetin-chargen.

(97) WORLD HEALTH ORGANIZATION. *WHO model list of essential medicines - 21st list, 2019* [online]. 23 July 2019, 12:00 [viewed 28 March 2024]. Available from: https://iris.who.int/bitstream/handle/10665/325771/WHO-MVP-EMP-IAU-2019.06-eng.pdf?sequence=1.

(98) WORLD HEALTH ORGANIZATION - PREQUALIFICATION UNIT - MEDICINES. *Update on N-nitrosamine impurities* [online]. 18 September 2023, 12:00 [viewed 27 March

2024]. Available from: https://extranet.who.int/prequal/news/update-n-nitrosamine-impurities.

(99) WORLD HEALTH ORGANIZATION. *Draft working document for comments: WHO good manufacturing practices 4 considerations for the prevention and 5 control of nitrosamine contamination 6 in pharmaceutical products* [online]. 2 April 2024, 12:00 [viewed 30 May 2024]. Available from: https://cdn.who.int/media/docs/default-source/medicines/norms-and-standards/current-projects/qas24_943_gmp_nitrosamines_forpublic52f95166-1c71-4cc2-a499-05bc4967bf14.pdf?sfvrsn=2140e3f4_2.

(100) BAYNE, Anne-Cécile V., Zdravka MISIC, René T. STEMMLER, Marc WITTNER, Margarita FRERICHS, Julia K. BIRD, and Ahmed BESHEER. N-nitrosamine Mitigation with Nitrite Scavengers in Oral Pharmaceutical Drug Products [online]. *Journal of Pharmaceutical Sciences.* 2023, **112**(7), 1794-1800 [viewed 5 May 2024]. Available from: 10.1016/j.xphs.2023.03.022.

(101) COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE. *Camzyos* [online]. *EPAR - Public Assessment Report.* 24 July 2023, 12:00 [viewed 18 April 2024]. Available from: https://www.ema.europa.eu/en/documents/assessment-report/camzyos-epar-public-assessment-report_en.pdf.

(102) U.S. FOOD AND DRUG ADMINISTRATION. *AAM/CHPA/PhRMA Questions for May 4th FDA-Industry Meeting to Discuss Nitrosamine Impurities in Pharmaceuticals* [online] [viewed 6 January 2024]. Available from: https://www.fda.gov/media/150864/download.

(103) EUROPEAN FEDERATION OF PHARMACEUTICAL INDUSTRIES AND ASSOCIATIONS. *N-nitrosamine Impurities in Biological Medicinal Products* [online]. 11 November 2020, 12:00 [viewed 1 June 2024]. Available from: https://www.efpia.eu/media/580595/n-nitrosamine-impurities-in-biological-medicinal-products.pdf.

(104) 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* [online]. 07.2023 [viewed 6 January 2024]. Available from:

https://www.hma.eu/fileadmin/dateien/Human_Medicines/CMD_h_/Advice_from_CMDh/Nitrosamins/CMDh_412_2019_Rev.21_2023_07_clean_-_PG_to_MAHs_on_nitrosamines.pdf.

(105) SUN, Minghui, Lingling ZHAO, Kai WANG, Lida HAN, Jihao SHAN, Liming WU, and Xiaofeng XUE. Rapid identification of “mad honey” from Tripterygium wilfordii Hook. f. and Macleaya cordata (Willd) R. Br using UHPLC/Q-TOF-MS [online]. *Food Chemistry.* 2019, **294**, 67-72 [viewed 1 June 2024]. Available from: 10.1016/j.foodchem.2019.05.028.

(106) ZHU, Hongyan, Xintong WANG, Xiaoming WANG, Guixiang PAN, Yan ZHU, and Yuxin FENG. The toxicity and safety of Chinese medicine from the bench to the bedside [online]. *Journal of Herbal Medicine.* 2021, **28**, 100450 [viewed 1 June 2024]. Available from: 10.1016/j.hermed.2021.100450.

(107) LUYCKX, Valerie A. Nephrotoxicity of Alternative Medicine Practice [online]. *Advances in Chronic Kidney Disease.* 2012, **19**(3), 129-141 [viewed 1 June 2024]. Available from: 10.1053/j.ackd.2012.04.005.

(108) AKKARAJU, Harshita, Ritik TATIA, Sayalee Sanjay MANE, Amol B. KHADE, and Swapnil J. DENGALE. A comprehensive review of sources of nitrosamine contamination of pharmaceutical substances and products [online]. *Regulatory toxicology and pharmacology: RTP.* 2023, **139**, 105355. Available from: 10.1016/j.yrtph.2023.105355.

(109) WILLIAMS, D. L. H. *Nitrosation reactions and the chemistry of nitric oxide.* 1st ed. Amsterdam: Elsevier, 2004. 9780080473604.

(110) WHITE, Emil H. The Chemistry of the N-Alkyl-N-nitrosoamides. I. Methods of Preparation [online]. *Journal of the American Chemical Society.* 1955, **77**(22), 6008-6010. Available from: 10.1021/ja01627a063.

(111) PLANTE, Jeffrey, Beth A. CAINE, and Paul L. A. POPELIER. Enhancing Carbon Acid pKa Prediction by Augmentation of Sparse Experimental Datasets with Accurate AIBL (QM) Derived Values [online]. *Molecules (Basel, Switzerland).* 2021, **26**(4) [viewed 16 April 2024]. Available from: 10.3390/molecules26041048.

(112) PONTING, David J., and Robert S. FOSTER. Drawing a Line: Where Might the Cohort of Concern End? [online]. *Organic Process Research & Development.* 2023, **27**(10), 1703-1713. Available from: 10.1021/acs.oprd.3c00008.

(113) HAO, Gugu, Rong HU, Xiaoqin WANG, Pan GAO, Lin WANG, Manhua JIANG, Libo XIN, Guoying TAN, Yanping ZHAO, Fuzhou SUN, Dexin CHU, Jinqian LV, Jinsong YOU, Fangfang HUANG, and Xuezhi SONG. N-Nitrosodimethylamine formation in metformin hydrochloride sustained-release tablets: Effects of metformin and hypromellose used in drug product formulation [online]. *Journal of Pharmaceutical and Biomedical Analysis.* 2023, **222**, 115066. Available from: 10.1016/j.jpba.2022.115066.

(114) SCHLINGEMANN, Joerg, Celine BOUCLEY, Sebastian HICKERT, Laura BOURASSEAU, Matt WALKER, Caroline CELDRAN, Thibaut CHEMARIN, Celine PEGUES, Matthias FRITZSCHE, Judith KEITEL, Anja GOETTSCHE, Maic SEEGEL, Stefan LEICHT, Brunhilde GUESSREGEN, Philipp REIFENBERG, Stephanie WETZEL, Tim MÜLLER, Fanny SCHOOREN, Tobias SCHUSTER, Mike LIEBHOLD, Annette KIRSCH, Phillip KRUEGER, Christoph SAAL, Bruno MOUTON, and Sandra MASANES. Avoiding N-nitrosodimethylamine formation in metformin pharmaceuticals by limiting dimethylamine and nitrite [online]. *International Journal of Pharmaceutics.* 2022, **620**, 121740. Available from: 10.1016/j.ijpharm.2022.121740.

(115) BOETZEL, Ruth, Joerg SCHLINGEMANN, Sebastian HICKERT, Christian KORN, Grace KOCKS, Bert LUCK, Giorgio BLOM, Mark HARRISON, Marc FRANÇOIS, Leonardo ALLAIN, Yongmei WU, and Youssi BOUSRAF. A Nitrite Excipient Database: A Useful Tool to Support N-Nitrosamine Risk Assessments for Drug Products [online]. *Journal of Pharmaceutical Sciences.* 2023, **112**(6), 1615-1624 [viewed 26 April 2024]. Available from: 10.1016/j.xphs.2022.04.016.

(116) MOSER, Justin, Ian W. ASHWORTH, Laurence HARRIS, Michael C. HILLIER, Kausik K. NANDA, and Garry SCRIVENS. N-Nitrosamine Formation in Pharmaceutical Solid Drug Products: Experimental Observations [online]. *Journal of Pharmaceutical Sciences.* 2023, **112**(5), 1255-1267. Available from: 10.1016/j.xphs.2023.01.027.

(117) MOORCROFT, M. J., J. DAVIS, and R. G. COMPTON. Detection and determination of nitrate and nitrite: a review [online]. *Talanta.* 2001, **54**(5), 785-803. Available from: 10.1016/s0039-9140(01)00323-x.

(118) ROK GRAHEK. *Mitigation Strategies for Nitrosamine Drug Substance Related Impurities: Quality and Bioequivalence Considerations for Generic Products* [online]. *Determination of nitrite in pharmaceutical excipients; air as source for higher nitrite*

levels. 6 December 2023, 12:00 [viewed 8 May 2024]. Available from: https://www.complexgenerics.org/education-training/mitigation-strategies-for-nitrosamine-drug-substance-related-impurities-quality-and-bioequivalence-considerations-for-generic-products/.

(119) GOLOB, Nejc, Rok GRAHEK, Malcolm ROSS, and Robert ROŠKAR. Nitrocellulose blister material as a source of N-nitrosamine contamination of pharmaceutical drug products [online]. *International Journal of Pharmaceutics.* 2022, **618**, 121687 [viewed 2 July 2024]. Available from: 10.1016/j.ijpharm.2022.121687.

(120) RYCHLÝ, Jozef, Agnes LATTUATI-DERIEUX, Lyda MATISOVÁ-RYCHLÁ, Katarína CSOMOROVÁ, Ivica JANIGOVÁ, and Bertrand LAVÉDRINE. Degradation of aged nitrocellulose investigated by thermal analysis and chemiluminescence [online]. *Journal of Thermal Analysis and Calorimetry.* 2012, **107**(3), 1267-1276 [viewed 2 July 2024]. Available from: 10.1007/s10973-011-1746-8.

(121) BOLTRES, Bettine. Evaluating Nitrosamines from Elastomers in Pharmaceutical Primary Packaging [online]. *PDA Journal of Pharmaceutical Science and Technology.* 2022, **76**(2), 136-150 [viewed 2 July 2024]. Available from: 10.5731/pdajpst.2021.012645.

(122) WORLD HEALTH ORGANIZATION. *Guidelines for drinking-water quality* [online]. 2017 [viewed 31 May 2024]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK442376/.

(123) CIOC, Răzvan C., Ciarán JOYCE, Monika MAYR, and Robert N. BREAM. Formation of N -Nitrosamine Drug Substance Related Impurities in Medicines: A Regulatory Perspective on Risk Factors and Mitigation Strategies [online]. *Organic Process Research & Development.* 2023, **27**(10), 1736-1750. Available from: 10.1021/acs.oprd.3c00153.

(124) LHASA LIMITED. *Lhasa Carcinogenicity Database* [online]. 8 January 2024, 12:00 [viewed 2 June 2024]. Available from: https://carcdb.lhasalimited.org/.

(125) INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE AND EUROPEAN MEDICINES AGENCY. *ICH M7(R2) Addendum on application of the principles of the ICH M7 guideline to calculation of compound-specific acceptable intakes* [online]. 18 July 2023, 12:00 [viewed 6 February 2024]. Available from:

https://www.ema.europa.eu/en/documents/scientific-guideline/ich-m7r2-addendum-application-principles-ich-m7-guideline-calculation-compound-specific-acceptable-intakes-step-5_en.pdf.

(126) NAOMI L. KRUHLAK, SUMAN CHAKRAVARTI, GOVINDARAJ KUMA, and ROUSTEM SAIAKHOV. *A New Structural Similarity Method to Identify Surrogate Compounds for Assessing the Carcinogenicity of Nitrosamine Impurities* [online] [viewed 6 February 2024]. Available from: https://multicase.com/wp-content/uploads/2023/06/SOT-2022.pdf.

(127) BERCU, Joel P., Melisa MASUDA-HERRERA, Alejandra TREJO-MARTIN, Priyanka SURA, Robert JOLLY, Michelle KENYON, Rob THOMAS, David J. PONTING, David SNODIN, Gregor TUSCHL, Stephanie SIMON, Kathleen de VLIEGER, Richard HUTCHINSON, Andreas CZICH, Susanne GLOWIENKE, M. Vijayaraj REDDY, Sandra JOHANSSEN, Esther VOCK, Nancy CLAUDE, and Richard J. WEAVER. Acceptable intakes (AIs) for 11 small molecule N-nitrosamines (NAs) [online]. *Regulatory toxicology and pharmacology : RTP.* 2023, **142**, 105415. Available from: 10.1016/j.yrtph.2023.105415.

(128) NUDELMAN, Raphael, Grace KOCKS, Bruno MOUTON, David J. PONTING, Joerg SCHLINGEMANN, Stephanie SIMON, Graham F. SMITH, Andrew TEASDALE, and Anne-Laure WERNER. The Nitrosamine “Saga”: Lessons Learned from Five Years of Scrutiny [online]. *Organic Process Research & Development.* 2023, **27**(10), 1719-1735. Available from: 10.1021/acs.oprd.3c00100.

(129) CHAKRAVARTI, Suman. Computational Prediction of Metabolic α-Carbon Hydroxylation Potential of N-Nitrosamines: Overcoming Data Limitations for Carcinogenicity Assessment [online]. *Chemical research in toxicology.* 2023, **36**(6), 959-970. Available from: 10.1021/acs.chemrestox.3c00083.

(130) HONMA, Masamitsu. An assessment of mutagenicity of chemical substances by (quantitative) structure-activity relationship [online]. *Genes and environment : the official journal of the Japanese Environmental Mutagen Society.* 2020, **42**(1), 23. Available from: 10.1186/s41021-020-00163-1.

(131) FINE, Jonathan, Leonardo ALLAIN, Joerg SCHLINGEMANN, David J. PONTING, Robert THOMAS, and George E. JOHNSON. Nitrosamine acceptable intakes should consider variation in molecular weight: The implication of stoichiometric DNA damage

[online]. *Regulatory toxicology and pharmacology : RTP.* 2023, **145**, 105505 [viewed 5 November 2024]. Available from: 10.1016/j.yrtph.2023.105505.

(132) EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE. *First OMCL analytical procedures for determination of nitrosamine drug substance-related impurities (NDSRIs) and intermediate-related contaminants published,* 28 Mar. 2024.

(133) JINGYUE YANG. *Mitigation Strategies for Nitrosamine Drug Substance Related Impurities: Quality and Bioequivalence Considerations for Generic Products* [online]. *Performance Characteristics of Mass Spectrometry-Based Methods for Quantitation of Nitrosamines: Insights from an Inter-laboratory Study.* 06.2023 [viewed 5 June 2024]. Available from: https://www.complexgenerics.org/education-training/mitigation-strategies-for-nitrosamine-drug-substance-related-impurities-quality-and-bioequivalence-considerations-for-generic-products/.

(134) EUROPEAN MEDICINES AGENCY. *ICH Topic Q 7 Good Manufacturing Practice for Active Pharmaceutical Ingredients* [online]. 11.2000 [viewed 6 May 2024]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-7-good-manufacturing-practice-active-pharmaceutical-ingredients-step-5_en.pdf.

(135) EUROPEAN MEDICINES AGENCY. *ICH Topic Q 6 A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances* [online]. 05.2000 [viewed 6 May 2024]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-test-procedures-and-acceptance-criteria-new-drug-substances-and-new-drug-products-chemical-substances-step-5_en.pdf.

(136) U.S. FOOD AND DRUG ADMINISTRATION. *Novel Drug Approvals for 2022* [online]. 18 March 2024, 12:00 [viewed 24 April 2024]. Available from: https://www.fda.gov/drugs/novel-drug-approvals-fda/novel-drug-approvals-2022.

(137) COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE. *Camzyos | European Medicines Agency* [online]. 7 August 2023, 12:00 [viewed 24 April 2024]. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/camzyos.

(138) KEAM, Susan J. Mavacamten: First Approval [online]. *Drugs.* 2022, **82**(10), 1127-1135. Available from: 10.1007/s40265-022-01739-7.

(139) COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE. *Camzyos* [online]. *EPAR - Medicine overview.* 24 July 2023, 12:00 [viewed 24 April 2024]. Available from: https://www.ema.europa.eu/en/documents/overview/camzyos-epar-medicine-overview_en.pdf.

(140) COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE. *Camzyos* [online]. *EPAR - Product Information.* 24 July 2023, 12:00 [viewed 25 April 2024]. Available from: https://www.ema.europa.eu/en/documents/product-information/camzyos-epar-product-information_en.pdf.

(141) Pyrimidinedione Compounds. Inventor: J. OSLOB, R. ANDERSON, D. AUBELE, M. EVANCHIK, J. C. FOX, B. KANE, P. LU, R. MCDOWELL, H. RODRIGUEZ, and Y. SONG. USOO9585883B2.

(142) GOLLA, Vijaya Madhyanapu, Mallika KALYAN, Upasana GHOLAP, Hara Prasad PADHY, Roshitha K. RAMACHANDRAN, and Gananadhamu SAMANTHULA. Discerning the stability behaviour of mavacamten availing liquid chromatography-mass spectrometry and nuclear magnetic resonance spectroscopy: In silico toxicity and mutagenicity prediction of degradation products [online]. *Journal of Mass Spectrometry.* 2024, **59**(3), e5007. Available from: 10.1002/jms.5007.

(143) BERARDI, Alberto, Maarten JASPERS, and Bastiaan H. J. DICKHOFF. Modeling the Impact of Excipients Selection on Nitrosamine Formation towards Risk Mitigation [online]. *Pharmaceutics.* 2023, **15**(8). Available from: 10.3390/pharmaceutics15082015.

(144) EUROPEAN FEDERATION OF PHARMACEUTICAL INDUSTRIES AND ASSOCIATIONS. *Workflows for Quality risk management of nitrosamine risks in medicines* [online]. 08.2022 [viewed 4 May 2024]. Available from: https://www.efpia.eu/media/676632/efpia-nitrosamines-quality-risk-management-workflows-sep-2022.pdf.

(145) INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE. *ICH Press Release* [online]. *ICH Assembly Meeting, Fukuoka, Japan, June 2024* [viewed 7 January 2024]. Available from: https://admin.ich.org/sites/default/files/inline-files/ICH48_Fukuoka_Meeting_PressRelease_2024_0612_1.pdf.

(146) INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE. *Outline of Concept Paper* [online].

Addendum to ICH M7 on Risk Assessment and Control of Nitrosamine Impurities 3 June 2024. 4 June 2024, 12:00 [viewed 1 July 2024]. Available from: https://nitrosamines.usp.org/uploads/short-url/nkdvqmtieqHnxaY440Ey95uWeT5.pdf.

(147) WORLD HEALTH ORGANIZATION - PREQUALIFICATION UNIT - MEDICINES. *Prequalified Active Pharmaceutical Ingredients | WHO - Prequalification of Medical Products (IVDs, Medicines, Vaccines and Immunization Devices, Vector Control)* [online] [viewed 30 May 2024]. Available from: https://extranet.who.int/prequal/medicines/prequalified/active-pharmaceutical-ingredients.

(148) WORLD HEALTH ORGANIZATION. *WHO Model List of Essential Medicines - 23rd list, 2023* [online]. 26 July 2023, 12:00 [viewed 28 March 2024]. Available from: https://iris.who.int/bitstream/handle/10665/371090/WHO-MHP-HPS-EML-2023.02-eng.pdf?sequence=1.

(149) HEALTH SCIENCES AUTHORITY SINGAPORE. *Recall of Ventolin 2mg Tablets* [online]. 9 December 2021, 12:00 [viewed 30 March 2024]. Available from: https://www.hsa.gov.sg/announcements/product-recall/recall-of-ventolin-2mg-tablets.

(150) WORLD HEALTH ORGANIZATION. *The selection and use of essential medicines* [online]. *Report of the WHO Expert Committee on Selection and Use of Essential Medicines, 2019 (including the 21st WHO Model List of Essential Medicines and the 7th WHO Model List of Essential Medicines for Children).* 30 January 2020, 12:00 [viewed 31 May 2024]. Available from: https://iris.who.int/bitstream/handle/10665/330668/9789241210300-eng.pdf?sequence=1.

Annex I: Nitrosation assay procedure (NAP test) (WHO, 1978)

The NAP test recommended by the WHO Expert Group should be conducted under the following conditions: (51)

- Concentration of drug: 10 mmol/I
- Concentration of nitrite: 40 mmol/I
- Reaction temperature: 370°C
- pH: 3-4
- Reaction times: 1 hour and 4 hours

Adequate excess of nitrite to facilitate the reaction is ensured by the relative concentrations of the reactants, while the absolute concentrations align with the sensitivity of available measurement methods for detecting the resulting N-nitroso compounds. Nitrous acid exhibits minimal decomposition at 37°C, and the pH range of 3-4, optimal for most nitrosation reactions, closely mirrors the stomach's pH during digestion. Reaction times of 1 and 4 hours are established for rapidly and slowly reacting compounds, respectively, ensuring the completion of complex reactions. Adjustments to reaction times may be necessary if certain products prove unstable, following a comprehensive examination of the chemical reactions.

In all instances, substrates subjected to the NAP test should be in the purest state attainable, and careful efforts should be made to eliminate preformed nitrosamines whenever possible.

The evaluation of combination products containing multiple amino groups may pose specific challenges. Likewise, when a compound easily metabolizes into various nitrosatable products, each of these products must be assessed separately. Although accelerators and inhibitors of N-nitrosation reactions can significantly influence yields, their non-selective action means they do not alter the relative ranking of nitrosatable drugs on a comparative scale.

Annex II: Timeline of nitrosamine detections and WHO's corresponding activities

Events are not exhaustive.

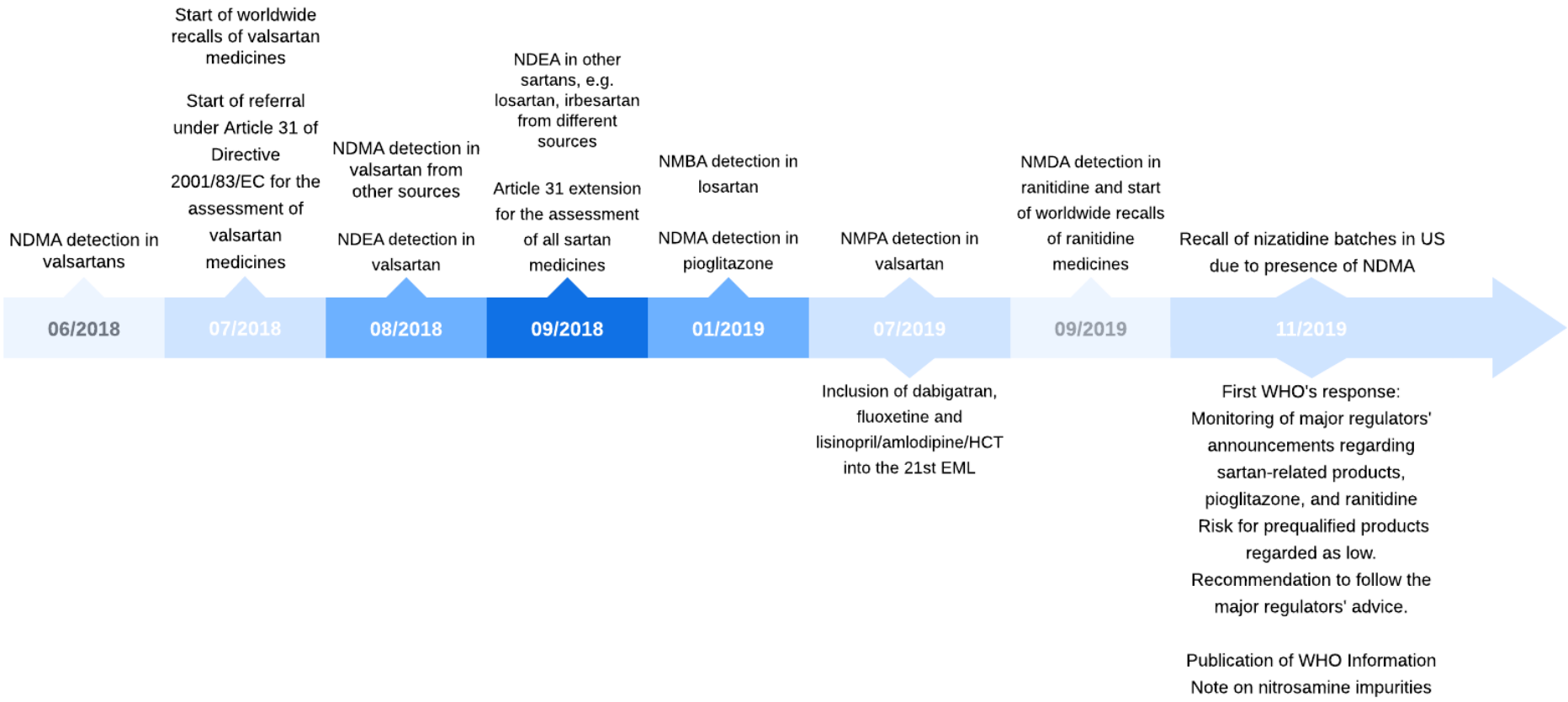
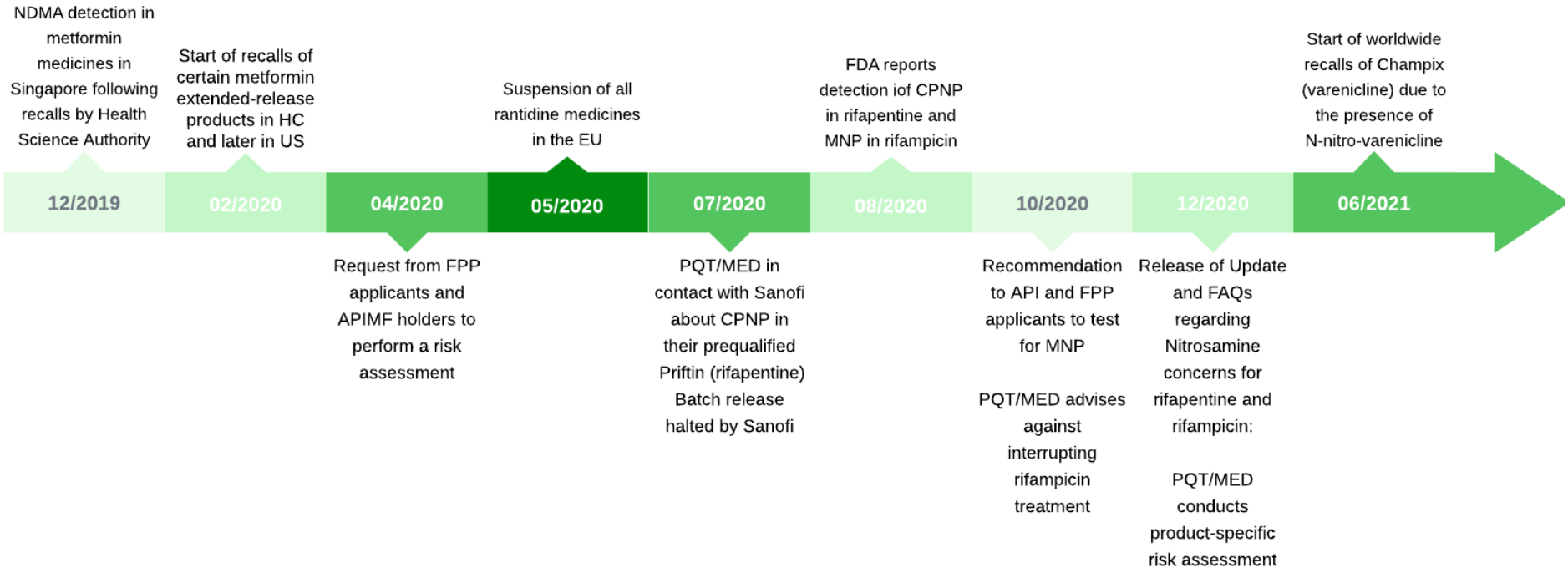


Figure 12-Timeline comparing nitrosamine detections and recalls (above the time bar) with WHO's corresponding activities (below the time bar)



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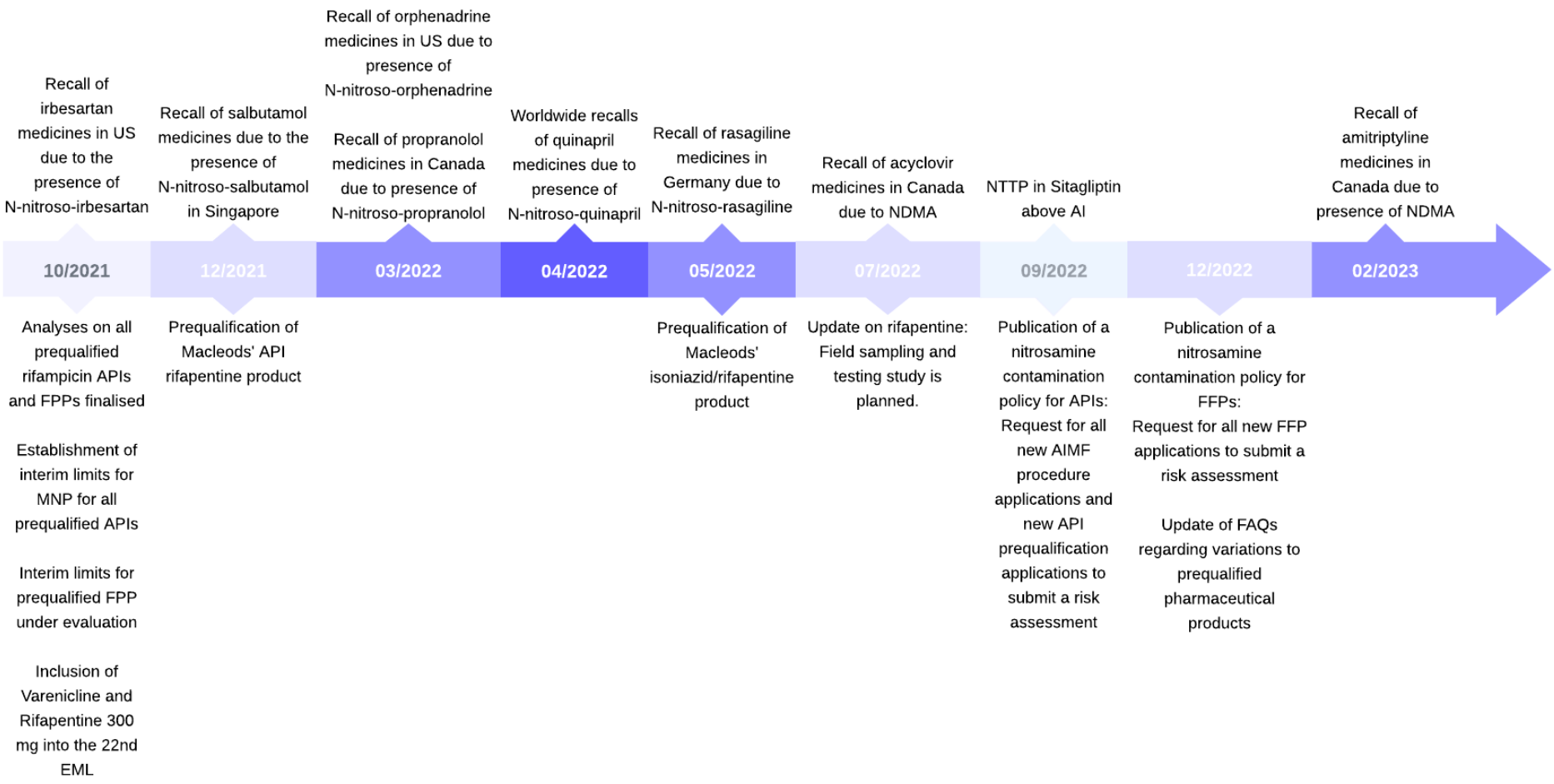


Figure 12-Timeline comparing nitrosamine detections and recalls (above the time bar) with WHO's corresponding activities (below the time bar)

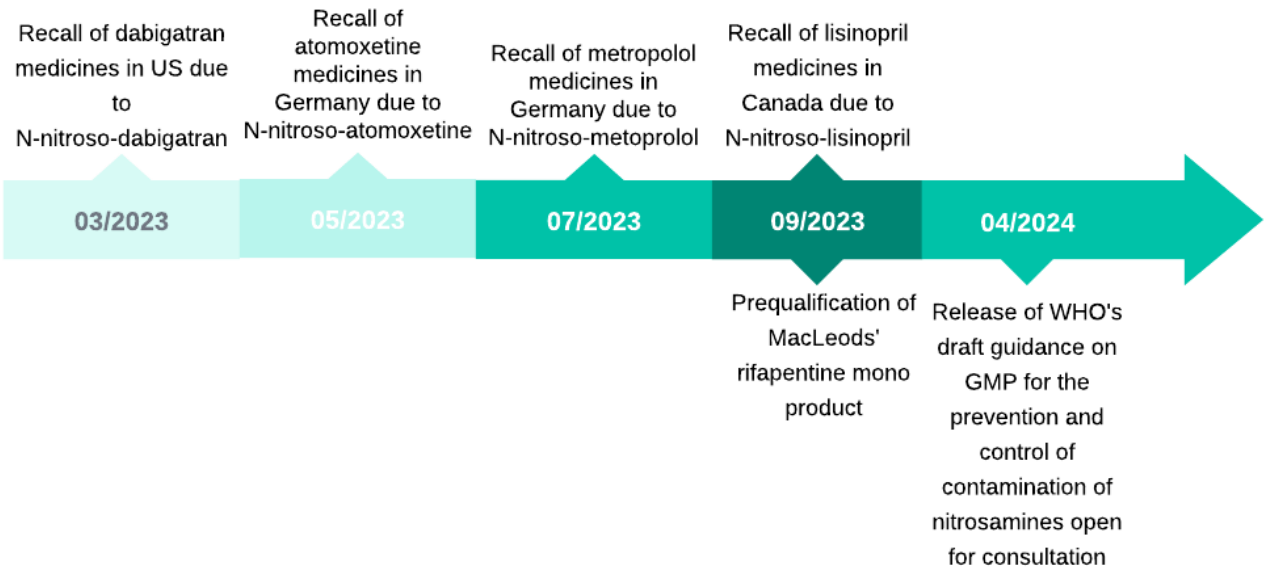


Figure 12-Timeline comparing nitrosamine detections and recalls (above the time bar) with WHO's corresponding activities (below the time bar)

Annex III: NA detection and essential drug and prequalification status

Table 4-Overview on detected NAs and their essential drug and prequalification status

Medicine, in which NAs were detected	Date of first NA detection	Recall	Detected nitrosamine	Prequalified FPP? When?(148, 67)	Essential drug status(149)?	Since when on EML?	Justification for essential drug status
Valsartan	June 2018(2)	Yes	NDMA, NDEA, NMPA(2)	No	No		
Losartan	September 2018(2)	Yes	NDEA, NMBA(2)	No	Yes	Before the NA crisis(65)	N/A
Irbesartan	September 2018(2)	Yes	NDEA(2)	No	No		
Pioglitazone	January 2019(2)	Yes	NDMA(2)	No	No		
Ranitidine	September 2019(2)	Yes	NDMA(2)	No	Yes	Before the NA crisis(65)	N/A
Nizatidine	November 2019(54)	Yes	NDMA(54)	No	No		
Metformin	December 2019(62)	Yes	NDMA(62)	No	Yes	Before the NA crisis(65)	N/A
Rifapentine	August 2020(62)	No	1-cyclopentyl-4-nitroso-piperazine(70)	Sanofi's Rifapentine 14.02.2017	Yes	150 mg: Before the NA crisis(65)	300 mg: adverse effects similar to those of the 150 mg strength
				Macleods' Isoniazid/Rifapentine 13.05.2022		300 mg: October 2021 (22nd EML)(68)	
				Macleods' Rifapentine mono 02.09.2023			

Table 4-Overview on detected NAs and their essential drug and prequalification status

Medicine, in which NAs were detected	Date of first NA detection	Recall	Detected nitrosamine	Prequalified FPP? When?(67)	Essential drug status(149)?	Since when on EML?	Justification for essential drug status
Rifampicin	August 2020(62)	No	1-methyl-4-nitroso-piperazine(70)	Several manufacturers (All mono products were prequalified before the NA crisis)	Yes	Before the NA crisis(65)	N/A
Varenicline	June 2021(76)	Yes	N-nitroso-varenicline(76)	No	Yes	October 2021 (22nd EML)(79)	Deadly consequences of tobacco consumption(79, 68)
Salbutamol	December 2021(150)	Yes	N-nitroso-salbutamol(150)	No	Yes	Before the NA crisis(65)	N/A
Orphenadrine	March 2022(83)	Yes	N-nitroso-orphenadrine(83)	No	No		
Propranolol	March 2022(96)	Yes	N-nitroso-propranolol(96)	No	No		
Quinapril	April 2022(6)	Yes	N-Nitroso-quinapril(6)	No	No		
Rasagiline	May 2022(7)	Yes	N-nitroso-rasagiline(7)	No	No		
Acyclovir	July 2022(92)	Yes	NDMA(92)	No	No		
Sitagliptin	August 2022(8)	No	NTTP(8)	No	No		
Amitriptyline	February 2023(91)	Yes	NDMA(91)	No	Yes	Before the NA crisis(65)	N/A
Dabigatran	March 2023(95)	Yes	N-nitroso-dabigatran(95)	No	Yes	July 2019 (21st EML)(98)	Favourable efficacy and acceptable safety(151)
Atomoxetine	May 2023(97)	Yes	N-nitroso-atomoxetine(97)	No	No		
Metoprolol	July 2023(89)	Yes	N-Nitroso-metoprolol(89)	No	No		
Fluoxetine	July 2023(93)	Yes	N-nitroso-fluoxetine(93)	No	Yes	July 2019 (21st EML)(98)	N/A
Lisinopril	September 2023(90)	Yes	N-nitroso-lisinopril(90)	No	Yes, in fixed-dose combination with amlodipine and HCT	July 2019 (21st EML)(98)	N/A

Annex IV: Analysis of APIs listed in the 23rd WHO EML (2023) for nitrosamine precursors

In order to determine the percentual number of APIs in the 23rd EML which are potential nitrosamine precursors, data from Schlingemann et al. were used as a basis. (9) According to their analysis there are 563 small molecules (APIs) in the 22nd EML from 2021 and 140 are potential nitrosamines precursors. 2023 the EML was updated, and 22 new small molecules were added to the list, whereas 4 substances were removed. That means that there are 581 small molecules included in the 23rd EML (2023). (149)

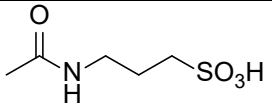
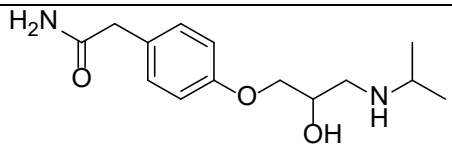
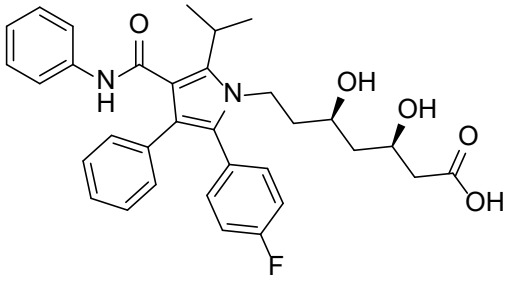
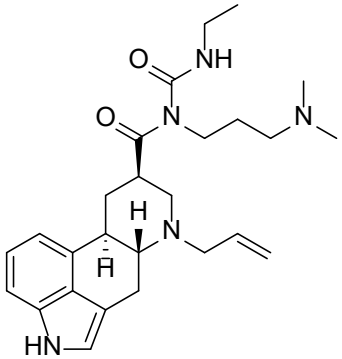
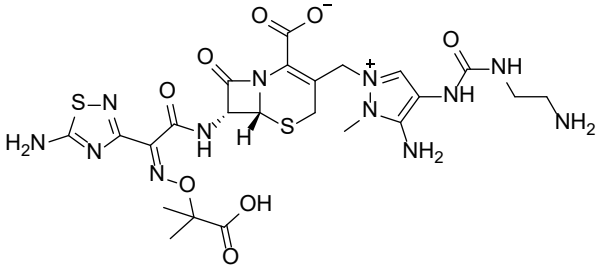
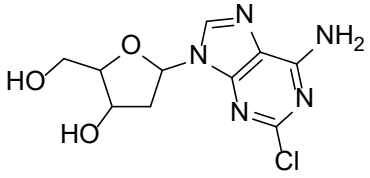
In a second step the added and removed structures were analysed for secondary and tertiary amine functionalities. For this purpose, rules from Schlingemann et al. were applied:

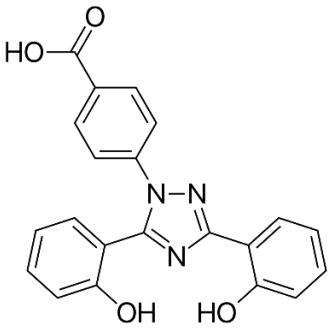
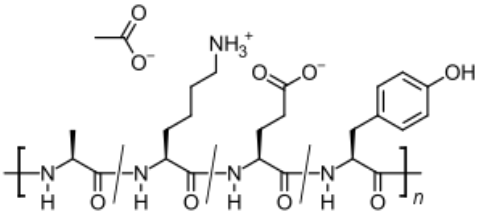
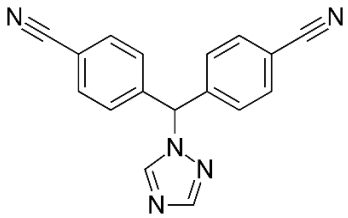
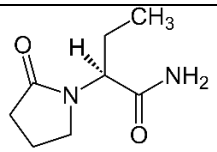
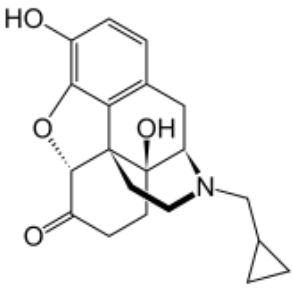
"For secondary amines, two carbons and one hydrogen must be bound to the amine nitrogen, and both carbons may only have single or aromatic bonds. This excludes enamines and amides. The bonds to amine nitrogen must be single bonds. This excludes nitrogen-containing aromatic rings […]. For tertiary amines, three carbons must be bound to the amine nitrogen. Two of them may only have single or aromatic bonds, while the third must only be single-bonded and be attached to at least one hydrogen. This hydrogen is required to allow for nitrosative cleavage of the residue." (9)

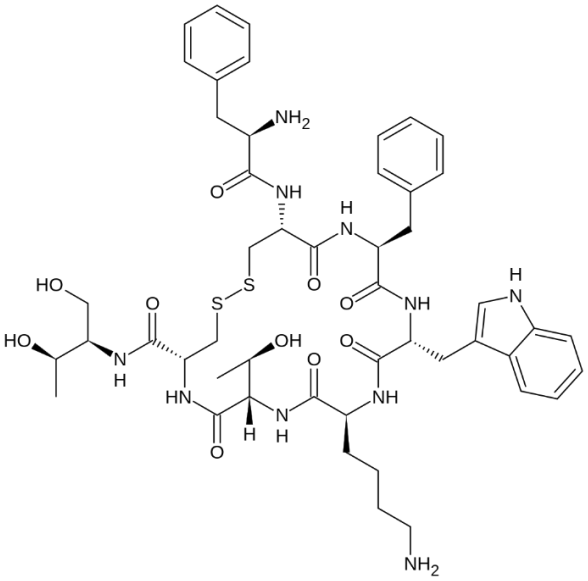
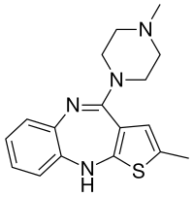
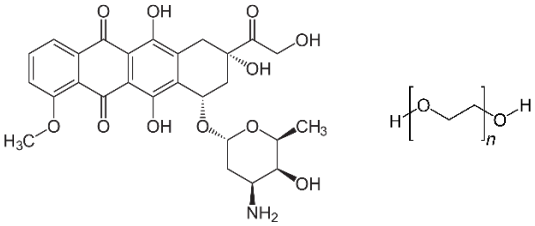
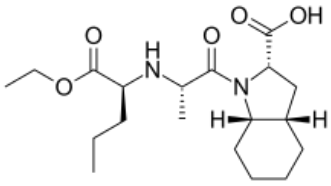
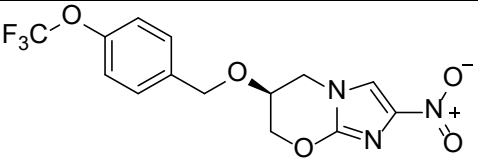
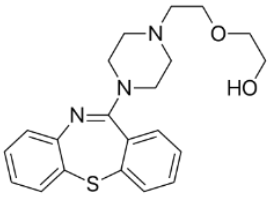
Results are provided in *Table 5* and *Table 6*. Nine vulnerable amines were added to the current EML, and two vulnerable amines were removed from the 22nd list which means that there are in total 147 new vulnerable amine in the current EML list. (149)

That is a percentage of 25,3%.

Table 5-Overview on newly added small molecule-APIs to the 23rd EML, their molecular structures and amine features

Small molecule	Molecular structure	Vulnerable 2nd amine	Vulnerable 3rd amine
Acamprosate		No	No
Atenolol		Yes	No
Atorvastatin		No	Yes
Cabergoline		No	Yes
Ceftolozane		No	No
Cladribine		No	Yes

Deferasirox		No	No
Glatiramer acetate		No	No
Letrozole		No	No
Levetiracetam		No	No
Naltrexone		No	Yes

Octreotide		No	No
Olanzapine		Yes	Yes
(Pegylated Liposomal) doxorubicin		No	No
Perindopril		Yes	No
Pretomanid		No	Yes
Quetiapine		No	Yes

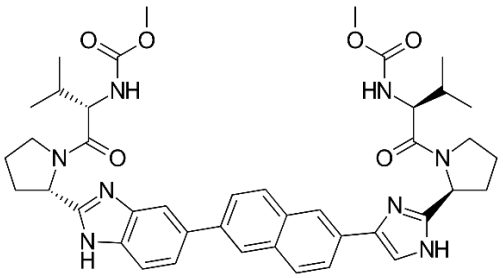
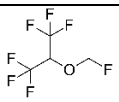
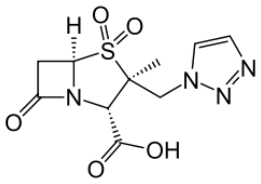
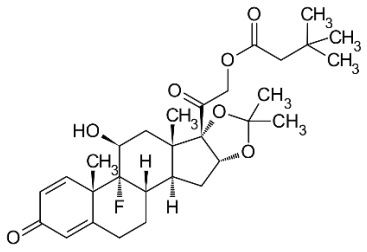
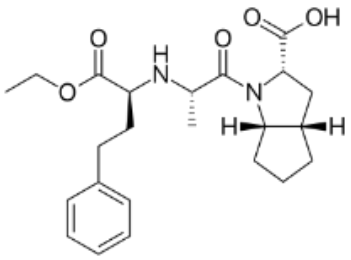
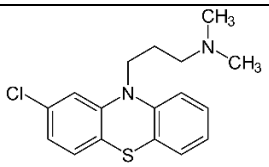
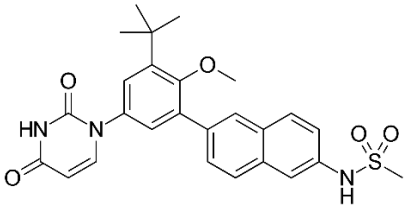
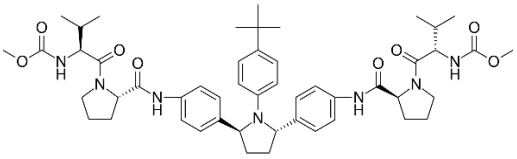
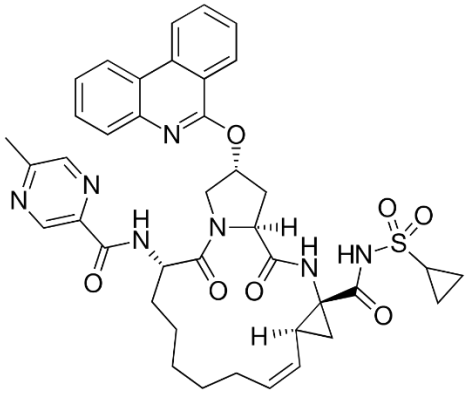
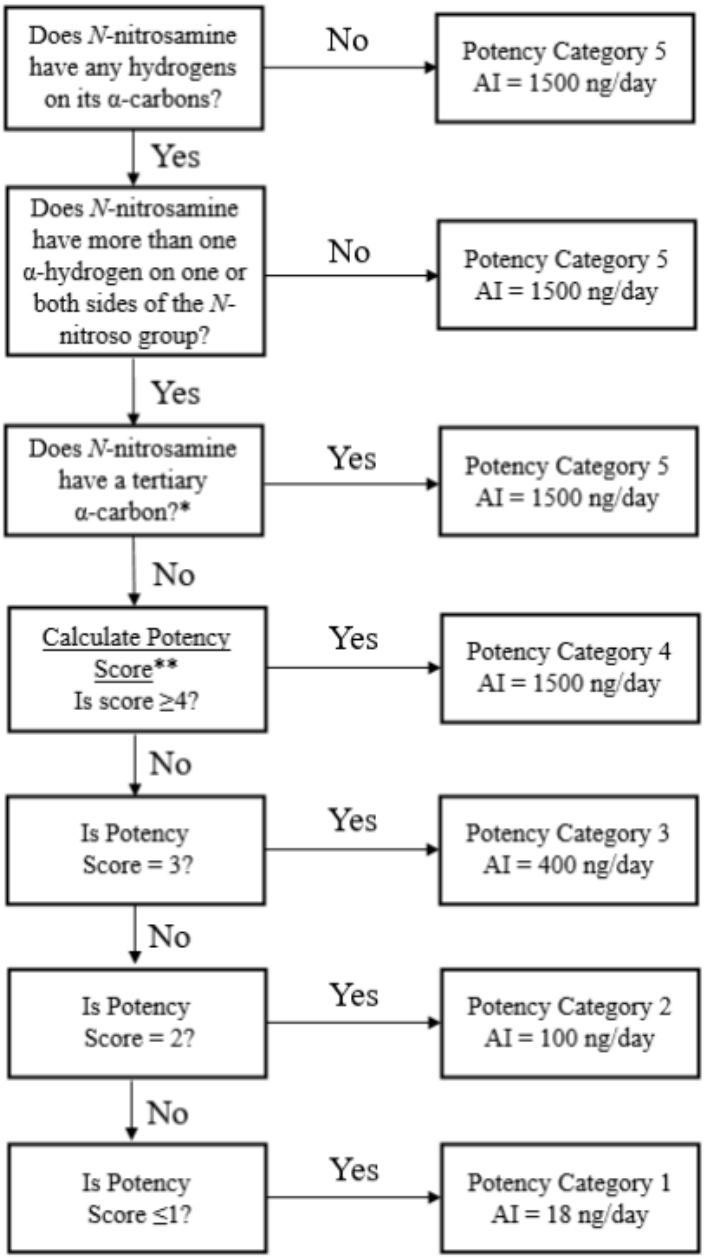
Ravidasvir		No	No
Sevoflurane		No	No
Tazobactam		No	No
Triamcinolone hexacetonide		No	No
Ramipril		1	

Table 6- Overview on removed small molecule-APIs from the 22nd EML, their molecular structures and amine features

Small molecule	Molecular structure	Vulnerable 2nd amine	Vulnerable 3rd amine
Chlorpromazin		No	Yes
Dasabuvir		No	No
Ombitasvir		No	Yes
Paritaprevir		No	No

Annex V: Carcinogenic potency categorization approach (CPCA)

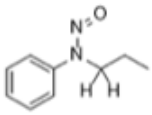
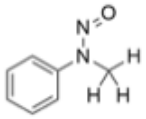
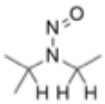
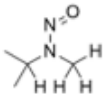
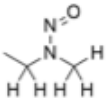
Figure 13-Flowchart for prediction of the Carcinogenic Potency Category of N-Nitrosamines (extract from Appendix 2 to EMA's Q&A document on nitrosamines) (134)



*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 nitrosamine) + **Activating Feature Score** (sum all scores for features present in the nitrosamine)

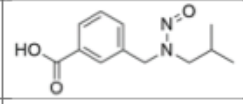
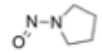
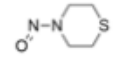
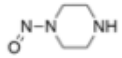
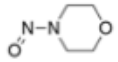
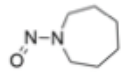
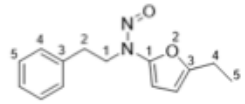
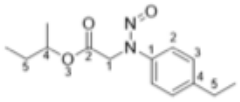
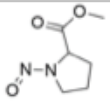
Table 7-Derivation of the α-Hydrogen Score (extract from Appendix 2 to EMA's Q&A document on nitrosamines) (134)

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

Count of hydrogen atoms on each a-carbon (lowest count first) and corresponding a-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 8-List of deactivating features and associated scores (extract from Appendix 2 to EMA's Q&A document on nitrosamines) (143)

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

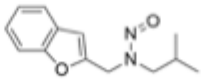
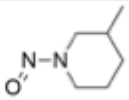
*Excludes examples where N-nitroso group is in a pyrrolidine ring, a 6-membered ring containing at least one sulphur atom or a morpholine ring (all counted separately).

**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 Structure-Activity Relationships for N-nitrosamine Activity, Comput Toxicol, 20:100186, where they are referred to as "β-carbon electron withdrawing groups."

***β-Carbon must be in a sp^3 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 9-List of activating features and associated scores (extract from Appendix 2 to EMA's Q&A document on nitrosamines) (134)

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

To calculate Activating Feature Score, sum the individual scores for all listed features present in the 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 VI: Determination of specification limits for option 2 of EMA's Q&A

According to EMA's Q&A document on nitrosamines, the calculation for total N-nitrosamines could be written as: (14)

$$\sum_{i=2}^{n} \frac{X_i}{AI_i} x100\% \leq 100\%$$

Where X_i is the amount of each single N-nitrosamine i in ppm and AI_i is the AI limit of each N-nitrosamine i in ppm.

For each batch, to determine whether the limit for total N-nitrosamines is met, the amount of each N-nitrosamine present (in ppm/ppb) should be converted to a percentage of its respective AI limit. The sum of % AI limits of specified N-nitrosamines should not exceed 100%.

Eidesstattliche Erklärung

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

Frankfurt a.M., 13.07.2024

Datum, Ort

Unterschrift