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Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Procedure under Article 5(3) of Regulation EC (No) 726/2004

Nitrosamine impurities in human medicinal products

Procedure number: EMEA/H/A-5(3)/1490

Note:

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

AI	Acceptable intake. In the context of this document, an intake level /limit associated with a theoretical excess lifetime cancer risk of 1:100,000 based on considerations in ICH M7(R1) for substances from the "cohort of concern"
API	Active Pharmaceutical Ingredient
AIM	Active Ingredient Manufacturer = active substance manufacturer
ALARA	as low as reasonably achievable
ALARP	as low as reasonably possible
BCNU	Bischloroethyl nitrosourea
CCNU	1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea
CHMP	Committee for Human Medicinal Products
CPDB	Carcinogenic potency database
CoA	Certificate of Analysis
CoC	Cohort of Concern Compounds
CEP	Certificate of Suitability to the monographs of the European Pharmacopoeia (CEP)
CRS	Chemical Reference Substance (official standard)
DIPNA	N,N-diisopropylethyl-N-ethylamine
DMF	Dimethylformamide
DI	Direct injection
DS	Drug substance
EDQM	European Directorate for the Quality of Medicines
EFSA	European Food Safety Agency
EIPNA	Ethylisopropyl-N-nitrosoamine
EMA	European Medicines Agency
ENOCs	Endogenously produced N-nitroso compounds
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
IARC	International Agency for Research on Cancer
IPC	In-process control
IR	Infrared Spectrometry
IU	International Units
LCDB	Lhasa Carcinogenicity Data Base
LOD	Limit of Detection
LTL	Less than Lifetime

LOQ	Limit of Quantification
MA	Marketing Authorisation
MAH	Marketing Authorisation holder
Methyl-CCNU	1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea
MGMT	Methyl-guanine-methyl-transferase
MNNG	N-Methyl-N´-nitro-N-nitrosoguanidine
MS	Mass Spectrometry
ND	Not detected
NDBzA	N-nitrosodibenzylamine
NDEA	N-Nitrosodiethylamine
NDELA	N-Nitrosodiethanolamine
NDiBA	N-Nitrosodiisobutylamine
NDMA	N-Nitrosodimethylamine
NDPA	N-Nitrosodi-n-propylamine
NDPhA	N-nitrosodiphenylamine
NHMTC	N-nitroso-2-hydroxymethyl-thiazolidine-4-carboxylic acid
NHPRO	N-nitrosohydroxyproline
NIAN	1-Nitrosoindole-3-acetonitrile
NLT	Not less than
NMEA	N-Nitrosomethylethylamine
NMNU	N-Methyl-N-nitrosourea
NMOR	N-Nitrosomorpholine
NMP	N-Methyl-2-pyrrolidone
NMT	Not more than
NMTCA	N-nitroso-2-methyl-thiazolidine-4-carboxylic acid
NMVA	N-Nitrosomethylvinylamine
NNK	4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone
NNN	N-Nitrosonornicotine
NOC(s)	N-nitroso compound
NPIC	N-nitrosopipecolic
NPIP	N-Nitrosopiperidine
NPRO	N-nitrosoproline
NPYR	N-nitrosopyrrolidine

NSAR	N-Nitrososarcosine
NTCA	N-nitroso-thiazolidine-4-carboxylic acid
NTHZ	N-Nitroso-thiazolidine
Ph. Eur.	European Pharmacopoeia
RH	Relative Humidity
RSD	Relative standard deviation
SAR	Structure-activity-relationship
SCCP	Scientific Committee for Consumer Products
SPC	Summary of Product Characteristics
TD50	Tumour dose 50 – the daily dose causing tumours in 50% of animals in a life time bioassay
TEA	Triethylamine
TTC	Threshold of Toxicological Concern
USDA	United States Department of Agriculture
USP/NF	United States Pharmacopoeia/National Formulary
UV	Ultraviolet Spectrometry
WFI	Water for Injection
WHO	World Health Organization

1. Information on the procedure

Building on the Article 31 referral on sartans with a tetrazole ring and the knowledge acquired on *N*-nitrosamines in medicinal products, EMA together with the EU Network and international partners has continued the review to identify if there are any consequences for medicinal products outside the class of sartans.

Taking into account that *N*-nitrosamines have been found in sartans with a tetrazole ring but also in other API/medicinal products (e.g. in some batches of pioglitazone and ranitidine) on 10 September 2019 the EMA's Executive Director initiated a procedure under Article 5(3) of Regulation EC (No) 726/2004, and requested the CHMP to further investigate the issues at stake and to give a scientific opinion on

- considerations for MAHs for medicinal products for human use containing chemically synthesised active pharmaceutical ingredients on the identification of the possible presence of *N*-nitrosamine impurities in their medicinal products ("call for review", Phase I); and
- all available scientific knowledge on *N*-nitrosamine impurities in human medicines containing chemically synthesised active pharmaceutical ingredients and their impact on the safe use of medicines. In this exercise the CHMP could seek the support of additional experts and stakeholders as needed. Such evaluation should include the need whether or not to broaden the scope, in a next phase, to products other than human medicines containing chemically synthesised active pharmaceutical ingredients (Phase II)

Therefore the scope of this procedure covers all medicinal products for human use authorised in the EU/EEA and UK, while the call for review above (phase I) is addressing medicinal products containing chemically synthesised active pharmaceutical ingredients, as well as biological medicinal products.

2. Scientific discussion

2.1. Introduction

Following the outcome of the Article 31 referral on sartans with a tetrazole ring[1] and the knowledge acquired on *N*-nitrosamines in medicinal products, EMA together with the EU Network and international partners has continued the review to identify if there are any consequences for medicinal products outside the class of sartans.

Taking into account that *N*-nitrosamines have been found in sartans with a tetrazole ring but also in other API/medicinal products in September 2019 the CHMP's opinion was sought by the EMA's Executive Director in accordance with Article 5(3) of Regulation (EC) No 726/2004 regarding the detection, management and prevention of presence of *N*-nitrosamines in medicinal products for human use.

2.2. Quality and safety aspects

Following the assessment of all available scientific knowledge on *N*-nitrosamines and their impact on the safe use of medicinal products this report addresses the following points:

Quality

[1] https://www.ema.europa.eu/en/medicines/human/referrals/angiotensin-ii-receptor-antagonists-sartans-containing-tetrazole-group

- Root causes for presence of *N*-nitrosamines and proposed measures to mitigate it.
- Consideration for analytical method development to identify and quantify *N*-nitrosamines in drug substances and medicinal products.

Satety

- Considerations for calculating risk for exposed patients in case of in case of detection of *N*-nitrosamines in medicinal product(s).
- Methodology for defining limits for N-nitrosamines in medicinal products.
- Consideration on epidemiological studies.

2.2.1. Root causes for presence of *N*-nitrosamines in medicinal products and measures to mitigate them

Presence, formation and regulation of N-nitrosamines in different areas

Environment

N-nitrosamines occur and are formed in the environment. In the air they form mainly by combustion processes and in water by biological processes in trace amounts. Control strategies are according to the as low as reasonably achievable (ALARA) or as low as reasonably practicable (ALARP) principles. Concentrations are considered to be higher in areas with less control and high air and water pollution.

Food

In food products formation of *N*-nitrosamines mainly occurs by reaction of nitrite and nitrosatable amines in meat, fish and other products at higher temperature. The formation and occurrence have raised major concerns in the seventies to nineties of the last century and measures had been undertaken to minimize formation by reducing the use of nitrate and nitrite in food production.

This has been reviewed by the European Food Safety Authority (EFSA) which refers to various surveys concluding that the exposure to volatile *N*-nitrosamines (NDMA plus NDEA) via processed meat as a main source of overall external exposure is 0.2 ng/kg/day in infants to 3.5 ng/kg/d in toddlers (see also section 2.4). Unpublished data on actual levels of nitrosamines in cooked/processed food analysed N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), NDIPA, NDPA but only found NDMA (and NPIP) at levels of 0.7-0.9 ppb or 14-17 ng/day consumption (Ad-hoc Expert Group Minutes). Regulations to minimize formation of *N*-nitrosamines in food, beverages and beer aim to minimize exposure and are based on the ALARA principles (EFSA, 2017).

Drinking Water

In Germany, control limits for NDMA in drinking water are 10 ng/l and considered as health-based according to the German Umweltbundesamt (UBA) for lifetime and less than lifetime exposure. California has set a public health goal of 3 ng/l for drinking water and New Jersey 0.7 ng/l for NDMA and 5 ng/l for NDPA in groundwater. The Environmental protection Agency (EPA) set health reference levels for NMBA (30 ng/l), NDEA (0.4 ng/l), NDMA (0.6 ng/l), NDPA (7ng/l), NMEA (3 ng/l), and NPYR (2 ng/l) (EPA, 2016).

Products from technical processes (e.g. pesticides, rubber, beer, cosmetics) Pesticides, Rubber, Beer, Cosmetics (products from technical processes)

The level of *N*-nitrosamines is minimized according to the ALARA principles in these areas. According to Directive 2009/48/EC on the safety of toys, levels of N-nitrosamines are limited to ≤10 µg/kg and

nitrosatable compounds to ≤100 µg/kg in toys made with elastomers, which can potentially be taken into the mouth. The German Bundesinstitut für Risikobewertung (BfR) calculated a yearly exposure of 50 or 68 ng (depending on the assessment strategy) assuming the maximum release limit of 0.05 mg/kg rubber is met, and the inflating time equates to 5 hours per year. In Cosmetics, the level of N-Nitrosamines should not exceed 50 µg/kg with specific recommendation for NDELA (SCCS 2011 and 2012).

Veterinary medicinal products (VMPs)

The Guideline on assessment and control of DNA reactive (mutagenic) impurities in veterinary medicinal products (EMA/CVMP/SWP/377245/2016) refers to mutagens of extremely high carcinogenic potency (cohort of concern), i.e., aflatoxin-like-, *N*-nitroso-, and alkyl-azoxy structures as follows: Intakes even below the TTC are theoretically associated with a potential for a significant carcinogenic risk and a case-by-case approach using e.g., carcinogenicity data from closely related structures, if available, should be developed to justify acceptable intakes for authorised VMPs. Principally, these substances should not occur as an impurity of an API or a VMP, due to their extremely high carcinogenic potency.

2.2.2. Presence and formation of *N*-nitrosamines in human medicinal products

2.2.2.1. Introduction on root-causes for the presence and formation of N-*nitrosamines in chemical API synthesis*

Initially in July 2018 a Referral Procedure (EMEA/H/A-31/1471) under Article 31 of Directive 2001/83/EC for sartans with tetrazole ring containing products (further referred to as " Sartans Referral Procedure") was triggered to assess the impact of *N*-nitrosamine impurities on the benefit-risk balance of valsartan medicinal products. It became evident that the detected levels of NDMA, and subsequently, other detected *N*-nitrosamines including NDEA, diisopropyl-*N*-nitrosamine (DIPNA), ethylisopropyl-*N*-nitrosamine (EIPNA) and 4-(methyl)(nitroso)amino)butanoic acid (NMBA) exceeded limits based on ICH M7(R1) principles for substances of the "cohort of concern" defined in this guideline and calculated considering a lifetime daily exposure, referenced there as "acceptable intake"(AI), further stated as "ICH M7(R1) limit" in this report. The procedure was extended in September 2018 to cover all EU authorised Angiotensin-II-receptor antagonists/blockers, possessing a tetrazole ring: i.e. candesartan, irbesartan, losartan, olmesartan and valsartan (further referred to as "sartans"), as synthesis steps of tetrazole rings common to those sartans led to nitrosamine formation. On 31 January 2019, EMA's CHMP concluded its Article 31 review setting temporary limits on the API applicable within a transition period of two years and defining stricter long-term requirements based on technical limits. Of note, at the time of the opinion of the sartans referral, only API root causes had been identified.

Subsequent to the Sartans Art. 31 referral, one API manufacturer informed the EU authorities and European Directorate for the Quality of Medicines (EDQM) that it had discovered NDMA in some batches of its pioglitazone API. The NDMA levels in the concerned pioglitazone batches were below the interim limits set for sartans (based on ICH M7(R1)) but the presence nonetheless of an *N*-nitrosamine in a non-sartan API was a significant finding. As a precaution, the EDQM reviewed immediately all Certificate of Suitability to the monographs of the European Pharmacopoeia (CEP) applications for this substance and in April 2019, EMA and National Competent Authorities (NCAs) requested that MAHs for pioglitazone who were using certain reagents in their manufacturing processes check their processes, to rule out the presence of *N*-nitrosamines.

In July 2019, EDQM received information on a new *N*-nitrosamine *N*-nitrosomethylphenylamine (NMPA) – in valsartan from another API manufacturer. The levels detected for products in the EU/EEA were below the ICH M7(R1) limit calculated for NMPA at the time based on methodologies referenced in the outcome of the Article 31 review.

In September 2019, at the request of the European Commission, an Article 31 review was initiated for ranitidine containing medicines (EMEA/H/A-31/1491) [2]after tests showed that some of these products contained NDMA, both in API and finished products. In a number of EU countries, national authorities initiated recalls of ranitidine medicines from pharmacies.

In May 2019, the Lessons Learnt Exercise was initiated within the European network to determine what lessons can be drawn from cases of unexpected presence of *N*-nitrosamine impurities in sartans. A final report for this exercise has been published on 24 June 2020[3]

2.2.2.1.1. Theoretically possible root-causes for *N*-nitrosamines in pharmaceutical products linked with water

When *N*-nitrosamines are present in raw materials, there is a risk that they are carried over in finished products. Similarly, if nitrites are present in raw materials, they could react with amines, ubiquitous in APIs, their precursors, reagents and many solvents, to form *N*-nitrosamines which could also be carried over in finished products.

NDMA can occur in drinking water as it is a by-product of several industrial processes and is a contaminant of certain pesticides. NDMA has recently been identified as a disinfection by-product of chloramination (by the reaction of monochloramine with dimethylamine, a ubiquitous component of waters impacted by wastewater discharges) and, to some extent, chlorination. NDMA can also be formed as a by-product of anion-exchange treatment of water. It is generally removed during water treatment by UV irradiation. The current WHO Guideline "Guidelines for drinking-water quality" (WHO/HSE/AMR/08.03/8; 4th edition, incorporating the 1st addendum) defines a limit for NDMA in drinking water of 0.1 µg/L , equivalent to 0.1 µg/kg = 0.1 ng/g = 0.1 ppb in case of ρ = 1 kg/L, due to different sources from the environment.

Maximum NDMA concentration levels were detected in different water samples from Australia and China [Krasner et al. (2013); NDMA 75 ng/L equivalent to < 75 ng/kg = 0.075 ng/g= 0.075 ppb]. The solubility of NDMA in water is high (290 g/L at 20 °C) [Alaba et al. (2017)], however considering the overall low levels at which it is found in water, it is concluded that NDMA from water highly probably does not represent a realistic source for NDMA contamination of APIs.

However, disinfection procedures may lead to significant *N*-nitrosamine generation as by-products, in case certain active substances are present [Parr et al. (2019)]. Shen et al. (2011) have investigated the susceptibility of 20 active substances to *N*-nitrosamine formation after exposure to water disinfected by chloramine. Molar yields higher than 1% were observed for eight pharmaceutical substances, with ranitidine showing the strongest potential to form NDMA. Despite lower molar turnover, similar results were reported for ranitidine when treated with water disinfected by ozonation [Lv J. (2017)]. For further information on ranitidine degradation and NDMA formation please refer to the ranitidine referral under Article 31 of Directive 2001/83/EC[2].

Nitrites have been observed in various reagents, often when sodium nitrite has been used in their preparation (for example, sodium azide). This is another route by which nitrites can be inadvertently

[2] https://www.ema.europa.eu/en/medicines/human/referrals/ranitidine-containing-medicinal-products
[3] https://www.ema.europa.eu/en/documents/report/lessons-learnt-presence-n-nitrosamine-impurities-sartan-medicines_en.pdf

introduced into a synthetic process. Since azides can be depleted by nitrites as outlined below in the sartan case, the relevance of this observation remains to be clarified.

2.2.2.1.2. Theoretically possible root-causes for *N*-nitrosamines in pharmaceutical products linked with solvents, reagents, catalysts

In the case of sartans, solvents such as dimethylformamide (DMF), *N*-methylpyrrolidone (NMP) and triethylamine (TEA) represent sources of amines such as dimethylamine (DMA), methybutylamine (MBA) and diethylamine (DEA), susceptible to *N*-nitrosamine formation. In addition, the solvent/reagent TEA is able to form NDEA by nitrosative dealkylation.

Based on evaluation of available literature information, the (potential) presence of secondary/tertiary amines and NOX in solvents listed in the ICH Q3C (R7) Guideline was assessed. The main outcome is summarized as follows:

Similar to DMF, dimethylacetamide is produced on industrial scale by reaction of dimethylamine with acetic acid, acetic anhydride, or acetate esters indicating dimethylamine to be an expected impurity [Le Berre et al. (2013)]. Due to high structural and functional similarity, both carboxylic acid derivatives possess comparable chemical properties, e.g. liberation of dimethylamine upon hydrolysis. It is concluded that the ICH Q3C (R7) solvent dimethylacetamide represents –in addition to DMF and NMP- an additional source of secondary amines susceptible to NDMA formation in combination with nitrosating agents.

The ICH Q3C (R7) solvent TEA is frequently used as reagent or solvent in organic synthesis and API manufacture. According to Spiegelhalder et al. (1978), commercially available lots of secondary/ tertiary amines were found to be contaminated with the corresponding *N*-nitrosamines, showing levels to range between 0.03 - 53.0 ppm. For example, NDMA was quantified to contaminate DMA solution significantly, ranging from 0.65 – 17.3 ppm. The highest *N*-nitrosamine concentration was detected in pyrrolidine (i.e. 53.0 ppm), while 0.03 ppm NDEA were found in TEA. Comparable results were reported one year later by Bontoyan et al. (1979). The relevance of these results, discovered 40 years ago in secondary and tertiary amines of unknown quality, is currently considered unknown.

The phase transfer catalysts TEA HCl and tetrabutylammonium bromide (TBAB) were identified as precursors of *N*-nitrosamines such as NDEA and *N*-nitrosodibutylamine (NDBA). Basically, the susceptibility of ammonium salts to form *N*-nitrosamines was discovered without clarifying reaction mechanism as shown above [Fiddler et al. (1972)]. Considering that quaternary alkyl ammonium salts are derived from the corresponding secondary and tertiary amines, these precursors represent potential impurities [Roose et al. (2015)], having also the potential to react with nitrosating reagents.

Based on information from literature, nitroalkanes such as 2-nitropropane and nitromethane were used to act as a source of nitrous acid in combination with certain oxidants/catalyst and to form *N*-nitrosamine in combination with secondary and tertiary amines [Franck et al. (1970); Potturi et al. (2012); Zhang et al. (2013)]. According to S. B. Markovsky [Ullmann's Encyclopedia of Industrial Chemistry (2012)], the ICH Q3C (R7) solvent nitromethane is usually produced on industrial scale by high temperature vapour-vapour-phase nitration of propane with nitric acid, followed by aqueous working-up and drying procedures before being separated by fractional column distillation. Consequently, low-level contamination with nitric acid, nitrous acid and nitrogen oxides etc. seems to be unlikely, but cannot be ruled out *per se*. During ranitidine synthesis, the precursor 1,1-bis(methylthio)-2-nitroethene is produced by reaction of dimethyl-*N*-methylcarbonimidodithionate with nitromethane, before being incorporated into ranitidine drug substance [Kleemann, Engel 2019].

Thus, nitromethane and its potential oxidative degradation to nitrosating agents cannot be excluded currently as a contributing factor in this case.

Some ranitidine HCl batches were found to be contaminated significantly with NDMA. For more information, please refer to the ranitidine referral under Article 31 of Directive 2001/83/EC.

2.2.2.2. Confirmed root-causes identified in the sartans referral

Further to the sartans Article 31 review and based on the reviews of responses from API manufacturers and assessments of CEP dossiers by EDQM in relation to *N*-nitrosamine impurities, 11 sartan CEPs out of a total of 125 were suspended, including 7 valsartan CEPs, 2 irbesartan CEPs and 2 losartan potassium CEPS, triggering recalls of the concerned medicinal products by the responsible NCAs. However, it has to be noted that the vast majority of sartan CEPs (i.e. > 90 %) were not affected, indicating high probability of process-specific route causes.

Olmesartan and candesartan CEPs were considered valid by EDQM both throughout the Referral Procedure and until now, enabling the corresponding medicinal products to remain available as alternative medicines.

In general, 5-substituted-1H-tetrazoles (further referred to as "tetrazoles"), known to exist in equilibrium of the 1H and 2H-tautomeric forms [Wittenberger, S. J., (1994)], can be synthesized by various procedures [Benson F.R., (1947); Herr R.J., (2002)] including by the reaction of organic nitriles with inorganic and organometallic azide reagents via a concerted 1,3 dipolar cycloaddition reaction or closely related ionic mechanisms. To avoid the use of toxic and explosive hydrazoic acid (HN_3), alternative reagents or reagent / catalyst combinations such as sodium azide (NaN_3), tributyltin azide (Bu_3SnN_3), triethylammonium chloride (TEA HCl)/ sodium azide, tributyltin chloride (Bu_3SnCl) / sodium azide or zinc bromide ($ZnBr_2$) / sodium azide etc. are frequently preferred for synthesizing tetrazoles [Herr R.J. (2002), Himo, F et al. (2003)]. Regarding active substance dossiers, most processes for tetrazole synthesis were developed by manufacturers of starting materials, intermediates and active substances on the basis of genotoxic azide reagents.

In order to speed up reactions and to shift the equilibrium of the cyclization reaction towards the product, it is required to add catalysts (phase transfer, Lewis acids), to apply reagents in excess and/or to perform the reactions at high temperatures in suitable solvents with higher boiling points [e.g. *N*,*N*-dimethylformamide (DMF), dimethylsulfoxide (DMSO) and *N*-methylpyrrolidone (NMP)] over several hours [Herr, R.J., (2002)].

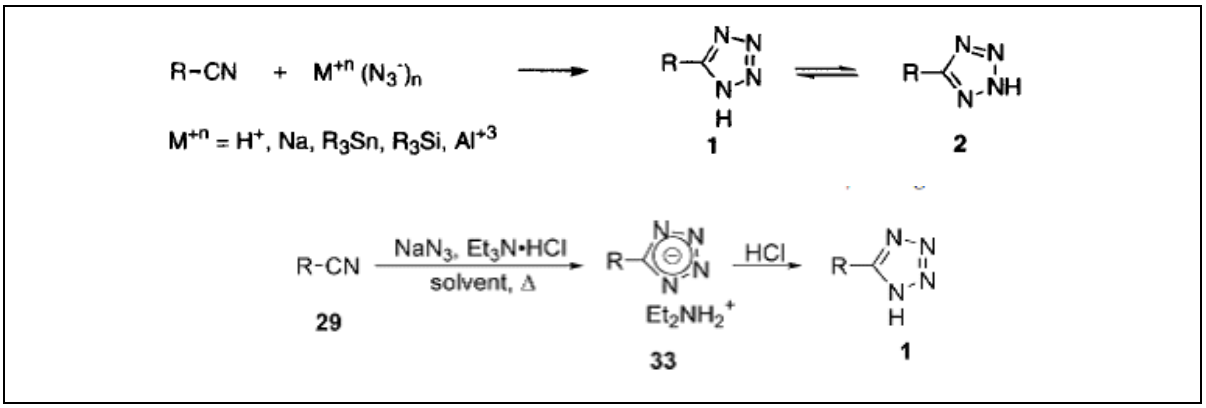


Figure 2.2.2.2-1 Basic structural features and standards synthesis of tetrazoles [Wittenberger, S. J., (1994); Herr, R.J., (2002)]

A typical drawback of these azide reagents and azide reagent /catalyst combinations is that upon hydrolytic work-up under acidic conditions, required for isolation and extraction of acidic tetrazoles, hazardous hydrazoic acid is liberated [Wittenberger, S. J., (1994)]. Residual quantities of azide reagents can be decomposed to gaseous by-products such as nitrogen (N_2) and dinitrogen oxide (N_2O) by the addition of sodium nitrite ($NaNO_2$), as recommended for mother liquors in the industrial production of sodium azide [Bräse et al. (2015)]. Considering that for sartans, azide reagents are used typically in excess, at least equimolar quantities of sodium nitrite in relation to the azide reagent are required to enable complete depletion by redox reaction.

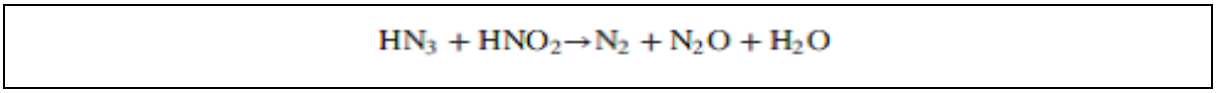


Figure 2.2.2.2-2 Depletion of hydrazoic acid by sodium nitrite [Bräse et al. (2015)].

In contrast, active substance manufacturers did not make use of the option to deplete -in turn- residual nitrite levels by addition of more or less non-toxic reducing agents such as e.g. urea and amidosulfonic acid [Laue et al. (2013)].

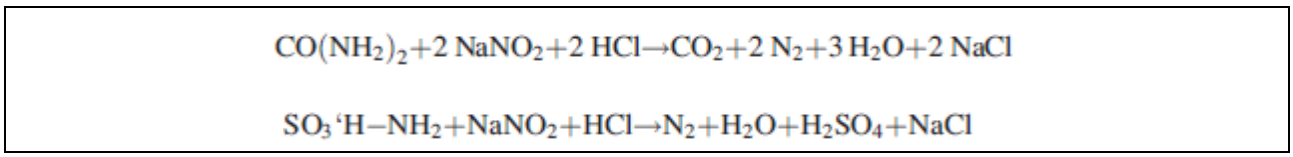


Figure 2.2.2.2-3 Depletion of nitrite by urea and amidosulfonic acid [Laue et al. (2013].

In principle, quenching procedures in the cyclization step of sartan processes can be performed in the presence or in the absence of APIs and intermediates, i.e. after phase separation of liquids or after separation of solids from the mother liquor by filtration. Quenching procedures in the presence of product (i.e. before separation procedures) enhance process safety but possess concomitantly the risk of significant by-product formation. In contrast, quenching procedures in the absence of product (i.e. after separation procedures) bear an inherent process safety risk but reduce the risk of deleterious side reactions with the product significantly. Any re-extraction or concentration followed by precipitation/crystallization from the quenched mother liquor to increase the overall yield of the process step can also lead to an elevated contamination of product with by-products formed during the quenching operation.

According to the CHMP Assessment Report for the sartans referral [4], different solvents, reagents and catalysts were used in the tetrazole forming cyclization step. Toluene, xylene, DMF and NMP as well as corresponding solvent mixtures (also with water and alcohols) were selected as high boiling solvents for the cyclization reaction, while products were often extracted with co-solvents such as EtOAc, CH_2Cl_2 during working-up procedures. In addition to tributyltin azide, sodium azide alone or in combination with tributyltin chloride and bis(tributyltin)oxide [$(Bu_3Sn)_2O$] were frequently chosen as the azide source. Some processes required the addition of auxiliary bases such as triethylamine (TEA) and diisopropylethylamine (DIPEA). Zinc bromide ($ZnBr_2$), triethylammonium chloride (TEA HCl), and tetrabutylammonium bromide (TBAB) represented typical catalysts to accelerate reaction rates.

During review of API manufacturing data, it became evident that $NaNO_2$ was added only in the minority of cases, i.e. mostly in large manufacturing processes. Regarding manufacturing processes for tetrazole-containing sartans, this reagent is not required in the cyclization step, but used only for

[4] https://www.ema.europa.eu/en/documents/variation-report/sartans-article-31-referral-chmp-assessment-report_en.pdf

depletion of residual quantities of azide reagents. In some cases, inconsistencies were identified in the dossiers, regarding the regulatory status of sodium nitrite (included in eCTD Module S.2.3 but omitted from the reaction scheme and the process description in S.2.2). It is reminded that all materials used in the manufacturing process should be included in the documentation, irrespective of their intended use.

In sartan CEPs suspended by the EDQM, a few API manufacturing processes were changed over the time by adding $NaNO_2$ into the processes as part of scale up activities. In some cases, these changes of processes were introduced by variation procedures on already granted CEP dossiers, in order to ensure process safety for large-scale production by azide quenching, to minimize genotoxic azide impurities, to treat waste streams and/or to optimize economic efficiency and reduce costs, suggesting the need to improve control, review and assessment of manufacturing processes as they are scaled up.

According to the CHMP Assessment Report for the sartans referral, *N*-nitrosamines such as NDMA, NDEA, DIPNA, EIPNA and NMBA were also identified as impurities in tetrazole-containing sartans. These impurities were generated in the same step or sub-step of manufacturing processes, considering that the most relevant way of *N*-nitrosamine formation is related to the simultaneous presence of amines and nitrosating agents as outlined below. However, carry-over of amine and NOx sources from previous into subsequent steps can also lead to *N*-nitrosamine formation as documented in the Pioglitazone case (see below). The use of solvents/reagents/catalysts sourced as "fresh" and "recovered" from third party suppliers was identified as another root cause of *N*-nitrosamine contamination (see above). In addition, GMP non-compliance such as cross-contamination in multi-purpose facilities and operator-related issues such as inadequate phase separations also led to *N*-nitrosamine contamination of APIs (see above).

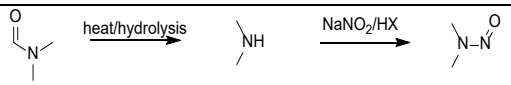
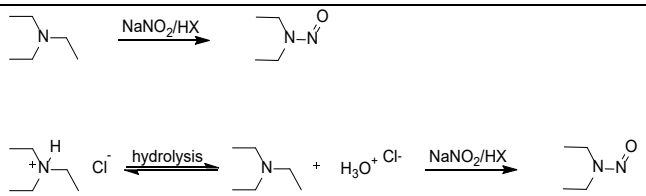
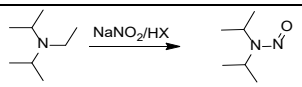
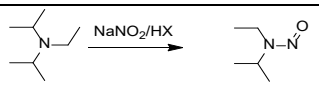
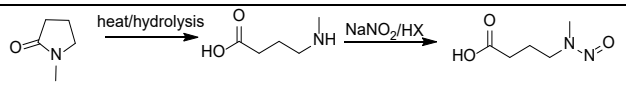
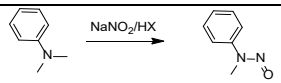
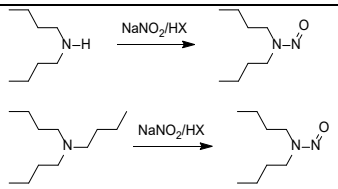
Subsequent to the sartans Referral, *N*-nitrosodibutylamine (NDBA) and *N*-nitrosomethylphenylamine (NMPA) were shown to be additional (potential) *N*-nitrosamine impurities. The phase transfer catalyst tetrabutylammonium bromide (TBAB) and the solvent/reagent *N*,*N*-dimethylaniline (*N*,*N*-DMA) are expected to be potential sources for secondary and tertiary amines such as dibutylamine, tributylamine, dimethylamine and *N*-methylaniline. Based on the identified routes of formation discussed above, and the fact that quaternary alkylammonium salts and tertiary amines have also been found to be directly susceptible to nitrosative de-alkylation [Fiddler et al. (1972)]. NDBA, NMPA and NDMA would likely be formed in combination with nitrosating agents such as $NaNO_2$.

In the sartans Referral API manufacturers identified deliberately added $NaNO_2$ as the common source of NOx in tetrazole containing sartans. In contrast, different sources were stated to be responsible for the concomitant presence of secondary and tertiary amines in the processes. Solvents such as DMF and NMP were reported to be sources for secondary amines such as *N*,*N*-dimethylamine (DMA) and for 4-methylaminobutyric acid (MBA). Reagents such as the tertiary amines TEA and DIPEA were identified to be the origin of the secondary amines *N*,*N*-diethylamine (DEA), diisopropylamine and ethylisopropylamine respectively. The phase transfer catalyst TEA HCl was identified as the source of the tertiary amine TEA and the secondary amine DEA.
According to the CHMP Assessment Report of the sartans referral, *N*-nitrosamine formation was caused by the reaction of $NaNO_2$ as the common NOx with different sources of secondary and tertiary amines as illustrated below. Two different routes of *N*-nitrosamine generation were identified which can be classified into two main reaction types:

1. Hydrolytic and/or thermal degradation of the solvents DMF and NMP to give the secondary amines DMA and MBA respectively, followed by subsequent *N*-nitrosation, finally yielding NDMA and NMBA

2. *N*-nitrosative de-alkylation of the reagents TEA, DIPEA and *N*,*N*-DMA (trialkyl amines), finally yielding NDEA, DIPNA, EIPNA and NMPA; hydrolytic dissociation of the catalyst TEA HCl (quaternary ammonium salt) to give the tertiary amine TEA, followed by *N*-nitrosative de-alkylation, finally yielding NDEA.

Table 2.2.2.2-1 *N*-nitrosamine formation routes and classification of reaction types

N-Nitrosamine	Route of Formation	Reaction type classification
NDMA		Hydrolytic and/or thermal degradation of DMF to give DMA and subsequent *N*-nitrosation
NDEA		*N*-nitrosative de-alkylation of the tertiary amine DIPEA Hydrolysis of quaternary ammonium salt TEA HCl to give the tertiary amine TEA and subsequent *N*-nitrosative de-alkylation
DIPNA		*N*-nitrosative de-alkylation of the tertiary amine DIPEA
EIPNA		*N*-nitrosative de-alkylation of the tertiary amine DIPEA
NMBA		Hydrolytic and/or thermal degradation of NMP to give the secondary amine MBA and subsequent *N*-nitrosation
NMPA		*N*-nitrosative de-alkylation of the tertiary amine *N*,*N*-DMA
NDBA		Nitrosation of the secondary amine DBA and/or *N*-nitrosative de-alkylation of the tertiary amine TBA

It became evident, however, that the proposed root cause analysis focussed only on degradative mechanisms for amine generation and *N*-nitrosamine formation, not taking into account the potential presence of secondary and tertiary amine impurities in the applied solvents, reagents and catalysts. According to the final report of the Lessons Learnt Exercise, the ICH Q3C (R7) solvent DMF represents a source of DMA due to the production process and due to thermal and hydrolytic degradation. The ICH Q3C (R7) solvent NMP is a source of 4-methylaminobutyric acid due to the production process and due to hydrolytic degradation. Furthermore, monoalkylamines, dialkylamines, trialkylamines and quaternary alkyl ammonium salts are potential sources of secondary and tertiary amines due to their industrial production processes.

In most cases, drug substance manufacturers propose test parameters and acceptance criteria for solvents, reagents and catalysts, usually based on suppliers' specifications but frequently delineated simply from Ph. Eur. reagent specifications defined for use in different analytical procedures according to the Ph. Eur. general notices. Often, the impurity profiles of the raw materials remain undisclosed. A literature survey on the industrial production and on the quality specifications of solvents, reagents and

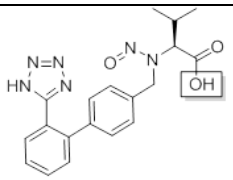
catalysts such as DMF, NMP, TEA, DIPEA, *N*,*N*-DMA, TEA.HCl and TBAB has been conducted, showing production specific impurity profiles (secondary and tertiary amines). Specifications have been established by chemical industry, covering in some cases test parameters and acceptance criteria for secondary and tertiary amines in these chemicals, suggesting the need to define adequate limits in section S.2.3 of the dossier.

Solvents, reagents and catalysts such as DMF, NMP, TEA, DIPEA, *N*,*N*-dimethylaniline (*N*,*N*-DMA), TEA HCl and TBAB have been observed to generate *N*-nitrosamines when combined with the nitrosating reagent $NaNO_2$ in the same step or sub-step of an API manufacturing processes. In line with literature data, *N*-nitrosamine formation is expected to be caused by hydrolytic and/or thermal degradation of solvents (DMF/NMP) and subsequent *N*-nitrosation of the released secondary amine and by *N*-nitrosative dealkylation of tertiary amines (TEA/DIPEA/*N*,*N*-DMA) or quaternary ammonium salts (TEA HCl/TBAB). However, physico-chemical studies on the parent reaction mechanism have not been conducted by MAHs or active substance manufacturers so far, suggesting the need to initiate research on this area.

In the case of valsartan, a single active substance manufacturer disclosed the (potential) presence of two valsartan specific *N*-nitroso compounds, i.e. derivatives of valsartan intermediates/impurities. The N-nitrosamine-carboxylic acid has been tested AMES negative. Due to the fact that the N-nitrosamine-benzyl ester cannot be considered a classical or a non-classical bio-isoster of the N-nitrosamine-carboxylic acid [Meanwell, N.A., (2011)], extrapolation of the negative AMES test result by referring simply to structural similarity was further evaluated by EDQM within a CEP procedure. It was confirmed that N-nitrosamine-benzyl ester is fully converted to N-nitrosamine-carboxylic acid (Ames negative) and this was supported by a spiking-purging study (purge factor > 10000) and by batch data showing that N-nitrosamine-benzyl ester is consistently below 0.03 ppm in valsartan, which was considered satisfactory.

However, both *N*-nitroso compounds are regarded recent examples of *N*-nitrosamines formed within a manufacturing process by nitrosation, suggesting the need for thorough nitrosatability testing of intermediates/impurities, as discussed in the aminophenazone case below.

N-nitrosamine-carboxylic acid



N-nitrosamine-benzyl ester

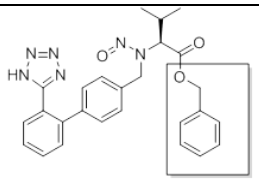
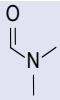
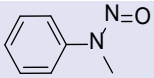
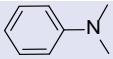
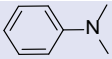
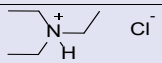
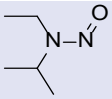
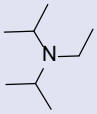
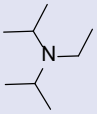
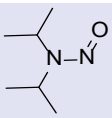
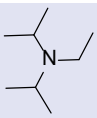
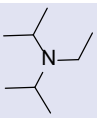
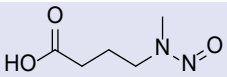
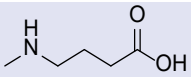
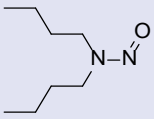
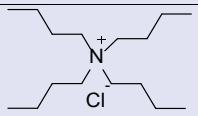
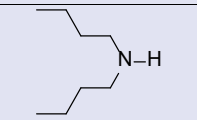
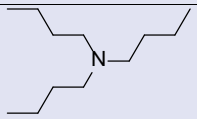


Figure 2.2.2.2-4 Valsartan specific *N*-Nitroso compounds (potentially) present in API from a single active substance manufacturer.

Table 2.2.2.2-2 Critical Compound Combinations responsible for *N*-nitrosamine formation in sartans

N-Nitrosamine	NOX Source	Amine Source	Amine nitrosated by NOX	Critical Compound Combination
 NDMA	$NaNO_2$	 DMF	 DMA	reagent/solvent
 NMPA	$NaNO_2$	 *N*,*N*-DMA	 *N*,*N*-DMA	reagent/solvent
 NDEA	$NaNO_2$	 TEA	 DEA	reagent/reagent
		 TEA HCl	 TEA	reagent/catalyst
 DIPNA	$NaNO_2$	 DIPEA	 DIPEA	reagent/reagent
 EIPNA	$NaNO_2$	 DIPEA	 DIPEA	reagent/reagent
 NMBA	$NaNO_2$	 NMP	 MBA	reagent/solvent
 NDBA	$NaNO_2$	 TBAB	 DBA	reagent/catalyst
			 TBA	

2.2.2.3. Root-causes identified in the pioglitazone case

In January 2019, NDMA was reported in some batches of pioglitazone HCl, in what was the first such report for a non-sartan medicinal product since June 2018. The manufacturer of the pioglitazone API concerned proposed that the preliminary root cause was the use of $NaNO_2$ and HBr in an early step of the process, followed by the use of DMF and HCl in a later step. This root cause requires either sodium nitrite or another form of nitrosating agent (NOx) to be carried over across several steps before DMF is introduced (e.g. as the nitrous acid salt of the pyridine moiety). Other root causes were also considered, including the use of solvents (e.g. DMF) contaminated with NDMA. However, it was not possible to investigate some of these other causes as no retained samples of raw materials were available.

This case was also the first time a *N*-nitrosamine has been detected in an API when its formation does not occur in the final synthetic step, and where sources of nitrite would need to be carried over across multiple unit operations including aqueous work-ups and crystallizations. A joint EDQM/EU inspection

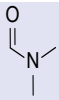
of the manufacturing site concluded that the proposed root cause was plausible. According to Kleemann, Engel (2019), two synthetic routes are usually applied to manufacture pioglitazone HCl on commercial scale. Pioglitazone HCl, contaminated with NDMA, was manufactured by a process similar to synthetic route I, making use of sodium nitrite for nitrosative diazotation of an aniline derivative. In contrast, synthetic route II enables manufacture of pioglitazone HCl without applying any nitrosating agent in the entire process.

Due to the fact that the detected NDMA levels were found to be constantly kept below the limit of ≤ 1.935 ppm [NDMA ≤ 96.0 ng/day; MDD = 49.6 mg pioglitazone HCl] recalls of corresponding medicinal products were not initiated. Despite the additional possibility to avoid NDMA formation in the entire process e.g. by replacing the solvent DMF and herewith DMA as its nitrosatable impurity/degradation product, the API manufacturer has decided voluntarily to withdraw two CEPs on 30 July 2019.

Following the above mentioned inspection, the manufacturing routes of all sources of pioglitazone HCl in the EU were assessed for the risk of *N*-nitrosamine formation. The MAHs using pioglitazone HCl from these manufacturers were subsequently requested to provide risk assessments for potential *N*-nitrosamine formation and batch analysis data on batches of their APIs. At this moment, seven CEPs for pioglitazone HCl remain valid according to the EDQM database, indicating with high probability a process specific route cause in this case.

In summary, use of $NaNO_2$ and DMF in different but subsequent synthetic steps according to route I represents the critical compound combination responsible for *N*-nitrosamines generation and contamination in the entire pioglitazone HCl manufacturing process.

Table 2.2.2.3-1 Critical Compound Combinations responsible for *N*-nitrosamine formation in pioglitazone HCl

N-Nitrosamine	NOX Source	Amine Source	Amine nitrosated by NOX	Critical Compound Combination
 NDMA	$NaNO_2$	 DMF	 DMA	reagent/solvent

2.2.2.4. Root-causes identified in the ranitidine case

A review of ranitidine medicines (EMEA/H/A-31/1491) was initiated on 12 September 2019 at the request of the European Commission, under Article 31 of Directive 2001/83/EC[5]. This was in response to detection of NDMA in batches of active substance and finished product from a series of different manufacturers above the temporary limits defined in the sartans referral. After the finalisation of the review, final conclusions and any conditions and recommendations will be published on the EMA website.

As a result of the discovery of NDMA in some ranitidine medicines, all CEPs for ranitidine HCl API were suspended by EDQM, indicating with high probability common process specific and/or common API specific root-cause(s) in these cases. In particular, NDMA formation from ranitidine over shelf life is being considered as a potential root cause[6] .

[5] https://www.ema.europa.eu/en/medicines/human/referrals/ranitidine-containing-medicinal-products
[6] https://www.ema.europa.eu/en/documents/referral/ranitidine-article-31-referral-chmp-list-questions_en.pdf

2.2.2.5. The historical aminophenazone case

2.2.2.5.1. Confirmed root-causes in the aminophenazone case

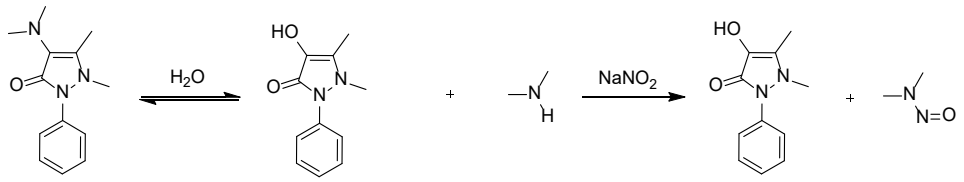
In 1977, the German BGA (Bundesgesundheitsamt, former German Federal Health authority, predecessor of BfArM, PEI) released a recommendation to withdraw aminophenazone (i.e. amidopyrine) preparations from the market [Eisenbrand et al. (1979); BGA Press release Nr. 16/77 (1977)]. This market withdrawal was linked to the fact that even aminophenazone API batches were found to be contaminated significantly with NDMA (NDMA levels up to 340 µg/kg = 340 ng/g = 340 ppb). The final decision was made to supersede a previous recommendation [Eisenbrand et al. (1979); BGA Press release Nr 13/75, (1975)] to re-formulate aminophenazone preparations by adding ascorbic acid as anti-oxidant to prevent nitrosation and NDMA formation. Such NDMA formation from aminopyrine was previously discovered in-vitro and in-vivo by Lijinsky et al. (1973) and associated with liver tumours in rats.

As consequence, the aminophenazone monograph was deleted from pharmacopoeias including Ph. Eur. It is noted that a revision is currently under consideration for aminophenazone in Pharmacopea Italica, and an NDMA specification limit for API has been introduced in any remaining medicinal products containing aminophenazone in Italy.

According to Mirvish et al. (1974), the formation of NDMA in aminophenazone API has been related to the reaction with nitrous acid anhydride (N_2O_3) and subsequent formation of the corresponding 4-hydroxypyrazol-3-one derivative. According to Lijinsky et al. (1973), NDMA is formed by direct reaction of aminophenazone API with nitrous acid (HNO_2) and subsequent formation of the corresponding 4-hydroxypyrazol-3-one derivative. At that time, aminophenazone API was manufactured by two similar processes, both utilizing sodium nitrite for a nitrosation procedure, followed by subsequent reduction and methylation reactions [Kleemann, Engel (1978)].

Aminophenazone has a non-aromatic pyrazolone ring, substituted with a dimethylamine group at the 4-position. Hydrolytic degradation leads to the generation of the corresponding 4-hydroxypyrazol-3-one derivative and the release of DMA [Reisch et al. (1969); Reisch et al. (1967)]. In case of sodium nitrite carry-over from the previous manufacturing step, formation of NDMA has to be expected.

Eisenbrand et al. (1979) stated that NDMA formation was caused by carry-over of sodium nitrite into the final step of aminophenazone synthesis, leading to the conclusion that NDMA was formed as an API degradation product via hydrolysis and subsequent nitrosation as shown below.

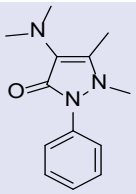
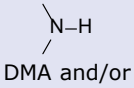
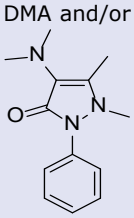


Scheme 2.2.2.5.1-4 NDMA formation in aminophenazone API via hydrolytic degradation and subsequent nitrosation [Eisenbrand et al. (1979); Reisch et al. (1969); Reisch et al. (1967)]

In summary, NDMA was generated in aminophenazone API by a critical compound combination of a labile dimethylamino substance with sodium nitrite carried over as the NOx source. Although the origin of NDMA is the same as observed for valsartan, i.e. the combination of sodium nitrite and DMA, the cases differ in the origin of DMA (solvent degradant vs. API degradant). With regard to the

manufacturing processes for aminophenazone conducted at that time, the use of sodium nitrite for nitrosation seems to be unavoidable for synthesis of the intermediate 4-Amino-2,3-dimethyl-1-phenylpyrazol-5-one ("Aminoantipyrin") at first sight. However, this intermediate was synthesized via nitration and subsequent hydrogenation processes previously [Thoms et al. (1923); Waser, E., (1925); Hamel et al. (1970) and recently Amanchi et al. (2019)].

Table 2.2.2.5.1-1 Critical Compound Combinations responsible for *N*-nitrosamine formation in aminophenazone

***N*-Nitrosamine**	**NOX Source**	**Amine Source**	**Amine nitrosated by NOX**	**Critical Compound Combination**
 NDMA	$NaNO_2$	 aminophenazone	 DMA and/or  aminophenazone	reagent/API

2.2.2.5.2. Further historical literature data on potential root causes

Following the aminophenazone findings, investigation of *N*-nitrosamine impurities in other APIs and FPs was performed. In contrast to aminophenazone, [Eisenbrand et al. (1979)], Krull et al. (1979) found none of the 73 products tested within their study to contain NDMA. However, *N*-nitrosamine levels were detected in disulfiram FP, ranging from 94 – 980 ppb for NDEA [Castegnaro et al. (1981)] and in piperazine formulations, up to 20 ppm mononitroso-piperazine [Bellander et al. (1985)].
Concentration ranges in APIs and FP were described in the following publications Castegnaro et al. (1981); Taylor et al. (1980); Dawson et al. (1987); Bellander et al. (1985). The chemical structures of APIs reported in literature to contain NDMA are shown in figure 2.2.2.5.2-1 [Parr et al. (2019)].

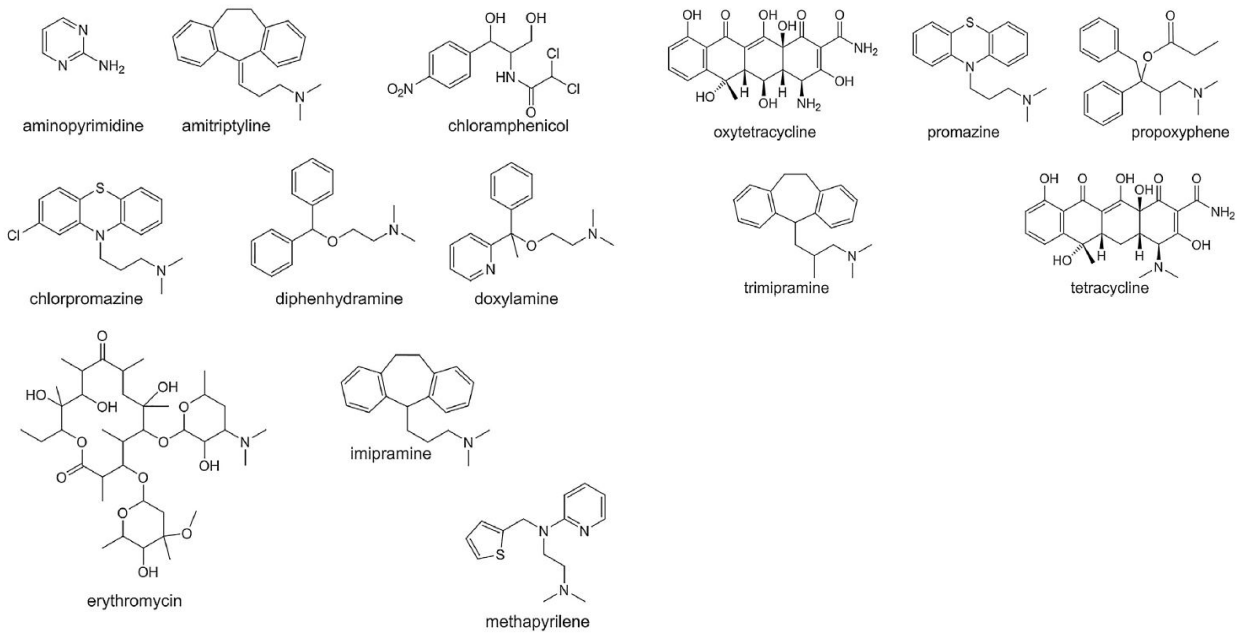


Figure 2.2.2.5.2-1 Chemical structures of APIs reported in literature to contain NDMA [Parr et al. (2019)].

In 1978, the WHO Expert Group suggested the nitrosation assay procedure (NAP test) as a general *in vitro* test system under standard conditions (10 mmol/l drug, 40 mmol/l nitrite, 37ºC, pH 3-4, with reaction times 1-4h) to study the nitrosation ability of drug substances, [IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Some pharmaceutical Drugs, (1980)].

At that time, aminophenazone was found to show the highest relative *N*-nitrosation of selected APIs. The authors stated that *"Since the endogenous formation of N-nitroso compounds from nitrosatable amine precursors and nitrosating agents, such as nitrite or nitrous gases, is not usually taken into account in carcinogenicity tests of the parent compound, additional investigations are necessary to evaluate this possible hazard."*

In 2007, Brambilla et al. (2007) summarized the genotoxic and carcinogenic risk to humans caused by drug-nitrite interactions in a review article. By referring to the IARC conclusion from 1980 mentioned above, these authors stated that "*in spite of this recommendation, guidelines for genotoxicity testing of pharmaceuticals do not indicate the need of performing adequate tests in order to assess whether a nitrosatable drug may undergo endogenous nitrosation to a genotoxic NOC*" (i.e. *N*-nitroso compound).

As outlined above, NDMA formation in aminophenazone has been linked to nitrosative degradation of the API in response to sodium nitrite carry over during drug substance synthesis [Eisenbrand et al. (1979)].

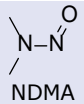
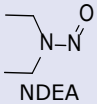
2.2.3. Confirmed root-causes for the formation of *N*-nitrosamines in medicinal products with regard to excipients and as contaminants from primary packaging

Nitrates and nitrites can be found in many excipients at parts per million levels. Sodium starch glycolate, croscarmellose sodium, pre-gelatinized starch, polyvinylpyrrolidone (PVP), cross polyvinylpyrrolidone (cPVP), and lactose are excipients that might carry trace levels of nitrate or nitrite

impurities [Wu et al. (2011)]. The exact sources of these trace impurities have not been investigated, but it is possible that they come from process water, processing steps requiring acid titration, bleaching, and potentially from oxidation in air as the excipient is being heated in a drying process. Some reports on formation of *N*-nitrosamines in medicinal products have been received suggesting a link to interaction of APIs with nitrites in excipients but not with nitrates. Accordingly, this possibility is considered a probable root-cause, suggesting, the need to initiate research e.g. in collaboration with academia.

In September 2019, a new root-cause for contamination of medicinal products with NDMA/NDEA was identified and reported to the authorities. NDMA/NDEA appear to have been formed during printing of the lidding foils and that formation of *N*-nitrosamines was caused by reaction of nitrocellulose in the lidding foil with amine containing printing ink [dimethylamine (DMA) and diethylamine (DEA)] and transferred to the finished product during heat-sealing blistering process via vaporization and condensation on the finished product. Since the deflagration temperature of plasticized nitrocellulose chips e.g. lies above 180 °C, generating different nitrogen oxides upon thermally induced decomposition according to Balser et al. [Ullmann's Encyclopedia of Industrial Chemistry (2012)], liberation of nitrogen oxides from nitrocellulose and subsequent nitrosating of amines in the printing drug ink is regarded plausible.

Table 2.2.3-1 Critical Compound Combinations responsible for *N*-nitrosamine contamination of finished medicinal products

N-Nitrosamine	NOX Source	Amine Source	Amine nitrosated by NOX	Critical Compound Combination
 NDMA	Nitrocellulose (on lidding foil)	Printing ink (on lidding foil)	 DMA	Lidding foil/printing ink
 NDEA	Nitrocellulose (on lidding foil)	Printing ink (on lidding foil)	 DEA	Lidding foil/printing ink

In summary, the formation of *N*-nitrosamines is linked in this case to a critical compound combination (CCC), consisting of the concomitant presence of secondary/tertiary amines in printing ink and nitrocellulose as nitrosating agent in the lidding foil during printing/packaging, which was considered plausible so far. This root-cause can be eliminated by replacing nitrocellulose based lidding foils by nitrocellulose-free lidding foils.

Considering that nitrocellulose represents a widely used primary packaging material for finished medicinal products, this root cause should be investigated by MAHs for their medicinal products packaged in blisters.

2.2.4. Discussion on root causes and strategies to mitigate the presence of *N*-nitrosamines in human medicinal products

NDMA and other *N*-nitrosamines from water are unlikely to represent a realistic source for contamination of APIs..

In summary, NDMA and other N-nitrosamines from water are unlikely to represent a realistic source for contamination of APIs. However, drug substance degradation processes caused by the use of disinfected water cannot be excluded as a contributing factor. So far, neither N-nitrosamines from water nor N-nitrosamines from API degradation processes linked with impurities in water have been identified as contributing factors.

In addition, secondary/tertiary alkylamines and quaternary alkyl ammonium salts were found to be contaminated with *N*-nitrosamines, having detected levels in the low ppm range. The relevance of these results, discovered 40 years ago in secondary and tertiary amines of unknown quality, is currently considered unknown. The ICH Q3C (R7) solvent dimethylacetamide represents –in addition to DMF and NMP- an additional source of secondary amines susceptible to NDMA formation in combination with nitrosating agents. The ICH Q3C (R7) solvent nitromethane cannot be excluded to act as nitrosating agent in combination with certain oxidants/catalyst and to form *N*-nitrosamines in combination with secondary and tertiary amines.

N-nitrosamine impurities e.g. in sartans could be linked directly to the simultaneous presence of the reagent $NaNO_2$ and of solvents, reagents and catalysts as sources of secondary and tertiary amines. These compound combinations are considered critical and present a high risk of *N*-nitrosamine formation and should be avoided or strictly monitored if it is justified adequately to be unavoidable in the entire API manufacturing processes.
In addition, the potential formation of Cohort of Concern Compounds (CoC compounds) such as *N*-nitrosamines should be evaluated by the MAHs/Applicants during manufacturing process development.

MAHs/Applicants are reminded that all materials used in the manufacturing process should be included in the dossier, irrespective of their intended use.

From the pioglitazone case, it was concluded that NDMA formation / contamination of this API manufactured in line with good manufacturing practice is considered principally avoidable by eliminating DMF as nitrosatable solvent from synthetic route I and by replacing it with a non-nitrosatable solvent as part of a variation procedure. In addition, selecting synthetic route II for production of pioglitazone HCl under good manufacturing practice offers the possibility to exclude *N*-nitrosamine formation in general. The option, to choose between two different manufacturing processes, demonstrates the need for thorough justification of the entire synthetic route during manufacturing process development.

Also, the aminophenazone case showed that the option to choose between two different manufacturing processes, demonstrates the need for thorough justification of the entire synthetic route during manufacturing process development.

According to Brambilla et al. (2007), 173 APIs have been found to form N-nitroso compounds such as *N*-nitrosamines upon reaction with nitrite under *in vitro* conditions. Therefore, it is recommended that the WHO NAP test be conducted on starting materials, intermediates and APIs during manufacturing process development. In case of positive findings, further investigations such as AMES testing etc. may be required in accordance with the ICH M7 Guideline.

In view of finished product manufacturing, it is concluded that *N*-nitrosamine formation in and contamination of finished products during primary packaging, performed in line with good

manufacturing practice, is considered principally avoidable by eliminating nitrocellulose as the responsible nitrosating agent in the lidding foil. This case demonstrates once again the need for thorough risk assessment on impurities such as CoC compounds within pharmaceutical process development.

Overall, the interactions between starting materials, intermediates, drug substances, solvents, reagents and catalysts should be thoroughly investigated during manufacturing process development, taking into account relevant ICH Guidance (Q3A, Q3C, Q3D, Q7, Q9, Q11, M7) and the EMA Guideline on the Chemistry of Active Substances.[7]

Based on the above considerations and the feedback from QWP and the Ad-hoc expert group, the following strategies to mitigate the presence of *N*-nitrosamines in human medicinal products should be considered:

- Strive for designing / adapting manufacturing processes to prevent the formation of or contamination with *N*-nitrosamines.
- Risk assessment of route of synthesis, starting materials, intermediates, raw materials (solvents, reagents, catalysts, etc.) and finished product manufacturing process (raw materials, packaging etc.) in consideration of the potential and confirmed root causes for the formation and contamination of *N*-nitrosamines in API synthesis and in finished product as detailed in 2.2.2 and 2.2.3 above.
- In case of identification and confirmation of any risk for the presence of N-nitrosamine through testing, a change of manufacturing process, starting materials and intermediates, raw materials or primary packaging in order to avoid use of nitrosating agents should be considered.
- If combination of nitrosating agents with solvents, reagent and catalysts have been justified to be unavoidable in the entire process, adequate control measures should be implemented. This must be reflected in the control strategy of the API and finished product as appropriate.
- The control point for nitrosamines should be selected in such a way that it will give assurance of presence of the impurity below the acceptable limit in the finished product.
- ICH M7(R1) provides the option for skip testing, which can be applied also in the case of a single nitrosamine impurities, as long as it can be shown that levels of the single mutagenic impurity in the drug substance are consistently less than 30% of the ICHM7(R1) limit for the respective N-nitrosamine, and provided the root cause of a detected nitrosamine is well-known and well-controlled as advised by QWP.
- CHMP further agreed with the 2nd QWP response that to justify omission from the specification, it has to be demonstrated that the level of the respective single nitrosamine is consistently at or below 10 % of the ICH M7(R1) limit, and the LOQ will need to be set at least this level. (Of note, levels below 10% of the limit would translate into a theoretical excess life time cancer risk of less than 1:1,000,000)

[7] https://www.ema.europa.eu/en/chemistry-active-substances-chemistry-new-active-substances

2.3. Consideration for analytical method development to identify and quantify N-nitrosamines in APIs and finished products

This section is based on all available data, including a literature survey on analytical methods for *N*-nitrosamines and addresses current analytical methods for *N*-nitrosamines recommended by the OMCL network and EDQM.

2.3.1. Analytical Methods

Since the awareness of the carcinogenic potential of *N*-nitrosamines, many detection methods have been developed in the field of food, cosmetic, rubber, pharmaceutical/toxicological, and environmental analysis.

While the early analytical methods employed had been polarography, spectrophotometry, and thin-layer chromatography (TLC), gas chromatography (GC) with a special chemiluminescence detector (also called thermal energy analyser (TEA)) was commonly used for about three decades. This detector catalytically pyrolysis the *N*-nitrosamines previously separated by GC. The N-NO bond is cleaved releasing the nitrosyl radical NO• that is separated from organic fragments and other gaseous products typically by cold traps. The nitrosyl radical is then oxidised with ozone leading to electronically excited nitrogen dioxide (NO_2^*) that decays back to the ground state emitting a characteristic wavelength in the near IR (NIR). The TEA is highly selective and sensitive down to the picomole range. However, organic nitrites, *N*-nitramines, C-nitroso, nitrates, and inorganic nitrite may also respond, too [Perera (2006)]. Thus, subsequent confirmation is needed to exclude false-positive results.

The use of mass-selective (mass-spectrometry, MS) detection in conjunction with GC or (ultra) high-performance liquid chromatography ((U)HPLC) allows analyte-specific detection based on both retention time and structurally specific fragmentation information in conjunction with high sensitivity. Therefore, GC-MS, GC-MS/MS, and LC-MS/MS are nowadays commonly used for analysis of *N*-nitrosamine in all types of materials.

Nevertheless, a recent publication [Kodamantani et al. (2018)] described a special version of the TEA detector in an LC system with a post-column anion exchange module followed by photochemical reactor and chemiluminescence detection (HPLC-AEM-PR-CL) for wastewater analysis looking for *N*-nitrosamines. Without any pre-concentration a LOQ of about 1 ng/L (depending on the concrete analyte) using 200 µL sample volume (i.e. about 0.2 pg absolute) was reached. The anion exchange module was used to generate hydroxide ions for the photochemical reactor from anions present in the eluate.

The method of choice has to guarantee the unambiguous determination of *N*-nitrosamine in accordance with scientifically recognized guidelines [ICH Q2(R1) (1995); EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2 (2012).]. Due to their physicochemical properties and, in some cases, their low molecular weight, thoroughly performed method evaluation is necessary to discriminate *N*-nitrosamine from other compounds. Considering specificity and selectivity of the method, identification criteria should be gained during the determination procedure as much as possible to corroborate the presence of *N*-nitrosamine.

Published review articles about recently implemented *N*-nitrosamine analytical procedures particularly highlight the benefits of combining chromatographic separations with highly sensitive detection methods determining *N*-nitrosamine in traces [Perera (2006); Wiltschko et al. (1998); Parr et al. (2019)]. Their character as volatile or non-volatile molecules make *N*-nitrosamines more or less suitable for different chromatographic techniques (LC, GC) . The main candidates of interest, namely

N-Nitrosodimethylamine (NDMA) and *N*-Nitrosodiethylamine (NDEA), with their volatile properties, can be well separated from confounding molecules under gas chromatographic or liquid chromatographic conditions. The latter also extends the range of analytes by including non-volatile or thermally instable *N*-nitrosamine (e.g. *N*-nitrosodiphenylamine), which can be measured by GC after time-consuming derivatisation steps only. Additionally, all review articles list adequate pre-purification and enrichment steps of *N*-nitrosamine from respective matrices, e.g. food, water, drug substances, etc., to reduce the complexity of compounds prior to analysis. Recently, a review was published summarising analytical methods utilised for the determination of *N*-nitrosamine in pharmaceuticals [Parr et al. (2019)], and information from the article can be gleaned to support guidance on analytical aspects.

A rather new approach has been published in May 2019 describing the simultaneous determination of related substances and N-nitrosamines in valsartan and losartan [Schmidtsdorff et al. (2019)] in one chromatographic run only using supercritical fluid chromatography (SFC) with modifier (methanol and formic acid), combined with UV and electrospray ionisation (ESI) MS detection. This may have some advantages for routine control of *N*-nitrosamine.

2.3.2. Sample Preparation Procedures

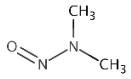
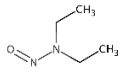
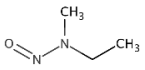
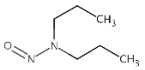
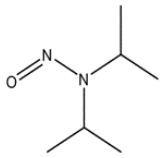
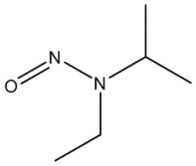
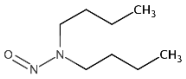
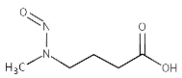
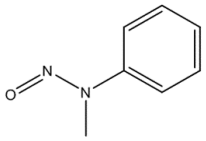
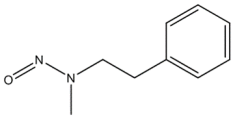
Special emphasis should be placed on the workup procedures prior to injection of the sample solution in either GC or LC in order to reduce potential interferences. While older literature describes laborious liquid-liquid extractions followed by concentration steps (with the risk to lose volatile *N*-nitrosamines), combined extraction and concentration can be achieved with solid-phase extraction (SPE) or solid-phase micro extraction (SPME).

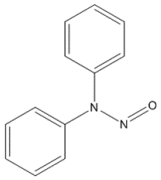
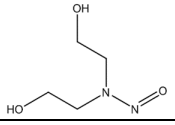
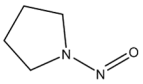
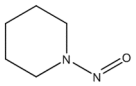
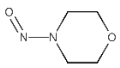
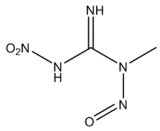
Several materials can be used for SPE. Published review articles [Perera (2006); Boyd et al. (2011)] discuss the advantages and disadvantages of different materials, either used alone or in combination. Activated carbon adsorbents are considered as suitable in general while reversed phase materials are deemed less effective. However, a combination of both increases the recovery of *N*-nitrosamines.

SPME extracts the volatile or semi-volatile analytes from solutions with fused-silica fibre coated with a polymeric liquid phase. After equilibration, the adsorbed or absorbed analyte on the fibre is thermally desorbed in a hot injector port of a gas chromatograph or in an appropriate interface of a liquid chromatograph [Perera (2006)].

The hydrophilicity and consequently the solubility of the target analyte in water or other solvents should be taken into account, when elaborating the workup procedure. While NDMA and NDEA show a high-water solubility, the water solubility of NDPA and NDBA is lower. Some characteristics are given in table 2.3.2-1. Typical solvents used for extraction of *N*-nitrosamines are dichloromethane, methanol and acetone.

Table 2.3.2-1 Physicochemical properties of some N-nitrosamines commonly studied (*: predicted, meridian; data obtained from US-EPA, CompTox Chemistry Dashboard, https://comptox.epa.gov/dashboard)

***N*-Nitrosamine**	**Abbreviation**	**Structure**	**CAS**	**Log K_{ow}**	**Water solubility**
N-nitrosodi-methylamine	NDMA		62-75-9	-0.57	13.5 mol/L
N-nitrosodi-ethylamine	NDEA		55-18-5	0.48	1.04 mol/L
N-nitrosomethyl-ethylamine	NMEA		10595-95-6	0.04	3.4 mol/L
N-nitrosodi-propylamine	NDPA		621-64-7	1.36	0.0999 mol/L
N-nitrosodi-isopropylamine	NDIPA / DIPNA		601-77-4	1.38	0.0999 mol/L
N-nitrosoethyl-isopropylamine	NEIPA / EIPNA		16339-04-1	0.9	0.199* mol/L
N-nitrosodi-butylamine	NDBA		924-16-3	2.63	0.008 mol/L
N-nitrosomethyl-amino butyric acid	NMBA		61445-55-4	-0,4*	2.29 mol/L*
N-nitrosomethyl-phenylamine	NMPA / PMNA		614-00-6	1.49*	0.0494 mol/L*
N-nitrosomethyl-2-phenylethylamine			13256-11-6	1.55*	0.0212 mol/L*

***N*-Nitrosamine**	**Abbreviation**	**Structure**	**CAS**	**Log K_{ow}**	**Water solubility**
N-nitrosodi-phenylamine	NDPh		86-30-6	3.13	0.000177 mol/L
N-nitrosodi-ethanolamine	NDELA		1116-54-7	-1.29*	7.45 mol/L
N-nitrosopyrrolidine	NPYR		930-55-2	-0.19	9.99 mol/L
N-nitrosopiperidine	NPIP		100-75-4	0.36	0.67 mol/L
N-nitrosomorpholine	NMOR		59-89-2	-0.44	8.61 mol/L
N-nitrosomethyl-nitroguanidine	NMNG / MNNG		70-25-7	-0.809*	1.29 mol/L*

2.3.3. Potential causes of erroneous analytical results

Depending on the sample matrix, artificial formation of *N*-nitrosamines during extraction and clean-up is possible when *N*-nitrosamine precursors (secondary amines and nitrites) are present in relevant quantities in the sample, in particular if acidic solutions are used. Activated carbon materials (used for SPE) should be treated with caution as they may lead to the formation of *N*-nitrosamines during the processing if sufficient precursors are present in the matrix [Boyd et al. (2011)]. Several inhibitors were used for workup procedures described in the literature, including sulfamic acid, ascorbic acid, and tocopherol [Perera (2006)]. Workup using sodium hydroxide is known to reduce artificial formation of *N*-nitrosamines, too [Huang et al. (2013)]. Artificial formation of *N*-nitrosamines should be taken into consideration during analytical validation to exclude any significant impact on the results. However, considering the typically low levels of *N*-nitrosamines in pharmaceuticals, recovery rates in the range of 80 – 120 % are deemed acceptable.

Based on the available information so far, as per the current Questions and Answers document[8], interference in analytical methods can also be caused by:

- presence of trace amounts of nitrosamines in testing materials utilised (e.g. water, airborne sources, plastics products, rubber/elastomeric products));
- in situ formation of nitrosamines (as seen e.g. with ranitidine at high temperature conditions).);
- identification of the specific peak of a certain nitrosamine (e.g. DMF co-eluting with NDMA).

[8] https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-questions-answers-information-nitrosamines-marketing-authorisation_en.pdf

2.3.4. Internal Standards

Internal standards with high purity grades should be employed to account for any possible losses during workup or due to the thermal instability inherent to several *N*-nitrosamines. While analysis with conventional detectors (TEA, FID, UV-Vis, etc.) commonly utilise a synthetic *N*-nitrosamine (i.e. *N*-nitrosodiisopropylamine, NDIPA) that does not occur in nature, the typical internal standard for MS detection is an isotopic standard (e.g. NDMA-d_6 or NDEA-d_{10}) that show almost identical physicochemical behaviour during analysis as the analyte of interest. Non-isotopically labelled analogues should not be present in the internal standards to exclude false positive *N*-nitrosamine determinations by means of MS. Additionally, it should be noted that NDIPA has been found in valsartan of one CEP holder [RIS World-Online (2018)]. Therefore, any internal standard should be carefully chosen.

2.3.5. Advantages of mass spectrometric detection devices

Most of the utilized GC and LC laboratory systems in the OMCL network are equipped with mass spectrometric (MS) devices. The superior properties of GC- or LC-MS devices to provide molecular structure information by simultaneously maintaining highly sensitive detection limits meet the pinpointed analytical criteria and requirements for *N*-nitrosamine detection. For LC-MS applications, all devices were equipped with atmospheric pressure chemical ionization (APCI) sources to obtain tremendously higher ionization rates for NDMA and NDEA. It is worth mentioning that the ionisation principle used for MS plays a relevant role with regard to sensitivity and detectability. Electrospray ionisation (ESI), commonly used for LC-MS analysis of *N*-nitrosamines in the years after 2000, can be hampered by ion suppression due to matrix effects [Lee et al. (2013)]. Therefore, APCI (positive mode) is commonly used for analysis of *N*-nitrosamines nowadays where this effect is less relevant.

2.3.6. Currently used methods in OMCLs

Table 2.3.6-1 below gives an overview of the currently used methods in the OMCL network and in jurisdictions outside Europe. Details can be found on the website of the EDQM. Most of the laboratories use a direct extraction of the respective drug substance (DS) or drug product (DP) with a subsequent dilution and filtration step. Afterwards, the extracted supernatants are transferred to GC- or LC-MS (partly LC-UV) devices and measured via direct injection (DI). Another common method is GC headspace (HS)-MS dissolving the sample directly in either *N*-methylpyrrolidine (NMP) or dimethyl sulfoxide (DMSO). These short workup procedures were chosen to minimise any loss due to the volatile character of NDMA and NDEA.

Table 2.3.6-1 Published methods of OMCLs to determine NDMA or NDEA. DCM = dichloromethane; DE = direct extraction; DI = direct injection; DMSO = dimethyl sulfoxide; DP = drug product; DS = drug substance; HS = headspace; LLE = liquid-liquid extraction; MeOH = methanol; NaOH = 1 M sodium hydroxide solution; NMP = N-methyl pyrrolidine

Analytical technique	GC-MS/MS (DI)	GC-MS (HS)	LC-MS/MS	HPLC-UV	High throughput RapidFire®-MS
Analyte(s)	NDMA, NDEA	NDMA, NDEA	NDMA, NDEA	NDMA, NDEA	NDMA, NDEA
Sample amounts (DS and/ or DP)	250-500 mg DS or DP containing 250 mg of DS	50-500 mg DS or 50-250 mg DP; 'one tablet'	50-100 mg DS or DP containing 50-100 mg of DS	62-320 mg DS	DS (unknown)
Workup procedure	DE with MeOH or DCM; LLE with NaOH and DCM	Direct HS-analysis after dissolution in NMP or DMSO	DE with MeOH	DE with MeOH/, H2O (35:65 V/V)	DE with MeOH
DS	valsartan irbesartan losartan candesartan olmesartan	valsartan irbesartan losartan candesartan olmesartan	valsartan irbesartan losartan ranitidine	valsartan losartan irbesartan candesartan olmesartan	losartan
NDMA – LOD	0.002-0.01 ppm (DS)	0.005-0.04 ppm (DS)	0.010-0.15 ppm (DS)	0.02-0.10 ppm	10 ppm
NDMA – LOQ	0.005-0.05 ppm (DS)	0.1 ppm (DS)	0.08-0.5 ppm (DS)	0.04-0.25 ppm	25 ppm
NDEA – LOD	0.002-0.01 ppm (DS)	0.02 ppm (DS)	0.006-0.02 ppm (DS)	0.04-0.10 ppm	25 ppm
NDEA – LOQ	0.007-0.03 ppm (DS)	0.05-0.08 ppm (DS)	0.02-0.15 ppm (DS)	0.08 – 0.50 ppm	50 ppm
Details	https://www.edqm.eu/en/ad-hoc-projects-omcl-network				

2.3.7. Sensitivity of the analytical methods

To date, limits of detection (LOD) and limits of quantitation (LOQ) as low as 2 ppb and 5 ppb, respectively, could be reached for NDMA (NDEA: 2 ppb and 7 ppb, respectively) with respect to the amount of API. It should be noted that the LOQ is more relevant as it describes the threshold above that reliable quantitative results can be obtained. These results can be used to evaluate the need for routine control of any contaminant found.

Nevertheless, both LOD and LOQ parameters correlate directly with the chosen and implemented workup procedure. The more API or finished product is extracted, purified, and concentrated, the higher is the possibility of reaching low LOD and LOQ values. Hence, a precise and detailed description of the individual laboratory processing of API and/or FP is necessary.

Due to lack of general *N*-nitrosamine determination methods for any kind of API or FP, the workup procedures for specific API, e.g. 'sartans' or ranitidine, or drug formulations have to be re-evaluated when additional compounds get into the focus of analysis. To overcome time-consuming efforts for routine screenings on NDMA or NDEA, novel high throughput MS applications were already tested to analyse great numbers of samples (e.g. RapidFire-MS). Unfortunately, the desired LOD and LOQ levels have not yet been reached so far.

It is important to note that results, LODs and LOQs for a certain finished product should always be reported in relation to the declared amount of API in this finished product since the declared amount of API is used for estimation of exposure to *N*-nitrosamines. The actual weight of a dosage unit is usually unknown to either the patient or the physician. Thus, results or LOQs reported in relation to the weight of the dosage unit are meaningless for estimation of exposure.

2.3.8. Discussion on analytical aspects

The CHM considers that analytical procedures for *N*-nitrosamines should be carefully chosen taking into account:

- potential presence of precursors (secondary amines; nitrite) in the sample
- workup procedures must be validated for any potential interferences
- hydrophilicity / lipophilicity as well as volatility / non-volatility of the target analyte
- use of an adequate internal standard with high purity grades to overcome any loss during sample preparation and to assure accurate quantitation
- LOQ provides the minimum level at which an analyte can be quantified with acceptable accuracy and precision and is thus preferred over LoD for impurity testing and decision-making
- LOQ should be minimum at or sufficiently below the toxicologically required limit, taking into account the purpose of testing (e.g. routine testing, justifying skip testing, justifying omission of specification)

Since different matrices and target analytes are possible, a universally applicable sample preparation method and the use of either HPLC or GC cannot be recommended in general. However, the sample preparation performed by the Swiss OMCL using suspension of the sample in sodium hydroxide solution followed by liquid-liquid extraction with dichloromethane may be applicable to various APIs and finished products. Nevertheless, specific validation is necessary in each case.

2.4. Considerations for calculating risk for exposed patients in case of detection of N-nitrosamines in medicinal product(s)

2.4.1. Background exposure to *N*-nitrosamines

2.4.1.1. Exposure to exogenous N-nitrosamines

N-nitrosamines are considered as a serious health risk in all products with consumer/patient exposure and remaining levels in these are limited by the ALARA/ALARP principles.

As concluded in the Sartan referral, the intake of NDMA and NDEA should be seen in relation to the overall intake of genotoxic carcinogens, e.g. as benzo[a]pyrene and other PAHs and also other nitrosamines that are present in common food sources such as grilled meat. As it is beyond the scope

of this referral to cover all non-nitrosamine substances, only *N*-nitrosamines are covered in this section.

There exist various estimates on the background exposure to exogenous *N*-nitrosamines (either for specific or the sum of n>1 *N*-nitrosamines in various sample types). The *N*-nitrosamine sources of exposure include endogenous generation from nitrite present in water and foods (e.g. drinking water contaminated with nitrite, eating processed foods), processed drinking water, drinking water from drinking water treatment plants (DWTPs) that use chlorination or chloramination processes, direct and indirect tobacco product exposure (e.g. smoking), contact with some latex and rubber products etc (see table below for estimates in some of these sources). Overall, it is considered likely that food is a major if not the dominant source of human exposure to exogenous *N*-nitrosamines, but the sum of the other exposure sources may also be substantial (at least for some *N*-nitrosamines).

Table 2.4.1.1-1 Some estimates in academic reports on exogenous *N*-nitrosamine-levels (specific and total levels, TNA) in various samples (some of which may exceed current EU limits).

Source	**Nitrosamine range detected**	**References (PubMed ID)**
Processed foods (cured, cooked, smoked)	Across food groups: TNA: range 0.4-35.6µg/kg NDMA: mean 2.2µg/kg NDBA: mean 1.5µg/kg NPYR: mean 1.5µg/kg NDEA: mean 0.9-1.5µg/kg NPIP: mean 0.5µg/kg NMOR: mean 0.05µg/kg	Lee 2019 PMID: 30208538 Gushgari & Halden 2018
Personal care products (cosmetics, hair products, lotions, shampoos, soaps)*	Hair gel (NDELA): 7644µg/kg Lotions (NDELA): 22-230µg/kg Soap (NDELA): 26-75µg/kg Shampoo (NDELA): 46-1287µg/kg Shower gel (NDELA): 46-3746µg/kg	Joo et al. 2015 Schothorst & Stephany 2001 Schothorst & Somers 2005
Tobacco product exposure (cigarettes, cigars, chewing tobacco, snuff)	Cigarettes: NNN: 0.306-7.4µg/g tobacco NNK: 0.194-3.2µg/g tobacco NAT: 0.32-4.6µg/g tobacco NAB: 0.021-0.95µg/g tobacco	Gushgari & Halden 2018 Edwards et al. 2017
Rubber and latex products[9]	*Condoms release to artificial sweat or saliva*:	Altkofer et al. 2005

	German samples: TNA <10-660µg/kg (up to 1.4µg per unit) including NDMA, NDBA and NDEA (range 3.8-31ng/g). Chinese samples (saliva): TNA range 5.3-1289.8µg/kg with NDMA N.D. to 36.5µg/kg, NDEA N.D. to 277.8µg/kg and NDBA 3.49-556.3µg/kg. Chinese sample (sweat): TNA range 15.6-792.9µg/kg with NDMA N.D. to 14.5µg/kg, NDEA 7.3-648.9µg/kg and NDBA N.D. to 299.1µg/kg. *Balloon release to artificial saliva:* German samples: TNA <10-380µg/kg (up to 0.46µg per unit) including NDMA, NDBA and NDEA.	Biaudet et al. 1997 Feng et al. 2010

*Cosmetic products containing nitrosamines including NDELA are no longer allowed on the EU/EEA market under the Cosmetics Directive 76/768/EEC (limit of 50 µg/kg (50 ppb) for nitrosamines).

Food and water: Nitrosamines, primarily NDMA, can be introduced into drinking water supplies when chloramine/chlorine disinfection of DW is used, but also to some extent by ozonisation. Generally, the average or median NDMA levels in drinking water seem to be around or below 10-20 ng/L.

Based on the literature overview for all food-nitrosamine studies before 2017 by Gushgari & Halden (2018), the food groups with the highest average total nitrosamine (TNA) concentrations are:

- Fats, oils and sweets (n=21 studies): TNA average 8.92 µg/kg
- Meats (n=118 studies): TNA average 8.10 µg/kg
- Fish (n=59 studies): TNA average 5.55 µg/kg
- Vegetables (n=21 studies): TNA average 5.35 µg/kg

The most commonly measured nitrosamines in food products according to Gushgari & Halden (2018) were NDBA, NDEA, NDMA, NDPA, NMEA, NMOR, NPIP and NPYR. NDMA was the most common nitrosamine across food groups with an average of 2.2 µg/kg whereas NDEA had an average of 0.9 µg/kg. A similar literature overview on nitrosamines focused on meat products and was published by Lee (2019), covering 25 studies reported between 1985 and 2018 (where the nitrosamine levels reported were also adjusted for the number of samples). The nitrosamines NDBA, NDEA NDMA, NPIP and NPYR were the most commonly ones present in nitrite/nitrate treated meat and poultry products (although at different proportions in different meat products). In most cases (i.e. food samples measured in studies), the average or median levels of TNA are around or <10 µg/kg in foods with the exceptions of fried foods such as fried bacon (average 35.6 µg/kg), fried pork (average 25.9 µg/kg) and fried chicken (average 22.4 µg/kg). Heat-treatment generally increases nitrosamine levels (especially frying, grilling and less so boiled cooking) – in particular in processed meat products. It can be noted that volatile *N*-nitrosamines in cured meat seem to be present at lower amounts than non-volatile *N*-nitrosamines. The level depends on the market e.g. higher levels have been observed in Belgium than in Denmark [Herrmann et al. (2015a], study (period of time) and products (cured meat, smoked meat, etc.). Some non-volatile *N*-nitrosamines may also be formed in raw cooked sausage depending on nitrite added such as *N*-nitrosohydroxyproline (NHPRO), *N*-nitrosoproline (NPRO), *N*-

nitroso-thiazolidine-4-carboxylic acid (NTCA) and *N*-nitroso-2-hydroxymethyl-thiazolidine-4-carboxylic acid (NMTCA). These non-volatile *N*-nitrosamines are however considered of low concern based on the data available and/or structure-activity-relationship (SAR) considerations (EFSA, 2017). The Lee-study estimates for NDMA and NDEA in various meat/poultry products (spanning different processing and cooking methods) is 0.3 to 5.7 µg/kg and 0.2-1.0 µg/kg, respectively. Both the Gushgari & Halden study and the Lee-study indicate that TNA levels in most food groups are <10 µg/kg (many in the range of 5-10 µg/kg) and that the overall TNA levels have decreased since the 1970s and 1980s.

A smaller review by Herrmann et al. (2015), who reviewed publications of the period 1990–2010 in three European countries (Finland, the Netherlands and Germany), reported mean estimates of exposure to volatile *N*-nitrosamines from all foods ranging from 1.0 ng/kg bw/day (NDMA only) to 12 ng/kg bw/day (sum of NDMA, NPYR, NPIP). Furthermore, it was estimated that the classical volatile *N*-nitrosamines (NDMA, NPYR, NPIP, NDEA) accounted for approximately 90% of the total exposure to volatile *N*-nitrosamines with NDMA and NPYR contributing to 40–50%. In Switzerland, the exposure to *N*-nitroso compounds (NOCs) via food (excluding drinking water) is estimated to be around 1,000 ng/day (20 ng/kg bw/day) with NDMA contributing up to 200 ng [Tricker et al. (1991), Lutz (1999)].

According to an evaluation of the European Food Safety Authority (EFSA), the combined daily intake of NDMA and NDEA via processed meat ranges from 0.5 to 1.7 ng/kg on average in adults in Europe (EFSA, 2017). Based on the evaluated studies, the EFSA panel noted that NDMA was the main compound contributing to mean overall human *N*-nitrosamine exposure. NDMA accounts to approximately 90% and NDEA to approximately 10% of human exposure to volatile *N*-nitrosamines.

An assessment assumed an, on average, 100 – 1,000 ng/day corresponding to 2 – 20 ng/kg bw/day exposure to NDMA from contaminated beverages and food, air and water pollution (Keszei et al. 2013, WHO). Other volatile *N*-nitrosamines found were NPIP, NDBA, NPYR.

Unpublished data on actual levels of nitrosamines in cooked/processed food analysed NDMA, NDEA, NDIPA, NDPA but only found NDMA (and N-nitrosopiperidine, NPip) 0.7-0.9 ppb or 14-17 ng/day consumption according to the Ad-hoc Experts Group.

Human urine samples: Assessment of nitrosamine levels in urine samples also provides an indication of overall (exogenous and endogenous) nitrosamine exposure. Based on published literature (1994 to 2016), the mean NDMA range in urine samples (from European and non-European countries) ranges between <10 ng/L and 1,920 ng/L (Krauss et al. 2009). For studies from European countries (Switzerland, Netherlands), the NDMA range was 83-1,134 ng/L based on average urinary volume of 1.5L per day which, assuming that there is not a 100% correlation between exposure/uptake and elimination, would indicate that the total NDMA exposure is very likely larger than ~1 µg/day for at least a part of the population. Considering that NDMA is often found as a proportion of the TNA measurements (e.g. average 2.2 µg/kg NDMA across food groups where the TNA levels are ~5-9 µg/kg across food groups in the Gushgari & Halden study), this would indicate that the TNA levels are also greater in urine samples. However, the reliability of the measures is unclear as is the relationship between external exposure and endogenous generation of NDMA specifically and volatile nitrosamines in general. Estimations are based on simulation model estimates, but the endogenous part also may be substantial.

2.4.1.2. Exposure to endogenous N-nitrosamines

Exposure to endogenously produced *N*-nitroso compounds (ENOCs) raises an equal concern as exposure to exogenous NOCs. Formation of ENOCs in the upper gastrointestinal tract from nitrosatable amines depends on the simultaneous presence of nitrite. Sufficient nitrite ingestion and formation of nitrite from ingested nitrate is a key factor for nitrosation. There is, however, no experimental proof for

the formation of ENOCs from exposure to nitrite or nitrate under normal diet conditions so far. In addition, the impact of potential formation of ENOCs in the body under inflammatory conditions and the amounts found in urine is currently unclear. There are no data available re the metabolic activation of NOCs outside the liver in humans. Surrogate models have so far been used mainly evaluating NDMA and NDEA formation in the upper gastrointestinal tract (see table 2.4.1.2-1).

Table 2.4.1.2-1 Some estimates on endogenous nitrosamine exposure using artificial *in vitro* models.

Source	Range detected	
Endogenous generation from nitrites.	Artificial stomach models (static and dynamic) [at low pH, defined levels of nitrite or nitrate, amine rich foods]: # Static model (2h, pH2) :): 6-18ug NDMA # Dynamic model (rapid pH shift 2.5->1.7, 3h) – cumulative mean 2.3–422ug NDMA. # Dynamic model (slow pH shift 3->1.7, 3h) – cumulative mean 1.8–42.7ug NDMA. # Extrapolation model based on Dutch food consumption data - 4ng/kg BW NDMA in young children and 0.4ng/kg BW NDMA in adults.	Krul et al (2004) Groenen et al (1980) Zeilmaker et al. (2010)

Zeilmaker et al. (2010) estimated the NDMA exposure of adults to NDMA formed after ingesting fish and nitrate rich vegetables to be 0.4 ng/kg bw/day. This has already been summarized and critically discussed in the CHMP Art 31 referral on sartans. The EFSA panel used the model described in the Guideline for Canadian Drinking Water Quality (Health Canada 2013) to calculate potential exposure by formation of ENOCs using NDMA as representative. The calculated NDMA exposure from endogenous formation was calculated by 0.064 ng/kg bw/day at the acceptable daily intake of nitrite ion of 0.07 mg/kg bw/d (EFSA 2017). The Panel also noted that this calculation included conservative assumptions like the availability of sufficient amounts of nitrosatable substrates and complete reaction of all nitrite with only these substrates at all time to produce only carcinogenic ENOCs.

2.4.2. Mutagenicity and carcinogenicity of *N*-nitrosamines

Most *N*-nitrosamines are considered to be mutagenic and carcinogenic, at least in animals, and with extensive difference in potency between the most and least potent nitrosamines. IARC has classified those with animal data available as class 2A or 2B. Currently, only some tobacco-related *N*-nitrosamines are classified as class 1 (e.g. NNN, NNK) although sufficient substance specific human data are lacking. Those with insufficient data from animals are classified as class 3. As outlined already in the Art 31 referral on sartans, the mechanistic principles of *N*-nitrosamine mutagenicity and carcinogenicity are also considered relevant in humans. Of highest concern with respect to mutagenic and carcinogenic potential are some of the so called volatile *N*-nitrosamines potentially formed in food (EFSA, 2017) such as *N*-nitrosodimethylamine NDMA, *N*-nitrosodiethylamine (NDEA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosopiperidine (NPIP) Nitroso-*N*-methyl-*N*-(2-phenyl)ethylamine (NMPEA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosomorpholine (NMOR), *N*-nitrosomethylethylamine (NMEA) and *N*-nitroso-di-n-propylamine (NDPA).

2.4.2.1. Reactive metabolites

N-Nitrosamines need to be activated metabolically to form different diazonium ions (e.g. methyldiazonium, ethyldiazonium etc.). Alkydiazonium ions are precursors of reactive electrophilic carbenium ions, which directly react with DNA thereby forming stable adducts mainly with nitrogen and oxygen of guanine, cytosine and thymidine. The structure and number of diazonium ions formed from a specific *N*-nitrosamine depends on the chemical structure of each individual *N*-nitrosamine. The resulting DNA-adducts depend on the nature of the formed diazonium ion. These different adducts are repaired by different cellular repair mechanisms with different capacity, velocity and accuracy. For the high mutagenic and carcinogenic potential of some volatile *N*-nitrosamines, the key step is metabolic activation by α-hydroxylation which subsequently leads to formation of diazonium ions. Lower number of α–hydrogens in alkyl-*N*-nitrosamines and substitution of the α-hydrogen may reduce the mutagenic potential. This consideration would lead to a ranking of the relative mutagenic potency of alkyl-*N*-nitrosamines: dimethyl, diethyl…> methyl nitroso…butanoic acid (one α–hydrogen)…>> tertiary-butyl (no α-hydrogen). Other substituents may also reduce or even eliminate the mutagenic and carcinogenic potential of *N*-nitrosamines such as branched, bulky or un-metabolisable groups at or near the α-carbon preventing metabolic activation [Benigni et al. (2005)]. Another factor for mutagenic potency is the stability of the diazonium ion formed. Usually, the stabilizing effect of the substituent is expected to decrease in the order isopropyl >carboxypropyl> ethyl > methyl, resulting in thermodynamic stabilities as follows: isopropyldiazonium ion >carboxypropyldiazonium ion >ethyldiazonium ion >methyldiazonium ion.

Assuming a nucleophilic substitution 2 (Sn2) reaction mechanism for alkylation of DNA by alkyldiazonium ions, steric hindrance has to be taken into account as an additional factor in reactivity estimation. Accordingly, kinetic reactivity of alkyldiazonium ions towards nucleophils via Sn2 reaction is expected to increase as follows: isopropyl diazonium ion <carboxypropyldiazonium ion <ethyldiazonium ion <methyldiazonium ion.

As shown in Figure 2.4.2.1-1, these theoretical considerations are supported by experimental data on relative alkylation rates / hydrolysis rates for alkyldiazonium ions: propyldiazonium ion <ethyldiazonium ion <methyldiazonium ion [Manso et al. (2008)]. Considering that mutagenic potency also determines carcinogenic potency, this ranking would also resemble the carcinogenic potency of *N*-nitrosamines.

Table 3. Alkylating potential of alkyldiazonium ions, expressed as the alkylation rate/hydrolysis rate ratio

Alkyldiazonium ion	k_{alk}/k_{H_2O} (35 °C)[a]
Methyl	12878 ± 102
Ethyl	10809 ± 179
Propyl	4691 ± 61
Butyl	3204 ± 117
Allyl	8169 ± 167

[a] Values given with their standard deviations.

Figure 2.4.2.1-1 Relative alkylation rates / hydrolysis rates for alkyldiazonium ions [Manso et al. (2008)].

However, the alkylating potential alone does not only determine the mutagenic and therefore carcinogenic potency. *N*-Nitrosamines with more bulky side chains (e.g. NPYR, NPIP) do have higher TD_{50} values as compared to those with small side chains. They are nevertheless high potential cohort of concern carcinogens with a TD_{50} below 1.5 mg/kg/day.

2.4.2.2. DNA repair processes

Among the genotoxic *N*-nitrosamines, different nitrosamines (or more specifically their reactive metabolites) can generate different types of DNA lesions. For instance, NDMA has so far been linked to different alkyl-group types of guanine adducts, adenine adducts, and thymine adducts whereas tobacco-related *N*-nitrosamines such as NNN and NNK are primarily linked to so called bulky DNA adducts on the different nucleosides. At the same time, there is only a limited capacity of DNA repair systems for different DNA lesions. This would imply that that to some degree, *N*-nitrosamine exposure from pharmaceutical impurities is integrated into the more general *N*-nitrosamine exposure as long as there is some commonality in the type of DNA lesions.

The full DNA lesion profiles of potent nitrosamines such as NDMA and NDEA remain unclear but it is well established that they generate pro-mutagenic 0^6-alkylguanine adducts (i.e. the modification of guanine through the addition of small alkyl-groups such as e.g. $-CH_3$, $-C_2H_5$) which are most commonly repaired via dealkylation by DNA alkyl transferases (AGT, also known as methyl-guanine-methyl-transferase, MGMT). Other nitrosamines such as NDBA, NDELA, NMEA, NNK and NNN are also known to generate 0^6-alkylguanosine adducts (Dennehy & Loeppky 2005, Coulter et al 2007, Kotandeniya et al 2013, von Hofe & Kleihues 1986, Bonfanti M et al. 1990). The activity of this enzyme is considered to have a high variability within humans. In a small study by Lees et al. 2002, the interindividual variability of MGMT activity in human colon mucosa was 6- to 7-fold. In a larger cohort this variability can be expected to be even greater. In rodent cell lines (e.g. rat liver), MGMT repair enzymes have been reported to be inducible in contrast to human cell lines. This may indicate potential differences in detoxification between rats and humans (Fritz et al. 1991, Grombacher & Kaina 1995, Fritz & Kaina 1992)

2.4.3. *N*-Nitrosamine carcinogenicity in animals

Animal data generated in lifetime bioassays are the most reliable source to conclude on the carcinogenicity of chemicals and human relevance. Reliable human data are currently not available for most chemicals and are also lacking for *N*-nitrosamines. The characterization and ranking of the carcinogenic potency based on animal data is difficult and only possible in a limited fashion. There are several aspects to be considered. One major factor is that it is experimentally practically impossible to reduce the exposure or dose beyond a certain level as one has to separate increasingly small chemical carcinogenesis effects from the background levels of target organ relevant neoplasms. In other words, to detect increasingly subtle toxicological neoplasms against the background levels of neoplasms in a given organ as the exposure decreases, the size of the experimental groups needs to increase substantially. The largest mammalian chemical carcinogenesis study so far used ~24,000 animals[10] – far more than all nitrosamine carcinogenicity studies reported so far - and only reached a sensitivity of 1 in 100 cases for liver and bladder neoplasms. This has to be considered in relation to the theoretical 1 in 100 000 excess risk for oncogenesis that serves as reference for establishing levels of mutagenic impurities that are expected to pose negligible carcinogenic risk according to ICH M7(R1), meaning that all estimates of human risk from animal data are based on a theoretical linear extrapolation (generally considered the most conservative extrapolation approach) from a higher animal dose to a

[10] Bruce R.D. et al (1981), Fund. Appl. Toxicol, 1 (1981), pp. 67-80.

very low dose (see sections 2.4.5. and 2.5 of the report). An additional factor is that most of the lifetime bioassays have been performed in rats and mice. However, laboratory rodent strains used were different. It is well known that various strains often display different sensitivities even to the same chemical.

The most comprehensive source for animal carcinogenicity data is the Carcinogenic Potency Database (CPDB, 2007). For this database, 6540 long-term animal cancer studies of 1547 chemicals were evaluated and the dose causing cancer in 50 percent of the animals (TD_{50}) was calculated by a mathematical model. For *N*-nitrosamines mentioned in this report, the TD_{50} values found in CPDB are listed in table 2.4.3-1. sorted by their descending carcinogenic potency (harmonic mean TD_{50}).

Table 2.4.3-1 TD_{50} values found in the CPDB for some *N*-nitrosamines considered of relevance (EFSA 2017, IARC)

Agent	**Abbre-viation**	**IARC Group**	**TD_{50} [mg/kg/ day] harmonic mean rat, CPDB**	**TD_{50} [mg/kg/day] most relevant study, sensitive species (tissue), CPDB**	**TD_{50} [mg/kg/day] other species, CPDB**	**Mutagenicity**
Nitroso-*N*-methyl-*N*-(2-phenyl)ethylamine	NMPEA		0.00998 male only	0.00788, rat (ugi), Lijinsky et al 1982		Ames test positive (CPDB)
N-Nitrosodiethylamine	DENA, NDEA	2A	0.026	0.05, rat (liv), Peto et al 1991b; 0.026, rat (eso), Lijinsky et al 1981	0.00725, cynomolgus; 0.012 bush babies; 0.054, rhesus (harmonic means)	Ames test positive (CPDB)
N-Nitrosomethylethylamine	NMEA	2B	0.053 (1 dose group)			Ames test positive (CPDB)
N-Nitrosodimethylamine	DMN, NDMA	2A	0.096	0.04 rat (liv), Peto et al 1991b; 0.06, rat (liv), Lijinsky et al 1984	0.189, mouse (harmonic mean)	Ames test positive (CPDB)
N-Nitrosonornicotine	NNN	1	0.096 (1 dose group)		10.8, hamster (harmonic mean)	Ames test positive, Padma et al 1989
4-(*N*-Nitrosomethylamino) -1-(3-pyridyl)-1-butanone	NNK	1	0.0999	0.182, rat (lun), Rivenson et al 1988		

N-Nitrosomorpholine	NMOR	2B	0.109	0.127, rat (liv), Lijinsky et al 1988	3.57, hamster (harmonic mean)	Ames test positive (CPDB)
N-nitrosomethylaniline	NMA, NMPA		0.142 (2 dose groups)		0.034 rat, Schmahl et al 1976	Positive in the hisG428 Salmonella strain TA104
N-Nitrosodi-n-propylamine	NDPA	2B	0.186 (1 dose group)		0.012 rhesus (liv)	Ames test positive (CPDB)
Nitrosodibutylamine	NDBA	2B	0.691 (1 dose group)		1.09 mouse (liv)	Ames test positive (CPDB)
N-nitrosopyrrolidine	NPYR	2B	0.799		1.7 rat (liv), Gray et al; 2.43 rat (liv), Berger et al 1987 0.697 mouse (harmonic mean)	Ames test positive (CPDB)
N-Methyl-*N´*-nitro-*N*-nitrosoguanidine	MNNG	2A	0.803	0.284 rat (pyl), Zaidi et al 1993	2.03 mouse (harmonic mean)	Ames test positive (CPDB)
4-methyl)(nitroso)amino)butanoic acid	NMBA		0.982 (1 dose group)			AMES test negative (CPDB) Ames test positive, Inami et al 2013
N-Nitrosopiperidine	NPIP	2B	1.43	1.31 rat (eso), Gray et al 1991	1.3 mouse (harmonic mean)	Ames test positive (CPDB)
N-Nitrosodiethanolamine	NDELA	2B	3.17	0.19 rat (liv) Lijinski et al 1985		Ames positive (CPDB)
N,*N*-diisopropylethyl-*N*-ethylamine	DIPNA		none		positive male only no TD_{50} calculated	Ames test negative, Kameswar et al 1979
N-nitrosodiphenylamine	NDPhA	3	167 (2 dose groups)		mouse, no positive	Ames test negative (CPDB)

Abbreviations: CPDB, carcinogenic potency database; eso, oesophagus; liv, liver; lun, lung; pyl, pylorus; ugi, upper gastrointestinal tract.

The accuracy of the TD_{50} strongly depends on study quality and size. Confidence intervals of TD_{50} values from studies with only one or two dose groups are higher as compared to studies with more dose groups. Studies with four or more dose groups are however exceptions. Only in the case of NDMA and NDEA are comparable studies in the same rat strain with the same number of dose groups available. The CPDB reports for each chemical species-specific harmonic means of the TD_{50} using the TD_{50} values of the most sensitive tumour target of each positive study. Most of the *N*-nitrosamine studies reported in the CPDB only have one or two dose groups, which are included in the reported harmonic mean TD_{50}. On the one hand, this approach gives weight to studies with few dose groups but on the other hand includes the variable sensitivity of different rodent strains. Therefore, neither study specific TD_{50} values nor harmonic mean TD_{50} values provide an accurate measure for real potency.

Using only the most extensive rat carcinogenicity study done for NDMA and NDEA [Peto et al. (1991)] the TD_{50} for liver tumours would be 42 µg/kg/day for NDMA and 50 µg/kg/day for NDEA resembling the alkylating potency in figure 2.4.2.1-1. The harmonic mean TD_{50} calculated using all rat studies in the CPDB database is 96 µg/kg/day for NDMA and 26.5 µg/kg/day for NDEA, respectively. In addition, other ranking systems like the EPA cancer slope factor also depend on data quality with respect to accuracy. The US Department of Agriculture (USDA, 2014) has calculated the oral slope factor for carcinogenic potency of some volatile and non-volatile *N*-nitrosamines in pork bacon (table 2.4.3-2). They ranked NDEA > NDMA > NPIP > NDBA > NPYR whereas non-volatile *N*-nitrosamines such as NPRO, NTCA and NTHZ where ranked as non-carcinogenic. The calculated values with 150 $(mg/kg/day)^{-1}$ for NDEA and 51 $(mg/kg/day)^{-1}$ for NDMA resembles the potency ranking by using the harmonic mean TD_{50} of the CPDB.

Table 2.4.3-2 Oral slope factors for carcinogenicity of selected *N*-nitrosamines (USDA/FSIS/OPHS/RAAS 2014)

Nitroso-	**Oral slope factor** $(mg/kg/day)^{-1}$	**Notes**
--dimethylamine (NDMA)	51	Source:EPAIRIS
--pyrrolidine(NPYR)	2.1	Source:EPAIRIS
--piperidine(NPIP)	9.4	Source:CaliforniaOEHHA
--diethylamine(NDEA)	150	Source:EPAIRIS
--dibutylamine(NDBA)	5.4	Source:EPAIRIS
--proline(NPRO)	-	IARC Group3 ("not classifiable as to its carcinogenicity to humans") referred to as non-carcinogenic by Lijinsky (1979).
--thiazolidine-4-carboxylicacid(NTCA)	-	Not listed by IARC. NTCA is "likely to be of little importance as far as its oncogenic properties are concerned." (Lin and Gruenwedel1990).
--thiazolidine(NTHZ)	-	Not listed by IARC. Referred to as non-carcinogenic by Lijinsky(1979).

The ranking of *N*-nitrosamines by their carcinogenic potency by using data of lifetime rodent carcinogenicity studies depends on the method used and varies. However, it seems to be relatively reliable by means of potency ranking when studies with multiple dose groups and a sufficient number of animals are available. It also seems that most non-volatile *N*-nitrosamines are of less concern with regard to carcinogenic potency (table 2.4.3-1).

The prime target organs for oral *N*-nitrosamine exposure in animals are liver (rat, monkey), oesophagus (rat), lung (rat), bladder (rat) and others with liver being mostly affected. Liver can also be expected as target organ with regard to its metabolic competence. These organs might also be considered as human relevant targets although sufficient data from humans are currently not available.

In the single exposure carcinogen database [Calabrese & Blain (1999)] 35 *N*-Nitrosamines are included that gave positive results with a single dose application. However, extrapolation of risks for low dose chronic exposure using those studies is problematic and no established methods are available.

In table 2.4.3-1 above, *N*-nitrosamines with a TD_{50} below 1.5 mg/kg/day (cohort of concern chemicals) are listed according to their TD_{50} as follows:

NMPEA>NDEA>NDMA>NMEA>NNK>NNN>NMOR>NMA>NDPA>NDBA>NPYR>MNNG>NMBA>NPIP

2.4.4. Use of *in vitro* mutagenicity data for carcinogenicity potency ranking of *N*-nitrosamines

Using *in vitro* mutagenicity data for potency ranking of *N*-nitrosamines appears problematic. Available Ames assay data are highly predictive for a qualitative prediction of carcinogenicity in rodent studies but problematic for estimation of carcinogenic potency [McCann et al. (1988), Bogen (1995)]. Major identified problems are summarised below:

- *N*-nitrosamines need to be activated metabolically and the artificial rat liver S9 mix used for simulation of metabolism in *in vitro* assays only provides limited metabolic competence,
- available Ames assays use different doses and also the strains used are often not the same. It is known that the quantitative results in Ames assays vary from laboratory to laboratory and also intra-laboratory variations are not negligible [Honma et al. (2019)],
- published Ames data are highly variable in quality,
- the four to five Salmonella and one E. Coli strains used in standard GLP Ames assays have different sensitivities and specificities for mutagenicity,
- all standard Ames strains are alkyl transferase proficient and effectively repair alkylated guanine caused by small alkyl-*N*-nitrosamines.

A publication by Wagner et al. (2012) used the alkyl-transferase deficient Salmonella strain YG7108, which is specifically sensitive for *N*-nitrosamine mutagenicity. The mutagenicity ranking for NDMA, NPIP, NMOR and NPYR was NDMA>NPIP>NMOR>NPYR, NDPHA was found not to be mutagenic. Ranking based on carcinogenic potency using the TD_{50} of rat carcinogenicity studies listed in table 2.4.3-1 is however NDMA>NMOR>NPYR>NPIP>NDPHA. The same authors also developed a single cell comet assay version with specifically optimized S9 mix to enhance sensitivity to *N*-nitrosamines in Chinese hamster ovary (CHO) cells. With this assay the ranking of mutagenicity in CHO cells was NDMA>NPIP>NMOR, NPYR was found not to be mutagenic.

This demonstrates that *in vitro* assays in bacteria like the Ames assay or tests in mammalian cells cannot be used as a quantitative surrogate for carcinogenic potency. They only might serve as a qualitative read out for a mutagenic potential.

2.4.4.1. Discussion on exposure to N-Nitrosamines and their carcinogenic potential

Based on available data, the CHMP noted that estimates on the exogenous exposure to *N*-nitrosamines vary. Assessments for food-mediated intake range between average 0.5-1.7 ng NDMA + NDEA/kg food (EFSA, 2017; processed meat only) and 1 ng NDMA/kg bw (Herrman et al, 2010b; ~50-100 ng NDMA/d per adult person from across food groups) to average 2.2 µg NDMA + 0.9 µg NDEA/kg food across food groups (Gushgari & Halden, 2017). Some processed food types such as fried foods are likely to contain >10 µg NDMA/kg food. Water intake is likely to represent an additional ~10-20 ng NDMA/L. Based on only NDMA measurements in urine samples, the NDMA exposure is likely to be >1-2 µg/d for subsets of the population (which may or may not represent a large endogenous NDMA production and generation.).

This does also not cover for all additional exposures that are very likely to have a far greater individual consumer and societal variability (e.g. smoking, contact exposure with rubber, personal care products etc.) and also not the total nitrosamine exposure (TNA) and non-nitrosamine exposure of relevance (e.g. all agents that generate the same DNA-lesions as pharmaceutical nitrosamines and are handled by the same cellular defence systems; see section 2.4.2.2.). Individual dietary habits are important, and it can for instance be assumed that exposure of individuals living on some types of diet (e.g. vegetarian), is significantly lower.

Exposure to endogenously produced *N*-nitrosamines raises an equal concern as exposure to exogenous *N*-nitrosamines. However, the exposure to endogenously produced *N*-nitrosamines is hardly quantifiable. No reliable data are currently available to draw firm conclusions on the impact of endogenously produced *N*-nitrosamines to overall *N*-nitrosamine exposure. In view of the uncertainties with respect to exposure levels, additional exposure should be limited as much as possible.

In conclusion, the carcinogenic potential is determined by multiple factors and depends on:

- The ability of the *N*-nitrosamine to be metabolically activated
- The metabolic competence and capacity of the tissue to form diazonium/carbenium ions
- The nature and stability of the diazonium/carbenium ion and the DNA-adducts formed
- The capacity, velocity and accuracy of the different cellular repair mechanisms responsible for the repair of the different DNA-adducts in tissues
- susceptibility (metabolic and proliferative) of the tissues exposed

It is therefore prudent to consider all *N*-nitrosamines containing a α-hydrogen that can be metabolically activated as potentially mutagenic and carcinogenic to humans, however with different potencies depending on nature of the functional group, specifics of metabolic activation and repair efficiency and capacity.

For NMEA, NNN, NMA, NDPA, NDBA, and NMBA, the TD_{50} has been calculated from studies with one or two dose groups only and therefore the reliability of the TD_{50} should be handled with caution. For NDMA and NDEA the respective harmonic mean TD_{50} was used in the CHMP Art 31 referral on sartans to calculate limits. Indeed, the number and extent of rat carcinogenicity studies were considered robust enough for calculating limits based on ICH M7 principles. For NDBA and NMBA the TD_{50} listed in the CPDB were not judged as robust enough because they were derived from one study with one dose group only. The study published for DIPNA was not listed in the CPDB and is assumed as not reliable enough to calculate any point of departure. CHMP has therefore recommended to use the AIs for NDMA or NDEA based on SAR considerations for NMBA and DIPNA, NDBA, respectively (EMA/351053/2019 rev 1).

It is recommended that for NMEA, NNN, NMA, NDPA and any other *N*-nitrosamine with no or non-reliable toxicity studies which may be identified in the future in pharmaceuticals, a similar approach based on SAR considerations using the AI of the closest related *N*-nitrosamine for which a robust AI could be calculated should be chosen.

In conclusion, CHMP considers that the primary attention with respect to risk by exposure of patients should be paid to the highly carcinogenic *N*-nitrosamines such as NMPEA, NDEA, NDMA, NMEA, NNK, NNN, NMOR, NMA, NDPA, NDBA, NPYR, MNNG, NMBA, NPIP and closely related molecules for which no data are available.

In addition, *in vitro* assays in bacteria like the Ames assay or tests in mammalian cells cannot be used as a quantitative surrogate for carcinogenic potency. They only might serve as a qualitative read out for a mutagenic potential.

2.4.5. Generic methodology to calculate excess risk for humans

As outlined in the CHMP Art 31 referral on sartans the generic and internationally agreed methodology for calculation of excess risk is the linear extrapolation using the TD_{50} calculated of animal cancer studies as the point of departure as described in ICH M7(R1). The linear extrapolation framework is a conservative/precautionary regulatory risk assessment approach for genotoxic carcinogens that stipulates that i) there are no toxicological thresholds (no 'dose' is safe, which is debated with regard to biological plausibility), ii) the exposure-outcome relationship must always be monotonic (also subject to debate but a precautionary and pragmatic premise), iii) any other biological variables must always be insignificant in relation to the exposure (which is very questionable as one reaches very low or high exposure levels) and iv) that the 'risk-per-unit-dose' is always constant (also debatable at very low or high exposures). These premises create a theoretical conservative framework where risk in relation to exposure is considered additive.

The methodologies to calculate the excess risk for humans and guiding decision making on immediate market actions in case of nitrosamine contaminations has followed so far the ICH M7(R1) approach for defining an AI. The AI in the context of ICH M7 is defined as an intake level that poses negligible cancer risk, or for serious/life-threatening indications where risk and benefit are appropriately balanced. The approach recommended in ICH M7(R1) is to use the TD_{50}, as the point of departure for the calculation of excess cancer risk and calculating the dose associated with a theoretical excess cancer risk of 1:100,000 as the AI from which the limit is calculated based on the maximum daily dose of the medicinal product. A well acknowledged and accepted source for TD_{50} values from cancer studies is the CPDB. The TD_{50} calculated in the CPDB provides a robust reference value as long as the studies are well described and are multiple dose group studies with a minimum of 3 dose groups and 50 animals per dose per sex. The extrapolation to the excess risk level for cancer is performed by linear back extrapolation to the dose theoretically causing a 1:100,000 risk by dividing the TD_{50} by 50,000 (50% or 0.5 x 100,000). For a person with a bodyweight of 50 kg the AI level is then calculated as AI = 50 x (TD50/50,000).

The ad-hoc expert group expressed preference for the $BMDL_{10}$ model to define usable point of departure metrics, stating however that in certain cases the TD_{50} model could be used. This approach is also harmonised across PROAST and BMDS software for quantal cancer bioassay data. The model averaging approach was also considered more suitable and easier and could overcome considerations with model selection. The BMDL is calculated as lower confidence limit (usually 90%) of a dose corresponding to a defined increase of a toxicological effect compared to controls. This increase is called benchmark response (BMR) or critical effect size (CES e.g. 5 or 10%). The corresponding dose is called Benchmark dose (BMD). Major limitations were identified by the Ad-hoc expert group with the

TD_{50} approach. The '1 in 100,000' risk of cancer is the chance of the rodent species developing cancer, not humans. There are no adjustment factors for extrapolation from animal to humans in this calculation. This has been accepted over the years, as the linear extrapolation provides such a low number, but this should be considered when interpreting this value and performing risk evaluations. BMDL methodologies have the advantages that confidence intervals are used, whole dose response is considered, and covariate analysis can be applied. The BMDL analysis is accessible (e.g. online PROAST) and makes no assumptions about linearity or threshold. The use of this method would ensure a harmonised approach with the method used by EFSA .

The use of $BMDL_{10}$ as point of departure instead of TD_{50} was extensively discussed by the CHMP in the Art 31 referral on sartans and the conclusion to use TD_{50} remains unchanged for the moment as there is still no internationally agreed methodology and the need for extensive multiple dose groups studies is much more essential for BMDL10 as for the TD_{50} approach. The ad-hoc meeting group acknowledged that a harmonized global approach is needed before the $BMDL_{10}$ approach can be used. The ad-hoc expert group also concluded that harmonization of methodology for using $BMDL_{10}$ would be possible with manageable effort (see section 3.1.). Another possibility would be using the cancer slope factor described by US EPA. However, the problems are basically the same as those identified for the $BMDL_{10}$ approach.

As a conclusion, based on all available data, the CHMP recommends using the TD_{50} approach. The insufficiencies and shortcomings of human data available are described below.

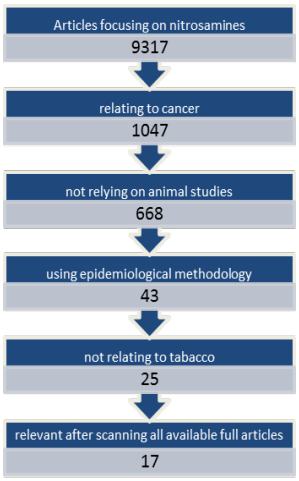
2.4.6. Literature review of epidemiological studies

Several *N*-nitrosamines are known to be potent mutagenic carcinogens in various animal species and therefore have been classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic in humans. In the following, we focus on *N*-nitrosamines which have been classified at least as probable carcinogens (Group 2A) by the IARC or are mentioned in EFSA (2017).

The search was done in PubMed and Embase databases for articles published in English mentioning "nitrosamine" or "nitroso" in the title. Any articles published until October 2019 are considered. For inclusion in our review, an article had to meet the following additional criteria:

- Mention of the term "nitrosamine", "nitroso" or any name or synonym of *N*-nitrosamine in the title
- At least one occurrence of "cancer", "malignant neoplasm" or "carcinoma" in the title or abstract
- Description of a "cohort" or "case-control" study design or the occurrence of the terms "population" or "epidemiology" in the title or abstract
- No reference to "animal" OR "rat" OR "mouse" OR "hamster" OR "rodent" OR "cat" OR "monkey"
- No mentioning of "smoking" OR "tobacco"

After removing duplicates, 33 full articles published in English language have been scanned if they address the association of individual cancer risk with the exposure to *N*-nitrosamines. 17 articles addressing the association of carcinogenesis with *N*-nitrosamines have been identified.



List of epidemiological studies identified

Authors	Title	comment
Catsburg et.al. (2014)	Dietary sources of *N*-nitroso compounds and bladder cancer risk: Findings from the Los Angeles bladder cancer study	cited
Chen et al. (2019)	Carcinogenic risk of *N*-Nitrosamines in Shanghai Drinking Water: Indications for the Use of Ozone Pre-treatment	not mentioned: no individual cancer risk has been evaluated
De Stefani et al. (2001)	Dietary nitrosamines, heterocyclic amines, and risk of gastric cancer: A case-control study in Uruguay	cited
De Stefani et al. (1996)	Meat consumption and risk of stomach cancer in Uruguay: A case-control study	not mentioned: study population overlapped with other study
Hidajat et al. (2019)	Lifetime exposure to rubber dusts, fumes and *N*-nitrosamines and cancer mortality in a cohort of British rubber workers with 49 years follow-up	cited
Jakszyn et al. (2006)	Endogenous versus exogenous exposure to *N*-nitroso compounds and gastric cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST) study	cited
Keszei, Goldbohm, et al. (2013)	Dietary *N*-nitroso compounds, endogenous nitrosation, and the risk of oesophageal and gastric cancer subtypes in the Netherlands Cohort Study	cited
Keszei, Schouten, et al. (2013)	Meat consumption and the risk of Barrett's oesophagus in a large Dutch cohort	not mentioned: study population overlapped with other study
Knekt et al. (1999)	Risk of colorectal and other gastro-intestinal cancers after exposure to nitrate, nitrite and *N*-nitroso compounds: A follow-up study	cited
Larsson, Bergkvist, and Wolk (2006)	Processed meat consumption, dietary nitrosamines and stomach cancer risk in a cohort of Swedish women	cited
La Vecchia et al. (1995)	Nitrosamine intake and gastric cancer risk	cited

Palli, Russo, and Decarli (2001)	Dietary and familial determinants of 10-year survival among patients with gastric carcinoma	cited
Pobel et al. (1995)	Nitrosamine, nitrate and nitrite in relation to gastric cancer: A case- control study in Marseille, France	cited
Pottegård, A et al. (2018)	Use of *N*-nitrosodimethylamine (NDMA) contaminated valsartan products and risk of cancer: Danish nationwide cohort study	cited
Song, Wu, and Guan (2015)	Dietary nitrates, nitrites, and nitrosamines intake and the risk of gastric cancer: A meta-analysis	cited
Zheng et al. (2018)	Dietary *N*-nitroso compounds and risk of pancreatic cancer: Results from a large case-control study	cited
Zhu et al. (2014)	Dietary *N*-nitroso compounds and risk of colorectal cancer: A case-control study in Newfoundland and Labrador and Ontario, Canada	cited

2.4.6.1. Epidemiological studies on the association of potent mutagenic N-nitrosamines in medicinal products with the risk of cancer

Currently, only one epidemiological study on the effect of *N*-nitrosamines in medicinal products on the risk of cancer has been published ` This study, based on Danish nationwide health registries, did not detect an increase in overall cancer risk or in any of the examined cancer types in patients that have been exposed to NDMA contaminated Valsartan. However, the study sample size is limited to 5,150 patients with overall 302 cases of cancer, and the average follow-up time is limited to 4.6 years. The limited sample size prevented the study from detecting possible smaller effects. To detect small short-term effects, a larger study population is necessary. Furthermore, longer follow-up times are needed to assess an excess lifetime risk.

CHMP was also informed about unpublished preliminary data from a cohort study on 780,000 patients using valsartan with over 400,000 potentially exposed to NDMA by contaminated valsartan batches. The preliminary results suggest an increase in liver cancer diagnosis of 16% in potentially NDMA exposed patients, however a meaningful evaluation was not possible due to the limited information available.

2.4.6.2. Epidemiological studies on the association of potent mutagenic N-nitrosamines from dietary sources with the risk of cancer

The effect of *N*-nitrosamines on the risk of cancer has been the subject of epidemiological research in the last few decades. The association of *N*-nitrosamines on subsequent carcinogenesis has been studied mostly via monitoring of dietary intake with few exceptions studying for example exposure by rubber dust (e.g. Hidajat et al. (2019)). In the following, studies on the association of *N*-nitrosamines and cancer risk in the context of dietary *N*-nitrosamine intake are summarised.

The associations of the risk of several cancer types with dietary intake of *N*-nitrosamines have been the subject of epidemiological studies. By recording dietary habits with questionnaires and classifying all nutritional components, the studies aimed to investigate associations of *N*-nitrosamines with the risk of cancer. Dietary habits were either recorded prospectively or, in the case of case control designs, retrospectively. In the latter design, cases refer to patients with incident cancer and controls are collected from the base population. Regarding the influence of *N*-nitrosamines, these studies mostly focussed on the association of cancer risk after the dietary intake of NDMA.

Keszei et al. (2012) found an association of oesophageal cancer with dietary intake of NDMA (HR for NDMA 1.15; 95% CI: 1.05 - 1.25) in a Dutch cohort of 120,852 participants. The participants were aged 55–69, recruited in 1986 and followed for 16.3 years.

The association of pancreatic cancer after dietary intake of NDEA and NDMA (OR for NDEA 2.28, 95% CI: 1.71 - 3.04, and OR for NDMA 1.93, 95% CI: 1.42 - 2.61) has been studied by Zheng et al. (2018) in a US-based case control study comprising 957 cases and 938 controls from genetically unrelated family members which were recruited between 2002 and 2009.

Catsburg et al. (2014) investigated the association of bladder cancer after dietary intake of NDMA (OR for *N*-nitrosamines 1.03, 95% CI: 0.78 - 1.36) in a case control study with 3,246 participants from the US which were recruited between 1987 and 1996.

Two studies focused on colorectal cancer. A cohort study by Knekt et al. (1999) based on 9,985 Finnish participants which were followed for 24 years found a RR after dietary intake of NDMA of 2.12 (95% CI: 1.04 - 4.33). A case control study by Zhu et al. (2014) with 4,241 Canadian participants aged between 20 and 74 years found a RR after dietary intake of NDMA of 1.42 (95% CI: 1.03 - 1.96).

Rectal cancer has been studied by Loh et al. (2011) who found a HR of 1.46 (95% CI: 1.16, 1.84) after dietary intake of NDMA in a UK based cohort of 23,363 participants who were recruited in 1993–1997 and followed for 11.4 years. A comparable RR of 1.61 (95% CI: 1.11 - 2.35) was found for rectal cancer after dietary intake of NDMA by Zhu et al. (2014) who employed a case control design with 4,241 Canadian participants.

The main body of research based on monitoring of dietary intake of *N*-nitrosamines is focused on gastric cancer. A meta-analysis by Song, Wu, and Guan (2015) comprises all recent studies concerning stomach cancer after dietary intake of NDMA and found an elevated relative risk of 1.34 (95% CI: 1.02 - 1.76). Included were cohort studies (Knekt et al. (1999), Jakszyn et al. (2006), Keszei et al. (2012)), of which only Larsson, Bergkvist, and Wolk (2006) showed a significant association. The included case-control studies [De Stefani et al. (1998); La Vecchia et al. (1995); Palli, Russo, and Decarli (2001); Pobel et al. (1995)], however, showed very heterogeneous results with exceptionally high effect sizes for the moderately sized studies of Pobel et al. (1995) and De Stefani et al. (1998). A subset of studies with at least 2000 participants, omitting De Stefani et al. 1998; Palli, Russo et al 2001; Pobel et al. (1995) showed no significant heterogeneity and no significant relative risk of gastric cancer with an RR of 1.12 (95% CI: 0.97 - 1.39) after dietary intake of NDMA. The heterogeneity between included studies in the meta-analysis by Song, Wu, and Guan (2015) emphasizes the challenge of attributing cancer risk to single ingredients in nutritional epidemiology [Schoenfeld and Ioannidis (2013), Egger, et al (1998)]. Even though those studies adjusted at least for the main confounders such as age, sex and total energy intake, residual confounding cannot be ruled out. For example, the intake of other carcinogens such as polyaromatic hydrocarbons has not been taken into account. Additionally, patient specific factors such as the intake of vitamins and ethanol, or the CYP2E1 metabolism might further modify effects [Toshihiko et al. (2005); Peto et al. (1991b); Stephens et al. (1994); Trafalis et al. (2010)].

Although, these studies suggest an association of dietary intake of NDMA with some types of cancer, definite conclusion cannot be drawn at this stage and further confirmation is required, as reported associations, effect sizes and especially dose-response relations should be interpreted with great caution. Further research based on large sample sizes and better control for confounding is needed.

2.5. Methodology for defining limits for N-nitrosamines

The methodology guiding decision making on immediate market actions in case of nitrosamine contaminations has so far followed the ICH M7(R1) approach. The generally accepted approach recommended for compound specific limits in ICH M7(R1) is to use the TD_{50} as the point of departure for the calculation of excess cancer risk and to calculate the AI / the dose associated with a theoretical excess cancer risk of 1:100,000 to define the limit. A well acknowledged and accepted source for TD_{50} values from cancer studies is the CPDB.

The extrapolation to the excess risk level for cancer is performed by linear back extrapolation to the dose theoretically causing a 1:100,000 risk by dividing the TD_{50} by 50,000 (50% or 0.5 x 100,000). For a person with a bodyweight of 50 kg, the AI level is then calculated as AI = 50 x (TD_{50}/50,000).

This approach has been extensively discussed for NDMA and NDEA already in the CHMP Art 31 referral on sartans.

In cases where robust TD_{50} values as point of departure for excess cancer risk calculations are not available, the SWP recommends using a class specific threshold of theoretical concern (TTC) of 18 ng/d as default option with the possibility to justify a higher limit based on the structure-activity-relationship (SAR) approach described in the ICH M7(R1). Of note, the class specific AI of 18 ng/d for nitrosamines recommended by SWP as outlined below was determined using a novel methodology not widely used at this stage.

The SAR approach has already been used for setting limits for NDBA, NMBA, DIPNA and EIPNA following the sartan referral. When using the SAR approach, the TD_{50} of the structurally closest related *N*-nitrosamine for which robust data are available to calculate a reliable TD_{50} should be applied to the nitrosamine in question, and as the methodology is established, the use of SAR can be considered an alternative approach to the TTC above.

2.5.1. Limits for individual nitrosamines, for multiple *N*-nitrosamines and less than lifetime (LTL) approach

The *N*-nitrosamines listed in table 2.4.3.1 with a TD_{50} below 1.5 mg/kg/day belong to the cohort of concern as defined in ICH M7(R1) and are:

NMPEA, NDEA, NDMA, NMEA, NNK, NNN, NMOR, NMA, NDPA, NDBA, NPYR, MNNG, NMBA, NPIP.

It is recommended that the primary attention with respect to risk for patients should be on these highly carcinogenic *N*-nitrosamines and closely related molecules for which no sufficient data are available. Limits for individual N-nitrosamines should be set using the compound-specific ICH M7(R1) approach considering a lifetime daily exposure.

For individual nitrosamines with sufficient animal carcinogenicity data, limits can be calculated using the risk-based approach and methodology described in ICH M7(R1) with TD_{50} as the PoD. This approach is already described in section 2.4.5.

Using the risk-based approach described in ICH M7(R1), it may be possible to accept the presence of multiple, highly carcinogenic *N*-nitrosamines within one medicinal product as long as the excess lifetime risk of the total nitrosamine content is kept below a risk of 1:100,000. As discussed above, the environmental background exposure levels to *N*-nitrosamines are difficult to estimate.

Amounts of NDMA, NDEA, NPIP, and NPYR in medicinal products at their AIs would, in a 'worst case' assumption, theoretically result in a patient exposure of up to 2.351 µg/day (96 ng +26 ng + 799 ng +

1430 ng) to those cohort of concern *N*-nitrosamines. As described in section 2.2 there are already cases where up to three different *N*-nitrosamines have been found in one pharmaceutical indicating that exposure to multiple *N*-nitrosamine via pharmaceuticals, although rare, may occur. In addition, patients may take more than one *N*-nitrosamine-containing medicine, which would further increase their daily *N*-nitrosamine intake. As outlined in paragraph 2.4, the risk is considered additive. Environmental exposure varies based on lifestyle. Patients with a "healthy" lifestyle may potentially have lower than average exposure to volatile *N*-nitrosamines. When taking pharmaceuticals containing *N*-nitrosamines controlled at limits calculated based on ICH M7(R1) approach, these patients may be exposed to higher exposures compared to the average population. The ad-hoc expert group suggested an approach to not exceed the acceptable risk level in case of more than one *N*-nitrosamine being present in a finished product. In case more than one *N*-nitrosamine occurs in manufacture it may be acceptable to limit the sum of *N*-nitrosamines to the limit of the most potent one found. Acceptability of such a concept may depend on the capability of effective control (see section 3.1). The SWP suggested a different approach to reach the goal of not exceeding the anticipated risk level of 1:100,000. When multiple *N*-nitrosamines are present in a single product and the total risk does not exceed the 10^{-5} tumour risk level, the proposed limits are considered acceptable. The SWP recommends setting a specific limit for each nitrosamine with the sum of all detected nitrosamines not exceeding the total risk level of 1 in 100,000 (see section 3.5.2), which is considered an acceptable alternative approach, as it also ensures an excess lifetime risk below 1:100.000.

The general paradigm of linearity of life-time dose and increase of cancer risk in the low dose range for mutagenic carcinogens is derived from rodent life-time bioassays. For NDMA and NDEA the studies used and cited are the studies by Peto (1991). There, linearity for the dose response in the low dose range was considered demonstrated for liver (with regard to the lowest dose tested in the experiment and reaching a sensitivity of ~5 cancer cases per 100 animals) but not for oesophageal neoplasms. The mathematical explanation the authors offer is that "*the appropriate low-dose dose-response relationships predict that if there is an appreciable background of "spontaneous" neoplasms of whatever type is of interest, then the dose-response relationship is likely to be simply linear at dose rates so low that the induced risk does not greatly exceed the background risk. No general predictions can be made of the shape of the dose-response relationship at low doses if the spontaneous rate is immeasurably small, as for oesophageal neoplasms in the present study. Consequently, it is unsurprising to note that at low doses the onset rate of oesophageal cancer appears not to be simply proportional to dose. For liver cancer, however, the background rates are about 8%, which is appreciable*". Furthermore, the relationship of dose and time to tumour onset is not linear and seems different between tumour entities.

In this respect it also needs to be noted that the background rates for liver tumours in humans is far lower than that for rats and significantly different between different age groups. According to the Ad-hoc expert group there also seem to be significant doubts among experts on whether this linearity for some tumours in rodents is transferable to humans. This is also stated by Peto et al. (1991a) in their publication: "*This provides us with what is probably a reasonably reliable estimate (despite the practical impossibility of direct confirmation) of the effects of ppb nitrosamine concentrations on rats under these experimental circumstances, but it does not provide reliable information as the effects of ppb nitrosamine concentrations on humans, and it would be a serious distortion of these experimental results to suggest otherwise.*"

Therefore, although according to ICH M7(R1) a simple linear extrapolation of accumulated lifetime acceptable dose is made with some additional adjustment factor, it is currently not recommended to generally accept the 'less-than-life' time concept in ICH M7(R1) for setting higher N-nitrosamine limits in drug products taken less than lifetime. Accepting the LLT approach could lead to high acute nitrosamine intake, especially with medicines given at high doses and for a short period of time.

When the limit calculated based on ICH M7(R1) principles for a lifetime exposure is exceeded, a thorough benefit/risk assessment performed by the authorities is needed. Such assessment will need to take into consideration the criticality of the medicine (medical need, treatment alternatives, patient risk related to drug shortage etc.) in order to decide on a case by case whether higher limits for *N*-nitrosamines can be accepted temporarily together with further measures to lower the contamination and potentially control in the long term below the limit calculated based on compound-specific ICH M7 principles for a lifetime exposure.

The LTL concept in ICH M7(R1) may be used as a guide for setting temporarily limits in such cases to prevent drug shortages where it would raise a public health issue.

ICH M7(R1) may be interpreted in a way that the LTL approach is generally acceptable for all mutagenic carcinogens, including the cohort of concern mutagens. CHMP supports a re-evaluation and clarification whether the LTL concept is relevant for the cohort of concern mutagens. This should be performed as part of interactions with ICH, also taking into account the criticism regarding the LTL approach for cohort of concern mutagens expressed by the Ad-hoc expert group and the CHMP.

It should also be noted that some experts of the ad-hoc expert group supported the concept of biological mechanism supporting a practical threshold even for mutagenic carcinogens. Such concepts are being investigated, and the body of knowledge is increasing. These concepts are based on the dose response of key cellular biochemical reactions such as e.g. DNA repair by MGMT and others important in chemical carcinogenesis of small alkylating N-nitrosamines. However, these concepts suggest that risk only starts to increase at exposures where the capacity of defence mechanisms such as DNA-repair is exceeded, but, above this level, may increase overproportionally. Thus, these concepts are not compatible with the assumption of a linear dose-response-relationship underlying the LTL approach, and would lead to particular concerns with nitrosamine doses exceeding individual repair capacities. Although such threshold concepts may appear interesting, defining a threshold is considered extremely difficult due to many unknown factors and interindividual variability, e.g.in terms of nitrosamine intake from other sources and differences in individual repair capacities. Overall, these concepts are not supported by robust scientific evidence at this stage and therefore should not be used as basis for regulatory decisions

2.5.2. Limits for nitrosamines without sufficient substance specific data

The TD_{50} calculated in the CPDB provides a robust reference value as long as the studies are well described and are multiple dose group studies with a minimum of 3 dose groups and 50 animals per dose per sex. Studies not meeting these requirements need to be assessed for robustness on a case by case basis, e.g. a higher number of dose groups may compensate for fewer animals per dose group. This approach was used for NDBA, NMBA and DIPNA for which the harmonic mean TD_{50} values listed in the CPDB were not judged as robust enough because they were derived from one study with one dose group only. The study published for DIPNA was not listed in the CPDB and is assumed not to be reliable enough to calculate any point of departure. CHMP has therefore recommended setting limits for NMBA and DIPNA, NDBA based on SAR considerations for NDMA or NDEA respectively (EMA/351053/2019 rev 1).

Therefore, for nitrosamines with insufficient data (e.g. NMEA, NNN, NMA, NDPA, MeNP), a similar approach based on SAR considerations can be used. The TD_{50} of the structurally closest related N-nitrosamine for which robust data are available to calculate a reliable TD_{50} should be applied.

SWP recommends using a class specific TD_{50} as default option with the possibility to justify higher limits based on SAR considerations. This approach sets the same TD_{50} for all nitrosamines where sufficient data to calculate a substance TD_{50} do not exist. Setting of a class specific TD_{50} is discussed in

the SWP response section. SWP derived a class specific TD_{50} by using the TD_{50} data of all nitrosamines listed in Lhasa carcinogenicity potency database (LCDB) and use of the lower 5th percentile. This TD_{50} is then used to calculate the excess risk which would in theory not exceeded with 95% probability by any nitrosamine. The extrapolation to the excess risk level for cancer is then done by linear back extrapolation to the dose theoretically causing a 1:100,000 risk by dividing the TD_{50} by 50.000 (50% or 0.5 x 100,000). This results in a class specific TTC of 18 ng/d ensuring with 95 % probability for any nitrosamine that a theoretical excess cancer risk of 1 in 100,000 will not be exceeded. The CHMP agreed that alternatively to the above TTC an SAR approach can be used as discussed in 2.5 above.

2.5.3. Other risk management approaches

Nitrosamines were first detected in sartans containing a tetrazole ring. The API manufacturing process was identified as the main source of those nitrosamine impurities and nitrosamine formation could be prevented by changes to the manufacturing processes. Therefore, the most stringent approach, an "avoidance" strategy, was chosen as outcome of the sartan referral.

However, since the sartan referral, other medicinal products have been found to contain nitrosamine impurities originating from various root causes and, in some cases, still unknown root causes. The variety of the root causes and the possibility of multiple sources of contamination renders the outcome of the sartan referral not generalisable to all cases where nitrosamines are identified. In this context it should be mentioned that the nitrosamine impurities found in sartans, especially in valsartan from one API manufacturer, were considerably higher than those found in most other medicinal products later on. Subsequent to the sartan referral, low level presence of nitrosamines in some finished product batches from one MAH were attributed to the primary packaging. Setting limits in the API would not have addressed this particular root cause.

The knowledge acquired since the sartan referrals confirmed that the root causes can be numerous, concomitant, at any stage of the production or storage of the medicinal product and cannot always be characterised. Therefore, a general "avoidance" strategy is not considered a realistic and feasible goal and would foreseeably lead to shortage problems of critical medicinal products. Therefore, CHMP was concerned with striking a viable balance in the best interest of patients, taking into account drug safety and ensuring availability of drugs that are important to human health.

The as-low-as-reasonably-possible (ALARP) approach is applied in fields outside of medicinal products and is mainly understood as reaching the lowest possible level that is feasible in terms of manufacturing capability for the majority of the operators (e.g. in the rubber industry), and at the same time is safe enough for consumers.

Whilst acknowledging the suitability of this approach in the fields where it is applied, the CHMP identified issues specific to medicinal products that do not render this approach suitable for the pharmaceuticals. There is no consistent and transparent way for regulators to determine when a pharmaceutical company has reduced the nitrosamine levels to an as far as reasonably possible level for a given medical product. It is also unclear how this can be handled in a regulatory manner so that MAHs and their medicinal products are treated in a consistent way, independent of the type of procedure (centralised, decentralised and national) and the relevant assessing competent authorities. It can be foreseen that the ALARP approach would result in inconsistent setting of specifications for the same products across the European Union due to subjectivity of the assessment related to this concept. The ALARP concept is based on the criterion of 'reasonably practicable' which is understood as the cost involved in reducing the risk further would be grossly disproportionate to the benefit gained.

Whilst this approach is adapted to certain sectors, this is not advisable for the purpose of defining a limit for medicinal products, as establishing a ratio between industrial and public health factors for

manufacturing of medicinal products would further substantially vary between operators, leading to different limits for each medicinal product and for the same product across the EU that would not be acceptable from a public health point of view. As the numerator of this ratio differs for each product of each MAH, harmonisation of the limits defined based on this approach cannot be achieved. Lastly, this ratio depends on economic factors that by nature are very volatile and may therefore lead to frequent changes in limits, which would create a substantial unpredictability in the limits fixed.

Furthermore, no clear benefit is expected as the difference in theoretical excess cancer risk between the ALARA/ALARP vs. the approach defined in sections 2.5.1 and 2.5.2 is considered to be negligible, owing to the little numerical difference between the limits set by these two approaches.

For all these reasons, CHMP decided to recommend the approach defined in sections 2.5.1 and 2.5.2 for setting limits for nitrosamines, i.e. based on toxicological considerations as outlined in the internationally agreed ICH Guideline M7(R1) on mutagenic impurities. This approach is considered sufficiently conservative from the safety point of view. Of note, the ICH M7(R1) allows for additional risk management approaches where appropriate.

Assessment of human risk stemming from potent nitrosamines such as NDMA and NDEA (classified as probable human carcinogens) is very difficult because exposure levels are far below those than can be experimentally tested and verified in animal studies and available field studies (e.g. epidemiological studies) are inconclusive. There is no empirical way to determine the actual risk from nitrosamine impurities in pharmaceuticals in relation to the background nitrosamine exposure levels (neither for specific nitrosamines such as NDMA or the total sum of nitrosamines) and to quantify the difference in risk when using ALARP approach vs. approach defined in sections 2.5.1 and 2.5.2. There has been some support for using ALARP approach, including from QWP, SWP, ad-hoc expert group, in this referral procedure based on a precautionary principle approach to reduce as much as possible the amount of nitrosamines in medicines.

Having considered that applying the ALARP approach for manufacturing of medicinal products for setting limits may in particular not lead to sufficiently clear and predictable limits, and the absence of clear benefit of this approach in terms of risk reduction, the CHMP concluded that this approach is not adequate for setting limits for N-nitrosamines in medicinal products and that the approach *as per* ICH M7(R1)for CoC substances is sufficiently conservative to ensure patient safety and allows the setting of clear and predictable limits.

2.5.4. Comparison of different options for setting limits

The advantages and disadvantages of the various options for setting limits of N-nitrosamines as considered by CHMP are summarized in the table 2.5.4-1 below.

Table 2.5.4-1 Advantages and disadvantages of different regulatory approaches for setting nitrosamine limits:

Regulatory Approach	Pros	Cons
Limit based on analytical capability	• Leads to lowest technically possible limit (and usually lower than those based on ICH M7 (R1) approach, depending on the maximum daily dose) • Enables classification of nitrosamines as non-acceptable impurities to be avoided unless justified sufficiently, similar to the ICH Q3C approach. In addition, future classification of other cohort of concern compounds as acceptable impurities in medicinal products is prevented.	• Does not take into account toxicological data, only based on analytical capability • Analytical limits may be different for different nitrosamines • Leads to different actual exposures depending on the daily dose of the medicinal product. • For high daily doses, the technical limit could be above the limit based on ICH M7(R1) approach. • If strictly applied, it may lead to unnecessary drug shortage creating public health issues for critical products whereas the difference in theoretical excess cancer risk *versus* ICH M7 (R1) approach is negligible. • Limit may not be sufficiently predictable, as state-of-the art methodologies evolve and lower analytical limits for a given API/product may be achieved.
Limit based on ICH M7(R1) methodology	Based on toxicology data with limits corresponding to the same level of theoretical risk. • Based on a lifetime of exposure estimate (70 years). • conservative linear risk extrapolation and potency handled by compound specific thresholds based on carcinogenicity data & adequate extrapolation to humans • Allows to take into account the actual exposure (daily amount	• Limits based on ICH M7 (R1) are usually higher than analytical limits (unless a medicinal product has a large maximum daily dose).

	and duration) • Allows consistent and harmonized implementation and follow-up for all medicinal products • Ensures consistency as toxicological risks are always assessed for all active substances/ medicinal products (i.e. the same limit for products containing the same active substance, depending only on the maximum daily dose). • ICH M7 methodology has already been developed with risk through polypharmacy integrated, resulting in very conservative safety margins	
ALARP	• Allows to take all elements (technical feasibility, toxicological risk calculations etc.) for a specific operator (e.g. MAH/API manufacturer) into consideration for determining realistically achievable nitrosamine reductions. • Provides the lowest case specific limit reasonably achievable for every single finished product	• Subjective assessment, as effort to reduce nitrosamines as 'reasonably practicable' cannot be measured in an objective, comparable way. • Based on the criterion of 'reasonably practicable' which is understood as the cost involved in reducing the risk further would be grossly disproportionate to the benefit gained. This is not adapted for the purpose of defining a limit in medicinal products which should be preferably based on toxicological data rather than a ratio between industrial and public health factors. • Would lead to setting of different limits between manufacturers that would lead to different risks that cannot be justified based on toxicological data. • Elements for a specific operator (in terms of effort needed to

		reduce nitrosamines) are susceptible to frequent changes. • Implementation of a harmonized approach across the EU not feasible as based on subjective criteria. • Time- and resource consuming with difference in theoretical excess cancer risk *versus* ICH M7 approach being negligible
Factor to address Polypharmacy	• Additional factor could ensure excess cancer risk to remain < 1:100.000) in case of exposure to multiple products contaminated with nitrosamines • Lowers the risk for patients potentially more vulnerable	• The overall probability of being exposed to two or more nitrosamine-contaminated products at the same time is not possible to estimate, as well as the average level of contamination • Additional factor would be arbitrary and not evidence-based and scientifically justified
Less than lifetime (LTL)	• Keeps excess lifetime cancer risk 1:100.000 proportionate to the treatment duration • Consistent with ICH M7 (R1) as it is based on the theory underlying the linear extrapolation calculations used for AI estimates.	• In products especially with short treatment durations and high daily doses, LTL-adjusted limits may be many-fold higher than limits calculated for lifetime exposure and may acutely overwhelm the repair capacity of human DNA. • Relies on strict linearity of the dose response even in the higher dose ranges, which is unproven.

Taking into account all arguments above , CHMP agreed that implementation of limits for N-nitrosamines in medicinal products based on ICH M7 principles for substances of the "cohort of concern" and calculated considering a lifetime daily exposure would provide the most appropriate and science-based approach that would also be consistent with the general handling of mutagenic impurities according to ICH M7(R1) and would also allow for the protection of public health. Indeed, this approach is considered sufficiently conservative to protect public health, especially with the additional safe-guard of not allowing a less-than-lifetime approach that could theoretically lead to high acute exposure to nitrosamines from medicinal products used for only short periods of time.

As explained above, this is considered a conservative and robust risk-based approach relying on toxicological data. It is based on a lifetime estimate of the theoretical excess risk for patients and considers substance specific data on carcinogenic potency as far as such data are available in contrast to an analytical limit which is based on technical capability of analytics only. This approach also allows a consistent implementation and follow-up for all medicinal products regardless of advances in technical development of analytical methods. It would be in line with the general approach for mutagenic impurities described in ICH M7(R1) and ensures consistent and risk-based regulation of all pharmaceutical products by controlling *N*-nitrosamines to the same limit in all products.

CHMP therefore recommends setting limits for N-nitrosamines in human medicinal products based on ICH M7 principles for substances of the "cohort of concern" and calculated considering a lifetime daily exposure.

2.6. Consideration for further studies

2.6.1. Consideration for further non-clinical/clinical studies

Non-clinical studies are only meaningful when adding to the weight of evidence for quantitative risk assessment. Further lifetime cancer bioassays in rodents should be avoided due to the long time needed (3 years including evaluation) and high costs. In addition, such studies probably would not add any further scientific value due to the high amount of already available carcinogenicity studies for many *N*-nitrosamines. Studies measuring mutations *in vivo* such as the transgenic rodent bioassays (TGR) to determine robust points of departure for mutations as the most important pre-cancerous insult are considered the best choice. However, these studies are relatively insensitive to low dose exposure and extensive studies would be needed to enable a robust calculation of benchmark doses as point of departure for risk calculation. Studies would also be needed for all *N*-nitrosamines considered relevant. Whether this is ethically acceptable especially with regard to the 3R principle is questionable.

Clinical studies under real life conditions to evaluate the risk of endogenous formation of *N*-nitrosamines from authorised compounds with a suspected potential to form *N*-nitrosamines endogenously may be helpful to confirm or refute such risk.

2.6.2. Consideration for further epidemiological studies

Although many epidemiological studies already investigated the association between *N*-nitrosamines and cancer, studies on the association of *N*-nitrosamine-contaminated medicines and cancer are rare. Due to increasing reporting of *N*-nitrosamine contamination in medicinal products and the fact that *N*-nitrosamines are potent mutagenic carcinogens, further studies are required to assess the risk of cancer after exposure to potentially contaminated medicinal products.

N-nitrosamines exposure is suspected to increase the risk of several different cancers. Therefore, a composite endpoint may be considered as the primary endpoint in further studies (e.g. any cancer or a combination of cancer types of special interest). However, individual cancer types should always be examined as secondary endpoints. As the liver represents the main target organ of *N*-nitrosamines toxicity in animals, future studies should in any case investigate the risk of liver cancer more closely. Besides liver cancer, cancers of the upper gastrointestinal tract, the lung and the bladder are also of interest due to biological plausibility and experiences from previous animal and nutrition studies (refer to section 2.4). If possible, outcome identification should be verified by additional procedures and/or medical treatment.

The suitability of the study population should be considered carefully. Study designs should focus on patient groups that differ – as much as possible - only in the contamination status of the examined medicinal product. As cancer occurrence is rare among younger adults, inclusion of mainly young adults may lead to studies that require an unrealistic sample size, especially in the case of uncommon cancers. For example, a cohort study would have to observe about 250,000 patient years per group to exclude a 2-fold increased risk of liver cancer in adult patients (20 to 85+ years of age) with a power of 80% (significance level 5%) if the two groups to be compared are equally represented. However, raising the lower age limit to 40 leads to a reduction in the number of patient years required by about 70,000 per group. The total sample size would even increase if the relation between both group sizes is unbalanced. This example is based on incidence data from the German Centre for Cancer Registry Data (ZfKD) for the calendar year 2014[11]. In the case of rare events, case control studies might be a suitable alternative to cohort studies.

Apart from the patient population to be included, a study should cover a sufficiently long observation time to enable the assessment of both, early and late cancer risks. Different lag-times should be considered in the analyses, as it is unlikely that very recent *N*-nitrosamines exposure affects an individual's risk of receiving a cancer diagnosis. Statements about required study periods are difficult, but a study period of at least 10 years is recommended in order to adequately address the risk of cancer. As long-term follow-up may increase imbalances between treated and untreated patients in cohort studies, risk factors for cancer that change over time – such as age – should be considered as time-dependent variables in the analyses to reduce time-varying confounding.

The observation of large populations over a long time period is challenging and may lead to biased results in case of excessive loss to follow-up. Therefore, studies using routinely collected data over time including larger sample sizes are expected to be more promising than studies with primary data collection. Appropriate data sources may be, for example, nationwide registries or large healthcare databases. Nevertheless, it should be considered that routinely collected data were not designed to answer the study question at hand. Depending on the initial purpose of the data sources, information on relevant variables may be missing. For example, administrative claims data are routinely collected for billing purposes and drugs that are not reimbursed by insurance companies or purchased without a prescription (over the counter, OTC) are rarely covered in administrative databases. To assess the risk of cancer associated with the use of OTC drugs is therefore challenging or even impossible. Furthermore, information on cancer risk factors may be limited or lacking in healthcare databases, such as nutrition, smoking, radiation exposure, alcohol consumption, obesity, the socioeconomic status or family history of cancer. One of the most serious events experienced by cancer survivors is cancer recurrence or the diagnosis of a second cancer. If possible, patients with records of previous cancer should be excluded or stratified analyses should be considered. However, information on previous cancer may be missing in several data sources. Especially, if the primary cancer has occurred long ago, such as childhood cancer. Despite the lack of information on individual cancer risk factors in healthcare databases, it should be considered that most cancer risk factors are unlikely to be associated with the contamination status of the drug of interest. Therefore, the chance for confounding is considered to be low in studies observing a uniform patient population, such as valsartan users only. However, if exposure to different types of drugs is compared in a study, confounding is more likely to be present, as the respective target populations may differ in their cancer risk factors.

The definition of exposure displays a major problem in the conduct of future studies, especially if the onset, the extent or the cause of the contamination is unclear or cannot be precisely determined. Non-exposed subjects may be categorized as exposed and/or patients treated with contaminated

[11] taken from: https://www.krebsdaten.de/Krebs/EN/Database/databasequery_step1_node.html, accessed on 18/11/2019)

medications may be categorized as non-exposed. Misclassification of exposure may bias the results leading to an underestimation or overestimation of the risk. Therefore, the use of dispensing data is preferred to the use of prescription data, as in the latter case it is uncertain whether the prescription was also filled. However, it should be considered that even if it is known that a prescription has been filled, information is missing whether the patient has actually taken the drug or not. In addition, the impact of individual contaminated batches is hardly observable, as information on dispensed batch numbers is not routinely collected in most healthcare databases.

If possible, stratified analyses should be performed according to predefined categories of cumulative exposure to examine a potential dose-response relationship. The cumulative amount of applied contaminated medication and the treatment duration should be considered to check the impact of both long-term exposure and treatment intensity. However, in case of slightly increased risks and/or low precision of the point estimates, or settings where only minimum thresholds need to be reached to observe risks, a dose-response relationship may not be observable. In order to get an impression of the clinical relevance of the observed risk estimates, the population attributable risk (i.e. an estimate of the excess risk) should be calculated to determine the proportion of cancer cases attributable to *N*-nitrosamines contamination. Finally, sensitivity analyses are recommended to check the robustness of the primary findings.

Further epidemiological studies to assess the association between the intake of potentially *N*-nitrosamines-contaminated drugs and risk of cancer are desirable, but their conduct is challenging. This is mainly due to the difficulty of reliably determining the exposure. Irrespective of the study design applied, potential data sources should contain sufficient patient numbers, should cover a sufficiently long observation time and should contain the required variables to answer the study question, i.e. information on exposure, outcome and important covariates. Prospective study designs using primary data collection are obviously not feasible for the examination of the association of *N*-nitrosamines-contaminated drugs and cancer risk. Therefore, using nationwide registries or large healthcare database might be the most promising approach for the conduct of further studies, despite the mentioned limitations. If a single data source does not contain all the necessary information, data linkage to other data sources that may contain the missing information should be checked prior to study initiation. Furthermore, the possibility of a meta-analytical approach may be considered in case of insufficient patient numbers in a given data source.

2.7. Relevance for the CHMP opinion on medicinal products containing sartans with a tetrazole ring

As a final outcome of the Art 31 referral on medicinal products containing sartans with tetrazole ring, CHMP required the following actions:

1. Obligatory risk assessments to be performed for manufacturing processes of the drug substances in order to evaluate the theoretical risk of N-nitrosamine formation and contamination

2. Implement a control strategy to detect and control *N*-nitrosamine impurities in the API.

Specifically, CHMP considered that NDMA and NDEA long-term limits in the API should be as low as technically possible. In this regard, a limit of quantification of 0.03 ppm for NDMA and NDEA was considered achievable according to the available data on analytical methods.

Limits for NDMA and NDEA for the API based on AI calculated according to ICH M7 were considered acceptable for a transitional period of 2 years. Thereafter, a technical limit for NDMA and NDEA of 0.03 ppm should be implemented.

The technical long-term limit for NDMA and NDEA in the API set for sartans was considered feasible by CHMP since the source of *N*-nitrosamine impurities was identified in the active substance manufacturing process and thought to be avoidable by introducing reasonable changes.

Having considered the knowledge acquired on the presence of nitrosamines in medicinal products since the sartans referral and taking into account the data assessed within the current review, in particular related to the root causes where it became clear that the root causes can be numerous, concomitant, at any stage of the production or storage of the medicinal product and cannot always be characterised, the CHMP considered that the outcome of the sartan referral should be reconsidered in light of the outcome of this Art. 5(3) referral. However, it is not within the competence of the CHMP to make changes to a legally binding decision. Instead CHMP invites the European Medicines Agency to inform the European Commission about these considerations.

3. Expert consultations

3.1. Ad-hoc expert group

The ad-hoc expert group meeting took place on Feb 27th and 28th 2020 at the EMA.

An extensive list of questions regarding the five topics most important for pharmaceutical quality, control of pharmaceutical quality, exposure to N-nitrosamines, cancer risk and risk assessment of nitrosamines was issued to the experts several weeks before the meeting. A summary of the conclusions of the expert group to the topics is provided here. The full meeting minutes with all questions and detailed answers is provided as Annex 1.

Chemistry

The experts consider the root-causes identified and confirmed so far plausible but emphasize that it would be beneficial to have more details from companies in general, outlining the rationale for their conclusions.

Additional potential root causes for N-nitrosamine contamination of API and drug product have been proposed by the experts. Most of the additional root-causes are based on theoretical considerations and might affect either drug substance or drug product.

The experts confirmed that polar aprotic solvents such as Dimethylformamide (DMF), Dimethylacetamide (DMAc) and N-Methyl-2-pyrrolidone (NMP) bear a risk of nitrosamine formation and they recommend avoiding these, if possible. Nitroalkanes such as Nitromethane are also known nitrosating agents. Furthermore, appropriate measures are recommended in case these solvents/reagents are used and unavoidable.

Furthermore, experts considered how nitrosamine impurities can form in solid oral dosage forms when all components are ostensibly in the solid state. It was suggested that grinding surfaces together during e.g. granulation or compression could lead to reactions on surfaces. This could be investigated by applying pressure and can be followed by IR or x-ray crystallography. Alternatively, adding a small amount of water to a granulation process could result in relatively high local concentrations of nitrites which are highly soluble, thus leading to rapid reactions with nitrosatable amines, if present.

It was also highlighted that exposure of the drug product to heat during e.g. formulation, storage and shipping might play a role in nitrosamine formation.

More extensive and carefully designed experiments are considered to be useful on API and finished products and additional considerations for such experiments were made by the experts in general as well as specifically for ranitidine and metformin.

The experts highlighted the importance of manufacturing process development and manufacturing processes designed to avoid generation or presence of nitrosamines. When avoidance is not possible, risk mitigating actions should be adopted. It was suggested that certain raw materials (e.g. DMF) should be avoided unless there is no alternative.

If, the presence of nitrosamines cannot be avoided (e.g. in case of no alternative synthetic step or if use of certain excipients or solvents cannot be avoided), it is expected that a suitable control strategy to reduce nitrosamine impurities (e.g. purification steps) is implemented. This should include adequate control of materials, process controls and suitable analytical controls based on regulatory guidance already in place. Furthermore, appropriate risk mitigation measures should be adopted.

In addition, the information to be submitted in an application file should be more detailed taking account this new aspect. So, for instance the description of a manufacturing process of an API should also describe more comprehensively, including a risk management about potential side reactions, steps which do not necessarily relate directly to the synthesis of the API itself but for instance description of workups, purifications and steps to deplete certain reagents (e.g. use of sodium nitrite to deplete excess of NaN_3 used in the tetrazole formation).

Furthermore, experts recommend that theoretical purge calculations should be confirmed with analytical data for nitrosamines. Feasibility in routine and at industrial scale of purging steps should be evaluated and adequately validated. In addition, experts recommended conducting steps at risk of generating nitrosamines early in the manufacturing process to have sufficient opportunities for purge downstream. In contrast, telescoped reaction sequences with minimal isolations reduce purge capability and increase risk of carry-over.

Analytical methods

Sample preparation is considered the main difficulty in establishing analytical procedures for nitrosamines. The experts considered that due to the presence of multiple excipients in different combinations in different products, the development of methods for finished products is more challenging than for APIs.

However, it is regarded possible to develop analytical procedures to measure the most commonly encountered volatile nitrosamines (NDMA, NDEA, NDBA, NEiPA (=EIPNA), NDiPA (=DIPNA), NDPA), even in a single method. Methods currently used by the OMCL network are GC or LC coupled with mass spectrometers of sufficient sensitivity. Due to its non-volatility and chromatographic properties, NMBA requires a separate LC-MS determination.

Due to the trace analytical level (e.g. 30 ppb), special care should be taken to avoid interference, matrix effects or artefacts generated during work up and measurement and conducting control experiments to identify any artefacts, for example, running orthogonal methods or carrying out confirmatory testing using a second method is deemed essential.

For pharmacopoeial purposes, analytical procedures should be developed based on commonly available instruments but ensuring sufficient sensitivity and specificity. Overall, experts support this approach for standardisation purposes.

The experts also noted that analytical methods have been published in Pharmeuropa 32.2, recently: 2.4.36. N-Nitrosamines in active substances

Exposure

Experts were asked on reliable estimates for intake of likely carcinogenic nitrosamines (such as NDMA and NDEA) from food and other sources in the EU as well as reliable estimates for endogenously formed nitrosamines under physiological conditions.

Experts agreed that the available data is mainly regarding food rather than other sources which makes it impossible to estimate an overall exposure with any degree of certainty.

At the moment there is insufficient data to respond to this question with any degree of specificity. There is a high level of uncertainty and high inter-individual variability which is dependent on lifestyle and culture. The assessment report already provides an overview on the different estimates on food derived exposure levels and the experts also concludes that food is the most important source of nitrosamine intake and personal lifestyle is a dominating factor in exposure especially from food.

The same is valid to an even greater extent for endogenous formation of nitrosamines. Data are very scarce, and the conclusions confirmed that reliable quantification is not possible.

Risk assessment

Experts were asked to provide their views and recommendations on

- which nitrosamines are considered most important for human safety?
- the route of exposure most relevant for carcinogenic risk of nitrosamines in humans
- is the cancer risk the same from exogenous and endogenous nitrosamines?
- toxicological tools for assessment of carcinogenic risk when (i) animal carcinogenicity data are available and (ii) no animal carcinogenicity data are available. How should less-than-lifetime (LTL) exposure is taken into account for risk assessment
- the value of epidemiological data on nitrosamine exposure and carcinogenic risk in humans and appropriate design of potential future studies to be undertaken
- consideration of exposure to multiple nitrosamines for risk assessment

Experts stated that there is sufficient research to support a stratification in terms of potency of mutagenicity, but its implementation would need further elaboration. Different groups in potency can be considered and this grouping can be explored in future studies. Alkylating nitrosamines with clearly defined mechanisms for mutation through well characterised adduct spectrums and repair mechanisms were considered most important. Smaller chain versus longer chain alkylating groups may also be important due to differences in reactivity and efficiency of repair systems. The experts however concluded that whilst there is good scientific data and evidence to make a stratification, it is very difficult to have different approaches for each type of nitrosamine and it is questionable whether this stratification is useful for regulatory purpose. The experts recommended to focus on the most predominant types of nitrosamines and adjust tests and try to rank them. Dimethyl- and Diethyl-groups were considered the more important in terms of mutagenic/carcinogenic potency compared to longer chain and cyclic compounds.

Experts considered oral intake and inhalation as the main routes of exposure but did not recommend differentiating between any routes of exposure with respect to risk assessment due to the risk inherent to nitrosamines. Experts also did not recommend differentiating exogenous and endogenous exposure with respect to risk assessment but also highlighted differences in metabolic and repair competence between rat and humans and between different organs as well as inter-individual differences (5-10 fold) for MGMT a major repair enzyme.

The main tools in risk assessment in the different areas were discussed. In food, food contact material, toys and cosmetics the ALARA approach is pursued as risk management option as a safe limit cannot be established. EFSA uses a > than 10,000-fold margin of exposure as an indicator for low concern. Experts favoured the benchmark dose lower confidence limit (BMDL) model with critical effects size (CES) or benchmark response (BMR) of 10% for calculation of the benchmark dose (BMD) and the $BMDL_{10}$ as tool for calculating a point of departure (PoD) that can be used in risk assessment. This approach is recommended for substances with sufficient animal carcinogenicity data available. In view of the use of TD_{50} as PoD for calculation of theoretical cancer risk in humans in ICH M7, the experts highlighted that the model is used to calculate a 1 in 100,000 cancer risk in humans when in fact it only calculates a theoretical risk in a rodent species without any adjustment for human to rodent differences. The pertinence of extrapolating a 1 in 100,000 risk from 25 animals out of 50 getting cancer was considered questionable. Biological thresholds were also discussed and might be justified through understanding of the relevant mechanisms in DNA repair (e.g. MGMT). However, this would require statistical models for which the relevant basic information is still missing. Experts recommended the use of $BMDL_{10}$ model when sufficient carcinogenicity data are available and any small issues remaining could be overcome easily.

Experts agreed that risk assessment is much more difficult for nitrosamines without animal carcinogenicity data. Use of chemical read across and AMES data may be an option but still be difficult as even isomers with very similar result in AMES mutagenicity assay show considerable difference in carcinogenic potency (e.g. cis- and trans-Nitroso-3,5-dimethylpiperidine). The best way would be comparison by data of the same in vivo assays (e.g. transgenic in vivo rodent mutation assays OECD TG488). AMES data are considered useful only for qualitative but not quantitative risk assessment.

The majority of experts agreed that LTL as use in ICH M7 is not useful for applying this to cohort of concern chemicals. Attention should be focused on short-term/high exposure scenarios where repair mechanisms might become saturated. Reference was made instead to the EFSA model of dose addition to be used in health protection.

Epidemiological studies are not yet providing convincing evidence for a quantification of the carcinogenic potential of Nitrosamines in humans. Epidemiological studies up to now only provide evidence for an association of processed meat and/or food intake and some cancers in humans (e.g. GI tract, bladder). Experts agreed that in an epidemiological study for medicinal products the most relevant outcomes would be liver cancer, GI including lower GI and bladder. They also suggest for further studies to do data utilization studies upfront to power study design.

For exposure to multiple nitrosamines expert recommend doing a dose addition approach as used by EFSA. A pragmatic and conservative approach in this respect could be to take the sum of all nitrosamine impurities in a medicinal product and then apply the PoD for the most potent nitrosamine.

Regulatory considerations

Experts were asked to discuss the different approaches (e.g. avoid, minimize, control) for regulation of nitrosamines in the different areas (e.g. water, food, beer, cosmetics, plastic/rubber toys, occupational exposure, etc.). Experts were further asked to provide their views on the different strategies for setting limits to control nitrosamines with considering that patients may be exposed to the same or multiple nitrosamines from the same product or different product:

- Setting a limit based on toxicological data ('Acceptable Intake', AI; Less-Than-Lifetime exposure, LTL)
- Setting a limit based on technical capability (Limit of Quantification, LoQ)
- As Low As Reasonably Achievable/Practicable (ALARA/ALARP)

For nitrosamines the ALARA principle is followed by EFSA (product-specific assessment). It is not a quantitative risk-assessment, due to the high level of inter-individual variability in exposure. The margin of exposure (MOE) approach is used by EFSA to perform an assessment. A margin of exposure (= BMDL10 /exposure) greater than 10,000 is considered as indicating low concern. An example for minimizing exposure is for food where there are strict limits for nitrates (E251-E252)/nitrites(E249-E250) intentionally added to food for preservation with respect to the food categories in which they can be added and their maximum levels. There are several restrictions in place to reduce the level of nitrates (see EFSA guideline). Over the years, steps have been taken to reduce the amount of nitrite used in meat curing processes to a minimum – however, it cannot be removed completely so limits are in place. It should also be considered that the limits are for the raw product and that cooking will likely increase the nitrosamine content due to application of heat. Levels may vary depending on the method and extent of cooking. For risk calculations experts were favouring using more detailed adjustment factors. The BMDL in place of the TD_{50} would be preferred, and more in line with the biological response and dose response assumptions for the well characterised alkylating agents NDMA and NDEA. For the occupational exposure in Germany, the tolerated exposure (excess cancer risk level of 4 in 10,000 for working time exposure) is presently 0.75 microgram/m^3 air and the acceptable exposure (excess cancer risk level of 4 in 10,000 for working time exposure) was 0.075 microgram/m^3 until 2018 and is presently 0.0075 microgram/m^3 (excess cancer risk level of 4 in 100,000 for working time exposure) in air for NDMA. These values are also applicable for the sum of all carcinogenic nitrosamines. Due to many potential sources of nitrosamines, measures to minimise the levels of nitrosamines should be implemented for all of them to avoid exceeding cumulative acceptable levels.

When deriving such limits, exposure to nitrosamines from other sources should be considered and a factor of 10% of AI may be appropriate to address polypharmacy. The AI should not be understood as a safe threshold.

Experts recommended not to use LoD for setting limits and not to use technical limits as nitrosamines may not be avoidable completely in many cases. The ALARP approach would imply some subjectivity in the decision-making and may not be very useful for medicinal products; however, it may be possible in specific cases to minimize risk if exposure cannot be avoided.

The experts stressed that, in cases where the root cause(s) for the presence of nitrosamines in medicinal products is identified, measures to avoid or mitigate can be taken. However, this is not always the case, which makes the control of the risk more complex. The critical need of medicinal products needs to be balanced against the risks when setting acceptable limits. The impact on drug supply needs to be considered when setting limits.

For deriving acceptable intakes, the $BMDL_{10}$ approach was favoured. TD_{50} should only be used in case linear extrapolation from animal data to human can be applied. For nitrosamines without carcinogenicity data a class specific approach was discussed in using e.g. the 95% CI from nitrosamines with data or using the AI calculated for the most potent nitrosamine. The AI as acceptable intake would apply to the finished product as this is what patient are using. When using TD_{50} for calculation of toxicological limits (AI) divergent opinions were expressed on how to potentially factor in the impact of animal to human differences (e.g. metabolism and repair) and some experts suggested a limit of 10 -50 ng/d for nitrosamines in drug products. However, at this stage, this is only an expert estimate and not based on specific toxicological data or well acknowledged toxicological calculation models.

Overall, the experts agreed that risks should be managed by defining acceptable control limits and to incorporate these into a decision tree.

3.2. BWP consultation

Overall, it can be concluded that there is a very low risk of nitrosamines being present as impurities in biological medicinal products, although it can't be completely ruled out. Types of biological substance at higher risk would be those containing chemically synthesized fragments, where similar risk factors to chemically synthesized active substances should be considered, or those packaged in blister packs containing nitrocellulose. Therefore, consideration should be given to extending the risk evaluation process to these classes of biological product and in particular, to assess the synthetic processes of any small molecules subsequently appended to biological molecules. In addition, as for chemically synthesized APIs, processes where nitrosating reagents are deliberately added should be considered at risk.

3.3. QWP consultations

3.3.1. Summary of QWP responses to first CHMP LoQ

CHMP has consulted QWP with a list of questions adopted by CHMP on 12 December 2019, regarding its view on risk assessment of and setting limits for nitrosamines:

1. For highly mutagenic nitrosamines (e.g. NDMA, NDEA), does QWP consider a technical limit (based on current analytical capability) or a limit based on acceptable intake as the most appropriate way of setting long-term limits in medicinal products? Please provide detailed reasoning for your choice.
2. In case a limit based on acceptable intake is considered appropriate,
    a) Should an additional "safety margin" be included considering that patients may be exposed to different nitrosamines within the same medicinal products or from different products?
    b) Should it be adjusted for non-chronic use?
    c) How should we deal with situations where the calculated acceptable intake is below quantification limits of sensitive state-of-the art assays?
3. In case a technical limit is considered appropriate,
    a) Should this be "frozen" (e.g. 0.03 ppm applied to tetrazole sartans) despite the fact that the analytical methods (e.g. LC/GC mass spectrometry instruments, protocols) are becoming increasingly more sensitive? If not, when/how often should the limit be revisited?
    b) A technical limit may lead to different actual exposures depending on the daily dose of the medicinal product. For example, for high daily doses, the technical limit could lie above the limit based on acceptable intake. How should this be dealt with?
    c) Should a technical limit be applied to all medicinal products including those intended for short-term administration?
4. Which tools are available to assess whether the amount of effort used for control of nitrosamines is proportional to the significance of the risk?

5. Which aspects would be important for a decision-tree to guide assessment of benefit/risk in cases where nitrosamine impurities are present?

6. During the course of investigations into nitrosamine contamination, regulatory actions have had to be taken without full information on where the nitrosamine contamination is coming from. What are the state of the art methods that could be employed to help identify root causes in the formation of impurities and inform risk assessments?

In response to the above, the ALARP approach is favoured by QWP and steps to avoid generating nitrosamines should be taken by the MAH to ensure that medicines aren't unnecessarily contaminated with these impurities. However, QWP would agree with an acceptable intake for nitrosamines defined by SWP. In case safe limits can be set by SWP, QWP would also agree to allow adjustment factors derived from ICH M7 in order to apply for less than lifetime use in line with ICH M7.

QWP has concerns regarding analytical methods and assumes that analytical methods cannot be developed for all API (or finished product) with a LoQ of 0.03 ppm or lower – this will depend on the API physicochemical properties and any interference with other components in the finished product.

For a decision tree to guide assessment of benefit/risk in cases where nitrosamine impurities are present – and particularly required when ALARP principle will be followed -, QWP has briefly drawn up a number of tools to consider for assessment whether the amount of effort used for control of nitrosamines is proportional to the significance of the risk.

3.3.2. Summary of QWP responses to second CHMP LoQ

CHMP has consulted QWP with a second list of questions adopted by CHMP on 30 April 2020, regarding its opinion on additional aspects relevant for testing and setting specifications:

The CHMP requests the opinion of the QWP on:

1. Is skip-testing (in API or FP) appropriate for nitrosamines if a risk has been identified (or should routine testing be carried out)? Under what circumstances?

2. Should testing be conducted on FP, API, or an intermediate?

3. What constitutes a representative number of batches for testing to demonstrate absence (3 batch as is generally standard)?

4. Which batches are considered representative (pilot/lab/production scale, commercial manufacturer)?

5. What should be the required sensitivity for analytical methods (e.g. threshold, LoQ)?

6. When multiple nitrosamines are detected in 1 product/API:

    a) At what level is a nitrosamine considered to be present. Above limit of detection? But if so, based on what method?

    b) How low would the method LoQ have to be as a result? (Need to be able to quantify impurities to a lower level than AI if multiple present. Also consider that the method would need to be selective enough for baseline separation of low-level impurities.)

In response to the above, QWP considered that the testing frequency (i.e. routine testing or skip-testing) for nitrosamines depends on the identified source of contamination and the level of risk. Skip testing is possible, provided the root cause of a detected nitrosamine is well-known and well-

controlled. If the actual source or at what stage the contamination enters the process is not identified or proven, a routine test in the finished product is expected.

The control point (finished product, API or an intermediate) for nitrosamines should be selected in such a way that it will give assurance of presence of the impurity below the acceptable limit in the finished product.

QWP suggested the number of batches to be tested should be commensurate with the risk in line with ICH M7. A distinction has to be made between new MA applications (1) and already marketed products (2).

1) If the source of risk has been identified and is well understood test results from a minimum of 6 pilot scale batches or 3 production scale batches should be provided with the MAA. Depending on the risk factors for nitrosamine formation (e.g. the closer the risk factors are to the finished product), more batches may need to be tested.

2) For marketed products, test results from 10% of annual batches, or 3 per year, whichever is highest, should be submitted. If fewer than 3 batches are manufactured annually, then test results from all manufactured batches should be submitted.

If multiple manufacturers, manufacturing processes and/or sources of at-risk raw materials are used, (or were used historically for batches still within expiry date), then testing of additional batches would be necessary to cover these risk factors.

Regarding analytical tests, QWP preferred LoQ over the LoD as a decision-making criterion since it provides the minimum level at which the analyte can be quantified with acceptable accuracy and precision (see ICH Q2 (R1).

To perform testing, the applied analytical method should be sufficiently sensitive. The LoQ should be at or below the acceptable limit for the respective nitrosamine impurities. Importantly, the purpose of testing also needs to be taken into account (routine testing, justification for skip testing or for omission of testing)

If testing is performed in order to justify skip testing, this implies demonstration that levels of the respective nitrosamines are consistently at or below 30% of the relevant acceptable limit for the respective nitrosamine impurities. Hence the LOQ should be at or below 30 % of the acceptable limit.

If testing is performed to justify omission from the specification, it has to be demonstrated that the levels of the respective nitrosamines are consistently at or below 10 % of the acceptable limit. Hence, generally the LOQ should be at or below 10% of the acceptable limit. Exceptions may be needed depending on the maximum daily dose of the medicine in question or if more than one nitrosamine is expected to be presence.

Different analytical methods could be used for determination of multiple nitrosamines. If the same analytical method is used for multiple nitrosamines, then the selectivity of the method should be demonstrated at the LoQ for each nitrosamine.

3.4. SWP consultations

3.4.1. Summary of SWP responses to first CHMP LoQ

CHMP has consulted SWP with a list of questions adopted by CHMP at the December 2019 plenary, regarding SWP opinion on risk assessment of and setting limits for nitrosamines. A summary of the SWP response to the specific questions is provided below.

1. *Question 1*: It is recognised that not all nitrosamines are mutagenic. Is it possible to identify those of high toxicological concern (e.g. NDMA, NDEA)? Should they be considered separately from less mutagenic nitrosamines (e.g. in ICH M7), and if so, how? Which approach should be taken for nitrosamines with negative mutagenicity data and no carcinogenicity data?

Summary of the SWP response: The toxicological concern for N-nitrosamines is based on their carcinogenic potential, which is driven by a DNA-reactive genotoxic mechanism. The proximate mutagens formed from N-nitrosamines cause mutations in the DNA, which initiate and contribute to the process of carcinogenesis. Consequently, mutagenic N-nitrosamines are of high toxicological concern and non-mutagenic N-nitrosamines are of lesser toxicological concern.

Differences in carcinogenic potency exist among the mutagenic N-nitrosamines. Where reliable carcinogenicity data exist, the TD_{50} (reflecting carcinogenic potency) of each N-nitrosamine is used to calculate an acceptable intake for the impurity. Any difference in potency is reflected in the calculated acceptable intake. Thus, although N-nitrosamines can be ranked as potent or less potent carcinogens, there is no need to use different methodological approaches for risk assessment.

Non-mutagenicity should be established by reliable data from a well-performed Ames test, as explained in ICH M7(R1). If a N-nitrosamine is shown to be non-mutagenic, it is considered a Class 5 impurity and should be managed following the concepts of ICH Q3A(R2) and ICH Q3B(R2).

2. *Question 2:* Which precautionary approach should be used in future should any new nitrosamines with insufficient toxicological data be identified as impurities? What are the minimum in-silico and/or empirical criteria for applying the same calculated limits to novel nitrosamines as to well-known nitrosamines (e.g. the calculated acceptable intake for NDEA applied to DIPNA, EIPNA)?

Summary of the SWP response: In the absence of sufficient compound-specific data for new N-nitrosamines, the SWP sees two potential approaches to deal with them. Both approaches have their advantages and limitations. One approach is a class-specific Threshold of Toxicological Concern (TTC); the other is a read-across approach making use of compound-specific data for N-nitrosamines with a similar structure. Both options are considered viable approaches. In any case where either of these approaches is used, the chosen approach needs to be justified on a case-by-case basis. Before a class-specific TTC for N-nitrosamines can be applied the value of this TTC needs to be determined.

3. *Question 3:* Is there a need for revising the ICH M7 guideline to achieve further clarity how to deal with the cohort of concern impurities (i.e. high potency mutagenic/carcinogenic compounds) in general? In particular, is the compound-specific acceptable intake approach considered adequate when suitable toxicological data is available?

Summary of the SWP response: The compound-specific acceptable intake (AI) approach for setting limits for impurities in the cohort of concern (i.e. high potency mutagenic/carcinogenic) as indicated in ICH M7 guideline, is still considered adequate. However, following the regional and global discussions on N-nitrosamines, it needs to be considered to add a chapter on N-nitrosamines to the addendum of ICH M7(R1).

4. *Question 4*: What are the strengths and limitations of the linear extrapolation model when determining acceptable intakes as outlined in ICH M7 guideline? How can data using this model be interpreted?

Summary of the SWP response: For mutagens, it is assumed that a no effect dose (or threshold) at the cellular or molecular level does not exist and there is a linear relationship between tumour incidence and dose that goes through a zero dose. This concept is debated scientifically, and several arguments have been presented against this view. Detoxifying and repair mechanisms can be effective as long as

these are not saturated. Different molecules may be subjected to different ADME mechanisms and lead to mutations requiring different types of repair. Nevertheless, as long as thresholds have not been clearly demonstrated, which still is the case for the majority of genotoxic carcinogens, a linear low dose-response extrapolation appears to be a conservative, but adequate, approach for regulatory purposes. Low dose linear extrapolation can lead to difficulties in risk communication. Many people are likely to consider an increased risk of 1 in 100,000 to be completely unacceptable, e.g. the population in the EU was calculated to be 512.4 million in 2019. An increased risk of 1 in 100,000 would result in over 5,000 extra cancers. Instead, taking into account the generally accepted conservatism of the linear extrapolation method, it should be interpreted as an exposure level at which there is a negligible and therefore acceptable cancer risk in the context of the use of medicinal products.

5. *Question 5*: For highly mutagenic nitrosamines (e.g. NDMA, NDEA), does SWP consider a technical limit (based on current analytical capability) or a limit based on acceptable intake as the most appropriate way of setting long-term limits in medicinal products? Please provide detailed reasoning for your choice.

Summary of the SWP response: The majority of the SWP members considers that compound-specific AI calculated as outlined in ICH M7(R1) is the most appropriate starting point for setting limits for mutagenic carcinogenic N-nitrosamines. However, there may be reasons to set a lower or a higher limit. Lower when the presence of the N-nitrosamine can, with a reasonable effort, be avoided or reduced and the principle of As Low As Reasonably Practicable (ALARP) is followed; higher when it is not technically feasible to impose a limit based on AI and overriding reasons are identified that can support a limit above the one set on AI (e.g. drug shortages, clinical need, disadvantages of alternative treatment; see answer to Q8). In addition, a limit based on AI may be adjusted using the less-than-lifetime (LTL) approach (see answer to Q6b).

6. *Question 6*: In case a limit based on acceptable intake for nitrosamines is considered appropriate,

    a) Should an additional "safety margin" be included considering that patients may be exposed to different nitrosamines within the same medicinal products or from different products?

Summary of the SWP response: When the same N-nitrosamine is present in multiple products, the assessment for each product is not affected by the composition of other products. A patient treated with multiple products may be exposed to multiple risks from different products, but likewise benefits also from multiple products.

When multiple N-nitrosamines are present in a single product, the total risk needs to be considered. When the total risk does not exceed the 10^{-5} tumour risk level, the proposed limits could in principle be considered acceptable.

    b) Should limits based on acceptable intake be adjusted for non-chronic use?

Summary of the SWP response: When using the LTL approach and multiple posologies exist for the product, the indication with the longest duration of exposure should be selected to calculate an LTL-adjusted AI. Examples of clinical use scenarios with different treatment durations for applying LTL-adjusted acceptable intakes are provided in Note 7 in ICH M7(R1). It is conceivable that using LTL-adjustment of the AI leads to relatively high limits, especially when products are considered only used for short durations. The calculated limit could be especially high when the daily dose of the product is relatively low. It is therefore important to take ALARP principles into account as explained in the answer to Q5.

    c) How should we deal with situations where the calculated acceptable intake is below

quantification limits of sensitive state-of-the art assays?

Summary of the SWP response: Setting limits lower than LoQ this limit is technically not feasible. If no further reductions of this technical limit are possible the final option would be to calculate what the risk level would be when the limit would be equal to the technical limit. The calculated risk should then be considered from a risk/benefit perspective. Risk/benefit assessment is further discussed in the answer to Q8.

d) For medicinal products administered parenterally, should acceptable intake be calculated using points of departure derived from data with orally administered nitrosamines?

Summary of the SWP response: Assuming that for both oral and parenteral routes of administration, the metabolism of N-nitrosamines is efficient, the risks following bioactivation to the proximate mutagens is not expected to differ to a large extent, obviating the need for correction factors. In addition, omitting further correction factors is also based on the assumption that GI absorption of N-nitrosamines is efficient.

7. *Question 7*: In case a technical limit for nitrosamines is considered appropriate,

a. A technical limit may lead to different actual exposures depending on the daily dose of the medicinal product. For example, for high daily doses, the technical limit could lie above the acceptable intake. How should this be dealt with?

Summary of the SWP response: The SWP considers the use of the technical limit as a starting point for setting limits for N-nitrosamines not appropriate. The technical limit may however be considered following ALARP principles as explained in the answer to Q5. The situation where a technical limit would lie above the AI is discussed in the answer to Q6c.

b) Should a technical limit be applied to all medicinal products including those intended for short-term administration?

Summary of the SWP response: No.

8. *Question 8*: Which aspects would be important for a decision-tree to guide assessment of benefit/risk in cases where nitrosamine impurities are present?

Summary of the SWP response: The clinical need for the product; the indication and characteristics of the intended clinical population; the availability and potential disadvantages of alternative treatments; the technical feasibility and effort needed to reduce or eliminate the impurity; the degree of risk reduction attained by the proposed measures to reduce or eliminate the levels of N-nitrosamine impurities in medicinal products; and the balance between what can be seen as reasonably practicable and grossly disproportionate are elements that are important for (a) decision-tree(s) to guide assessment of benefit/risk in cases where N-nitrosamine impurities are present. Assessment of benefit/risk in cases where N-nitrosamine impurities are present may be needed in various scenarios.

9. *Question 9*: What is expected for products falling outside the scope of ICH M7 guideline, i.e. drug substances and drug products intended for advanced cancer indications (as defined in the scope of ICH S9), or when the drug substance is itself genotoxic at therapeutic concentrations?

Summary of the SWP response: The SWP recommends adhering to current guidance and considers this guidance applicable to N-nitrosamines.

10. *Question 10*: What is the SWP's opinion on the setting of a temporary limit above the acceptable intake to avoid potential shortages of essential medicines? What multiple of the acceptable intakes could be accepted under these circumstances and for what duration?

Summary of the SWP response: Setting of a temporary limit above the AI to avoid potential shortages of essential medicines is in line with the ALARP principle as explained in the answers to Q5 and Q8. To set temporary limits above the AI the less than lifetime (LTL) approach can be used, which would keep the estimated tumour risk below the level of 10^{-5}.

3.4.2. Summary of SWP response to second CHMP LoQ

CHMP has consulted SWP with a list of remaining questions adopted by CHMP at the April 2020 plenary, regarding SWP opinion on setting limits for nitrosamine without sufficient toxicological data and limits in products with more than one nitrosamine identified. A summary of the SWP response to the specific questions is provided below.

1. What should be the preferred approach to calculate a compound specific AI for nitrosamines without sufficient data, based on the two options previously advised by the SWP as presented below?

    a. Structure-activity-relationship considerations as performed for DIPNA, EIPNA, MeNP, NDBA and NMBA.

    b. A class specific threshold of toxicological concern (TTC) values.

In response to the above, it is the SWP view that not either one of the two options mentioned should be considered the preferred approach, but rather maintains that both options are feasible. As an initial approach to set limits for N-nitrosamines for which insufficient compound-specific data are available, the SWP proposes that by default a class-specific TTC of 18 ng/day can be used. Since the proposed class-specific TTC for N-nitrosamines of 18 ng/day is considered a conservative value being sufficiently protective to be used for any N-nitrosamine, the SWP considers it appropriate to use this class-specific TTC for N-nitrosamines without available toxicological data as a default approach.

The SWP recommended to use the Lhasa carcinogenicity database (LCDB) TD_{50} values, based on a reproducible method for calculating TD_{50} values from the CPDB data set, by using stringent criteria according to Thresher et al. (2019).These are: removal of datasets where no dose-response or non-linear dose-response curves exist; exclusion of data sets with a single dose group; removal of TD50 values of 1,000,000 mg/kg and above); and consistently applying the same methodology (discarding the method depending on lifetable tumour data) in contrast to CPDB also using lifetable tumour data. According to Peto et al (1984), not using lifetable tumour data (i.e. the data are not actuarially adjusted) may lead to an underestimation of the real tumour incidence, especially at high doses where other toxicity may have affected survival. Nevertheless, the correlation between CPDB and LCDB (not using lifetable data) TD_{50} values is high (Pearson's correlation coefficient 0.926 and 0.979 for all individual datasets and for all compounds, respectively).

Consequently, it may be expected that the LCDB would provide a reliable TTC for N-nitrosamines. Yet, the number of TD_{50} values for N-nitrosamines in the LCDB is very small (45), which increases the chance that the calculated TTC may shift when more data would become available. The expected lognormal distribution of TD_{50} values is well reflected by the CPDB data set. However, the LCDB set lacks compounds in the upper range, as compared to the CPDB set. The highest TD_{50} value in the LCDB set is 6.04 mg/kg bw/day for N-Nitrosopiperazine and N-Nitroso-2,3-dihydroxypropylethanolamine. The respective TD_{50} values for these compounds in the CPDB set are 8.78 and 5.98 mg/kg bw/day. In the CPDB set another 11 N-nitrosamines are listed with higher TD_{50} values, ranging from 9.66 to 167 mg/kg bw/day. The lower TTC derived from the LCDB set (18 ng/day) as compared to the TTC derived from the CPDB set (38 ng/day) may be due to this difference between the data sets. The lower number of TD_{50} values in the LCDB set has, however, not led to a broader CI.

Deviation from this default approach may however, be justified on a case-by-case basis. This justification may be based on structure-activity-relationship considerations and should adhere to basic principles for a read-across approach as already addressed by the SWP response to the first list of CHMP questions (section 3.4.1). SWP indicated that conservative expert knowledge/review by comparing compounds with similar structures is a potential way for predicting carcinogenic potency for new N-nitrosamines with insufficient toxicological data, by using scientifically based assumptions and available data from other analogous substance(s). However, it was also suggested that important principles for this read-across approach, similar to those developed within the Read-Across Assessment Framework (RAAF)framework by ECHA, should be followed. These principles were:

- The main prerequisite for using read-across for the prediction of carcinogenic potency of N-nitrosamines is that any read-across approach must be based on structural similarity between the source and target substances.
- However, structural similarity alone is not sufficient to justify the possibility to predict carcinogenic property(ies) of the target substance by read-across. A read-across hypothesis needs to be provided. This hypothesis establishes why a prediction for carcinogenic property is possible and should be based on recognition of the structural aspects the chemical structures have in common and the differences between the structures of the source and target substances. There may be several lines of supporting evidence used to justify the read-across hypothesis, with the aim of strengthening the case.
- The differences in the chemical structures should not influence absorption, distribution, metabolism and excretion (ADME) properties to a significant extent or do so in a regular pattern. The additional use of in silico predictive tools for ADME may be of help to support overall predictions made.
- The read-across approach must be justified scientifically and documented thoroughly.

It was concluded that the advantage of read-across is that all available data that can be related to the carcinogenic potency of the *N*-nitrosamine for which no reliable carcinogenicity data exist are used to provide an informed judgement on the carcinogenic potency. A disadvantage is that often insufficient data are available to fill the data gap for the new N-nitrosamine making a prediction impossible or rendering too much uncertainty to be of any use.

2. How to deal with medicinal products that contain more than one nitrosamine? Accept, if the 1:100.000 additional risk level as outlined in ICH M7(R1) is not exceeded:

    a. Use AI of the most potent nitrosamine identified (with sum of nitrosamine levels required to be below AI)

    b. Total risk level of the sum of all detected nitrosamines not to exceed 1 in 100.000

The SWP favours option b, meaning that the calculated risks associated with the proposed limits for each N-nitrosamine impurity should be added. When the cumulative risk does not exceed the 10^{-5} tumour risk level, the proposed limits can be accepted.

3.5. PRAC consultation

CHMP has consulted PRAC with a request for PRAC advice adopted by CHMP on 13 January 2020, regarding its view on need for further epidemiological studies and the questions on epidemiological aspects posed to the Ad Hoc expert group:

1. Is there a need for further studies to better evaluate a potential relationship between exposure to nitrosamines in medicinal products and cancer risk in humans? If the answer is yes
   a. What is your view on the most promising and feasible study type and design to receive meaningful and interpretable data?
   b. Which would be the most appropriate study population (e.g. age limit)?
   c. Would comparator(s) be adequate (if yes which ones?)
   d. Would you recommend specific data sources to be used?
   e. Which would be the most important endpoints (e.g. incidence of overall cancer vs. composite of specific cancer types vs. specific cancer)?
   f. Which important potential confounders would need to be considered?
   g. Considering cancer or specific cancer types as outcome, what would be the minimum time of exposure that should be evaluated within a study considering lag-times of specific cancer types?
   h. Considering rarity of events, what should be the minimum detectable increase in risk to be excluded

2. Additionally, CHMP is seeking expert advice on questions relating to existing epidemiological data and further studies evaluating the relationship between nitrosamine exposure and cancer risk in humans. Does PRAC have any comments on the questions proposed to the expert group regarding epidemiological data (please see respective questions on epidemiology aspects in LoQ to experts).

The PRAC considered in response to the questions above that, in principle, a study may in some specific settings be feasible, however a general conclusion on feasibility cannot be reached. Furthermore, a number of critical challenges have been identified, which leads to a conclusion that it is unlikely possible to design a study which can achieve meaningful results.

One overarching challenge concerns estimation of exposure to nitrosamines from medicinal products, of various reasons. Firstly, there would be a need having reliable information on not just brand name, but also at the batch level. Such data are rarely available. Furthermore, nitrosamines are present in a number of sources to which daily, chronic exposure occurs, such as from diet and the environment, and often at higher concentrations than estimated for medicinal products so far. In addition, individuals may be exposed to carcinogens other than nitrosamines e.g. through food. Furthermore, a long induction time is anticipated for an outcome such as cancer, which also enhances problems with reliable estimation of exposure over a long time period.

Taken together, since nitrosamines are common in daily life and varying in concentrations (e.g. geographically, depending on life-style) any assessment of exposure is likely being imprecise, regardless of availability of exact information regarding exposure through medications.

In case an epidemiological study would be considered, it was suggested that potential objectives of such study would need to refer to "confirm the risk" rather than "quantify the risk" due to the existing uncertainties.

PRAC noted that the study by Pottegård, et al. (2018)(1) did not identify an increase in total cancer cases in NDMA-exposed patients. For single cancer outcomes, increases in risk were observed for colorectal cancer and for uterine cancer, although with wide confidence intervals that included the null.

PRAC was made aware of that in France, currently consideration is given to the feasibility of conducting a cohort study on the risk of cancer following exposure to nitrosamines from medicines using SNDS

(French Health Insurance claims database). The PRAC was also informed about a cohort study performed on an insurance database, examining the association between potentially NDMA-contaminated valsartan and cancer risk. Further consideration to these data should be given once complete results are available.

4. Conclusions

4.1. Root causes for presence of N-nitrosamines

The CHMP agreed that risk of presence of nitrosamines must be evaluated by the MAHs/Applicants. All the root causes for the presence of nitrosamines in medicinal products recognised so far from the available data and the outcome of expert consultations are presented below and should be considered for the risk evaluation:

- Use of sodium nitrite ($NaNO_2$), or other nitrosating agents in the presence of secondary or tertiary amines or quaternary ammonium salts, or in combination with reagents, solvents and catalysts, which are susceptible to degradation to secondary or tertiary amines.
- Use of contaminated raw or recovered materials e.g. solvents, reagents and catalysts (GMP issue, API).
- Use of nitrosamine-contaminated starting materials or intermediates (API).
- Cross-contaminations (related to GMP) due to different processes run on the same line and due to operator-related errors such as inadequate phase separations (API).
- Degradation processes of starting materials, intermediates and drug substances, including those induced by inherent reactivity in combination with carry-over of sodium nitrite ($NaNO_2$), or other nitrosating agents.
- Contamination from blister packaging materials.

The following theoretically possible root-causes that could also lead to nitrosamine formation in and contamination of medicinal products:

- Additional GMP issues may include cross contamination during medicinal product manufacture e.g. due to contaminated solvents or process equipment.
- Formulation in general: nitrites from excipients could react with amines in APIs, or low molecular weight amine impurities. The experts were therefore in favour of testing of excipients for nitrite. Since excipients are generally the greatest component of medicinal products, high amounts of nitrite could be present as a reaction partner. Contamination of excipients with nitrosamines was also seen as a theoretical possibility.
- Storage conditions of APIs (e.g. impact of container).
- Packaging composition (e.g. use of nitrocellulose beyond blister packaging).
- Additional degradation pathways: subsequent degradation of a nitrosated API or nitrosated impurity to smaller nitrosamines.
- Storage conditions after packaging.
- Water quality: nitrosamines in treated water as a result of the use of chloramine (or chlorine which can form chloramines with any amines present) and further reaction to nitrosamines. Other oxidants (e.g. ozone) can lead to NOx formation which could then react with amines to

generate nitrosamines.

- Possible reactions of volatile low molecular weight amines occurring in the manufacturing process (solvents, raw materials, in combination with nitrosating agent). Their volatility means they could potentially carry over during e.g. distillation processes.
- Structure inherent to the API molecule or to intermediates in its synthesis, including presence or generation of amines susceptible to nitrosation in the manufacturing process.
- Reactions of quaternary amines in addition to secondary and tertiary amines (e.g. use of Tetrabutylammonium bromide (TBAB) which could give rise to N-nitrosodibutylamine (NDBA) although the lack of an available electron lone pair makes this an unlikely mechanism, nevertheless, NDBA has been found in some instances. Nitrosamine formation seems in general however more likely to be a result of tertiary and secondary amine impurities. Also, dimethylacetamide was mentioned as a reaction partner with nitrosating agents.
- Nitroalkanes are also known nitrosating agents. Other potential root causes might include emissions from vulcanisation processes (rubber, also when it is in contact e.g. with product) and carbon capture technology.

Biological products

- The BWP concluded that there is only a very low risk of nitrosamines being present as impurities in biological medicinal products. At higher risk would be biological products containing chemically synthesized fragments, where risk factors similar to chemically synthesized active substances should be considered, or biologicals packaged in blister packs containing nitrocellulose. Consideration should be given to extending the risk evaluation to classes of biological product (see section 4.3.) using processes where nitrosating reagents are deliberately added. The CHMP agrees with the BWP advice and considers that a risk evaluation/risk assessment for biological medicinal products should be performed taking into consideration the abovementioned risk factors.

As confirmed by QWP and other experts, most of the currently identified root-causes support the conclusion that risks can be substantially reduced by thorough process design, adequate process development and technical measures, which should be supported by suitable specifications of N-nitrosamines in the finished product to ensure that the medicinal product taken by the patient is within the above limits of ICH M7(R1). The control point for nitrosamines selected in such a way that it will give assurance of presence of the impurity below the limit in the finished product. In addition, based on the QWP feedback, the options ICH M7(R1) provides for skip testing or omission from a specification subject to specific requirements as outlined above would also be considered acceptable.

4.2. Analytical methods for N-nitrosamines

On 30th March 2020, three analytical methods for quantifying N-nitrosamines were published in Pharmeuropa 32.2, 2.4.36, which are stated there as method A (LC-MS), method B (GC-MS, and method C (GC-MS).

The combination of these three methods allows to analyse the following N-nitrosamines: N-nitroso-dimethylamine (NDMA, methods ABC); N-nitroso-diethylamine (NDEA, methods ABC); N-nitroso-dibutylamine (NDBA, method C); N-nitroso-N-methyl-4-aminoburyric acid (NMBA, method A); N-nitroso-diisopropylamine (NDiPA or DIPNA, methods AC); N-nitroso-ethyl-isopropylamine (NEiPA or EIPNA, methods AC) and N-nitroso-dipropylamine (NDPA, method C) in sartan-containing products and is considered suitable for additional APIs and finished products. A specific validation of the analytical

procedure is necessary in each case as indicated in the above mentioned monograph (*'When a procedure is applied to substances outside of the scope covered by the initial validation(…) or to medicinal products or is used quantitatively, then it must be validated'*).

Sufficient limit of detection (LOD), and limit of quantitation (LOQ) including adequate recovery is clearly influenced by the appropriate sample weight used for sample preparation and is subject of the entire analytical procedure.

LOQ provides the minimum level at which an analyte can be quantified with acceptable accuracy and precision and is thus preferred over LOD for impurity testing and decision-making. LOQ should be at minimum at or sufficiently below the toxicologically required limit, taking into account the purpose of testing (e.g. intended for routine testing, for justifying skip testing, for justifying omission of specification)

4.3. Setting Limits for N-nitrosamines in human medicinal products

The advantages and disadvantages of the various options for setting limits have been thoroughly discussed by CHMP and are reflected in section 2.5.4. The CHMP considers that setting limits for individual nitrosamines in human medicinal products based on ICH M7 principles for substances of the "cohort of concern" and calculated considering a lifetime daily exposure is recommended as the best option after careful consideration of patient safety and regulatory practical requirements. This would ensure constant supply of safe medicinal products, as well as consistent and transparent decisions. Use of ICH M7 methodology with TD_{50} as a PoD is internationally agreed and harmonized in contrast to the use of $BMDL_{10}$ as suggested by the AHEG. Using $BMDL_{10}$would also in most cases not lead to lower limits.

The approach suggested by the SWP to use an as-low-as reasonably practicable (ALARP) concept for N-nitrosamines is considered an alternative potential concept for setting limits but was not seen as an adequate approach for medicinal products by the AHEG and by the CHMP.

Applying the ALARP approach for manufacturing of medicinal products to set limits may in particular not lead to sufficiently clear and predictable limits, and the absence of clear benefit of this approach in terms of risk reduction, the CHMP concluded that this approach is not adequate for setting limits for N-nitrosamines in medicinal products

The approach in the current procedure to set limits for N-nitrosamines evolved from the earlier decision for sartan-containing products where a technical limit for the API was defined after a transitional period. CHMP considered at the time a technical limit to be feasible for sartans and this was set on the API since the source of nitrosamine impurities was identified in the active substance manufacturing process and thought to be avoidable by introducing reasonable changes. The knowledge acquired since the sartan referral has made clear that the root causes can be numerous, concomitant, at any stage of the production or storage of the medicinal product and cannot always be characterised. Therefore, a general "avoidance" strategy is not considered a realistic and feasible goal and would foreseeably lead to shortage problems of critical medicinal products. Therefore, CHMP was concerned with striking a viable balance in the best interest of patients, taking into account drug safety and ensuring availability of drugs that are important to human health.

In order to allow a consolidated approach, and to take account of the changes in scientific understanding the CHMP considered that the outcome of the sartans referral should be reconsidered in light of the outcome of this Art. 5(3) referral (see section 3.7). As this not within the competence of the CHMP to make changes to a legally binding decision, the Agency will inform the European Commission about these considerations.

4.3.1. Presence of more than one *N*-nitrosamine

Exceptionally, the presence of more than one nitrosamine may be identified in a given finished product.

For such cases, the ad-hoc expert group suggested the following approach, i.e. to limit the sum of N-nitrosamines to the limit of the most potent one found. Feasibility of such a concept may depend on the capability of effective control.

The SWP advised on an alternative approach that, in case more than one N-nitrosamine is present in a medicinal product, the total risk should be considered. Limits for individual nitrosamines could in principle be acceptable as long as the total risk does not exceed the 10^{-5} tumour risk level. SWP favoured setting a specific limit for each nitrosamine which represents a fraction of the acceptable 1:100,000 tumour risk level. The sum of all nitrosamine specific risk levels should not exceed a total risk level of 1 in 100,000. This also means that controlling the sum of all nitrosamines to the limit of the most potent one, as suggested by the ad-hoc expert group, will automatically keep the total nitrosamine specific risk level to ≤1:100,000, and both options are therefore equally acceptable for CHMP

4.3.2. N-nitrosamines with insufficient toxicological data

When N-nitrosamines were identified with insufficient substance specific data (e.g. NMEA, NNN, NMA, NDPA), a read-across approach based on SAR considerations was used to derive a substance specific AI. The TD_{50} of the structurally closest related N-nitrosamine for which robust data are available to calculate a reliable TD_{50} was used as the point of departure.

SWP recommended (see section 3.5.2) the use of a class specific TTC as the default option for nitrosamines with insufficient substance specific data and to use the TD_{50} values reported in the Lhasa carcinogenic potency database (LCDB).To derive a sufficiently conservative TTC, the lower 5^{th} percentile of the TD_{50} data of all nitrosamines were used. This results in an estimated daily lifetime dose of 18 ng/d, which in theory is not exceeding a theoretical excess cancer risk of 1 in 100,000 with a 95% probability for any N-nitrosamine. Where an applicant or MAH however intends to set higher limit, this needs to be duly justified. The justification can be based on a read-across (SAR) approach and should adhere to the principles outlined in the SWP response document and used for DIPNA, EIPNA, NMBA, NDBA and MeNP.

CHMP agrees with the recommendation made by SWP. The class specific TTC is derived from well acknowledged methodology and not only an expert estimate. Nevertheless, the value for the class specific TTC lies well within the range of the estimate of the ad-hoc expert group.

4.3.3. Less-than-lifetime (LTL) approach

As a precautionary measure, CHMP does not recommend to generally applying the LTL approach to *N*-nitrosamine impurities. The concept of adjusting acceptable daily intake levels for mutagenic impurities for the expected (less than lifetime) duration of use is outlined in ICH M7(R1) was supported by SWP (however only in combination with the ALARP principle) but was rejected by the ad-hoc expert group because of the potential risk of exceeding individual DNA repair capacity with exposure to high acute nitrosamine doses that may result from implementation of the LTL approach, especially for medicinal products with only short-term use.

However, in case nitrosamines are newly detected in a medicinal product above limits calculated as per sections 2.5.1 and 2.5.2, the LTL approach may serve as a first estimate of risk and, dependent on the criticality of the medicinal product, as a guide for determining an interim limit, until the root cause(s) has(have) been identified and - as far as possible - eliminated with the goal of achieving nitrosamine levels below the abovementioned limits. In case limit as defined in sections 2.5.1 and 2.5.2 is not reasonably achievable, and the affected product is critical for public health, higher limits may be acceptable in exceptional cases, based on a thorough benefit-risk balance assessment.

CHMP also considered necessary to review the general acceptability of the LTL concept to all mutagenic carcinogens, especially the cohort-of-concern mutagens, by the ICH M7 EWG in light of the international concerns with this concept in the nitrosamine case.

4.4. Considerations on epidemiological studies

Further insights into the association between the intake of potentially *N*-nitrosamines-contaminated drugs and risk of cancer are required but conduct of epidemiological studies for this purpose is challenging. This is mainly due to the difficulty of reliably determining exposure. Irrespective of the study design applied, potential data sources should contain sufficient patient numbers, should cover a sufficiently long observation time and should contain the required variables to answer the study question, i.e. information on exposure, outcome and important covariates. Using nationwide registries or large healthcare database might be the most promising approach for the conduct of further studies, despite the mentioned limitations. If a single data source does not contain all the necessary information, data linkage to other data sources that may contain the missing information should be checked prior to study initiation. Furthermore, the possibility of a meta-analytical approach may be considered in case of insufficient patient numbers in a given data source. The PRAC and CHMP suggested that potential objectives of such studies would need to refer to "confirm the risk" rather than "quantify the risk" due to the existing uncertainties.

5. Recommendations

Based on the above assessment of the available data, the CHMP recommends that:

1. The presence of N-nitrosamines in human medicinal products shall be mitigated as much as possible and shall be at or below a limit based on ICH M7(R1) principles for substances of the "cohort of concern" defined in this guideline and calculated considering a lifetime daily exposure.

   This should be achieved by an appropriate control strategy and by the design or adaptation of the manufacturing processes aiming to prevent formation of and contamination with nitrosamines whenever possible.

2. The risk of presence of nitrosamines must be evaluated by the MAHs/Applicants. In case of risk, confirmatory testing must be performed.
   - A risk evaluation/risk assessment for the presence of nitrosamines must be submitted for new marketing authorization applications at the time of submission, and for already authorized medicinal products containing chemically synthesised active pharmaceutical ingredients (APIs) as per the 'call for review'12 and for biological medicinal products in a

[12] https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-information-nitrosamines-marketing-authorisation-holders_en.pdf

similar exercise as per instructions to be published in a Questions and Answers document[13].

- The approach for risk evaluation/risk assessment should cover manufacturing processes of active substance and finished product in consideration of the root-causes, and subsequent confirmatory testing in the finished product.in case a risk is identified
- Although the overall risk of presence of nitrosamines in biological medicinal products is considered very low, the following risk factors should be taken into consideration: biologicals containing chemically synthesized fragments, where risk factors similar to chemically synthesized active substances are present, biologicals using processes where nitrosating reagents are deliberately added, or those packaged in certain primary packaging material, such as blister packs containing nitrocellulose.

3. Where a nitrosamine has been detected, a limit based on the above mentioned ICH M7(R1) principles for "cohort of concern" substances considering a lifetime daily exposure should be calculated.

   The following limits have been established for some specific N-nitrosamines and should be applied:

***N*-Nitrosamine (CAS number)**	**ng/day*** **
NDMA* (62-75-9)	96.0
NDEA*(55-18-5)	26.5
EIPNA**(16339-04-1)	26.5
DIPNA**(601-77-4)	26.5
NMBA**(61445-55-4)	96.0
MeNP**(16339-07-4)	26.5
NDBA**(924-16-3)	26.5

   These limits are applicable only if a finished product contains a single N-nitrosamine.

   * Limit calculated on the basis of harmonic mean TD50 derived from carcinogenic potency database (CPDB)

   **Limit derived using SAR/read-across approach

   ***The conversion to a specification limit in ppm for a particular medicinal product is calculated by dividing the respective above limit (ng) by the maximum daily dose (mg) of a given product as reflected in the SmPC .

   - The limit as calculated above will usually need to be included in the finished product specification.
   - Skip testing is only justified if it can be shown that the levels of the respective nitrosamine are consistently ≤ 30% of the limit defined above and the root cause is identified and well-understood.

[13] https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-questions-answers-information-nitrosamines-marketing-authorisation_en.pdf

- Omission from the specification is only justified if it can be shown that the levels of the respective nitrosamine are consistently ≤ 10% of the limit defined above and the root cause is identified and well-understood.

4. If more than one N-nitrosamine is identified in a given finished product (or its API), it must be ensured that the total risk level of the sum of all detected N-nitrosamines does not exceed 1 in 100,000 life-time risk. An alternative approach where the sum of all detected N-nitrosamines does not exceed the limit of the most potent N-nitrosamine identified may also be used. The approach chosen for a particular case needs to be duly justified by the applicant/MAH.

5. Exceptionally, when a single N-nitrosamine cannot be kept below the limit defined in 3. or the total risk level of the sum of more than one detected N-nitrosamine cannot be kept below a 1 in 100,000 life-time risk, the MAH should submit to the relevant competent authorities forthwith an investigation report including the potential/identified root cause(s), preventive/corrective actions and a thorough discussion on the impact on the benefit/risk balance including all relevant considerations (e.g. medical need, daily dose, duration of administration and treatment alternatives, potential patient risk in case of drug shortage). Acceptability of limits higher than those defined in 3 and 4. is then decided by the relevant competent authorities on a case-by-case basis, after having performed a benefit/risk evaluation. In such instances, the "less-than-lifetime" (LTL) concept in ICH M7(R1) may be considered by the competent authorities for the range of a temporarily acceptable exposure until further measures can be implemented to reduce the contaminant at or below the limits defined in point 3. and 4 .

6. Exceptions to sections 3. and 4. include some products falling outside the scope of the ICH M7(R1) guideline, i.e. certain active substances and finished products intended for advanced cancer indications or when the active substance is itself genotoxic. For finished products intended only for advanced cancer, N-nitrosamine impurities should be controlled according to ICH Q3 A(R2) and ICH Q3B(R2), as specified in the Q&A document to ICH S9. When the active substance itself is genotoxic at therapeutic concentrations, N-nitrosamine impurities could be controlled at limits for non-mutagenic impurities according to ICH M7(R1).

7. When N-nitrosamines are identified with sufficient substance specific animal carcinogenicity data to calculate a reliable TD50 then this should be used to derive a substance specific limit for lifetime exposure as recommended in ICH M7(R1).

8. When *N*-nitrosamines are identified with insufficient substance specific data to derive a substance specific limit for lifetime exposure as recommended in ICH M7(R1), a class specific TTC for nitrosamines of 18 ng/d can be used as default option. This TTC has been derived from the Lhasa carcinogenic potency database and is considered a conservative and acceptable approach. If a MAH intends using a higher limit than 18 ng/day, an approach based on structure-activity-relationship (SAR) considerations is acceptable. The approach taken needs to be duly justified by the applicant/MAH.

9. MAHs should implement a control strategy regarding N-nitrosamines for their active substances and finished products, which should include current and prospective measures to minimise the risk of generation/contamination with any nitrosamine (e.g. change of manufacturing process, introduction of appropriate specifications and development of appropriate methods, measures related to the premises and equipment e.g. cleaning procedures, environmental monitoring,…) and control any future change that may impact on this risk (e.g. change of supplier, change of manufacturing process, change of packaging…)

   In order to fulfil their obligations above, MAH/applicants shall:

- carry out risk evaluation/risk assessment of manufacturing processes of API (route of synthesis, starting materials, intermediates, raw materials) in view of potential formation of or contamination with N-nitrosamines, taking into account potential and confirmed root causes for the presence of N-nitrosamines in APIs.
- carry out risk evaluation/risk assessment of finished product (degradation of API, primary packaging material, excipients, etc.), taking into account the root-causes for the presence of *N*-nitrosamines in finished products.
- ensure that, in accordance with Article 23 and Annex I of Directive 2001/83/EC and Article 16 of Regulation (EC) No 726/2004, their medicinal products are manufactured and controlled by means of processes and methods in compliance with the latest state of scientific and technical progress. As a consequence, MAHs/ Applicants shall design their manufacturing processes and controls to prevent if possible or mitigate as much as possible the presence of *N*-nitrosamines in their API and finished product(s) and shall introduce any subsequent changes to their manufacturing process as needed.
- ensure that active substances and excipients used in their finished products are manufactured in compliance with good manufacturing practices as laid down in Article 46(f) of Directive 2001/83/EC.
- MAHs'/Applicants' compliance with the above-mentioned obligations is subject to regular controls by competent authorities including during inspections.

10. With regard to the analytical method(s) employed the following is advised:
    - The limit of quantitation (LoQ) provides the minimum level at which an analyte can be quantified with acceptable accuracy and precision and should thus be used to define the required analytical sensitivity for impurity testing.
    - If quantitative testing is performed as a routine control, the LoQ should be at or below the limit for the respective nitrosamine impurity defined in 3.
    - If quantitative testing is performed to justify skip testing, the LoQ of the analytical procedure employed should be ≤ 30% of the limit defined in 3.
    - If quantitative testing is performed to justify omission of specification, the LoQ of the analytical method employed should be ≤ 10% of the limit defined in 3.
    - Higher sensitivity of analytical methods may be needed for medicinal products used at high daily doses (, or in case more than one nitrosamine is anticipated or identified in a given medicinal product. Such cases should be discussed with the relevant competent authority/ies.
    - Different analytical methods may be used for determination of multiple nitrosamines. If the same analytical method is used for multiple nitrosamines, the selectivity of the method should be demonstrated at the LoQ for each nitrosamine.
11. Although further epidemiological studies would be useful to better characterize the relationship between nitrosamine exposure from medicinal products and cancer risk, critical challenges have been identified such as large sample size, long study duration, determination of exposure, identification of confounding factors and adequate control group which would be necessary to achieve meaningful and interpretable results. Nationwide registries or large healthcare database might be the most promising approach but may not contain all important information. In such cases, data linkage to other data sources that may contain the missing information should be

checked prior to study initiation. Furthermore, the possibility of a meta-analytical approach may be considered in case of insufficient patient numbers in a given data source.

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