AGENZIA ITALIANA DEL FARMACO

The case of nitrosamines

Luca Ginnari Satriani

12/05/2023

Dichiarazione di trasparenza/interessi*

Le opinioni espresse in questa presentazione sono personali e non impegnano in alcun modo l'AIFA

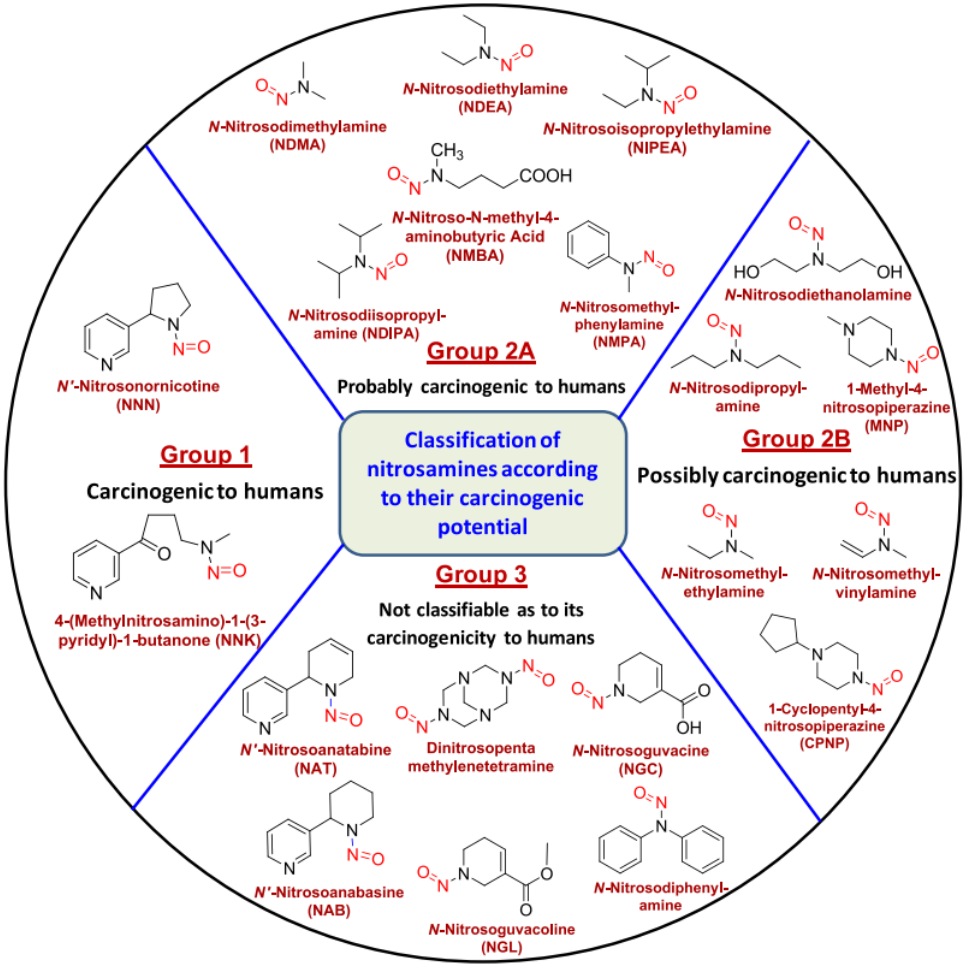
Interessi nell'industria farmaceutica	NO	Attualmente	Da 0 a 3 anni precedenti	oltre 3 anni precedenti
INTERESSI DIRETTI:				
1.1 Impiego per una società: Ruolo esecutivo in una società farmaceutica	X	☐	☐	☐ obbligatorio
1.2 Impiego per una società: Ruolo guida nello sviluppo di un prodotto farmaceutico	X	☐	☐	☐ obbligatorio
1.3 Impiego per una società: altre attività	X	☐	☐	☐ facoltativo
2. Consulenza per una società	X	☐	☐	☐ facoltativo
3. Consulente strategico per una società	X	☐	☐	☐ facoltativo
4. Interessi finanziari	x	☐	☐	☐ facoltativo
5. Titolarità di un brevetto	X	☐	☐	☐ facoltativo
INTERESSI INDIRETTI:				
6. Sperimentatore principale	X	☐	☐	☐ facoltativo
7. Sperimentatore	X	☐	☐	☐ facoltativo
8. Sovvenzioni o altri fondi finanziari	X	☐	☐	☐ facoltativo
9. Interessi Familiari	x	☐	☐	☐ facoltativo

* Luca Ginnari Satriani, secondo il Regolamento per la disciplina dei conflitti di interesse all'interno dell'Agenzia Italiana del Farmaco approvato dal CdA AIFA con Delibera n. 37 del 13 ottobre 2020.

N.B. Il compenso ricevuto per questo intervento è regolato dalla contrattazione collettiva.

Nitrosamines

- Le N-Nitrosamine sono molecole contenenti il gruppo funzionale N-Nitroso. Si tratta di inquinanti ambientali ubiquitari presenti a livelli di concentrazione generalmente bassi (da ppm a ppb).
- Nel 1956 è stata osservata per la prima volta la carcinogenicità di NDMA in un gran numero di specie animali.
- Molte nitrosamine si ritiene che siano mutagene e cancerogene con forti differenze tra le diverse classi.
- Lo IARC ha classificato quelle per le quali si dispongono di dati su animali nelle classi 2A, «probably carcinogenic» e 2B «Possibly carcinogenic»



J. Med. Chem. 2021, 64, 2923−2936

Nitrosamines: potential sources

Food and beverages

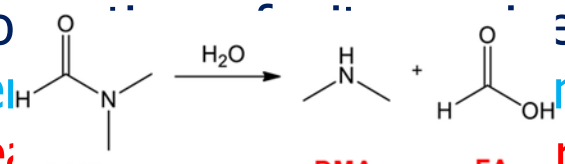
Cosmetics

Medicines

Nitrosamines formation

Potential sources of sec./tert. ammines:

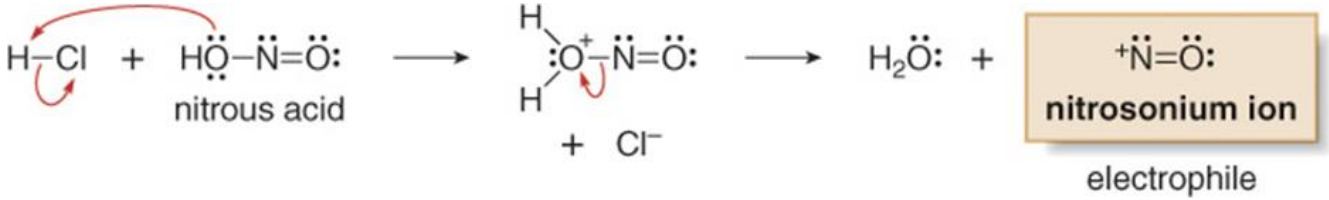
e.g. Ammides (eg. DMF, DMA)



Fo es is possible in the presence of secondary, ter mmonium salts and nitrite salts under acidic re rally pH≤3).

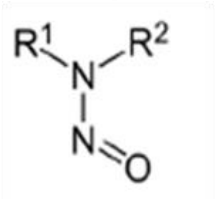
Other potential nitrosant agents:

Nitrogen Oxides (eg. N_2O_3, N_2O_4)
Alkyl Nitrites
Hydrazins in oxidating conditions
Nitrosonium salts
Nitroso halides



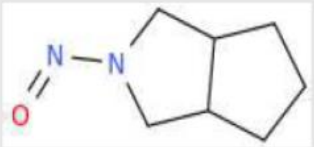
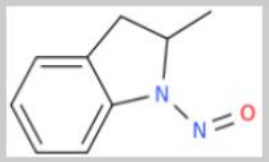
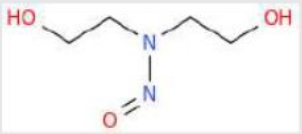
Diazonium salts ← + primary amines

+ sec./tert. amines →



Nitrosamines

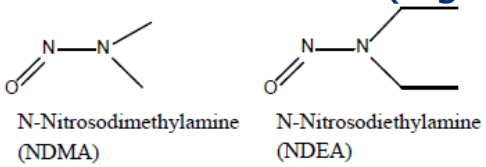
Nitrosamines in medicines…before the Valsartan "case"

API	Impurezza	Limiti	Struttura
Clopamide	cis-2,6-dimethyl-1-nitrosopiperidine	Absent (from risk assessment)	
Gliclazide	2-nitroso-octahydrocyclopenta [c] pyrrole (impurity B)	2 ppm	
Indapamide	(2RS)-2-methyl-1-nitroso-2,3-dihydro-1H-indole (impurity A)	5 ppm	
Trolamine (Triethanol amine)	N-nitrosodiethanolamine (impurity C)	24 ppb	

Nitrosamines in sartan medicines

In June 2018, a manufacturer detected *N*-nitrosodimethylamine (NDMA) in valsartan active substance batches.

Nitrosamines contamination (NDMA e NDEA) was found in other Sartans (e.g. Losartan, Irbesartan, etc.).



On 31 January 2019, EMA recommended that companies making sartan medicines review their manufacturing processes so that they do not produce nitrosamine impurities.

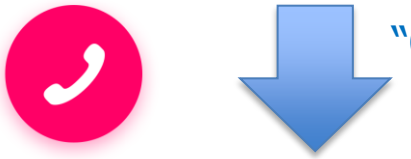
These recommendations follow EMA's review of NDMA and N-NDEA, which are classified as probable human carcinogens (substances that could cause cancer)

Companies had a transition period to make any necessary changes, during which strict temporary limits on levels of these impurities would have been applied.

Companies have been requested to demonstrate that their products have no quantifiable levels of these imp. before they can be used in the EU.

CHMP's Article 5(3) referral on nitrosamine impurities

On 10 September 2019, a referral according to Article 5(3) of Regulation (EC) No 726/2004 was triggered by the EMA Executive Director (ED) requesting the CHMP to conduct a scientific evaluation on the presence of nitrosamine impurities in human medicines containing chemically synthesised active pharmaceutical ingredients (APIs)



"Call for review to MAH"

As a result of the first phase of the referral, a "call for review" to MAHs was launched on 19 September 2019 requesting MAHs for human medicines containing chemically synthesised APIs to review their medicines for the possible presence of N-nitrosamines, to test all products at risk and to introduce changes to the marketing authorisations (MAs) within 3 years.

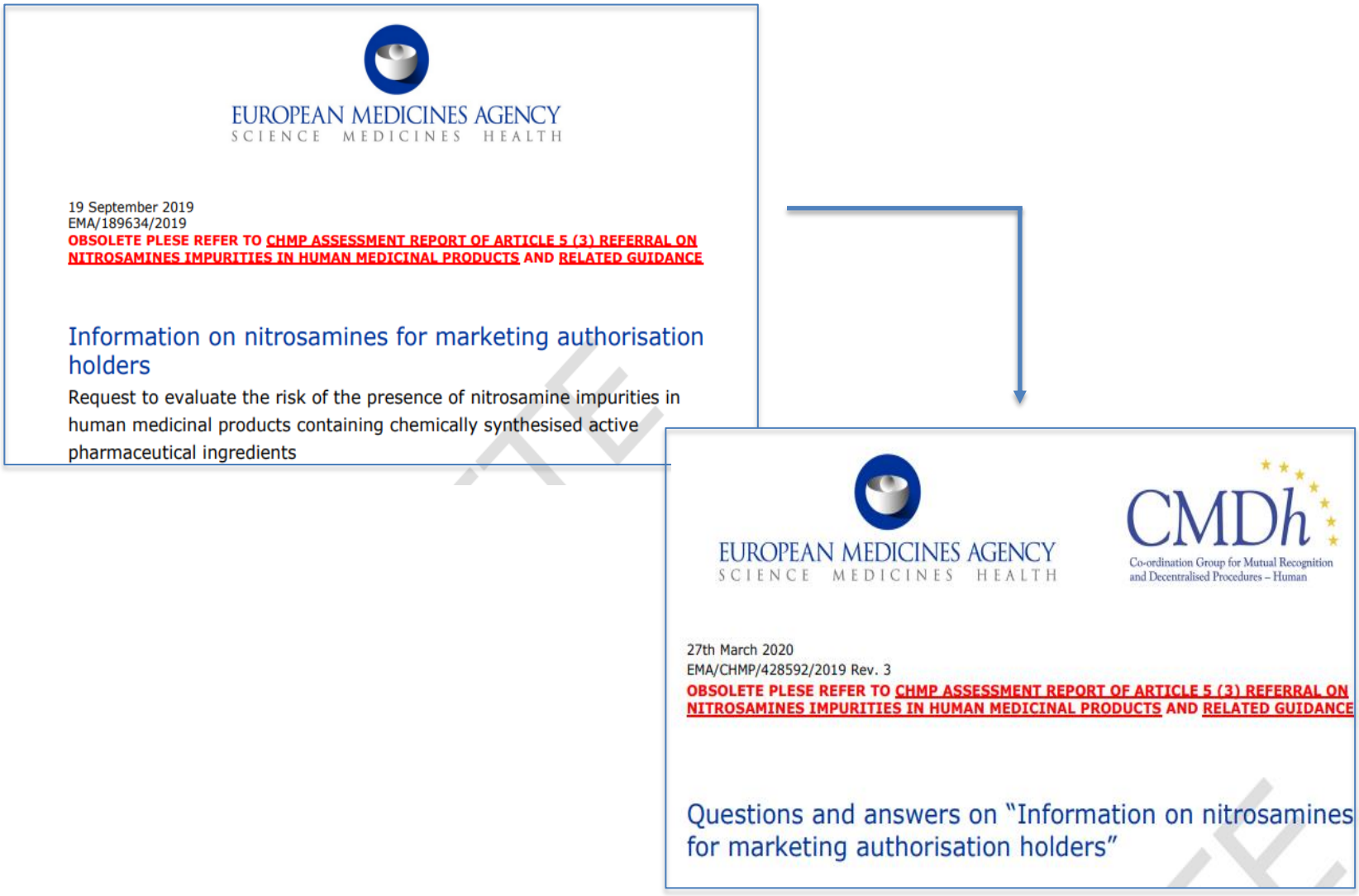
AR of the CHMP's Article 5(3) opinion on nitrosamine impurities

In June 2020, the CHMP finalised its review according to Article 5(3)

1) The AR provides general guidance and recommendations on mitigating and preventing the presence of nitrosamines in human medicinal products. All MAHs/Applicants of human medicinal products should work with the manufacturers of their APIs and FPs in order to ensure that the presence of nitrosamine impurities in their medicinal products is mitigated and controlled at or below a limit defined based on ICH M7(R1) principles and calculated considering a lifetime daily exposure and kept as low as possible and that appropriate risk mitigating measures are taken.

2) The "call to review to MAHs" has been extended to include not only chemicals but also biologicals

The "Call for review" to MAHs



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

19 September 2019
EMA/189634/2019
OBSOLETE PLESE REFER TO CHMP ASSESSMENT REPORT OF ARTICLE 5 (3) REFERRAL ON NITROSAMINES IMPURITIES IN HUMAN MEDICINAL PRODUCTS AND RELATED GUIDANCE

Information on nitrosamines for marketing authorisation holders

Request to evaluate the risk of the presence of nitrosamine impurities in human medicinal products containing chemically synthesised active pharmaceutical ingredients

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

CMDh
Co-ordination Group for Mutual Recognition and Decentralised Procedures – Human

27th March 2020
EMA/CHMP/428592/2019 Rev. 3
OBSOLETE PLESE REFER TO CHMP ASSESSMENT REPORT OF ARTICLE 5 (3) REFERRAL ON NITROSAMINES IMPURITIES IN HUMAN MEDICINAL PRODUCTS AND RELATED GUIDANCE

Questions and answers on "Information on nitrosamines for marketing authorisation holders"

Q&A for MAHs/Applicants on the CHMP Opinion for the Article 5(3) on nitrosamine impurities in human medicinal products (1/2)

EMA/409815/2020 Rev. 15 Revision history

Revision	Summary of changes made	Date
0	Replace obsolete Q&A published in 2019 to support the initial "call for review" with a new version reflecting the main principles agreed as part of the Article 5(3) referral which concluded in July 2020.	03rd August 2020
1	Update to Q&A3 in order to clarify products in scope of the call for review. Update to Q&A 4 in order to add the link to the outcome of the referral under article 3 of Directive 2001/83/EC for ranitidine.	29th January 2021
2	Update to Q&A3 on indicating testing timeline at the time of step 1 "risk identified" reporting.	24th February 2021
3	Update to Q&A 3 on the approach for non-marketed medicines. New Q&A 19 on the requirements for line extensions and variation applications.	15th April 2021
4	Update to Q&A3 on combining step 2 response for multiple products from the same MAH.	18th May 2021
4*	Updates to Q&As3 on when to perform step 2 confirmatory testing in order to meet the established deadline for step 3. Update and Q&A10 to add an AI for NMOR.	29th June 2021
5	Update to Q&A10 to add an AI for NNV.	21st September 2021
6	Guidance on confirmatory testing requirements for marketed (Q&A 8) and on-going applications (Q&A 14) to include cases where a potential nitrosamine impurity cannot be synthesized, and when a product is available in multiple strengths of the same dosage form.	14th October 2021
7	Inclusion of additional guidance on control strategies for products containing more than one nitrosamine impurity including examples (Q&A 10) and a decision tree (Annex I).	31st January 2022
8	Update to guidance on root causes and risk factors for nitrosamine contamination (Q&A 4) and on policy for confirmatory testing (Q&A 8) and dossier requirements (Q&A 15) to allow testing of intermediates, raw materials or API under certain circumstances.	24th March 2022

Q&A for MAHs/Applicants on the CHMP Opinion for the Article 5(3) on nitrosamine impurities in human medicinal products (2/2)

Rev.	Summary of changes made	Date
9	New Q&A 20 providing clarifications on what are the regulatory steps for dealing with scenario A cases and update Q&A10 with new AIs (N-nitrosomethylphenidate, N-nitrosopiperidine, N-nitrosorasagilene, 7-Nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo-[4,3-a]pyrazine, N-nitroso-1,2,3,6-tetrahydropyridine, N-nitrosonortriptyline, N-methyl-N-nitrosophenethylamine) and guidance on use of Ames test.	20th May 2022
10	Update to Q&A 5 to provide clarifications on the expectation for MAHs to continue to re-visit risk evaluations when new information becomes available with specific reference to API-nitrosamine risk. Update to Q&A 10 to include newly adopted AI for *N*-nitrosodabigatran and to indicate APIs where related nitrosamines have been identified. Clarification of how to set limits for products containing salt, hydrate or solvate forms of the API. Update to Q&A 14 to reference the new risk evaluation template for use in marketing authorisation applications.	23rd June 2022
11	Update to Q&A 3 on submission of amended step 1 response and extension of Step 3 deadline for chemical medicines.	29th July 2022
12	Update of Q&A 10 to add nitrosoduloxetine and introduction of Q&A 21 on approach to control presence of nitrosamine while the AI is being established.	10th October 2022
13	Update of Q&A 10 to add N-nitrosofluoxetine, N-nitrosoparoxetine, N-nitrosodiphenylamine, N-nitroso-mefenamic acid, N-nitrosopyrrolidine and N-nitrosodiethanolamine.	5th December 2022
14	Introduction of Q&A 22 on approach to control presence of N-nitrosamine exceeding the AI while CAPAs are being implemented. Update of Q&A 20 to consider the possibility of an interim limit based on the LTL approach during CAPA implementation. Update of Q&A 21 for increased clarity on the application of the temporary universal limit.	22nd December 2022
15	Amendment of Q&A 22 to indicate that no variation should be submitted to implement temporary above AI limits in specifications.	30th March 2023

Q&A for MAHs/Applicants: Q2

Q2. What is the 'call for review'?

The call for review consists of 3 steps:

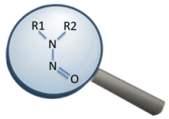
- Step 1: MAHs to perform a risk evaluation to identify if APIs and/or FPs could be at risk of presence of nitrosamine;

- Step 2: if a risk is identified, MAHs to proceed with confirmatory testing in order to confirm or refute the presence of nitrosamines. MAHs should report outcomes as soon as possible;

- Step 3: if the presence of nitrosamine(s) is confirmed, MAHs should implement effective risk mitigating measures through submission of variation.

Q&A for MAHs/Applicants: Q3

Q3. For the 'call for review' for chemically synthesised and biological medicinal products, when and how should MAHs report steps 1 and 2 to competent authorities?



Submission of step 1 outcome:

For product containing:

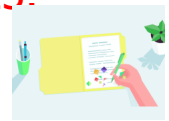
Chemically synthesised APIs -> latest by 31st March 2021.

Biological APIs -> latest by 01st July 2021.

Submission of step 2 outcome:

Chemically synthesised APIs -> latest by 26th September 2022.

Biological APIs -> latest by 01st July 2023.



Submission of any changes required to MA (Step 3):

Chemically synthesised APIs -> latest by 01st October 2023.

Biological APIs -> latest by 01st July 2023.

Q&A for MAHs/Applicants: Q4

Q4. What are the currently identified root causes for presence of nitrosamines?

Classification of the root causes

Risk factors

- Manufacture of the active substance (AS)
  - AS Manufacturing process/syntesis
  - Solvents, catalyst, reagents
  - Carry-over from raw materials
- Finished product
  - Storage
  - FP manufacturing process
  - Excipients
  - Packaging materials - Inks
- GMP aspects
  - Poor-controlled processes
  - Cross-contamination
  - Recovered/recycled solvents/materials

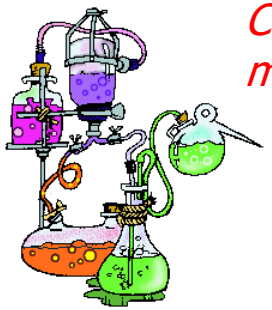
Q&A for MAHs/Applicants: Q4

Risk factors related to the manufacture of the AS (1/2)

1. Use of nitrite salts and esters (e.g. $NaNO_2$, alkyl nitrites), or other nitrosating agents (e.g. nitroso halides, nitrosonium salts, nitrogen oxides, nitro alkanes, halogenated nitro alkanes, Fremy's salt, nitroso sulfonamides), in the presence of secondary or tertiary amines within the same or different steps of the manufacturing process. Sources for secondary or tertiary amines can also be starting materials, intermediates, reagents, solvents (e.g. DMF, DMAc and NMP) and catalysts, which contain amine functionality, amine impurities (e.g. quaternary ammonium salts) or which are susceptible to degradation to reveal amines.

Nitrosating agents +

Sources for/or secondary or tertiary amines



Contaminated raw/ starting materials

Solvents (DMF, DMAc, NMP)

Q&A for MAHs/Applicants: Q4

Risk factors related to the manufacture of the AS (2/2)

2. Nitrite formation by oxidation of hydroxylamine or nitrite release from nitro-aromatic precursors (e.g. by fluoro de-nitration), in the presence of secondary or tertiary amines within the same or different steps of the manufacturing process (see 1).

3. Use of disinfected water (chlorination, chloro-amination, ozonisation) in the presence secondary or tertiary amines within the same or different steps of the manufacturing process (see 1).

4. Oxidation of hydrazines, hydrazides and hydrazones by hypochlorite, air, oxygen, ozone and peroxides in the manufacturing process or during storage.

5. Use of contaminated recovered or recycled materials (e.g. solvents, reagents and catalysts).

6. Use of contaminated starting materials and intermediates supplied by vendors who use processes or raw materials which may contain residual nitrosamines or nitrosating agents.

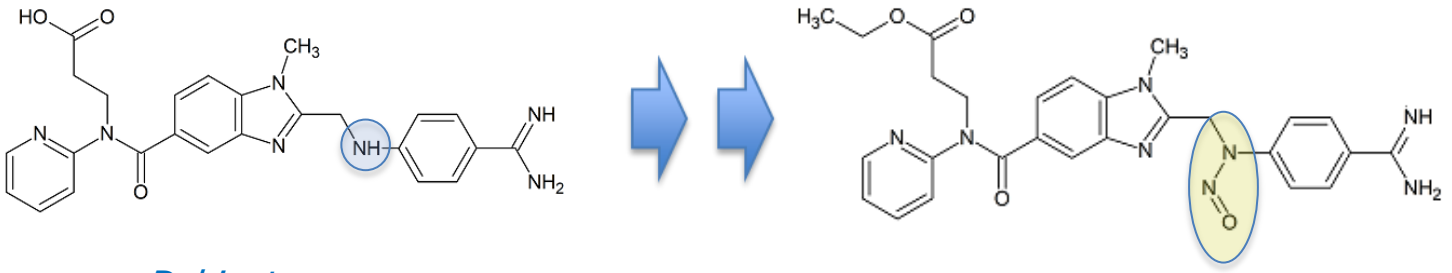
7. Carry-over of nitrosamines deliberately generated (e.g. as starting materials or intermediates) during the manufacturing process.

Q&A for MAHs/Applicants: Q4

Risk factors also related to the finished product (1/5)

8. Reaction of nitrosatable nitrogen functionality in APIs or their impurities/degradants with nitrosating agents present in components of the FP during formulation or storage.

- API-NO formation:"*A particular risk of formation of nitrosamines should be noted for active substances that contain a nitrosatable amine functional group*"



Dabigatran *N-Nitroso Dabigatran*

Q&A for MAHs/Applicants: Q4

Risk factors also related to the finished product (2/5)

- Exipients: "*Nitrites have been identified as impurities in many common excipients*"

Sr. No.	Excipients	HCHO	Hydrogen peroxide	NO_2	NO_3
1.	Microcrystalline cellulose, PH102	0.51 ± 0.5	<2	9.4	23
2.	Lactose monohydrate	1.4	<2	0.28 ± 0.5	0.77 ± 0.5
3.	Lactose anhydrous	2.68 ± 0.5	<2	1.20 ± 0.5	1.20 ± 0.5
4.	Pre-gelatinized starch	2.13 ± 0.5	<2	1.90 ± 0.5	4.45 ± 0.5
5.	Povidone	–	24.74 ± 0.5	0.42 ± 0.5	0.35 ± 0.5
6.	Crospovidone	22.83 ± 0.5	2.12 ± 0.5	4.73 ± 0.5	15.55 ± 0.5
7.	Sodium Starch Glycolate	2.19 ± 0.5	<2	4.52 ± 0.5	46.52 ± 0.5
8.	Croscarmellose Na	0.07 ± 0.5	<2	0.70 ± 0.5	9.54 ± 0.5
9.	Magnesium stearate	0.07 ± 0.5	<2	2.26 ± 0.5	4.59 ± 0.5
10.	Hydroxypropyl Cellulose	1.41	13	0.9	3.5

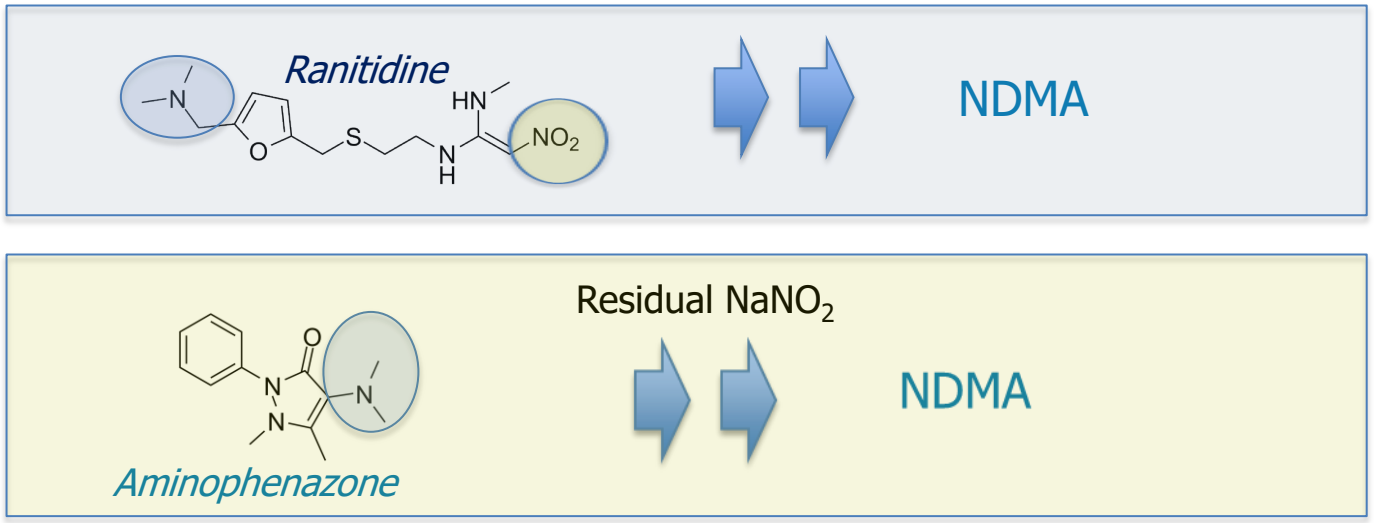
Akkaraju et al. 2023

- API degradation: "*Vulnerable amines could be formed by degradation (e.g. hydrolysis) during formulation or storage.*"

Q&A for MAHs/Applicants: Q4

Risk factors also related to the finished product (3/5)

9. Degradation processes of active substances, including those induced by inherent reactivity (e.g. presence of nitro-alkyl, oxime, or other functionality) or by the presence of an exogenous nitrosating agent. This could potentially occur during both active substance and finished product manufacturing processes or during storage and could be influenced by crystal structure, crystal habit and storage conditions (temperature, humidity etc.).



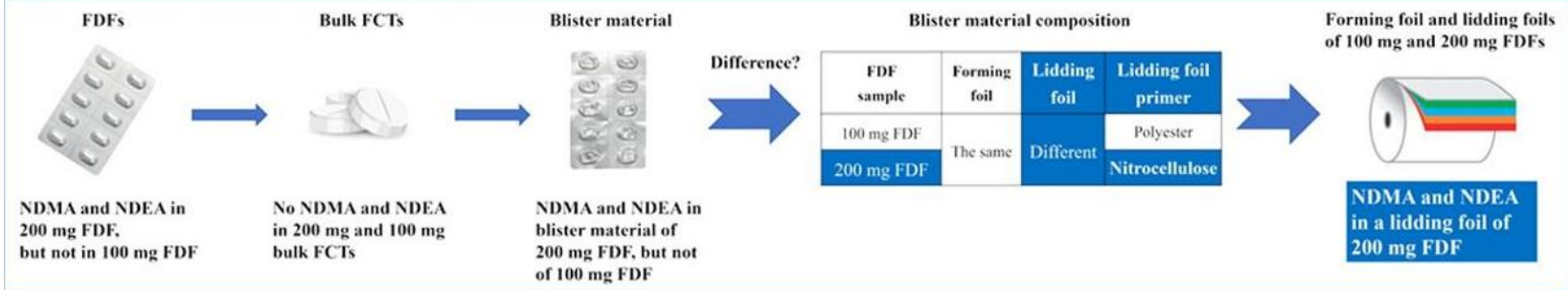
Q&A for MAHs/Applicants: Q4

Risk factors also related to the finished product (4/5)

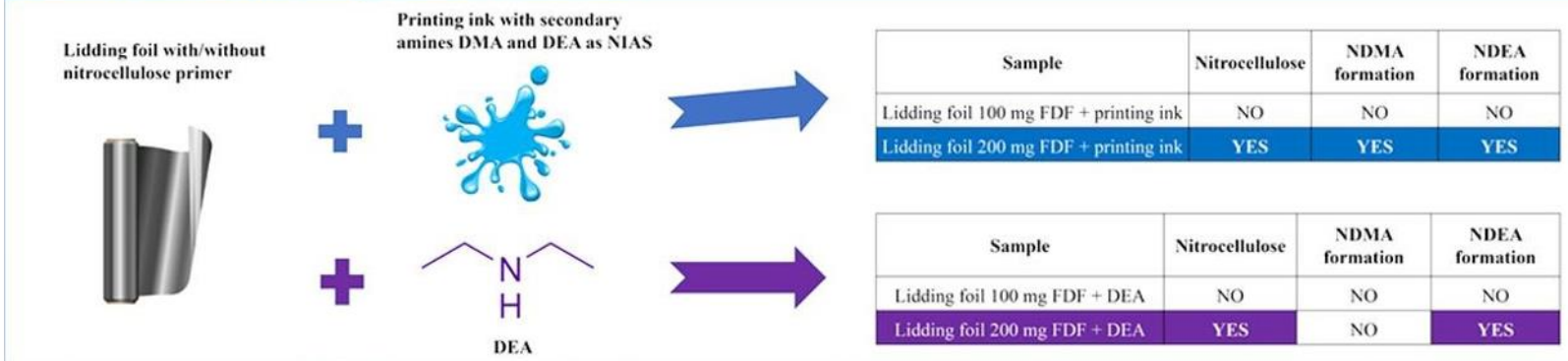
10. Oxidation of hydrazine or other amine-containing functional groups present in active substances or their impurities/degradants (e.g. from hydrazones and hydrazides), either in active substance manufacturing processes or during storage. This root cause has also been observed during manufacture and storage of finished products containing such functional groups. Potential oxidants include oxygen and peroxides (common impurities in some excipients)

11. Use of certain packaging materials. Relevant nitrosamine contamination has been observed in primary packaging of finished products in blister with lidding foil containing nitrocellulose. During the blister heat-sealing process, nitrogen oxides can be generated thermally from nitrocellulose. Under these conditions, nitrosamines have been shown to form from low molecular weight amines present either in printing ink or in the finished product and to transfer to the product and/or to the cavity via evaporation and condensation.

Detection of nitrosamines in FDF and root cause investigation



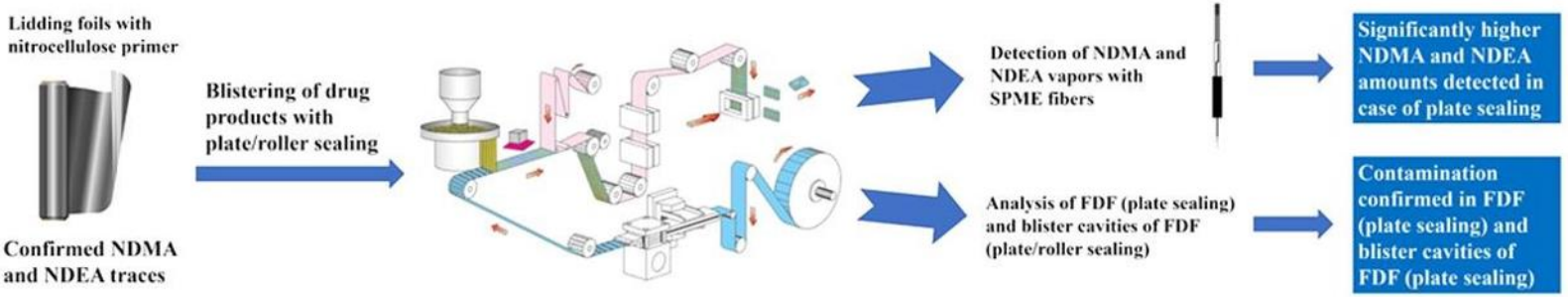
Formation of nitrosamines in a lidding foil containing nitrocellulose



Sample	Nitrocellulose	NDMA formation	NDEA formation
Lidding foil 100 mg FDF + printing ink	NO	NO	NO
Lidding foil 200 mg FDF + printing ink	YES	YES	YES

Sample	Nitrocellulose	NDMA formation	NDEA formation
Lidding foil 100 mg FDF + DEA	NO	NO	NO
Lidding foil 200 mg FDF + DEA	YES	NO	YES

Transfer of nitrosamines from lidding foil to drug product during blistering operation



''Nitrocellulose blister material as a source of N-nitrosamine contamination of pharmaceutical drug products'' Nejc Golob, Rok Grahek, Malcolm Ross, Robert Roškar; Int. J .Pharm., 2022 Mar 18.

Q&A for MAHs/Applicants: Q4

Risk factors also related to the finished product (5/5)

12. Reaction of amines leaching from quaternary ammonium anion exchange resins (e.g. used for purification steps) with nitrosating agents present in the liquid phase.

Water production

A recent example of this was in the production of water for injections where residual chloramine used to disinfect incoming water reacted with dimethylamine leaching from the anion exchange resin used in the demineralisation step to form NDMA.

The same risks could be associated with active substances or finished products manufactured using water purified using similar resins.

Q&A for MAHs/Applicants: Q4

Risk factors related to GMP aspects

13. Cross-contamination due to different processes being run successively on the same manufacturing line.

e.g. caused by ineffective cleaning procedures

14. Carry-over of impurities between process steps due to operator-related errors or insufficiently detailed batch records such as inadequate phase separations during work-up procedures.

15. Use of contaminated recovered or recycled materials (e.g. solvents, reagents and catalysts)

e.g. caused by ineffective/non-validated recovery procedures

Cross-contamination between different processes

Q&A for MAHs/Applicants: Q6

Q6 What factors should be considered in prioritising the risk evaluation?

When conducting the risk evaluation and risk assessment, MAHs should use a risk-based approach to prioritise products for evaluations and confirmatory testing.

MAHs may consider factors such as:

- the maximum daily dose taken for the concerned medicinal product,
- duration of treatment,
- therapeutic indication,
- number of patients treated.

For example, medicinal products with higher daily dose and those for chronic use may take priority.

Q&A for MAHs/Applicants: Q7

Q7 How should the risk evaluation be performed? (1/2)

- ICH Q9 guideline MAHs/Applicants in collaboration with API, FP manufacturers and their raw material suppliers are required to perform risk evaluations using quality risk management principles, as outlined in ICH Q9 guideline.

- Q4 of the Q&A. MAHs/Applicants and manufacturers should consider as part of the risk evaluation all potential sources of contamination or formation of nitrosamine, notably the root causes listed under Q4.

If, after completion of the risk evaluation, a risk is identified in the API and/or the FP, MAHs/applicants must notify the competent authorities of the identified risk, proceed without further delay with confirmatory tests and introduce any necessary changes to the dossier.

Q&A for MAHs/Applicants: Q8

Q8 How should confirmatory tests be conducted by MAHs and manufacturers? (1/4)

For the purpose of confirmatory testing as part of step 2 of the call for review to MAHs, testing should generally be carried out on the FP.

FP testing →

However, some root causes may only be linked to the API manufacturing process (see Q&A 4). In these cases, testing of the API or intermediates upstream of the active substance could be used as a surrogate for testing the finished product, provided that the risk assessment performed on the FP concluded no additional risk factors for formation of nitrosamine impurities in the finished product (see Q&A 4, risk factors related to the finished product).

API/ intermediates testing →

In any case, if the control point of nitrosamines is not in the finished product, the responsibility for quality lies with the MAH.

Q&A for MAHs/Applicants: Q8

Q8 How should confirmatory tests be conducted by MAHs and manufacturers? (2/4)

The number of batches to be tested should be commensurate with the risk. MAHs and manufacturers should test a representative number of batches of FP and the relevant SM, intermediates, API or raw materials as applicable.

- If the source of risk has been identified and is well understood (e.g. by spike and purge studies) such that impurity levels are expected to be consistent from batch to batch, testing should be conducted on 10% of annual batches, or 3 per year, whichever is highest. This includes testing not only of newly produced batches but also retained samples of batches still within expiry date.

- If fewer than 3 batches are manufactured annually, then all batches should be tested.

If multiple manufacturers, manufacturing processes and/or sources of at-risk raw materials are used, then testing of additional batches would be necessary to cover these risk factors.

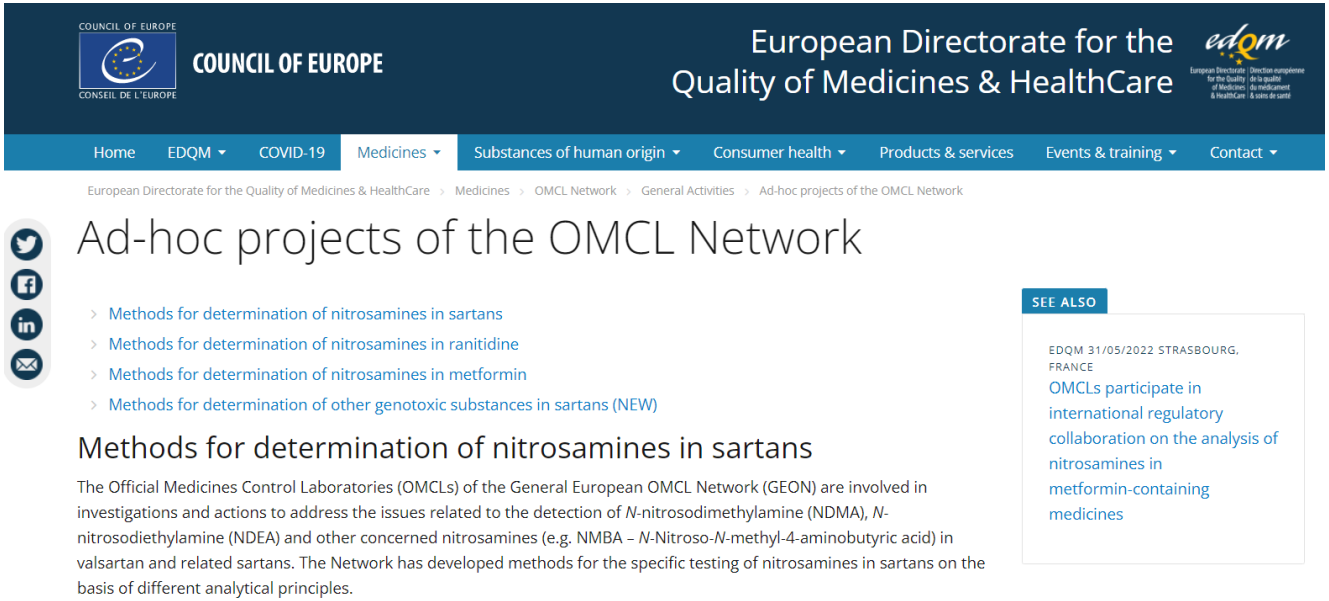
If a product is available in multiple strengths of the same dosage form with the same risk factors applicable to each, then testing could be rationalised by testing only the worst-case scenario strength.

The worst-case approach should be justified by the MAH on a case by case basis.

Q&A for MAHs/Applicants: Q8

Q8 How should confirmatory tests be conducted by MAHs and manufacturers? (3/4)

Methods for determination of various nitrosamines in sartans with a tetrazole ring, metformin and ranitidine have already been developed by the Official Medicines Control Laboratories and are available for reference on the European Directorate for the Quality of Medicines & HealthCare (EDQM) website. These may serve as a starting point for the development and validation of analytical methods for testing other APIs/FPs.



Q&A for MAHs/Applicants: Q8

Q8 How should confirmatory tests be conducted by MAHs and manufacturers? (4/4)

Given the trace levels of nitrosamines to be measured, the following technical aspects should be considered when developing analytical methods:

- Interference caused by presence of trace amounts of nitrosamines in testing materials utilised (e.g. water, airborne sources, plastics products and rubber/elastomeric products);
- Contamination during sample preparation (avoiding cross contaminations from gloves, membranes, solvents etc.) which could lead to false positive results;
- In situ formation of nitrosamines during analysis;
- Use of accurate mass techniques are required (MS/MS or high-resolution accurate mass systems) in order to overcome interference in the identification of the specific peak of a certain nitrosamine (e.g. false positives have been observed from DMF co-eluting with NDMA)

Q&A for MAHs/Applicants: Q9

Q9 What are the requirements of the analytical method(s)?

The analytical methods need to be sufficiently sensitive in order to adequately detect and quantify trace levels of nitrosamine impurities.

nitrosamines quantity ($10^{-6} \div 10^{-9}$)

- The limit of quantification (LoQ) provides the minimum level at which an analyte can be quantified with acceptable accuracy and precision and should thus be used for impurity testing and decision-making;

If quantitative testing is performed:

1. as a routine control -> LoQ ≤ of the acceptable limit (AL)*;
2. to justify skip testing -> LoQ ≤ 30% of the AL;
3. to justify omission of specification -> LoQ ≤ 10% of the AL.

**The AL should be based on the relevant acceptable intake (AI) for the respective nitrosamine impurity*

Q&A for MAHs/Applicants: Q10

Q10 Which limits apply for nitrosamines in medicinal products? (1/5)

ICH M7 (R1) guideline defines N-nitrosamines as substances of the "cohort of concern" for which limits in medicinal products refer to the so-called substance-specific acceptable intake (AI) (the Threshold of Toxicological Concern, TTC, value of 1.5 ug/day cannot be applied) which is associated with a negligible risk (theoretical excess cancer risk of <1 in 100,000 over a lifetime of exposure). The calculation of AI assumes a lifelong daily administration of the maximum daily dose of the medicinal product and is based on the approach outlined in the ICH M7 (R1) guideline as well as the principles described in relation to the toxicological evaluation in the assessment report of the CHMP's Article 5(3) opinion on nitrosamine impurities in human medicinal products.

Q&A for MAHs/Applicants: Q10

Q10 Which limits apply for nitrosamines in medicinal products? (2/5)

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

N-Nitrosamine (CAS number)	ng/day[1,*]	Source[2]
N-Nitrosodimethylamine, NDMA[3,4] (62-75-9)	96.0	
N-Nitrosodiethylamine, NDEA[3,4] (55-18-5)	26.5	
N-Nitrosoethylisopropylamine, EIPNA[3,5] (16339-04-1)	26.5	
N-Nitrosodiisopropylamine, DIPNA[3,5] (601-77-4)	26.5	
N-Nitroso-*N*-methyl-4-aminobutyric acid, NMBA[3,6] (61445-55-4)	96.0	
1-Methyl-4-nitrosopiperazine, MeNP[5] (16339-07-4)	26.5	Rifampicin
N-Nitroso-di-*n*-butylamine, NDBA[3,5] (924-16-3)	26.5	
N-Nitroso-*N*-methylaniline, NMPA[3,4] (614-00-6)	34.3	
N-Nitrosomorpholine, NMOR[3,7] (59-89-2)	127	
N-Nitrosovarenicline, NNV[8]	37.0	Varenicline
N-Nitrosodipropylamine, NDPA (621-64-7)[3,5]	26.5	
N-Nitrosomethylphenidate[9], NMPH, (55557-03-4)	1300	Methylphenidate
N-Nitrosopiperidine[3] (100-75-4)	1300	

Q&A for MAHs/Applicants: Q10

Q10 Which limits apply for nitrosamines in medicinal products? (3/5)

API-NO

N-Nitrosamine (CAS number)	ng/day[1,*]	Source[2]
N-Nitrosorasagiline[10]	18	Rasagiline
7-Nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo-[4,3- a]pyrazine[11]	37	Sitagliptin
N-Nitroso-1,2,3,6-tetrahydropyridine, NTHP[3] (55556-92-8)	37	
N-Nitrosonortriptyline[12]	8	Amitriptyline, nortryptyline
N-Methyl-*N*-nitrosophenethylamine, NMPEA[3] (13256-11-6)	8	
N-Nitrosodabigatran[10]	18	Dabigatran
4-(Methylnitrosoamino)-1-(3-pyridinyl)-1-butanone (NNK)[7]	100	
N-nitrosoduloxetine[13]	100	Duloxetine
N-nitroso-fluoxetine[13]	100	Fluoxetine
N-nitrosoparoxetine[9]	1300	Paroxetine
N-nitroso-diphenylamine NDPh[14] (86-30-6)	78000	
N-nitroso-mefenamic acid [15]	78000	Mefenamic acid
N-nitroso-pyrrolidine NPYR[3,7] (930-55-2)	1700	
N-nitroso-diethanolamine NDELA [3,7](1116-54-7)	1900	

Q&A for MAHs/Applicants: Q10

Q10 Which limits apply for nitrosamines in medicinal products? (4/5)

Calculation of the limit when a new nitrosamine is identified
Two scenarios are foreseen :

- If N-nitrosamines are identified with sufficient substance specific animal carcinogenicity data, TD50 should be calculated and used to derive a substance specific limit for lifetime exposure as recommended in ICH M7(R1) guideline,

- If N-nitrosamines are identified without sufficient substance specific data to derive a substance specific limit for lifetime exposure as recommended in ICH M7(R1) guideline.
  - a class specific TTC for nitrosamines of 18 ng/day (derived from the Lhasa carcinogenic potency database) can be used as default option can be used as default option.
  - an approach based on SAR considerations to derive an acceptable intake limit is acceptable, if appropriately justified.

The approach taken needs to be duly justified by the MAH/Applicant.

In all above cases, the MAH/Applicant is required to liaise with the relevant competent authorities in order to verify acceptability of the approach taken

Q&A for MAHs/Applicants: Q10

Q10 Which limits apply for nitrosamines in medicinal products? (5/5)

For determining limits in the case of presence of more than one nitrosamine, two approaches are considered acceptable in order not to exceed the acceptable risk level of 1:100,000 as outlined in ICH M7(R1) guideline:

1. The total daily intake of all identified N-nitrosamines not to exceed the AI of the most potent Nitrosamine identified, or
2. Total risk level calculated for all identified N-nitrosamines not to exceed 1 in 100,000.

The approach chosen needs to be duly justified by the MAH/Applicant.

Acceptability of a negative in vitro bacterial reverse mutation test

At present, negative in vitro bacterial reverse mutation tests are not accepted by the EU network and international regulators as sole evidence for lack of mutagenic potential for nitrosamines and classification as Class 5 impurities according to ICH M7. This is because some nitrosamines, which have elicited negative tests, have been shown to be carcinogenic in vivo and there are concerns that experimental conditions such as choice/concentration of solvent and metabolic activation system are not optimal for formation of activated species extracellularly which may also not be stable enough to reach DNA.

Q&A for MAHs/Applicants: Q12

Q12 Which are the measures to mitigate the risk of presence of nitrosamines ?

The presence of N-nitrosamines in the FP shall be mitigated and shall be at or below the limit.

MAHs shall design or adapt the manufacturing process of their FPs to prevent formation of and contamination with nitrosamines.

Some examples:

- Changes in manufacturing process,
- Changes in SM/raw material/excipients quality,
- Changes in suppliers of raw materials/starting materials/excipients;
- Segregating some production steps in dedicated equipment (in order to avoid cross contamination),
- Avoiding the use of recycled/recovered materials;
- Changes in packaging systems/materials;
- Changes in formulation;
- Changes in storage conditions of DS/DP.

Q&A for MAHs/Applicants: Q14

Q14 What is the approach for new and ongoing marketing authorisation applications (MAA)?

At the submission stage:

Step 1

The risk evaluation should be submitted as an attachment to Module 1 with a corresponding reference in Module 3.2 of the MA dossier.

To supplement the detailed risk evaluation, the template located on the CMDh nitrosamine website could also be submitted: https://www.hma.eu/human-medicines/cmdh/advice-from-cmdh/nitrosamine-impurities.html.

The template is optional for CAPs. For NAPs, and DCPs, the template is mandatory and the CMDh practical guidance located in the same section of the same website should be followed.

Step 2

If a risk of presence of nitrosamines in the medicinal product is identified, applicants are required to provide the risk assessment outlining the impact on the benefit-risk balance of the product and a risk mitigation strategy. Applicants should also submit confirmatory testing plans or confirmatory testing data as mentioned in step 2 (see Q&A 2).

Q&A for MAHs/Applicants: Q14

Q14 What is the approach for new and ongoing marketing authorisation applications (MAA)?

Risk evaluation CMDH template

May 2022
CMDh/439/2022

	Currently identified risk factors for presence of nitrosamines (Q4 of EMA/409815/2020)	Evaluated? (Yes / No)						Reference to annexed background documents
		DS manuf. 1	DS manuf. 2	DS manuf. xxx	DP manuf. 1	DP manuf. 2	DP manuf. xxx	
	Risk factors related to the manufacture of the active substance:							
1	Use of nitrite salts and esters (e.g. $NaNO_2$, alkyl nitrites), or other nitrosating agents (e.g. nitroso halides, nitrosonium salts, nitrogen oxides, nitro alkanes, halogenated nitro alkanes, Fremy's salt, nitroso sulfonamides), in the presence of secondary or tertiary amines within the same or different steps of the manufacturing process. Sources for secondary or tertiary amines can also be starting materials, intermediates, reagents, solvents (e.g. DMF, DMAc and NMP) and catalysts, which contain amine functionality, amine impurities (e.g. quaternary ammonium salts) or which are susceptible to degradation to reveal amines.				NA	NA	NA	
2	Nitrite formation by oxidation of hydroxylamine or nitrite release from nitro-aromatic precursors (e.g. by fluoro de-nitration), in the presence of secondary or tertiary amines within the same or different steps of the manufacturing process (see 1).				NA	NA	NA	
3	Use of disinfected water (chlorination, chloro-amination, ozonisation) in the presence secondary or tertiary amines within the same or different steps of the manufacturing process (see 1).				NA	NA	NA	
4	Oxidation of hydrazines, hydrazides and hydrazones by hypochlorite, air, oxygen, ozone and peroxides in the manufacturing				NA	NA	NA	

Q&A for MAHs/Applicants: Q14

Q14 What is the approach for new and ongoing marketing authorisation applications (MAA)?

For new and on-going marketing authorisation applications, the number of batches to be tested as part of any confirmatory testing should be commensurate with the risk in line with ICH M7(R1) guideline.

The source of risk has to be well understood (e.g. by spike and purge studies) such that impurity levels are expected to be consistent from batch to batch. Test results from a minimum of 6 pilot scale batches or 3 production scale batches may be sufficient. Depending on the risk factors for nitrosamine presence, e.g. with risk factors being closer to the FP, more batches may need to be tested.

If multiple manufacturers, manufacturing processes and/or sources of at-risk raw materials are used, then testing of additional batches would be necessary to cover these risk factors.

Q&A for MAHs/Applicants: Q15

Q15 When should a test for nitrosamines be included in the MA dossier?

When a nitrosamine is identified after Step 2 confirmatory testing, a limit will usually need to be included in the specifications of the finished product and the product must comply if tested.

If the root cause has been identified in the finished product manufacturing process or storage, or nitrosamines have been detected in the finished product, but the actual source of contamination remains unclear, routine testing of the finished product is required by default.

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 based on acceptable intake (AI) in the finished product.

Testing is usually expected to be carried out in the finished product, however if the source of a nitrosamine impurity is identified in the active substance manufacturing process, control options 1 to 3 as stated in ICH M7(R1) guideline could be used to demonstrate that the nitrosamine will not be present above the acceptable limit based on AI in the finished product.

Q&A for MAHs/Applicants: Q15

Q15 When should a test for nitrosamines be included in the MA dossier?

Testing of raw materials (e.g. excipients) should also be considered if these are potential sources of nitrosamine impurities.

Exceptions from routine testing may be possible, if the root cause of contamination is demonstrated to be well-understood:

- Only if the amount of nitrosamine present is consistently below 10% of the acceptable limit based on AI in the API or in the finished product, then a test for the nitrosamine could be omitted from the specification. → *No testing*

- Only if levels of a single nitrosamine are consistently below 30% of the acceptable limit based on AI in the API or the finished product, skip-testing according to the ICH Q6A definition could be acceptable. → *Skip testing*

Q&A for MAHs/Applicants: Q21

Q21 What is the approach to control the presence of nitrosamines until a substance specific AI is established?

To protect public health, to inform decisions on required market actions while ensuring at the same time availability of medicines while a formal AI is established, a temporary AI (t-AI) of 178 ng/day (total nitrosamines) can be adopted by the relevant authorities for marketed medicines identified to contain one or more nitrosamines exceeding the TTC of 18ng/day.

This t-AI has been derived using TD50 values calculated in the Lhasa carcinogenic potency database and is based on a probabilistic approach that there is a 33% risk that the "true" AI is below the t-AI. It is expected that the t-AI would be used for a period of less than 12 months, as an exposure over this period of time is not expected to increase the theoretical overall lifetime risk above 1:100,000.

In practice, this means that when competent authorities are notified about a product containing a new N-nitrosamine exceeding the TTC limit of 18 ng/day, no market actions may be required for batches with N-nitrosamine levels ≤178 ng/day at the MDD pending the agreement of the AI. The adoption of the t-AI is not automatic and is evaluated by the relevant authorities at the time of notification. Use of the t-AI beyond 12 months will require additional consultation with competent authorities.

Q&A for MAHs/Applicants: Q22

Q22 What is the approach to control presence of Nnitrosamine exceeding the AI during CAPA implementation?

In accordance with the regulatory steps taken by authorities following the identification of an N-nitrosamine exceeding the AI and outlined in Q&A20, the less-than lifetime (LTL) concept or the use of interim limits may be considered by the lead authority and NCAs on a temporary basis in order to inform market actions and at the same time ensure availability of medicines.

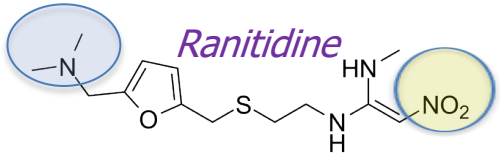
The approach is applicable to all authorised products that have:

- a duration of treatment not exceeding 10 years;

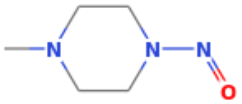
- CAPA implementation timeline of up to 3 years from the establishment and publication of the AI (nevertheless MAHs are expected to expedite CAPAs implementation).

Nitrosamines in other medicines: regulatory actions

April 2020 - Suspension of Ranitidine-containing medicines: NDMA was found in these medicines may form from the degradation of ranitidine itself with increasing levels seen over its shelf life.
It is not clear whether NDMA can also be formed from ranitidine inside the body -> the CHMP has recommended a precautionary suspension of these medicines in the EU.



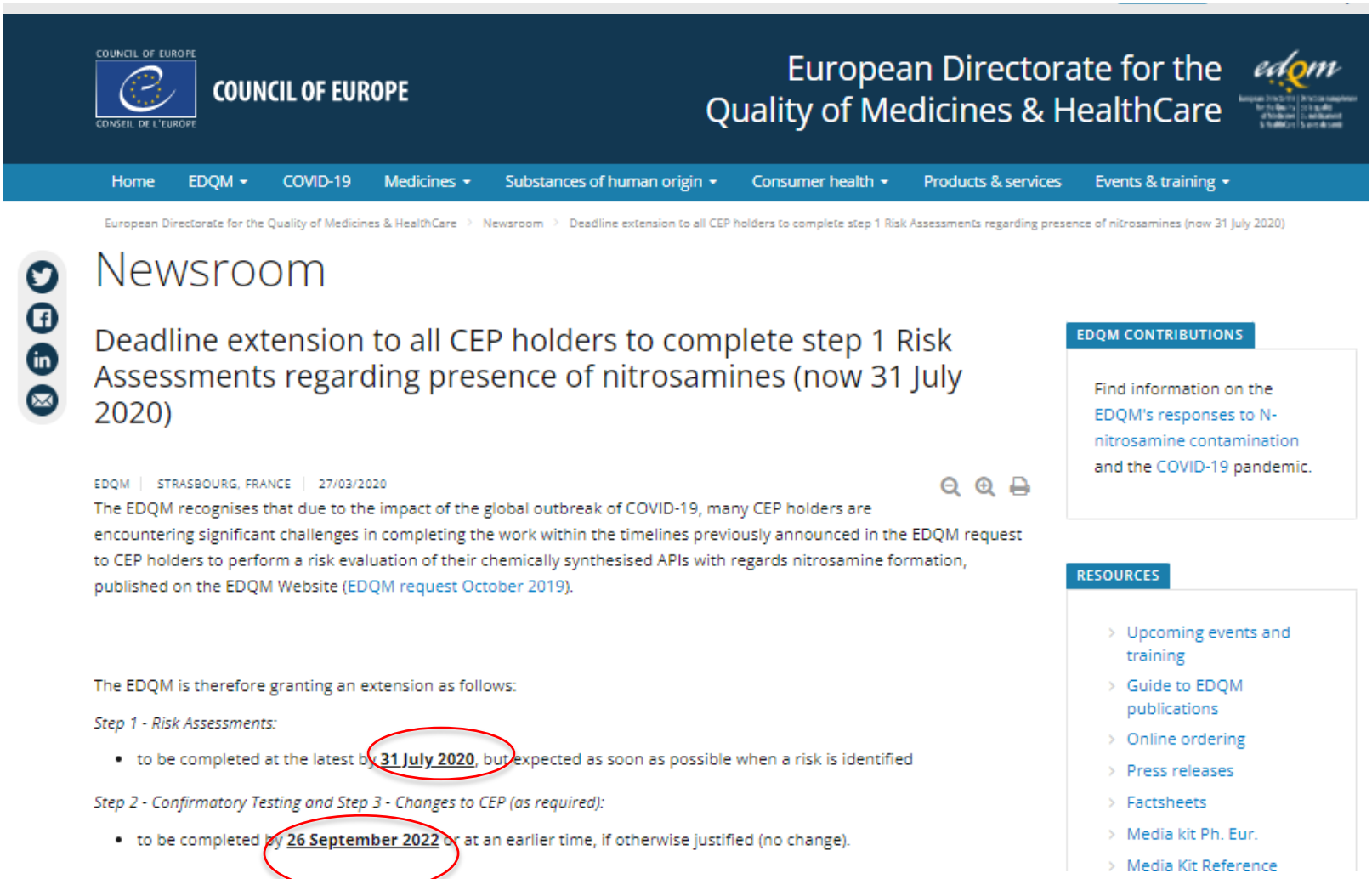
February 2021 national competent authorities are asking marketing authorisation holders for rifampicin-containing medicines to test their medicines before releasing them onto the market -> 1-nitroso-4-methyl piperazine was found in these medicines. Rifampicin is a first-line treatment for tuberculosis.



1-nitroso-4-methyl piperazine

In 2019 NDMA was found in some EU batches of metformin-containing medicines, used for the treatment of diabetes. In October 2020, EMA and the NCAs are asking MAHs for metformin-containing medicines to test their medicines before releasing them onto the market. As metformin is considered a critical medicine, EMA and NAs are cooperating closely to avoid possible shortages so patients can continue to get the treatments they need.

The call for review for CEP Holder



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European Directorate for the Quality of Medicines & HealthCare > Newsroom > Deadline extension to all CEP holders to complete step 1 Risk Assessments regarding presence of nitrosamines (now 31 July 2020)

Newsroom

Deadline extension to all CEP holders to complete step 1 Risk Assessments regarding presence of nitrosamines (now 31 July 2020)

EDQM | STRASBOURG, FRANCE | 27/03/2020

The EDQM recognises that due to the impact of the global outbreak of COVID-19, many CEP holders are encountering significant challenges in completing the work within the timelines previously announced in the EDQM request to CEP holders to perform a risk evaluation of their chemically synthesised APIs with regards nitrosamine formation, published on the EDQM Website (EDQM request October 2019).

The EDQM is therefore granting an extension as follows:

Step 1 - Risk Assessments:

- to be completed at the latest by **31 July 2020**, but expected as soon as possible when a risk is identified

Step 2 - Confirmatory Testing and Step 3 - Changes to CEP (as required):

- to be completed by **26 September 2022** or at an earlier time, if otherwise justified (no change).

EDQM CONTRIBUTIONS

Find information on the EDQM's responses to N-nitrosamine contamination and the COVID-19 pandemic.

RESOURCES

- Upcoming events and training
- Guide to EDQM publications
- Online ordering
- Press releases
- Factsheets
- Media kit Ph. Eur.
- Media Kit Reference

Eur. Ph. monographs changes

In February 2021, the Ph. Eur. Commission revised the five monographs on sartans with a tetrazole ring, namely:

- Valsartan (2423),
- Losartan potassium (2232),
- Irbesartan (2465),
- Candesartan cilexetil (2573),
- Olmesartan medoxomil (2600),

A reference to general chapter 2.5.42. N-Nitrosamines in active substances was introduced in the Production section to assist manufacturers. The five revised monographs become legally binding on 1 April 2021.

2.5.42 Eur. Ph. monograph

2.5.42. *N*-NITROSAMINES IN ACTIVE SUBSTANCES

This chapter describes analytical procedures for the detection of various *N*-nitrosamines in particular active substances. Procedures A and B have been validated as limit tests (30 ppb) and procedure C has been validated as a quantitative test. The scope of each procedure is defined in Table 2.5.42.-1. With these three procedures, it is possible to analyse the following *N*-nitrosamines: *N*-nitroso-dimethylamine (NDMA); *N*-nitroso-diethylamine (NDEA); *N*-nitroso-dibutylamine (NDBA); *N*-nitroso-*N*-methyl-4-aminobutyric acid (NMBA); *N*-nitroso-diisopropylamine (NDiPA); *N*-nitroso-ethyl-isopropylamine (NEiPA) and *N*-nitroso-dipropylamine (NDPA).

Procedure A uses deuterated *N*-nitroso-diethylamine (NDEA-d_{10}) as internal standard. Procedures B and C use *N*-nitroso-ethylmethylamine (NEMA) as internal standard.

When a procedure is applied to substances outside of the scope covered by the initial validation (see Table 2.5.42.-1) or to medicinal products or if procedure A or B is used quantitatively, then it must be validated.

Table 2.5.42.-1. – *Scope of the validation*

isopropylamine CRS). In a single volumetric flask, dilute 300 µL of each of these CRS to 50.0 mL with *methanol R3*. Dilute 300 µL of this solution to 100.0 mL with *methanol R3*.

Test solution. Suspend 150.0 mg of the substance to be examined in 0.5 mL of *methanol R3*. Add 0.5 mL of the internal standard solution. Mix thoroughly for 5 min and sonicate for 15 min. Add 4.0 mL of *water for chromatography R*. Mix thoroughly for 5 min and sonicate for 15 min. Centrifuge at about 3000 *g* for 5 min. Filter the supernatant through a membrane filter (nominal pore size 0.20 µm). Use the filtrate.

Spiked solution. Suspend 150.0 mg of the substance to be examined in 0.5 mL of the *N*-nitrosamines spiking solution. Add 0.5 mL of the internal standard solution. Mix thoroughly for 5 min and sonicate for 15 min. Add 4.0 mL of *water for chromatography R*. Mix thoroughly for 5 min and sonicate for 15 min. Centrifuge at about 3000 *g* for 5 min. Filter the supernatant through a membrane filter (nominal pore size 0.20 µm). Use the filtrate.

Reference solution. Dilute 0.5 mL of the *N*-nitrosamines spiking solution with 0.5 mL of the internal standard solution. Mix thoroughly for 5 min and sonicate for 15 min. Add 4.0 mL of *water for chromatography R*. Mix thoroughly for 5 min and sonicate for 15 min. Centrifuge at about 3000 *g* for 5 min. Filter through a membrane filter (nominal pore size 0.20 µm). Use the filtrate.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 40 °C.

Procedure A=LC-MS/MS
Procedure B=GC-MS
} Validated as limit test (30 ppb)
Procedure C=GC-MS/MS → Validated as a quantitative test

Table 2.5.42.-1. – *Scope of the validation*

Active substance (monograph number)	NDMA	NDEA	NDBA	NMBA	NDiPA	NEiPA	NDPA
Candesartan cilexetil (2573)	A*BC	ABC	C	A	AC	AC	C
Irbesartan (2465)	A*BC	ABC	C	A	AC	AC	C
Losartan potassium (2232)	A*BC	ABC	C	A	AC	AC	C
Olmesartan medoxomil (2600)	A*BC	ABC	C	A	AC	AC	C
Valsartan (2423)	A*BC	ABC	C	A	AC	AC	C

* In procedure A, the presence of dimethylformamide (DMF) in the substance to be examined may interfere with the detection of NDMA.

EDQM and CEPs actions

All CEPs for ranitidine hydrochloride are suspended, as the EDQM was informed about the presence of low levels of NDMA in medicinal products containing this active substance.

The EDQM has reviewed the CEPs for metformin active substance and it has been concluded that the presence of nitrosamines is not related to the active substance but to the medicinal product: no action has therefore been taken with regard to CEPs for metformin.

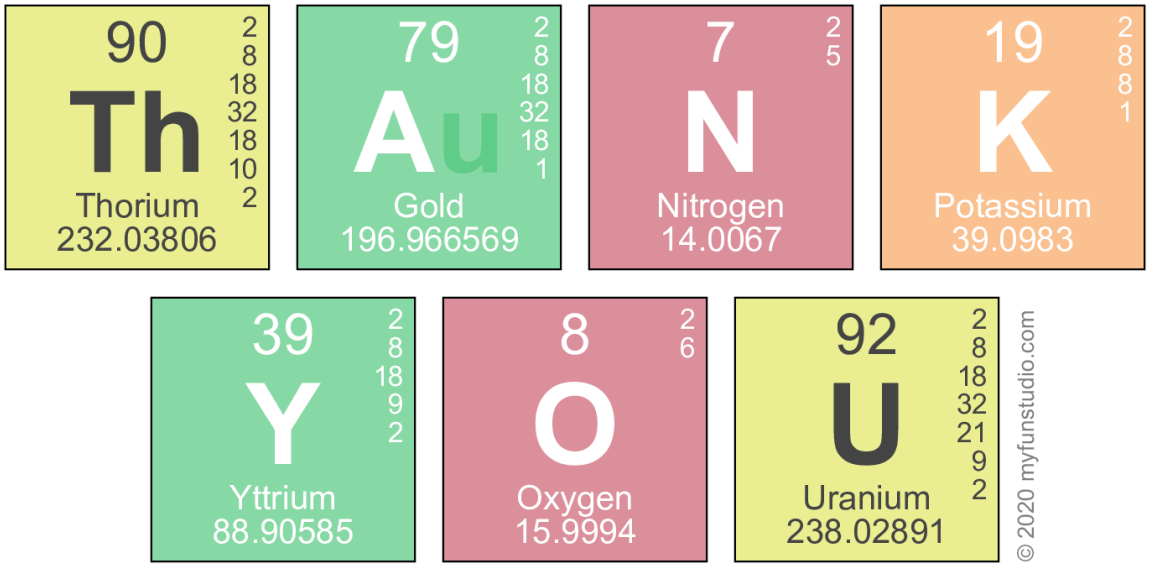
Controls for nitrosamine impurities have also been introduced in certain CEPs for pioglitazone hydrochloride, rizatriptan benzoate, prednisolone, clarithromycin and tigecycline.

The Nitrosamine Implementation Oversight Group (NIOG)

The Nitrosamine Implementation Oversight Group (NIOG) oversees the harmonised implementation of the CHMP's Article 5(3) opinion on nitrosamines.

It was set up by the European medicines regulatory network under the February 2021 implementation plan, and reports on progress to EMA's Management Board and the Heads of Medicines Agencies (HMA).

The group contains representatives from the CHMP, CMDh, EMA working parties, EDQM and EMA staff. It also acts as the main interface for the pharmaceutical industry stakeholders to discuss regulatory and scientific developments on nitrosamines with EMA and the European medicines regulatory network.



Un ringraziamento particolare alla Dott.ssa Laura Galatti!

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