Nitrosamines as Impurities in Drugs; Health Risk Assessment and Mitigation Workshop Day 1

March 29, 2021

House Rules

Muting Phones: If you are not speaking please keep your phone on mute.

Note: All attendees will be muted, only panelists have the ability to unmute.

Video Feature: Only the panelists engaging in the discussions will have their video feature turned on. If you are not speaking, please turn the video feature off.

Questions & Discussion:

- Submit any questions that you would like answered by using the “Q&A” feature to the bottom center of your screen in Zoom.
- If you are a panelist who will be speaking, please check your “Chat” feature as the host will prompt you when it is time for you to present.
- Please use the “Q&A” box so that the moderator and workshop hosts can work together to monitor the questions and make sure they are addressed as time allows during the workshop. Panelists will answer questions live as time permits, you will not receive an answer in the “Q&A” box.

Purpose and Goals of the Workshop

Dr. Aisar Atrakchi, FDA

N-Nitroso Compounds (NOC) in the Human Environment: Lessons and Issues

Gerhard Eisenbrand

Senior Research Professor for Food Chemistry and Toxicology

TU Kaiserslautern (Retired)

Heidelberg, Germany

Discovery and early research

Freund HA (**1937**)

Clinical manifestations and studies in parenchymatous hepatitis.

Ann. Internal. Med. 10: 1144-1155

Barnes JM, Magee PN (**1954**)

Some toxic properties of dimethylnitrosamine

Brit. J. Ind. Med. 11: 167-174

Magee PN, Barnes JM (**1956**)

The production of malignant primary hepatic tumours in the rat by feeding dimethylnitrosmine.

Brit. J. Cancer 10: 114-122

Schmähl D, Preussmann R (**1959**)

The carcinogenic effect of nitrosodimethylamine in Rats.

Naturwissen. 46: 175

Schmähl D, Preussmann R, Hamperl H (**1960**)

Leberkrebserzeugende Wirkung von Diethylnitrosamin nach oraler Gabe bei Ratten.

Naturwissen. 47: 89

Magee PN, Farber E (**1962**)

Toxic liver injury and carcinogenesis. Methylation of rat liver nucleic acids by dimetylnitrosamine *in vitro.*

Biochem. J. 83: 114-124

Pivotal publications: dose response & structure/activity

Zeitschrift für Krebsforschung 69, 103—201 (1967)

Organotrope carcinogene Wirkungen bei 65 verschiedenen N-Nitroso-Verbindungen an BD-Ratten* **

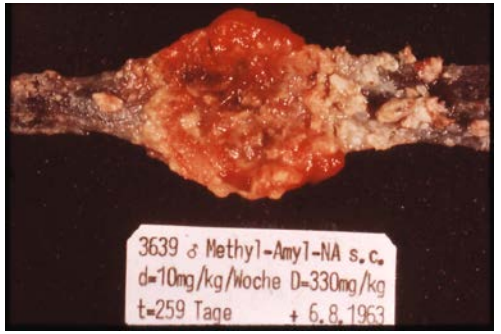
H. Druckrey, R. Preussmann, S. Ivankovic und D. Schmähl***

unter Mitarbeit von

J. Afkham*, G. Blum*, H. D. Mennel*, M. Müller
P. Petropoulos und H. Schneider

Forschergruppe Präventivmedizin am Max-Planck-Institut für Immunologie in Freiburg i. Br.

Eingegangen am 26. Oktober 1966

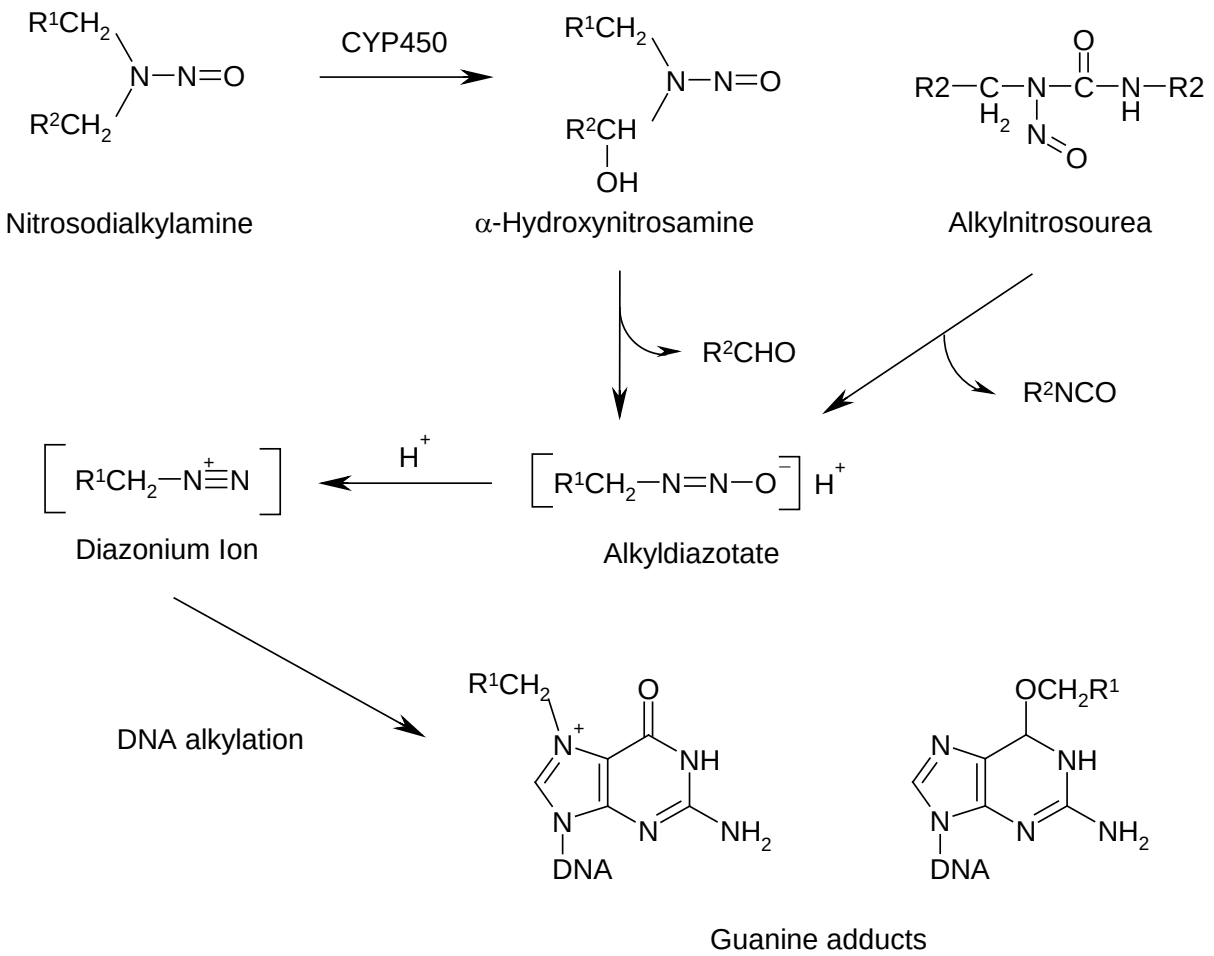


Peto R, Gray R, Brantom P, Grasso P (1991). Effects on 4080 rats of chronic ingestion of N-nitrosodiethylamine or N-Nitrosodimethylamine:
A Detailed Dose-Response Study. Cancer Res 51: 6415-6451.-

The biological activities of NOC

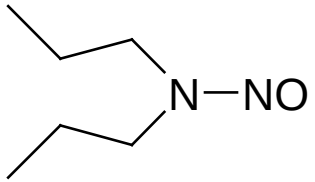
- Over 90% of > 300 NOC known are carcinogenic in animal experiments.
- NDMA, NDEA and tobacco specific NOC like NNK exert carcinogenic effects in a very wide spectrum of animal species, up to subhuman primates. No species found to be resistant.
- Structure-activity studies have helped to identify structural elements responsible for carcinogenicity, and vice-versa, those abrogating carcinogenicity.
- The organotropic action of NOC is a characteristic feature of this class of compounds: tumors can be specifically induced in target organs, including lung, nasal cavity, esophagus, stomach, pancreas, colon, urinary bladder, CNS.
- Bioactivation of NOC and their interaction with critical cellular targets proceeds basically similar in animal and human tissues.

Metabolic activation of N-nitroso compounds



Structure and carcinogenic activity

Carcinogenic



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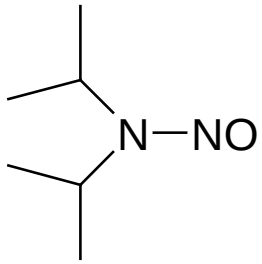

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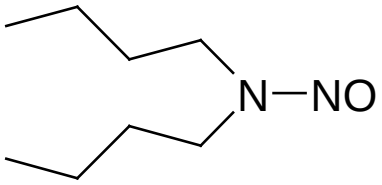

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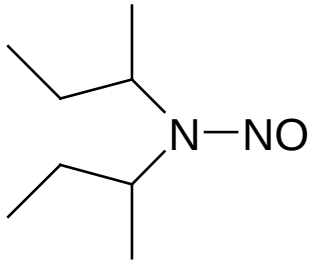

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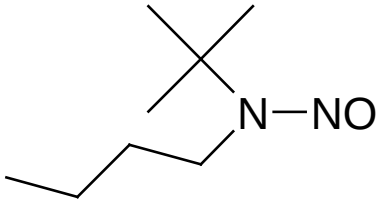
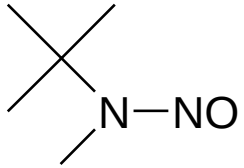
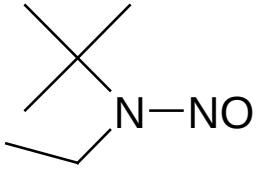
Noncarcinogenic



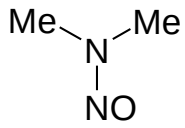




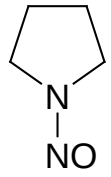
Human NOC exposure (selection)

Food/consumer products/drugs/occupational
(tobacco not considered)

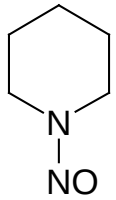
Major volatiles



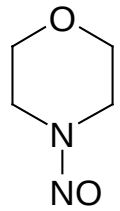
NDMA
(N-nitroso-dimethylamine)



NPYR
(N-nitroso-pyrrolidine)



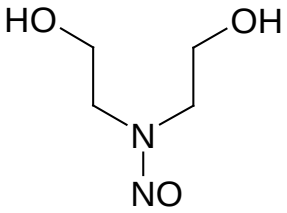
NPIP
(N-nitroso-piperidine)



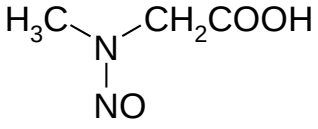
NMOR
(N-nitroso-morpholine)

Carcinogenic

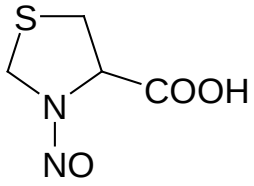
Major non-volatiles



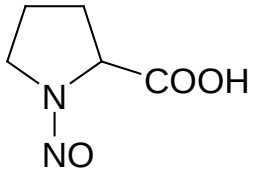
NDELA
(N-nitroso-diethanolamine



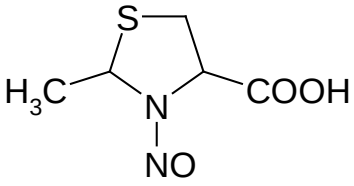
NSAR
(N-nitroso-sarcosine)



NTCA
(N-nitroso-thiazolidine-4-carboxylic acid)



NPRO
(N-nitroso-proline)

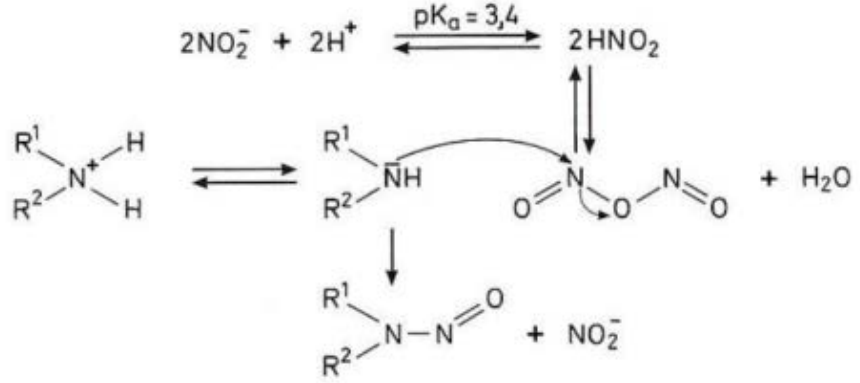


NMTCA
(N-nitroso-2-methylthiazolidine-4-carboxylic acid)

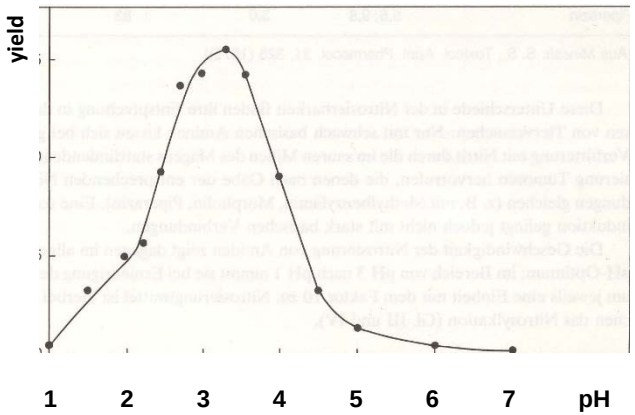
Carcinogenic **Noncarcinogenic**

Basics of NOC formation

Nitrosation of secondary amines



NDMA formation is dependent of pH



Rate constants of nitrosation some secondary amines at optimal pH (3.0 - 3.4)

Amines	pK_a	optimal pH	Rate constants K_2 (Mol $^{-2}$ • sec $^{-1}$)
Dimethylamine	10.7	3.4	0.00045
N-Methylbenzylamine	9.5	3.0	0.013
Morpholine	8.7	3.4	0.42
Piperazine	5.6; 9.8	3.0	83

Modified from Mirvish, 1975

Nitrosation of amines

$$2\,HNO_2 \rightleftharpoons N_2O_3 + H_2O$$

$$R_2NH + N_2O_3 \longrightarrow R_2NNO + HNO_2$$

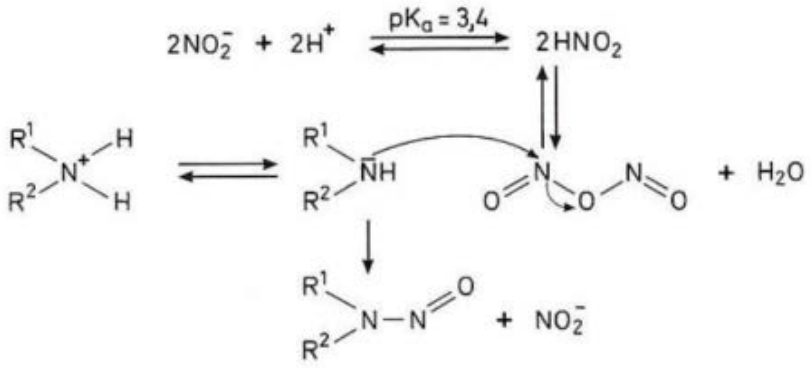
Nitrosation of amides

$$HNO_2 + H^+ \rightleftharpoons H_2NO_2^+ \rightleftharpoons NO^+ + H_2O$$

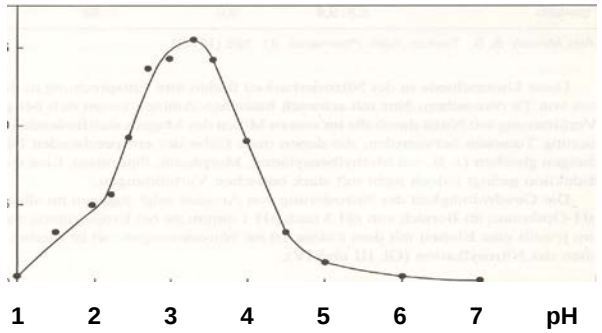
$$RNHCOR' + NO^+ \longrightarrow RNNOCOR' + H^+$$

Basics of NOC formation: mechanisms and kinetics

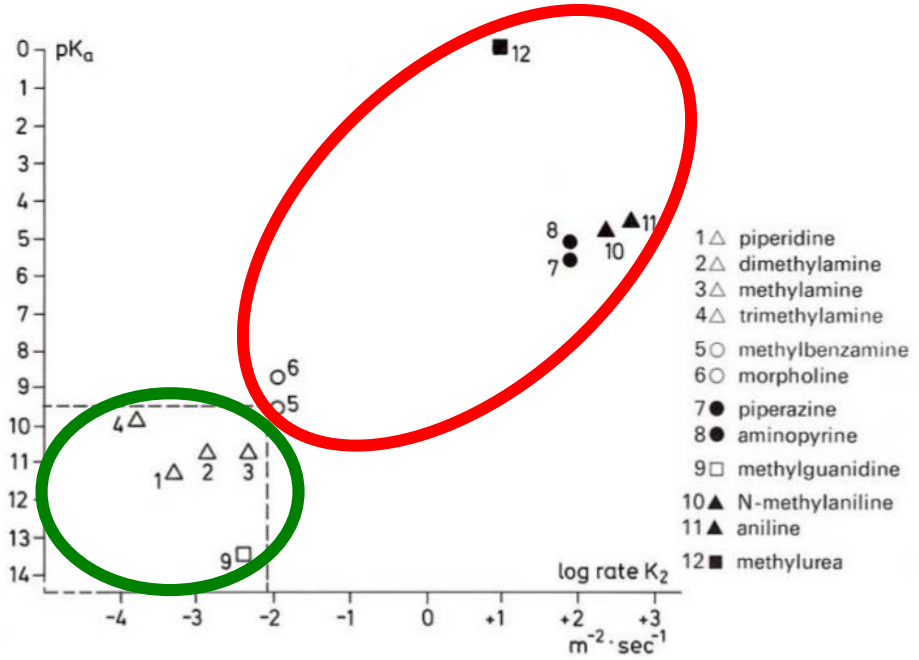
- **Mechanism of nitrosation of secondary amines**



NDMA formation in dependence of pH



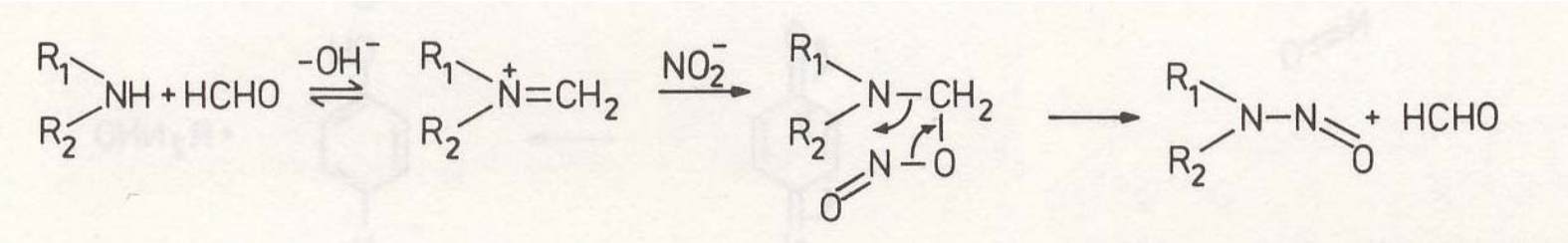
Estimated rate constants and pK_a values



- No tumors formed in feeding experiments with nitrite
- Tumor formation by gastric NOC synthesis

Formaldehyde-catalyzed nitrosation of secondary amine

(pH 6-11)



Keefer LK, Roller PP (1973)

Basics of NOC formation: primary, secondary, tertiary amines, catalysts, inhibitors

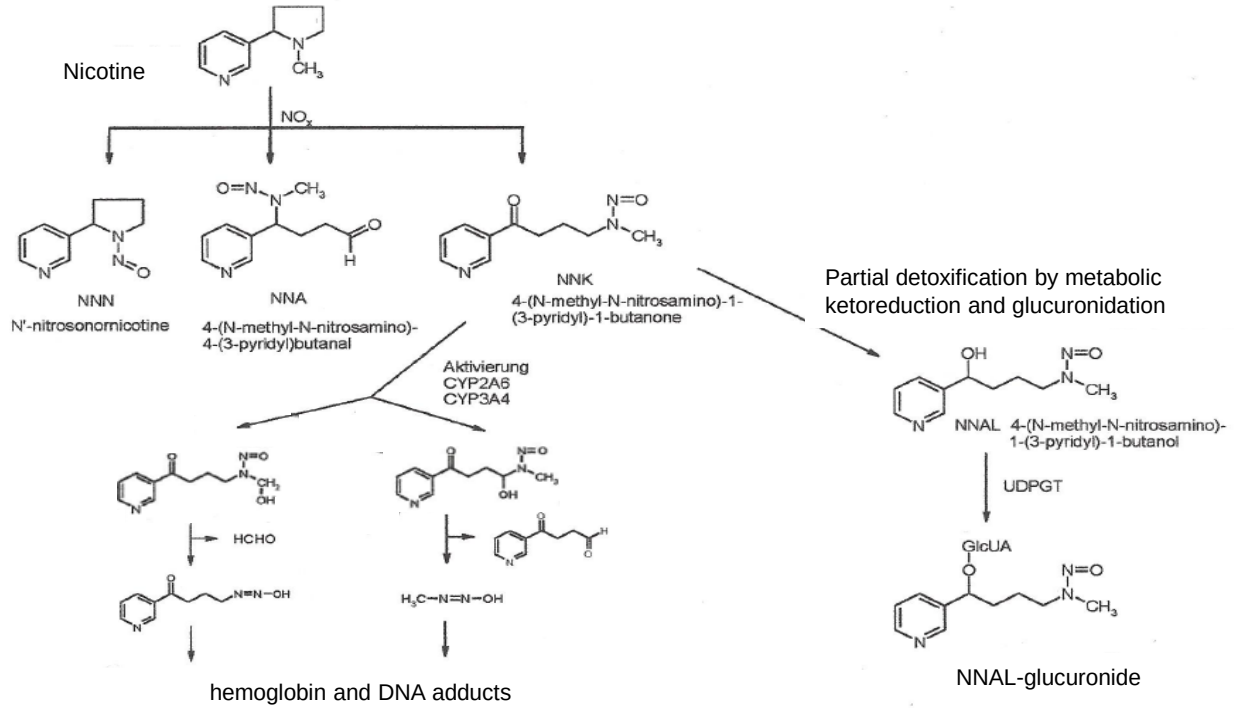
- **Primary amine**s → diazonium ions→ alcohols *(aqueous acidic medium, reaction may be used to scavenge nitrosating agents, however rates slower than those of secondary amines)*
- **Secondary amines** → rapid N-nitrosation

  (rates depend on pK value of the amine)
- In basic or nonaqueous media → rapid reaction with NOx

  (since protonation not competing)
- Most **tertiary amines** also form NOC, by dealkylating nitrosation

  (with few exceptions at much smaller rates)
- **Catalysts**: formaldehyde (& certain other carbonyl compounds), halogenides, SCN^- ;
- **Inhibitors**: ascorbates, tocopherols, phenolics (flavanoids)

NNN, NNA, NNK: tobacco-specific NOC from Nicotine



NNK

- Systemic lung carcinogen in rats, mice, hamsters, and ferrets (rats: as well pancreas, nasal cavity, liver)

  Considered to cause lung, oral cavity and pancreatic cancer in people exposed to tobacco products *(Hecht,SS CRT 11: 559 (1998); Nature Rev. Cancer 3: 733 (2003)*

NNK & NNN: Group 1 (IARC) (Carcinogenic to humans)

- Sufficient evidence for carcinogenicity in animals
- Strong mechanistic evidence in exposed humans

(IARC Monographs, Vol 89 (2007)

Exposure biomarker: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides in urine in exposed nonsmokers

Passive exposure to cigarette smoke shown for

- transplacental (amniotic fluid/ urine of newborns)
- environmental tobacco-smoke (ETS) exposure

  (men in exp. chamber, hospital workers, women living with smokers, elementary-school children and people in a gambling casino)
- Typical levels of NNAL and NNAL-glucuronides in exposed non-smokers are about 1–5% of those in smokers. For ETS exposure, these data are consistent with a causal link between exposure and lung cancer

  (*Hecht S.S., Nature Rev. Cancer 3: 733 (2003)*

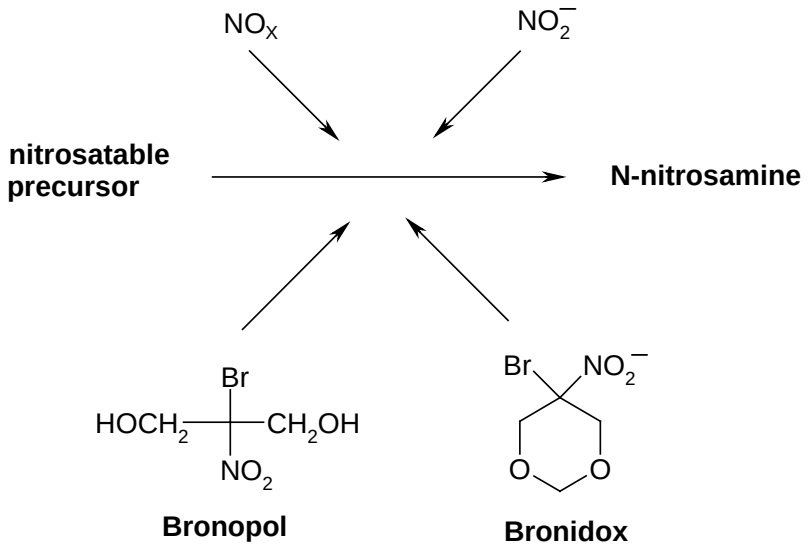
Non-food products: Cosmetics, personal care

NOC found in cosmetics/personal care/ consumer products

(predominant: NDELA, sporadically NDEA,NDPA, NMOR & long chain N-nitroso-methylalkylamines)

- Use of secondary amines
- Use of low purity materials, often with NOC contamination
- contact with nitrosating agents (NOx) during production and/or storage

Potential ways of formation of N-nitrosamines in cosmetic products



Mitigation of NOC contamination in cosmetics

EU Cosmetics Directive (Annex II); Opinion of the SCCS SCCS/1486/12, 2012

Purity specifications for basic materials, such as mono- and trialkanolamines:

use of secondary amines not allowed;

max content of secondary amines : $\leq$ 0.5%

max content of nitrosamines : $\leq$ 50 µg/kg

N-nitrosatable compounds not allowed if nitrosating agents in the product

(e.g. the preservative Bronopol)

→ Estimated systemic average consumer exposure to NOC from dermal application of cosmetics $\leq$ 0.05 µg NDELA/person/d

(Janzowski et al. 2000)

Occupational exposure: dermal and /or inhalative to cutting fluids in metal working industries

Contamination of synthetic cutting fluids based on aqueous solutions of alkanolamines with N-Nitrosodiethanolamine (NDELA), N-Nitrosomorpholine (NMOR) and N-Nitroso-1.3-oxazolidines *(Fajen et al. 1977; Stephany et al. 1978; Ellen et al. 1982)*

NOC contamination levels in cutting fluids in Europe *(modified from Eisenbrand et al. 1996*)

	Contaminated Samples [%]	
Concentration [µg/kg]	**NDELA**	**NMOR**
< 20	22	43
20-500	46	6
> 1000	32	18

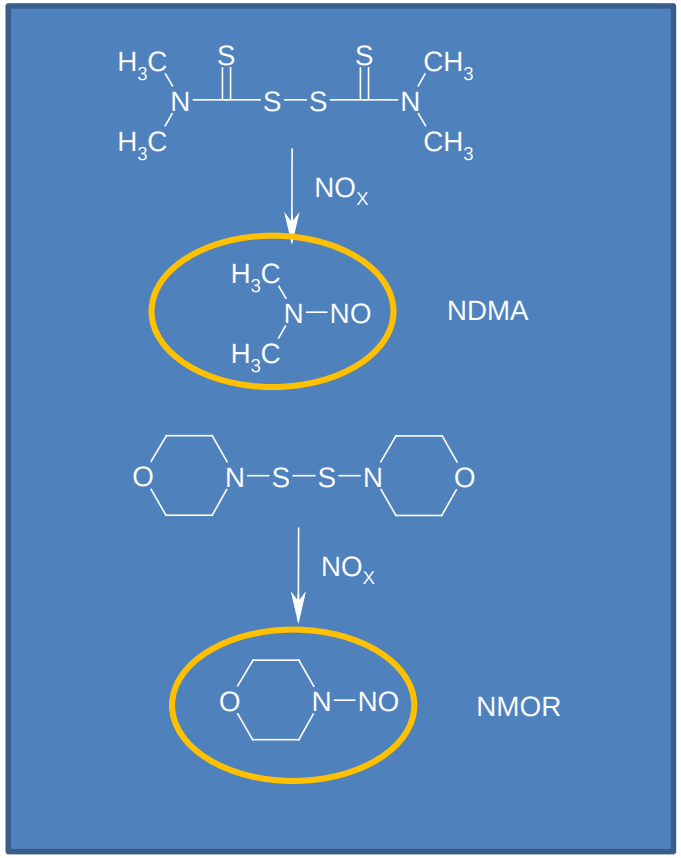
Mitigation measures

(TRGS 522/ technical rules for hazardous substances 2018)

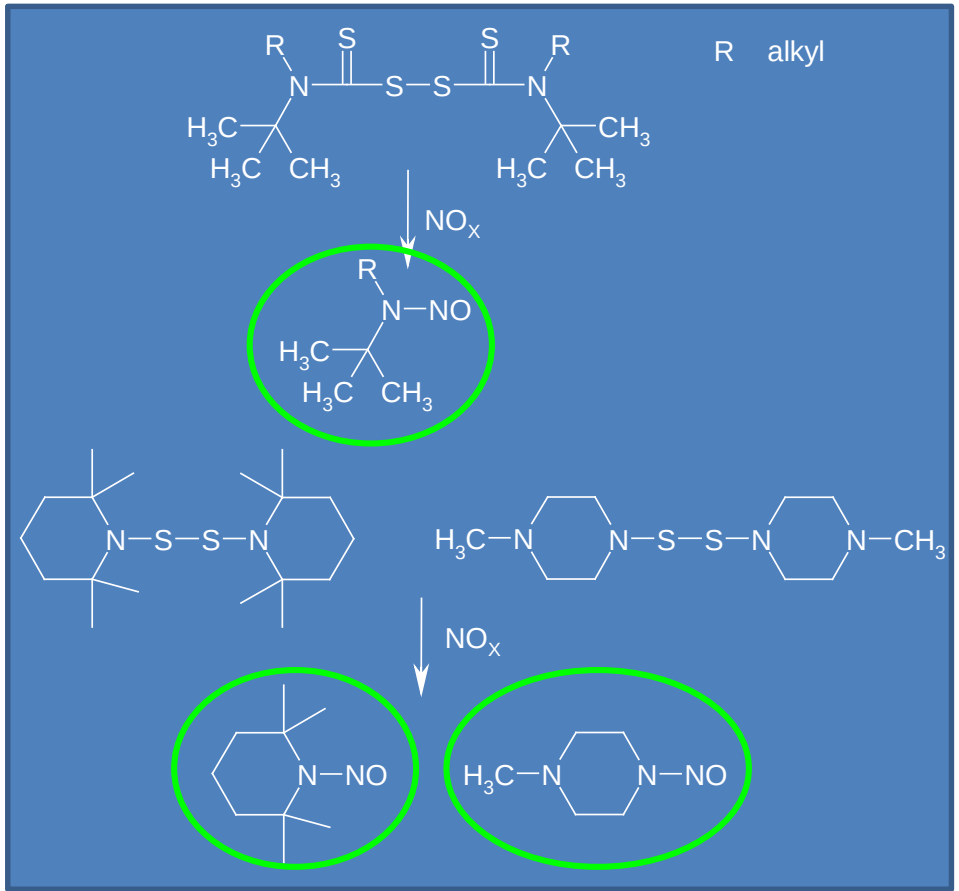
- no use of nitrite as corrosion inhibitor, no use of nitrosating preservatives, minimize contact with NOX during all operations
- Recommended : use of nitrosating inhibitors /scavenging agents to prevent NOC formation
- replace chemicals giving rise to carcinogenic NOC by those that do not (eg „safe amines“)

Example: The concept of „Safe Amines“ for vulcanization accelerators/x-linkers ⇨ non-carcinogenic NOC *(Wacker et al,1987)*

Carcinogenic NOC



Non- Carcinogenic NOC



Technical rules for hazardous substances: air at working places in specific industries (TRGS 552, 2018)

Industry	NOC	Critical working places	Tolerance/ acceptance conc. Indiv.NOC or Σ
Metal	**NDELA** / **NDMA** / **NMOR**	Cutting, grinding, drilling	0.75/ 0.075 µg/m^3
Rubber	**NDMA** / **NDEA** / **NMOR** / **NPIP**	Vulcanisation ad subsequent processes; storage	0.75/ 0.075 µg/m^3
Chemical	**NDMA** / **NDEA** / **NMOR** / **NPIP**	Use of dimethylformamide /tank/ reactor filling	0.75/ 0.075 µg/m^3

Food: Nutritional Exposure to NOC

- Isolation and identification of a hepatotoxic factor in herring meal produced from sodium nitrite preserved herring *(Ender F, Havre GN, Helgebostad A, Koppang N, Madsen R, Ceh L , 1964 Naturw. 51: 637-638)*

  ⇨ Poisoning of sheep after feeding nitrate-treated fish meal → liver toxicity

- Identification of NDMA as the most probable causative agent
- ⇨ Analytical studies to uncover the presence and formation of NOC in food in the early 1970s → lack of specifcity and sensitivity
- ⇨ Development of ultrasensitive and highly specific analytical instrumentation, → thermal energy analyzer TEA by Fine et al. (1975)
- Present methodology: preferentially LC/ GC MS/MS/MS

Major processing methods prone to cause NOC contamination in food
→ mitigation

- Addition of nitrate/nitrite for curing of meat (bacon etc)

  → NO_2/NO_3 reduction; adding inhibitors like ascorbic acid, tocopherol; lowering of NOx in smoke (smoked food)

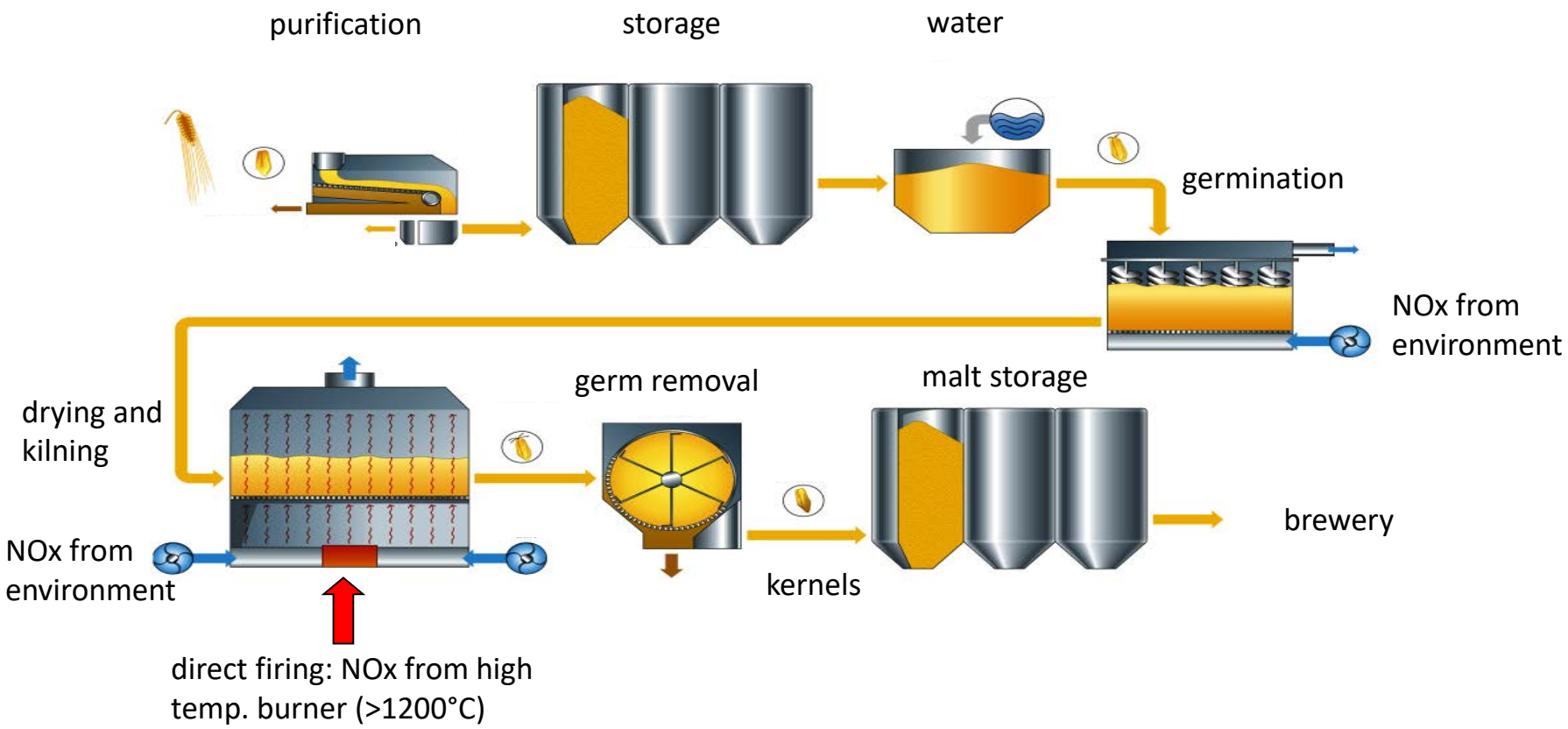
- Drying/ kilning of malt by direct firing techniques

  production of beer → NOx minimization

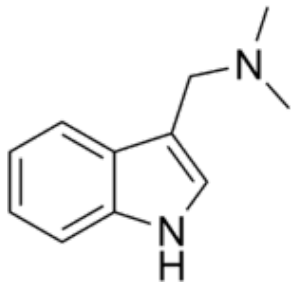
- Packaging (rare cases) → avoidance of migration into foods (NOC or Nox)

One example: The kilning process

NOC formation by reaction of malt amines with NO_x



NDMA formation from the precursor gramine in barley



+ NOx → NDMA

Gramine

(Ahmad et al , 1987)

Estimated daily dietary intake of NDMA

(Taken from Hrudley et al, 2013)

US.: 0.03 -0.06 µ g/day, (0.08 µ g/day with beer consumption) (Hrudley et al, 2013); US : 0.11 µ g/day (Fristachi & Rice, 2007)

West Germany: 0.17 - 0.28 µ g/day (Tricker et al 1991);

France: 0.19 µ g/day (Biaudet et al, 1994);

Finland: 0.05 µ g;/day (0.12 µ g/day with beer) (Dich et al 1996)

Spain: 0.114 µ g/day in Spain (Jakszyn et al 2004);

Australia: 0.4–3.1 µ g/day (Schäfer et al, 2010)

Endogenous formation of NOC in the GI tract : the pioneers

Sander and Bürkle (1969)

Induction of malignant tumors in rats by simultaneous feeding of nitrite and secondary amines*, Z Krebsforsch* ***73****,54-66*

Lijinsky, W. (1974)

Reaction of drugs with nitrous acid as a source of carcinogenic nitrosamines

Cancer Res ***34****, 255-58*

„ Many of the drug structures currently in focus related to NOC contamination have been investigated for nitrosatability and biological effects of the respective NOC by Lijinskys pioneering research “

Loeppky, R. (1990)

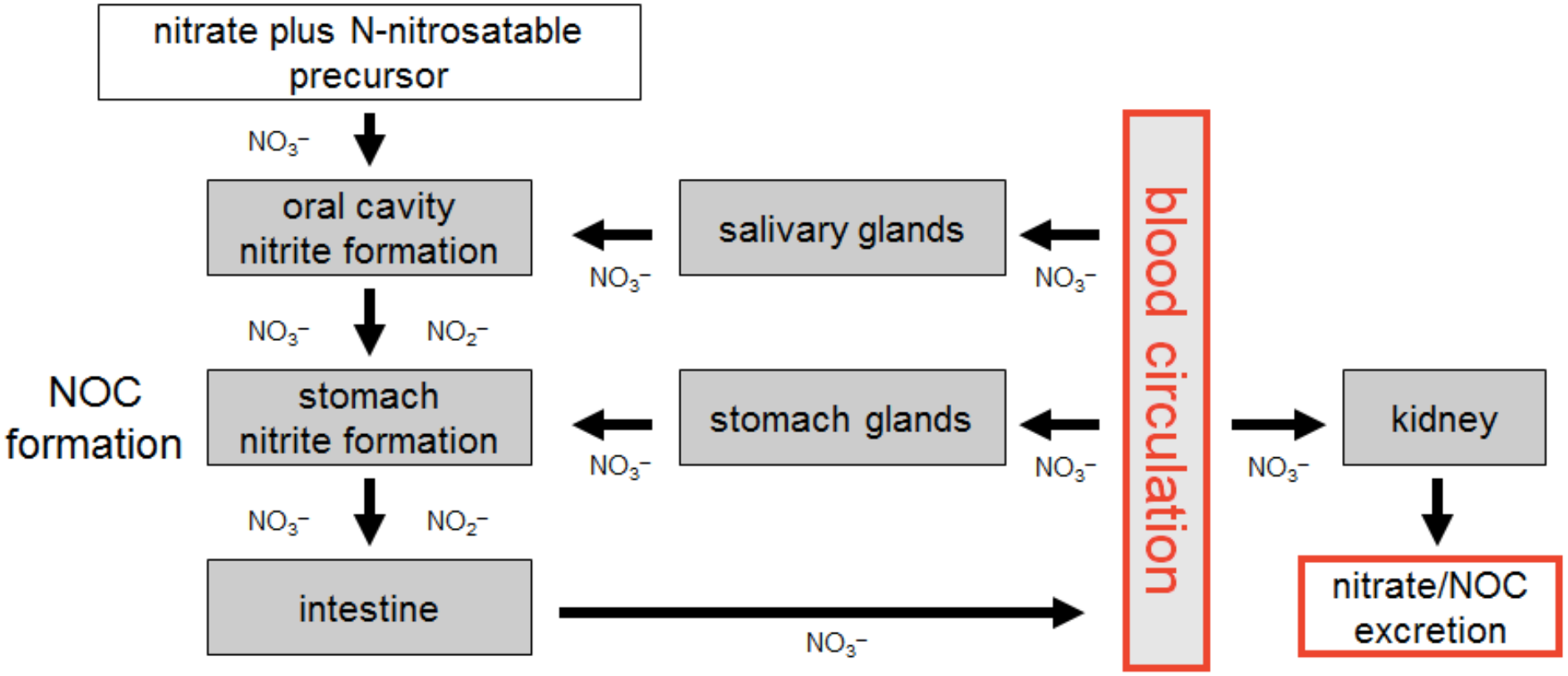
Dealkylating nitrosation of tertiary amines *(Smith, Loeppky, JACS , 1967)*

Rapid nitrosamine formation from tertiary nitrogen compounds: an overview,

in: Drug Dev.& Eval.,Eisenbrand et al, (Eds),G Fischer

Mechanistic insights into nitrosation chemistry, especially of tertiary amines

Flow chart of gastrointestinal nitrate circulation.



From: Nitrate and Nitrite in the Diet: An approach to assess Benefit and Risk for Human Health, Opinion of the DFG Senate commission on Food Safety, Deutsche Forschungsgemeinschaft www.dfg.de/sklmDFG

Endogenous Nitrate, nitrite and nitrogen monoxide

- In humans nitrate excreted in urine reported to exceed the amount ingested by a factor of 4 ; endogenous nitrate biosynthesis in humans found to reach about 10 µmol/kg bw/d= about 0.7 mg/kg bw/d or **50 mg/d for a 70 kg** person *(Green et al 1981; Tannenbaum et al 1978)*
- A variety of enzymes and proteins act as reductases giving rise to NO from nitrate/nitrite, including flavoproteins and cytochrome P-450, deoxygenated hemoglobin/ myoglobin, xanthine oxidase and mitochondrial respiratory chain enzymes, among others *[Reutov, Sorokina, 1989; Zhang et al, 1989; Shiva et al, 2007; Crosby et al, 2003; Millar et al 1997)*
- The signalling molecule NO is generated from L-arginine by a family of oxygen-dependent NO synthases *(Marletta et al, 1998; Moncada, Higgs, 1993; Hattori et al 1994*
- NO is also a key component formed in response to bacterial infections and /or during inflammatory reactions *(Stuehr, Marletta, 1985; Hussein et al, 2008)*

- Dietary nitrate uptake: **average 175 mg/d**, mainly from vegetables (EFSA, 2008)

Metabolic relationship : NO, Nitrite, Nitrate

Nitrate, nitrite and NO are metabolically interconvertible

[Weitzberg, Lundberg 1998; Lundberg, Govoni, 2004)

- NO can endogenously be oxidized to nitrate and nitrite
- *[Marletta et al 1998; Leaf et al, 1989)*
- the latter two can undergo reduction and cycling back to bioactive NO in blood and tissues before terminal excretion, e.g. as urinary nitrate *(van den Brandt,et al, 1989).*

Estimated endogenous formation of NDMA

- Endogenous NDMA exposure was estimated based on measured human blood levels *(Gough et al, (1983) Simenhoff et al, (1982), Dunn et al.(1986)),* assuming steady state and a clearance rate in humans of 3.45 L/min *(Gombar et al 1990)*
  → to range between 100 to nearly 2,500 µg/day = 1.4 to 35 µg/kg bw/day (based on 71.5 kg bw) *(Hrudley et al, 2013)*

- A similar mean value of 18 µg/kg/day (ca.1,360 µg/day) was estimated based on levels of O^6 -MeG in leukocytes *(Giorgiadis et al 2010; Hrudley et al, 2013)*

NDMA estimates are based on analytical data of uncertain reliability
→ confirmation/validation required

The WHO Nitrosation assay procedure (NAP Assay)

Coulston & Dunne,eds.1980 in: *The Significance of N-Nitrosation of Drugs*
Drug Dev.& Eval.,Eisenbrand et al, (Eds),G Fischer1990

Assay procedures

The nitrosation assay procedure known as the NAP test has been recommended by an expert group as a WHO meeting report (Coulston & Dunne,

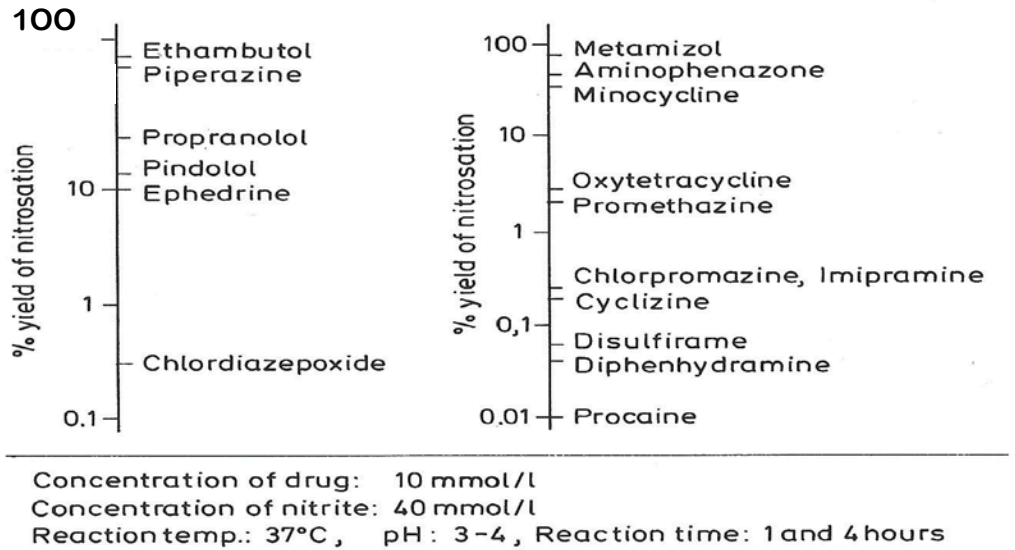


Fig. 13: Relative nitrosatability of selected drugs in the NAP test

Examples of In-vivo formation of NOC by endogenous nitrosation of drugs

In patients with parasitic infections: Endogenous formation of N-nitrosamines from piperazine and their urinary excretion following antihelmintic treatment with piperazine citrate

Tricker et al. (1991) Carcinogenesis 12, 1595-9

Influence of dietary nitrate on in-vivo nitrosation of amidopyrine in humans: use of „ethanol effect“ for biomonitoring of NDMA in urine

Spiegelhalder B. (1990), In: Eisenbrand et al, (Eds.)1990,The Significance of N-Nitrosation of Drugs; Drug Dev.Eval. Vol. 16, G.Fischer Verlag, New York

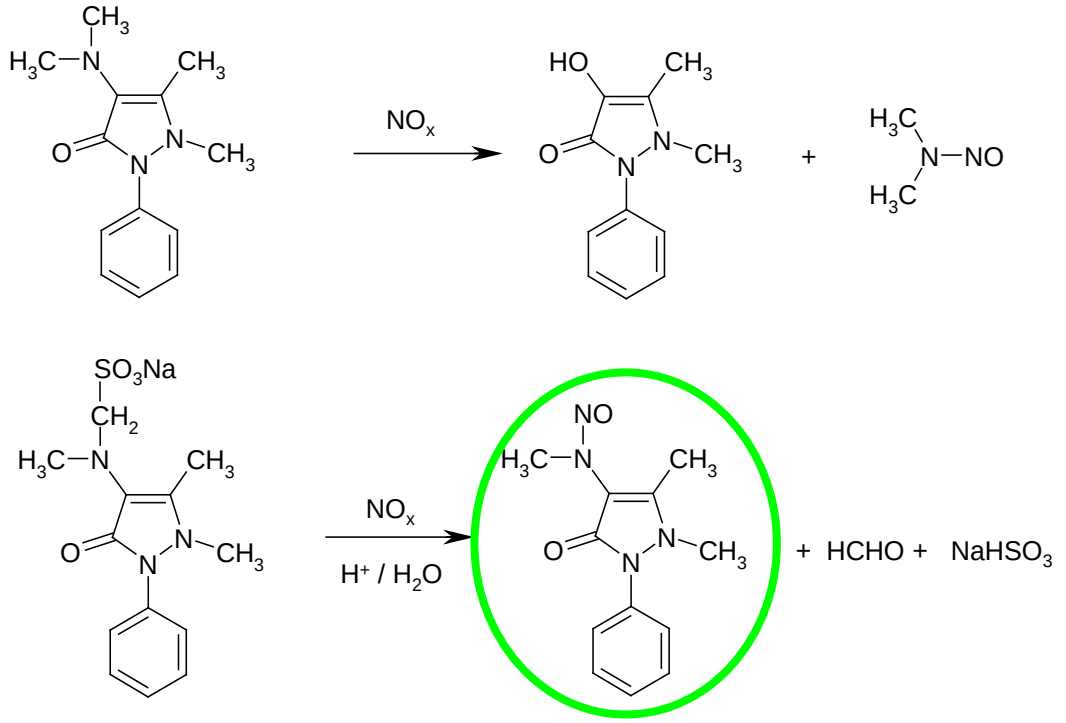
NOC from amidopyrine and metamizol

Several prescription/ OTC products reported to contain various NOC,

in particular NDMA (Krull et al. 1979)

→ Regular NDMA contamination of AP (Eisenbrand et al. 1979)

→ AP withdrawn from market



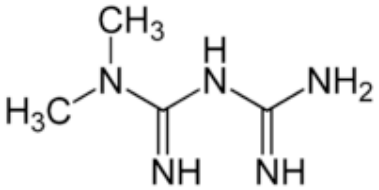
Noncarcinogenic

Berscheid et al (1978)

NDMA in Sartans, Metformin… *(EMA, 2018)*

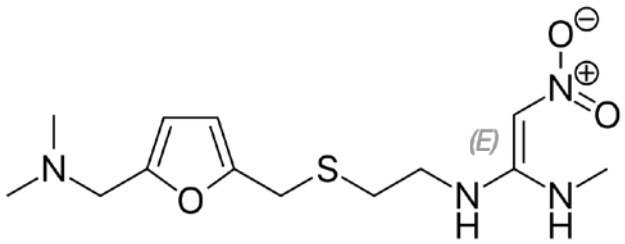
NDMA identified as a process related contaminant in Valsartan
NDMA may have been present in batches reaching back to 2012 when the synthesis was changed to a process using dimethylformamide (DMF) as solvent and $NaNO_2$ as a quenching agent to destroy azide (sodium azide, used for tetrazole synthesis)
All Sartans whith a tetrazole ring system synthesized by this technology expected to be contaminated

Metformin
(1,1 Dimethylbiguanide)



NDMA source?

Ranitidine



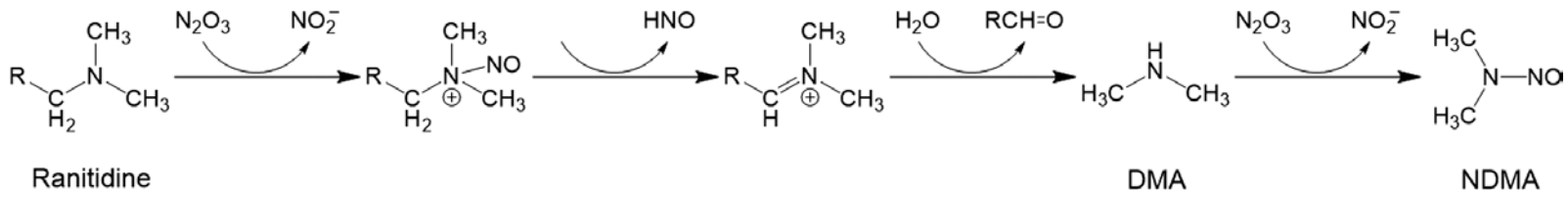
Synthesis (Wikipedia)

- Dimethyl-*N*-methylcarboimidodithionate + **nitromethane** → *N*-Methyl-1-methylthio-2-nitroetheneamine (1) ; 1 + dimethylaminomethylfurfurylthioether (2)→Ranitidine;
- Mechanism of NDMA formation? Nitromethane as nitrosating agent?
- Risk of in-vivo-nitrosation?

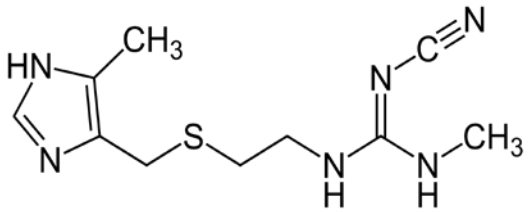
*In 10 human volunteers, following ranitidine intake, the urinary NDMA excreted over 24 h increased **400-folds** from 110 to 47 600 ng, while total N-nitrosamines increased 5-folds"*

Zeng and Mitch (2016):
Oral intake of ranitidine increases urinary excretion of N-nitrosodimethylamine",

Mechanism proposed:



Cimetidine



+ NOX → **N-Nitrosocimetidine**

Biological properties

in-vitro: mutagenic (Ames test), methylating DNA , inducing DNA SSB & DNA repair, SCE and cell transformation)

in-vivo: noncarcinogenic (rats), no DNA methylation
→ metabolically denitrosated by GSH/GSTs, cysteine & hemoglobin-SH groups, CYP450;

(Habs et al, 1982; Jensen, Magee, 1990)

Defining limits: EMA/369136/2020

Approach recommended in ICH M7(R1): use the TD50 as POD for the calculation of excess cancer risk; calculate dose associated with a theoretical excess cancer risk of 1:100,000 as the acceptable intake (AI) from which the limit is calculated based on the maximum daily dose of the medicinal product.

***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.

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 TD50 by 50,000 (50% or 0.5 x 100,000). For a person of 50 kg bw the AI level is:

AI = 50 x (TD50/50,000)

BMDL10 (mg/kg bw/d): NDMA : 0,027; NDEA : 0,018; (Opinion of SCCS/1486/12, 2012)

Open Questions/ knowledge gaps/ research needs

1. Exposure

exogenous (from diet) → database update→ suitable reference concerning risk assessment of other exposure pathways like contaminated drugs ?

Endogenous (in-vivo) → validated analytical methodology (specificity, absence of artifacts) ?

PBBK-based estimates of human endogenous exposure?

predictivity of biomarkers of in-vivo nitrosation: urinary excretion of N-Nitrosoproline /nitrosoamino acids → surrogate biomarker(s) for total NOC (including carcinogenic NOC) ?

2. Mitigation

Structure-based (in silico) screening of APIs → prediction of N-nitrosatability and/ or propensity to form NOC/electrophiles?

Given pharmacological/ toxicological tolerance → explore possibilities to replace critical structural elements (successfully achieved in other areas)

Nitrosamine Contamination of Drug Products

Sruthi King, Ph.D.
Associate Director of Pharmacology/Toxicology
Office of Generic Drugs
Center for Drug Evaluation and Research
U.S. Food and Drug Administration

FDA Nitrosamine Workshop
March 29-30, 2021

Disclaimer

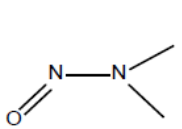
This presentation reflects the views of the author and should not be construed to represent FDA's views or policies.

Nitrosamines in Drugs: Scope and Impact

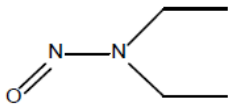
- Nitrosamines (NA) are in food, water, tobacco, consumer goods, environment.
- Chemistry is not new; toxicity and potency is not new.
  - Potent rodent carcinogens and probable human carcinogens
- NA presence in drug products was first reported to FDA in 2018. NA contamination affected products *globally*, resulting in recalls of vital medications.
- Detection and quantification of NA requires development of highly sensitive analytical methods.
- Root cause analysis identified risk factors for NA formation.
- Managing the NA contamination in drug products requires multidisciplinary approaches to conduct risk:benefit assessments, collaboration with industry and international regulatory partners, and effective communication with public.

NAs in Drug Products

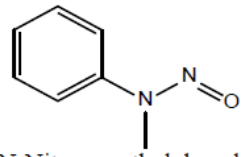
- CDER/Office of Generic Drugs (OGD) initially alerted of presence of NDMA in valsartan, which is in the angiotensin II receptor blockers (ARB) class of drugs
- NAs have been identified in active pharmaceutical ingredients (API) and finished drug products (DP). Both generic and brand drugs have been affected, although the effect has been greater on generic drugs.
- Multiple NAs in DPs have now been identified. Control strategies are needed for single and multiple NAs in the DP.
- Despite nearly 2.5 years into the contamination issue, many challenges remain.



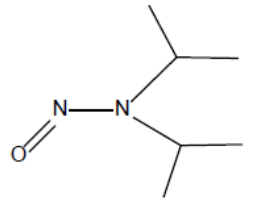
N-Nitrosodimethylamine (NDMA)



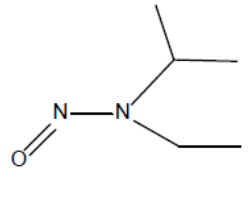
N-Nitrosodiethylamine (NDEA)



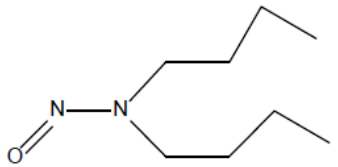
N-Nitrosomethylphenylamine (NMPA)



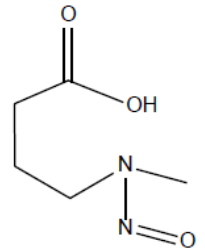
N-Nitrosodiisopropylamine (NDIPA)



N-Nitrosoisopropylethylamine (NIPEA)



N-Nitrosodibutylamine (NDBA)



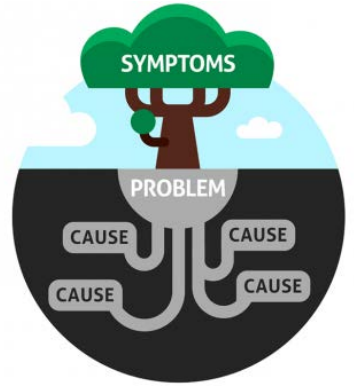
N-Nitroso-N-methyl-4-aminobutyric Acid (NMBA)

1-methyl-4-nitrosopiperazine (MNP) and 1-cyclopentyl-4-nitrosopiperazine (CPNP) have also since been identified in rifampin and rifapentine.

Risk Assessment Considerations

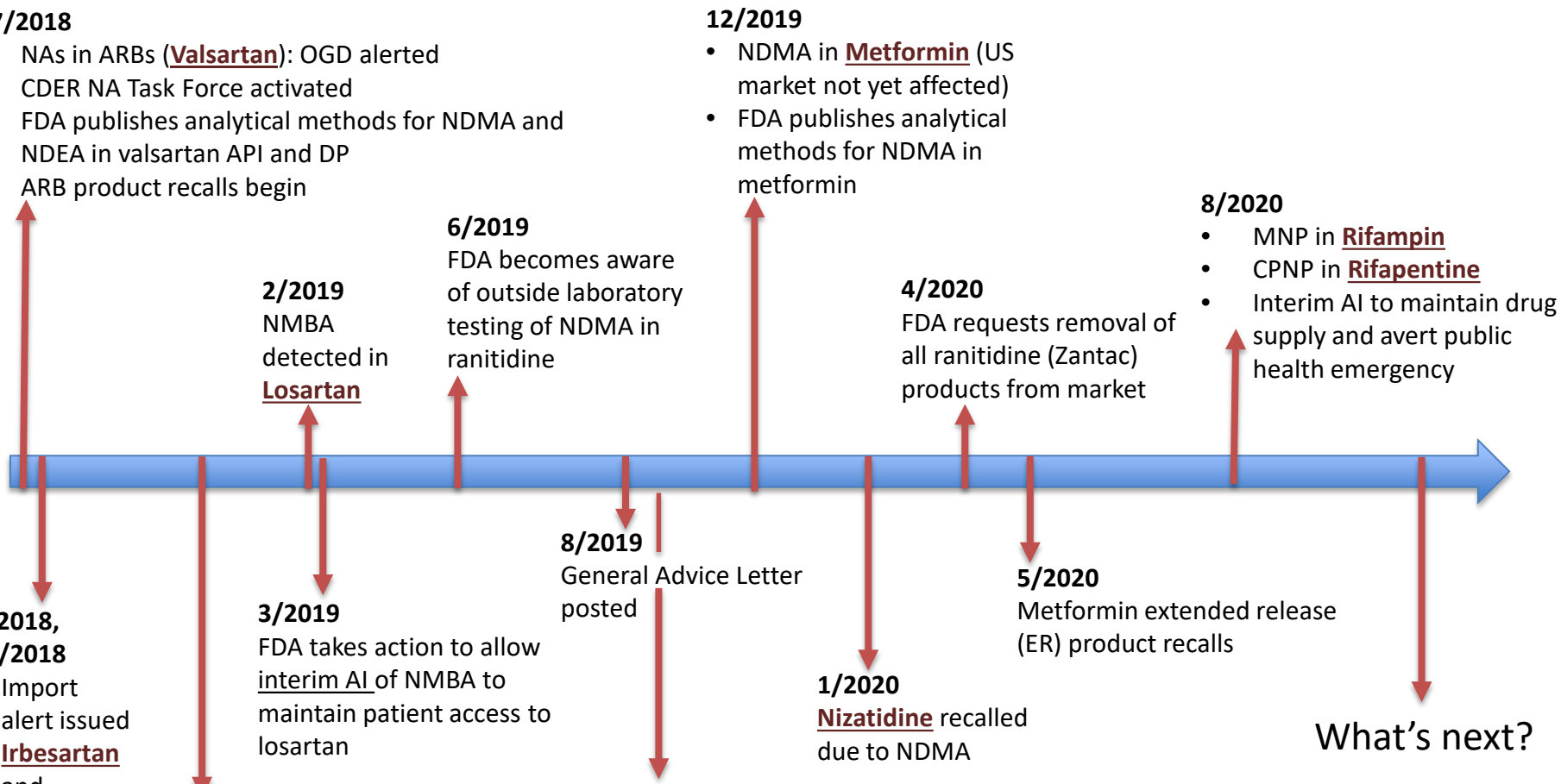
- Which NA identified? Are there multiple NAs?
  - Risk of formation or levels detected?
  - Analytical method sufficiently sensitive?
  - Root cause investigation?
  - How many lots affected?
  - API or DP or both affected?

- What is the acceptable intake (AI) for the NA?
  - Are nonclinical data available?
- Which products are affected? Are they medically necessary?
  - Are batch levels above AI?
  - Should drug be recalled?
- What is the drug supply?
  - Will recall precipitate drug shortage?
- Are therapeutic alternates available?



Timeline: Key Events and Affected Products

https://www.fda.gov/drugs/drug-safety-and-availability/information-about-nitrosamine-impurities-medications

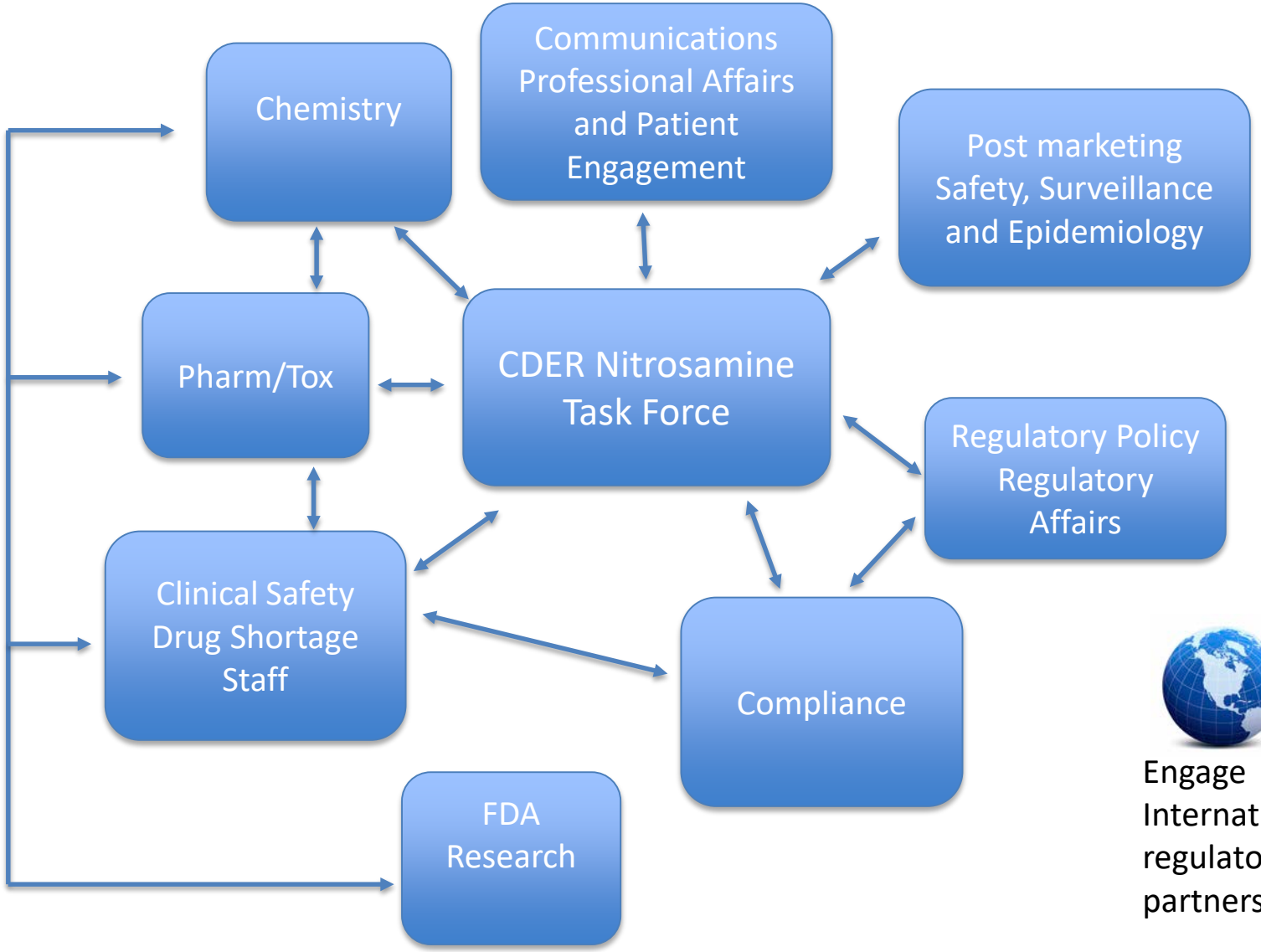


Key Disciplines engaged within CDER

- July 2018: Dr. Janet Woodcock, CDER director, activated the CDER Nitrosamine Task Force to manage the contamination incident, with CDER Office of Counter Terrorism and Emergency (CTECS) Coordination at the helm

- Over 100 subject matter experts from across CDER and FDA meet regularly to discuss and propose recommendations to mitigate risk of NA in drug products and maintain patient access to critical medications
  - Discuss and propose recommendations and update senior management on strategies to mitigate risk and maintain patient access
  - Engage international regulators to discuss harmonized approaches for addressing NA contamination (risk assessments, marketing actions, communications, information sharing, etc.)

Multidisciplinary Coordination



Engage International regulatory partners

What are the complexities?

Quality Issues

- Root cause investigations are necessary to inform control strategies
  - Process related: starting materials, intermediates, API itself
  - Supply chain: recycled or recovered materials used in the synthesis
  - Product stability: excipients in formulation, drug substance (DS) and/or drug product (DP) stability
    - Process impurities or degradants or both?

- Highly sensitive analytical methodologies to identify and quantify NA
  - Sampling and testing: necessary to identify levels of NA that pose a safety concern→ inform recall decisions
  - Facilitate risk assessment of manufacturing process

- Establish risk assessment expectations for NAs in DP (pending and approved products)

What are the complexities?

Safety Considerations: Risk assessment of products with NA

- NAs are “cohort of concern” (CoC) compounds needing tighter control because they pose greater risk than other compounds
  - Lifetime exposure is calculated based on an increase of 1 case of cancer in 100,000 people: acceptable level of risk
  - Balance risk of exposure to NA with risk of no access to medically necessary drug

- NA potency varies across compounds
  - General agreement across regulatory authorities that NAs should be avoided or tightly controlled (if unavoidable) in drug products

- Calculate acceptable intake (AI) using approaches in ICH M7 guidance using mutagenicity and rodent carcinogenicity information for the NA
  - AI informs analytical sensitivity and recall decisions

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/m7r1-assessment-and-control-dna-reactive-mutagenic-impurities-pharmaceuticals-limit-potential

Safety Considerations (Contd)

- Identify TD_{50}, which is the dose that produces tumors in 50% of animals in a dosing group from animal carcinogenicity data
  - AI = (TD_{50} (mg/kg/day)÷50,000) x 50 kg = dose of NA in mg/day
  - AI = daily dose of NA, when taken over a lifetime (~70 years) represents a risk of 1 additional case of cancer in 100,000 patients

- Consideration when selecting a TD_{50}: are the data robust?
  - Animals per dosing group
  - Treatment regimen, dosing frequency
  - Toxicology assessments and data presentation
  - Number of studies available
  - Relevance of species and tumor

- Collaboration with Pharm/Tox experts in OGD and Office of New Drugs (OND) to establish AI

Compound-specific data

- Not all NAs have robust carcinogenicity data in the literature
- Some nitroso compounds have no data
- Surrogate compounds may be useful to inform AI
  - ICH M7 states, “Although the principles of this guidance can be used, a case-by-case approach, using, e.g., carcinogenicity data from closely related structures, if available, should usually be developed to justify acceptable intakes for pharmaceutical development and marketed products.”
- Which surrogate is best?
    - Robustness of data
    - Structural similarities

What are the complexities?

Risk:Benefit Assessment

- Wide range products effecting large numbers of patients with serious medical conditions: Hypertension, Diabetes, Heartburn, Tuberculosis (anti-infectives)

- Maximum daily dose (MDD): calculate acceptable intake based on MDD
  - Inform analytical control limits to facilitate risk assessment of manufacturing process

- Medical necessity evaluation: Maintain patient access while balancing risk of exposure to NA

- Product recalls: Multidisciplinary coordination
  - Are there alternate therapeutic options for patients?
  - Will recalls precipitate drug shortage?
  - Strategies to address shortage

Interim AIs

- Offer flexibility when patient access to medically necessary drugs may be affected if all product is recalled
  - Industry is a key partner
- Offer a *short term strategy* to maintain patient access while process changes are instituted to remove/reduce NA formation
- Require multidisciplinary discussion and consensus
  - Pharm/Tox and Clinical (OGD and OND), Office of Pharmaceutical Quality (OPQ), Drug Shortage Staff (DSS), Office of Compliance (OC), Office of Communications, CTECS, and other experts as needed
- Applied in several cases to mitigate shortage: Losartan, Rifampin, Rifapentine

Short-term vs Long-term Exposure to NAs

- ICH M7 allows for adjustments based on duration of use for mutagenic impurities
- NA risk assessments consider lifetime exposure limits
  - Potent rodent carcinogens, probable human carcinogens
  - Uncertainty associated with simple adjustment to AI
- ICH M7 states, “Compounds from some structural classes of mutagens can display extremely high carcinogenic potency (cohort of concern), i.e., aflatoxin-like, N-nitroso-, and alkyl-azoxy structures. If these compounds are found as impurities in pharmaceuticals, acceptable intakes for these high-potency carcinogens would likely be significantly lower than the acceptable intakes defined in this guidance.”
- Flexibility (Interim AI) used as *short-term strategy* to maintain patient access to medically necessary drugs and avoid/mitigate shortage
  - Adjustments based on duration of use are not considered in determining Interim AI for a specific product

Communicating Risk

- https://www.fda.gov/drugs/drug-safety-and-availability/information-about-nitrosamine-impurities-medications
  - Inform industry: analytical methods, sampling and testing, risk assessment strategies
  - Alert patients, care providers, patient advocacy groups, pharmacies/suppliers/distributors
  - Recalled products
  - Alternate treatment options

- Address media concerns, citizens petitions, Congressional inquiries

- Global engagement: discuss risk assessment strategies and regulatory actions to harmonize approaches

Nitrosamine Guidance

- https://www.fda.gov/regulatory-information/search-fda-guidance-documents/control-nitrosamine-impurities-human-drugs
- Addresses API and DP manufacturers: root cause investigations
  - Risk Assessment→ Confirmatory Testing if at risk→ Reporting Changes
  - Pending and Approved products
- Lists AI for NAs: NDMA, NDEA, NMBA, NMPA, NIPEA, NDIPA*
- Risk mitigation strategies: Control of single NA and control for multiple NAs
  - Single NA allowed up to its AI; Total nitrosamine exposure ≤ 26.5 ng/day
  - Analytical methods
    - Limit of quantitation (LOQ) ≤ 0.03 ppm
    - If MDD is high (e.g., > 1 g), LOQ and limit of detection (LOD) as low as reasonably practical
- Reporting timelines

* MNP and CPNP have since been identified and AI for each is posted here: https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-nitrosamines-rifampin-and-rifapentine

Ongoing Challenges

Quality Oversight

- Multiple factors can contribute to NA formation
  - Stability
  - Excipients
  - Storage conditions

- Risk assessment is key to understand whether
  - NAs can be completely eliminated or if control and monitoring are better options
  - Potential for API-related nitroso impurities to form and control strategies

- Method development and validation: How low is too low?
  - Improved methods→ more rapid and improved screening

Ongoing Challenges

Safety and Risk:Benefit Assessments

- NAs are everywhere (food, water, environment, tobacco, formed endogenously)
  - How does this compare to exposure from drug products?

- Quantity and quality of available nonclinical data varies for NAs
  - If data are not robust, identification of appropriate TD_{50} to calculate AI is challenging
  - Improved testing methods and risk assessments identifying previously uncharacterized nitroso impurities, with no published safety data
  - Surrogate compound assessment is useful but somewhat limited
    - Identify compounds with robust data
    - Chemical informatics approaches to inform potency: structural similarity, metabolic activation, size

Are we there yet?

We've learned a lot over the last 2.5 years…

•FDA researchers play an important role in addressing data gaps to inform regulatory recommendations.

•Collaboration with experts in academia and industry, along with international regulatory partners, is key to address current challenges and identify harmonized risk assessment strategies.

Acknowledgements

- Nitrosamine Safety Team
  - Robert Dorsam, Ph.D. (OGD)
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  - Timothy McGovern, Ph.D. (OND)
  - Karen Davis Bruno, Ph.D. (OND)

- CDER CTECS Drug Nitrosamine Task Force Members
  - CTECS collaborators
  - OPQ collaborators
  - OGD and OND collaborators
  - Drug Shortage Staff
  - Office of Communications
  - Office of Compliance

Question 1
Exposure and Risk Assessment

What are the endogenous levels of nitrosamine formation in humans and rodents? Once formed, what is the rate/kinetics of elimination? What are the conversion rates in the liver, circulation levels in blood, and, normal variations? If this information is not available, can it be determined experimentally?


Presentation

Question 2
Exposure and Risk Assessment

Can nitrosamines be classified? If yes, what is the basis of their classification? e.g. could they be classified based on:

- Carcinogenic potency?
- Chemical structure e.g. aliphatic vs. cyclic?
- Chemical reactivity? Direct alkylating agents vs. indirect (require metabolism)? Adduct formed e.g. O6- , N7- methylation?
- Other
- Why would you choose this basis of classification?

If classification is not possible, is it feasible to calculate a “single” Acceptable Intake (AI) value for nitrosamines i.e. Class Specific Limit, using the existing carcinogenicity study results of 110+ nitrosamines (irrespective of study quality)?

Break

11:30 AM - 11:40 AM

Question 3
Exposure and Risk Assessment

The carcinogenic potential of nitrosamines is dose and duration dependent;

- Is there an in vivo exposure level for nitrosamines that could define low vs high risk for carcinogenicity? Is it appropriate to calculate a NOEL dose for carcinogenicity? What are the criteria to do so (Ames negative, in vivo mutation assay negative, other)?
- Can a less than lifetime (LTL) approach as described in ICH M7 Guidance be used to determine the AI, of a nitrosamine if a drug is indicated for a short duration of use?

Microsoft
werPoint Presentat

Lunch

12:30 PM - 1:00 PM

Question 4
Exposure and Risk Assessment

How would the risk assessment change when multiple nitrosamines are present in a drug product? What are the key variables to consider when conducting such risk assessment? (nonmutagenic carcinogen + mutagenic carcinogen; nonmutagenic carcinogen + weakly mutagenic carcinogen; multiple mutagenic carcinogens, etc.)

Day 1 Adjourn

Nitrosamines as Impurities in Drugs; Health Risk Assessment and Mitigation Workshop Day 2

March 30, 2021

House Rules

Muting Phones: If you are not speaking please keep your phone on mute.

Note: All attendees will be muted, only panelists have the ability to unmute.

Video Feature: Only the panelists engaging in the discussions will have their video feature turned on. If you are not speaking, please turn the video feature off.

Questions & Discussion:

- Submit any questions that you would like answered by using the "Q&A" feature to the bottom center of your screen in Zoom.
- If you are a panelist who will be speaking, please check your "Chat" feature as the host will prompt you when it is time for you to present.
- Please use the "Q&A" box so that the moderator and workshop hosts can work together to monitor the questions and make sure they are addressed as time allows during the workshop. Panelists will answer questions live as time permits, you will not receive an answer in the "Q&A" box.

Question 5
Exposure and Risk Assessment

Should the regulatory limits for nitrosamines listed for food and water or, amount formed endogenously, be considered in determining AI of nitrosamines in drugs?

Question 6
Chemistry

In the absence of data and based on identified differences in nitrosamine chemistries and reactivities, can read-across for structural similarity to related compounds be used for nitrosamines? What are the key parameters to consider when conducting (Q)SAR assessment for nitrosamines?

Break

11:00 AM - 11:10 AM

Question 7
Chemistry

Nitrosamines can be formed during manufacturing of the Active Pharmaceutical Ingredient (API), and/or Drug Product (DP). What are possible approaches to consider in order to reduce nitrosamine formation during manufacturing? Can nitrosamines be eliminated completely from API and/or DP?

Lunch

12:30 PM - 1:00 PM

General Discussion

Day 2 Adjourn


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