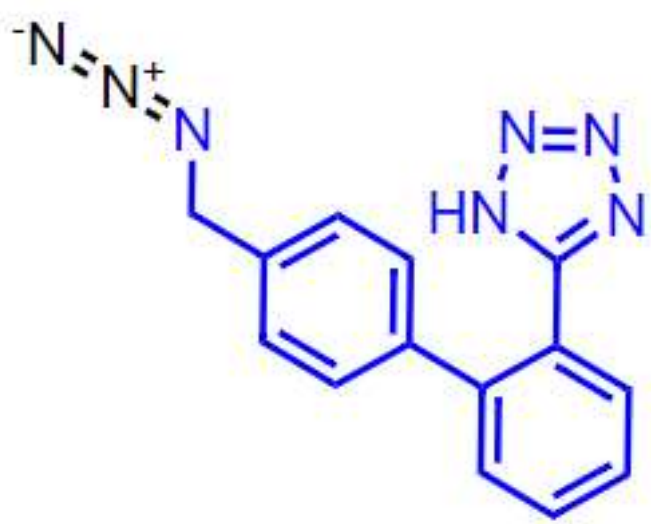
MUTAGENIC IMPURITIES

Vorasit Vongsutilers, Ph.D., R.Ph. Bodin Tuesuwan, Ph.D., R.Ph

Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University

Azidomethyl-biphenyl-tetrazole (AZBT), is a compound that can form during the manufacture of the active ingredient in some sartan medicines. It is known to damage DNA, and as a result long-term exposure over years may increase an individual's risk of developing cancer.



5-(4'-(azidomethyl)-[1,1'-biphenyl]-2-yl)-1H-tetrazole

TGA Published: 20 August 2021

https://www.tga.gov.au/alert/azide-impurity-sartan-blood-pressure-medicines#fn1s

Monitoring of sartan medicines stepped up: traces of a new foreign substance detected

Chemical compound azidomethyl-biphenyl-tetrazole (AZBT) detected in individual sartans

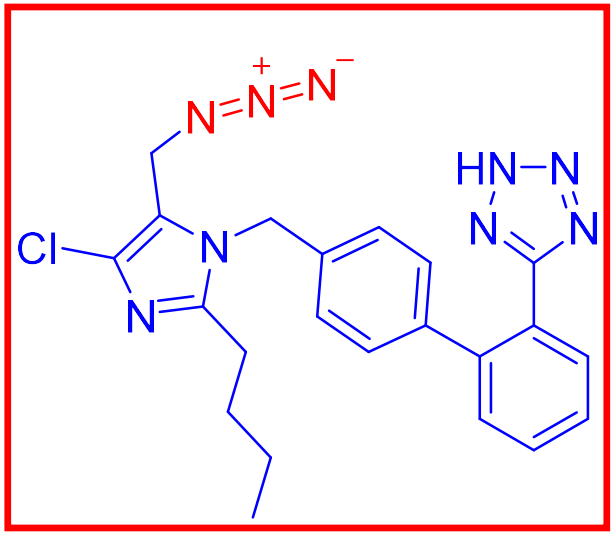
01.07.2021

Azide impurity in 'sartan' blood pressure medicines

TGA investigation - low levels of contamination with azidomethyl-biphenyl-tetrazole (AZBT)

Risk of presence of mutagenic azido impurities in sartan active substances with a tetrazole ring

Back



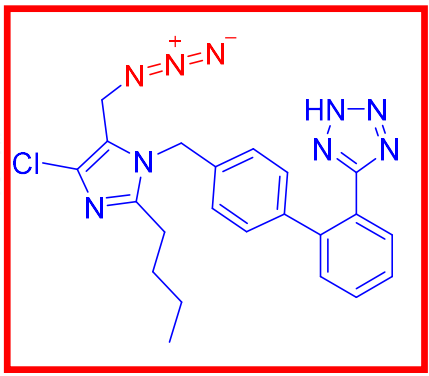
NEWS | 29 APRIL 2021 | STRASBOURG, FRANCE

Following recent investigations demonstrating the mutagenicity of an azido impurity in sartan active substances (APIs) with a tetrazole ring, the EDQM confirms that the CEP holders concerned have already been contacted and have addressed the issue as requested.

The review of the data received, performed according to the requirements of ICH M7, shows that only a few sources are concerned and appropriate actions are being taken to ensure that the level of azido impurities is below the threshold of toxicological concern (TTC) in these sources.

If confirmatory testing reveals that batches of APIs currently on the market exceed the applicable TTC limit, actions (e.g.

Risk of presence of mutagenic azido impurities in sartan active substances with a tetrazole ring



NEWS | 29 APRIL 2021 | STRASBOURG, FRANCE

Following recent investigations demonstrating the mutagenic of azido impurity in sartan active substances (APIs) with a tetrazole ring, the EDQM confirms that the CEP holders concerned have already been contacted and have addressed the issue as requested.

The review of the data received, performed according to the requirements of ICH M7, shows that only a few sources are concerned and appropriate actions are being taken to ensure that the level of azido impurities is below the threshold of toxicological concern (TTC) in these sources.

If confirmatory testing reveals that batches of APIs currently on the market exceed the applicable TTC limit, actions (e.g. suspension) may be taken by the EDQM for these CEPs.

The EDQM reminds CEP holders that they should provide the appropriate information relating to the risk identification they have performed for their CEP to their customers. The marketing authorisation holders will then be able to use this information to fulfil their legal responsibilities.

IMPURITIES

Proper Control Impurities Leads to Good Quality Medicines.

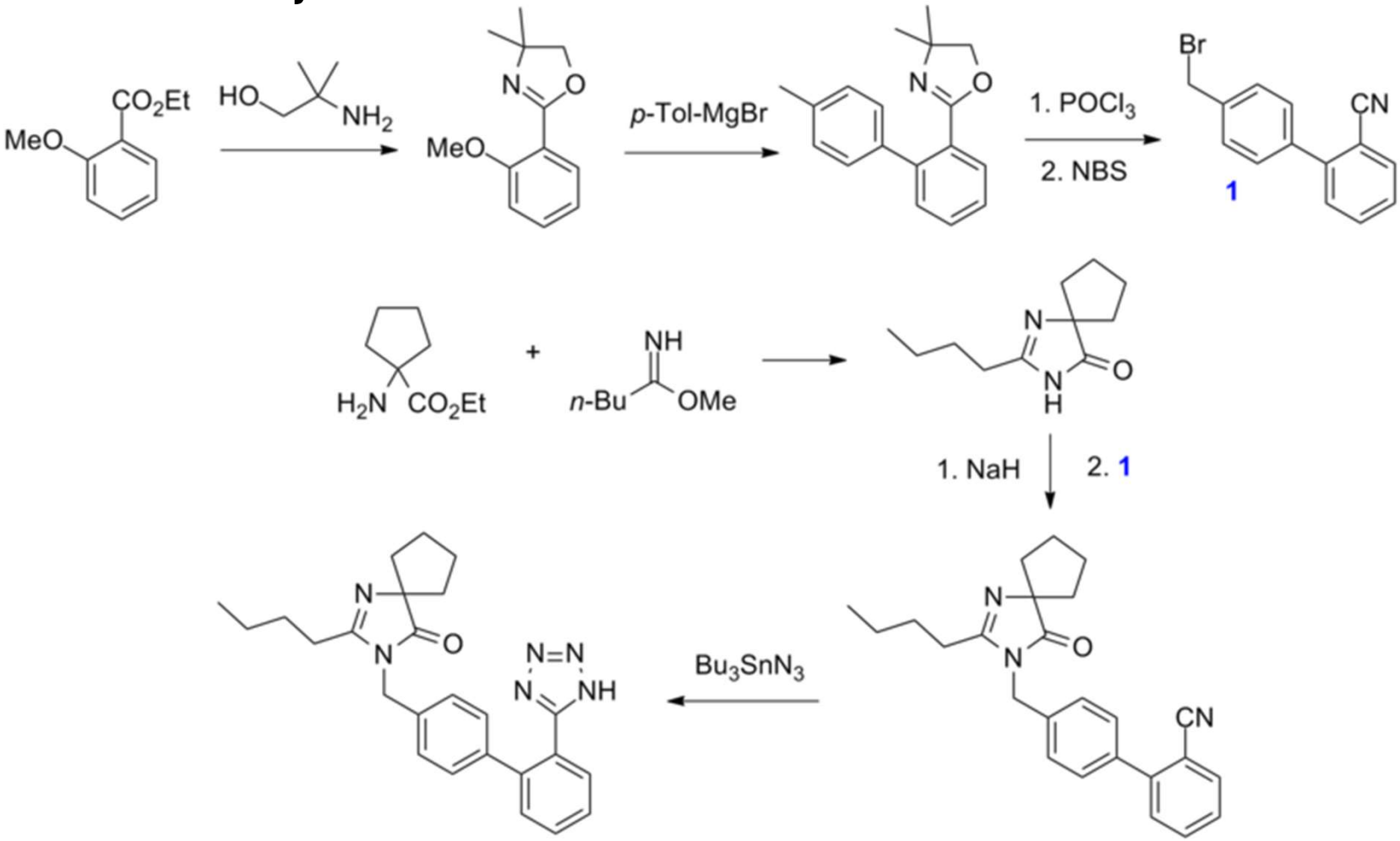
Source of Impurities: Drug substance synthesized via chemical route

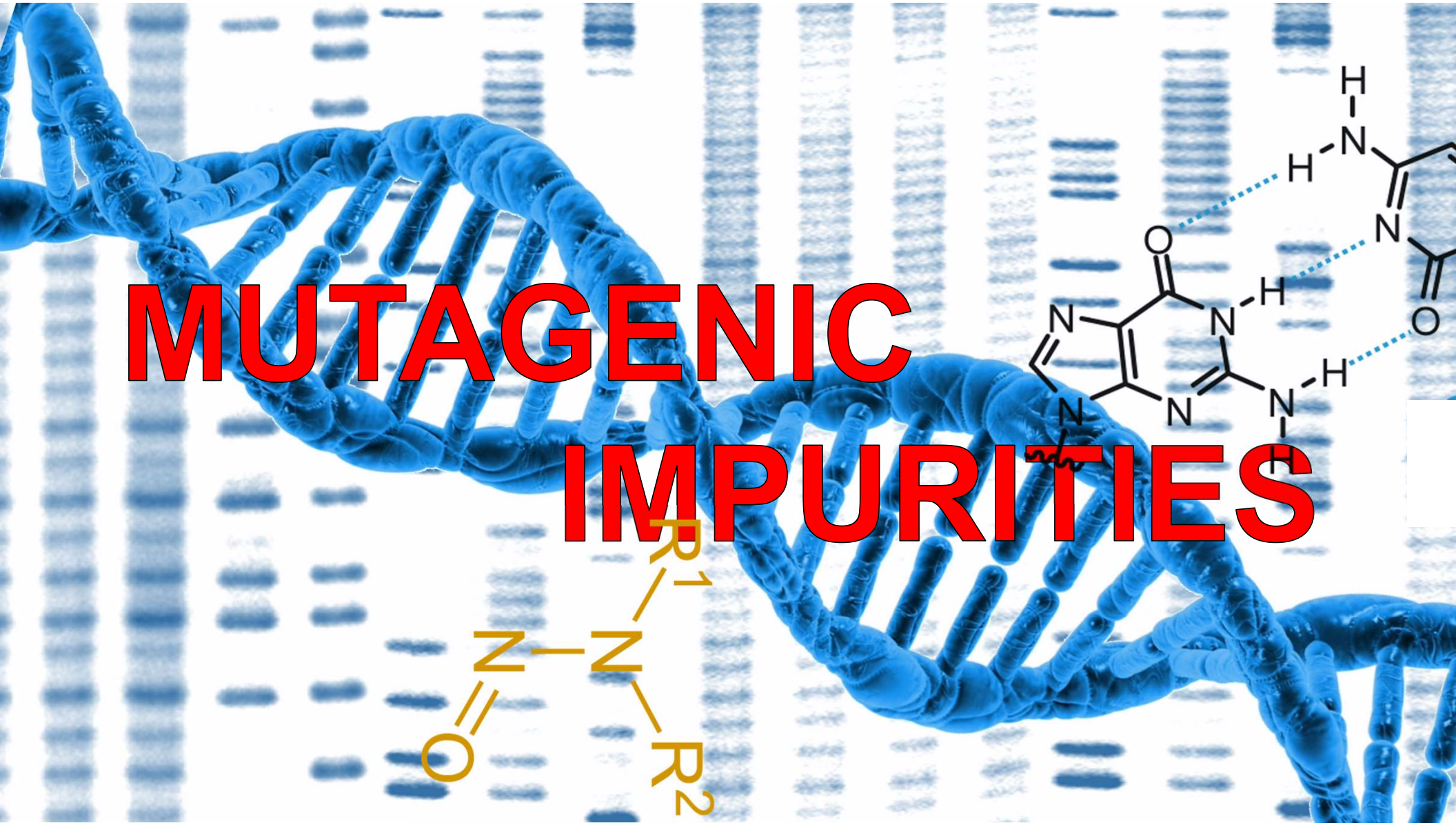
Source of Impurities: Raw material source, manufacturing

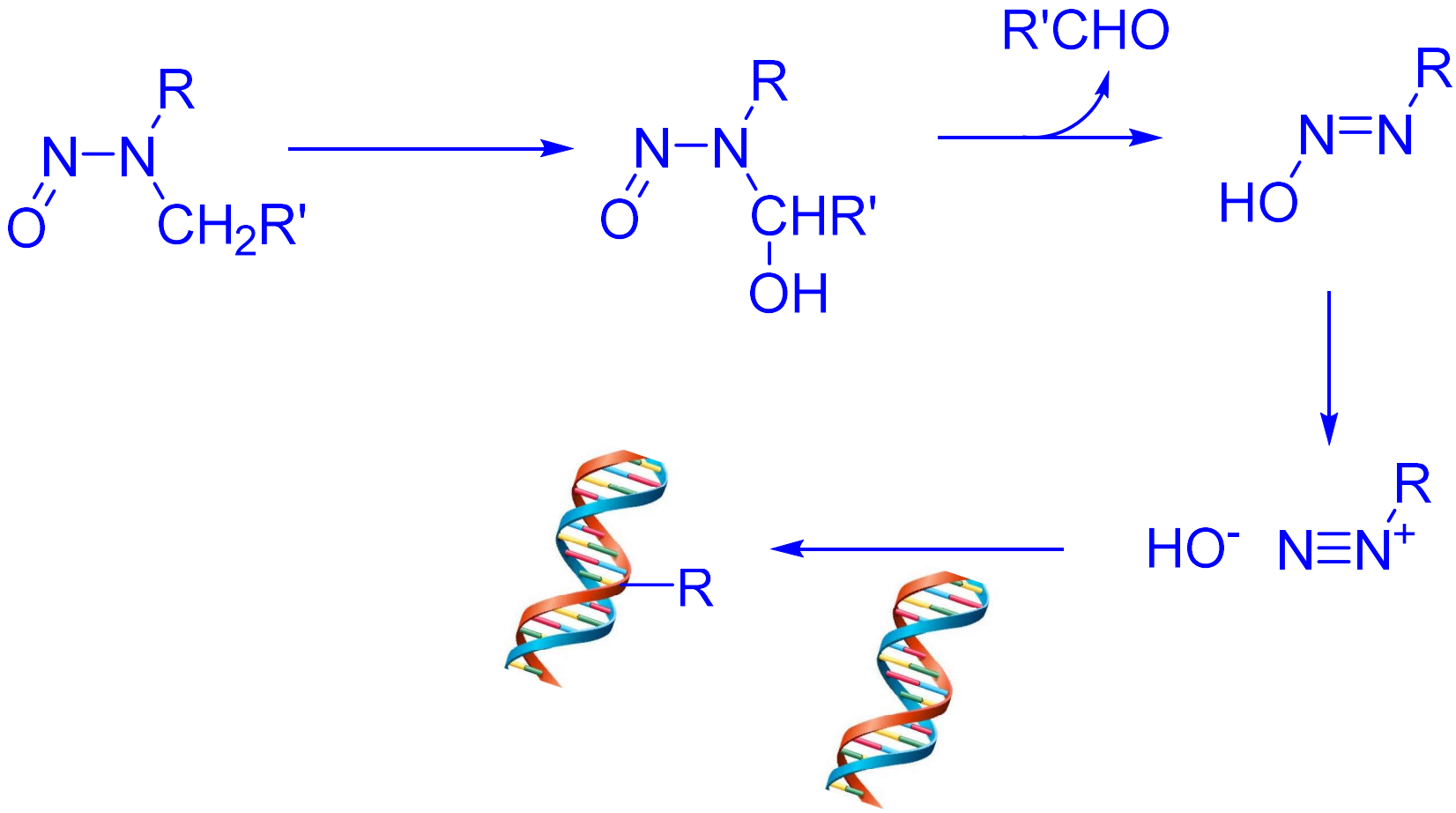
Source of Impurities: Chemical interaction between drug substance and excipient

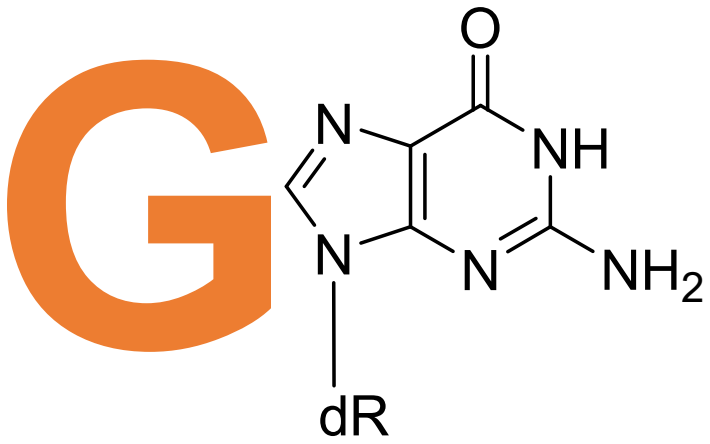
Source of Impurities: Drug product manufacturing process

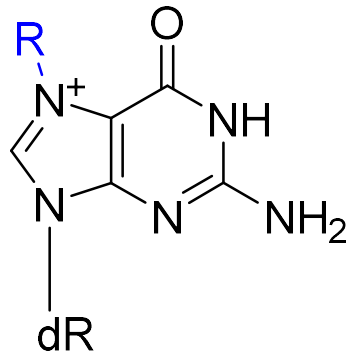
Example of Irbesartan Synthesis

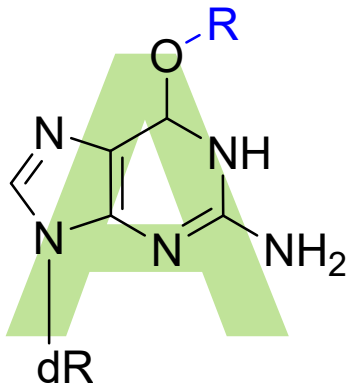


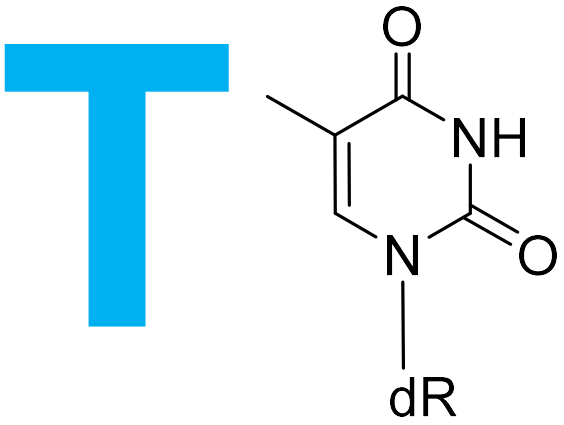

MUTAGENIC
IMPURITIES

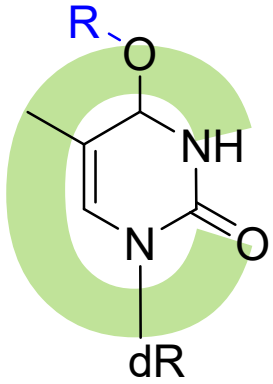

R
N–N
O
CH2R'
R
N–N
O
CHR'
OH
R'CHO
R
N=N
HO
R
HO- N≡N+
R


G
O
N
NH
N
N
NH2
dR

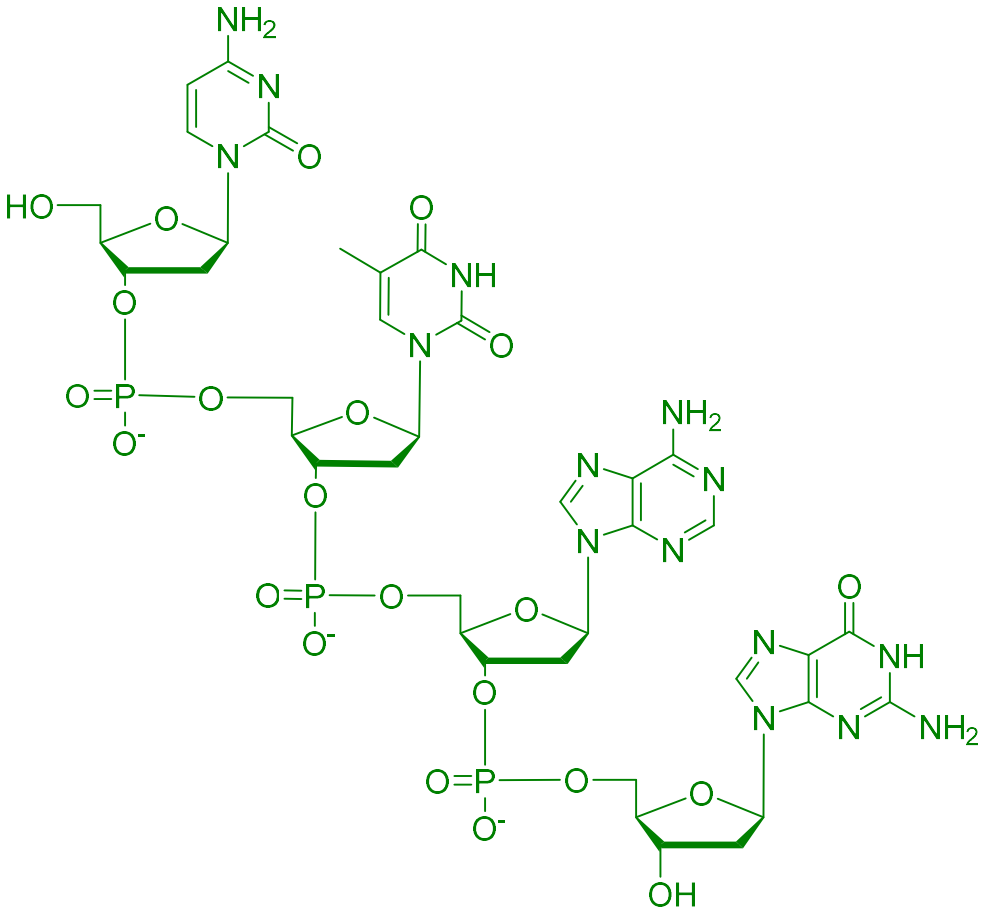

R
N+
O
NH
N
N
NH2
dR

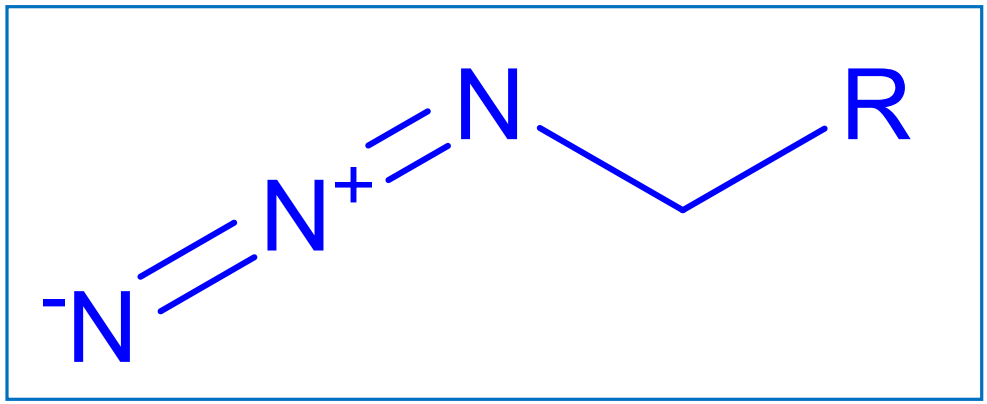

R
O
N
NH
N
N
NH2
dR

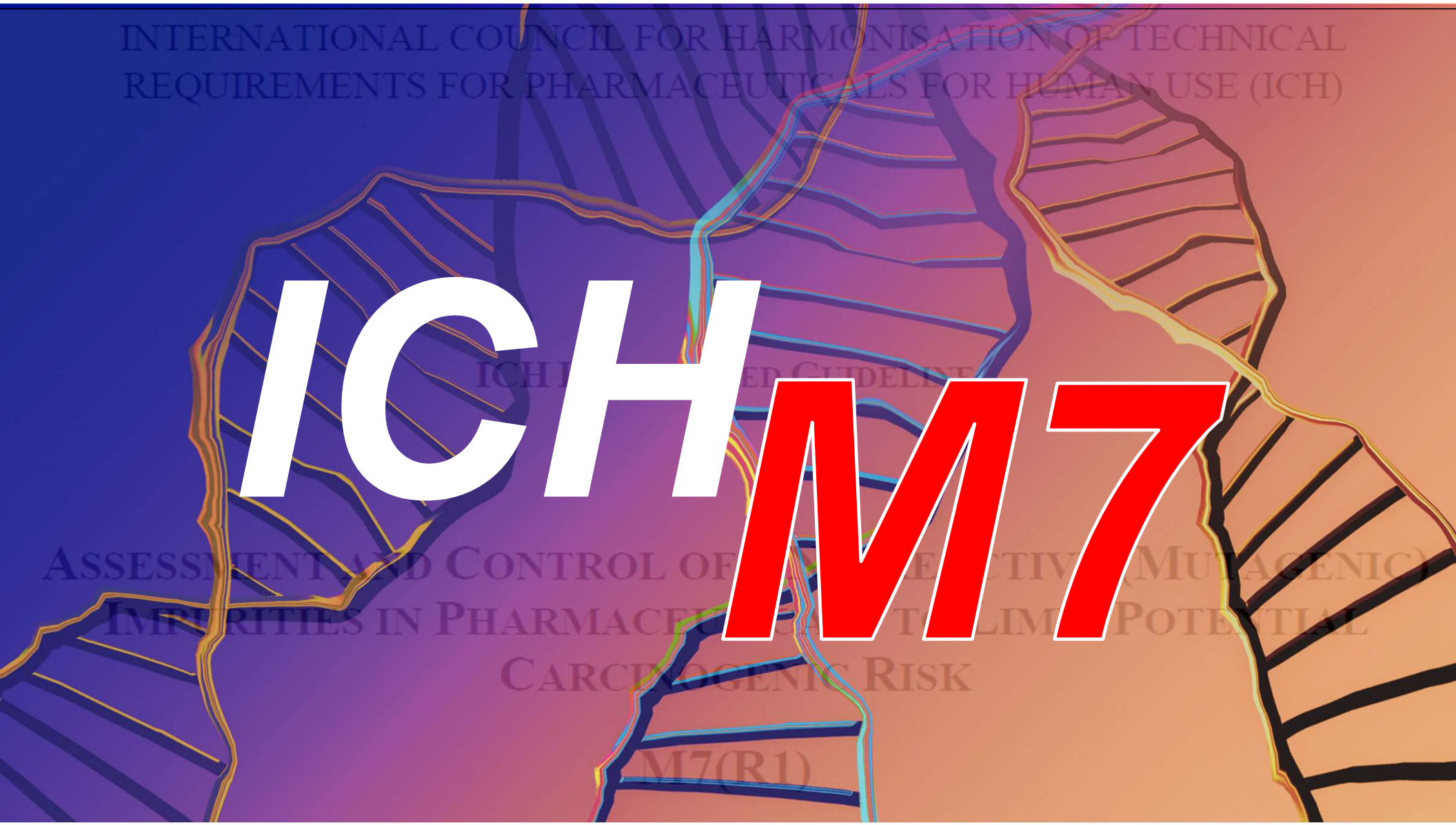

T
O
NH
N
O
dR


R
O
NH
N
O
dR

Azide DNA Damage






ICH
M7

INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE (ICH)

ICH HARMONISED GUIDELINE

ASSESSMENT AND CONTROL OF DNA REACTIVE (MUTAGENIC) IMPURITIES IN PHARMACEUTICALS TO LIMIT POTENTIAL CARCINOGENIC RISK

M7(R1)

Current *Step 4* version

dated 31 March 2017

INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE (ICH)

ICH HARMONISED GUIDELINE

APPLICATION OF THE PRINCIPLES OF THE ICH M7 GUIDELINE TO CALCULATION OF COMPOUND-SPECIFIC ACCEPTABLE INTAKES

Addendum to M7(R2)

Draft version

Endorsed on 6 October 2021

Currently under public consultation

Mutagenic/ Carcinogenic Genotoxic substances

Genotoxicity

A broad term that refers to any deleterious change in the genetic material regardless of the mechanism by which the change is induced. <*ICH M7*>

Mutagenic impurity

An impurity that has been demonstrated to be mutagenic in an appropriate mutagenicity test model, e.g., bacterial mutagenicity assay. <*ICH M7*>

Genotoxic carcinogen

a chemical capable of producing cancer by directly altering the genetic material

SCOPE & OBJECTION OF ICH M7

Focus on DNA reactive substances

Complement to ICH Q3A & Q3B

Example

In Q3B drug product (1 g/day) contain 0.2% impurity = 2 mg/day

Acrylonitrile PDE = 6 µg/day

SCOPE & OBJECTION OF ICH M7

Focus on DNA reactive substances

Complement to ICH Q3A & Q3B

Example

In Q3B drug product (1 g/day) contain 0.2% impurity = 2 **mg**/day

Acrylonitrile PDE = 6 **µg**/day

Impurities Classification with Respect to Mutagenic and Carcinogenic Potential and Resulting Control Actions

Class	Definition	Proposed action for control (details in Section 7 and 8)
1	Known mutagenic carcinogens	Control at or below compound-specific acceptable limit
2	Known mutagens with unknown carcinogenic potential (bacterial mutagenicity positive*, no rodent carcinogenicity data)	Control at or below acceptable limits (appropriate TTC)
3	Alerting structure, unrelated to the structure of the drug substance; no mutagenicity data	Control at or below acceptable limits (appropriate TTC) or conduct bacterial mutagenicity assay; If non-mutagenic = Class 5 If mutagenic = Class 2
4	Alerting structure, same alert in drug substance or compounds related to the drug substance (e.g., process intermediates) which have been tested and are nonmutagenic	Treat as non-mutagenic impurity
5	No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity	Treat as non-mutagenic impurity

*Or other relevant positive mutagenicity data indicative of DNA-reactivity related induction of gene mutations (e.g., positive findings in *in vivo* gene mutation studies)

ICH M7

Impurities Classification with Respect to Mutagenic and Carcinogenic Potential and Resulting Control Actions

Class	Definition	Proposed action for control (details in Section 7 and 8)
1	Known mutagenic carcinogens	Control at or below compound-specific acceptable limit
2	**Known mutagens with unknown carcinogenic potential (bacterial mutagenicity positive*, no rodent carcinogenicity data)**	**Control at or below acceptable limits (appropriate TTC)**
3	Alerting structure, unrelated to the structure of the drug substance; no mutagenicity data	Control at or below acceptable limits (appropriate TTC) or conduct bacterial mutagenicity assay; If non-mutagenic = Class 5 If mutagenic = Class 2
4	Alerting structure, same alert in drug substance or compounds related to the drug substance (e.g., process intermediates) which have been tested and are nonmutagenic	Treat as non-mutagenic impurity
5	No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity	Treat as non-mutagenic impurity

*Or other relevant positive mutagenicity data indicative of DNA-reactivity related induction of gene mutations (e.g., positive findings in *in vivo* gene mutation studies)

ICH M7

Risk characterization

TTC-based acceptable intakes

Acceptable intakes based on **compound-specific risk** assessments

Acceptable intakes in relation to **less-than-lifetime (LTL) exposure**

A Threshold of Toxicological Concern (TTC)

Define an acceptable intake for **any unstudied chemical** that poses a negligible risk of carcinogenicity or other toxic effects.

For application of a TTC in the assessment of acceptable limits of mutagenic impurities in drug substances and drug products, a value of **1.5 µg/day**

NOT FOR NITROSAMINES

LTL (Less Than Lifetime)

Acceptable Intakes for an Individual Impurity

Duration of treatment	≤ 1 month	>1 - 12 months	>1 - 10 years	>10 years to lifetime
Daily intake [µg/day]	120	20	10	1.5

Acceptable Total Daily Intakes for Multiple Impurities

Duration of treatment	≤ 1 month	>1 - 12 months	>1 - 10 years	>10 years to lifetime
Total Daily intake [µg/day]	120	60	30	5

LTL (Less Than Lifetime)

Acceptable Intakes for an Individual Impurity

Duration of treatment	**≤ 1 month**	**>1 - 12 months**	**>1 - 10 years**	**>10 years to lifetime**
Daily intake [µg/day]	120	20	10	1.5

Acceptable Total Daily Intakes for Multiple Impurities

Duration of treatment	**≤ 1 month**	**>1 - 12 months**	**>1 - 10 years**	**>10 years to lifetime**
Total Daily intake [µg/day]	120	60	30	5

Examples of clinical use scenarios with different treatment durations for applying acceptable intakes

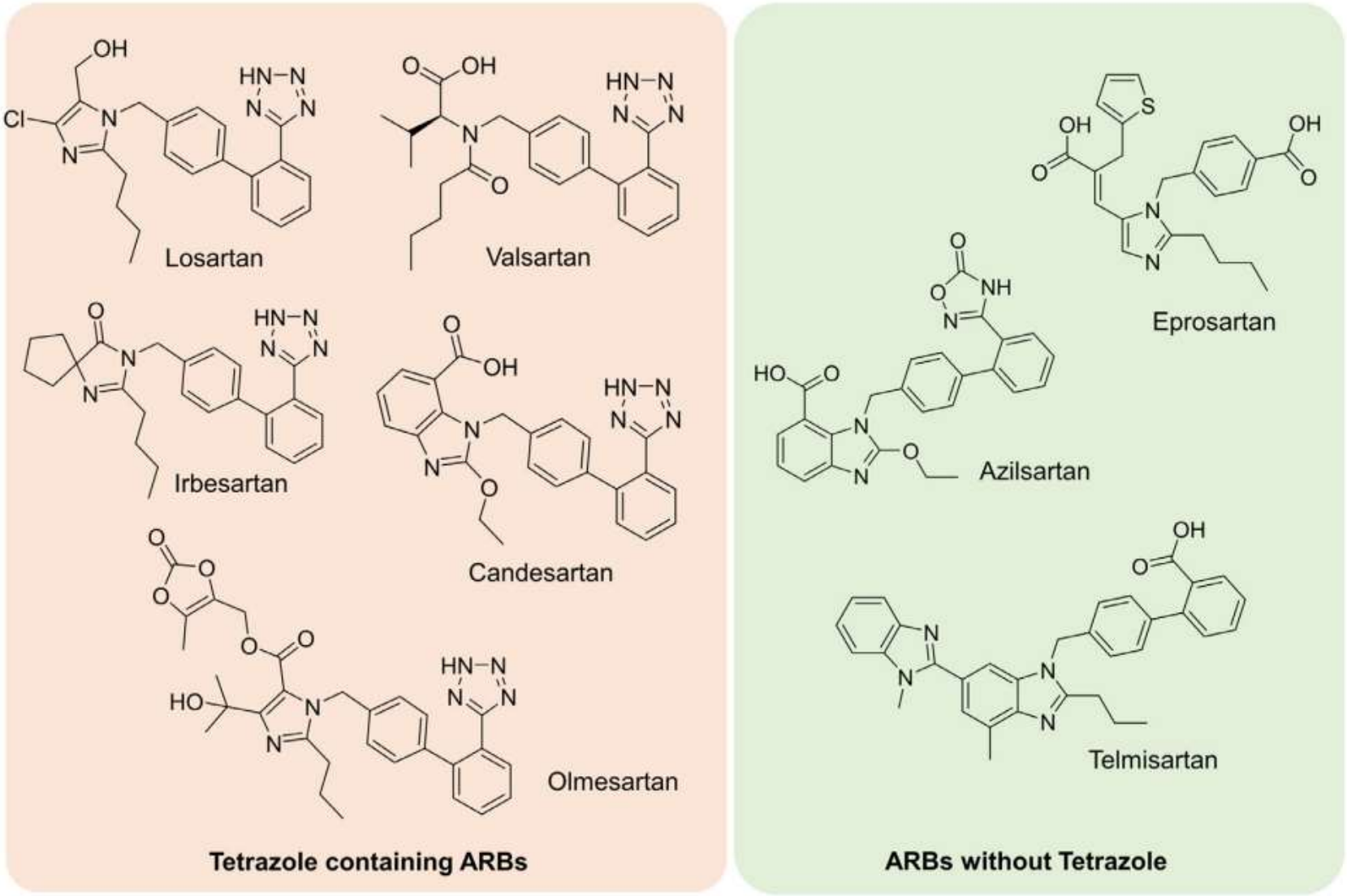
Scenario[1]	Acceptable Intake (µg/day)
Treatment duration of ≤ 1 month: e.g., drugs used in emergency procedures (antidotes, anesthesia, acute ischemic stroke), actinic keratosis, treatment of lice	120
Treatment duration of > 1-12 months: e.g., anti-infective therapy with maximum up to 12 months treatment (Hepatitis C Virus), parenteral nutrients, prophylactic flu drugs (~ 5 months), peptic ulcer, Assisted Reproductive Technology (ART), pre-term labor, preeclampsia, pre-surgical (hysterectomy) treatment, fracture healing (these are acute use but with long half-lives)	20
Treatment duration of >1-10 years: e.g., stage of disease with short life expectancy (severe Alzheimer's), non-genotoxic anticancer treatment being used in a patient population with longer-term survival (breast cancer, Chronic Myelogenous Leukemia), drugs specifically labeled for less than 10 years of use, drugs administered intermittently to treat acute recurring symptoms[2] (chronic Herpes, gout attacks, substance dependence such as smoking cessation), macular degeneration, Human Immunodeficiency Virus (HIV)[3]	10
Treatment duration of >10 years to lifetime: e.g., chronic use indications with high likelihood for lifetime use across broader age range (hypertension, dyslipidemia, asthma, Alzheimer's Disease (AD) (except severe AD), hormone therapy (e.g., Growth Hormone, Thyroid Hormone, Para Thyroid Hormone), lipodystrophy, schizophrenia, depression, psoriasis, atopic dermatitis, Chronic Obstructive Pulmonary Disease (COPD), cystic fibrosis, seasonal and perennial allergic rhinitis	1.5

Ref.: M7(R1) , Table 4

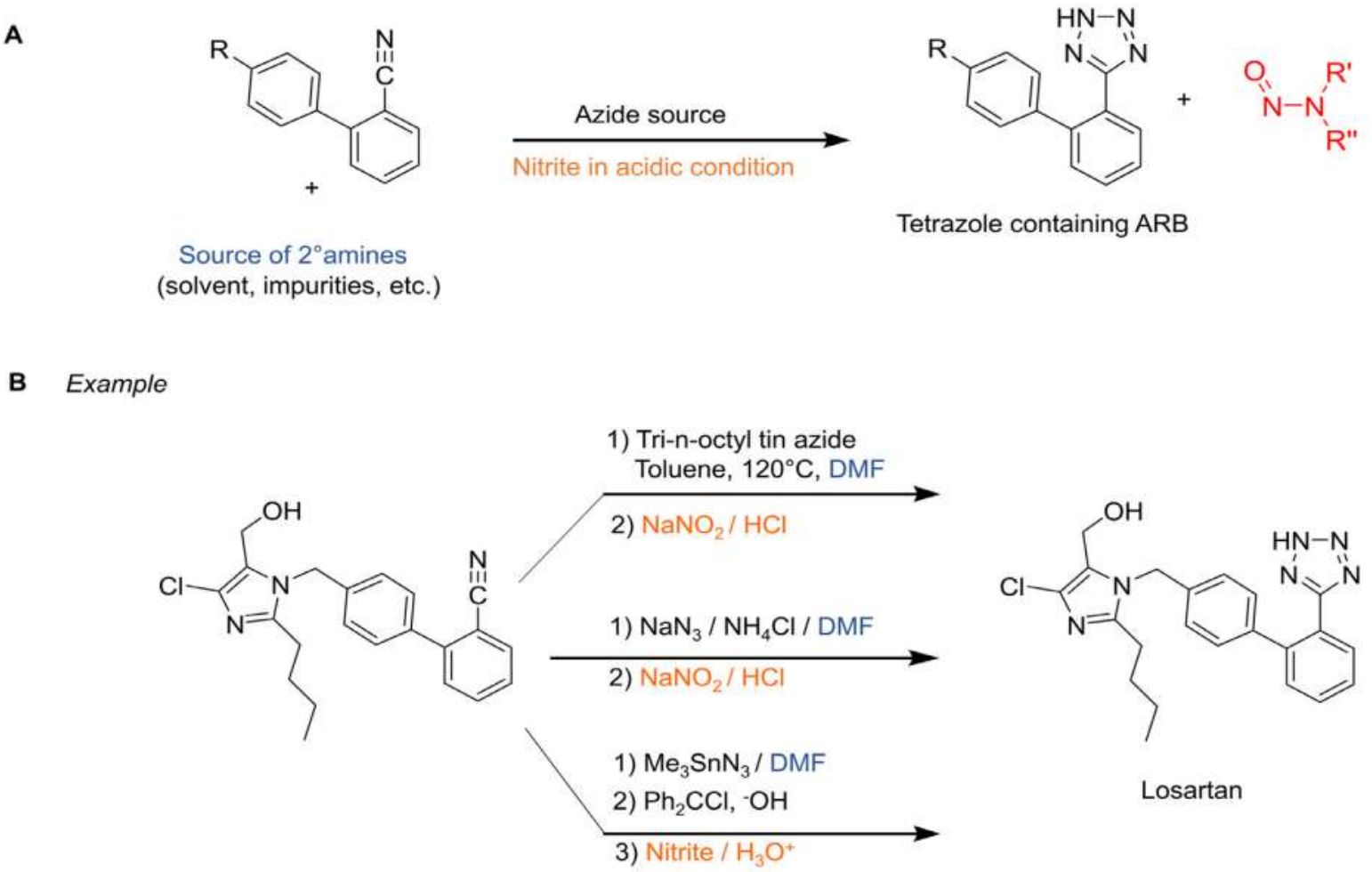
[1] This table shows general examples; each example should be examined on a case-by-case basis. For example, 10 µg/day may be acceptable in cases where the life expectancy of the patient may be limited, e.g., severe Alzheimer's disease, even though the drug use could exceed 10-year duration.

[2] Intermittent use over a period >10 yrs., but based on calculated cumulative dose, it falls under the >1-10 yr. category.

[3] HIV is considered a chronic indication, but resistance develops to the drugs after 5-10 years and the therapy is changed to other HIV drugs.



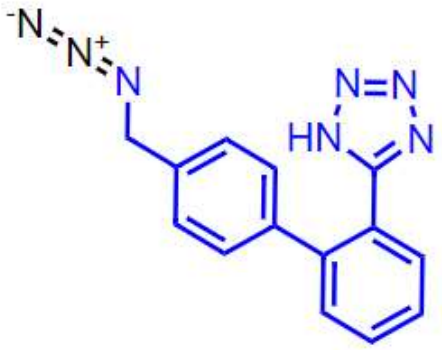
Tuesuwan, B.; Vongsutilers, V. Nitrosamine contamination in pharmaceuticals: threat, impact, and control. J. Pharm. Sci. 2021 https://doi.org/10.1016/j.xphs.2021.04.021

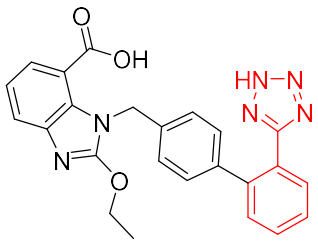


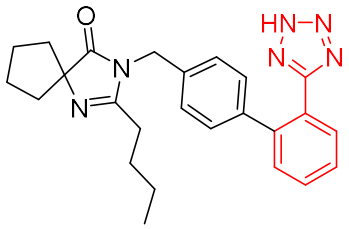
Tuesuwan, B.; Vongsutilers, V. Nitrosamine contamination in pharmaceuticals: threat, impact, and control. J. Pharm. Sci. 2021
https://doi.org/10.1016/j.xphs.2021.04.021

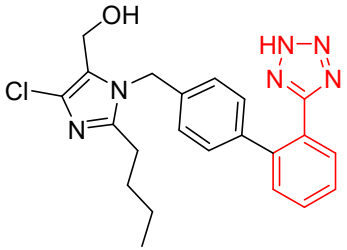
Limits for AZBT impurities

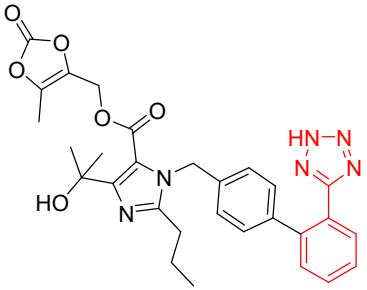
	AZBT	
Active substance (max daily dose)	**Maximum daily intake (ng/day)**	**Limit (ppm)**
Candesartan (32 mg)	1.5	46.8
Irbesartan (300 mg)	1.5	5.0
Losartan (150 mg)	1.5	10.0
Olmesartan (40 mg)	1.5	37.5
Valsartan (320 mg)	1.5	4.68

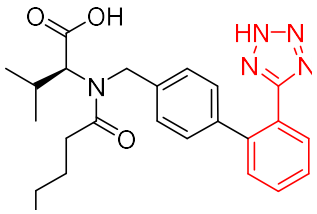




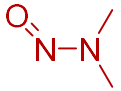
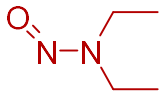




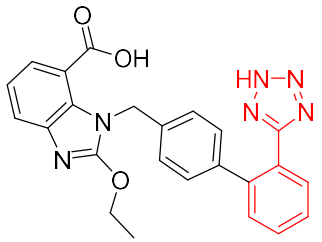
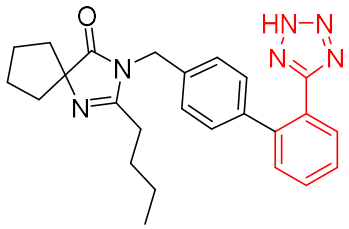
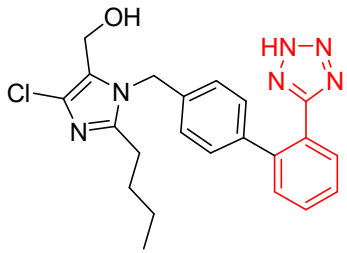
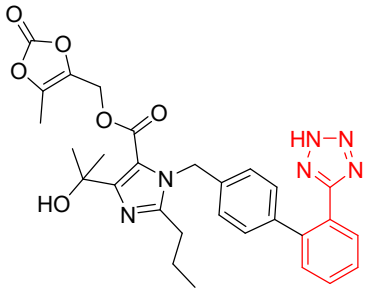
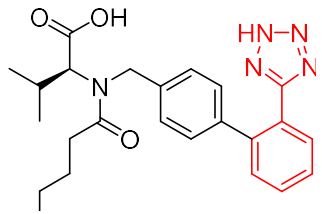




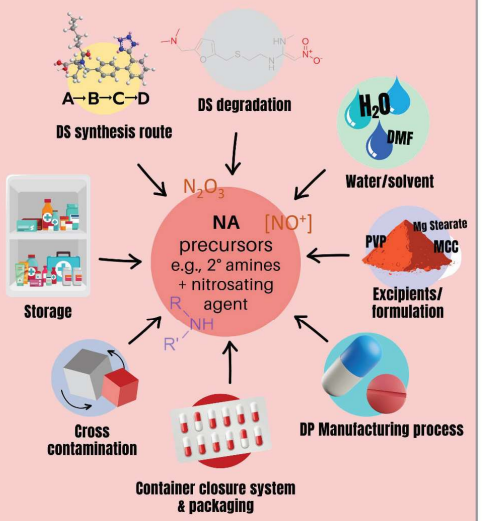
Temporary limits for NDMA and NDEA impurities

	NDMA 		NDEA 	
Active substance (max daily dose)	**Maximum daily intake (ng)**	**Limit (ppm)**	**Maximum daily intake (ng)**	**Limit (ppm)**
Candesartan (32 mg)	96.0	3.000	26.5	0.820
Irbesartan (300 mg)	96.0	0.320	26.5	0.088
Losartan (150 mg)	96.0	0.640	26.5	0.177
Olmesartan (40 mg)	96.0	2.400	26.5	0.663
Valsartan (320 mg)	96.0	0.300	26.5	0.082

Reference: EMA

Step 1
Risk evaluation

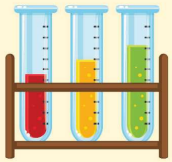


Estimation of reactivity of NA formation

Potential NA in drug products

Database of NA precursor e.g., nitrite in excipient database

Step 2
Confirmatory testing



- Mandatory method validation
- Published/Pharmacopoeial methods for known NA
- Method development & method validation for new NA in question
- LOQ must meet objective of testing of each product

Absence | Presence | Level of NA in DP

Toxicity of found NA

Previously reported data

Estimation by QSAR

LTL concept in ICH M7?

Step 3
Control/Change/Variation to mitigate risk of NA

- Depend on the level of contamination and root- cause
- **Above AI** → implement control strategy to keep NA under AI limit
  - **> 30% AI** → control NA in specification
  - **≤ 30% AI** → propose skip-testing of NA
  - **≤ 10% AI** → propose omission of NA testing
- All changes/variation must be reported to relevant regulatory agencies

✵ Aiding tools for nitrosamine risk evaluation

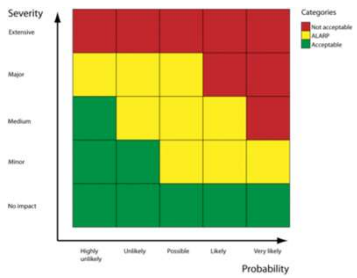
DS : Drug substances
DP : Drug products
NA : Nitrosamines

Tuesuwan, B; Vongsutilers, V., Current threat of nitrosamines in pharmaceuticals and scientific strategies for risk mitigation, *J Pham Sci., 112 (2023) 1192– 1209*

RISK ASSESSMENT

Identify

known and potential sources



Evaluate

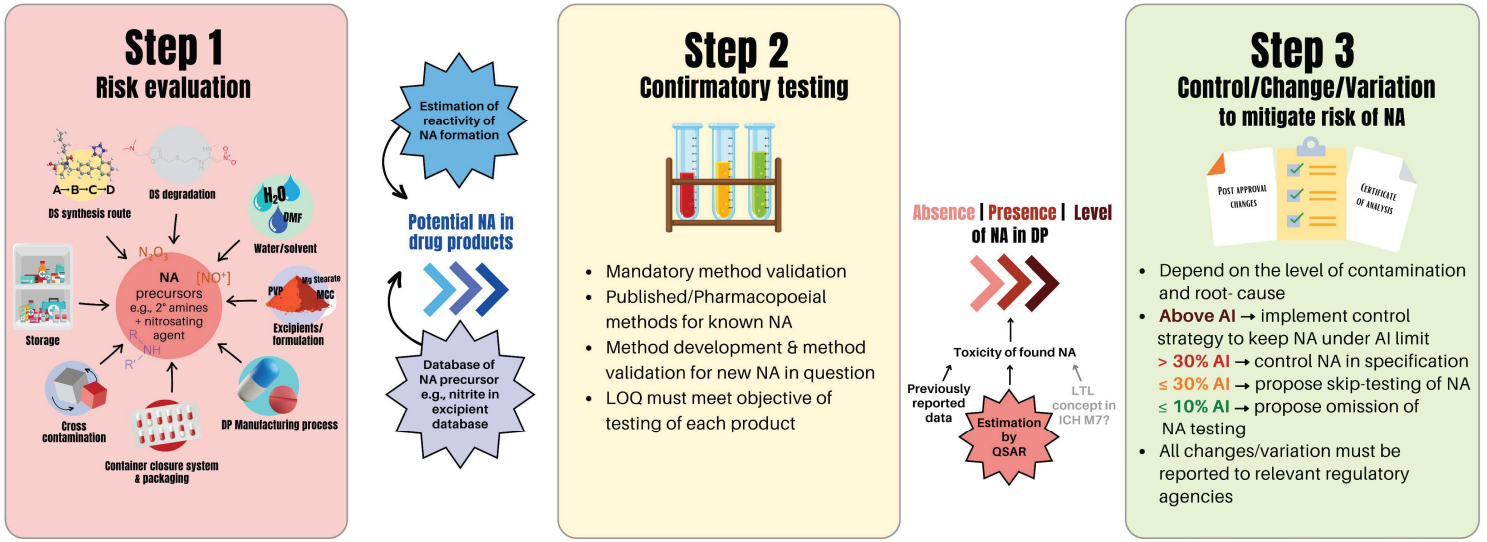
the presence of mutagenic impurity

Summarize & document

the risk assessment

Ass

port
nges
nented
thority



Aiding tools for nitrosamine risk evaluation

DS : Drug substances
DP : Drug products
NA : Nitrosamines

What should be appropriated control strategies?

API/Excipient manufacturers

Finished product manufacturers

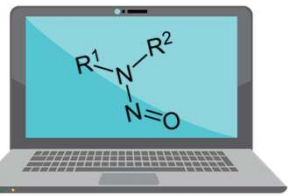
Regulatory Agency

Importer

Hospital

Others

What should be considered in dealing with **nitrosamine impurities** issues?



INFORMATION

is the key to assess risk

PRIORITIZING

- High dose medication
- Duration of treatment
- Therapeutic indication
- Number of patients treated

PHASING

e.g., EMA approach

The call for review consists of the following 3 steps:

- Step 1: MAHs to perform a risk evaluation to identify if APIs and/or FPs could be at risk of N-nitrosamine in accordance with the principles outlined in Q&A 7 of the Q&A for the implementation of the Article 5(3) referral:
  - to be undertaken before 31 March 2021 for human medicines containing chemically synthesised APIs;
  - to be undertaken before 1 July 2021 for human medicines containing biological act substances.
- Step 2: if a risk is identified, MAHs to proceed with confirmatory testing in order to co with the principles outlined in Q&A Q&A for the implementation of the Article 5(3) referral. MAHs should report the ou soon as possible.
- Step 3: the presence of nitrosamines above the AI MAHs should implement effe mitigating measures through the submission of variations.
  - to be undertaken before 26 September 2022 for human medicines containing che synthesised APIs;
  - to be undertaken before 1 July 2023 for human medicines containing biological a substances.

Patients taking sartan medicines should not stop their treatment without first consulting a doctor or pharmacist.

As the risks are very low and associated with long-term exposure, there is no immediate health risk.

TGA : 20 August 2021

What should health professionals do?

Please be aware of this issue and advise patients accordingly.

There is no reason to stop prescribing sartans.

However, there may be limited availability of some irbesartan and losartan brands due to the current shortage.

You may wish to remind patients of the importance of keeping their blood pressure under control and reassure them that the risk posed by the impurity at the trace levels observed to date is very low.

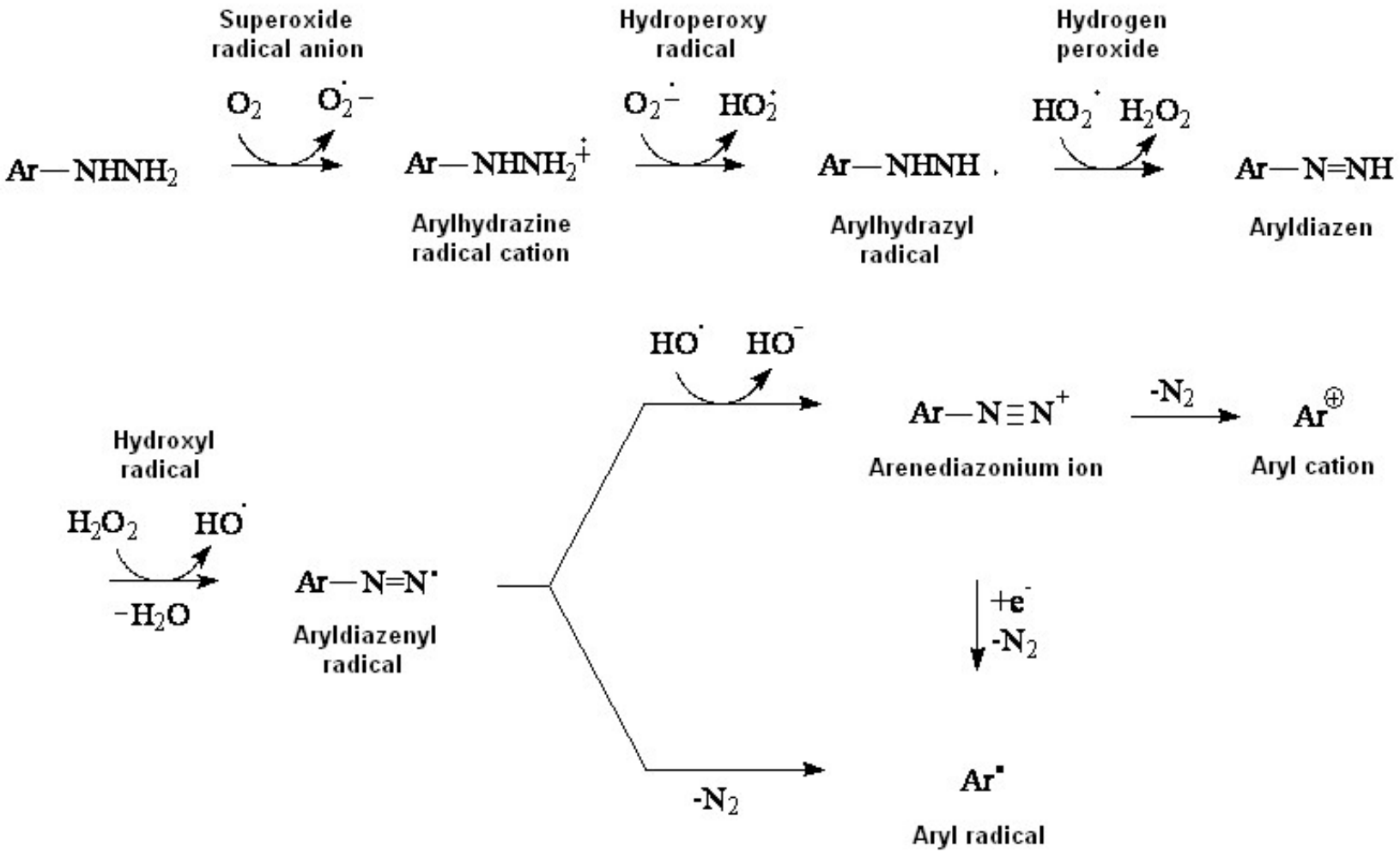
TGA : 20 August 2021

MUTAGENIC IMPURITIES

Vorasit Vongsutilers, Ph.D., R.Ph. Bodin Tuesuwan, Ph.D., R.Ph

Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University

MOA Hydrazine DNA Damage

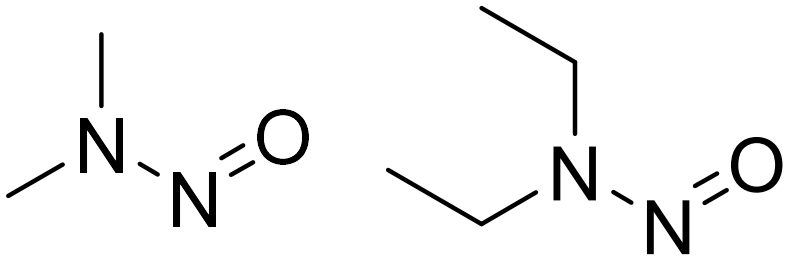


Public advisory

Multiple lots of irbesartan, losartan and valsartan drugs recalled

Starting date:	May 30, 2021
Posting date:	October 26, 2021
Type of communication:	Advisory
Subcategory:	Drugs
Source of recall:	Health Canada
Issue:	Product Safety

NDMA and NDEA



"probably carcinogenic to human"

International Agency for Research on Cancer (IARC), WHO (1987)

"reasonably anticipated to be human carcinogens"

National Toxicology Program, U.S. Department of Health and Human Services (2017)