Advanced Course in Toxicology: March 01, 2021

Application of SAR and modelling in pharmaceutical and medical device industry:

- Secondary Pharmacology Modelling — Andreas Czich
- Nitrosamines in the framework of ICH M7 — Andreas Czich
- Implication for risk assessment of Medical Devices — Susanne Dorn

SANOFI

Application of SAR and modelling in pharmaceutical industry: Secondary pharmacology modelling (e.g. CiPA initiative) and Nitrosamines in the context of ICH M7

Andreas Czich, R&D Preclinical Safety Frankfurt

In silico prediction activities in the regulatory context

EMA reflection paper and FDA considerations

- **EMA Content: Qualification of non-genotoxic impurities**
  - Defining the process of risk assessment
  - Risk assessment by including information from
    - toxicological databases (e.g. IMI eTOX database)
    - (Q)SAR approaches/tools
      → referring to in silico toxicology protocols regarding endpoints and method
    - read-across (RAX) approaches
    - in vitro data

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

15 November 2018
EMA/CHMP/SWP/545588/2017
Committee for Medicinal Products for Human Use (CHMP)

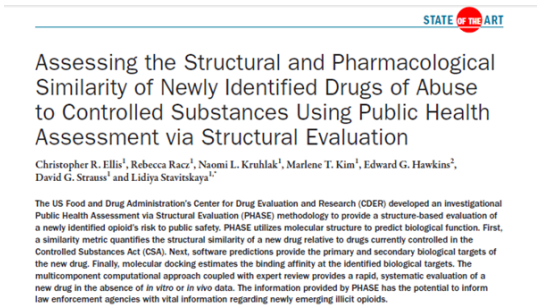
Reflection paper on the qualification of non-genotoxic impurities
Draft

Draft agreed by Safety Working Party	October 2018
Adopted by CHMP for release for consultation	15 November 2018
Start of public consultation	23 November 2018
End of consultation (deadline for comments)	30 September 2019

Comments should be provided using this template. The completed comments form should be sent to SWP-H@ema.europa.eu

Keywords	*Non-genotoxic impurities, pharmacology, toxicology, threshold of toxicological concern, read across, animal testing, in vitro testing, 3R's.*

- **FDA many activities in the area of 2nd pharmacology**
  - DALA (Drug Abuse Liability Assessment)
  - Cardiovascular Safety

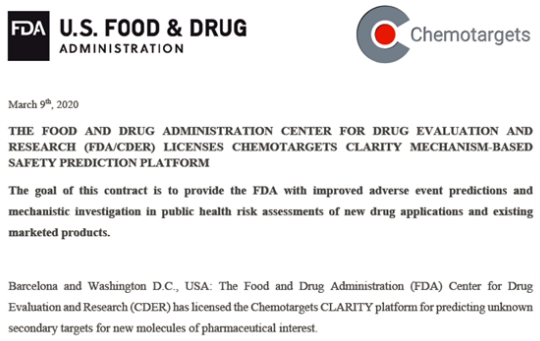


STATE OF THE ART

Assessing the Structural and Pharmacological Similarity of Newly Identified Drugs of Abuse to Controlled Substances Using Public Health Assessment via Structural Evaluation

Christopher R. Ellis[1], Rebecca Racz[1], Naomi L. Kruhlak[1], Marlene T. Kim[1], Edward G. Hawkins[2], David G. Strauss[1] and Lidiya Stavitskaya[1,*]

The US Food and Drug Administration's Center for Drug Evaluation and Research (CDER) developed an investigational Public Health Assessment via Structural Evaluation (PHASE) methodology to provide a structure-based evaluation of a newly identified opioid's risk to public safety. PHASE utilizes molecular structure to predict biological function. First, a similarity metric quantifies the structural similarity of a new drug relative to drugs currently controlled in the Controlled Substances Act (CSA). Next, software predictions provide the primary and secondary biological targets of the new drug. Finally, molecular docking estimates the binding affinity at the identified biological targets. The multicomponent computational approach coupled with expert review provides a rapid, systematic evaluation of a new drug in the absence of *in vitro* or *in vivo* data. The information provided by PHASE has the potential to inform law enforcement agencies with vital information regarding newly emerging illicit opioids.



FDA U.S. FOOD & DRUG ADMINISTRATION

Chemotargets

March 9th, 2020

THE FOOD AND DRUG ADMINISTRATION CENTER FOR DRUG EVALUATION AND RESEARCH (FDA/CDER) LICENSES CHEMOTARGETS CLARITY MECHANISM-BASED SAFETY PREDICTION PLATFORM

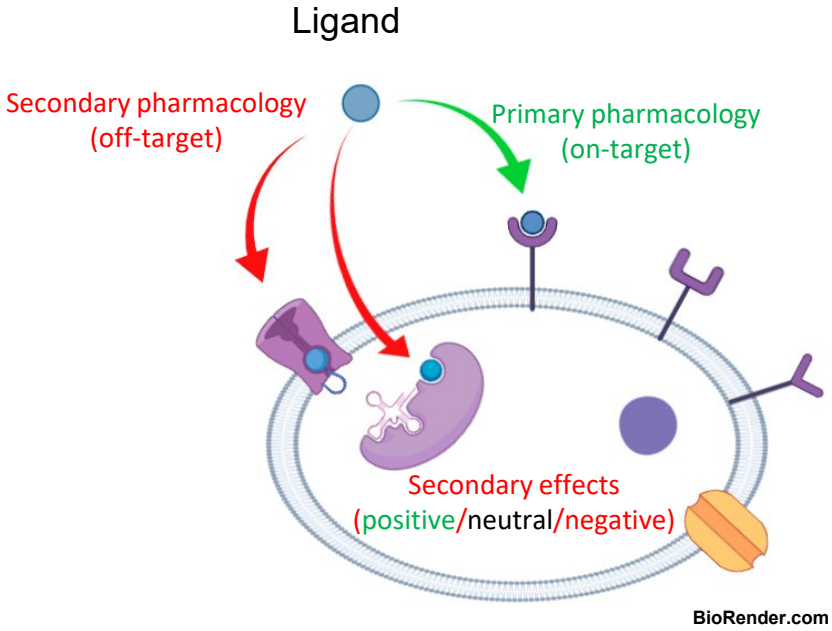
The goal of this contract is to provide the FDA with improved adverse event predictions and mechanistic investigation in public health risk assessments of new drug applications and existing marketed products.

Barcelona and Washington D.C., USA: The Food and Drug Administration (FDA) Center for Drug Evaluation and Research (CDER) has licensed the Chemotargets CLARITY platform for predicting unknown secondary targets for new molecules of pharmaceutical interest.

[6] European Medicines Agency "Reflection paper on the qualification of non-genotoxic impurities - Draft" *EMA/CHMP/SWP/545588/2017, 1-9,* 2018

Secondary Pharmacology (2ndP) / Off-Target

- Compounds and metabolites can trigger target-mediated secondary pharmacology
- Synthetic drugs cover a broad(er) target space
- Small molecules tend to be more promiscuous
  - A drug may hit 6.3 targets on average*
- More than 100 targets have been associated with clinical drug side effects already**
- Several Initiatives ongoing, oben source models available

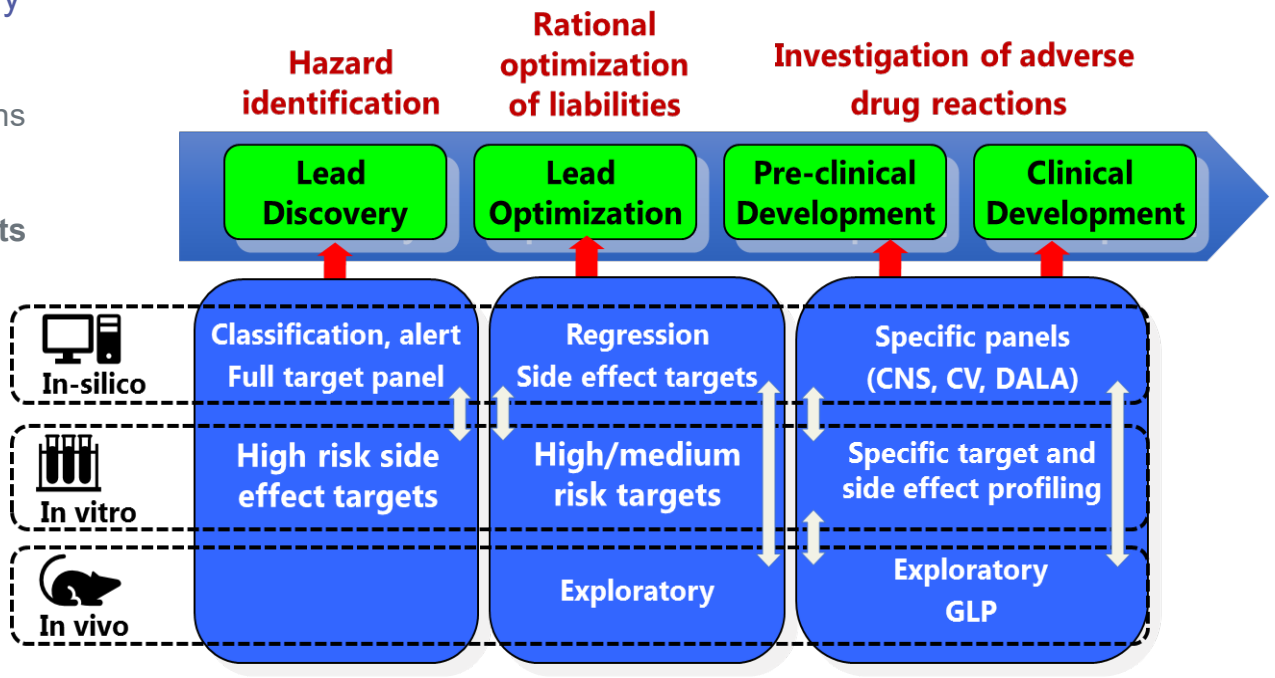


Adapted from JPTM 2020, 105:106869.
doi: 10.1016/j.vascn.2020.106869.

*Mestres et al., DDT 14 (9), 479-485.
** Bowes et al (2012) Nature Rev Drug Discov 11:909-922; Lynch et al (2017) JPTM S1056-8719(16):30147-30152.

Addressing Secondary Pharmacology (2ndP)

- Cascaded in silico / in vitro / in vivo investigations
- Role of computational Toxicology
  - Optimize screening throughput
  - Compound prioritization
  - Guiding more targeted investigations to safe resources and animals
  - **Fill data gaps, providing weight of evidence (2 regulatory requests in 2020, one will be presented at SOT 2021)**
  - Mechanism of toxicity support, creating hypothesis
  - New identified impurities
  - Regulatory questions
  - ……



*J Pharmacol Toxicol Methods. 2020 Apr 14:106869. doi: 10.1016/j.vascn.2020.106869.
A practical guide to secondary pharmacology in drug discovery.

CIPA Initiative

CiPA Comprehensive in vitro Pro-arrhythmia Assay

= initiative to propose a novel safety *in vitro* / *in silico* screening paradigm for the assessment of ventricular proarrhythmic liabilities [1]

- ➡ led by Cardiac Safety Research Consortium, HESI, FDA
- ➡ indented to revise the ICH S7B guideline and to eliminate the clinical thorough QT (TQT) study

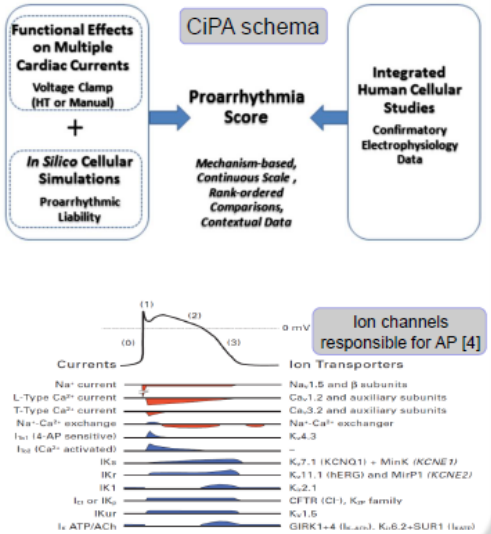
● **Schematic elements of CiPA** [2]

1) *in vitro* assays determining functional effects on 7 key cardiac ion channels
   - IKr (hERG)
   - IKs
   - Ito
   - IK1
   - ICaL (Cav1.2)
   - INa (Nav1.5: peak & late)

   outward currents / inward currents

2) *in silico* models simulating the cellular action potential (AP) based on O'Hara/Rudy type [3]

3) human stem cell-derived cardiomyocytes confirming proarrhythmic potential



Model available at the FDA Homepage

Validation demonstrated: CiPA model can be applied in early research to eliminate drug candidates with proarrhythmic liabilities

Depending on drug development phases different data is used for proarrhythmic risk assessment

- Inhibition (IC50) of the 3 ion channels hERG, Cav1.2 and Nav1.5 predicted by in silico
- Action potential (AP) prolongation simulated by the CIPA model or measured in the Purkinje fiber assay

1] Cavero I, Holzgrefe H. Comprehensive in vitro Proarrhythmia Assay, a novel in vitro/in silico paradigm to detect ventricular proarrhythmic liability: a visionary 21st century initiative. Expert Opin. Drug Saf. 13 (6), 745-758, 2014

[2] Sager PT, Gintant G, Turner JR, Pettit S, Stockbridge N. Rechanneling the cardiac proarrhythmia safety paradigm: A meeting report from the Cardiac Safety Research Consortium. Am. Heart J. 167 (3), 292-300, 2014

[3] O'Hara T, Virág L, Varró A, Rudy Y. Simulation of the undiseased human cardiac ventricular action potential: model formulation and experimental validation. PLoS Comput Biol. 7(5), e1002061, 2011

[4] Abriel H, Schläpfer J, Keller DI, Gavillet B, Buclin T, Biollaz J, Stoller R, Kappenberger., Swiss Med. Wkly. 134(47-48), 685-194, 2004

ICH M7 Guideline

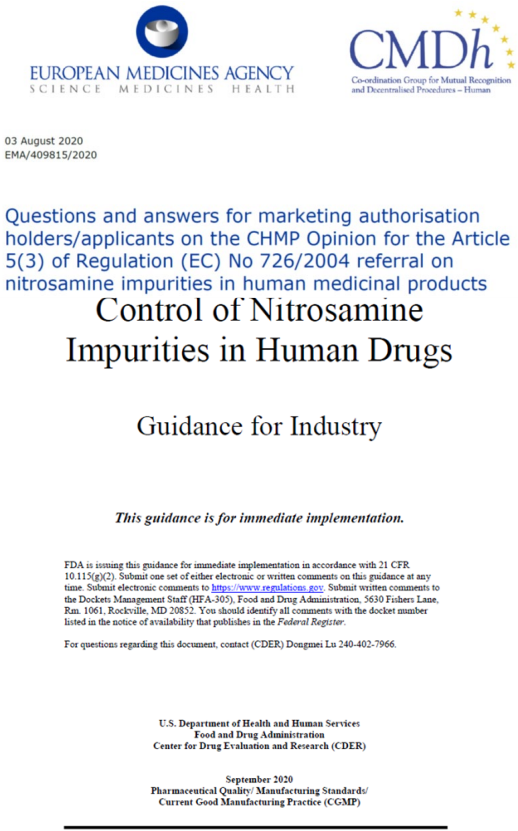
- ICH M7 implemented in 2014, providing a practical, harmonized framework for identification, categorization, qualification and control of mutagenic impurities
  - To limit potential carcinogenic risk
- DNA reactive substances with the potential to directly cause DNA damage / causing cancer
  - Applicable to clinical development and marketing
  - Provides recommendations relevant to both safety and analytics
- Identification of potential mutagenic impurities
  - Database and literature searches for carcinogenicity and bacterial mutagenicity
  - Assessment of (Quantitative) Structure-Activity Relationships (QSAR) that focuses on bacterial mutagenicity predictions
  - Two (Q)SAR prediction methodologies required in ICH M7: Expert rule-based &Statistical-based
  - Expert knowledge use for additional supportive evidence on relevance of any positive, negative, conflicting or inconclusive in silico prediction

Class	Definition	Action for Control
1	Known mutagenic carcinogens	≤ compound-specific limit Acceptable Intake (AI) or PDE
2	Known mutagens with unknown carcinogenic potential potential (bacterial mutagenicity positive, no rodent carcinogenicity data)	≤ appropriate Threshold of Toxicological Concern (TTC)
3	Alerting structure, unrelated to structure of drug substance (DS); no mutagenicity data	≤ appropriate TTC *or* conduct Ames test (negative = Class 5; positive = Class 2)
4	Alerting structure, same alert in DS or compounds related to DS (e.g., process intermediates) which have been tested and are non-mutagenic	Non-mutagenic impurity
5	No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity	

Regulation of *N*-Nitrosamine drug impurities

- ICH M7 Guidance describes assessment of impurities in pharmaceuticals
  - Nitrosamines are cohorts of concern (CoC)
  - For CoC the default Threshold of Toxicological Concern can not be used to generate a toxicology limit
  - Compound or a class-specific limit (Acceptable Intakes =AI) applied depending on available data
- Detection of *N*-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) in Sartans in 2018
- Detection of NDMA in pioglitazone and ranitidine 2019; EMA and other agencies requested a *N*-Nitrosamine risk assessments to be performed on every marketed pharmaceuticals!
- Immediate need to address the risk of *N*-Nitrosamine impurities in pharmaceuticals
  - regulatory agencies have provided provisional limits for *N* Nitrosamine impurities based on its structure activity relationship (SAR) with “close” analogs, mainly NDEA and NDMA

Current Guidance Documents for Limiting Nitrosamines



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

03 August 2020
EMA/409815/2020

Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products

Control of Nitrosamine Impurities in Human Drugs

Guidance for Industry

This guidance is for immediate implementation.

FDA is issuing this guidance for immediate implementation in accordance with 21 CFR 10.115(g)(2). Submit one set of either electronic or written comments on this guidance at any time. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this document, contact (CDER) Dongmei Lu 240-402-7966.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

September 2020
Pharmaceutical Quality/ Manufacturing Standards/
Current Good Manufacturing Practice (CGMP)

EMA (Aug 2020)	FDA (Sep 2020)
Biological Medicines in scope	Not mentioned, consistent with M7 (out of scope)
Less than lifetime approach not applicable for Nitrosamine	Follow ICH M7 procedure, consult with FDA
Default AI = 18 ng/day	Default AI = 26 ng/day
8 AI for Nitrosamine published, NMPA 34,3 ng/day	6 AI for Nitrosamines, NMPA 26 ng/day
For 2 or more impurities • Total daily intak of all identified Nitrosamine does not exceed AI of most potent Nitrosamine • Risk level not exceeds 1 in 100.000	Maximum Daily Dose (MDD) Approach if more then one Nitrosamine present; • MDD < 880 mg/day → Acceptable Limit 0,03 ppm • MDD > 880 mg/day → Acceptable Limit 26 ng/day as sum

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Creation of an ad hoc workgroup to study *N*-Nitrosamine Structure-Activity Relationships

- **In depth scientific investigations were initiated and are ongoing specific to N-Nitrosamine activity based on work already started by Joel Bercu and colleagues**
- **20 companies and universities participating, 46 scientists contributing**
- **Led by Leadscope Inc., (Kevin P. Cross, Ph.D.) and Lhasa Ltd (David Ponting, Ph.D.)**
- **5 separate teams addressing different scientific and regulatory issues**
  - Mutagenicity
  - Carcinogenicity
  - Data acquisition and sharing
  - SAR development
  - Risk assessment

Can we do better at predicting *N*-Nitrosamine carcinogenicity potency?

- **Can we define less potent subclasses?**
  - By investigating different reaction pathways
  - By investigating repair mechanisms
  - By defining alerts for classification by both structural similarity and mechanism
  - By defining categorical alerts to predict broad carcinogenic potency categories
- **Classifying a test compound based on mechanism prior to finding structure similarity analogs during read across**

Can we do better at predicting *N*-Nitrosamine carcinogenicity potency?

- Carcinogenicity TD50 values spread over several orders of magnitude
- NDEA is the most potent one, providing an acceptable intake (AI) of 26 ng / day
- Distribution into subclasses using SAR and mechanistic understanding should be feasible

A. Thresher et al.

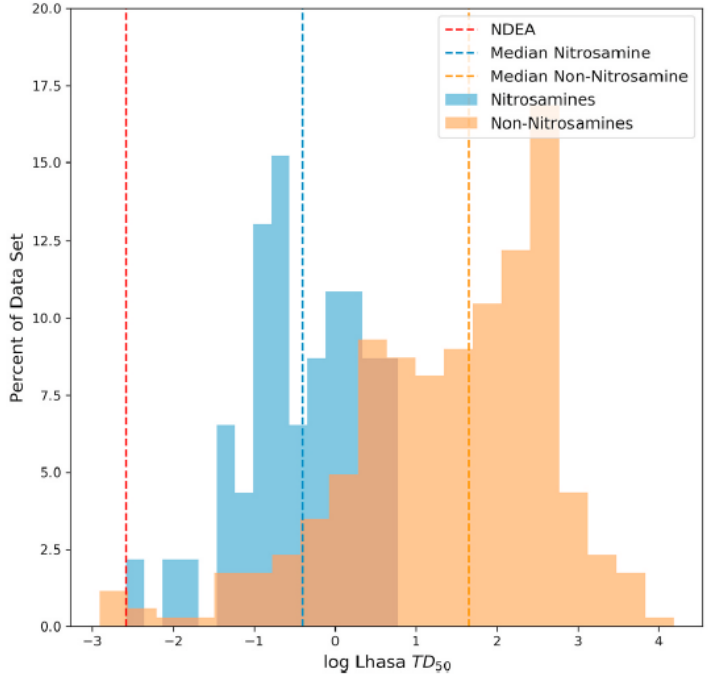


Fig. 5. Distribution of log Lhasa TD50 values for nitrosamine and non-nitrosamine compounds as a proportion of the respective data sets within the LCDB.

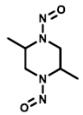
Can we do better at predicting *N*-Nitrosamine carcinogenicity potency?

https://www.lhasalimited.org/Public/Library/2020/Do%20all%20nitrosamines%20pose%20a%20significant%20level%20of%20genotoxic%20risk%20-%20Webinar%20Slides.pdf

Exploring the SAR

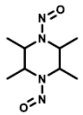
Piperazines

Metabolism possible



Mutagenic, Carcinogenic

Metabolism partially blocked



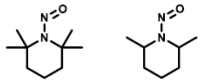
Non-mutagenic, Non-carcinogenic

Piperidines

Metabolism possible

Mutagenic, Carcinogenic

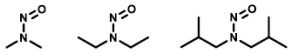
Metabolism partially blocked



Non-mutagenic, Non-carcinogenic

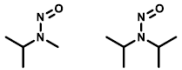
Dialkyl nitrosamines

Metabolism possible



Mutagenic, Carcinogenic

Metabolism partially blocked

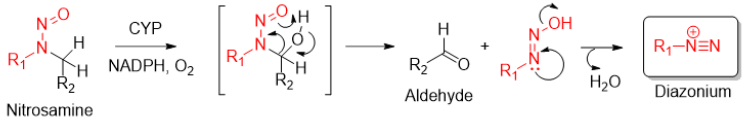


Non-mutagenic, Weak carcinogen

References: Rao et al, Mutation Research (1978) 57(2), 127; Rao et al, Mutation Research (1977) 56(2), 131; Rao et al, Mutation Research (1979) 66(1), 1.

Can we do better at predicting *N*-Nitrosamine carcinogenicity potency?

- **Exploring the metabolism / activation pathways**
  - Metabolic activation by CYP enzymes → Alkylnitrosamines (CYP2E1, 2A6 and others)



  - Consider other potential pathways as alternative toxification/detoxification pathways, eg transnitrosation, Beta-oxidation, Peroxidation or other routes to oxidative damage or even direct interaction (decomposition) ?
  - Understand differences in reactivity by using physicochemical parameters and Quantum Mechanical calculations of reactions

Finding Structure-Activity Relationships for *N*-Nitrosamines and grouping into potency categories

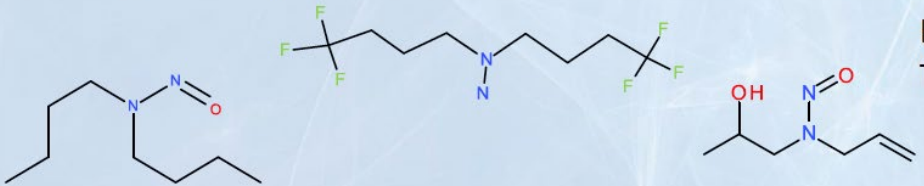
- *N*-Nitrosamine TD_{50} potency values span several orders of magnitude
  - Consider 4 logarithmic potency categories[17]

- Most methyl and ethyl substitutions yield very high potent carcinogens

NDMA NDEA

Very High Potency
TD_{50} 0-0.15 mg/kg/day

- Longer chains; steric bulk; distant electron-withdrawing groups

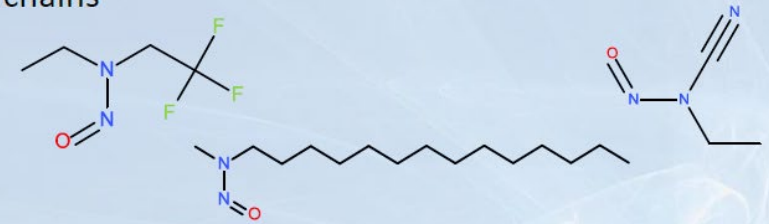


High Potency
TD_{50} 0.15-1.5 mg/kg/day

[17]Bercu, Compound- and Class-Specific Limits for Common Impurities in Pharmaceuticals in Genotoxic Impurities version 2, Teasdale, Ed. 2020

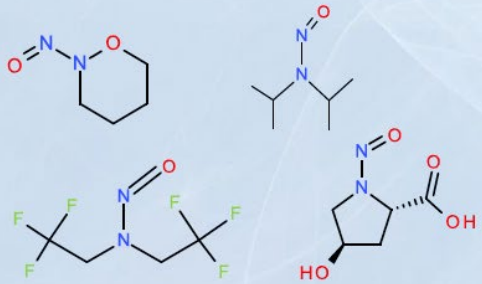
Finding Structure-Activity Relationships for *N*-Nitrosamines and grouping into potency categories

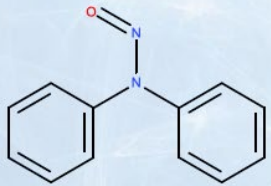
- Steric bulk near alpha-carbon; nearby electron-withdrawing groups; very long chains



Medium Potency
TD_{50} 1.5-15 mg/kg/day

- Lack of alpha-carbon hydrogens or only 1 alpha-carbon hydrogen; strong electron-withdrawing groups on (both) sides





not alpha-carbon hydroxylation mechanism

Low Potency
TD_{50} > 15 mg/kg/day

Summary

- **The number of in silico tools used for the hazard ID and risk assessment in the regulatory environment in pharmaceutical industry is increasing**
  - In 2020, Sanofi received 2 regulatory requests to fill data gaps for impurities using in silico prediction for multiple toxicological endpoint
  - Secondary Pharmacology prediction plays a key role in hazard ID and data gap filling
  - Tools regularly used for gentoxicity, phototoxiciy and DALA assessments
- **Identification of Nitrosamines and risk assessment according to ICH M7 for marketed drugs is a key activity for pharmaceutical industry**
  - Development of prediction models on mutagenic/carcinogenic potential to build subgroups for the diverse classes of nitrosamines is key for a scientifically based risk assessment
  - Harmonization of processes to set globally accepted limits for Nitrosamines
    - Default values
    - Less than Lifetime approach

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- David Ponting (LHASA Ltd.)