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answers



Genotoxic Impurities:

Overview of ICH M7 and Guidance for Nitrosamine Impurities

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Agenda

- What are genotoxic impurities?
- Why are they a concern?
- ICH M7 Guidance
- Hazard Assessment
- Guidance related to Nitrosamines and Nitrosamine Drug Substance Related Impurities (NDSRIs)
- The Enhanced Ames Test

Genotoxic Impurities

- What are they?
 - > Compounds, which are not the chemical entity defined as the drug substance, that are demonstrated to induce genetic mutations, chromosomal breaks, and/or chromosomal rearrangements.
 - > Can arise from the manufacturing synthesis process or during storage of drug substances
- Why the concern?
 - > Potential to cause cancer and/or genetic damage in humans
 - > Even low level exposures may present safety concerns
 - > The identification limits provided in ICH Q3A(R2) and ICH Q3B(R2) may not be acceptable for genotoxic or carcinogenic impurities
 - > Impurities in drug substances and products do not have beneficial effects and may impose a risk without associated benefit
 - > Can lead to clinical holds and delayed approval of drug products

International Conference on Harmonisation

- | | |
|------|---|
| 2010 | Accepted as a multidisciplinary topic (M7) |
| 2014 | ICH M7 Finalized: Assessment and Control of DNA reactive (mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk (26 pages) |
| 2015 | M7(R1) Addendum (Step 2): Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk |
| 2023 | M7(R2) and associated addendums finalized. Adds Acceptable intakes for an additional 7 commonly found genotoxic impurities, along with other revisions. |

ICH M7(R2): Scope

- New drug substances and new drug products in clinical development and subsequent application for marketing
- Certain post approval submissions of marketed products and new marketing applications (drug substance previously approved), with:
 - > Changes to the drug substance (new impurities)
 - > Changes to the drug product (new degradants)
 - > Changes in clinical use (changes in cancer risk)

ICH M7(R2): Scope Exclusions

- Drug substances and drug products for:
 - > biological/biotechnological, peptide, oligonucleotide, radio-pharmaceutical, fermentation products, herbal products, and crude products of animal or plant origin
- Drug substances and drug products intended for advanced cancer indications as defined in ICH S9
- Excipients used in existing marketed products, flavoring agents, colorants, and perfumes

ICH M7(R2): General Principles

- Focus on DNA-reactive impurities, i.e. mutagenic impurities
 - > Typically positive in the bacterial mutagenicity assay
 - > Other types of genotoxics that are non-mutagenic typically have threshold mechanisms and usually do not pose carcinogenic risk in humans at the level impurities are ordinarily present
- Threshold of Toxicological Concern (TTC) concept applies – if controlled to 1.5µg per day = $<1 \times 10^{-5}$ lifetime cancer risk
- Less than Lifetime (LTL) principle applies
 - > Clinical development and marketed products with shorter treatment durations have higher acceptable levels – extrapolated based on TTC
- Evaluate actual impurities plus risk-based subset of potential impurities

ICH M7(R2): Hazard Assessment

- Analysis of actual and potential impurities by conducting database and literature searches for carcinogenicity and bacterial mutagenicity data in order to classify per Table 1.
- Known mutagen - evaluate literature and databases
- Structure of unknown mutagenicity - perform a computational toxicology assessment using (Q)SAR methodologies that predict bacterial mutagenicity. Use **two** complementary (Q)SAR systems (Expert rule-based and statistical based)
 - > Apply expert knowledge to review outcomes as warranted
 - > Absence of structural alert from two (Q)SAR systems is sufficient to conclude that impurity is of no concern, and no further testing is required.

ICH M7(R2) Table 1: Impurity Classifications and Control Actions

Class	Definition	Proposed Action for Control
Class 1	Known mutagenic carcinogens	• $\leq$ compound-specific limit
Class 2	Known mutagens with unknown carcinogenic potential	• $\leq$ appropriate TTC
Class 3	Alerting structure, unrelated to structure of DS, no mutagenicity data	• $\leq$ appropriate TTC -or- • Conduct Ames test (non-mutagenic = class 5; mutagenic = class 2)
Class 4	Alerting structure, same alert in DS or compounds related to DS which have been tested and are non-mutagenic	• Non-mutagenic impurity (ICH Q3A/B)
Class 5	No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity	• Non-mutagenic impurity (ICH Q3A/B)

ICH M7: Hazard Assessment Follow up

- To follow up on a structural alert (Class 3):
 - > Control the impurity to less than threshold exposures, or
 - > Conduct a bacterial mutation assay (using neat impurity material)
 - Appropriately conducted negative bacterial mutation assay overrules any structure-based concern; no further genotoxicity assessments is recommended. These impurities are considered non-mutagenic (Class 5).
 - Option to follow-up with *in vivo* mutagenicity assay for Ames positive compounds that cannot be controlled

ICH M7: Exception in Approaches

- Higher acceptable intakes may be justified;
 - > When human exposure to the impurity is much greater from other sources, e.g., food, or endogenous metabolism (e.g., formaldehyde).
 - > Cases of severe disease, reduced life expectancy, late onset but chronic disease, or with limited therapeutic alternatives.
 - > Based on a risk/benefit analysis when control efforts cannot reduce levels below the acceptable limit and levels are As Low As Reasonably Practicable (ALARP)
- Lower acceptable intake may be warranted for some structural classes of mutagens, i.e., aflatoxin-like-, N-nitroso-, and alkyl-azoxy structures that display extremely high carcinogenic potency.

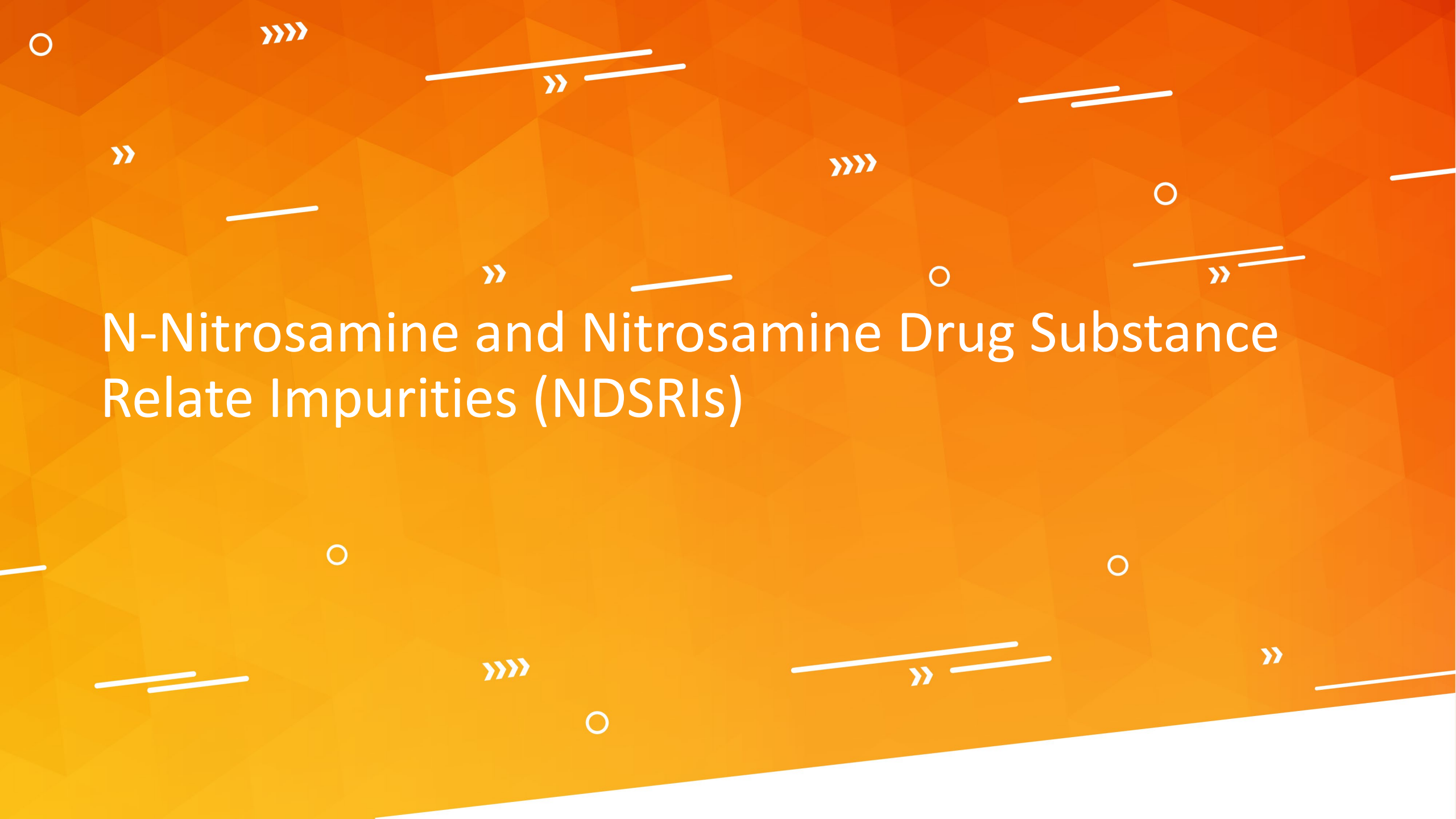
ICH M7: Note 2

- For impurities that are not feasible to isolate or synthesize or when compound quantity is limited, it may **not** be possible to achieve the highest test concentrations recommended for an ICH-compliant (OECD471) bacterial mutagenicity assay according to the current testing guidelines.
- In this case, bacterial mutagenicity testing could be carried out using a modified assay format with proven high concordance to the ICH-compliant assay to enable testing at higher concentrations with justification.
 - > Modified 2-strain (duplicate or triplicate plates)
 - > 6-well Ames with OECD compliant tester strains

ICH M7(R2) Note 3: *in vivo* tests for Ames positive impurities

<i>In Vivo</i> Test	Factors to Justify Choice of Test as Fit-for-Purpose
Transgenic mutation assays	• For any bacterial mutagenicity positive • Justify selection of tissue/organ
<i>Pig-a</i> assay	• For bacterial mutagenicity positive without S9*
Micronucleus assay	• For bacterial mutagenicity positive without S9 and compounds known to be clastogenic*
Rat liver unscheduled DNA synthesis (UDS) test	• For bacterial mutagenicity positive with S9 only • Responsible liver metabolite known: – to be generated in test species used – to induce bulky adducts
Comet assay	• Justification needed (chemical class-specific mode of action to form alkaline labile sites or single-strand breaks as preceding DNA damage that can potentially lead to mutations) • Justify selection of assay tissue/organ
Others	• With convincing justification

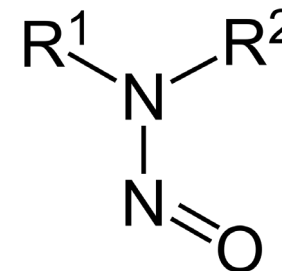
* For indirect acting mutagens (requiring metabolic activation), adequate exposure to metabolite(s) should be demonstrated.



N-Nitrosamine and Nitrosamine Drug Substance Relate Impurities (NDSRIs)

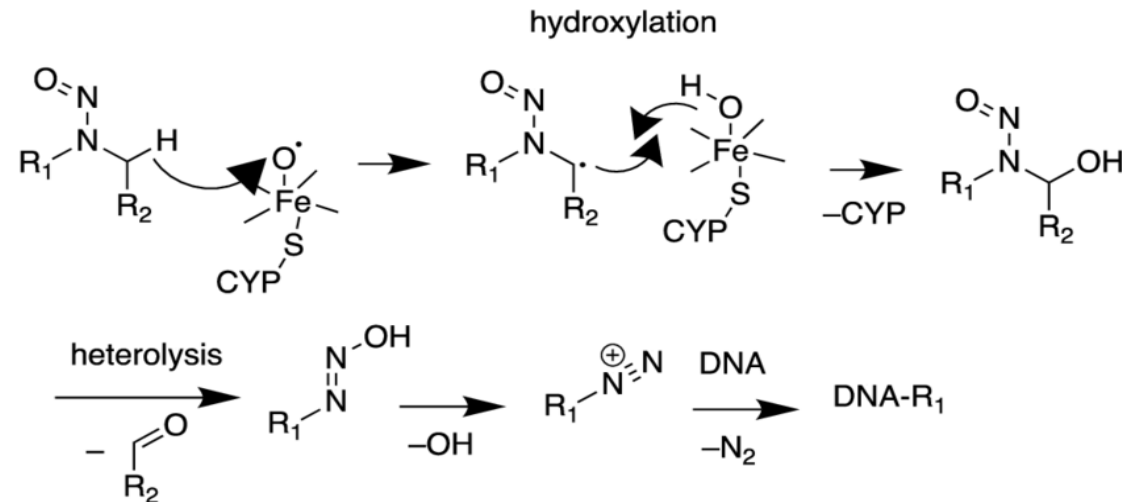
N-Nitrosamine and Nitrosamine Drug Substance Related Impurities (NDSRIs)

- Nitrosamines Drug Substance-Related Impurities (NDSRIs) may be formed under conditions related to formulation or manufacturing of APIs
 - > APIs with secondary amine or dimethyl tertiary amine centers at risk of forming NDSRIs
- Root cause in some cases may be presence of nitrite impurities in excipients or water
- Nitrosamines can also be formed as foods and drugs are processed in the body.
- Nitrosamines may be carcinogenic
 - > Class 1- Known mutagenic carcinogen (ICH M7)
 - > Class 2A- Probable carcinogen (IARC)
 - > Cohort of Concern (Coc) – per ICH M7, AI of < 1.5 µg/day
 - Prior to 2023 guidance from EMA and FDA, all new NDSRI controlled at 18 ng/day
- Challenges:
 - > Control to 18 ng/day or reformulate → \$\$\$
 - > Robust *in vivo* data for NDSRIs unavailable to set AI based on TD₅₀ or read-across
 - > Transgenic Rodent (TGR) assay availability → ⌚⌚⌚
 - > Standard Ames methodologies are not considered robust enough to detect mutagenic nitrosamines



Mutagenicity of N-Nitrosamines and NDSRIs

- Metabolic activation required for nitrosamines to be mutagenic
 - > Metabolic pathway requires formation of alkyldiazonium ion
 - > Not all NDSRIs will fall into CoC



Source: Kostal and Voutchkova-Kostal, Chem Res Toxicol. 2023 Feb 20

Guidance on N-Nitrosamine Impurities (2023-2024)

- Updated guidance from EMA (July 2023, since revised) and FDA (August 2023, updated Sept 2024) provided major update on AIs and testing for NDSRIs
 - > Carcinogenic Potency Characterization Approach (CPCA) approach to assign potency score with corresponding AI based on chemical structure (e.g. number of alpha-hydrogens and number of “activating” or “deactivating” structural features)
 - Potency score 4 or 5 → 1500 ng/day
 - Potency score 1, 2, or 3 → 18-400 ng/day
 - > Specification of Enhanced Ames Test (EAT) conditions
 - Negative EAT results could be helpful for CPCA category 1-3 compounds, to set higher AIs (EMA).
 - Negative EAT → 1500 ng/day
 - Positive EAT → AI based on CPCA or read-across

Comparison of Standard and Enhanced Ames Test

Specification	Standard OECD Ames Test	Enhanced Ames Test
Tester Strains	<i>Salmonella typhimurium</i>: TA98, TA100, TA1535, and TA1537 (or TA97 or TA97a) <i>Escherichia coli</i>: WP2A <i>uvrA</i> or WP2 <i>uvrA</i> (pKM101) <i>Salmonella</i> strain TA102 may be used in place of <i>e.coli</i>	<i>Salmonella typhimurium</i>: TA98, TA100, TA1535, and TA1537 <i>Escherichia coli</i>: WP2 <i>uvrA</i> (pKM101)
Plating Method	Plate Incorporation or Preincubation (20-60 min)	Preincubation (30 min)
Metabolic activation	- In the absence of metabolic activation - In the presence of 5-30% S9. mostly derived from livers of induced rats. S9 from other species acceptable	- In the absence of metabolic activation - In the presence of 30% induced rat S9 - In the presence of 30% induced hamster S9
Solvent	Water or organic solvent (eg DMSO)	Water or organic solvent. When organic solvent is used, lowest concentration in preincubation mixture to be used. Justification may be needed to justify solvent does not interfere with metabolic activation.
Positive Controls	Concurrent strain specific PCs	- Concurrent strain specific PCs - At least two N-nitrosamines known to be mutagenic in the presence of S9

Additional tests for Nitrosamine Impurities

- In addition to a negative Enhanced Ames Test, the 2024 Guidance from FDA requests additional data along with negative EAT results to support an AI of 1500 ng/day
 - > A second *in vitro* mammalian cell mutation assay
 - CHO HPRT or MLA
 - > *In vitro* metabolism data
 - *Could include human hepatocytes or microsomes*



**Nitrosamines
Research
Program**



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Government/ Regulatory

Agence nationale de
sécurité du
médicament et des
produits de santé
(ANSM)

Danish Medicines
Agency

European Medicines
Agency

Federal Institute for
Drugs and Medical
Devices
(Bundesinstitut für
Arzneimittel und
Medizinprodukte,
(BfArM)

Health Canada

Institut National de
la recherche
agronomique (INRA)
National Institute of
Health Sciences
(NIHS), Japan
National Institute for
Public Health and the
Environment (RIVM)
SwissMedic
US Food and Drug
Administration

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Private Industry

AbbVie
Amgen
AstraZeneca
BASF
Bayer
Boehringer Ingelheim
Bristol Myers Squibb
Charles River
Laboratories
Corteva Agriscience
Eli Lilly
Gilead
GSK
Inotiv
Janssen

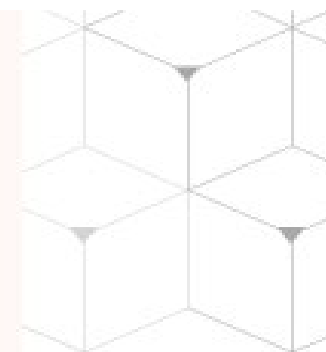
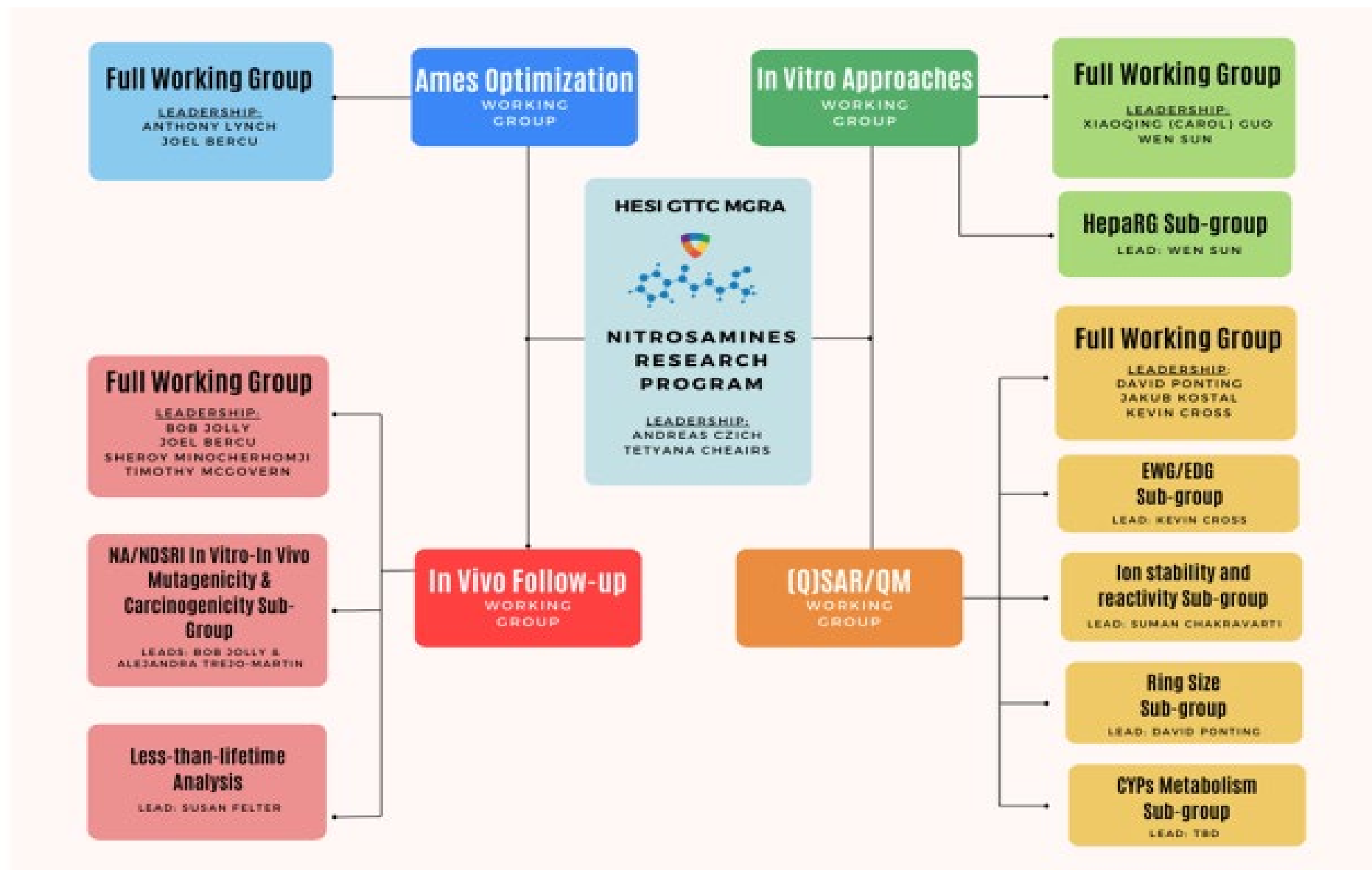
Labcorp
Leadscope/Instem
Litron Laboratories
Merck
Merck kGA
MultiCASE, Inc.
Novartis
PepsiCo
Pfizer
Proctor & Gamble
Roche
Sanofi
Teva Pharmaceuticals
Xenometrix

7

Academic, NGO, Consultants

FSTox Consulting
Lhasa Limited

George Washington
University
New York Medical
College
St. George's Medical
School, University of
London
Swansea University
University of
Colorado
University of
Minnesota



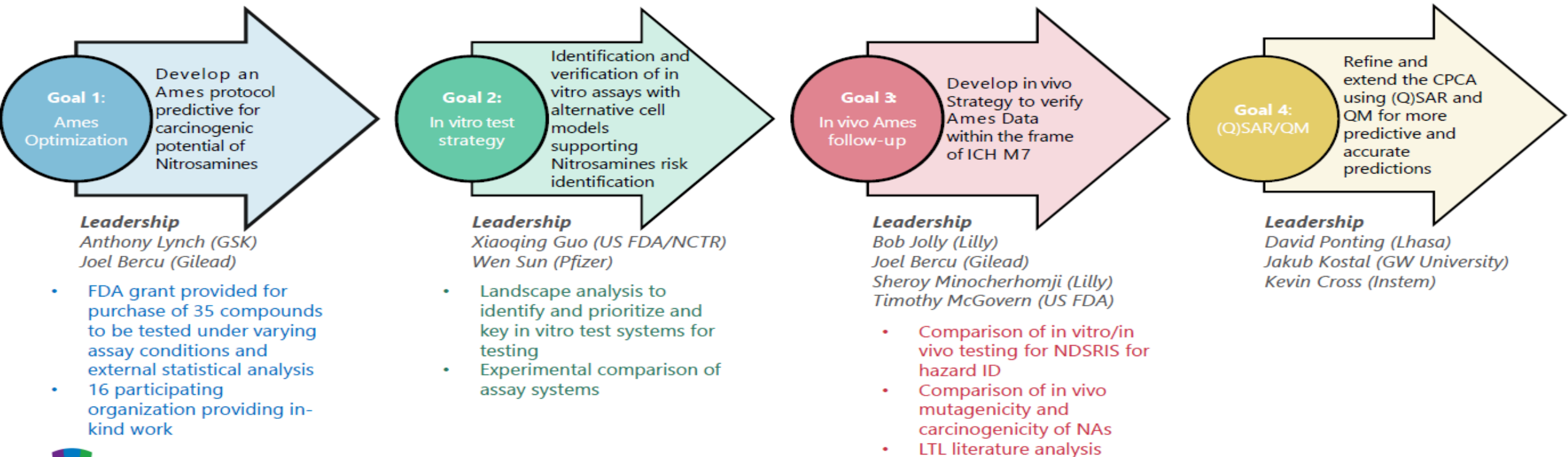
MGRA Nitrosamines =



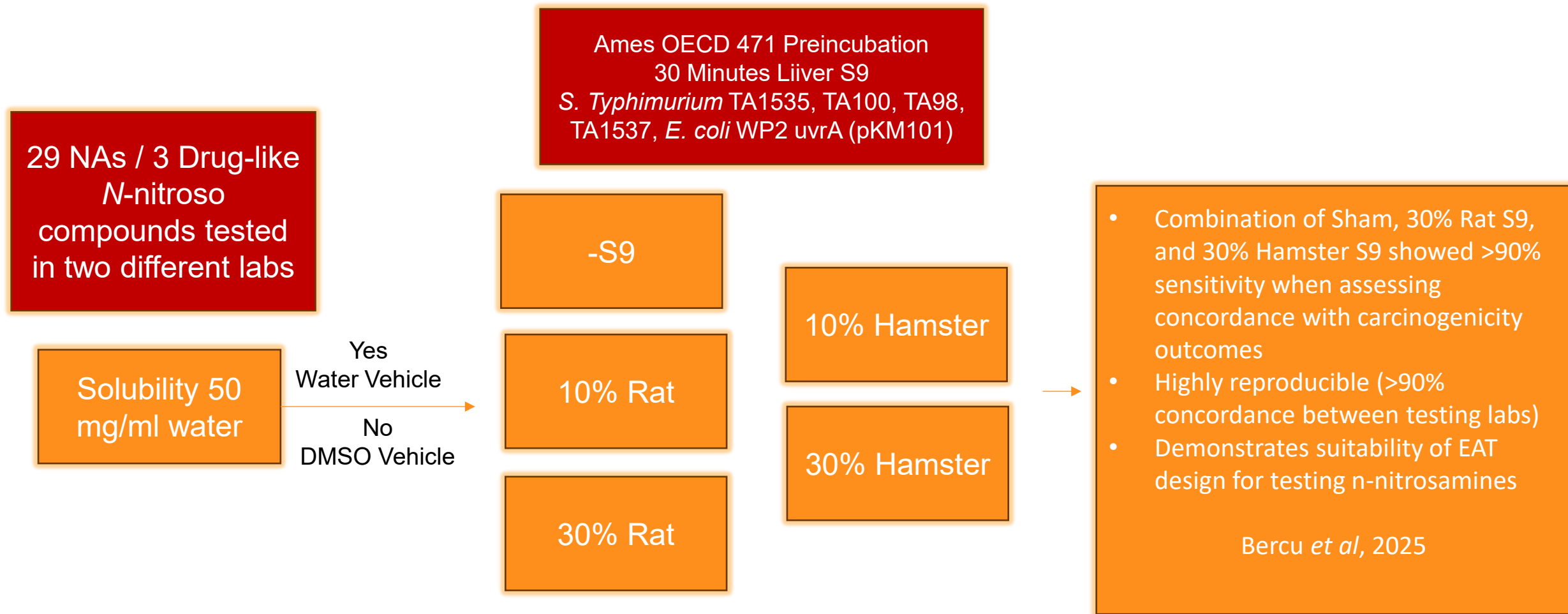
Nitrosamines
Research
Program

Leadership

- Tetyana Cheairs (New York Medical College)
- Andreas Czich (Sanofi)



HESI.



HESI Ames Optimization Ring Trial Study

The background is a vibrant orange with a subtle geometric pattern of overlapping triangles. Scattered across the surface are various white decorative elements: single lines, double lines, and groups of three or four chevron-like shapes pointing in different directions. There are also several small white circles. The overall aesthetic is modern and minimalist.

Questions?

