# Studies on tissue specific carcinogenicity and on metabolism of N-nitrosamines (SC05 to FWC EMA/2020/46/TDA/L1.02 – ROC38))

**First published:** 11/11/2025

**Last updated:** 14/10/2026

Study Finalised

## Administrative details

**EU PAS number**

EUPAS1000000819

**Study ID**

1000000819

**DARWIN EU® study**

No

**Study countries**

Germany

**Study description**

The MUTAMIND SC05 project investigates the major metabolic sites of the NDSRIs, which are presumed to impact the metabolic activations of these compounds. For this purpose, the MUTAMIND SC05 project will explore the (de)activation of NDSRIs using the in vitro human liver models. This aim to identify the major sites of the metabolic activation to study e.g. the hydroxylation sites.
Furthermore, the impact of the potential conjugation reactions, which may influence the reactivity of the NDSRIs and the general detoxification process will be addressed in in vitro studies. Additionally, in silico tools will be employed to predict the metabolites generated during this process. This will facilitate the evaluation of their applicability and predictivity for the upcoming projects related to NDSRIs.
This deliverable provides an overview of the approaches that will be employed throughout the course of the project. The project is categorized into two main objectives:
• Objective 1: Review of quantitative organ-specific carcinogenicity for N-nitrosamines.
• Objective 2: Mechanistic study on the metabolism of N-nitrosamines.

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**Study status**
Finalised

# Research institutions and networks

## Institutions

### Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM)

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Institution

## Contact details

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Primary lead investigator

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## Study timelines

**Date when funding contract was signed**

Planned: 05/08/2025

Actual: 05/08/2025

**Study start date**

Planned: 05/08/2025

**Data analysis start date**
Planned: 01/01/2026

**Date of interim report, if expected**
Planned: 06/08/2026

**Date of final study report**
Planned: 06/09/2026

Actual: 02/10/2026

## Sources of funding

- EMA

## More details on funding

SC05 to FWC EMA/2020/46/TDA/L1.02 – ROC38

## Study protocol

202510_EMA_SC05_D2_study_protocol_final.pdf (849.34 KB)

## Regulatory

**Was the study required by a regulatory body?**

Yes

**Is the study required by a Risk Management Plan (RMP)?**

Not applicable

# Methodological aspects

## Study type

**Study topic:**

Other

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**Study topic, other:**

Evaluate tissue specific carcinogenicity and on metabolism of N-nitrosamines

**Study type:**

Not applicable

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**Scope of the study:**

Method development or testing

**If ‘other’, further details on the scope of the study**

Research project to better define threshold for nitrosamine impurities

**Data collection methods:**

Combined primary data collection and secondary use of data

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**Study design:**

The project investigated the tissue-specific tumour formation by N-nitrosamines in existing in vivo cancer studies.
Further, metabolic activation and detoxification was investigated using in silico and in vitro methods.

**Main study objective:**

The Mutamind 5 project has two main objectives, each subdivided to several subobjectives. These are briefly described in the following:
Objective 1: Review of quantitative organ-specific carcinogenicity for N-nitrosamines
Objective 1a: Creation of a database of tissue-specific TD50s for N-nitrosamines
Objective 1b: Analyse any relationships between structure, functional groups

and/or physicochemical properties and tissue-specific carcinogenicity
Objective 1c: Analyse the contributions of organ-specific metabolism, tissue distribution, repair mechanisms and stability of the activated impurity to tissue-specific carcinogenicity
Objective 1d: Conclude on the implications of findings from objectives 1a, 1b and 1c on requirements for tissue sampling and analysis for in vivo mutagenicity studies with N-nitrosamines

Objective 2: Mechanistic study on the metabolism of N-nitrosamines
Objective 2a: Selection of NDSRIs for empirical evaluation
Objective 2b: Pre-experimental in silico based generation of metabolites from its structure
Objective 2c: Experimental phase to assess metabolism of NDSRIs and corresponding active substances
Objective 2d: In silico to in vitro comparison

## Study drug and medical condition

**Medicinal product name, other**

Several model compounds with nitrosmine substructure

## Population studied

**Short description of the study population**

none

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**Special population of interest**

Other

**Special population of interest, other**

no patients involved

**Estimated number of subjects**

0

# Study design details

**Data analysis plan**

Objective 1: Review of quantitative organ-specific carcinogenicity for N-nitrosamines

The project investigated the tissue-specific tumour formation by N-nitrosamines with diverse physicochemical properties.

A systematic review of peer-reviewed publications from 2010 onwards was combined with data from the (updated) Carcinogenic Potency Database (CPDB). Relationships between tissue-specific carcinogenicity and chemical structure, functional groups, CPCA categories, physicochemical properties and ADME parameters were analysed.

The analysis identified the most frequent target organs of Nitrosamines and examined the influence of study design and physicochemical properties on target-organ prevalence. It also evaluated the tissue-specific expression of phase I and II enzymes using data from the Human Protein Atlas and peer-reviewed literature.

Objective 2: Mechanistic study on the metabolism of N-nitrosamines

The study evaluated how metabolic activation and detoxification influenced the genotoxicity of NDSRIs using a diverse set of NDSRI compounds covering

different structural features and mutagenic potencies.
In silico tools predicted sites of metabolism, metabolite structures, and competing activation and detoxification pathways. Experimental studies incubated the selected NDSRIs with in vitro assays like S9 liver fractions or primary hepatocytes from humans/rats to identify metabolites and degradation products, including those formed through CYP-mediated activation, denitrosation and phase II conjugation.
Targeted LC–MS/MS methods quantified parent NDSRIs and selected metabolites over time, to calculate their metabolic half-lives and intrinsic clearance. Predicted and experimentally detected metabolites were integrated into compound-specific metabolic maps. Comparison of the in silico predictions with the experimental results assessed the performance of the prediction tools for phase I and II metabolism

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**Summary results**

N-Nitrosamine drug substance-related impurities (NDSRIs) pose considerable health risk due to their mutagenic and carcinogenic properties. One important aspects of NAs carcinogenicity is its tissue specificity. The analysis of prevalent target organs revealed liver as the predominant target organ following oral NAs exposure. Also organs of the upper aerodigestive tract, most notably the esophagus was observed. The organ level pattern aligned with cytochrome P450 (CYP) expression data, showing relevant CYP450 enzyme expression in the liver and esophageal tissue. These findings supported esophagus as target organ alongside the liver and offer suggestion for the tissue selection in the future toxicological in vivo studies of NDSRIs.
NDSRIs require a metabolic activation, which is influenced by their structural properties and enzyme accessibility. The second part of the project evaluated whether an integrated computational and experimental workflow could improve the prediction of NDSRI metabolism, with particular emphasis on cytochrome P450-mediated α-hydroxylation adjacent to the N-nitroso group. Seven

structurally diverse NDSRIs spanning N-nitrosamine carcinogenic potency categorization approach categories 1–5 and three negative controls were investigated. MetaSite predictions, molecular docking of six human CYP isoforms, and quantum mechanical approaches were combined with clearance and metabolite profiling in induced rat liver S9, human liver S9 and primary human hepatocytes. The approaches provided complementary information: MetaSite identified overall plausible sites and pathways of metabolism, docking evaluated whether α-carbons could adopt catalytically competent orientations, and quantum-chemical calculations characterised intrinsic reactivity and energetic feasibility. Experimental studies demonstrated marked compound- and system-dependent differences in clearance and metabolite formation and showed that α-hydroxylation was only one of several competing routes

# Data management

## ENCePP Seal

The use of the ENCePP Seal has been discontinued since February 2025. The ENCePP Seal fields are retained in the display mode for transparency but are no longer maintained.

## Use of a Common Data Model (CDM)

**CDM mapping**

No

## Data quality specifications

**Check conformance**

Unknown

**Check completeness**

Unknown

**Check stability**

Unknown

**Check logical consistency**

Unknown

## Data characterisation

**Data characterisation conducted**

No