EMA/2020/46/TDA, Lot 1 – ROC25 Refinement of enhanced Ames test conditions for N-nitrosamines MUTAMIND2

First published: 25/07/2025

Last updated: 14/10/2026

Study Finalised

Administrative details

EU PAS number

EUPAS1000000684

Study ID

1000000684

DARWIN EU® study

No

Study countries

Germany

Study description

Study protocols for the bacterial mutagenicity assay based on the current version of the EAT protocol for testing N-nitrosamines, including phenotyping the activation methodology of commonly used positive controls and selected NDSRIs.

Study status

Finalised

Research institutions and networks

Institutions

Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM)

First published: 01/02/2024

Last updated: 01/02/2024

Institution

Federal Institute for Drugs and Medical Devices (BfArM)

Germany

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Last updated: 30/04/2024

Institution | Regulatory Authority

ICCR-Roßdorf GmbH

Contact details

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Study timelines

Date when funding contract was signed

Planned: 28/11/2024

Actual: 28/11/2024

Study start date

Actual: 05/12/2024

Data analysis start date

Actual: 05/12/2024

Date of interim report, if expected
Actual: 05/04/2025

Date of final study report
Planned: 07/04/2026

Actual: 05/05/2026

Sources of funding

- EMA

Study protocol

EMA_SC04_D2_Study Protocol.pdf (1.06 MB)

Regulatory

Was the study required by a regulatory body?

No

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

Not applicable

Methodological aspects

Study type

Study type list

Study topic:

Other

Study topic, other:

Refinement of enhanced Ames test conditions for nitrosamines

Study type:

Not applicable

Scope of the study:

Method development or testing

Study design:

N-Nitrosamines (NAs) are classified based on their core structure as Cohort of Concern (CoC) compounds in the ICH M7 guideline. NAs represent a class of highly potent mutagenic carcinogens that requires strict controls to limit their amounts. Risk assessment of new NAs or NAs lacking robust in vitro

Main study objective:

Objective 1 will focus on the selection of NDSRI positive controls for the EAT and the evaluation of assay performance and sensitivity.
The selected NDSRI substances will be tested with 3 batches of hamster S9 at 5 concentrations and in compliance with the regulatory approach according to TG OECD471, five bacterial strains (TA1535, TA1537, TA98, TA100 and E. coli WP2 uvrA) will be included in the test design.
Phenotyping of NDSRI activation will be achieved by employing high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS), to follow the formation of drug and NDSRI metabolites. CYP dependent metabolism will be studied by incubations which S9 fraction, using CYP specific inhibitors and with recombinant human bactosomes®.
Objective 2 will evaluate the effect of solvent concentrations on the NDSRI

activity on the Ames test comparing the preincubation protocol and the plate incorporation protocol.
Benchmark dose (BMD) modelling will be utilized for sensitivity analyses and to compare different study conditions.
This project finally aims to define a sensitive Ames test design for NDSRIs that will lower the risk for false negative outcomes.

Study design details

Summary results

Key Findings:
NDSRI metabolic activation is CYP450-dependent but compound-specific. Several human CYP isoforms are involved, including CYP1A1, 2A6, 2B6, 2C8, 2C9, 2C19, 2E1 and 3A4. N-nitroso-varenicline was not proficiently metabolised by the tested enzymes, indicating that some NDSRIs may require other metabolic pathways.
The three tester strains TA1535, TA100 and E. coli WP2 uvrA were the most broadly responsive strains for detecting NDSRI mutagenicity. TA98 and TA1537 may add value for certain NDSRIs and can help interpret equivocal results.
DMSO concentration influenced both NDSRI metabolism and assay sensitivity; solvent levels therefore need to be carefully controlled and standardised.
The comparison of induced vs. non-induced hamster S9, and of plate incorporation vs. preincubation, provided the basis for defining the recommended EAT conditions.
Strain-specific NDSRI positive controls are proposed, based on reproducible positive responses and adequate fold changes under hamster S9 activating conditions.

Documents

Abstract of study report

EMA_SC04_D7_executive_summary.pdf (231.7 KB)

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