

# In vitro mutagenicity methodology for nitrosamines (InVitroNAmutagenicity)

**First published:** 18/10/2022

**Last updated:** 14/03/2024

Study

Finalised

## Administrative details

### EU PAS number

EUPAS49355

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### Study ID

49440

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### DARWIN EU® study

No

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### Study countries

- ☐ Germany
  - ☐ United Kingdom
  - ☐ United States
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## Study description

The project “In vitro mutagenicity methodology for nitrosamines” aims at generating a better understanding how the Ames test and the in vitro Comet assay can be methodologically optimized to reliably detect mutagenicity of different nitrosamines (NAs). A carefully selected set of reference NAs, active pharmaceutical ingredient (API)-derived NAs and supporting reference compounds will be used to demonstrate reproducibility, sensitivity (the proportion of genotoxic carcinogens that generate positive results), and specificity (the proportion of non-genotoxic compounds that generate a negative result) of each test model and provide data to estimate and compare the genotoxic potency of test compounds in the two different in vitro assays. One part of the project focuses on evaluation and optimization of the Ames test to improve its sensitivity in detecting the mutagenic potential of NAs with one focus on appropriate solvents and solvent concentrations as well as metabolising systems. The other part is dedicated to evaluation and optimization of the in vitro Comet assay with metabolically competent liver cell models (primary rat and human hepatocytes versus HepG2 cells) as a complementary or alternative assay for detection of potentially mutagenic/carcinogenic NAs and for respective risk assessment. Determination of compound solubility, compound purity and determination of metabolic competence of both S9 fractions and cell models will be part of the study.

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## 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

**First published:** 01/02/2024

**Last updated:** 30/04/2024

**Institution**

**Regulatory Authority**

## Swansea University Medical School

☐ United Kingdom

**First published:** 01/02/2024

**Last updated:** 01/02/2024

**Institution**

**Educational Institution**

**Hospital/Clinic/Other health care facility**

Federal Institute for Drugs and Medical Devices  
(BfArM) Bonn, Germany, Technical University  
Kaiserslautern Kaiserslautern, Germany, ICCR-  
Rossdorf Rossdorf, Germany, Swansea University  
Medical School Swansea, UK, Leadscope  
Columbus, Ohio, USA

## Contact details

### Study institution contact

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Study contact

[christina.ziemann@item.fraunhofer.de](mailto:christina.ziemann@item.fraunhofer.de)

### Primary lead investigator

Christina Ziemann

Primary lead investigator

## Study timelines

### Date when funding contract was signed

Planned: 09/08/2021

Actual: 09/08/2021

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**Study start date**

Planned: 01/01/2022

Actual: 01/01/2022

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**Data analysis start date**

Planned: 04/10/2022

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**Date of final study report**

Planned: 30/10/2023

Actual: 30/10/2023

## Sources of funding

- Other

## More details on funding

EMA

## Study protocol

[D2\\_Study Protocol\\_EMA\\_SC02\\_in vitro\\_final.pdf](#)(2.9 MB)

## Regulatory

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

Yes

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

Not applicable

# Other study registration identification numbers and links

SPECIFIC CONTRACT No. 02 implementing framework contract No.  
EMA/2020/46/L1.02

## Methodological aspects

### Study type

### Study type list

**Study type:**

Not applicable

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**Scope of the study:**

Assessment of risk minimisation measure implementation or effectiveness

Other

**If 'other', further details on the scope of the study**

Optimization of in vitro mutagenicity/genotoxicity assays for prediction of in vivo mutagenicity and cancerogenicity of nitrosamines

**Main study objective:**

Generating a better understanding on how the Ames test and the in vitro Comet assay can be methodologically optimized to reliably detect mutagenicity of different nitrosamines (NAs) and API-derived nitroso compounds.

### Study drug and medical condition

## **Anatomical Therapeutic Chemical (ATC) code**

(B03BB) Folic acid and derivatives

Folic acid and derivatives

## Population studied

### **Age groups**

Adults (18 to < 46 years)

Adults (46 to < 65 years)

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### **Estimated number of subjects**

0

## Study design details

### **Outcomes**

Experimental data on mutagenicity of nitrosamines and API-derived nitroso compounds in the Ames test under different conditions, experimental Comet assay data on induction of DNA damage by these compounds using in vitro liver cell models and data on solubility, purity and on metabolic competence of S9 fractions and cell models.

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### **Data analysis plan**

A carefully selected set of reference NAs, active pharmaceutical ingredient (API)-derived NAs and supporting reference compounds will be used to finally evaluate reproducibility, sensitivity (the proportion of genotoxic carcinogens that generate positive results), and specificity (the proportion of non-genotoxic compounds that generate a negative result) of each test model and provide data to estimate and compare the genotoxic potency of test compounds in the

two different in vitro assays. The study will be complemented by a small Comet assay round robin study with HepG2 cells to demonstrate reproducibility.

## Documents

### Study results

[EUPAS49355\\_Abstract\\_Study Results.pdf](#) (660.66 KB)

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## 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.

## Data sources

### Data sources (types)

[Other](#)

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### Data sources (types), other

The study will generate experimental data, which will be disseminated on conferences as talks and posters and will be published in peer-reviewed journals and as final report.

## Use of a Common Data Model (CDM)

**CDM mapping**

No

Data quality specifications

**Check conformance**

Unknown

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**Check completeness**

Unknown

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**Check stability**

Unknown

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**Check logical consistency**

Unknown

Data characterisation

**Data characterisation conducted**

No