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

Study ID

49440

DARWIN EU® study

No

Study countries

Germany

United Kingdom

United States

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 puritiy and determination of metabolic competence of both S9 fractions and cell models will be part of the study.

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

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Primary lead investigator

Christina Ziemann

Primary lead investigator

Study timelines

Date when funding contract was signed

Planned: 09/08/2021

Actual: 09/08/2021

Study start date
Planned: 01/01/2022

Actual: 01/01/2022

Data analysis start date
Planned: 04/10/2022

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

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

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)

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.

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)

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

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

Check completeness

Unknown

Check stability

Unknown

Check logical consistency

Unknown

Data characterisation

Data characterisation conducted

No