ENSURING RESEARCH INTEGRITY IN ONTOX THROUGH SYSTEMATIC AUDITS OF IN VITRO AND IN SILICO WORKFLOWS.

Kandarova H.[1], Jennen D.[2], Jiang J.[3], Marusczyk J.[4], Schwab C.H.[4], Krull C.[5], Pôbiš P.[1], Zajac D.J.[3], Gabrišová K.[1], Vinken M.[3]

1 Institute of Experimental Pharmacology and Toxicology, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovakia; 2 Department of Toxicogenomics, GROW School for Oncology and Reproduction, Maastricht University, Maastricht, The Netherlands, 3 Department of Pharmaceutical and Pharmacological Sciences, Vrije Universiteit Brussel, Brussel, Belgium, 4 Molecular Networks GmbH, Nuremberg, Germany, 5 University of Applied Sciences, HU, Utrecht, The Netherlands;

SUMMARY

The ONTOX project, funded under the European Union's Horizon 2020 programme, aims to advance innovative, animal-free approaches for chemical risk assessment. In its original proposal, ONTOX committed to ensuring that all research activities are conducted in compliance with Good Data Practices (GDP) and Good In Vitro Method Practices (GIVIMP), thereby guaranteeing scientific robustness, transparency, and regulatory relevance of the generated outputs. To deliver on this commitment, the consortium has established a dedicated audit framework to systematically evaluate the implementation of these practices across participating research teams. Two complementary audits will be performed. The first will focus on the in vitro experimental work, assessing adherence to OECD GIVIMP principles, with particular attention to quality assurance, traceability, documentation, and method performance standards in the application of advanced 3D cell and tissue models. This audit will support the reproducibility and reliability of in vitro findings and ensure their suitability for regulatory acceptance. In parallel, a second audit will target the in silico components of ONTOX, which include computational modeling, QSARs, and other predictive tools developed in accordance with OECD principles and guidelines for model validation and use. This process will evaluate data integrity, transparency of algorithms, reproducibility of predictions, and compliance with international standards, thereby strengthening confidence in the outputs of the modeling teams. Both audits will be conducted by a dedicated ONTOX quality teams, working closely with the respective research groups to identify strengths, gaps, and opportunities for continuous improvement. By applying harmonised audit strategies to both in vitro and in silico work packages, ONTOX sets a precedent for integrated quality management across diverse scientific approaches. Ultimately, this initiative ensures that ONTOX's research outputs are generated under a framework of accountability and best practice, reinforcing their scientific credibility and regulatory applicability. The dual audit system represents an innovative step towards building trust in New Approach Methodologies (NAMs) and highlights ONTOX's leadership in promoting a culture of quality in next-generation risk assessment.

Why an Audit?

Within the ONTOX project, the audit is a cornerstone of quality assurance:

- Verifies compliance with best practices in in vitro / in silico method development and application.
- Strengthens credibility of results, supporting regulatory acceptance.
- Promotes harmonisation across partners, enabling reproducibility and comparability of data.
- Identifies gaps in laboratory / computing practices and provides a roadmap for continuous improvement.

In Vitro Audit

- Evaluates compliance with OECD GIVIMP principles
- Focus on traceability, documentation, QA, and method performance in advanced 3D models
- Ensures reproducibility, reliability, and regulatory acceptance

In Silico Audit

- Reviews computational modelling, QSARs, and predictive tools
- Assesses data integrity, algorithm transparency, reproducibility
- Ensures compliance with OECD validation standards

Expected Impact

- Identifies strengths and gaps across research teams
- Harmonises quality management in both experimental and computational work
- Reinforces trust in NAMs and ONTOX's leadership in next-generation risk assessment

Quality is not optional –
it is the foundation of ONTOX innovation.

In Vitro Audit Questionnaire

1. Quality Management System

a. Do you have an established and documented Quality Management System (QMS)? Please upload 2–3 relevant documents (e.g., QMS manual, audit reports) as evidence.
b. Is the QMS compliant with relevant standards of ISO 9001, ISO 17025 or GLP?
If yes, please upload the certificate from autorised body.
c. Do you have an internal audit schedule for GIVIMP compliance?
If yes, please upload the relevant file.
d. Are corrective and preventive actions (CAPAs) documented and followed up on? Please upload audit schedules, CAPA reports, or management review minutes.

2. Organisation and Personnel

a. Are roles and responsibilities clearly defined and documented?
Please upload supporting files (organisation charts, project matrix).
b. Are personnel trained and competent to perform in vitro methods?
Please upload the training plan/schedule.

3. Facilities and Equipment

a. Do laboratory facilities meet the requirements for cell culture work?
Upload facility-related documents and a few photos as proof.
An annotated floor plan of the in vitro testing unit/facility.
b. Are equipment calibration and maintenance records available?
Upload an example of the calibration and maintenance record for your
- laminar hoods,
- CO2 incubators,
- Cell storage systems (N2 tanks or -80°C refrigerators),
- Pipettor calibration records,
- any other equipment identified as critical equipment in the process
c. Do you monitor environmental conditions (e.g., temperature, CO2 levels) in cell culture areas?
Provide monitoring logs.

4. System Procurement and Characterisation

a. Do you have a documented cell banking system (master and working cell banks)? Please upload cell banking SOPs and related records.
b. Are cell banks stored under controlled conditions and periodically tested for mycoplasma/other contamination?
Describe the system and upload recent contamination test results.
c. Are cell lines sourced from authenticated suppliers?
Upload certificates for the cell lines used in your laboratories
d. Is there a procedure for verifying cell line identity?
Upload the SOP and records.
e. Is there a system in place for monitoring cell passage number? Upload tracking sheets.
f. Are routine cell culture conditions (e.g., media composition, CO2 concentration, temperature) clearly defined and followed?
Upload SOPs/media preparation records.

5. Reagents and Materials

a. Are records of reagent lot numbers and expiry dates maintained?
Please upload an example of inventory logs.
b. Are reagents stored under appropriate conditions and within expiry dates?
Upload SOP for labelling and storage of materials.
c. Are reagents and media clearly labelled with identity, lot number and expiry date? Upload examples of labels or photos.
d. Are procedures in place for the disposal of expired reagents?
Upload relevant SOPs or describe the procedure in the facility.
e. Are all critical reagents validated before use?
Upload examples of validation records / alternatively, upload examples of certificates of Analysis from the supplier for the most critical materials.

6. Procedures and Method Implementation

a. Are Standard Operating Procedures (SOPs) in place for all test methods?
Upload your list of SOPs (only list)
b. Have all personnel performing the methods been appropriately trained and documented for each specific procedure?
Upload training certificates or records.
c. Are Standard Operating Procedures (SOPs) in place for all test methods?
List the methods used in your laboratory and SOP numbers.
Upload 2 SOPs as an example of procedural SOP
d. Do you periodically revise the SOPs?
Upload documentation that demonstrates the process of review.
e. Are method transfer (external training records) or validation reports available for the implemented test methods? Provide sample reports.
f. Are deviations from SOPs recorded and investigated?
Upload an example of a deviation report.

7. Data Recording and Reporting

a. Are raw data recorded contemporaneously and securely?
Provide examples of laboratory log books and/or a screenshot of databases of electronically stored raw data.
b. Have your devices active audit trial mode?
Upload a screenshot of the audit trail report.
c. Are electronic data backed up regularly?
Upload backup protocols or schedules.
d. Are report templates standardised?
Provide a sample template.

8. Archiving and Data Integrity

a. Are data protected against loss and unauthorised access?
Upload a description of the system implemented in the facility.
b. Is there a documented retention policy for raw data and records?
Upload retention policy.
c. Are archived records accessible only to authorised personnel?
Please upload a description of the archive practice.

In Silico Questionnaire

1. Purpose & Governance

What is the primary purpose of the AI/in silico model you contributed to? (Select all that apply)

Classification (e.g., toxic vs. non-toxic, hazard categorization)
Regression (e.g., predicting LD50, NOAEL, IC50)
Dose-response modeling (e.g., continuous toxicity scoring)
Read-across predictions (e.g., filling data gaps using analogs)
Adverse Outcome Pathway (AOP) mapping (e.g., linking molecular events to outcomes)
Ontology-driven predictions (e.g., using knowledge graphs for mechanistic insights)
Language model tasks (e.g., NLP for literature mining, data extraction, summarization)
Knowledge representation / ontology mapping
Generative chemistry (e.g., designing safer molecules or virtual libraries)
Uncertainty quantification (e.g., prediction confidence, applicability domain)
Other (please specify): _____

2. Which of the following OECD (Q)SAR principles are addressed in your model documentation? (Select all that apply)

Defined endpoint
Unambiguous algorithm
Defined applicability domain
Appropriate measures of goodness-of-fit, robustness, and predictivity
Mechanistic interpretation (if possible)
None of the above
Not applicable
Other : _____

3. Personnel & Training

Which of the following qualifications or experiences apply to your role in this project? (Select all that apply)

- ○ Degree in a relevant field (e.g., computer science, bioinformatics, cheminformatics, AI)
- ○ Completion of relevant online training or certification (e.g., Coursera, edX, Udacity, etc.)
- ○ Peer-reviewed publications related to AI/in silico modeling
- ○ Previous project experience in AI/ML or toxicity prediction
- ○ Experience in scientific software or pipeline development
- ○ None of the above
- ○ Other : _____

4. Data Management

Which of the following practices are followed to ensure data provenance and integrity? (Select all that apply)

- ○ Source of all input data is documented
- ○ All modifications to data are version-controlled
- ○ SOP exists for tracking data lineage
- ○ Data is regularly backed up
- ○ None of the above
- ○ Other : _____

How is data security and sharing handled in your workflow? (Select all that apply)

- ○ Data stored in secure repositories (e.g., GitLab, Zenodo, institutional servers)
- ○ Access is restricted via user authentication and permissions
- ○ Personal or sensitive data is anonymised
- ○ Consent for data reuse is documented when applicable
- ○ Compliance with GDPR and FAIR principles is ensured
- ○ None of the above
- ○ Other: : _____

5. Model Development & Auditability

Which aspects of the model development process are documented or version-controlled? (Select all that apply)

- ○ Workflow from data ingestion to deployment is documented in an SOP
- ○ Version control is applied to code and configuration files (e.g., Git)
- ○ Model training and evaluation scripts are reproducible
- ○ Schedule for retraining and updating models is defined
- ○ Drift monitoring procedures are in place
- ○ None of the above
- ○ Other: _____

What specifications of the development platform are recorded? (Select all that apply)

- ○ Hardware setup (e.g., cloud provider, CPU/GPU type)
- ○ Operating system and software dependencies
- ○ Python/R or other library versions used
- ○ Licensing details of third-party software or packages
- ○ None of the above
- ○ Other: _____

What components of audit trail and traceability are implemented? (Select all that apply)

- ○ Logs of all major changes to data and code (e.g., Git commits)
- ○ Model versioning and tracking (e.g., MLflow, DVC)
- ○ Metadata captured for each model version (e.g., dataset used, parameters, time)
- ○ Logs are stored in an accessible and searchable format
- ○ None of the above
- ○ Other: _____

6. Model Validation & Performance:

Which of the following methods have been used to evaluate the performance, reliability, and regulatory relevance of your AI/in silico model? (Select all that apply)

Statistical/Machine Learning Methods

AUC-ROC, Precision, Recall, F1-score
RMSE / MAE or similar regression error metrics
Cross-validation (e.g., k-fold, LOOCV, stratified CV)
Y-scrambling / permutation testing
Temporal validation (e.g., using historical vs. newer data splits)
None of the above
Other: _____

In Silico-Specific & Regulatory Practices

Applicability domain analysis (e.g., similarity, leverage, distance-based methods)
External validation with regulatory datasets (e.g., EPA ToxCast, ECHA REACH)
Benchmarking against existing (Q)SAR or QSAR Toolbox models
Alignment with Adverse Outcome Pathways (AOPs)
Uncertainty quantification (e.g., confidence intervals, conformal prediction)
None of the above
Other (please specify): _____

Have you assessed or considered the regulatory impact of prediction errors (e.g., false positives/negatives)?

Yes: Quantitatively (e.g., confusion matrix-based analysis)
Yes: Qualitatively (e.g., risk discussion or expert review)
No: Not applicable to this model
No: But plan to include this in future versions
Unsure

7. Transparency & Usability:

Which input and output formats does your model support? (Select all that apply)

Input formats

SMILES
SDF
Tabular data (e.g., CSV, XLSX)
Text-based descriptors
Omics data
Other: _____

Output formats

○Toxicity class (e.g., toxic / non-toxic)
○Continuous toxicity score
○Confidence intervals or probability scores
○Other: _____

Is your model interoperable with the following tools or platforms? (Select all that apply)

○OECD QSAR Toolbox
○KNIME
○Custom APIs
○Standardized file formats (e.g., SDF, CSV, JSON)
○Other: _____

8. Ethical & Legal

How is your model or software licensed and made accessible? (Select all that apply)

○MIT License
○GNU General Public License (GPL)
○Proprietary license
○Open-access (publicly available)
○Restricted-access (internal or partner use only)
○Includes third-party licensed components
○Other: _____

9. Documentation & Maintenance

Which of the following practices are used to ensure reproducibility of your AI/in silico model or workflow? (Select all that apply)

Containerization (e.g., Docker, Singularity)
Workflow management tools (e.g., KNIME, Nextflow, Snakemake)
Random seed logging for reproducible stochastic processes
Workflow documentation (e.g., Jupyter notebooks, scripts)
Environment specification (e.g., requirements.txt, Conda YAML)
Version control of data and code (e.g., Git, DVC)
Use of standardized model formats (e.g., SBML, PMML, OECD-compatible outputs)
QMRF or QPRF documentation for OECD-compliant QSAR reporting
FAIR metadata annotation (e.g., ISA-Tab or equivalent)
Other: _____

FUNDING

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 963845