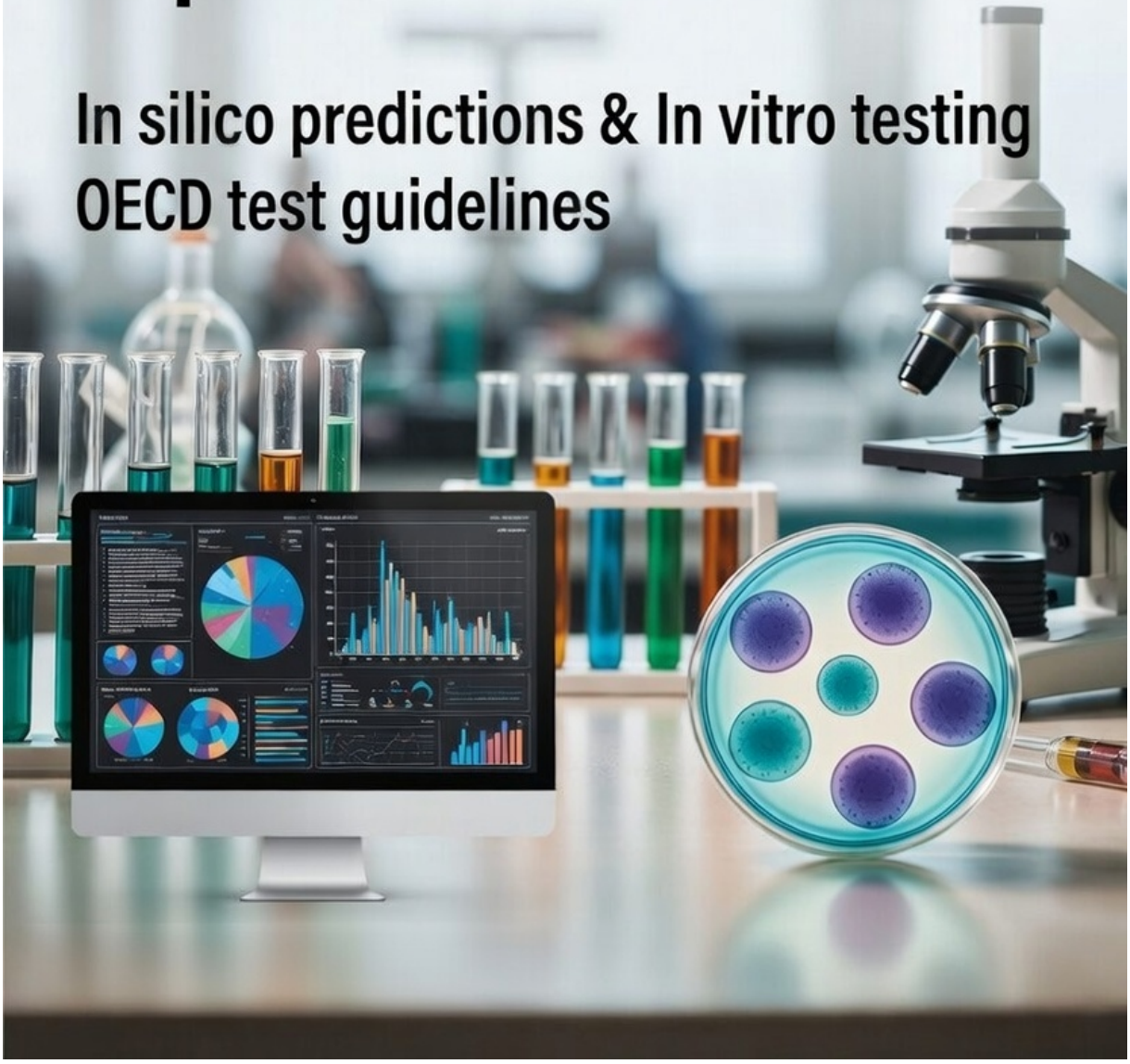
Animal Testing

Implementation Pathway for 100% Animal-Free Replacement of LD50 Test

In silico predictions & In vitro testing OECD test guidelines

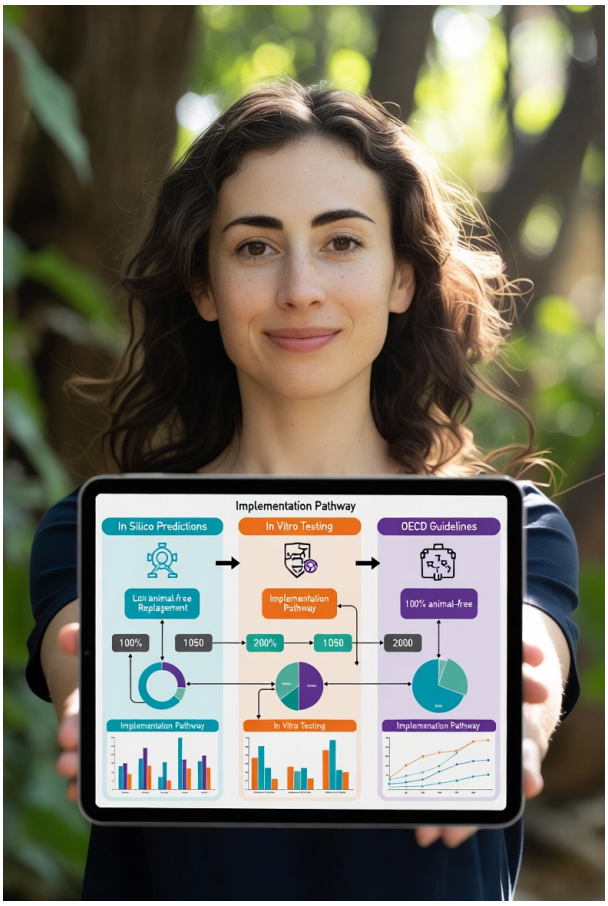


While the OECD has not established a single, standalone Test Guideline (TG) that fully replaces the traditional LD50 test (deleted in 2002 as OECD TG 401) with a 100% animal-free method for acute oral toxicity, it actively promotes the use of Integrated Approaches to Testing and Assessment (IATA) as a flexible, science-based framework to integrate non-animal methods for regulatory decision- making.

IATA allows for the combination of in silico, in vitro, and existing data in a weight-of-evidence (WoE) approach to predict acute toxicity, potentially avoiding animal testing entirely, especially for low-toxicity substances (e.g., LD50 > 2000 mg/kg) or under regulations like the EU cosmetics ban or REACH where animal data waivers are possible.

This aligns with OECD's Series on Testing and Assessment (e.g., No. 336 on Subacute Inhalation Toxicity, but extended conceptually to acute oral via case studies), emphasizing the 3Rs (Replacement, Reduction, Refinement) principles.

IATA is particularly suitable for GHS classification and labeling, where non-animal data can support hazard categorization without in vivo confirmation if the uncertainty is low. As of 2025, no fully validated animal-free OECD TG exists for comprehensive acute oral toxicity across all hazard categories, but IATA case studies and emerging methods like AcutoX demonstrate pathways for acceptance.



Below is a step-by-step implementation pathway, drawing from OECD guidance on IATA (e.g., Series No. 260), validated non-animal tools, and real-world examples. This is intended as a guide for researchers and for their own professional development.

Step 1: Problem Formulation and Hypothesis Development

- Define the regulatory context (e.g., GHS classification for acute toxicity categories 1-5 or "not classified" if LD50 > 2000 mg/kg).

- Formulate a hypothesis based on the chemical's structure, use, and expected exposure (e.g., "low acute oral toxicity due to poor bioavailability").

- Gather physicochemical data (e.g., molecular weight, logP) to inform predictions.

- **OECD Alignment**: This follows the initial phase of IATA as per OECD guidance, ensuring the approach is hypothesis-driven to minimize data gaps.

Step 2: Collection of Existing Information

- Review databases like ECHA's REACH dossiers, EPA's ToxCast/Tox21, PubChem, or OECD eChemPortal for historical data on the substance or analogs.

- Include human-relevant data from epidemiological studies or incident reports if available.

- OECD Alignment: OECD encourages using existing data first to avoid unnecessary testing, as outlined in IATA frameworks.

Step 3: In Silico Predictions

- Apply QSAR models (e.g., OECD QSAR Toolbox, Leadscope, or VEGA) to estimate LD50 or GHS categories based on structure-activity relationships.
- Use read-across from structurally similar compounds with known toxicity data.

- Incorporate machine learning tools trained on large datasets for higher accuracy (e.g., 80-95% correct or conservative predictions for GHS).

- OECD Alignment: QSAR and read-across are accepted under OECD principles (e.g., Series No. 69 on Validation of QSAR), and can form the basis of IATA submissions.

Step 4: In Vitro Testing

- Conduct cytotoxicity assays like the 3T3 Neutral Red Uptake (NRU) assay (OECD TG 129) using human or mammalian cell lines to estimate IC50 (inhibitory concentration 50%), which correlates with LD50.

- Use advanced models:
  - Human cell-based systems (e.g., HepG2 for liver metabolism) or organ-on-a-chip for systemic effects.

  - Methods like AcutoX, which uses human cells with/ without S9 liver extract to measure neutral red uptake and MTT, predicting GHS/EPA categories with 63-74% accuracy and 90-93% protectiveness.

- For metabolism-dependent toxicity, include S9 fractions or 3D liver models.

- OECD Alignment: TG 129 supports using in vitro data to inform acute toxicity, though primarily for starting doses; in IATA, it can contribute to full assessments for low-toxicity substances.

Step 5: Weight-of-Evidence Integration and Uncertainty Assessment

- Integrate all data using a structured WoE approach (e.g.,
scoring reliability and relevance per OECD criteria).

- Apply defined approaches (DAs) if available, with fixed data interpretation procedures (e.g., Bayesian networks for combining predictions).

- Assess uncertainty: If low (e.g., consistent predictions of LD50 > 2000 mg/kg), conclude without animal data; if high, iterate with additional non-animal tests.

- OECD Alignment: This core IATA step is detailed in OECD Series No. 260 and No. 336, allowing expert judgment for regulatory submissions.

Step 6: Regulatory Submission and Validation

- Document the IATA in a report, including rationale, data sources, and uncertainty analysis.

- Submit to regulators (e.g., ECHA for REACH, EPA for TSCA) who may accept it for waiver of in vivo tests.

- For OECD mutual acceptance, contribute to IATA Case Studies Project (e.g., Kao's 2020 studies on systemic toxicity using read-across and cell assays for chlorobenzenes/ alkylphenols, adopted by OECD).

- Seek validation through bodies like EURL ECVAM or ICCVAM for broader acceptance.

- OECD Alignment: OECD reviews IATA case studies annually (e.g., 9th cycle in 2023), leading to guidance updates; successful cases can inform future TGs.

Limitations and Considerations

- Currently best approach for low-toxicity chemicals; highly toxic substances may require more data or still need in vivo confirmation in some jurisdictions.

- Regulatory acceptance varies: Full replacement is common for cosmetics (EU Directive 1223/2009), but for pesticides/industrials, IATA supports reduction rather than total elimination.

- Ongoing advancements (e.g., AcutoX's 2025 validation) may lead to new OECD TGs by integrating high-throughput screening.

This pathway is an approach that supports ethical, human-relevant assessments while aiming for OECD compliance through IATA.

References:

Here is a suggested References list based on the key sources and citations implied or directly mentioned in the document (e.g., OECD guidelines, series, tools, databases, methods like AcutoX, Kao case studies, and regulations).

The references focus on official, verifiable sources where possible, using standard formats (similar to APA/Chicago for reports and publications). These align with the content on IATA, non-animal methods for acute oral toxicity, QSAR/read-across, in vitro assays, and regulatory frameworks.

URLs are included for easy access, publication years (based on original or latest versions), and brief notes on relevance.

If a specific series number (e.g., No. 336) doesn't perfectly match acute oral toxicity, it's because OECD IATA is applied flexibly across endpoints—use the general series or case study reports for broader support.

OECD. (2001). Guidance Document on Acute Oral Toxicity Testing (OECD Series on Testing and Assessment No. 24). OECD Publishing.
https://www.oecd.org/en/publications/guidance-document-on-acute-oral-toxicity-testing_9789264078413-en.html
(Provides background on acute oral toxicity testing, including historical context for the deletion of TG 401 and promotion of alternative approaches.)

OECD. (2010). Guidance Document on Using Cytotoxicity Tests to Estimate Starting Doses for Acute Oral Systemic Toxicity Tests (OECD Series on Testing and Assessment No. 129). OECD Publishing.
https://ntp.niehs.nih.gov/sites/default/files/iccvam/suppdocs/feddocs/oecd/oecd-gd129.pdf
(Supports the use of in vitro cytotoxicity assays like 3T3 Neutral Red Uptake (NRU) to inform acute toxicity assessments, relevant to Step 4 in your pathway.)

OECD. (2007). Guidance Document on the Validation of (Quantitative) Structure-Activity Relationship [(Q)SAR] Models (OECD Series on Testing and Assessment No. 69). OECD Publishing.
(Outlines principles for accepting QSAR and read-across in regulatory submissions, aligning with Step 3: In Silico Predictions.)

OECD. (2017). Guidance Document for the Use of Adverse Outcome Pathways in Developing Integrated Approaches to Testing and Assessment (IATA) (OECD Series on Testing and Assessment No. 260). OECD Publishing.
https://www.oecd.org/en/publications/guidance-document-for-the-use-of-adverse-outcome-pathways-in-developing-integrated-approaches-to-testing-and-assessment-iata_44bb06c1-en.html
(Core guidance on IATA frameworks, hypothesis-driven approaches, weight-of-evidence, and uncertainty assessment; supports overall pathway and Steps 1, 5.)

OECD. (Various years). OECD Series on Testing and Assessment. Multiple reports, including case studies on IATA (e.g., annual review cycles). OECD Publishing.
https://www.oecd.org/en/topics/sub-issues/testing-of-chemicals/publications-on-testing-and-assessment-of-chemicals.html
(General series encompassing IATA case studies, including extensions to acute endpoints; note: No. 336 relates to skin sensitisation defined approaches, but the series covers broader concepts.)

OECD. (Ongoing). OECD QSAR Toolbox. Free software tool for QSAR modeling, read-across, and chemical category formation.
https://qsartoolbox.org/
https://www.oecd.org/en/data/tools/oecd-qsar-toolbox.html
(Directly referenced for in silico predictions in Step 3.)

OECD. (Various). eChemPortal: The Global Portal to Information on Chemical Substances. OECD. https://www.echemportal.org/
(Database for existing data collection in Step 2.)
European Chemicals Agency (ECHA). (Ongoing). REACH Dossiers and Registration Database. https://echa.europa.eu/
(Source for historical data, read-across, and waivers under REACH in Steps 2 and 6.)

U.S. Environmental Protection Agency (EPA). (Ongoing). ToxCast/Tox21 Data.
https://www.epa.gov/chemical-research/toxcasttm-data
(High-throughput in vitro data for existing information in Step 2.)

National Center for Biotechnology Information (NCBI). (Ongoing). PubChem Database. https://pubchem.ncbi.nlm.nih.gov/
(Physicochemical and toxicity data resource in Steps 1–2.)

European Parliament and Council. (2009). Regulation (EC) No 1223/2009 on Cosmetic Products (EU Cosmetics Regulation). Official Journal of the European Union.
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32009R1223
(Bans animal testing for cosmetics, enabling full replacement via non-animal methods in limitations section.)

Kao Corporation. (2020). Case Studies on Systemic Toxicity Evaluations Using Non-Animal Methods (Chlorobenzenes and Alkylphenols). Adopted by OECD IATA Case Studies Project.
https://www.kao.com/global/en/newsroom/news/release/2020/20201210-002
(Real-world example of IATA using read-across and cell assays for systemic toxicity, referenced in Step 6.)

Sedgwick, P., et al. (2024). Predicting acute oral toxicity using AcutoX: An animal product-free human in vitro test method. Toxicology in Vitro.
https://pubmed.ncbi.nlm.nih.gov/39150754/
(Describes the AcutoX method using human cells, NRU/MTT assays, with/without S9, for GHS category prediction; aligns with Step 4 and emerging methods.)

OECD. (Ongoing). IATA Case Studies Project Reports (e.g., review cycles including 2023/2024). OECD Publishing.
https://www.oecd.org/en/topics/sub-issues/assessment-of-chemicals/integrated-approaches-to-testing-and-assessment.html
(Documents annual reviews and adoptions, such as Kao's 2020 case, supporting validation and regulatory acceptance in Step 6.)

Conclusion

The traditional LD50 test (OECD TG 401, deleted in 2002) has long been recognized for its ethical concerns and scientific limitations, prompting the OECD to champion Integrated Approaches to Testing and Assessment (IATA) as the primary pathway toward reducing—and ultimately replacing—animal use in acute oral toxicity evaluation.

This document outlines a suggested practical, step-by-step implementation pathway that leverages in silico predictions (e.g., OECD QSAR Toolbox and read-across), existing data sources (e.g., eChemPortal, REACH dossiers, ToxCast), in vitro cytotoxicity assays (e.g., OECD TG 129 3T3-NRU or advanced human-relevant methods like AcutoX), and structured weight-of-evidence integration to predict acute toxicity outcomes, particularly for low-toxicity substances (LD50 > 2000 mg/kg).

When uncertainty is low, this IATA-based approach can help fully support GHS hazard classification, labeling, and regulatory decisions without in vivo confirmation, as demonstrated in real-world case studies (e.g., Kao Corporation's read-across applications) and emerging tools.

While no single, fully validated standalone OECD Test Guideline yet exists for comprehensive animal-free acute oral toxicity across all hazard categories, significant progress continues. Annual OECD IATA case study review cycles (including the tenth cycle in 2024) and advancements in New Approach Methodologies (NAMs) are building confidence in non-animal predictions, especially for cosmetics (under EU bans) and REACH waivers.

These efforts prioritize human-relevant, mechanistic data over crude lethality endpoints.

In conclusion, full 100% animal-free replacement of acute oral toxicity testing is not only feasible but increasingly achievable for many substances through rigorous IATA application. As validation strengthens, regulatory acceptance broadens, and tools like AcutoX and high-throughput in vitro platforms mature, this pathway paves the way for more ethical, efficient, and scientifically robust chemical safety assessments.

Continued collaboration between scientists, regulators, and industry will accelerate the transition to a future where animal testing for acute toxicity becomes obsolete, protecting both human health and animal welfare.