In Silico Toxicology 101

Computational Tools for Chemical Hazard Characterization

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Inotiv

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Outline

Part 1

- Learning objectives
- Types of NAMs
- Methods for chemical hazard characterization: where do NAMs fit in?
- History of *in silico* NAMs and current trends

Part 2

- *In silico* NAMs overview
- Purpose, inputs, and outputs of *in silico* NAMs
  - QSAR
  - Read-across
  - PBPK
  - IVIVE
  - qAOP
  - Molecular modeling
  - AI/Machine learning/Deep learning
- Summary

Victoria Hull, M.S.

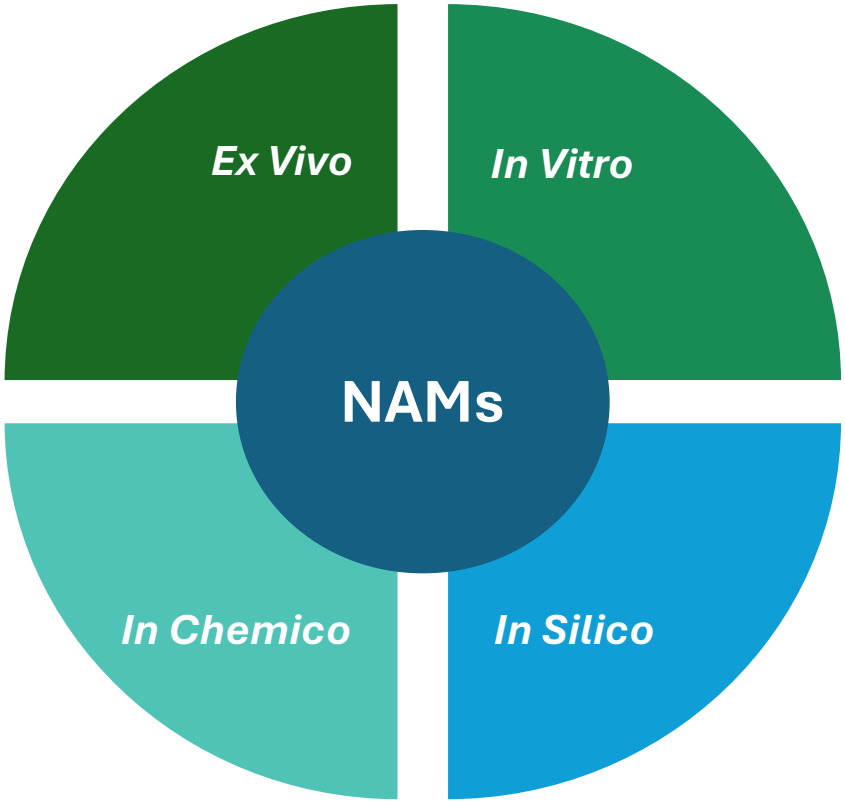
Part 1: Background and Introduction

Learning Objectives

- Describe some current uses of *in silico* NAMs and the state of their acceptance for regulatory applications.
- Explain how *in silico* methods can complement *in vitro* and *in vivo* data for chemical hazard characterization.
- Define important terms used to describe various aspects of *in silico* NAMs.
- List the purpose, inputs and outputs, and basic flow of information for common *in silico* NAMs.

What word or phrase do you associate with in silico NAMs?

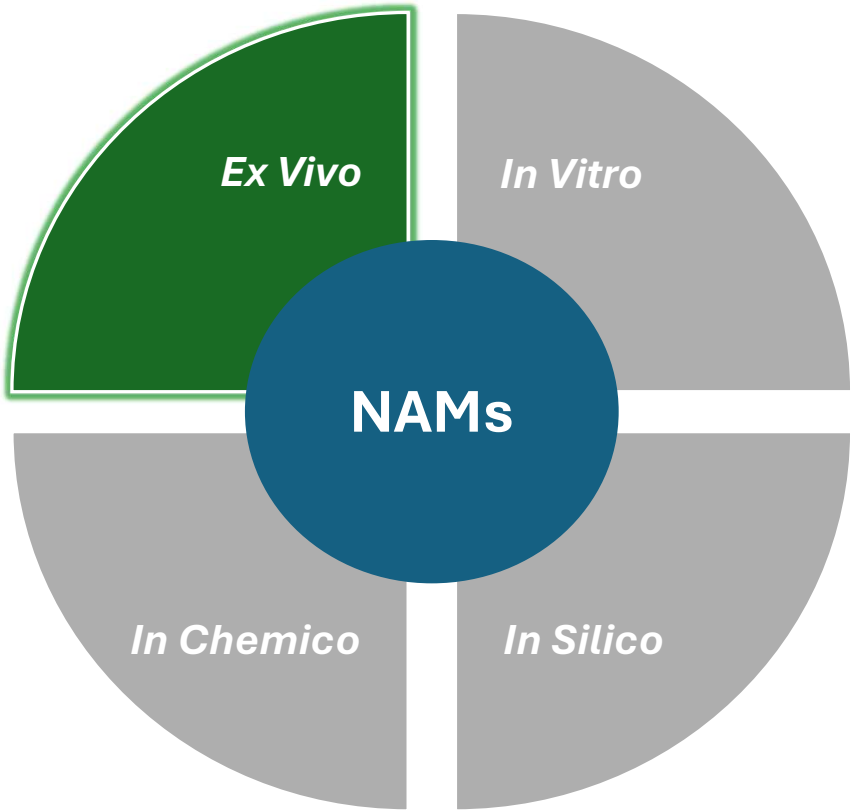
Types of NAMs



New Approach Methodologies (NAMs) is a broad term (often synonymous with Non-Animal Methods) referring to any technology used to assess chemical hazard without using intact animals.

Note: Words that are **bolded in orange** are defined in the webinar glossary

Types of NAMs

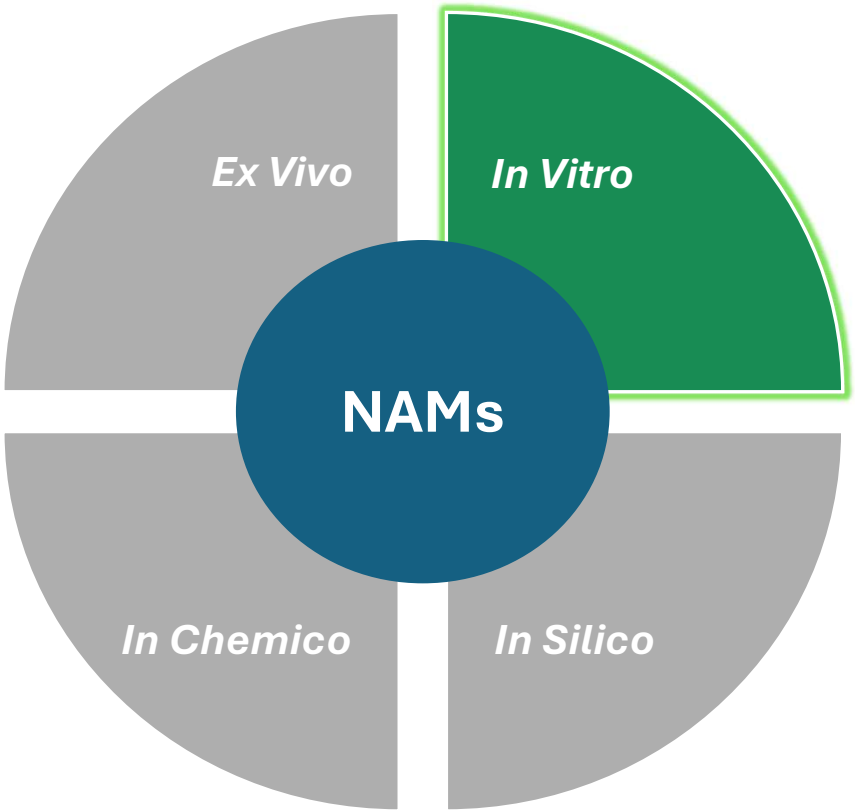


Ex vivo methods measure biological effects by using living tissues or organs.

Examples:

- tissue explants
- perfused organ models

Types of NAMs

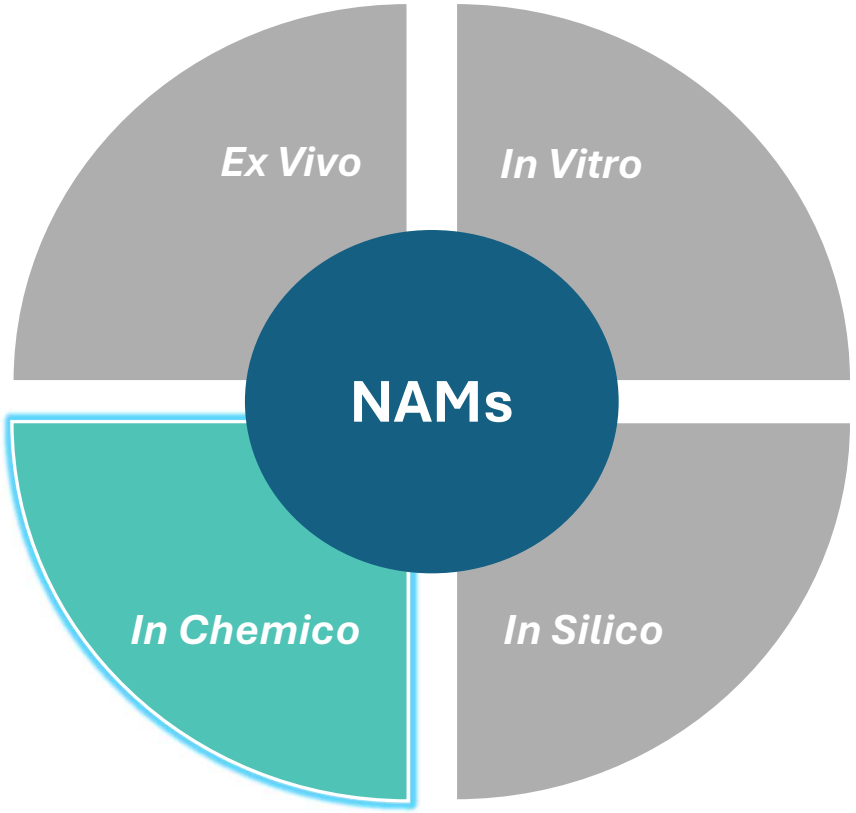


In vitro methods measure biological effects by using human or animal cells.

Examples:

- cell-based assays
- high-throughput screening assays
- organ-on-a-chip models

Types of NAMs

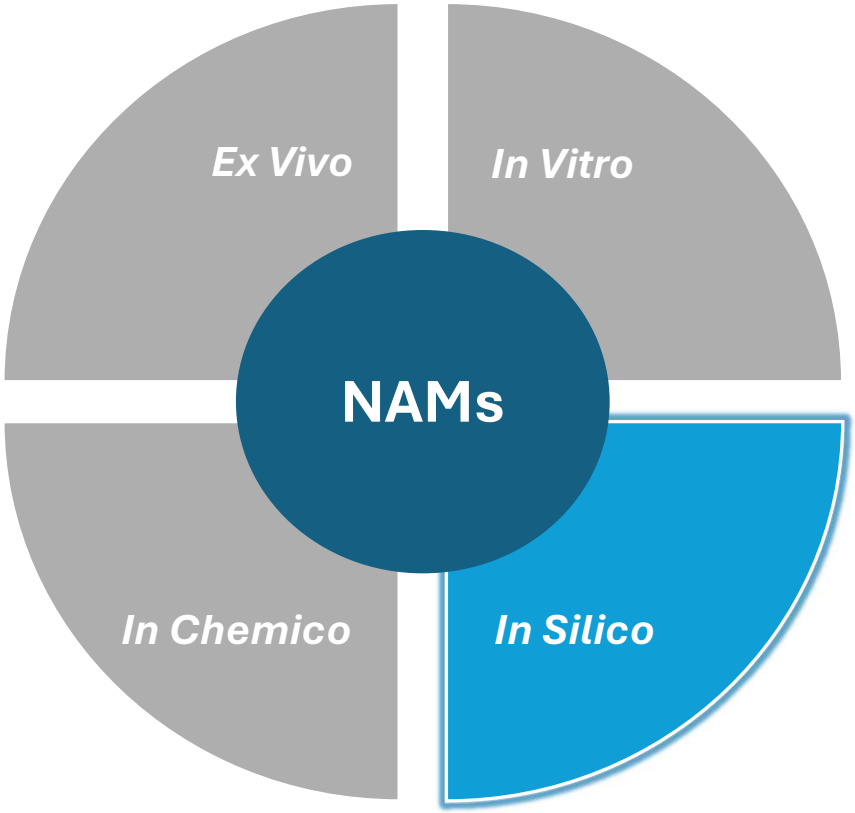


In chemico methods directly measure the chemical reactivity of a substance.

Examples:

- acellular assays
- protein/plasma membrane tests

Types of NAMs



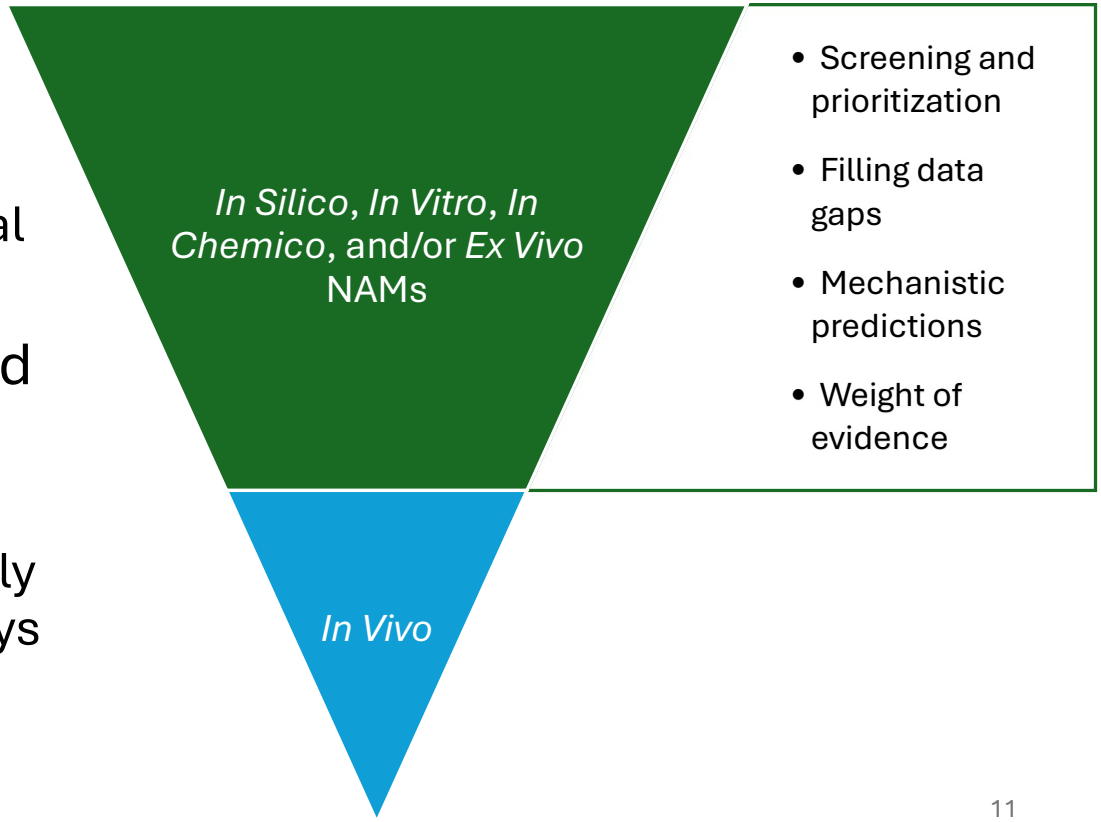
In silico methods predict the intrinsic properties or fate of a chemical using existing data and algorithms.

Examples:

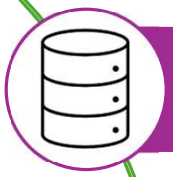
- quantitative structure-activity relationship models
- read-across
- molecular modeling
- machine learning
- artificial intelligence

Methods for chemical hazard characterization: where do NAMs fit in?

- Historically reliant on *in vivo* studies
  - Low throughput, high cost, ethical concerns, sparse human data
- Paradigm shift toward integrated approaches to testing and assessment
  - High throughput, low cost, broadly applied, targeted biological assays
  - Focus on 3Rs



Traditional Methods and NAMs Can Be Complementary



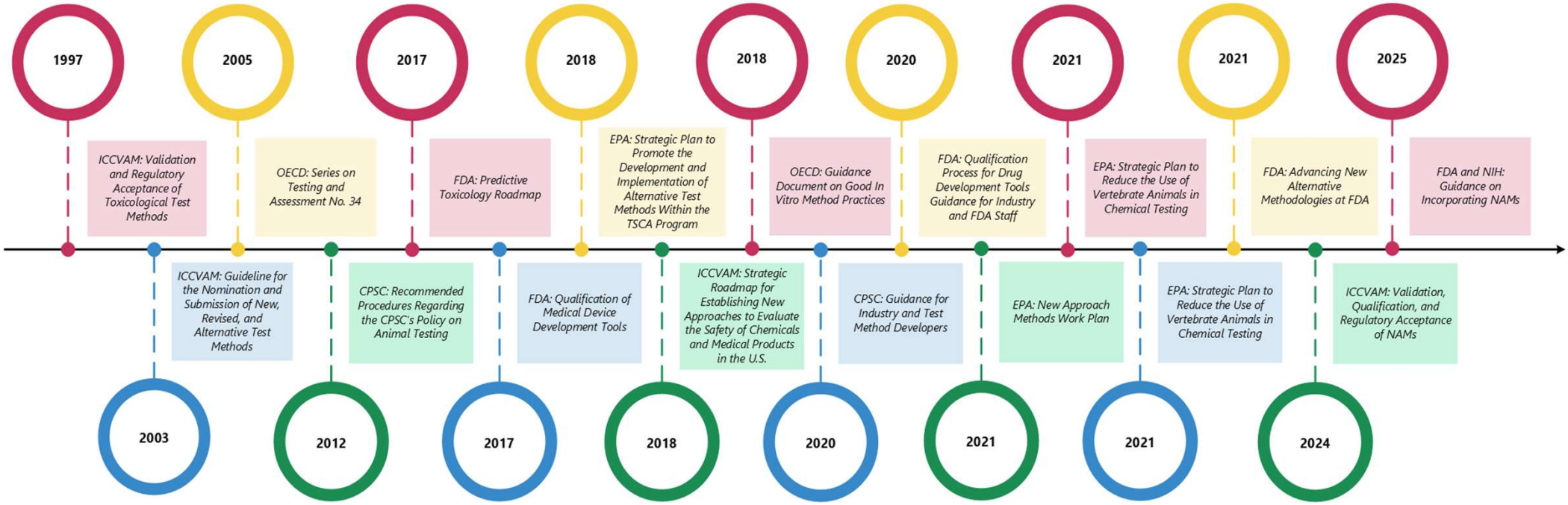
High-quality *in vivo* reference data is needed to train, test, and validate models.

Modelers need to work with traditional toxicologists to create methods that are biologically relevant.

NAMs can give important context to *in vivo* results, making them more human-relevant (*i.e.*, modeling population variability or demographic-specific results).

NAMs can be used for early-stage screenings, helping to focus test methods and reduce the number of animal tests.

Timeline of Select Milestones Regarding Regulatory Use of NAMs



ICCVAM: Interagency Coordinating Committee on the Validation of Alternative Methods
OECD: Organisation for Economic Co-operation and Development

CPSC: Consumer Product Safety Commission
FDA: Food and Drug Administration
EPA : Environmental Protection Agency
NIH: National Institute of Health

Source: 2024 ICCVAM Report

Progress Towards Regulatory Acceptance of *In Silico* NAMs

Acceptance

- Quantitative structure activity relationship (QSAR) models and read-across methods have regulatory acceptance for screening and filling data gaps.
- Physiologically based pharmacokinetic (PBPK) models are used in regulatory submissions to predict drug-drug interactions, tissues concentrations, and other pharmacokinetics.
- In vitro to in vivo extrapolations are used in submissions to put in vitro results into an in vivo, human-relevant context.

Emerging Trends

- Machine learning and artificial intelligence can be used to predict complex toxicities with greater accuracy.
- More complex in silico tools like Collaborative Acute Toxicity Modeling Suite (CATMoS) or SARA-ICE are working towards regulatory acceptance for acute oral toxicity and skin sensitization, respectively.
- There are ongoing efforts for global harmonization on validation protocols and data standards for in silico NAMS.

Resources

- For a list of NAMs accepted by US agencies, visit: https://ntp.niehs.nih.gov/whatwestudy/niceatm/accept-methods
- To see methods submitted in the EU Tracking System for Alternative methods towards Regulatory acceptance (TSAR), visit: https://tsar.jrc.ec.europa.eu/test-method/
- Pending interest, we will continue to add resources for regulatory guidance to the webinar's supplementary materials.

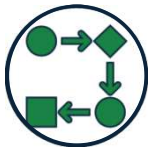
Case studies will be covered in next week's webinars!

Things to Consider

- Not every method or model works for every purpose!
- **Context of use** is the manner and purpose of use for a particular method, approach, or application. (What toxicological endpoint is the method testing for? What, if any, regulatory need does the method address?)
- **Applicability domain** is the chemical or biological space in which the model's predictions are considered accurate.

In silico NAMs Overview

Use computers to predict chemical properties, biological activity, and potential toxicity, eliminating the need for physical experiments



Models and algorithms predict outcomes based on chemical structure and existing data

Considered as an analytical layer that helps interpret and link data from *in chemico* and *in vitro* assays

Helps rapidly screen and prioritize chemicals and fill data gaps

Widely used to support 3Rs initiatives and are a key component of integrated approaches to testing and assessment (IATA)

Brad Reisfeld, PhD

Part 2: Overview of *In Silico* Methods

Featured Methods

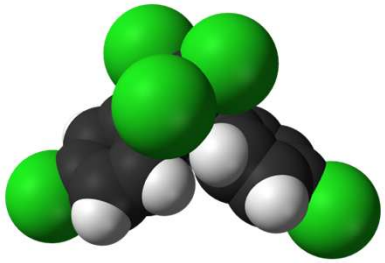
- **Quantitative structure-activity relationship (QSAR)**
- **Read-across**
- **Physiologically-based pharmacokinetic (PBPK) modeling**
- ***In vitro* to *in vivo* extrapolation (IVIVE)**
- **Quantitative adverse outcome pathway (qAOP)**
- **Molecular modeling**
- **Artificial intelligence/Machine learning/Deep learning**

Assess your knowledge I

QSAR

- Will the chemical be toxic to a specific endpoint (*e.g.*, mutagenicity)?
- What is the predicted potency or severity of an effect?
- How does structural modification affect toxicity?

Purpose: **Quantitative structure-activity relationships** (QSARs) relate structural properties of a molecule to its biological activity.



Molecular descriptors

molecular weight
polar surface area
number of H-bond donors
dipole moment
LUMO
number of aromatic rings
…

QSAR →

Biological activity

LD_{50}, IC_{50}
K_i, K_d
active vs. inactive
toxic vs. non-toxic
low, medium, high toxicity
…

Quantitative structure property relationships (QSPRs) is a similar technique, where the molecular structure is related to some chemical property, like solubility.

QSAR

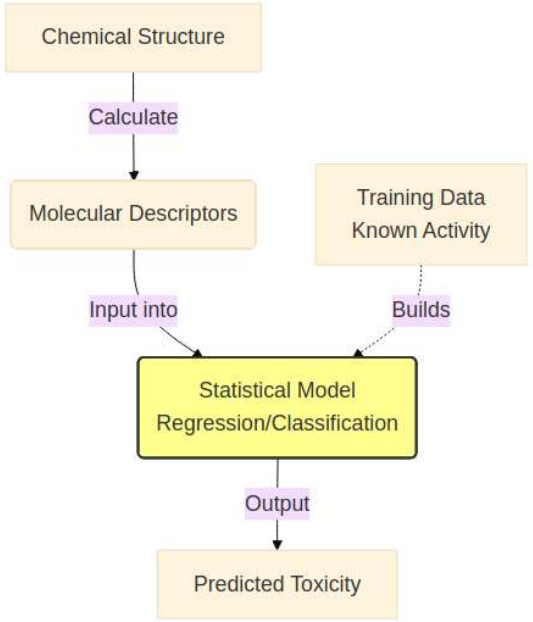
Inputs

- For model development: a list of chemical structures (to become sets of **molecular descriptors**) and some measure of their biological activity
- For model usage: the structure of a molecule for which the activity is unknown

Outputs

- The predicted biological activity in the form of a classification or a quantitative measure

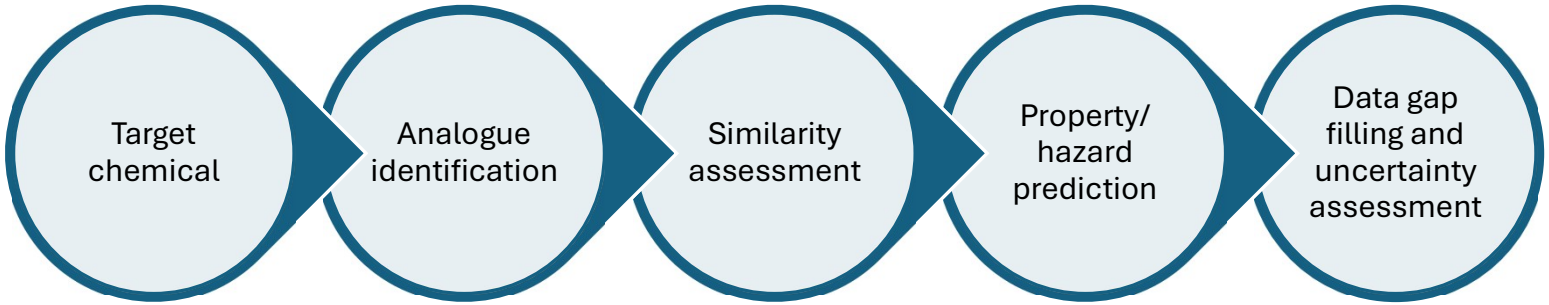
Information Flow



Read-across

- What is the likely toxicity of a data-poor chemical based on that of similar chemicals?
- Can we fill data gaps for our compounds of interest without additional testing?
- What is the rationale for similarity between chemicals in the application of interest?

Purpose: **Read-across** fills experimental data gaps by transferring toxicity information from similar chemicals (**chemical analogues**).



Read-across

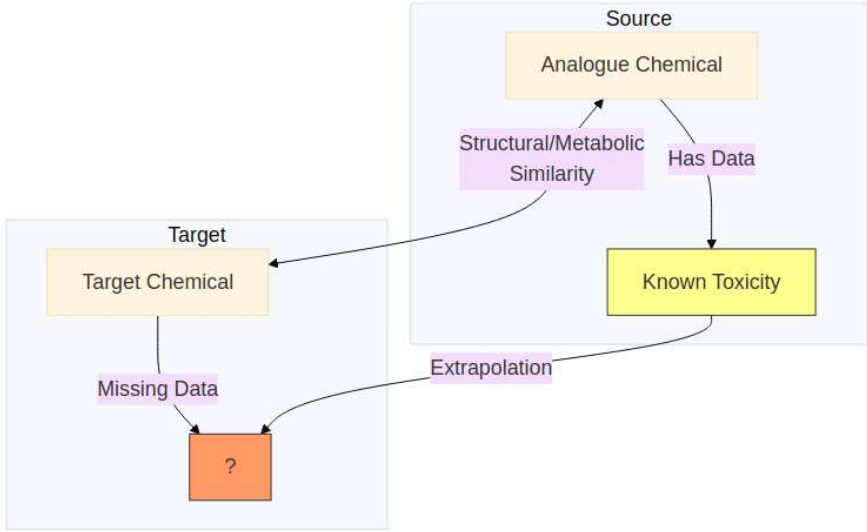
Inputs

- Chemical structures, molecular descriptors, physicochemical properties, toxicity, and mechanistic data

Outputs

- A quantitative prediction for the target chemical, justified by a measure of uncertainty or confidence in the result

Information Flow

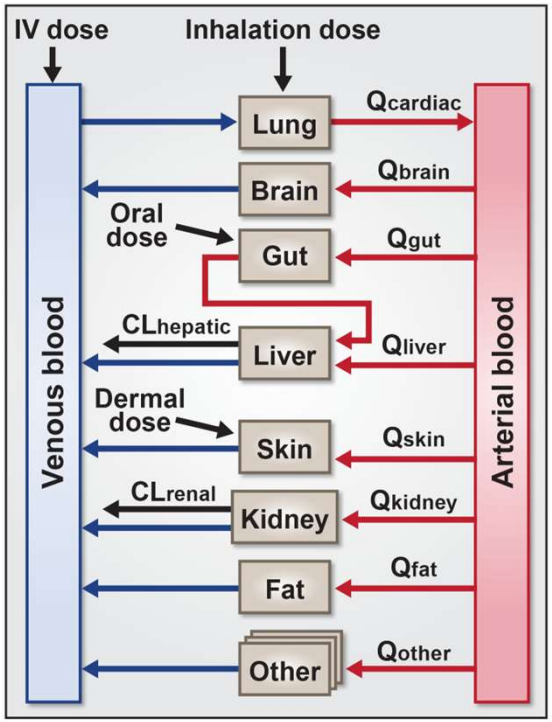


PBPK

- What are the internal tissue concentrations over time?
- How does exposure route affect target organ dose?
- Can we extrapolate between species, doses, or exposure scenarios?
- What is the relationship between external exposure and internal dose?

Purpose: Physiologically-based pharmacokinetic (PBPK) models predict chemical concentrations over time in specific tissue compartments for a given exposure scenario.

- The body is represented by relevant tissue compartments connected by major blood flows.
- Cross-species and exposure route extrapolations are possible.
- Mechanistic relationships can be included.



CL: clearance
Q: flow

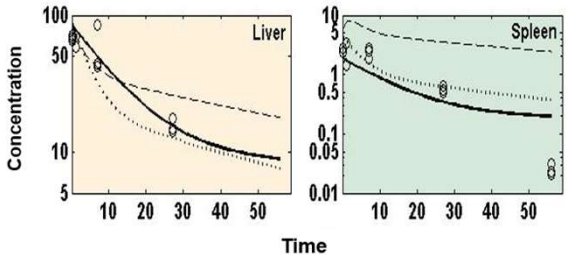
PBPK

Inputs

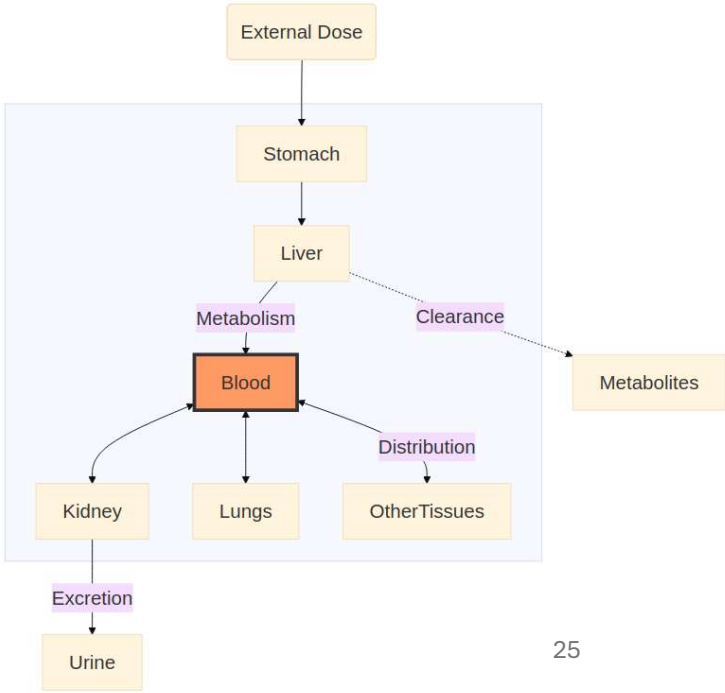
- Physiological parameters (*e.g.*, blood flow rates)
- Chemical-specific physicochemical (*e.g.*, logP) and **ADME** parameters (*e.g.*, intrinsic clearance)

Outputs

- Predicted concentration-time profiles of the chemical species in each compartment for a given dosing scenario



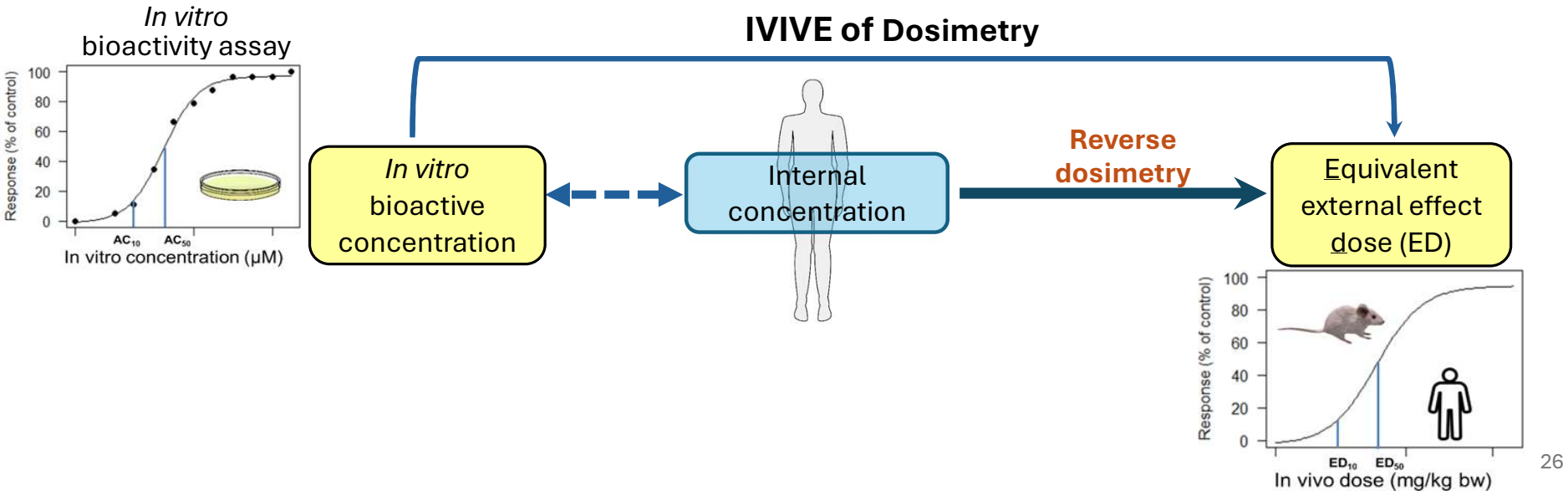
Information Flow



IVIVE

- What *in vivo* dose corresponds to an active *in vitro* concentration?
- Can cell-based assay results predict human health effects?
- What is the human equivalent dose for observed *in vitro* effects?

Purpose: ***In vitro* to *in vivo* extrapolation** (IVIVE) translates *in vitro* assay results (cell-based, biochemical) into predictions relevant to *in vivo* toxicity in whole organisms.



IVIVE

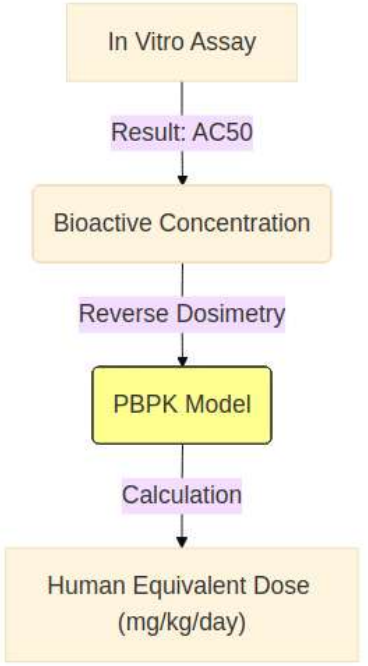
Inputs

- *In vitro* bioactivity data (*e.g.*, AC_{50}, EC_{50}, IC_{50})
- Chemical-specific ADME parameters Pharmacokinetic models or data
- Exposure estimates or biomonitoring data

Outputs

- Human equivalent doses or concentrations
- Bioactivity-to-exposure ratios (risk context)
- Points of departure for risk assessment

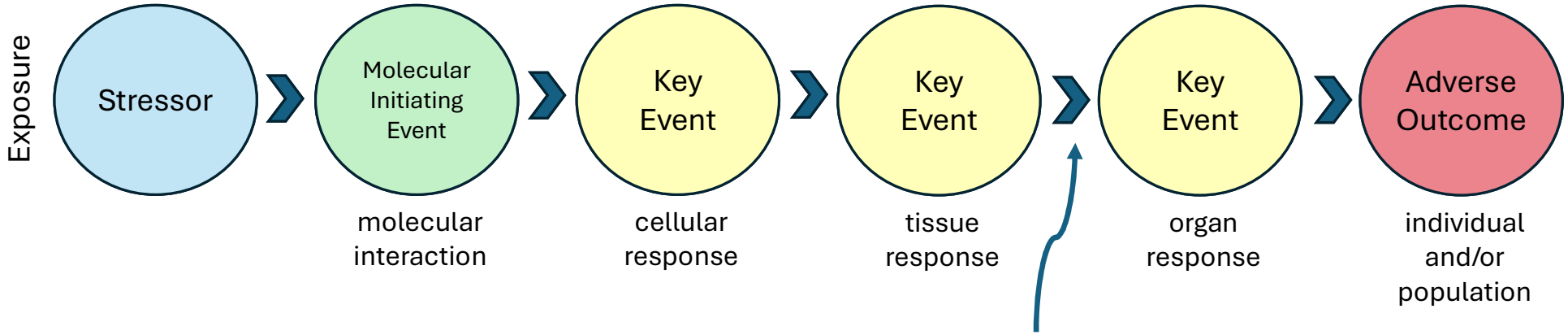
Information Flow



qAOP

- How does a molecular interaction lead to an adverse health outcome?
- What is the quantitative relationship between key events in the pathway?
- Can we predict apical toxicity from early mechanistic data?
- Where are critical points for intervention?

- **Purpose**: **Quantitative adverse outcome pathways** (qAOPs) connect **molecular initiating events** to **adverse outcomes** through a series of **key events**, with quantitative relationships between the key events.



Key event relationship: mechanistic, statistical, semi-empirical, probabilistic

qAOP

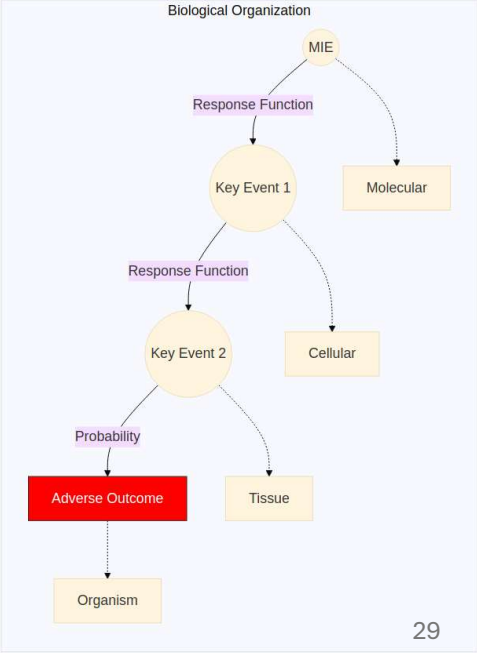
Inputs

- Molecular initiating event data
- Dose-response data at multiple biological levels
- Quantitative relationships between key events
- *In vitro*, *in vivo*, and computational data across levels of biological organization

Outputs

- Quantitative transition information between key events
- Predicted dose-response for adverse outcome
- Identification of critical key events for intervention
- Weight of evidence for pathway relevance

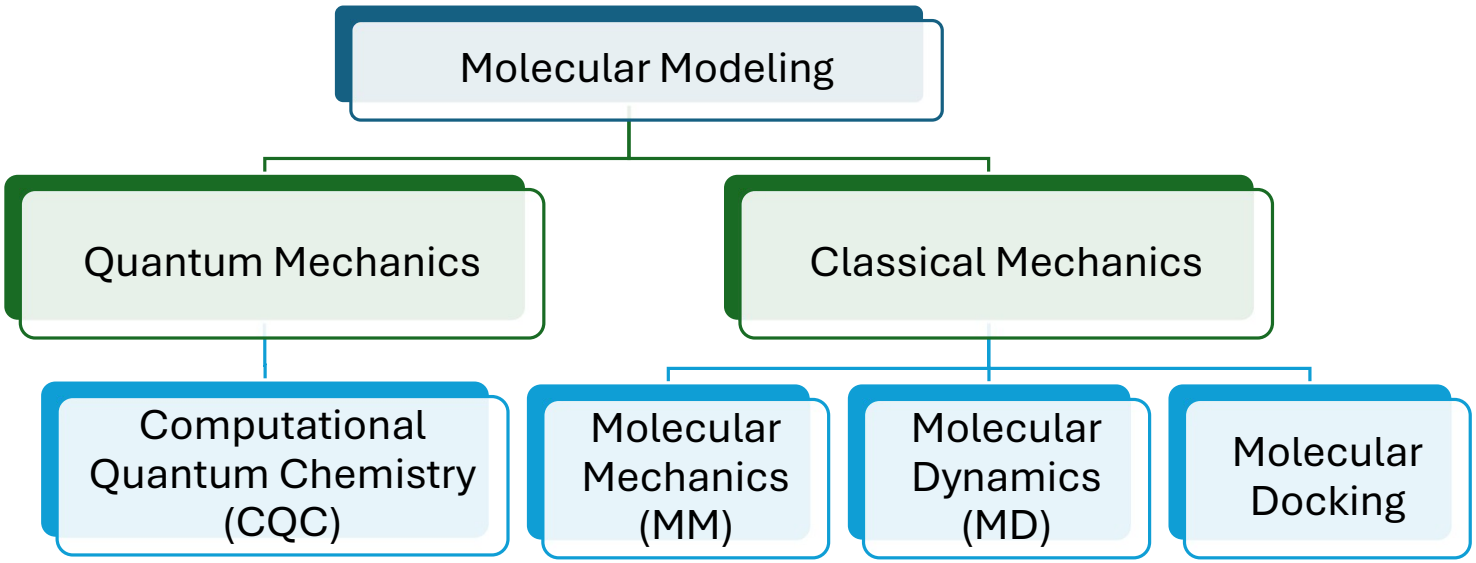
Information Flow



Molecular Modeling

- How does a chemical bind to a target protein or receptor?
- What are the key structural features for toxicity
- What is the preferred 3D conformation of the molecule?
- Can we predict metabolic transformations and rates?

- **Purpose**: Molecular modeling predicts molecular structures, properties, and interactions at the atomic and molecular level



Molecular Modeling

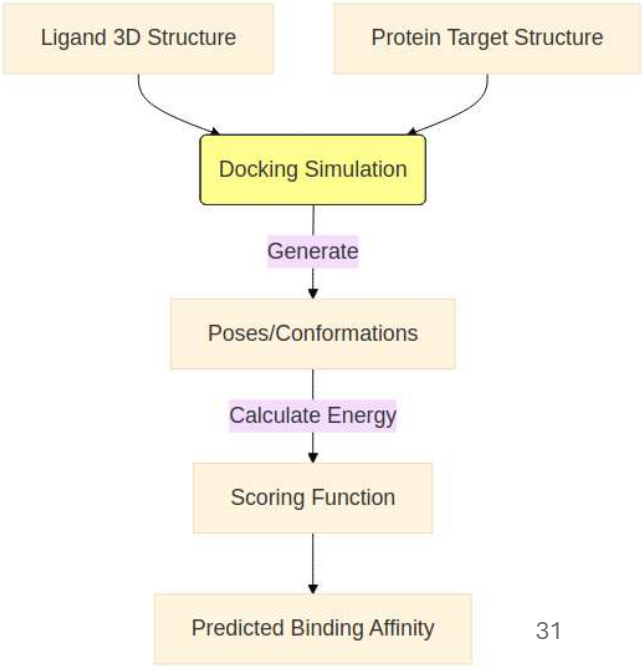
Inputs

- Chemical structure
- Target protein structure (X-ray crystallography, homology models)
- Force fields or quantum mechanical parameters

Outputs

- 3D molecular conformations (MM, CQC)
- Energetics, reactivity and mechanistic insights (CQC)
- Protein-ligand binding poses and affinities (docking)

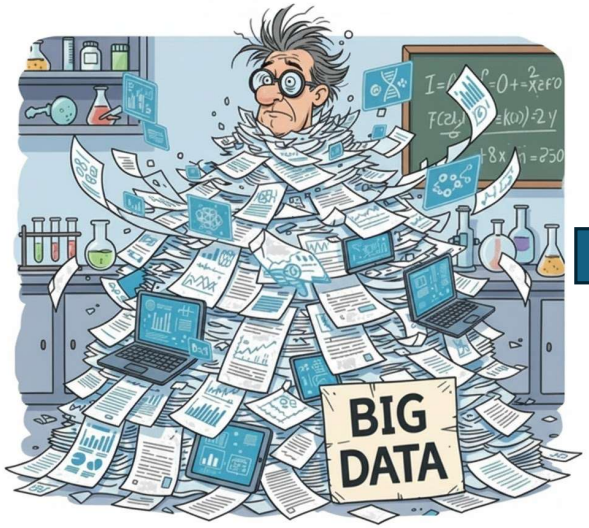
Information Flow for docking

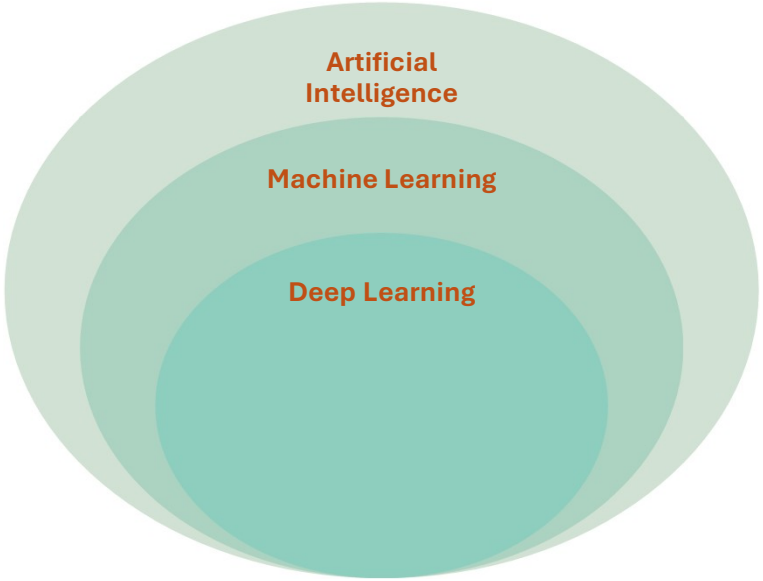


AI/Machine Learning/ Deep Learning

- Which chemicals should be tested first based on predicted hazard?
- Can we integrate multiple data types for better predictions?
- Can we identify unknown toxicity patterns in big datasets?
- How consistent are different data sources in pointing to the same hazard?

Purpose: AI/ML/DL can predict various measures related to toxicity by learning patterns from large datasets, including complex non-linear relationships not captured by traditional methods.





- Toxicity Predictions
- Molecular Properties
- Biological Targets
- Pathways & Mechanisms
- Omics Analysis
- Dose-Response
- Exposure & Fate
- Risk Prioritization

AI/Machine Learning/Deep Learning

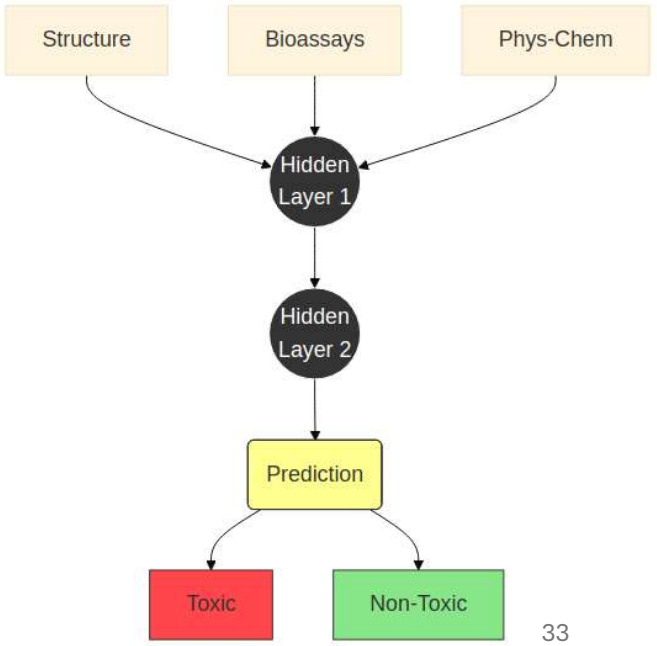
Inputs

- Large training datasets (chemical structures, assay data, -omics)
- Molecular descriptors or direct structural representations
- Multiple endpoint data for multi-task learning
- Test/Validation datasets

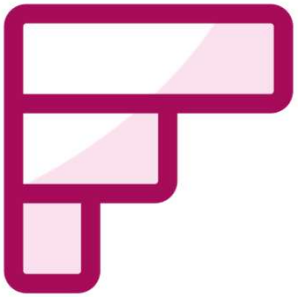
Outputs

- Toxicity predictions (quantitative, categorical)
- Feature importance rankings
- New, synthetic data generated by the model
- Metrics for model accuracy, sensitivity, selectivity, etc.

Information Flow



Assess your knowledge II



In terms of their relevance to your work and/or your level of interest, rank the top in silico method we've covered today?

Summary

- *In silico* approaches provide a valuable complement to *in vitro* and *in vivo* studies, enhancing the evidence base for chemical hazard characterization and risk assessment.
- Their use is expanding across multiple sectors, with growing acceptance for certain regulatory applications.
- This presentation highlighted seven *in silico* methodologies, outlining for each their intended purpose, required inputs, generated outputs, and overall information flow.

Questions?

Links to Webinar Materials

- *In Silico* Methods Quick Reference Sheet
- Glossary
- List of Additional Resources

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