In Silico Toxicology 101

Applications and Case Studies: Part II

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Inotiv

December 18, 2025

Outline

- Learning objectives
- Review of *in silico* NAMs
- Case studies
  - Quantitative AOP
  - Molecular modeling
  - AI/machine learning/deep learning
- Q&A

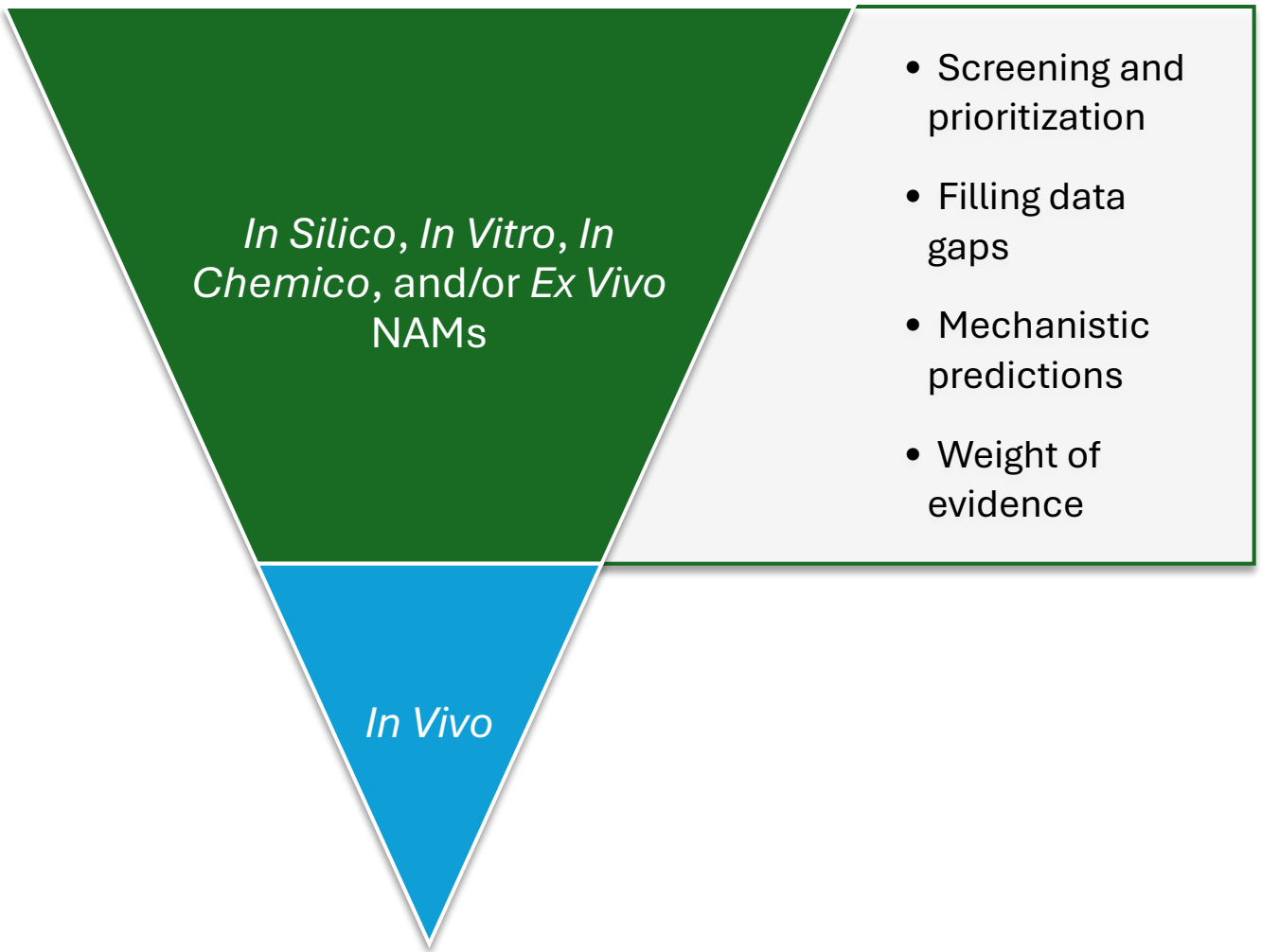
Learning Objectives

- Explain what is involved in developing, implementing, applying, and verifying *in silico* NAMs.
- Describe several ways in which an *in silico* NAM can be evaluated and validated.
- Summarize the drawbacks and benefits of the usage of *in silico* NAMs.
- Give examples of the types of data needed to support the development and testing of various types of *in silico* NAMs.
- Identify the strengths and weaknesses of *in silico* approaches relative to *in vivo* methods for the case studies presented (applicability, chemical space).
- Recognize some software used in the development and evaluation of NAMs.

Review

https://www.thepsci.eu/in-silico-tools-webinars/

YouTube



Paradigm shift toward integrated approaches to testing and assessment

- High throughput, low cost, broadly applied, targeted biological assays
- Focus on 3Rs

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

Review

- 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.

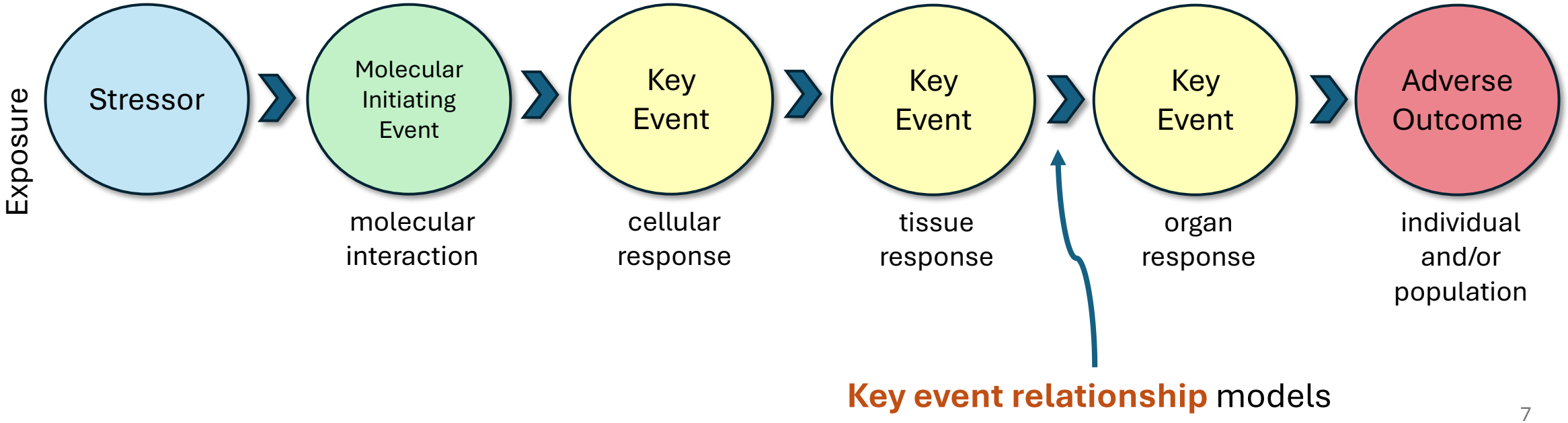
qAOP

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.



Do not edit
How to change the design

Which is an approach that can be applied to qAOP development?

ⓘ The Slido app must be installed on every computer you're presenting from

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qAOP

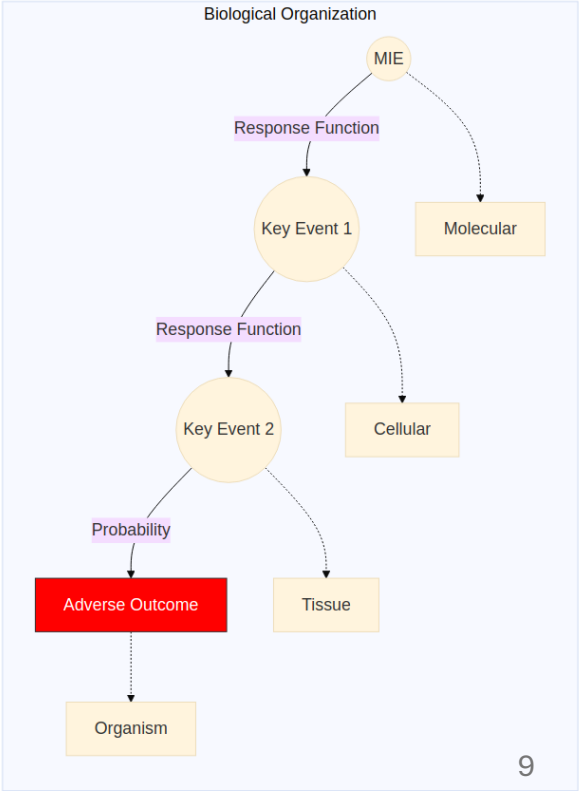
Inputs

- Molecular initiating event (MIE) data
- Dose-response data at multiple biological levels
- Quantitative relationships between key events (KEs)
- *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



qAOP Case Study

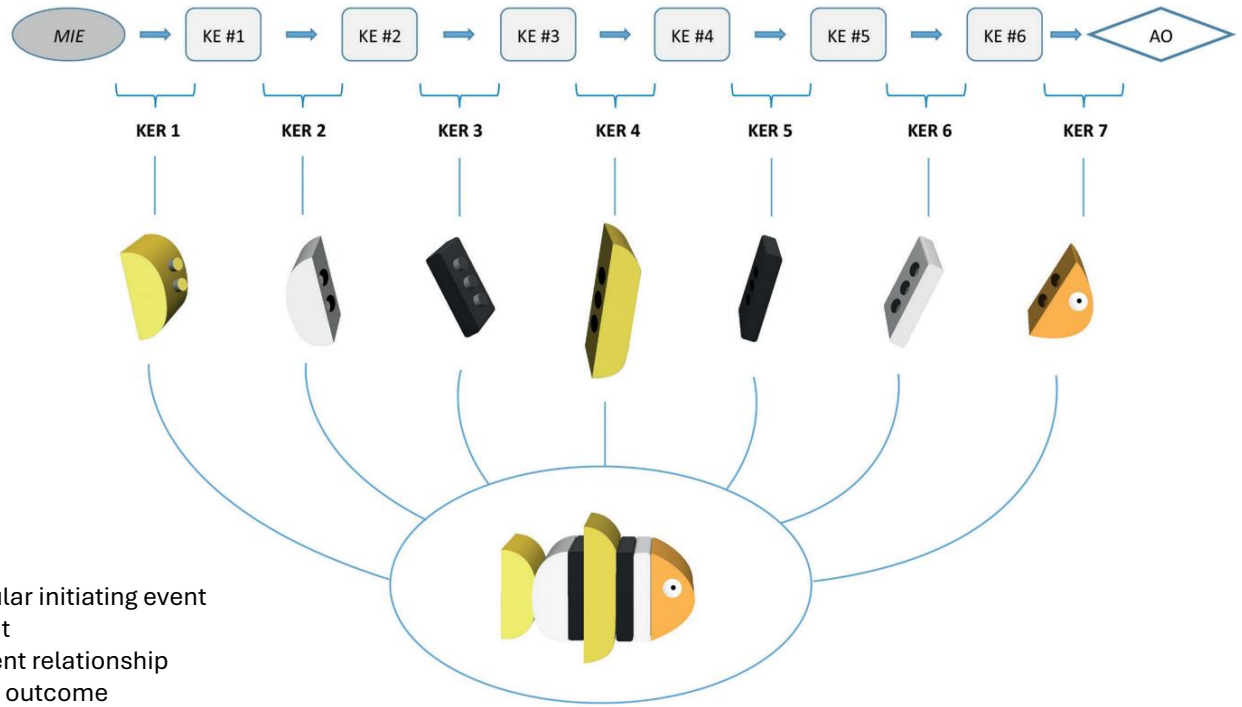
A modular approach for assembly of quantitative adverse outcome pathways

Foran et al., 2019

ALTEX

doi: 10.14573/altex.1810181

Can we use existing information to develop a modular qAOP to describe response relationships between key events for screening and prioritization?



MIE = molecular initiating event
KE = key event
KER = key event relationship
AO = adverse outcome

Models were developed and applied to data gathered for two different applications to address:

- Decreased fish populations (AOP Wiki #25)
- Impairment to learning and memory (AOP Wiki #48)

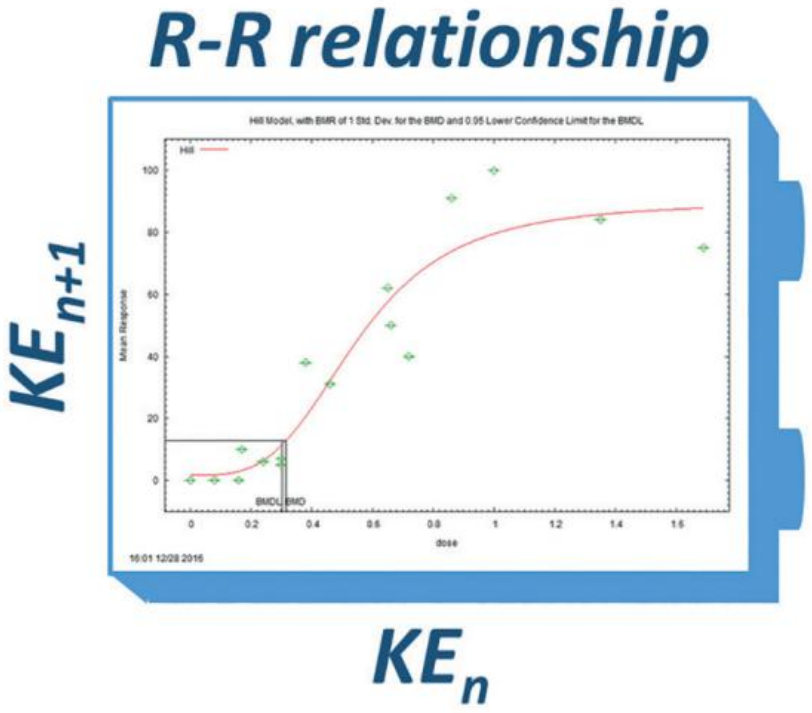
AOP Wiki

https://aopwiki.org/

qAOP Case Study

Methods:

1. Mine literature to gather data relating to two KEs
   - Empirical data and *in silico* predictions
2. Create modules for each response-response (R-R) relationship to facilitate linkages among KEs and adverse outcome
   - Detect changes in the MIE or KE and estimate changes to subsequent KEs
   - Depending on data availability, these can be adjacent (KE 1→2) or non-adjacent (KE 1→3)
   - U.S. Environmental Protection Agency's (EPA) Benchmark Dose Software or Excel

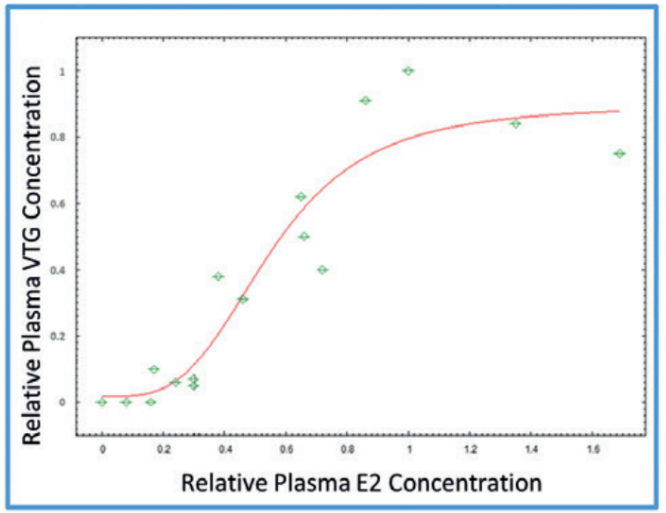


qAOP Case Study

Results: Decrease in fish populations

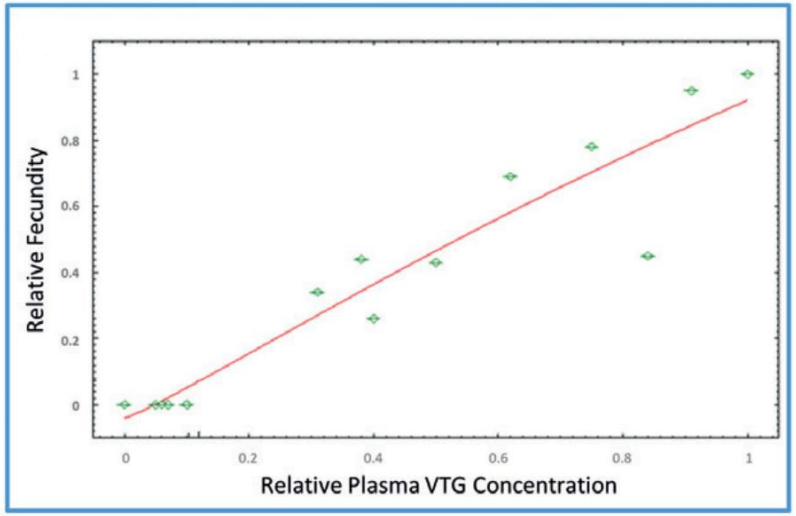
Data from known KEs were gathered from the literature:

- Where extracted values for the MIE were insufficient to develop a R-R relationship (*e.g.*, two data points), a quadratic equation was found to be the best fit.
- For other KEs, there were sufficient data to model R-R relationships.
- An *in silico* approach was found linking fecundity to population decline (AO).



Estrogen hormone concentrations related to egg yolk precursor protein in female fathead minnows.

Relating the same egg yolk precursor protein concentrations to fecundity. Based on these data, a different best fit model was selected.



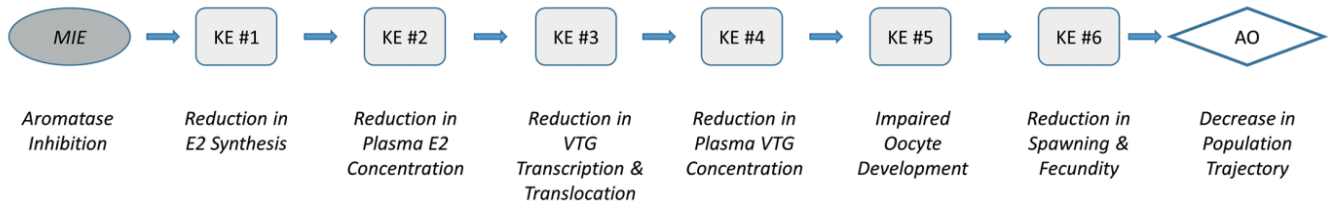
qAOP Case Study

Results: Decrease in fish populations

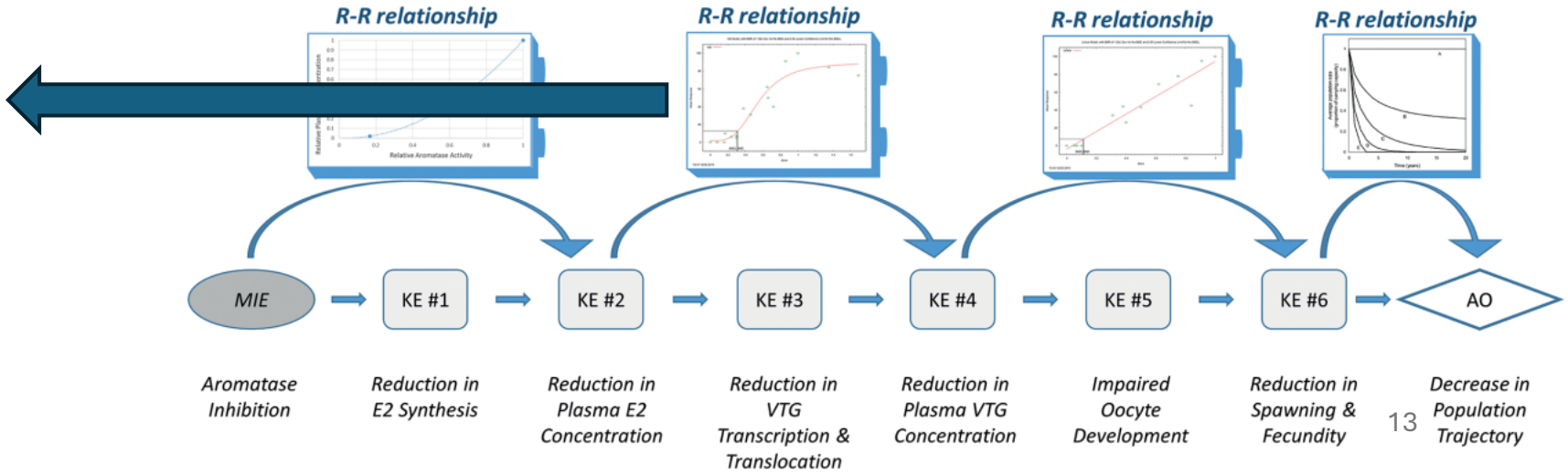
Linking these different modules and establishing the R-R relationships between the endpoints used to measure KEs, predictions leading up to the adverse outcome were established.

Using this model, we can estimate that 45% reduction in plasma estrogen hormone levels leads to a 53% reduction in egg yolk precursor protein.

Starting conceptual framework



Establishing quantitative relationships among the KEs

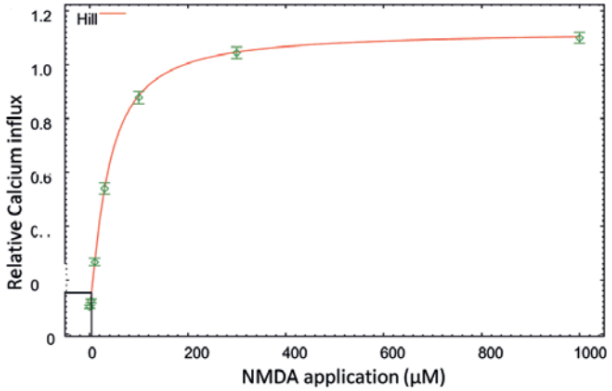


qAOP Case Study

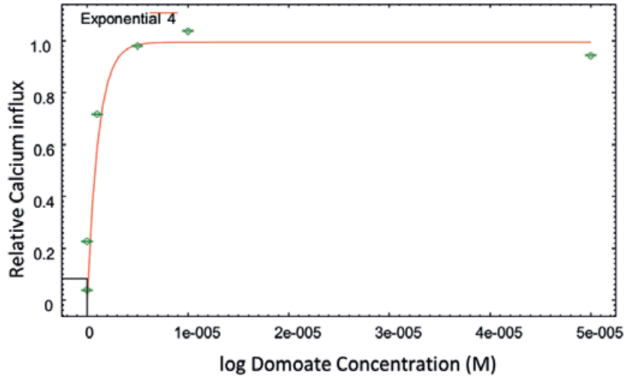
Results: Learning and memory impairments

- Data gathered for this qAOP focused on NMDA which responds to the MIE.
  - Data normalization was applied such as various mammalian species and were normalized from 0-1 based on response.
- To increase the predictivity of the different modules within this qAOP, due to complexity of the AOP and lack of data for some KEs, a specific neurotoxin was used.
  - This led to additional information for modeling the relationship between the MIE and KEs.

Activity of KE endpoint following addition of synthetic amino acid to stimulate receptor activity



Dose response model after neurotoxin exposure



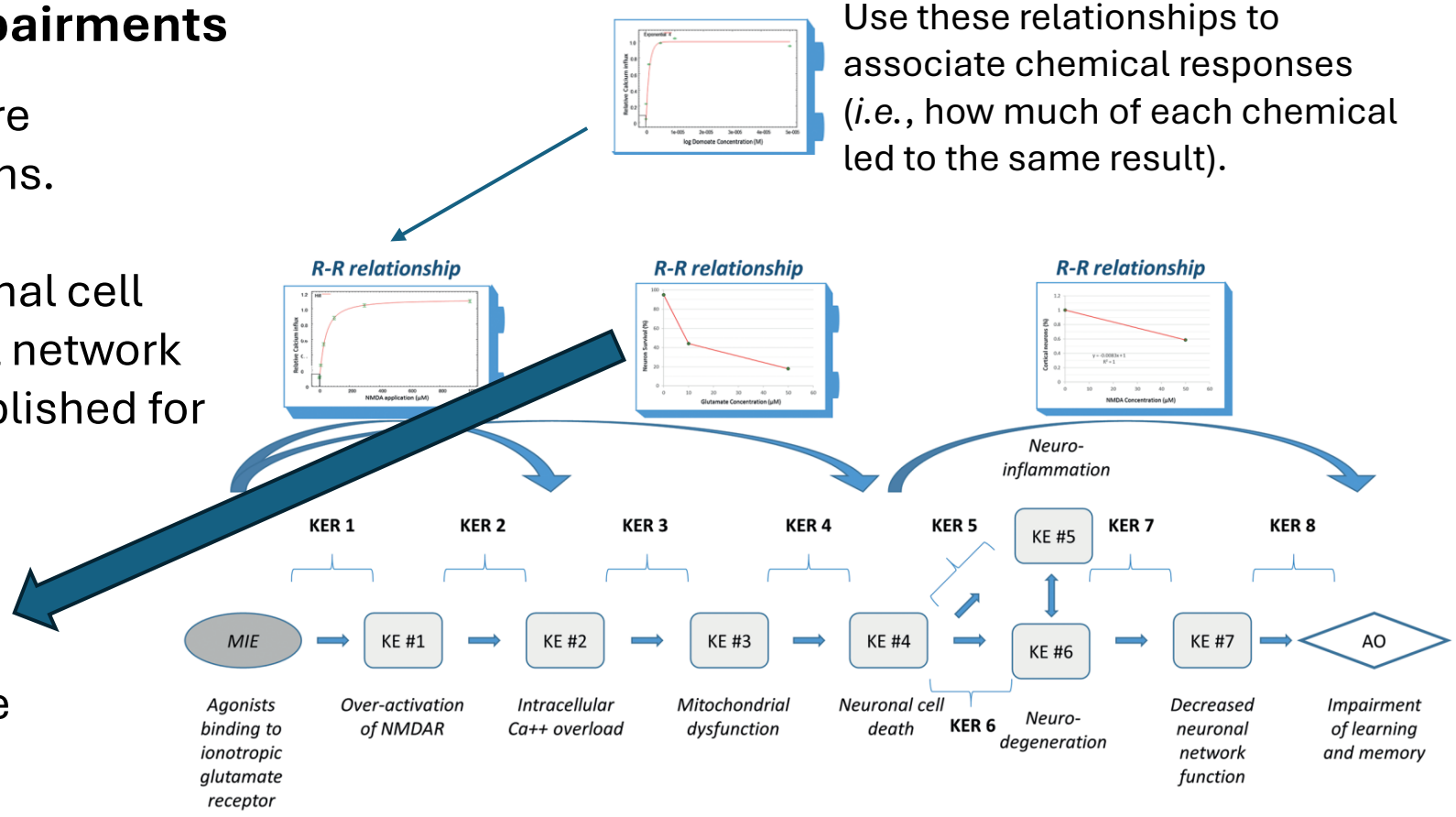
qAOP Case Study

Results: Learning and memory impairments

Non-adjacent KER R-R relationships were established and used to make predictions.

There were various ways in which neuronal cell death could lead to decreased neuronal network function. However, an R-R was not established for either one.

Calcium influx data were used to create this model, which were used to estimate the concentration of glutamate that will result in 50% neuronal survival.



Use these relationships to associate chemical responses (*i.e.*, how much of each chemical led to the same result).

qAOP Case Study

Summary: Creation of a modular approach to building a qAOP using existing information

Strengths:

- Demonstrated wide applicability of building qAOPs in this manner by using two different use cases and complexity
  - Showcased predictions across multiple KEs
- Chemical agnostic approach that can be built upon as more information is collected

Weaknesses:

- Uncertainty was not determined for the best fit curves
- Small datasets were used for some KERs, which can hinder the confidence of predictions

See further examples of qAOP approaches in the Additional Resources.

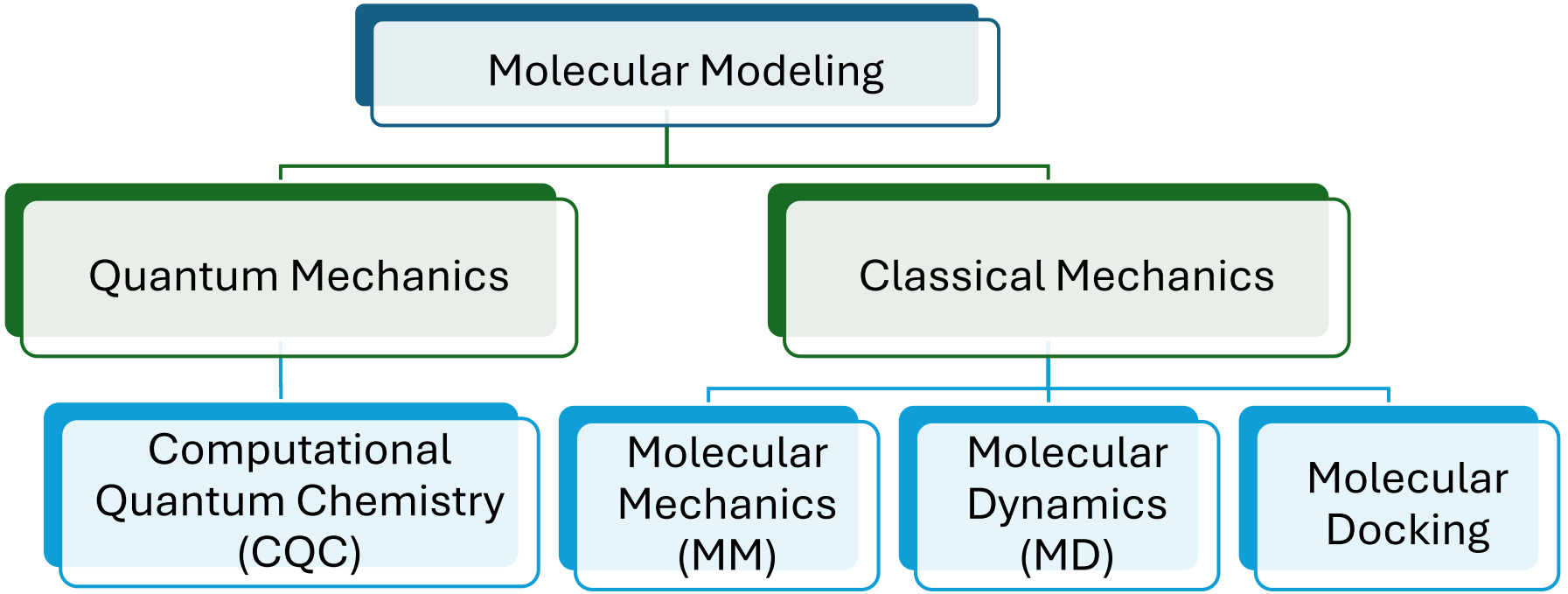
Molecular Modeling

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

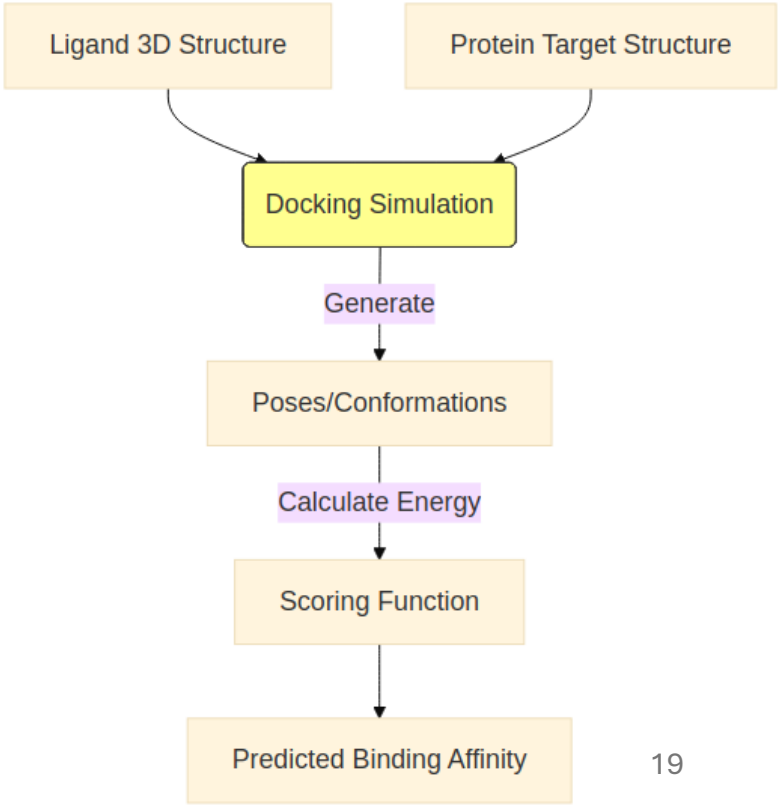
Inputs

- 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



Molecular Modeling: Docking

Use	Purpose
Binding affinity estimation	Ranking compounds by predicted binding strength
Virtual screening	Finding potential active compounds quickly from libraries
Predicting molecular initiating events (MIEs)	Linking chemical–protein binding to the first step in an adverse outcome pathway (AOP)
Chemical prioritization	Screening environmental or industrial chemicals for hazard potential
Predicting metabolic interactions	Estimating whether a compound binds to CYPs or other enzymes involved in metabolism or bioactivation
Supporting QSAR/ML models	Providing mechanistic descriptors to improve toxicity prediction

Molecular Modeling Case Study

Mechanism-based toxicity screening of organophosphate flame retardants using Tox21 assays and molecular docking analysis

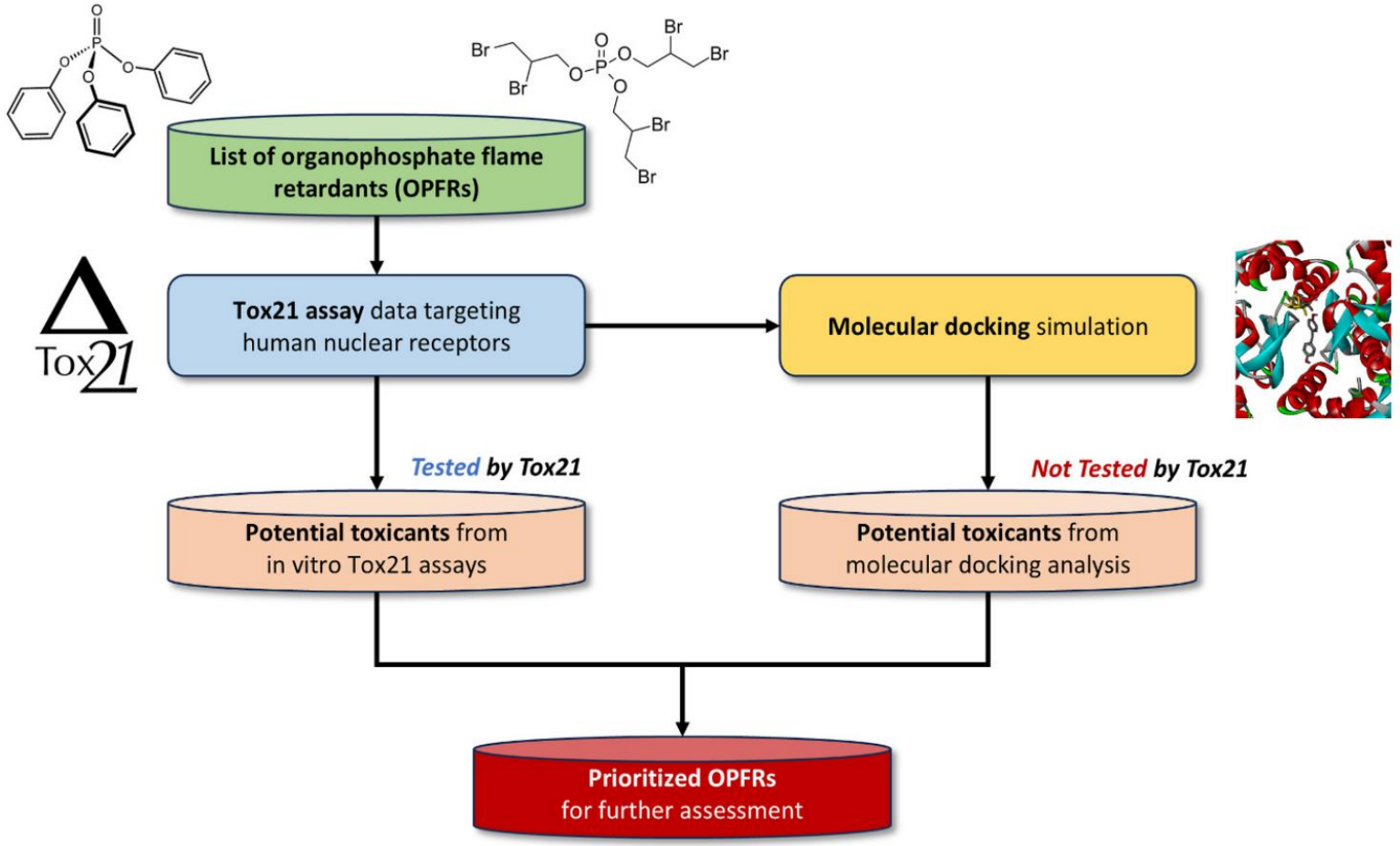
Kim et al., 2024
Chemosphere
doi: 10.1016/j.chemosphere.2024.143772

There is a **lack of sufficient, mechanism-informed toxicity data for organophosphate flame retardants (OPFRs):** chemicals that are increasingly used as replacements for brominated flame retardants.

- **Biological activity data: Tox21 assay hit-call data** targeting seven human nuclear receptors (ER, AR, GR, PPARs, PXR, CAR, FXR)
- **Protein data: Crystallographic structures of human nuclear receptors** were downloaded from the **Protein Data Bank (PDB)**.
- **External Data:** Existing ***in vivo*** **oral toxicity data** from the **EPA ToxValDB**

Molecular Modeling Case Study

To address these gaps, the authors propose a **mechanism-based integrated screening framework**.



https://doi.org/10.1016/j.chemosphere.2024.143772

Molecular Modeling Case Study

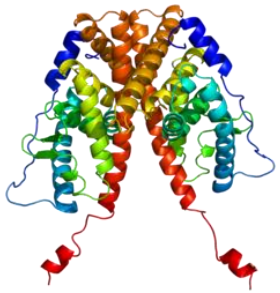
Details

- **Chemicals**: A **literature search** identified 48 OPFRs.
- **Assays**: The **EPA CompTox Dashboard** was searched for Tox21 high throughput assays related to **nuclear receptors** and the resulting list of assays **was filtered** based on whether they referenced an **adverse outcome pathway** (AOP).

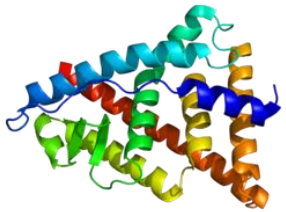
  - This resulted in 25 assays.
  - Out of the 48 chemicals, 26 were found to have been part of the assay battery.
  - **That left 22 whose activity had to be predicted.**

Molecular Modeling Case Study

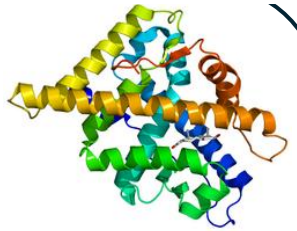
five of the nine receptors used for docking



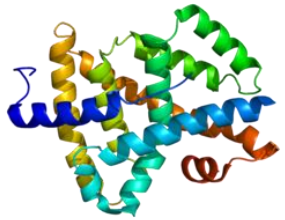
ER-α



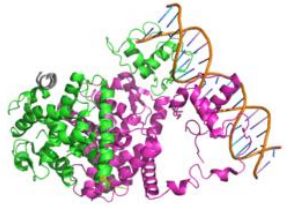
ER-β



AR

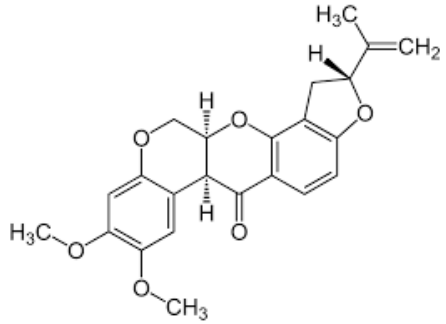


FXR

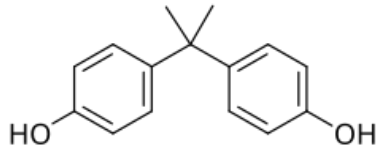


PPAR-γ

known positives



rotenone



bisphenol A

Software: **Autodock Vina** - https://vina.scripps.edu/

AutoDock Vina: Docking and virtual screening program

License Apache 2.0 | version 1.2.5 | docs passing | Downloads 256k

AutoDock Vina is one of the **fastest** and **most widely used open-source** docking engines. It is a turnkey computational docking program that is based on a simple scoring function and rapid gradient-optimization conformational search. It was originally designed and implemented by Dr. Oleg Trott in the Molecular Graphics Lab, and it is now being maintained and develop by the Forli Lab at The Scripps Research Institute.

- An OPFR was judged bioactive if its affinity was at least as strong as that of the known positive.
- Chemicals in the *in vitro* battery were not run through the docking procedure.

Do not edit
How to change the design

Does this method of predicting molecular activity seem reasonable (You can select more than one answer)?

ⓘ The Slido app must be installed on every computer you're presenting from

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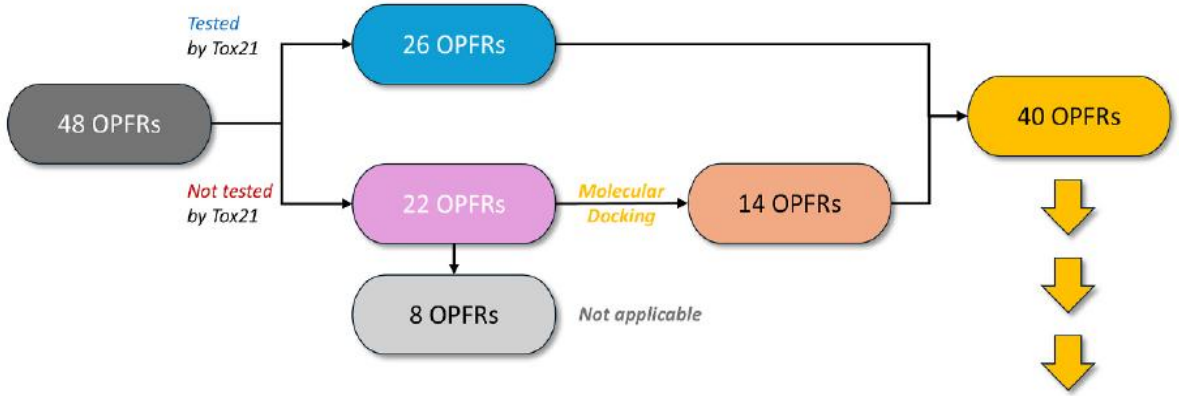
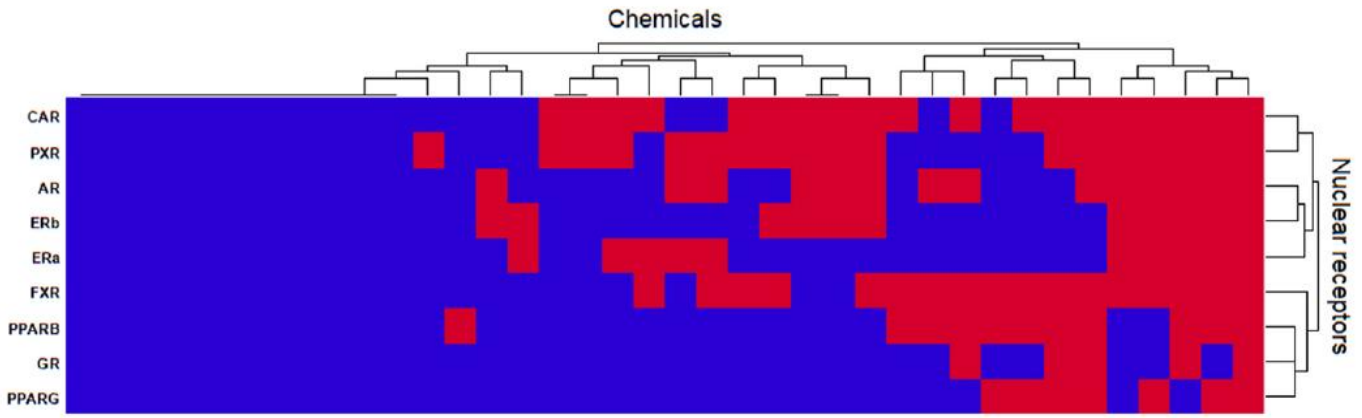
Molecular Modeling Case Study

Results



Pathways to obtaining bioactivity for the OPFRs



Bioactivity as a function of OPFR (horizontal axis) and receptor (vertical axis). Actives are in red; inactives are in blue.

https://doi.org/10.1016/j.chemosphere.2024.143772

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How to change the design

Based on the information provided, what do you see as either the major strength or major weakness of the use of molecular docking in this study?

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Molecular Modeling Case Study

Using molecular docking in this study

Strengths:

- Molecular docking is an appropriate **screening tool** for identifying likely binders.
- The use of molecular docking can provide mechanistic support for the **molecular initiating event** (MIE) in an AOP.

Weaknesses:

- **Docking was not used on the chemicals in the *in vitro* battery** to look for concordance between measured and predicted bioactivity. **Testing the method with 'knowns' is a critical step**.
- Only a small subset of Tox21 assays are truly binding assays, so combining these with docking-based activities raises questions about validity.

AI/Machine Learning/Deep Learning

AI/Machine Learning/Deep Learning

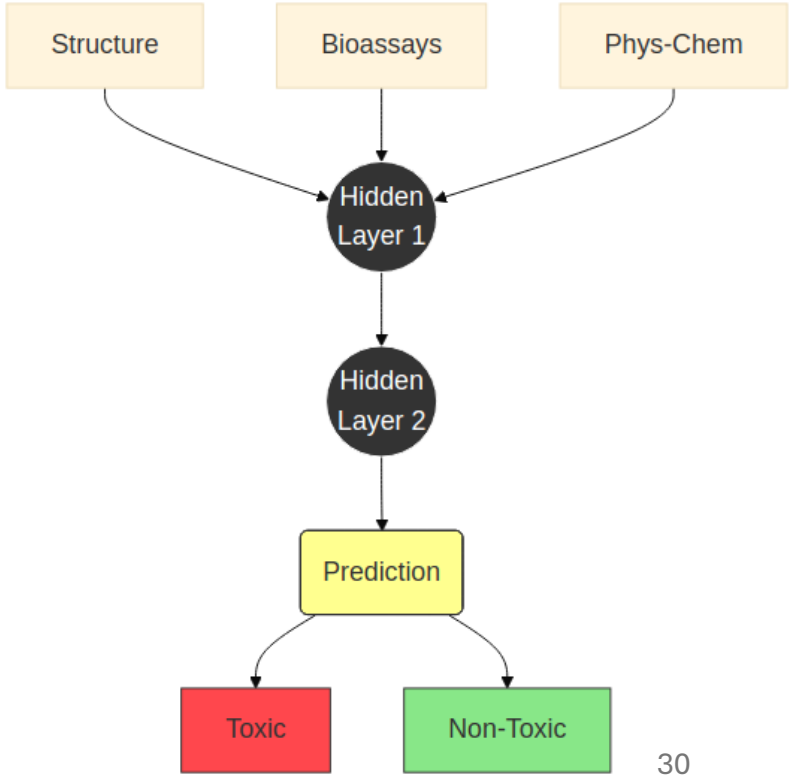
Inputs

- 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



Do not edit
How to change the design

What word or phrase comes to mind as you think about the use of AI in chemical hazard characterization and risk assessment?

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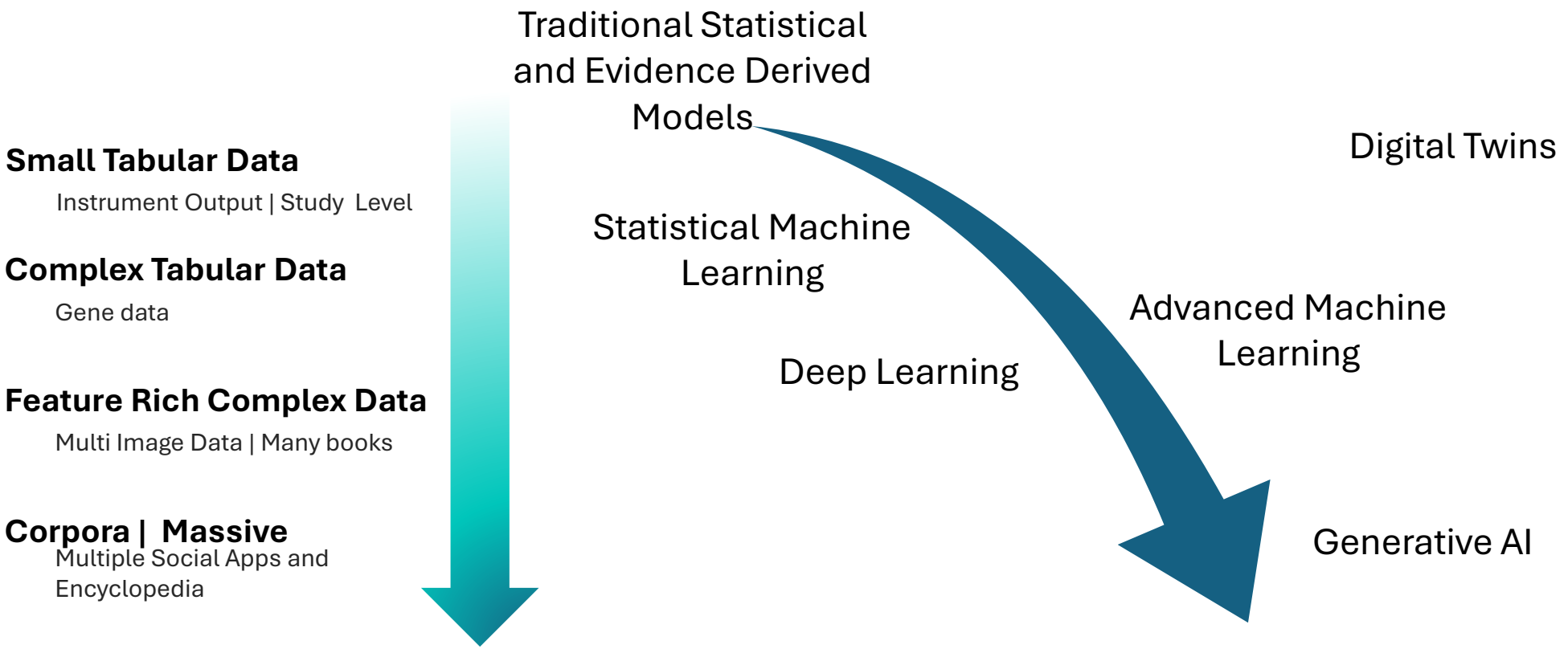
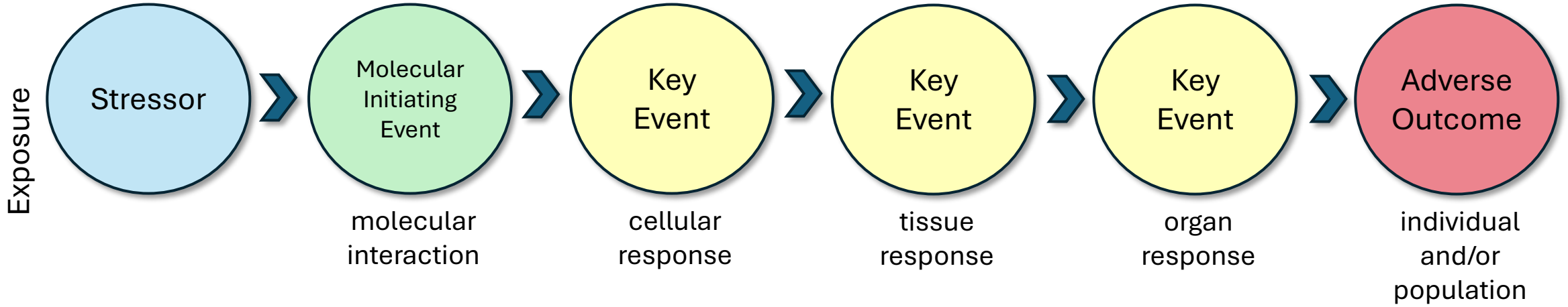
Artificial Intelligence (AI) is a broad concept with many competing definitions

Many models underlie "AI," but this term typically describes a tool that helps automate complex decision-driven action, whereas machine learning (ML) automates an information learning step like a classifier.



MIE defines the start of the chain of events



Molecular Initiating Event with Machine Learning

Machine learning approach to molecular initiating event prediction using high-throughput transcriptomic chemical screening data

Bundy et al., 2025

Journal of Chemical Information and Modeling

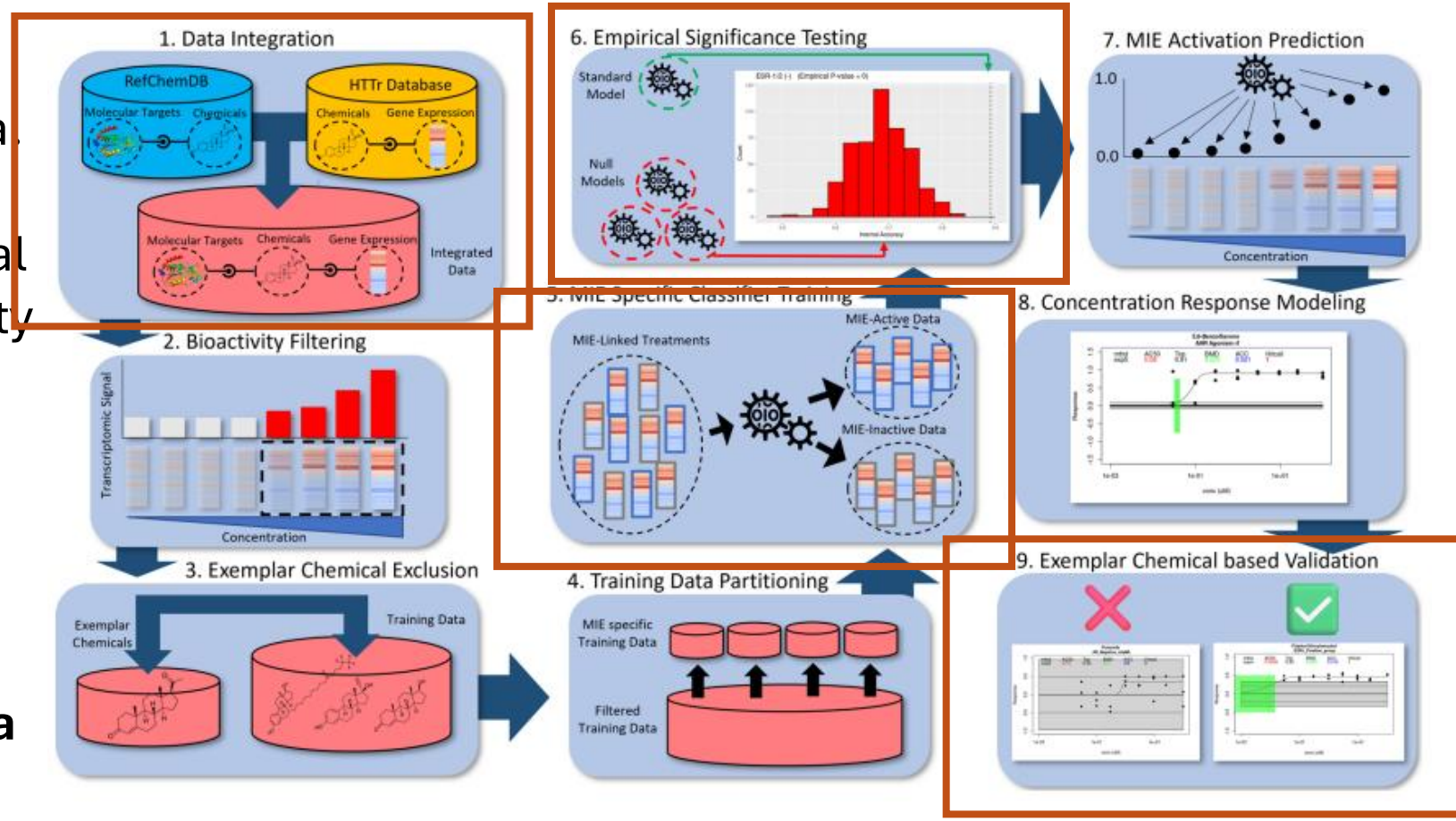
doi: 10.1021//acs.jcim.5c00699

The Problem:

- It is easy to generate massive amounts of gene expression data.
- Interpreting *cause* is difficult.
- The interaction between chemical target and MIE is central to activity annotation.
- Current methods rely on incomplete or non-specific databases.

The Approach:

- **Can we build an ML to classify a chemical's MIE?**



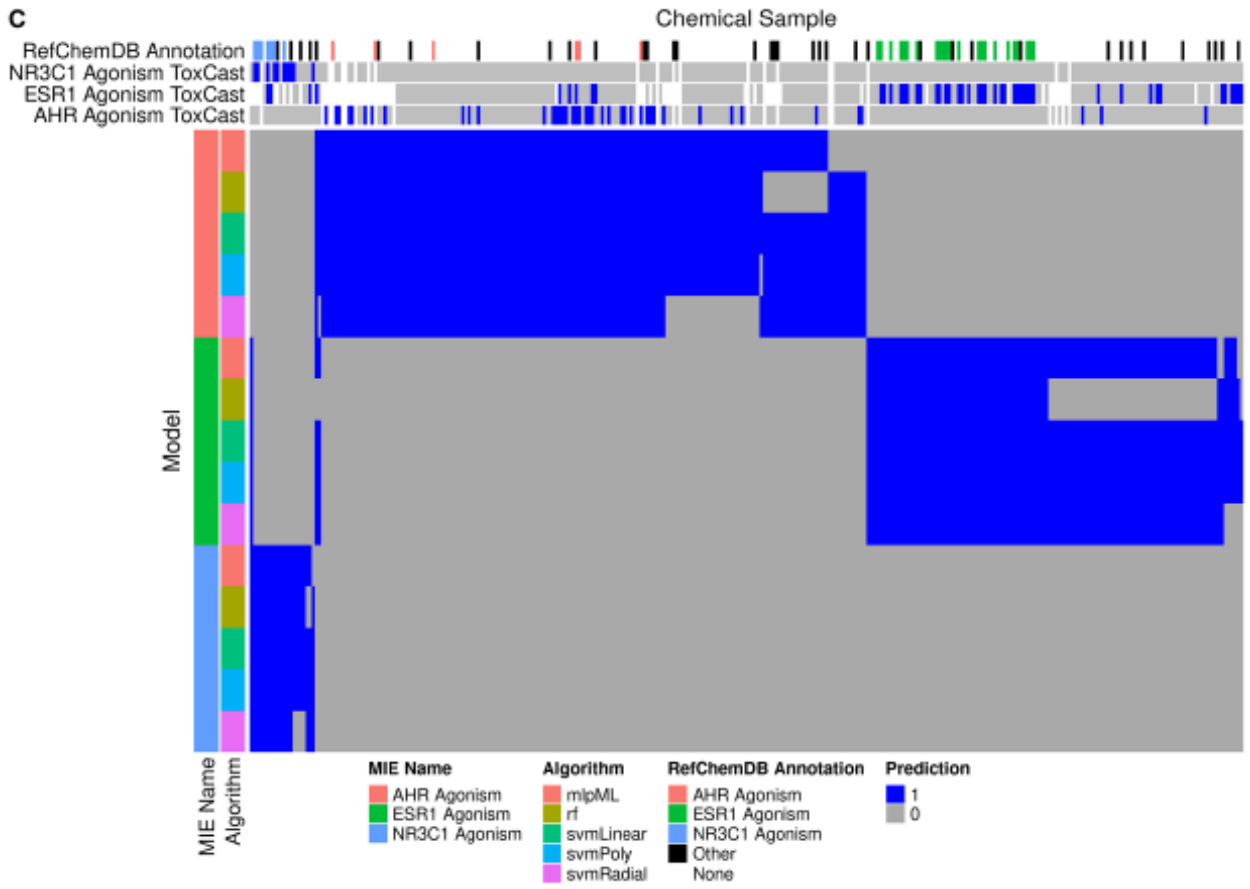
Molecular Initiating Event with Machine Learning

Key Strengths

- **Null Model Validation:** Prevents false pattern detection via rigorous testing.
- **Dose-Response Integration:** Models 8-point concentrations to ensure realistic chemical behavio
- **External Validation:** Cross-referenced against ToxCast wet-lab data for biological credibility.

Areas to Improve

- **Single-Cell Limitation:** Restricted to MCF-7 cells, missing tissue-specific targets.
- **Reference Data Requirements:** Needs five reference chemicals, limiting rare pathway analysis.



Predicting Active Concentrations

Transformers enable accurate prediction of acute and chronic chemical toxicity in aquatic organisms

Gustavsson et al., 2024

Science Advances

doi: 10.1126/sciadv.adk6669

The Problem:

- QSARs predict toxicity directly via structural relationships.
- Can suffer from low accuracy and a "narrow applicability domain"

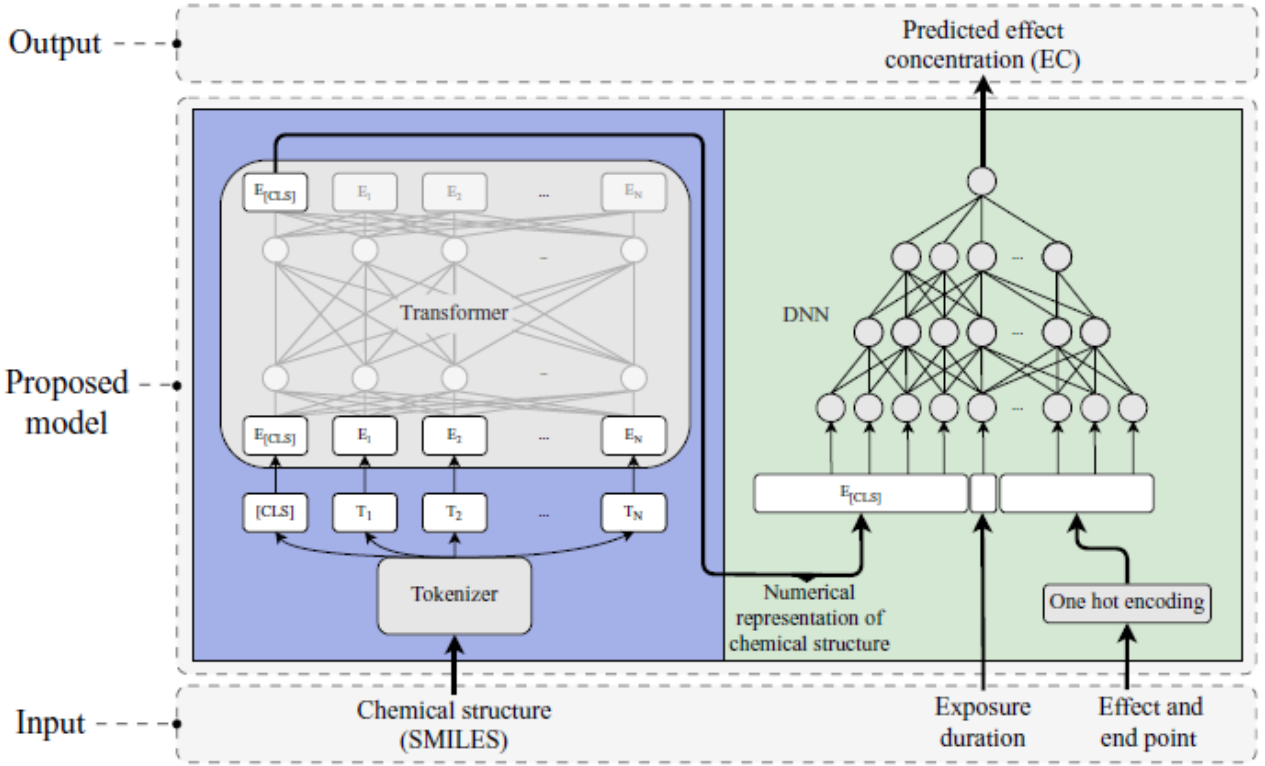
The Solution:

- Can we provide context using "ChatGPT"-like sentence

Context provides meaning:
"I saw her duck.":

I observed a woman lower her head quickly to avoid something.

I noticed a duck that belongs to her.



["I", "saw", "her", "duck", "by", "the", "river"]

[("I", "saw"), ("saw", "her"), ("her", "duck"), ("duck", "by"), ("by", "the"), ("the", "river")]

["I", "saw", "her", "duck", "by", "the", "river"]

Predicting Active Concentrations

Sources:

- REACH – European chemical regulation data
- ECOTOX – U.S. EPA database
- EFSA – European Food Safety Authority pesticide data

Training:

- The final training set consisted of **147,864 experimentally measured effect concentrations**

Organisms:

- Algae
- Aquatic invertebrates
- Fish

Endpoints:

- **EC_{50}** – Concentration causing an effect (*e.g.*, death or immobility) in 50% of the test population
- **EC_{10}** – Concentration causing an effect in 10% of the population

Table 1. Overview of the EC_{50} and EC_{10} datasets for fish, aquatic invertebrates, and algae. The datasets were used to train and validate the transformer-based model. The number of unique experimental setups is the number of unique combinations of chemicals, end points, effects, and exposure durations in each dataset. Effect abbreviations: DVP, development; GRO, growth; ITX, intoxication; MOR, mortality; MPH, morphology; POP, population; REP, reproduction.

Dataset	Organism group	End point	Effect	No. of data points	No. of unique chemicals	No. of unique experimental setups	No. of chemicals responsible for more than 50% of data	Concentration (mg/liter) mean (5–95%)*	Exposure duration (hour) mean and SEM*
Fish EC_{50}	Fish	EC_{50}	MOR	52,666	3542	8974	87	27.1 (0.005–155.0)	88 ± 0.63
Fish EC_{10}	Fish	EC_{00}–EC_{10}, NOEC	MOR, ITX, DVP, GRO, REP, MPH, POP	19,751	2321	7870	107	16.9 (0.0001–100)	621 ± 10.2
Aquatic invertebrates EC_{50}	Aquatic invertebrates	EC_{50}	MOR, ITX	34,820	3741	9116	98	23.1 (0.0007–140)	78 ± 0.61
Aquatic invertebrates EC_{10}	Aquatic invertebrates	EC_{00}–EC_{10}, NOEC	MOR, ITX, DVP, REP, MPH, POP	15,372	2647	6991	118	14.1 (0.0003–100)	311 ± 3.50
Algae EC_{50}	Algae	EC_{50}	POP	13,019	2843	4487	188	25.863 (0.008–144)	91 ± 0.82
Algae EC_{10}	Algae	EC_{00}–EC_{10}, NOEC	POP	11,830	2756	4180	184	16.6 (0.003–100)	131 ± 3.48

*These values were calculated from the $\log_{10}$-transformed data used to train the models.

Sheer scale of data needed

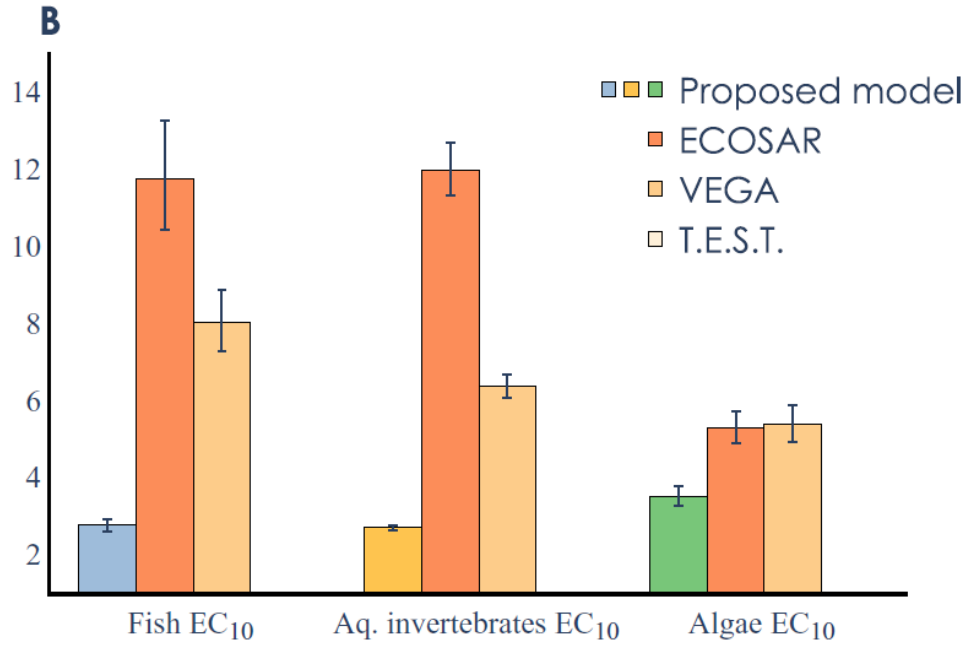
Predicting Active Concentrations

Strengths:

- Integrates EC_{50} and EC_{10} data to capture relationships between acute and chronic toxicity
- Predicts for many chemical structures, unlike tools that reject out-of-domain chemicals
- Learns toxic features automatically via context models, reducing reliance on fragments

Weaknesses:

- Black Box provides numeric predictions without mechanistic explanations
- Data Hungry; not suitable for rare endpoints



Questions?

Visit https://www.thepsci.eu/in-silico-tools-webinars/ for webinar materials

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

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