# Machine Learning Models to Address Cardiotoxicity Within an AOP Framework

## *An academic perspective*

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# Outline

- Paradigm shift in toxicology & the role of in silico approaches
- Drug safety
  - Cardiotoxicty and ALTERNATIVE approach
  - Modeling exercises
- Conclusions

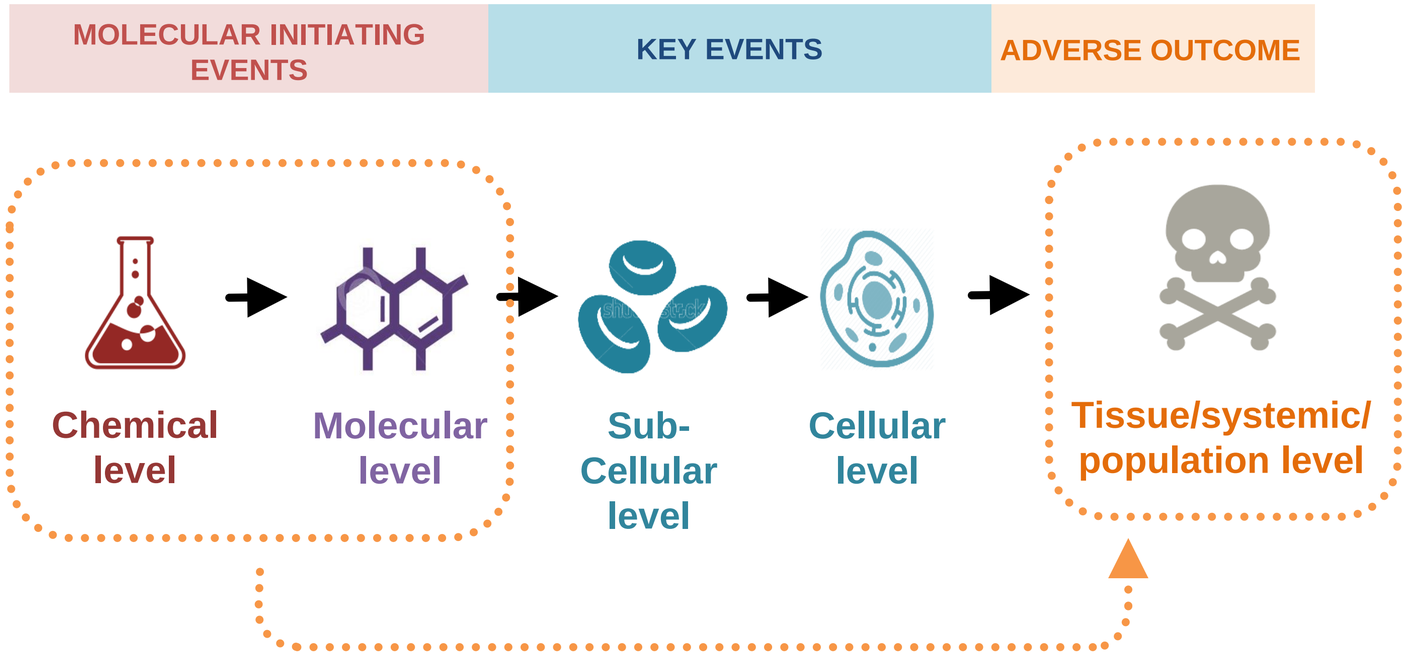
# Paradigm shift in toxicology in 21st century

Efforts to move from traditional animal-based testing to an increased use of new approaches

NAMs
"new approach methodologies" → any technology, methodology, approach, or combination that can be used to provide information on chemical hazard and risk assessment

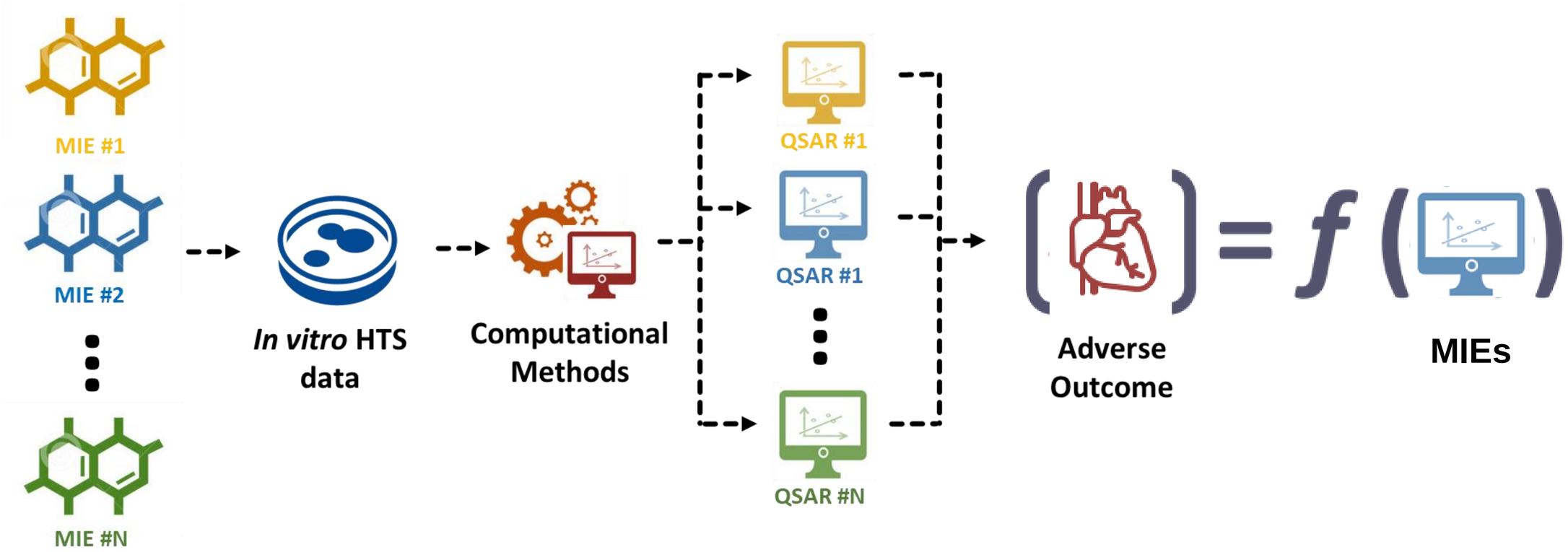
- Increasing understanding of key toxicity pathways and molecular mechanisms leading to the toxic effects

# Adverse outcome pathways




*AOPs link molecular initiating events (MIEs) to an adverse outcome (AO) through some key events (KEs)*

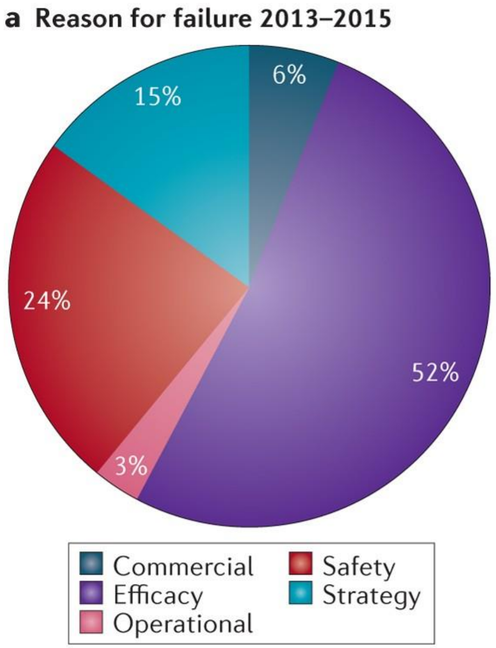
# Modelling MIEs with QSAR

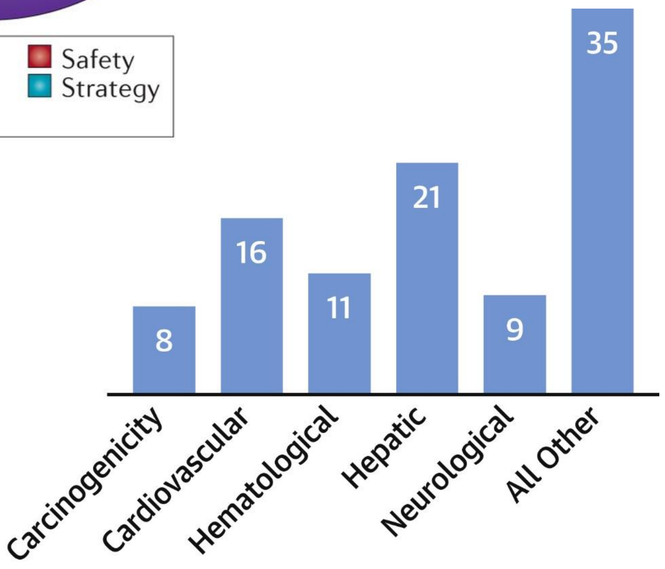



Identifying chemical stressors for AOPs through predicted activities

# Drug safety

- Drug safety accounts approx. for 25% of drug failure
- Multiple reasons for late discovery of drug toxicity
  - Off-target effects
  - ADME properties
  - Translational relevance to humans of preclinical safety studies in animals
  - Statistical analysis (sample size)
  - Standardized conditions vs. real life situation (genetic, age, comorbidity)



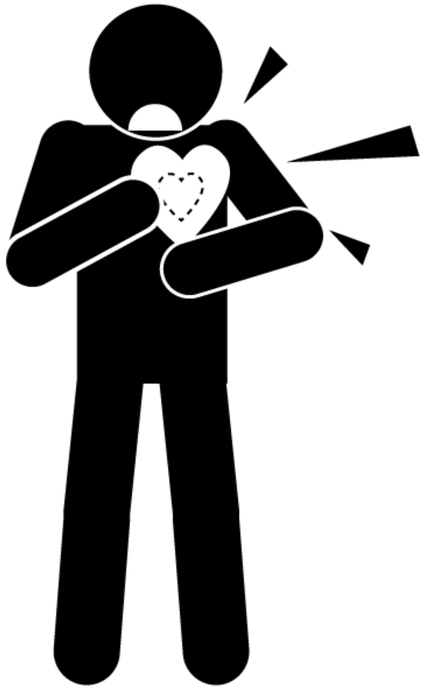




Harrison R. *Nat Rev Drug Discov* **15**, 2016
Van Norman GA, *JACC Basic* **4**, 2019

# Drug safety

- Room for the use of computational methods to anticipate drug toxicity
  - More limited experimental confirmation
  - Statistical assessment of models differs from hit or lead searching (costs of errors)
  - Effective to guide decision making

# Cardiotoxicity of chemical mixtures



**17.9 million**

Among non-communicable diseases (NCDs), cardiovascular diseases (CVDs) are the prominent cause of mortality each year

**85%**

due to heart attack and stroke

**Lifestyle**

**+**

**Environmental factors**



**Effects may be extremely marked in mature and aged population.**

ALTERNATIVE

# Regulatory guidelines and gaps for cardiotoxicity

## Pharmaceuticals

ICH S7A
ICHS7B
ICH M4 (R2)

### Current regulatory guidelines

***In vivo* testing**

Evaluation of heart rate, ECG, blood pressure, QT prolongation

***In vitro* testing**

hERG/$I_{KR}$ assay

### Knowledge gaps

Interspecies differences

*In vitro*: Missing assessment of structure & contractility

Limited predictivity of current methods

## Chemicals, Pesticides, Biocides

### Current regulatory guidelines

***In vivo* testing**

Evaluation of cardiac organ weight, pathology, histopathology, cardiac malformation

OECD Test Guidelines (TGs)
407, 408, 409, 453
412 & 413
426 & 443
414

### Knowledge gaps

Interspecies differences

MoA identification difficult

Large number of chemicals

**Mixtures & susceptible population: not sufficiently covered**

*ECG = Electrocardiogram (records electrical signal from heart to check for different heart conditions)*

*hERG/$I_{KR}$ assay = measures changes in activity of potassium channel, detects delayed ventricular repolarization (risk factor for arrythmias)*

*Schaffert and Murugadoss et al. 2023 (ALTEX)*

# Regulatory challenges

## Cardiotoxicity

- Not addressed as **separate** endpoint in case of chemicals, pesticides, and biocides
- **Limited regulatory experience** with cardiotoxicity of chemicals
- **Implications on limit values & classification** are not well known

## Mixtures

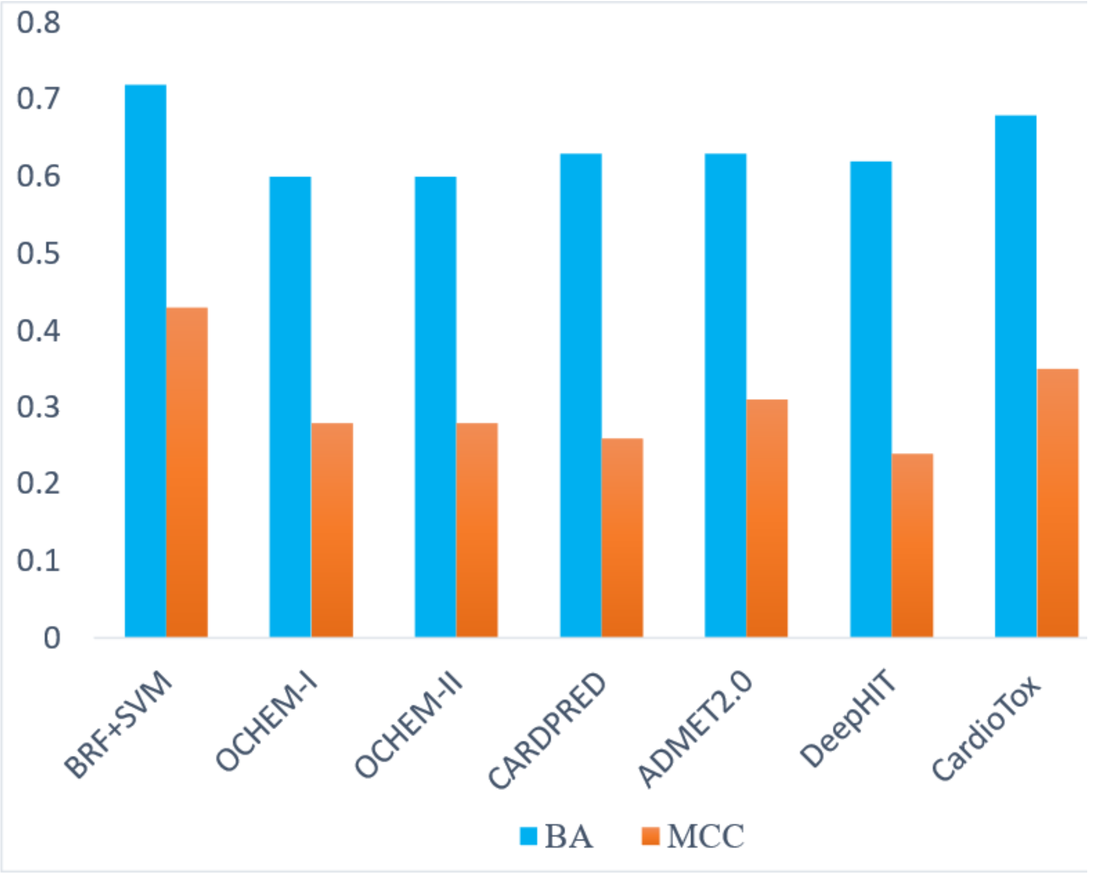
- **Too few mode of action data** for individual chemicals available to calculate potential mixture activity
- **Too large number of chemical mixtures** to test via animal tests expensive, time consuming, unethical

# Modeling for cardiotoxicity

- Initial focus on hERG inhibition (data rich)
- Characterize the chemical space covered by QSAR models for
  - prediction ability for drug and non-drug compounds (e.g.: pesticides, PAHs, etc.)
  - Profiling compounds according to most represented mechanisms of action for cardiotoxicity
- Modeling other mechanisms

# hERG QSAR models

- 7963 bioactivity data reported in ChEMBL.
- New QSAR models based on different thresholds (pIC50 = 6 or pIC50 = 5) with six machine learning algorithms (RF, KNN, GB, XGB, MLP, and SVM)
- Comparison with other tools




Delre P., Front Pharmacol 2022 Sep 5;13:951083

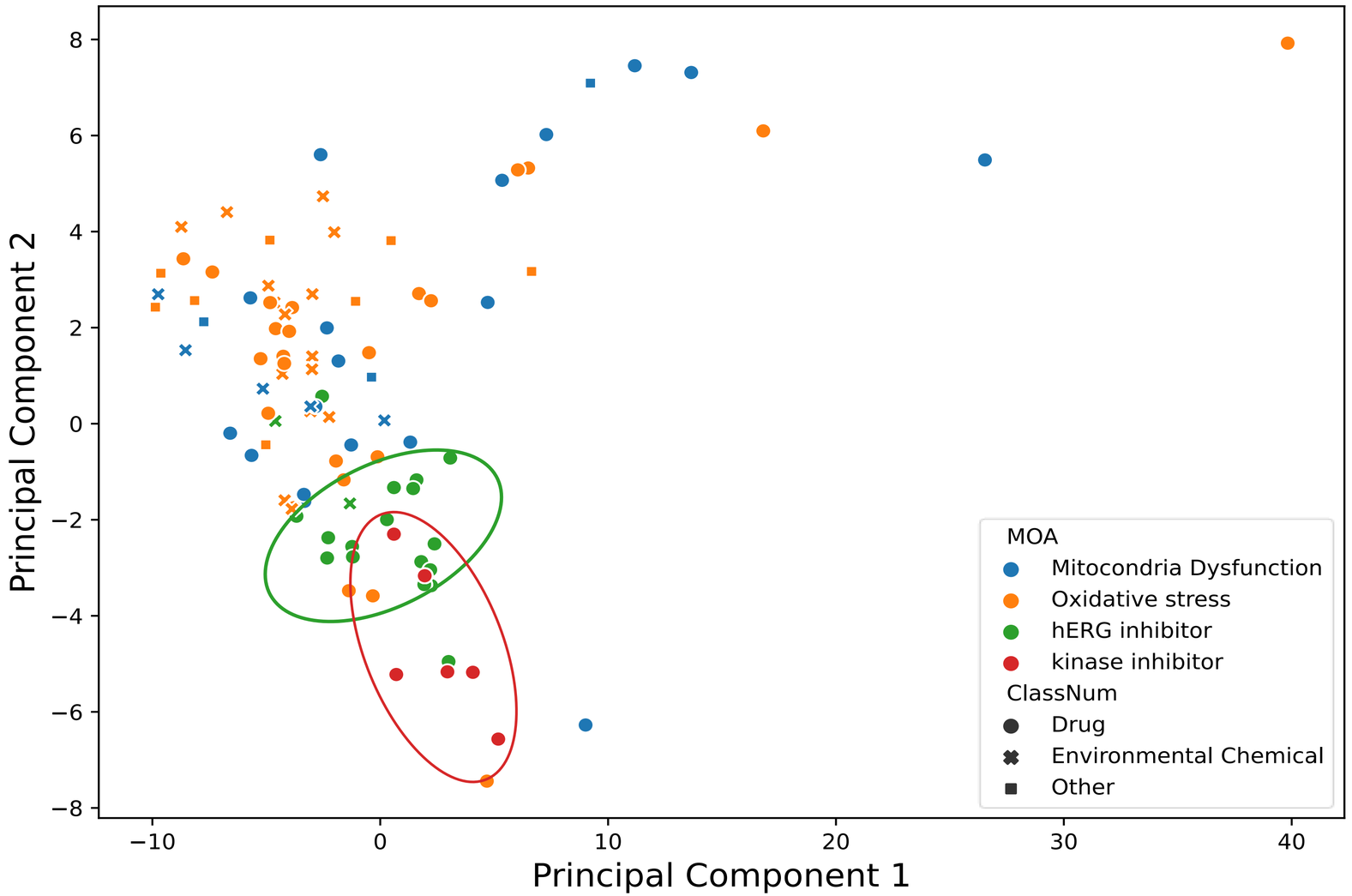
# Evaluation set

Literature review of cardiotoxic effects of chemicals (in vitro, in vivo, in humans)

- 280 compounds belonging to different classes (environmental chemicals, drugs or other)
- 220 of these compounds labelled with one or multiple modes of action

Shagun K et al. Chem Res Toxicol 34, 566, 2021.

# Evaluation set




**IRFMN hERG Models (th6)**

| Class | In AD | Out AD |
|---|---|---|
| All | 123 | 61 |
| Environmental | 31 | 36 |
| Drug | 79 | 10 |

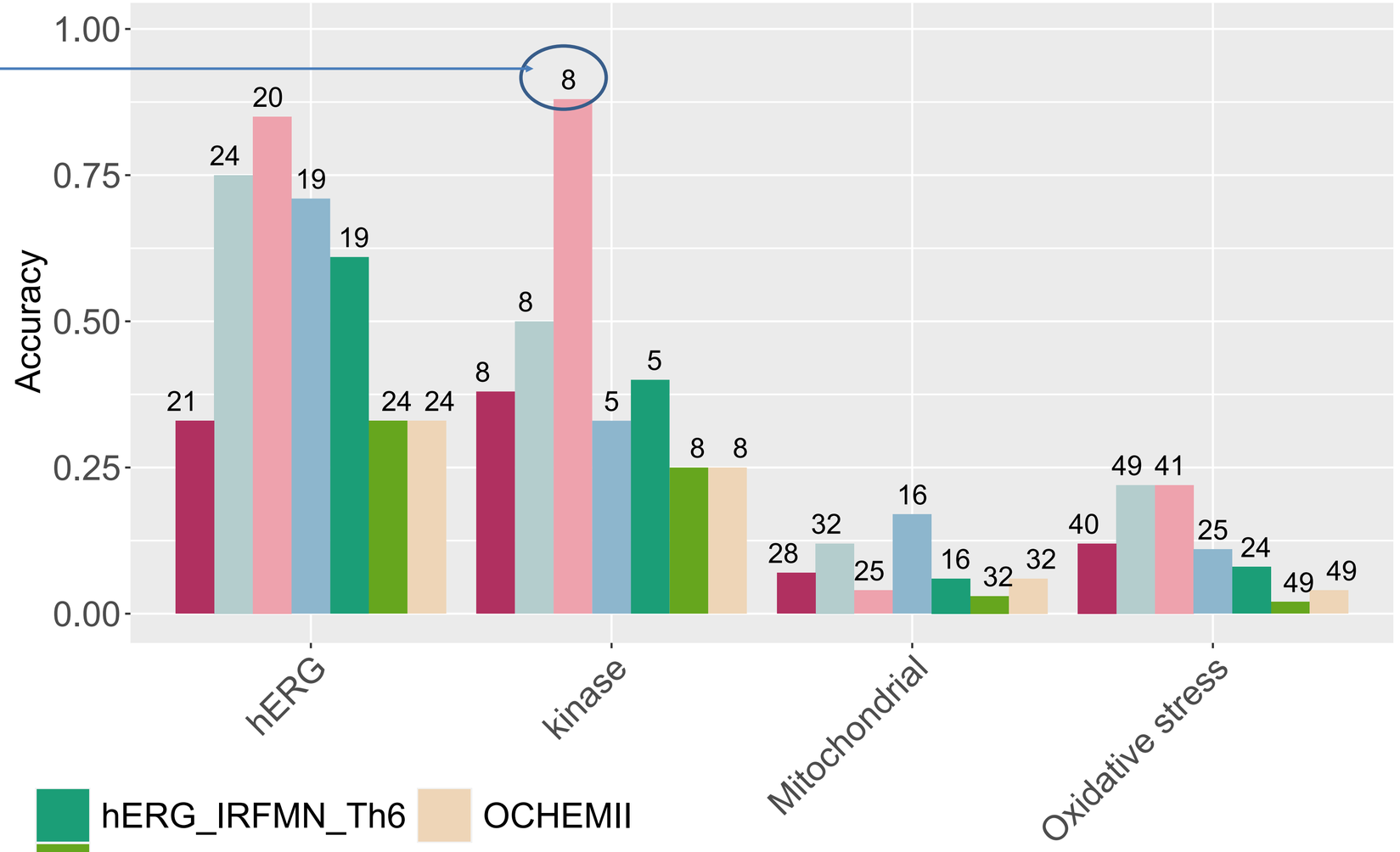
Score plot: hERG and kinase inhibitors compounds have similar chemical features

# Performance based on MoAs

4/8 kinase inhibitors with mixed MoAs (hERG inhibitors)

**IRFMN hERG Models (th6)**

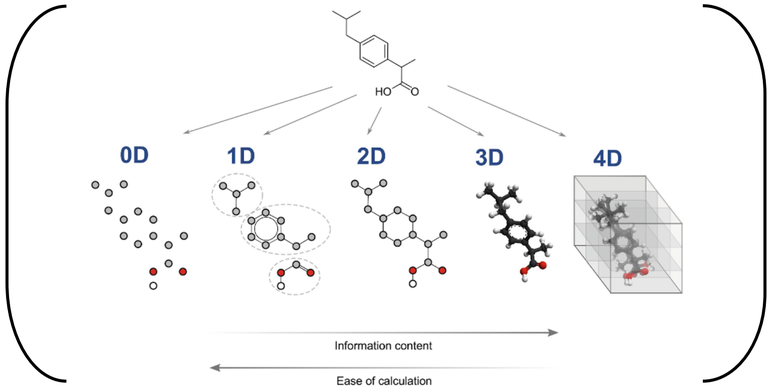
| Class | In AD | Out AD |
|---|---|---|
| Oxidative Stress | 17 | 8 |
| Mitochondrial Dysfunction | 8 | 8 |
| hERG inhibitor | 19 | 0 |
| Kinase inhibitor | 5 | 0 |



# Exploiting the AOP framework in silico

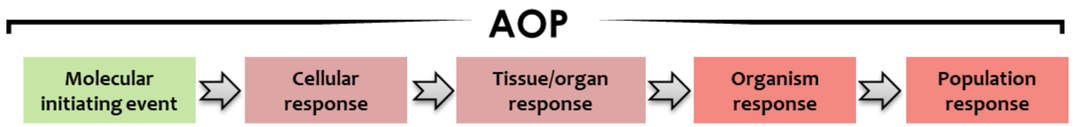
## *Quantitative Structure-Activity Relationship (QSAR)*

***Biological Activity*** = ***f***

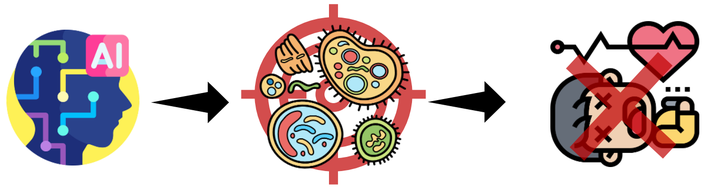



***To predict the biological activity of a compound based on its chemical structure and other related properties***

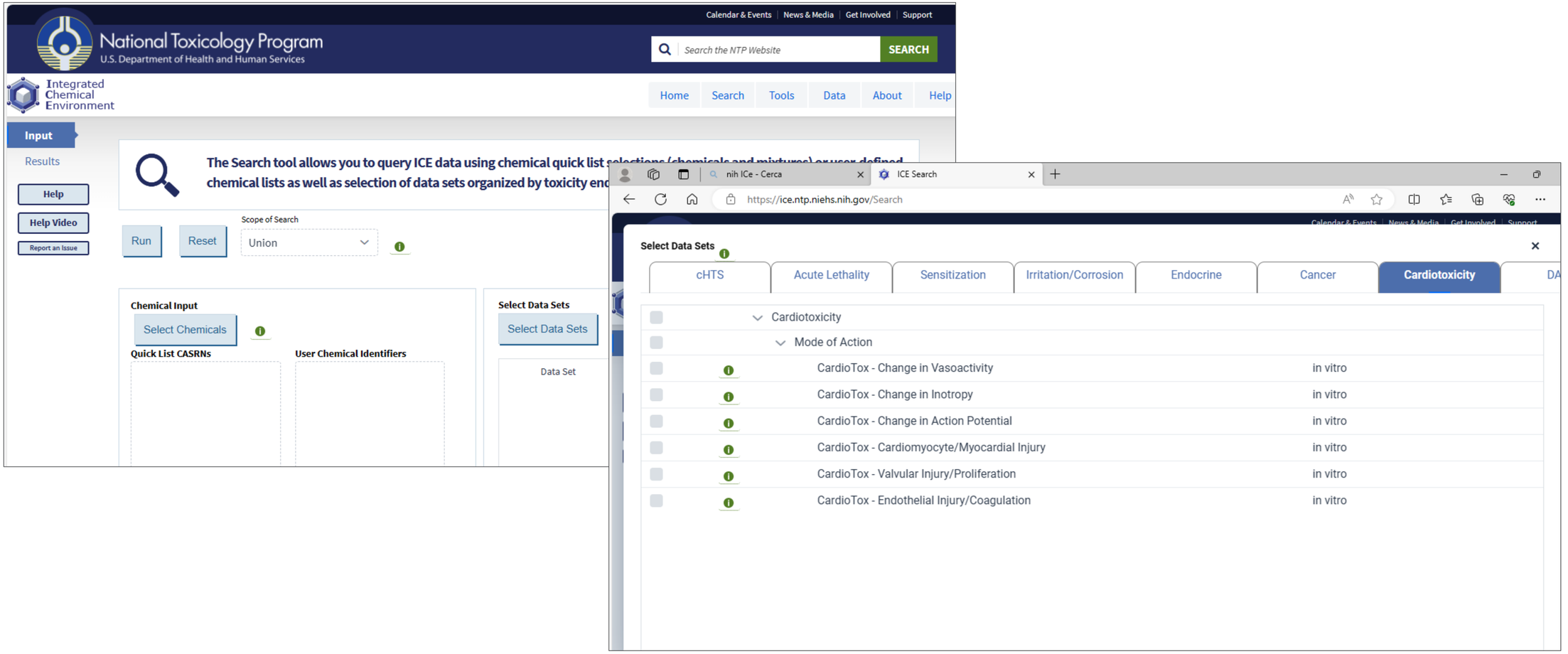
**Biological targets identified based on the AOPs for cardiotoxicity**




**Machine learning (ML) models to predict potential capability of compounds to interact with biological targets**



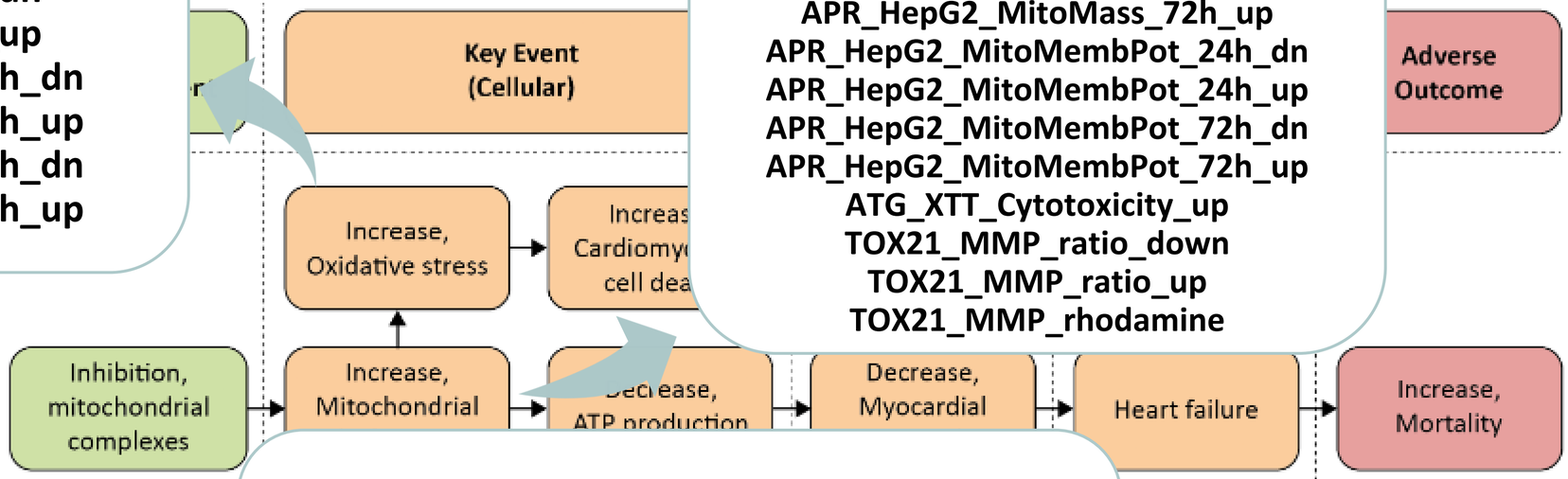
# Assays encoding AOP MIEs



# Assays encoding AOP MIEs

**Oxidative-Stress**

APR_HepG2_P-H2AX_24h_dn
APR_HepG2_P-H2AX_24h_up
APR_HepG2_P-H2AX_72h_dn
APR_HepG2_P-H2AX_72h_up
APR_HepG2_StressKinase_24h_dn
APR_HepG2_StressKinase_24h_up
APR_HepG2_StressKinase_72h_dn
APR_HepG2_StressKinase_72h_up



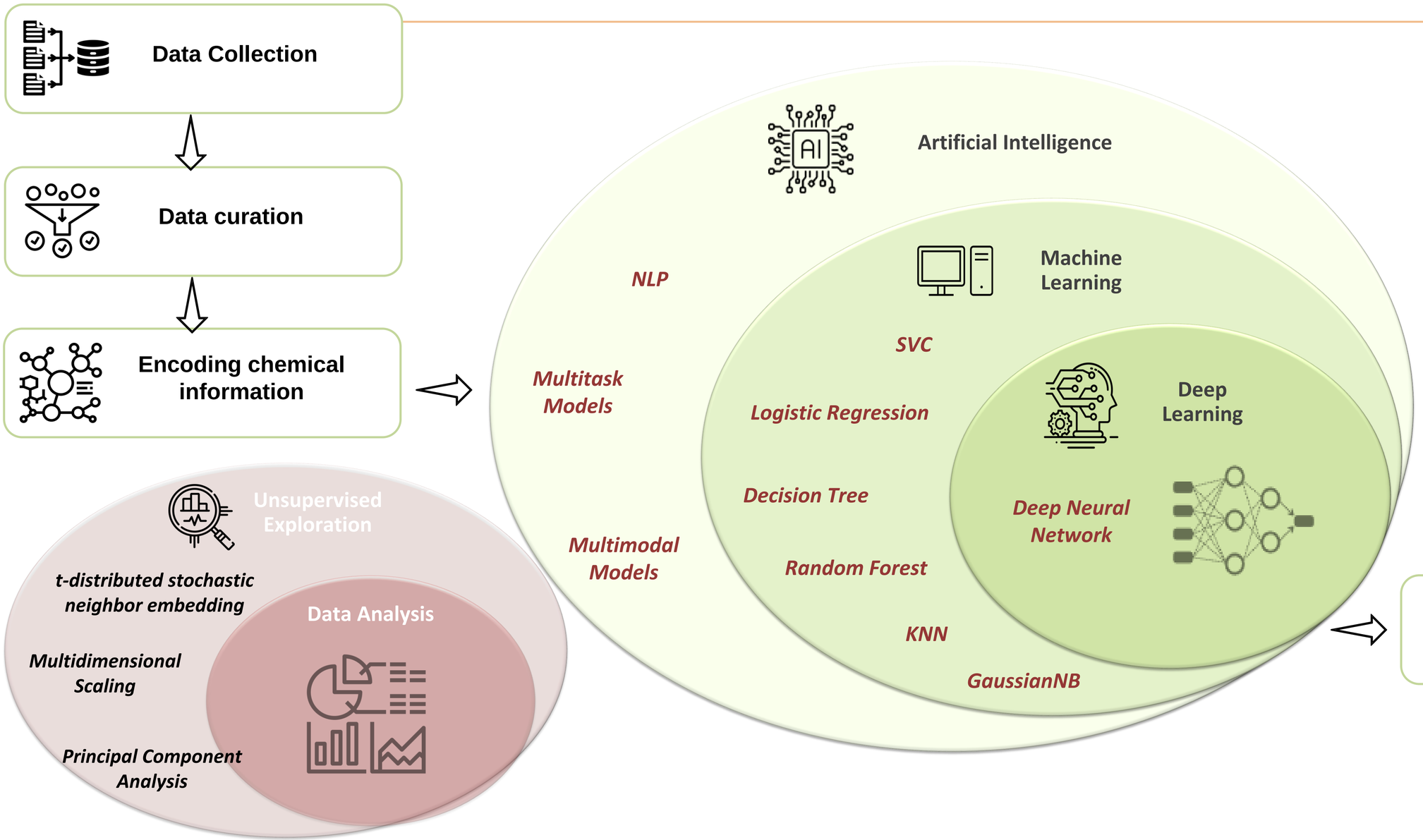

**Mitochondrial-Dysfunction**

APR_HepG2_MitoMass_24h_dn
APR_HepG2_MitoMass_24h_up
APR_HepG2_MitoMass_72h_dn
APR_HepG2_MitoMass_72h_up
APR_HepG2_MitoMembPot_24h_dn
APR_HepG2_MitoMembPot_24h_up
APR_HepG2_MitoMembPot_72h_dn
APR_HepG2_MitoMembPot_72h_up
ATG_XTT_Cytotoxicity_up
TOX21_MMP_ratio_down
TOX21_MMP_ratio_up
TOX21_MMP_rhodamine

**Inhibition mitochondrial complexes**

CCTE_Simmons_MITO_basal_resp_rate_OCR_dn
CCTE_Simmons_MITO_basal_resp_rate_OCR_up
CCTE_Simmons_MITO_inhib_resp_rate_OCR_dn
CCTE_Simmons_MITO_inhib_resp_rate_OCR_up
CCTE_Simmons_MITO_max_resp_rate_OCR_dn
CCTE_Simmons_MITO_max_resp_rate_OCR_up

# Modeling pipeline



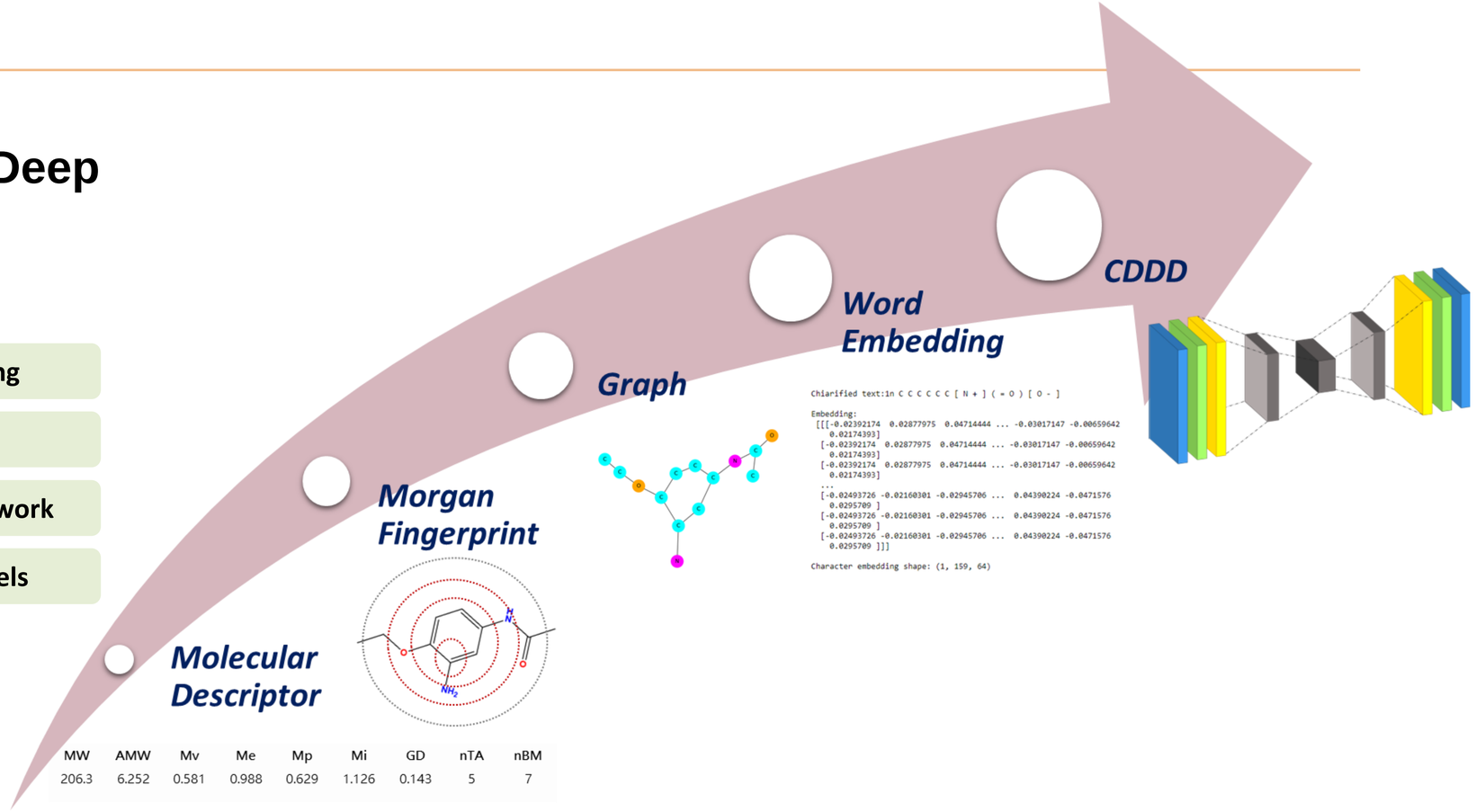
# Encoding chemical information

## Baseline Machine Learning Models

- SVC
- Logistic Regression
- Decision Tree
- Random Forest
- KNN
- GaussianNB

## Artificial Intelligence: Deep Learning

- Text Embedding
- GCNN
- Deep Neural Network
- Multitask Models




**We aimed to assess the best combination of model-descriptors for each endpoint by systematically evaluating all possible combinations of model types, descriptors, and biological targets.**

# Results of Data Collection and Curation process

## 1) Quality control:

**Only QC passed data was maintained in our dataset.**

## 2) Activity definition:

**A chemical is classified as active if it yields a positive result in at least one assay and as negative if all available results are negative.**

## 3) SMILES curation:

**Standard SMILES canonization, removed salts, concentrating on the largest fragments, excluded stereochemistry and removed the resulted duplicate structures.**

| AOP | Name | Number of compounds | Active | Inactive | Active% | Inactive% | number of assays used |
|---|---|---|---|---|---|---|---|
| MIE | Inhibition Mitochondrial complexes | 232 | 184 | 48 | **79** | 21 | 7 |
| KE1 | Increase Oxidative Stress | 636 | 191 | 445 | **30** | 70 | 7 |
| KE2 | Mitochondrial Dysfunctions | 5004 | 1147 | 3857 | **23** | 77 | 12 |

# Modeling Results (ML)

| | external test set | | 5-fold cross validation | | | | |
|---|---|---|---|---|---|---|---|
| | **Balance Accuracy** | **F1-Score** | **Balanced Accuracy** | **Model selected** | **OverSampling** | **Encoders** | **SPLIT%** |
| **Inhibition of mitochondrial complexes** | 0.721 | 0.865 | 0.833 | k-Nearest Neighbors | SMOTE | Mordred Molecular Descriptors | 90-10 |
| **Increase in Oxidative stress** | 0.720 | 0.605 | 0.748 | Logistic regression | SVM-SMOTE | Mordred Molecular Descriptors | 90-10 |
| **Increased Mitochondrial dysfunction** | 0.742 | 0.602 | 0.921 | Extreme Gradient Boosting | SMOTE | Latent Description CDDD | 90-10 |

https://doi.org/10.3390/toxics12010087

# Artificial Intelligence: Deep Learning

## Increased Mitochondrial dysfunction

| | External Test Set | | | | | | (cv) validation set | |
|---|---|---|---|---|---|---|---|---|
| | **Balanced Accuracy** | **Precision** | **Sensitivity** | **Specificity** | **MCC** | **F1-Score** | **Balance Accuracy** | **Encoder** |
| **Deep Neural Network** | 0.746 | 0.527 | 0.672 | 0.819 | 0.454 | 0.591 | 0.870 | Circular Fingerprint |
| **Deep Neural Network** | 0.700 | 0.446 | 0.638 | 0.762 | 0.358 | 0.525 | 0.737 | MACCs fingerprint |
| **Deep Neural Network** | 0.808 | 0.539 | 0.828 | 0.788 | 0.542 | 0.653 | 0.836 | Latent representation CDDD |
| **Deep Neural Network** | 0.774 | 0.471 | 0.828 | 0.720 | 0.470 | 0.600 | 0.811 | Molecular Descriptors |
| **Message Passing Neural Network** | 0.746 | 0.527 | 0.672 | 0.819 | 0.454 | 0.591 | 0.741 | Graph |
| **Neural Language Processing** | 0.780 | 0.551 | 0.741 | 0.819 | 0.510 | 0.632 | 0.753 | Text Vectorization and character embedding |
| **Neural Language Processing Augmented** | **0.815** | **0.616** | 0.776 | **0.855** | **0.585** | **0.687** | **0.886** | **Text Vectorization and character embedding** |
| **Multimodal** | 0.808 | 0.592 | **0.777** | 0.839 | 0.564 | 0.672 | 0.830 | All (no graph) |
| **Extreme Gradient Boosting (best ML model)** | 0.742 | 0.605 | 0.600 | 0.883 | 0.485 | 0.602 | 0.921 | Latent Description CDDD |

# Model performance comparison

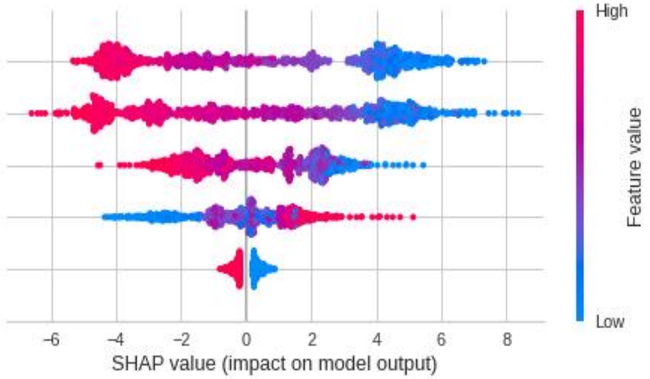
1. Gradually increasing the size of the training set, to gain insights into the **model behavior**.
2. The behavior of the multimodal approach aligns with the ideal scenario, **consistently improving its performance as the training set gets bigger.**

# A mechanistic interpretation: Model explainability

**We want to explore the reason why the models make decisions and perform assessments**

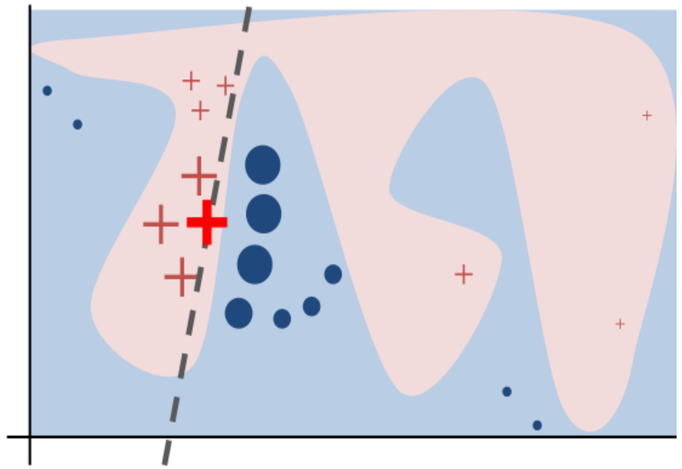
***Game Theory*** ⟶ **SHAP**

SHAP values interpret the impact of having a certain value for a given feature in comparison to the prediction we'd make if that feature took some baseline value.



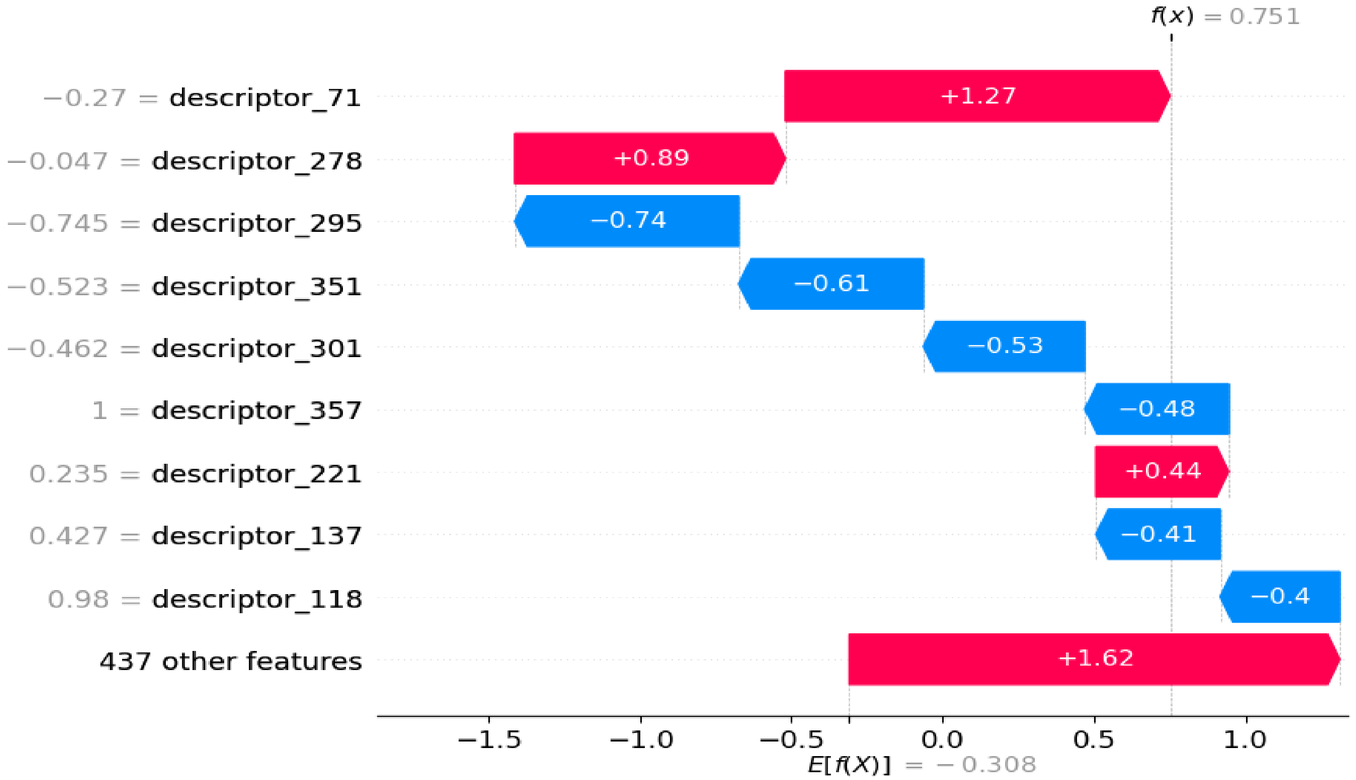

***Perturbative approach*** ⟶ **LIME**

While the model may be very complex globally, it is easier to approximate it around the vicinity of a particular instance. While treating the model as a black box, we perturb the instance we want to explain and learn a sparse linear model around it, as an explanation



# Descriptor’s importance

We explored the importance of descriptors for the assessment provided by ML models.
Example of results reported for increased mitochondrial dysfunction



# Take home messages

# Conclusions & perspectives

- ML and AI models to assess the potential cardiotoxic effects of chemicals belonging to different classes such as pesticides, drugs, and industrial compounds following the AOP developed specifically for cardiotoxicity
- They can serve as a first-tier component in the Integrated Approaches to Testing and Assessment (IATA) for cardiotoxicity
- Providing elements to inform decision makers (limitations and uncertainties, interpretability/explainability)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