Methods for Estimating Relative Potency Values

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- The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.
- We have no conflicts of interest to disclose.

EPA United States Environmental Protection Agency

Public Science Meeting on PCB Mixture Assessment Methods

- **Introduction to EPA's human health risk assessment practices for chemical mixtures**
  - *Glenn Rice, U.S. EPA*
- **Mixtures modeling: methods considered for the assessment of PCBs**
  - *Jeff Gift and Laura Carlson, U.S. EPA*
- **Methods for estimating relative potency values**
  - *Grace Patlewicz, U.S. EPA*
- **Overview of the Mixture Similarity Tool (MiST)**
  - *Graham Glen and Joanne Trgovcich, ICF*

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Talk Outline

- What does Computational Toxicology encompass?
- What are approaches that can be used to fill data gaps?
- How does this help us estimate relative potency values?
- Case example using PCB Neurotoxicity Data

Computational (*in silico*) Toxicology

- Existing information on the chemical of interest
- Predictions from (Q)SAR
- Thresholds for Toxicological Concern (TTC)
- Information from “similar” chemicals – grouping/read-across
- *In chemico* tests
- *In vitro* tests
- Molecular biology, -omics
- Exposure, (bio-)kinetics

Resources for Computational (*in silico*) Toxicology

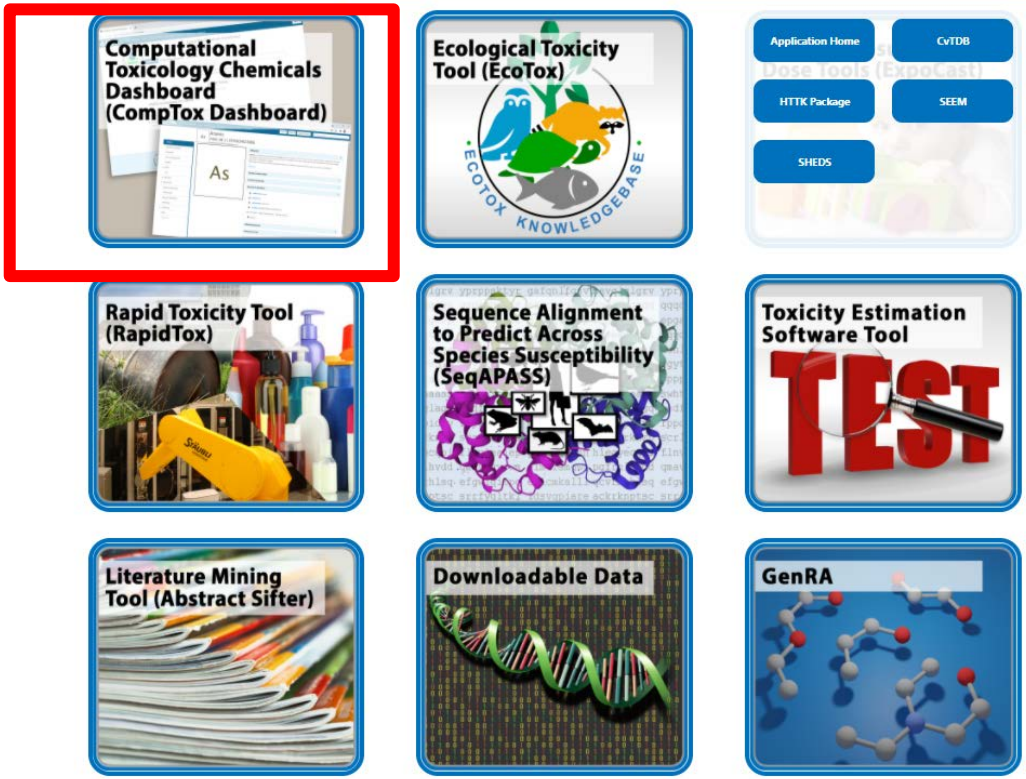
- The EPA CompTox Chemicals Dashboard is just one of many existing public resources that can be used to conveniently access information from traditional and novel technologies for a large number of substances.
  - Existing information on the chemical of interest
  - Predictions from (Q)SAR
  - Thresholds for Toxicological Concern (TTC)
  - Information from “similar” chemicals – grouping/read-across
  - *In chemico* tests
  - *In vitro* tests
  - Molecular biology, -omics
  - Exposure, (bio-)kinetics

EPA CompTox Chemicals Dashboard

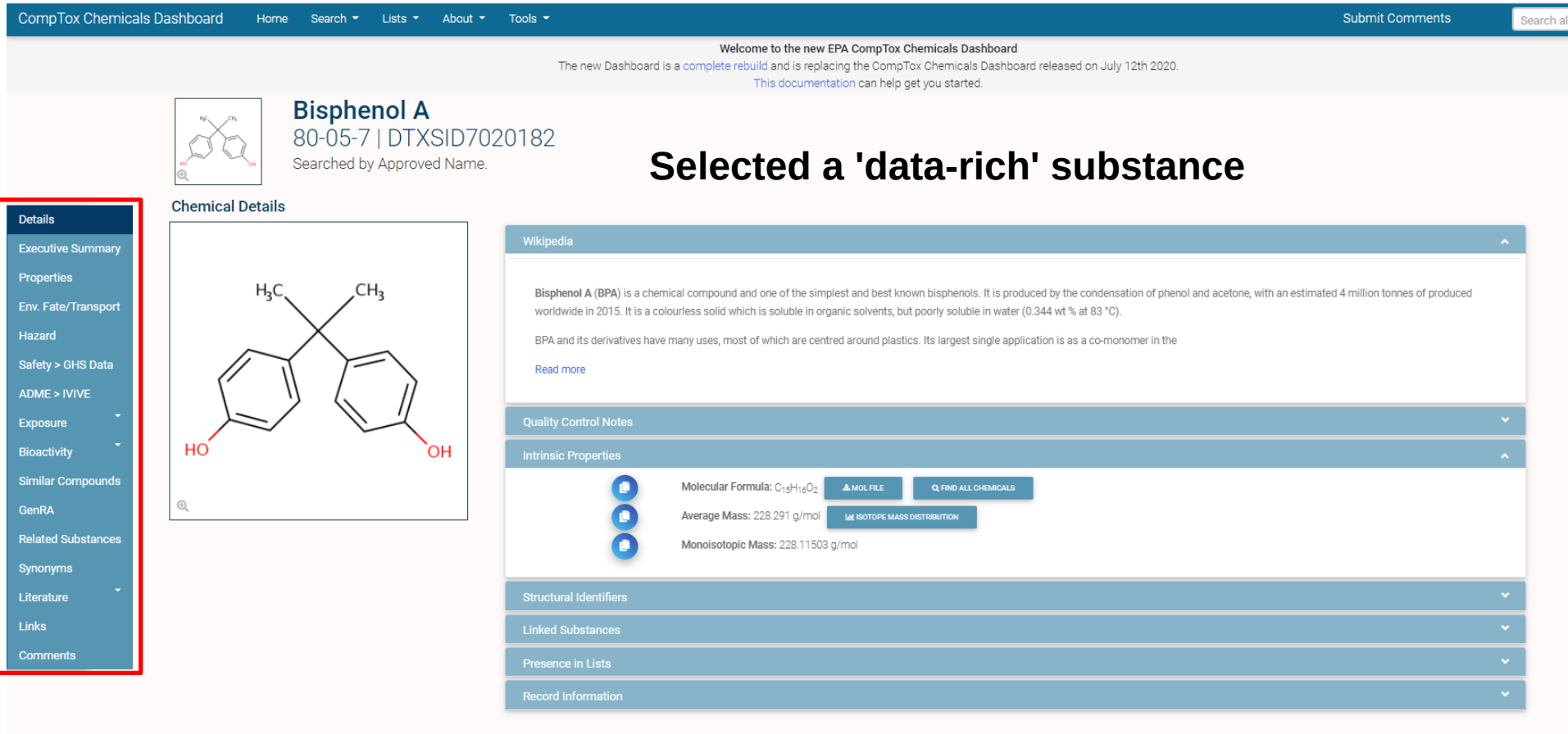
- A publicly accessible website delivering access to:
  - ~900,000 chemicals with related property data
  - Experimental and predicted physicochemical property data
  - Integration to “biological assay data” for 1000s of chemicals
  - Information regarding consumer products containing chemicals
  - Links to other agency websites and public data resources
  - “Literature” searches for chemicals using public resources
  - “Batch searching” for thousands of chemicals
  - DOWNLOADABLE Open Data for reuse and repurposing

https://comptox.epa.gov/dashboard/

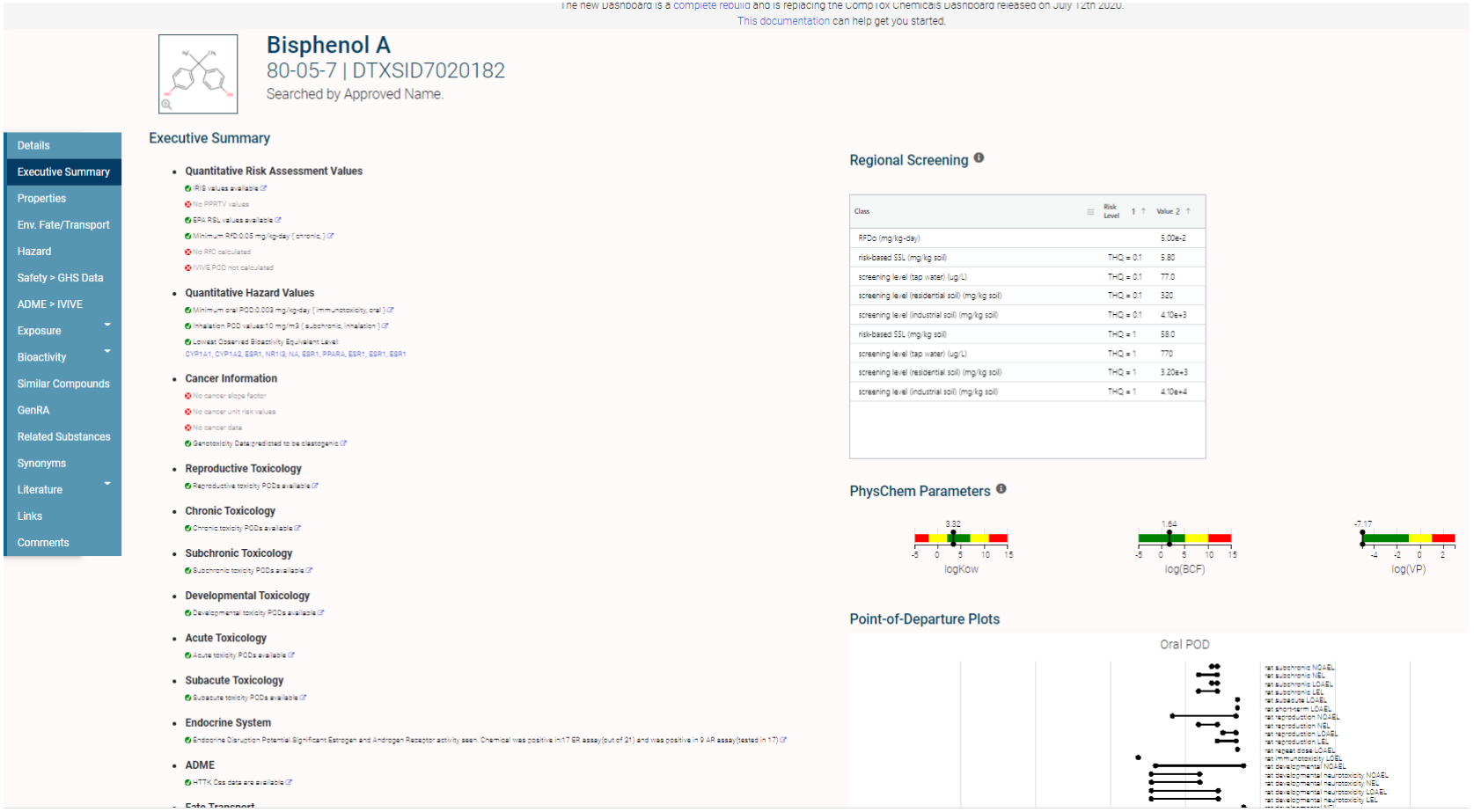
The EPA CompTox Portal
https://comptox.epa.gov/



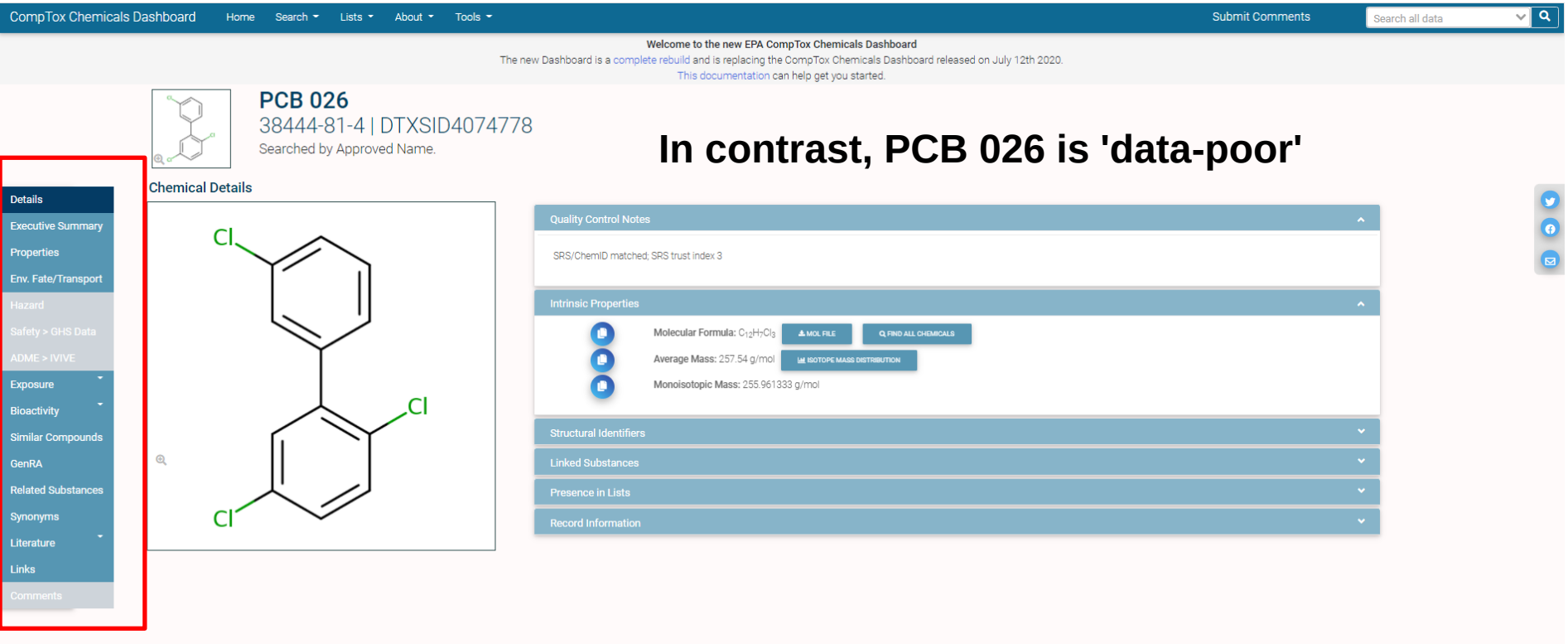
CompTox Chemicals Dashboard:
Landing Page for a specific chemical



CompTox Chemicals Dashboard:
Executive Summary of 'existing' data



CompTox Chemicals Dashboard:
Landing Page for a specific chemical



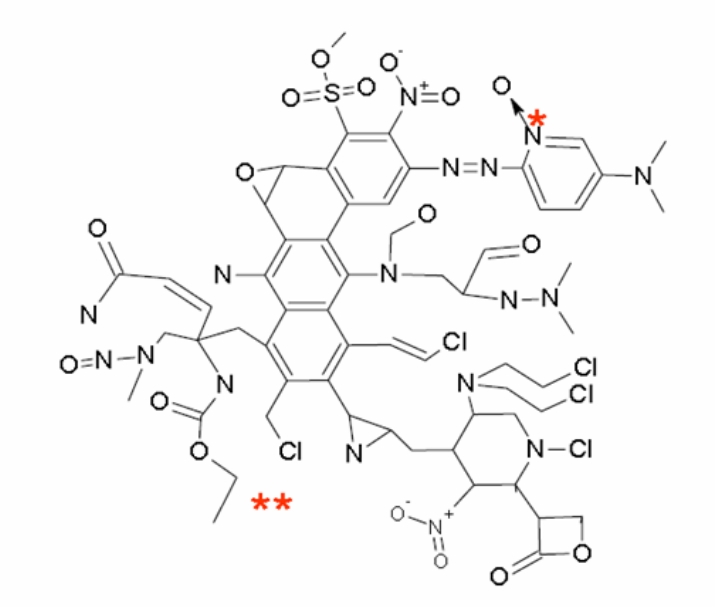
CompTox Chemicals Dashboard:
Executive Summary of 'existing' data

No existing (traditional) information….what are alternative sources of information that can be used to address data gaps?

Structural Activity Relationships (SARs) and Structural Alerts (SAs)

- A SAR (or SA) is a (qualitative) association between a chemical substructure and the potential of a chemical containing the substructure to exhibit a certain biological effect

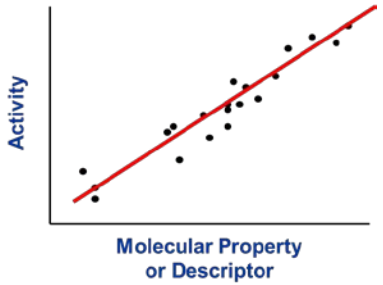
e.g. carcinogenicity alerts reflected in the Supramolecule from Ashby & Tennant (1988) Mut Res 204: 17-115

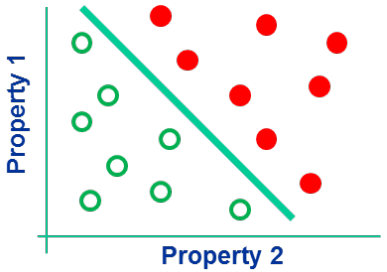


Quantitative Structure-Activity Relationships (QSARs)

- A (Q)SAR attempts to relate (statistically or otherwise) the activity of one or more molecules to their physico-chemical properties or structural descriptors
- QSAR can be used to predict:

Quantitative endpoints	Qualitative endpoints
e.g. potency	e.g. active / inactive





Collections of (Q)SARs

- An Expert System is a formalised system, usually computerised that enables an end-user to make rational predictions of toxicity based on structure alone
- Expert systems are typically categorised by whether they are underpinned by:
  - empirically based algorithms such as QSARs e.g., TEST, OPERA
  - knowledge bases such as SARs e.g., Derek Nexus, Toxtree
  - or a hybrid e.g., TIMES, ChemTunes

Regulatory Applications of (Q)SARs

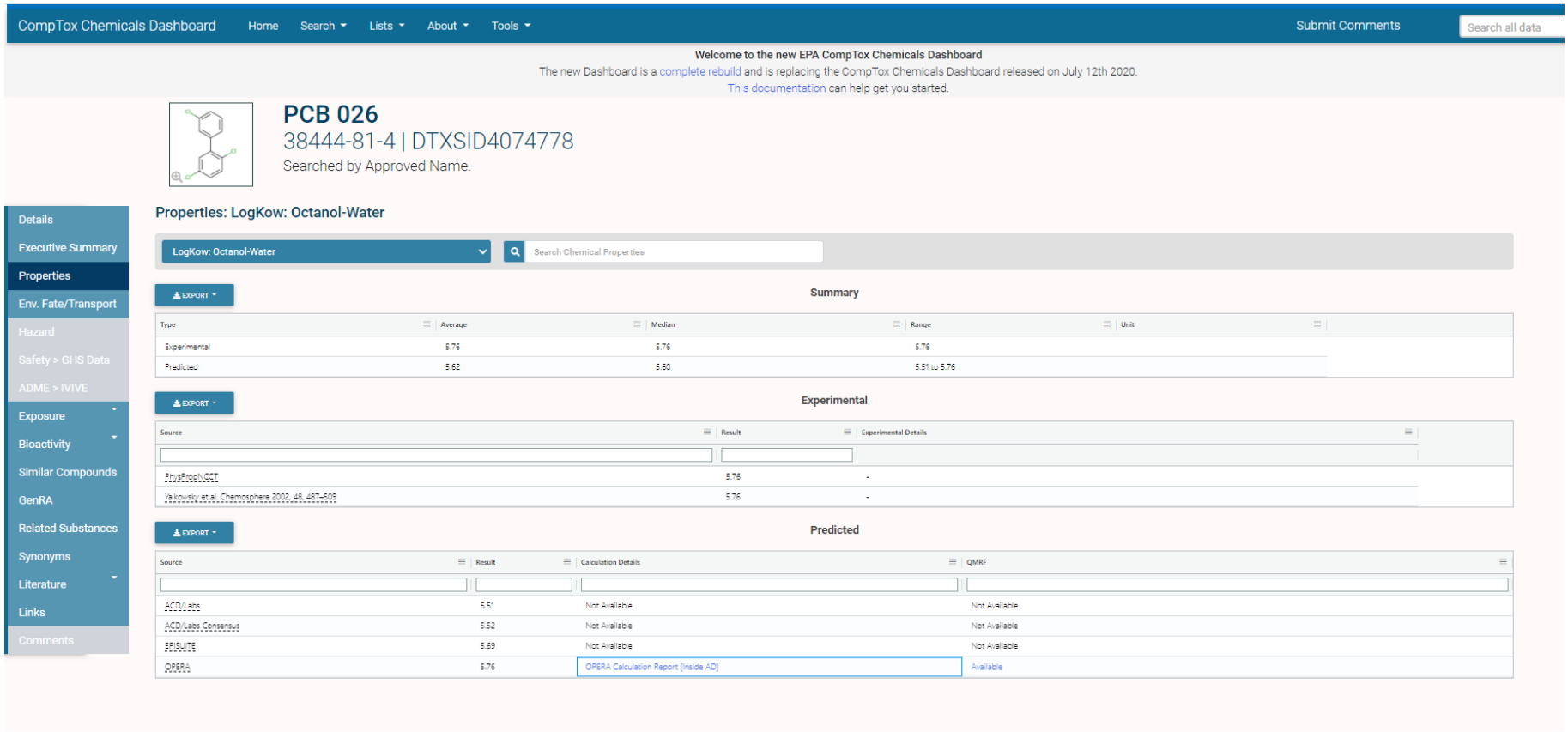
- “Packaged mature knowledge for systematic reuse”
- For data gap filling – to provide an estimate for a given (eco)toxicity/e-fate/phys chem endpoint in lieu of testing (replacement or supporting information)
- To rationalise spurious results in experimental data – since the (Q)SAR is based on a larger body of data, provides a more compelling Weight of Evidence (WoE) to rationalise the validity of a potential outlier
- Essential for category development and associated read-across justification - to provide a context of endpoint mechanistic similarity
- To add another line of evidence as part of a WoE within the context of an Integrated Approaches to Testing & Assessment (IATA)

Scientific Validity: OECD Principles for (Q)SAR Validation

- A (Q)SAR should be associated with the following information:
  - a defined endpoint
  - an unambiguous algorithm
  - a defined applicability domain
  - appropriate measures of goodness-of-fit, robustness and predictivity
  - a mechanistic interpretation, if possible
- Principles were agreed by OECD in 2004 and associated guidance was published in 2007

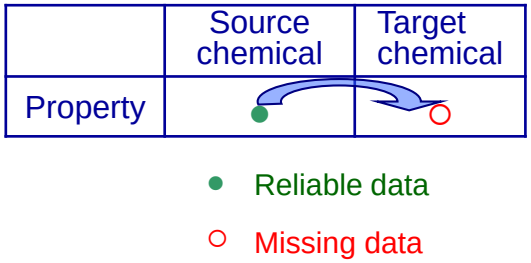
Many QSARs/Expert systems use these principles as a basis to demonstrate potential utility for application. Reporting Formats (QMRF and QPRFs) exist to help summarise model characteristics and substance specific predictions.

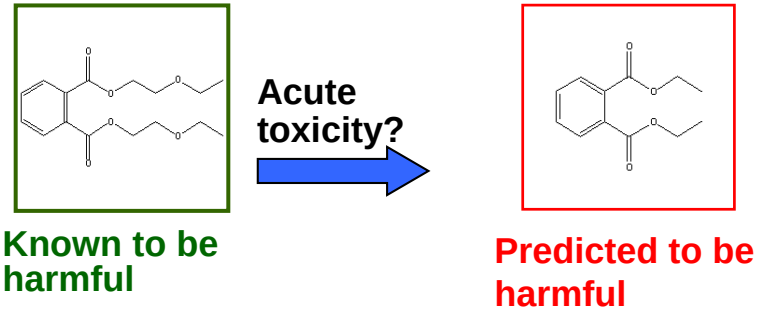
QSARs which provide physchem (property) information



Read-across

- Read-across describes the method of filling a data gap whereby a chemical with existing data values is used to make a prediction for a ‘similar’ chemical.
- Used within analogue and category approaches.
- A target chemical is a chemical which has a data gap that needs to be filled i.e. the subject of the read-across.
- A source analogue is a chemical that has been identified as an appropriate chemical for use in a read-across based on similarity to the target chemical and existence of relevant data.





Ongoing issues with read-across

- Although there is a wealth of technical guidance on how to develop read-across assessments, acceptance remains an issue. This is also not helped by the fact that read-across is typically an expert driven assessment.
- One issue impeding acceptance relates to the “uncertainty of the read-across prediction”.
- As such there have been many efforts to identify the sources of uncertainty in read-across, characterise them in a consistent manner and identify practical strategies to address and reduce those uncertainties.
- Notable in these efforts have been the development of frameworks for the assessment of read-across, evaluating the utility of New Approach Methods (NAMs).
- Quantifying uncertainty and performance of read-across is a need as are approaches to more effectively characterise similarity contexts beyond structure e.g., metabolism, reactivity etc.

Read-Across Software Tools

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Navigating through the minefield of read-across tools: A review of in silico tools for grouping

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ABSTRACT

Read-across is a popular data gap filling technique used within analogue and category a
regulatory purposes. In recent years there have been many efforts focused on the challe
in read-across development, its scientific justification and documentation. Tools have als
oped to facilitate read-across development and application. Here, we describe a number of
able read-across tools in the context of the category/analogue workflow and review th
capabilities, strengths and weaknesses. No single tool addresses all aspects of the workflow
how the different tools complement each other and some of the opportunities for their fu
ment to address the continued evolution of read-across.

Published by

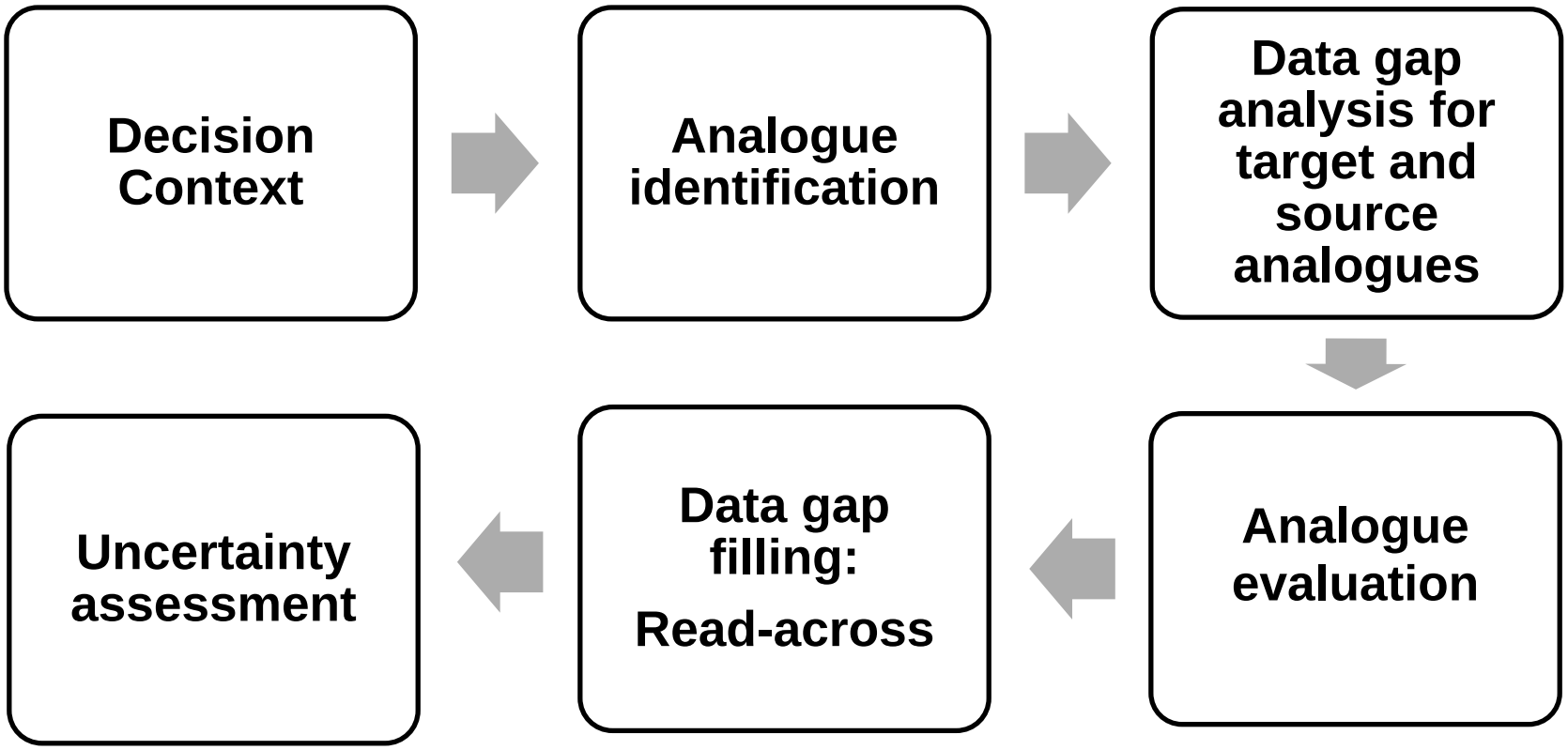
(Patlewicz et al., 2017)

Summary of key features of selected publicly available read-across tools.

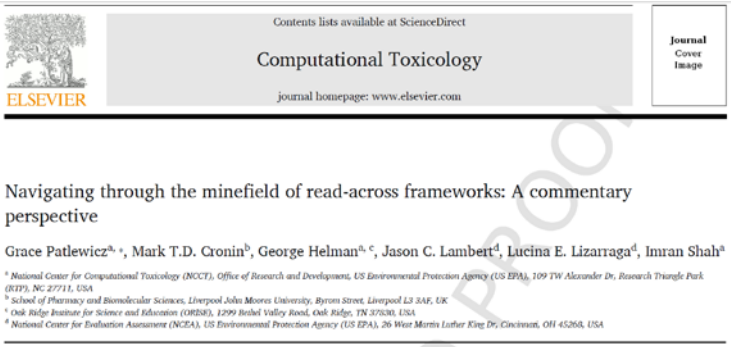
	AIM	ToxMatch	Ambit	OECD Toolbox	CBRA	ToxRead	CliPro
Development timeline	Java based version is dated 2012. Initial development of web version was 2005.	First public version released in Dec 2006	Original AMBIT tool was developed in 2004–2005	Proof of concept released in 2008	Implementation of the Low et al. [27]	Implementation of Gini et al. [22]	Implementation described in Russo et al. [45]
Type of Tool	Standalone	Standalone	Web-based and standalone	Standalone or Client/Server	Standalone	Standalone	Web-based
Latest Version	1.01 (Nov 2013) Static	1.07 (Jan 2009) Static	3.0.3 Ongoing Enhanced in 2013–2015	3.4 (July 2016) Version 4 released April 2017 Ongoing	0.75 First release	0.11 BETA Ongoing	First release
Developed by	SRC Inc	Ideaconsult Ltd	Ideaconsult Ltd	LMC, Bourgas	Fourches Lab at North Carolina State University	Istituto di Ricerche Farmacologiche Mario Negri	Zhu Research Group at Rutgers University
Available from	https://www.epa.gov/tsca-screening-tools/analog-identification-methodology-aim-tool	https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/toxmatch	http://cefic-lri.org/lri_toolbox/ambit/	www.qsartoolbox.org	http://www.fourches-laboratory.com/software	http://www.toxread.eu/	http://cilpro.rutgers.edu/
Accepted Chemical Input	CAS, Name, SMILES, structure drawing/import	CAS, Name, SMILES, InChI	Name, identifiers, SMILES, InChI	CAS, Name, SMILES, structure drawing, MOL, sdf	Mol file, descriptors as txt	SMILES	PubChem CID, CAS, IUPAC, SMILES, InChI
Endpoint Coverage	N/A	Any based on user input	IUCLID[a] 5-supported endpoints (43 total)	Any as per the regulatory endpoints	Any based on user input	Mutagenicity and Bioconcentration Factor (BCF)	Any based on user input
Analogue Identification Approach	Fragment matching	Distance and correlation based similarity indices based on descriptors or fingerprints	Substructure or similarity searching using structure, name, SMILES, InChI	Category definition followed by subcategorisations	Tanimoto distance using chemical and biological descriptors	VEGA similarity algorithm	Weighted Estimated Biological Similarity
Neighbour Selection	Automatic	Automatic	Manual	Automatic + Manual Filter	Automatic	Automatic	Automatic + Manual Filter
Data Source	Tool provides inventory index	User provided or tool provided	User and tool provided	User provided or tool provided	User provided	Tool provided as a result of the EU ANTARES project	User provided but tool provides PubChem *in vitro* data
Quantitative vs Qualitative	N/A	Both	User determined - Qualitative	Both	Qualitative	Qualitative for mutagenicity, quantitative for BCF	Qualitative
Visualisation	None	Standard 2D plots, histograms and similarity matrix	None	Standard 2D Plots	Radial plot of neighbours	Interactive Neighbour plot	Activity Plot
Output/Export	Output reports in the form of HTML, pdf or Excel	sdf or txt files of data, image files of plots	Assessment report as docx or xlsx, data matrix as xlsx	IUCLID format, pdf and rtf files of prediction report, text files of data, image files of plots etc	NA	Image file of plot	Tabulation of predictions and image of similarity plot

[a] IUCLID stands for International Uniform Chemical Information Database. IUCLID is a software program for the administration of data on chemical substances first developed to fulfill EU information requirements under REACH.

Generalised Read-Across Workflow



A Harmonised Hybrid Read-Across Workflow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Navigating through the minefield of read-across frameworks: A commentary perspective

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- Where do NAM data fit?
- How should we transition to data-driven approaches?
- Quantifying the uncertainty in the read-across predictions made?

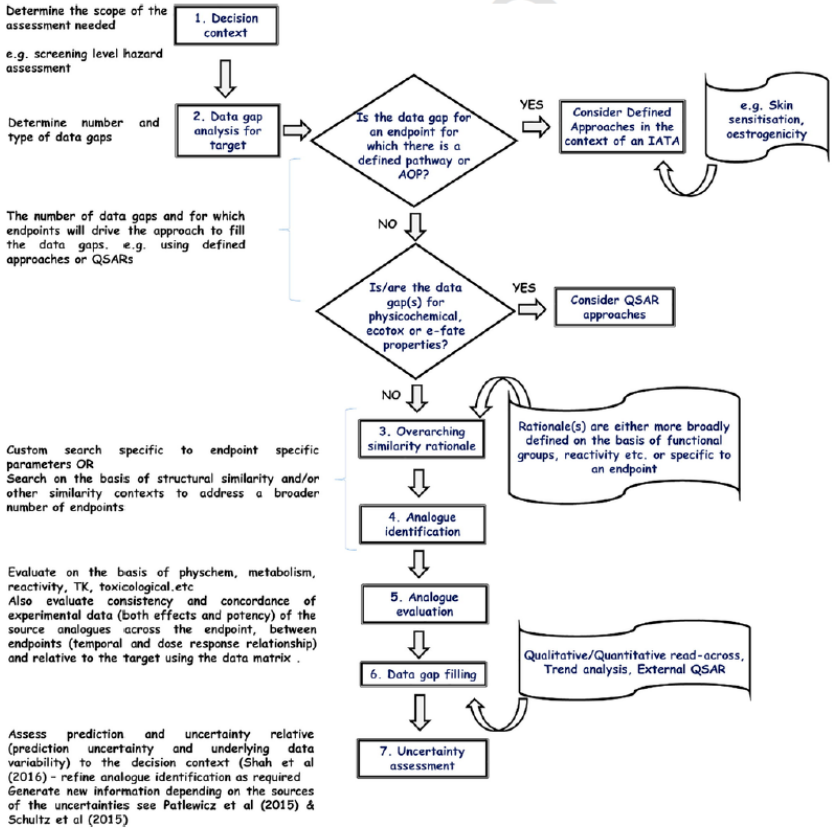


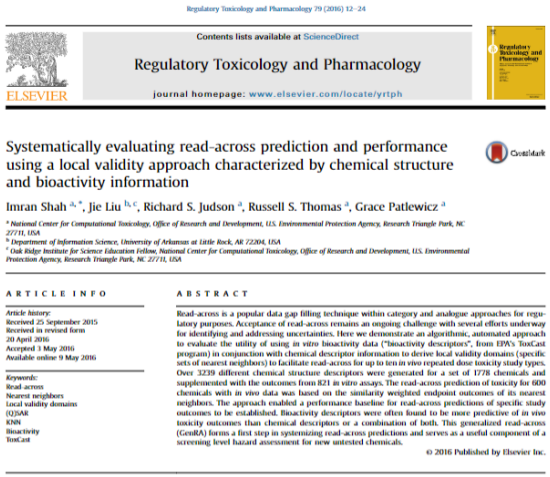
Fig. 9. A harmonised hybrid development and assessment framework.

Patlewicz et al., 2018

Data-driven read-across approaches

GenRA (Generalised Read-Across)

- Predicting toxicity as a similarity-weighted activity of nearest neighbours based on chemistry and bioactivity descriptors (Shah et al, 2016)
- Goal: To establish an objective performance baseline for read-across and quantify the uncertainty in the predictions made



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Systematically evaluating read-across prediction and performance using a local validity approach characterized by chemical structure and bioactivity information

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ABSTRACT

Read-across is a popular data gap filling technique within category and analogue approaches for regulatory purposes. Acceptance of read-across remains an ongoing challenge with several efforts underway for identifying and addressing uncertainties. Here we demonstrate an algorithmic, automated approach to evaluate the utility of using in vitro bioactivity data ("bioactivity descriptors", from EPA's ToxCast program) in conjunction with chemical descriptor information to derive local validity domains (specific sets of nearest neighbors) to facilitate read-across for up to ten *in vivo* repeated dose toxicity study types. Over 3239 different chemical structure descriptors were generated for a set of 1778 chemicals and supplemented with the outcomes from 821 *in vitro* assays. The read-across prediction of toxicity for 600 chemicals with *in vivo* data was based on the similarity weighted endpoint outcomes of its nearest neighbors. The approach enabled a performance baseline for read-across predictions of specific study outcomes to be established. Bioactivity descriptors were often found to be more predictive of *in vivo* toxicity outcomes than chemical descriptors or a combination of both. This generalized read-across (GenRA) forms a first step in systemizing read-across predictions and serves as a useful component of a screening level hazard assessment for new untested chemicals.

$$y_i^{\beta,\alpha} = \frac{\sum_j^k s_{ij}^{\alpha} x_j^{\beta}}{\sum_j^k s_{ij}^{\alpha}}$$

Jaccard similarity:

$$s_{ij} = \frac{\sum_l (x_{il} \wedge x_{jl})}{\sum_l (x_{il} \vee x_{jl})}$$

$\alpha \square \{chm, bio, bc\}$

$\beta \square \{bio, tox\}$

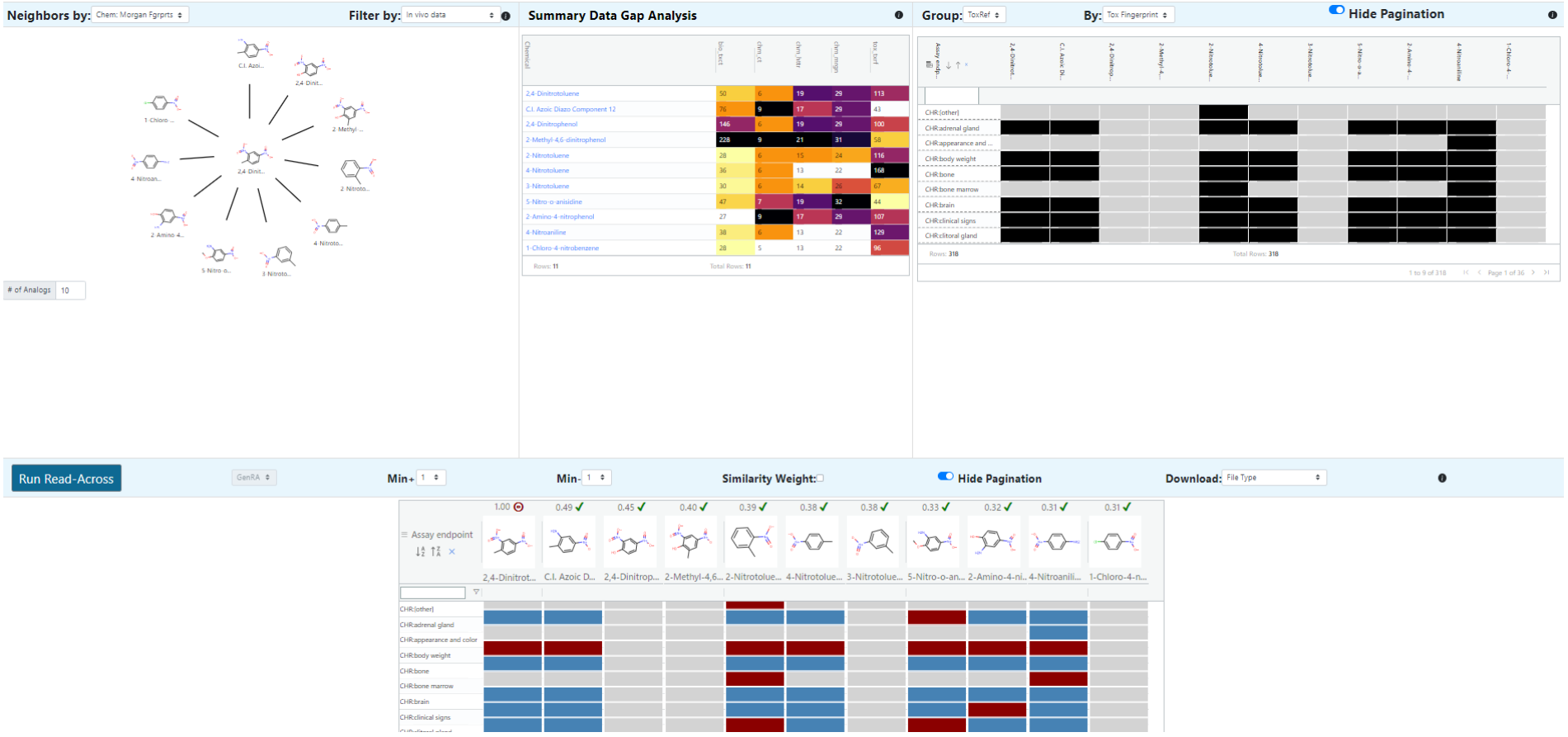
y_i = *predicted activity of chemical* (c_i)

x_j^{β} = *activity of* c_j in β

s_{ij}^{α} = *Jacccard similarity between* $x_i^{\alpha}, x_j^{\alpha}$

k = *up to* k *nearest neighbours*

GenRA v3 tool in practice

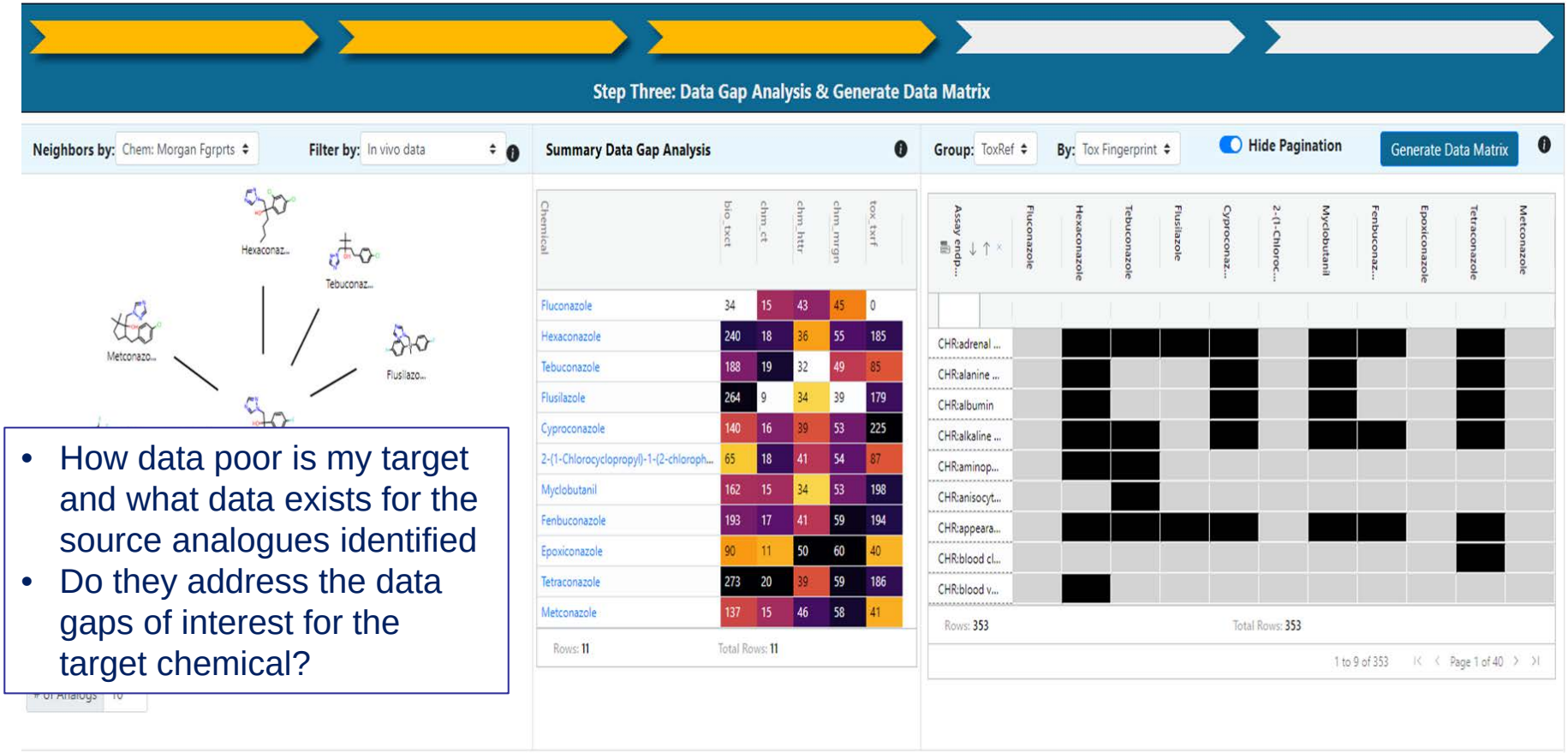


GenRA v3 tool in practice

Search for a chemical of interest (target) using the search box or the Ketcher drawing tool

Radial plot with target in the centre and source analogues (similar) ordered clockwise by decreasing similarity (Jaccard)

GenRA v3 tool in practice

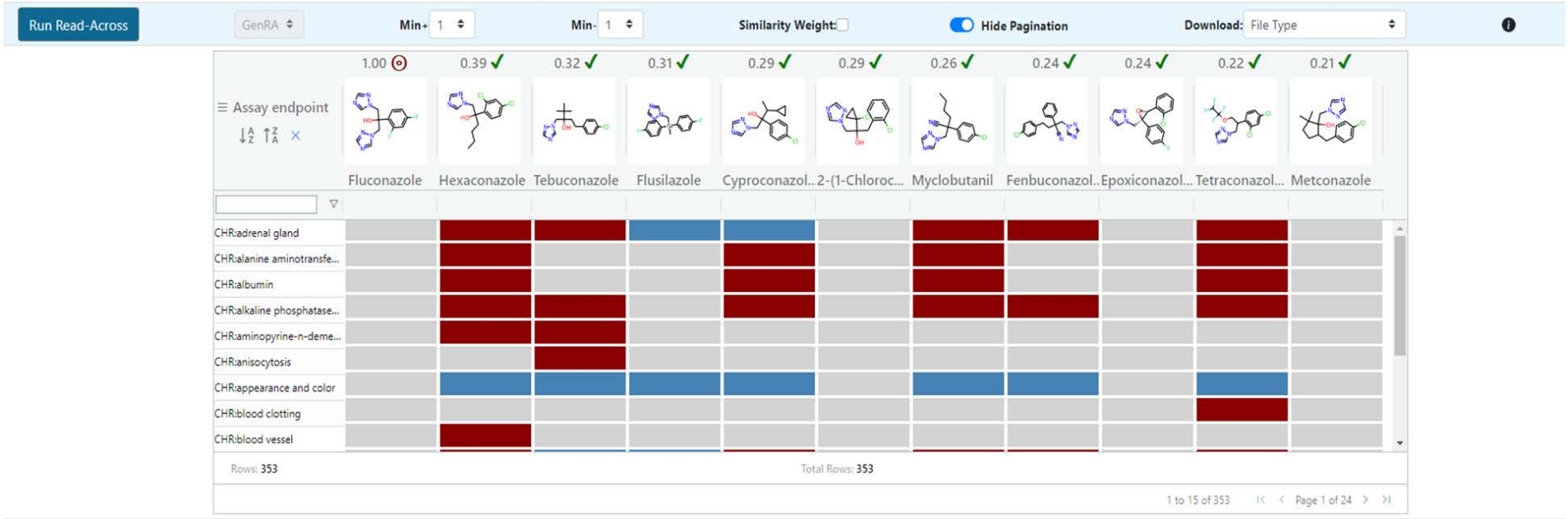


- How data poor is my target and what data exists for the source analogues identified
- Do they address the data gaps of interest for the target chemical?

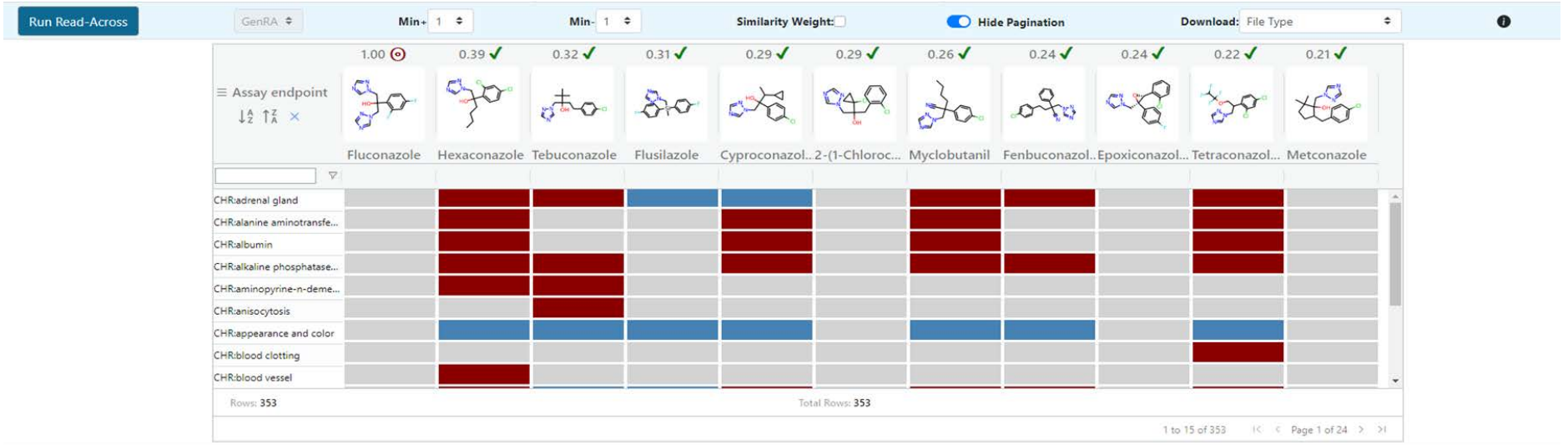
GenRA v3 tool in practice

What is the consistency and concordance across my source analogues?
Should I deselect analogues from consideration from the entire set of predictions?
Should I consider subcategorising the analogues selected?

Toxicity data represented as binary outcomes – red (positive), blue (negative), grey (no data)



GenRA v3 tool in practice



First column is updated with predictions

GenRA – Current research

- Consideration of other information to define and refine the analogue selection & evaluation
  - physicochemical similarity (Helman et al, 2018)
  - metabolic similarity (Boyce et al, 2022),
  - reactivity similarity (Nelms et al 2018)
  - transcriptomics similarity (Tate et al, 2021)
- Transitioning to quantitative predictions of toxicity
  - Using GenRA to predict Lowest Observed Adverse Effect Level (LOAEL), acute oral (median lethal dose) LD50 (Helman et al 2019a,b)
- Developing a compendium of expert driven read-across examples to investigate how data driven read-across with NAM data can mirror expert assessments (*in prep*)

EPA United States Environmental Protection Agency

Relative Potency Values

- Relative potency values have been applied in the assessment of mixtures as described in the first talk of this session. They represent a special type of grouping approach as described in the existing OECD grouping guidance.
- Well known examples include Toxic Equivalent Factors (TEFs) which have been used to assess mixtures of dioxins and furans.
- However, there are specific caveats and criteria for the use of these TEFs.
- TEFs and the estimation of toxic units for mixtures of chemicals which contribute to a biological effect through a common toxicity pathway.

Relative Potency Values

- In the TEQ approach, the most toxicologically relevant compound is used as the reference compound. Components of the mixture should act by the same single toxic pathway and be of the same compound type (structural/functional group similarity) as the reference.
- The components of the mixture are each assigned TEFs such that their individual toxicity is expressed as a fraction of the toxicity of the reference which is given a TEF of 1.

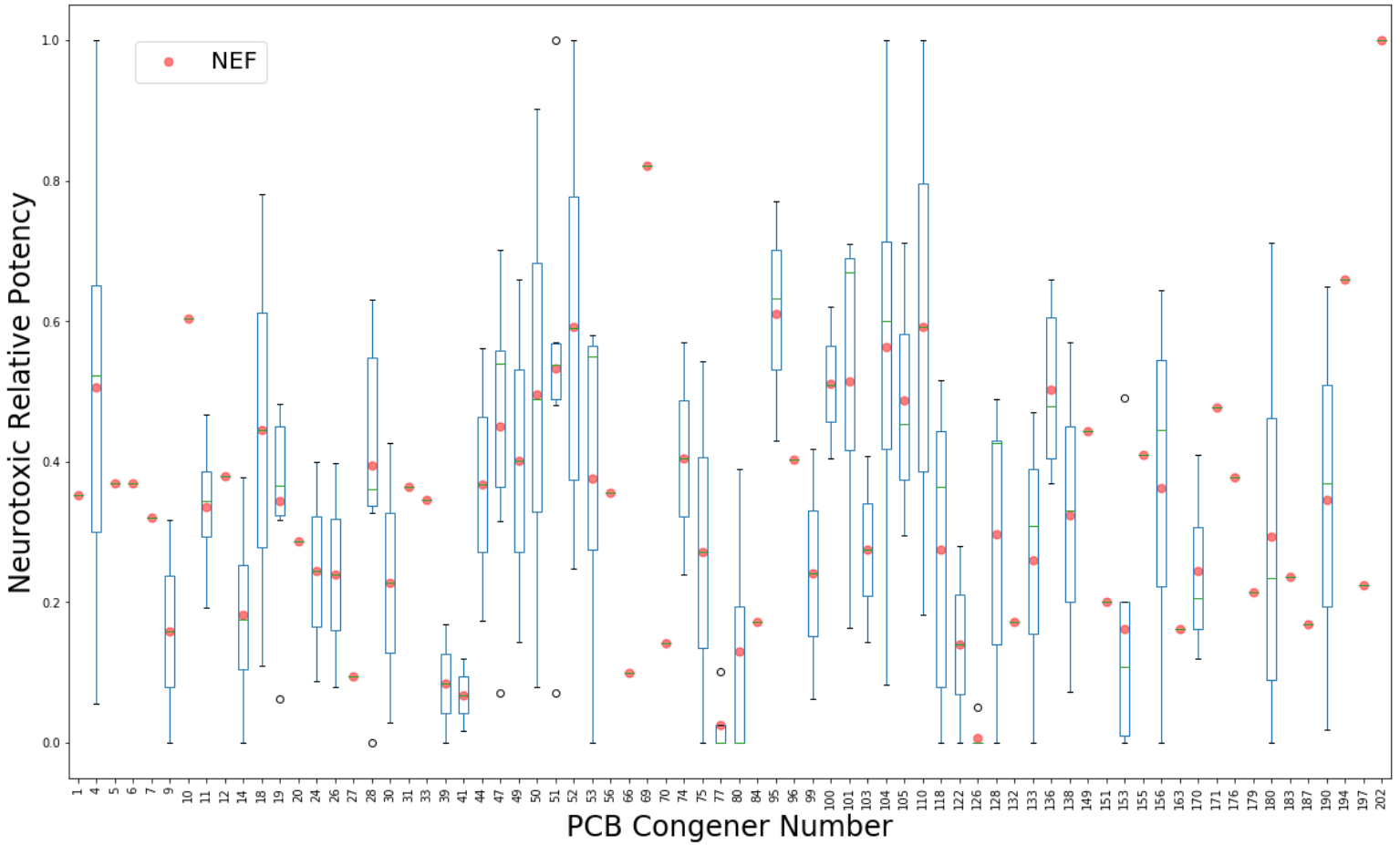
TEFs

- TEF (component A) = $\frac{\text{Reference effect value}}{\text{Component A effect value}}$
- An example of an effect value (or “effect level”) would be a LOAEL
- The amount of each component in the mixture is then multiplied by its respective TEF and the values for each component are summed to give an overall toxic equivalency relative to the reference compound
- TEQ = sum(concentration X TEF)
- But what if the effect value of Component A is missing?
  - This is where read-across, QSARs can play a role in filling in the missing gaps.

NEFs

- Same principle as TEFs but NEFs were derived for the neurotoxicity of PCBs
- First developed by Simon et al (2007) who developed neurotoxic equivalent values for a dataset of 87 PCB congeners of which 83 congeners had *in vitro* experimental data
- However, the data was taken from several different studies each of which measured different effects. A more flexible interpretation of the TEF approach. Subsets of the 83 PCB congeners did overlap in terms of their *in vitro* data.
- Pradeep et al (2018) sought 1) to re-evaluate an alternative NEF from an expanded dataset and 2) investigate the feasibility of developing new QSAR models to predict NEFs.
- The resulting model could then be applied to estimate NEFs of the remaining untested PCB congeners

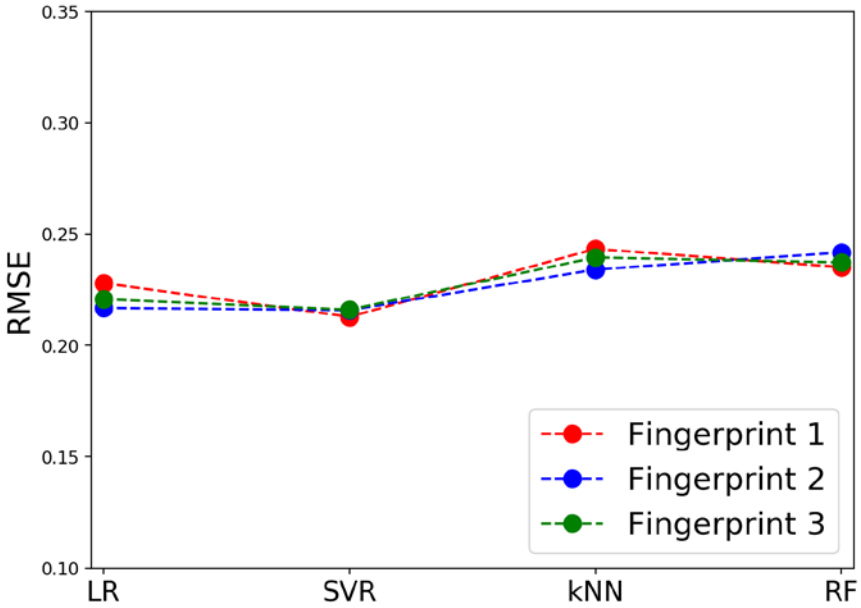
Variability of NEFs



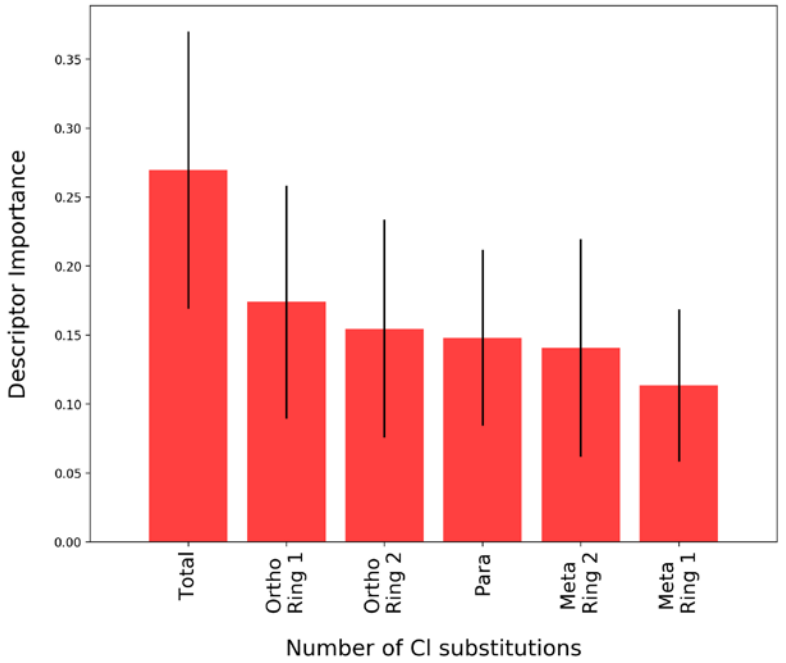
A QSAR model to predict NEFs

- Is there a mathematical relationship between chemical characteristics and NEFs ?
  - Step 1: Characterise the PCB congeners in terms of structural characteristics using custom fingerprints (encode the chlorine substitution pattern of PCBs as a bitstring of 1s and Os)
  - Step 2: Investigate the feasibility of using different approaches to build QSAR models that relate the calculated inputs from Step 1 to known NEFs.
  - Step 3: Evaluate the robustness and performance of any QSAR models
- QSAR models derived had low predictivity (RMSE ~0.24) which was largely attributed to the large uncertainties of the data and the associated NEF values.
- Nonetheless, in the absence of better information, the derived NEFs and the QSAR predicted NEFs could be helpful to fill data gaps if applied with caution.

A QSAR model to predict NEFs



4 different modelling approaches attempted



Which structural features were most influential in estimating the NEFs

EPA United States Environmental Protection Agency

Summary remarks

- Computational toxicology covers a broad spectrum of different approaches
- Have highlighted a few of the main approaches to provide context
- The EPA CompTox Chemicals Dashboard provides a wealth of information (predicted and experimental data for hazard and exposure) which is a relevant starting point in the assessment of any substance of interest.
- Relative potency values are a special case of grouping approaches (read-across).
- Illustrated one case study where a QSAR model was developed to predict relative potency values for neurotoxicity (extending the so-called NEFs that Simon et al established) using chemical structural characteristics which could be applied to estimate NEFs for untested PCB congeners.