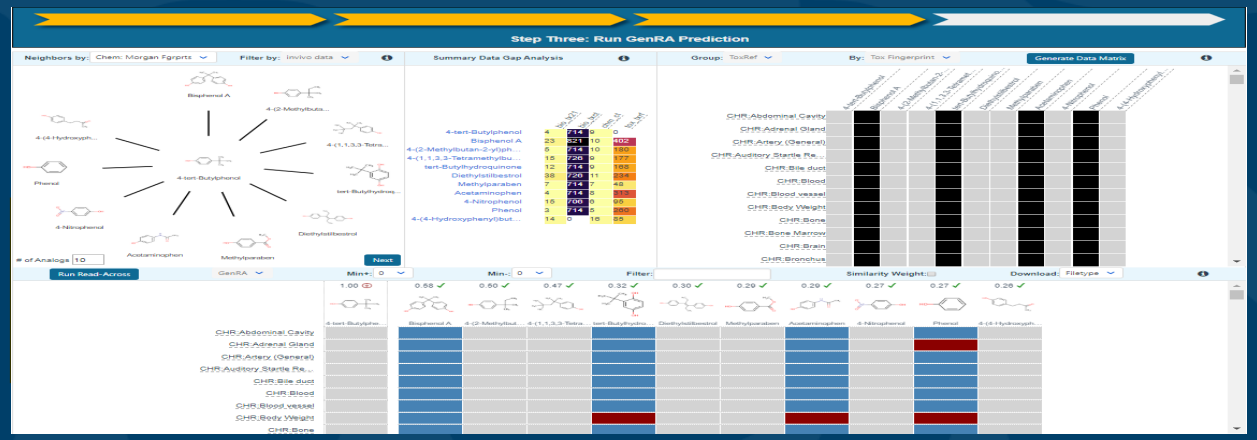
A Framework to Build Scientific Confidence in Read-across



Grace Patlewicz
National Center for Computational Toxicology (NCCT), US EPA

Abbreviations/Definitions

- Target – substance of interest, data poor
- Source – analogue with data which will be used to make the read-across prediction
- PMN – Premanufacture notice
- PPRTV - Provisional Peer Reviewed Toxicity Values (for Superfund)
- GenRA – Generalised Read-across

Talk Objectives

Understanding:

- Definitions of read-across, category & analogue approaches
- Read-across development and assessment frameworks
- Harmonised framework for read-across
- Selected read-across tools
- Ongoing issues with read-across
- Current directions towards quantifying read-across performance and its associated uncertainties
- Generalised Read-across (GenRA) – an approach and an application

Definitions: Chemical grouping approaches

- Read-across describes one of the techniques for filling data gaps in either the analogue or category approaches
- "Analogue approach" refers to grouping based on a very limited number of chemicals (e.g. target substance + source substance)
- "Category approach" is used when grouping is based on a more extensive range of analogues (e.g. 3 or more members)

A chemical category is a group of chemicals whose physico-chemical and human health and/or environmental toxicological and/or environmental fate properties are likely to be similar or follow a regular pattern as a result of structural similarity (or other similarity characteristics).

Uses of Read-across

	Chemical 1	Chemical 2	Chemical 3	Chemical 4
Property 1	●	○	●	○
Property 2	●	○	●	●
Property 3	○	●	●	○
Property 4	●	●	●	●
Activity 1	○	○	○	○
Activity 2	●	●	●	●
Activity 3	○	○	○	○
Activity 4	○	●	○	●

read-across

interpolation

extrapolation

Trend analysis or internal QSAR

● reliable data point

○ missing data point

Uses of Read-across

- Examples where "read-across" approaches are applied include:
  - US EPA Provisional Peer Reviewed Toxicity Values (PPRTVs) where data is lacking for a specific substance of interest
  - EPA Test Rules – Industry registrants providing information to satisfy a test rule
  - EPA Pre Manufacture Notifications (PMN) – QSARs such as those in Epiwin and ECOSAR are routinely used for e-fate and ecotox predictions but read-across is relied upon for non cancer endpoints
  - ASTDR Emergency response values – an accidental spill that requires an immediate assessment of acute toxicity for first responders
  - REACH registrations – addressing information requirements

Developing a read-across assessment

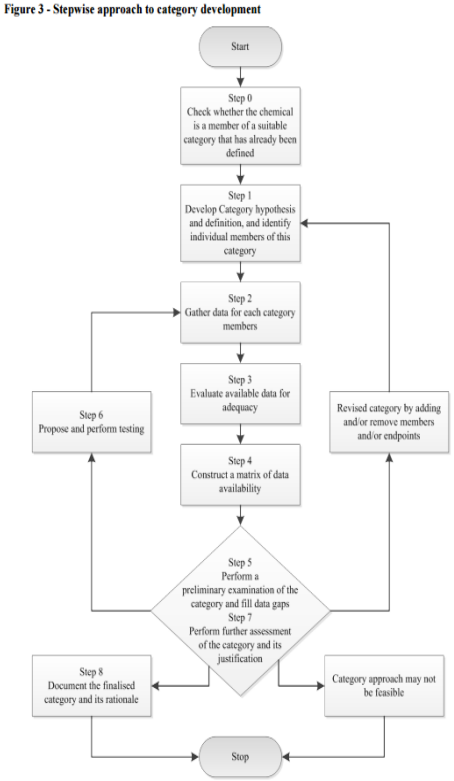
- Existing guidance and resources that can be helpful in developing a read-across assessment:
  - Technical regulatory guidance has been published by OECD and ECHA
  - OECD guidance from 2007 was updated in 2014
  - ECHA Chapter 6 QSARs and Grouping of Chemicals as well as practical guides
- However, many papers have been published that complement and augment the regulatory guidance for development of read-across
  - Wang et al (2012) Application of computational toxicological approaches in human health risk assessment. I A tiered surrogate approach (EPA PPRTVs)

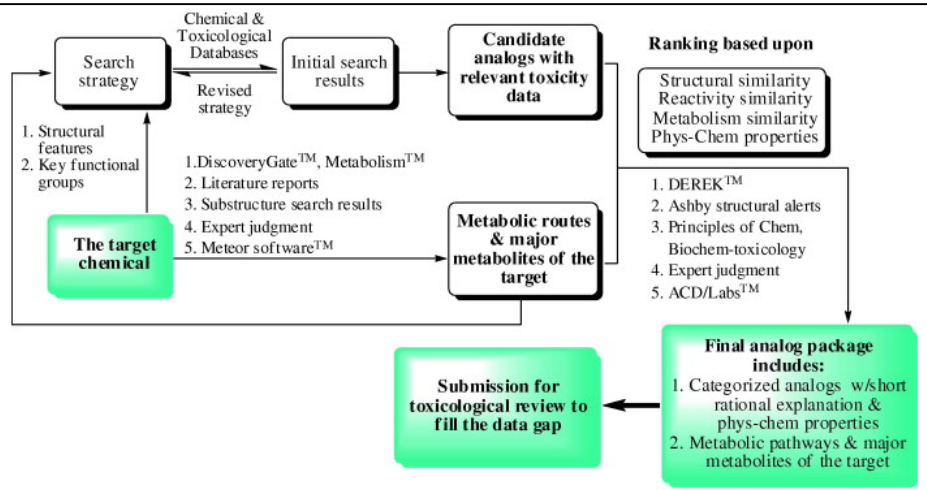
Developing a read-across assessment

- Selected literature include:
  - ECETOC TR116 category approaches, Read-across, (Q)SAR
  - Wu et al (2010) – Framework for using structural, reactivity, metabolic and physicochemical similarity to evaluate suitability of analogs for SAR based toxicological assessments
  - Patlewicz et al (2013) Use of category approaches, read-across and (Q)SAR general considerations
  - Patlewicz et al (2015) Building scientific confidence in the development and evaluation of read-across
  - Ball et al (2016) Towards Good Read-across Practice

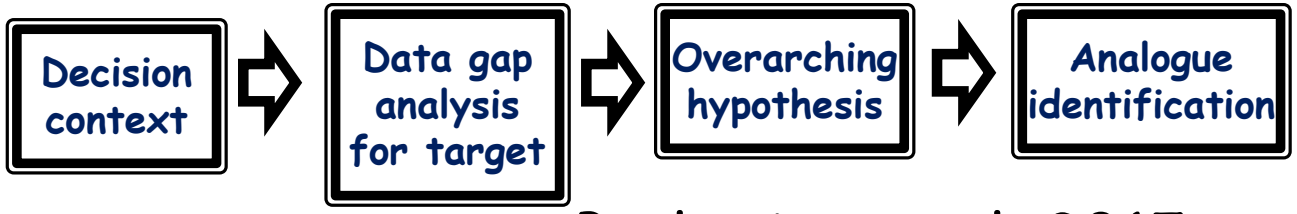
Frameworks for developing category/analogue approaches

OECD (2014)





Wu et al, 2010



Patlewicz et al, 2015

Summary highlights of read-across development frameworks

Framework	ECHA	OECD	Wu	Wang	Patlewicz
Context	REACH	International regulatory purposes	Product Stewardship	Quantitative risk assessment	Regulatory purposes/Product stewardship
Approach	Analogue/Category – aim is to fill an endpoint specific study. Focused on structural similarity as a starting point Approach is more hypothesis driven	Analogue/Category – a generalisation of the ECHA approach	Analogue Systematic stepwise evaluation of analogue suitability based on structure, reactivity, p-chem and metabolism	Analogue Approach is based on a WOE assessment from structure, ADME and toxicity considerations	Analogue Stepwise approach considering general (pchem, reactivity, metabolism) and endpoint specific considerations
Terms of reference	Target/Source	Target/Source	Substance of interest/Analogue	Chemical of Concern/Surrogate	Analogue/Category
Scope	Endpoint specific	Endpoint specific	Systematic stepwise evaluation of analogue suitability based on structure, reactivity, p-chem and metabolism Most sensitive/relevant endpoint – focused on repeated dose toxicity endpoints; quantitative risk assessment	Approach is based on a WOE assessment from structure, ADME and toxicity considerations. "Best" surrogate is selected from a set of candidates based on most similar and most conservative toxicity value	Approach is aimed to identify source analogues that can be used to address as many endpoints as appropriate, even though the read-across prediction itself is justified on an endpoint per endpoint basis and some source analogues might be excluded from the prediction itself if they are not appropriate for specific endpoints of

Reviewed in Patlewicz et al., 2018

Ongoing issues with read-across

- Although there is much guidance for developing read-across assessment, acceptance still remains an issue, especially for regulatory purposes.
- A key issue thwarting acceptance relates to the "uncertainty of the read-across"
- 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. These allow for a structured assessment of the read-across justification.

Sources of uncertainty in read-across

- Analogue or category approach? (no. of analogues)
- Completeness of the data matrix – no. of data gaps
- Data quality for the underlying analogues for the target and source analogues
- Consistency of data across the data matrix – concordance of effects and potency across analogues
- Overarching hypothesis/similarity rationale – how to identify similar analogues and justify their similarity for the endpoint of interest
- Address the dissimilarities and whether these are significant from a toxicological standpoint e.g. ToxDelta
- Presence vs. absence of toxicity
- Toxicokinetics

Frameworks for Assessing Read-across

- Blackburn & Stuard
- Patlewicz et al (2015)
- Schultz et al (2015)
- ECHA RAAF (2015, 2017)

- These aim to identify, document and address the uncertainties associated with read-across inferences/predictions

Frameworks for the assessment of read-across

United States Environmental Protection Agency

READ ACROSS UNCERTAINTY EVALUATION QUESTIONNAIRE FOR:
Target chemical (SOI) = *(list CAS#)*

INSTRUCTIONS
Complete the Questionnaire. Answer the questions for each endpoint where SAR was conducted, and follow instructions li
general, NO responses indicate potential areas of uncertainty in the proposed read across.)

Questions	Responses by Endpoint	
	Repeat Dose Toxicity	Reproductive Toxicity
Section I. Chemical similarity between source (analogue) and target (SOI)		
1. For each endpoint, list the CAS#s of the source (analogues) contributing the critical study for the read across		
	CAS# _	
2. What is the 'suitability rating' of the analogue?	____ Su ____ Su (skip to ____ Su (continu of the in	
3. Are any differences in functional groups and asso be more reactive than the target)?		
	____ YES ____ NO ____ UNKNOWN ____ No Differences	____ YES ____ NO ____ UNKNOWN ____ No Differences
	NOTES, if any:	NOTES, if any:

Suitability of Analogs contributing data

Are all features of SOI covered or differences in conservative direction

Table 2
Scientific confidence considerations in Read-across evaluation.

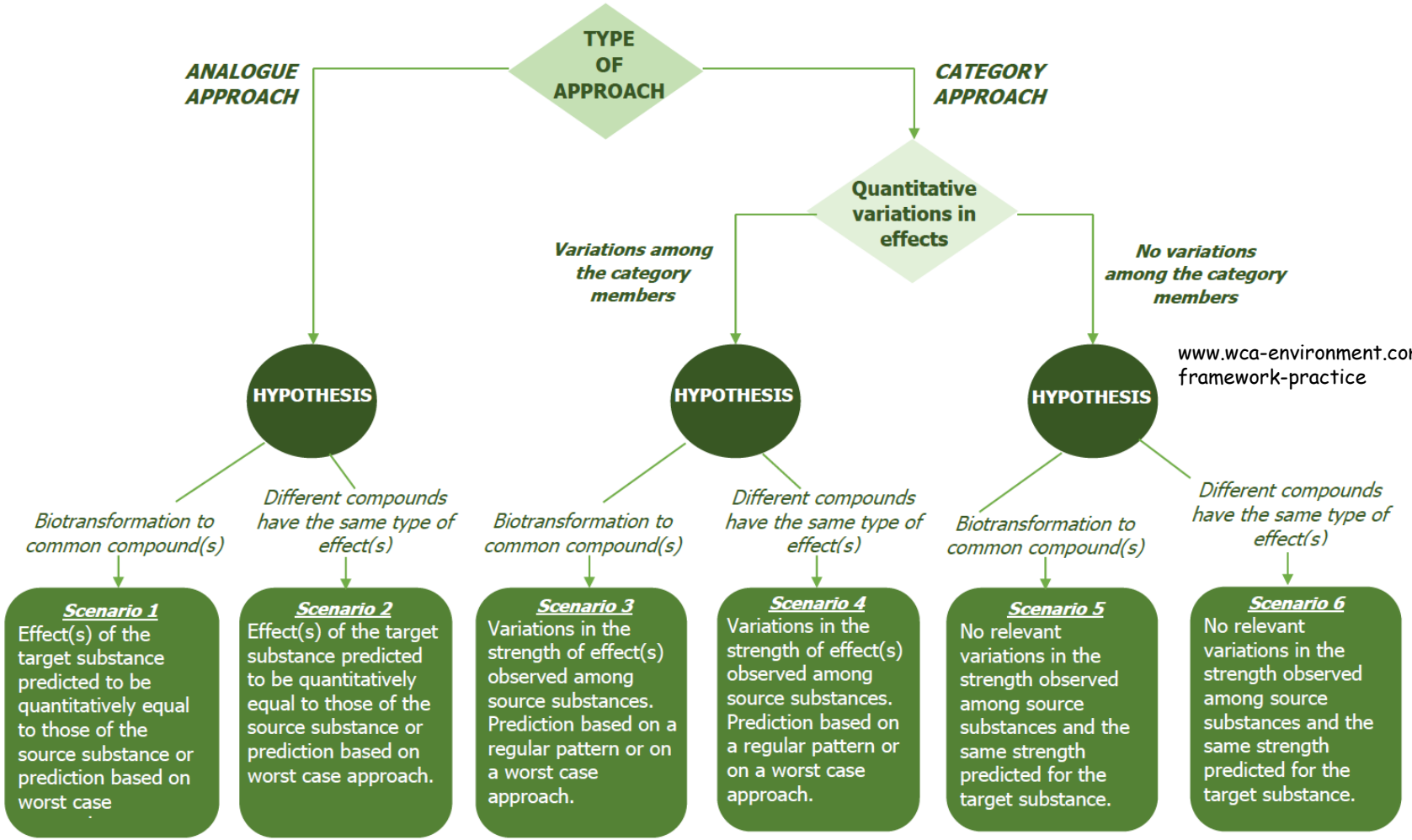
Data issues	Similarity rationale
Analogue/category approach	Similarity rationale/hypothesis that underpins the analogue/category approach – Metabolic transformation – Structural similarity
Completeness of data matrix – No of data gaps e.g. source analogue(s) have many data points to address, target substance has a handful of data gaps.	Analogue validity – Analogue similarity with respect to general and endpoint specific considerations – Rationalization of why structural differences do not impact the toxicity
Quality of data for source analogues – e.g. Klimisch scores of 1 or 2	Concordance of effects and potency (if relevant) per endpoint • Presence or absence of adverse effects • Type of read-across (Qualitative, Quantitative, Trend Analysis) Concordance of effects and potency (if relevant) across endpoints

Patlewicz et al (2015)

Frameworks for the assessment of read-across

- Schultz et al (2015)
- Outlined a strategy for structuring and reporting a read-across
- Defined different read-across scenarios
- Two main aspects tackled:
  - an assessment of the similarity of the source analogues
  - an assessment of the mechanistic relevance and completeness of the read-across (number of analogues, absence/presence of toxicity, quality of underlying data, temporal and dose response relationship between mechanistically relevant endpoints
- Three scale grading of the overall read-across confidence Low, Medium, High

Frameworks for the assessment of read-across: RAAF



www.wca-environment.com/blog/putting-read-across-assessment-framework-practice

Frameworks for the assessment of read-across: RAAF

- Six scenarios identified
- For each scenario there will be a number of scientific considerations
- Each is associated with an "assessment element" (AE)
- Each AE is scored from 1-5 where 5 is "acceptable with high confidence" to 1 is not acceptable
- These scores are termed Assessment Options (AO)
- A minimum score of 3 is needed for a read-across to be taken up and used to inform decision making
- There are common assessment elements e.g. reliability of the underlying data and there are scenario specific elements e.g. common underlying mechanism for scenario 2

Summary highlights of read-across assessment frameworks

Framework	ECHA RAAF (2017)	Blackburn and Stuard (2014)	Patlewicz et al (2015)	Schultz et al (2015)
Context	REACH	Product Stewardship	Regulatory purposes & Product stewardship	Regulatory purposes & Product stewardship
Scope	Analogue/Category	Analogue/Category	Analogue/Category	Analogue/Category
Framework	Scenarios addressing analogue (2) and category (4) approaches as described above Each scenario is associated with a number of assessment elements (AE) (both common and scenario specific).	Framework addresses 3 aspects: analogue suitability (covered in Wu et al, 2010); data quality of the analogues; consistency of the data across the analogues and relative to the target	Identifies the sources of uncertainty in relationship to the data and similarity context	Different scenarios are articulated to frame up to 11 different similarity criteria. factors proposed to evaluate mechanistic relevance and completeness of the read-across

A harmonised hybrid read-across workflow

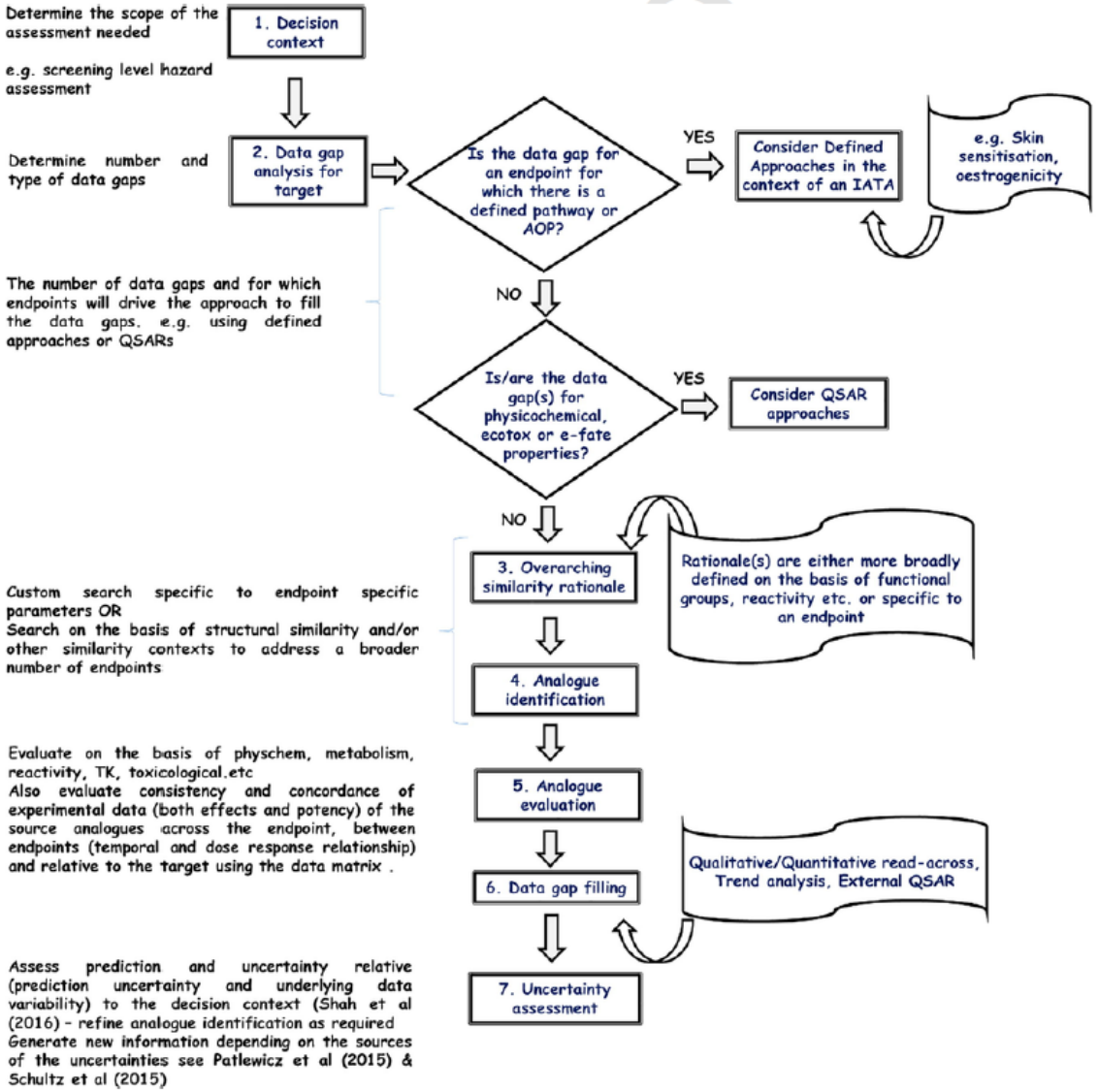


Fig. 9. A harmonised hybrid development and assessment framework.

Patlewicz et al., 2018

A harmonised hybrid read-across workflow

United States Environmental Protection Agency

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Navigating through the minefield of read-across frameworks: A commentary perspective

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Ongoing issues with read-across

- These frameworks allow for a structured assessment of the read-across justification.
- The next step is how those uncertainties can be addressed
- Blackburn and Stuard (2014) propose the use of assessment factors
- The RAAF and the work by Schultz et al (2015) advocate the use of New Approach Methods (NAM) (e.g. High Throughput Screening (HTS) data) to enhance the scientific confidence of a read-across
- Examples have been published by Schultz (2017) and colleagues
- Others such as Shah et al (2016) or Zhu et al (2016) have explored quantifying the uncertainties of read-across and using NAM data in conjunction with chemical structure information in a 'QSAR-like' read-across (Generalised Read-Across (GenRA)

Selected read-across tools

Tool
Analogue identification
Analogue Evaluation
Data gap analysis
Data gap filling
Uncertainty assessment
Availability

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journal homepage: www.elsevier.com/locate/comtox

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

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Nearest neighb

ABSTRACT

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

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Quantifying uncertainty & Assessing performance of read-across

- GenRA (Generalised Read-Across) is a "local validity" approach
- Predicting toxicity as a similarity-weighted activity of nearest neighbours based on chemistry and bioactivity descriptors
- Systematically evaluates read-across performance and uncertainty using available data

Jaccard similarity:

GenRA - Approach

I. Data

1,778 Chemicals
3,239 Structure descriptors (chm)
820 Bioactivity hitcall (bio) ToxCast

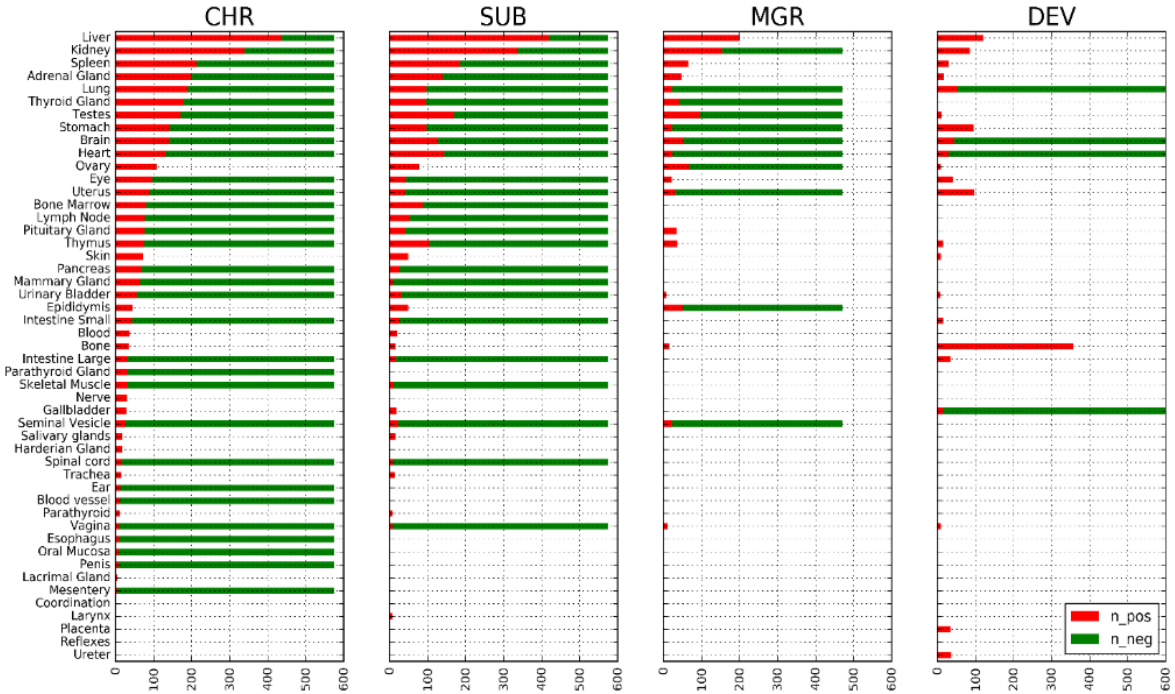
574 toxicity effects (tox) ToxRefDB

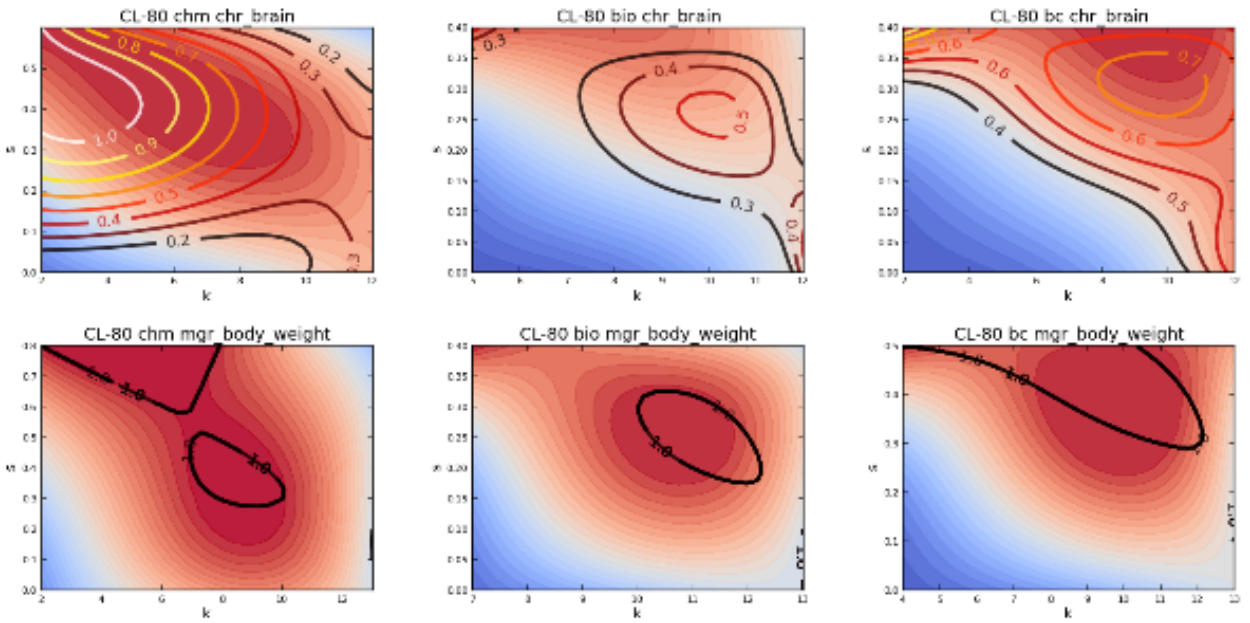
II. Define Local neighbourhoods

Use K-means analysis to group chemicals by similarity
Use cluster stability analysis
~ 100 local neighbourhoods

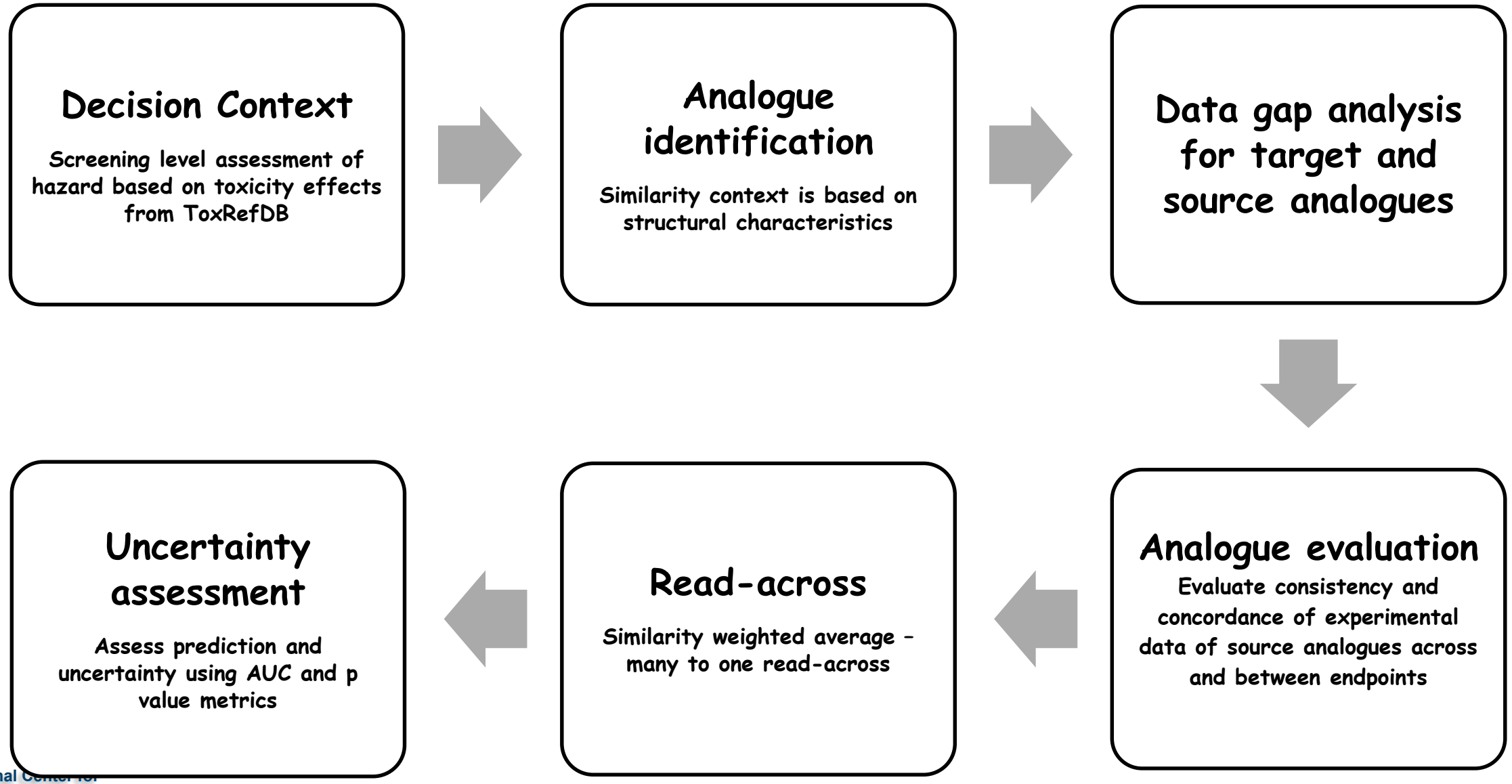
III. GenRA

Use GenRA to predict toxicity effects in local neighbourhoods
Evaluate impact of structural and/or bioactivity descriptors on prediction
Quantify uncertainty





Read-across workflow in GenRA



GenRA tool in reality

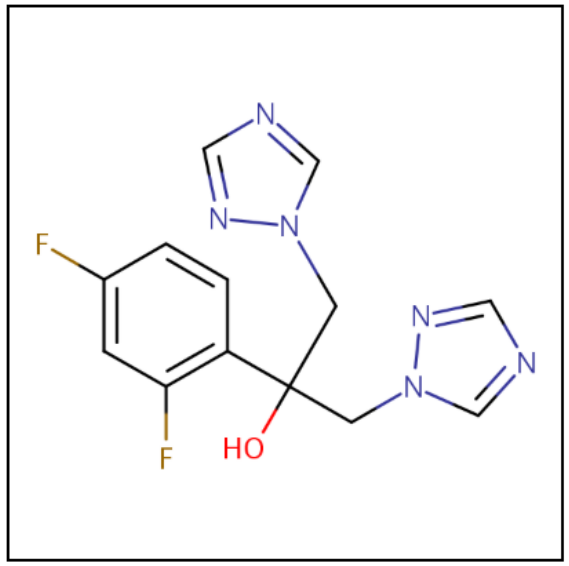
- Integrated into the EPA CompTox Chemicals dashboard

Fluconazole

86386-73-4 | DTXSID3020627

Searched by DSSTox Substance Id.

DETAILS
EXECUTIVE SUMMARY
PROPERTIES
ENV. FATE/TRANSPORT
HAZARD
ADME
EXPOSURE
BIOACTIVITY
SIMILAR COMPOUNDS
GENRA
RELATED SUBSTANCES
SYNONYMS
LITERATURE
LINKS
COMMENTS



Wikipedia

Fluconazole is an antifungal medication used for a number of fungal infections. This includes candidiasis, blastomycosis, coccidiodomycosis, cryptococcosis, histoplasmosis, dermatophytosis, and pityriasis versicolor. It is also used to prevent candidiasis in those who are at high risk such as following organ transplantation, low birth weight babies, and those with low blood neutrophil counts. It is given either by mouth or by injection into a vein.

Common side effects include vomiting

...

Read more

Intrinsic Properties

Molecular Formula: $C_{13}H_{12}F_2N_8O$ Mol File

Find All Chemicals

Average Mass: 306.277 g/mol Isotope Mass Distribution

Monoisotopic Mass: 306.104065 g/mol

Structural Identifiers

Linked Substances

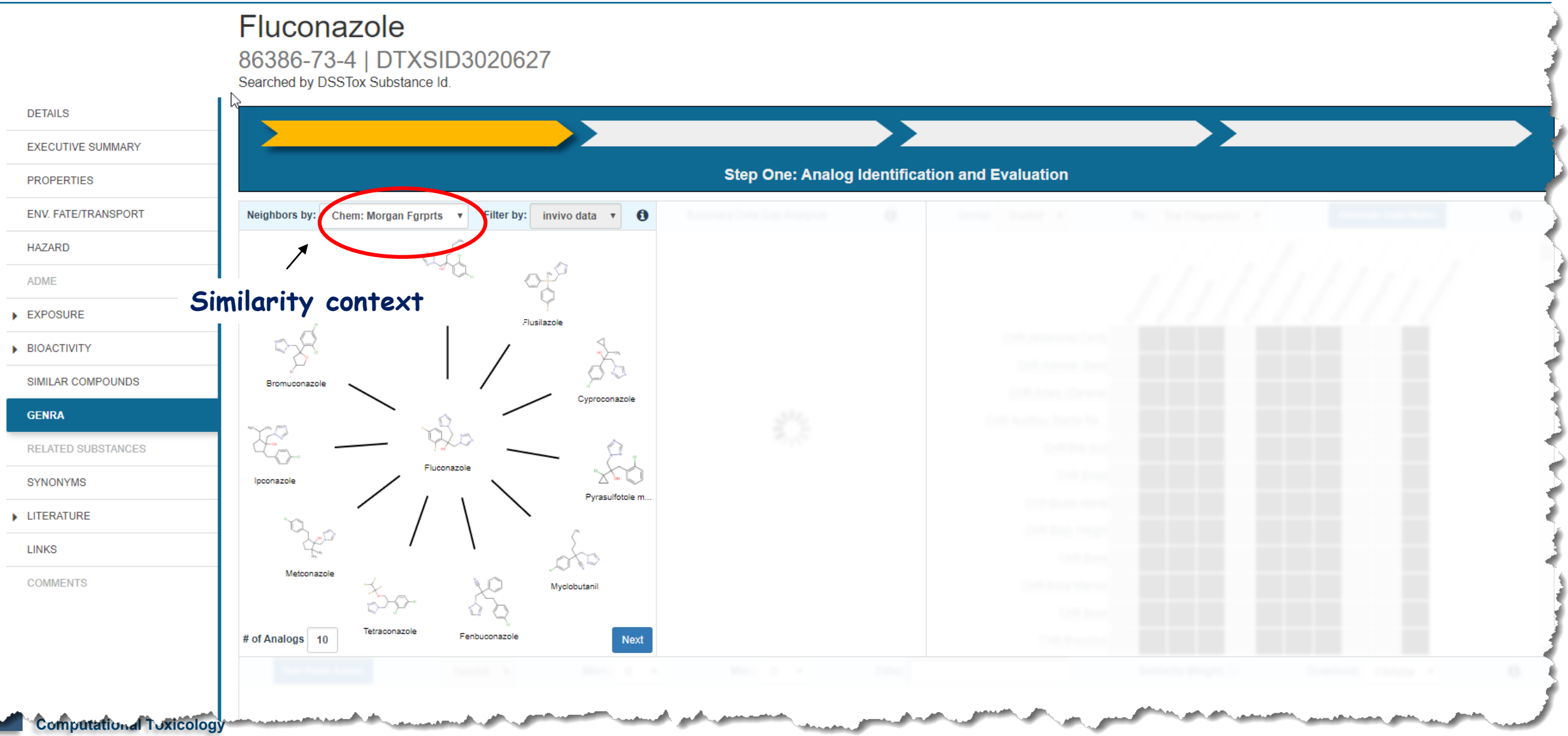
Presence in Lists

Record Information

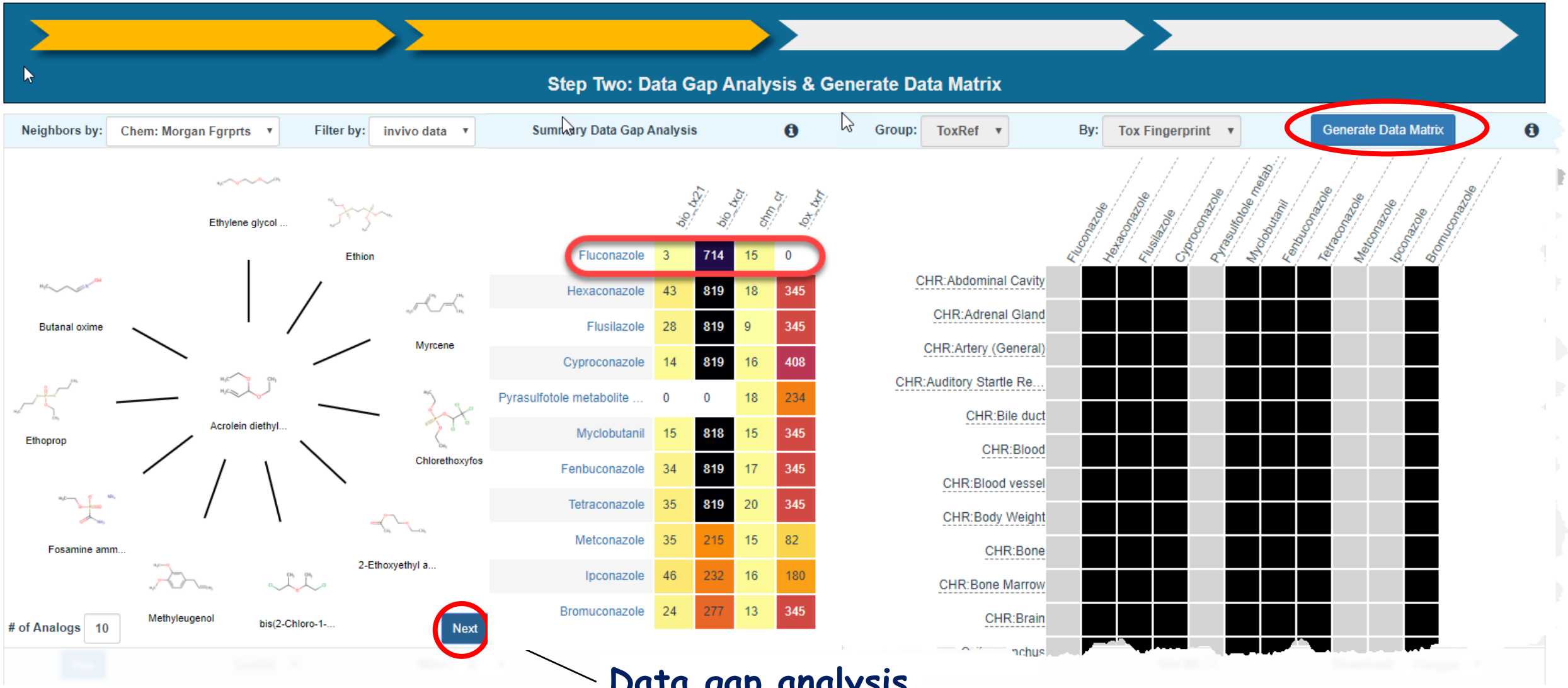
Quality Control Notes

GenRA tool in reality

- Structured as a workflow



GenRA tool in reality



Data gap analysis

GenRA tool in reality

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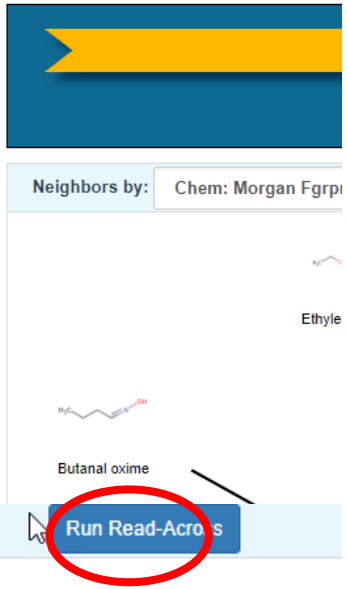

Run GenRA

Short Communication

Generalized Read-Across (GenRA): A workflow implemented into the EPA CompTox Chemicals Dashboard

George Helman[1,2], Imran Shah[2], Antony J. Williams[2], Jeff Edwards[2], Jeremy Dunne[2] and Grace Patlewicz[2]*

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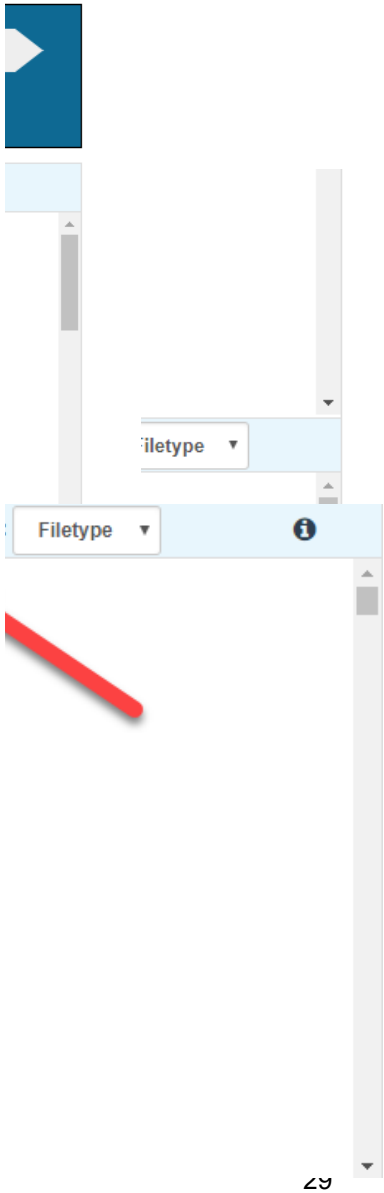


Abstract

Generalized Read-Across (GenRA) is a data driven approach which makes read-across predictions on the basis of a similarity weighted activity of source analogues (nearest neighbors). GenRA has been described in more detail in the literature (Shah et al., 2016; Helman et al., 2018). Here we present its implementation within the EPA's CompTox Chemicals Dashboard to provide public access to a GenRA module structured as a read-across workflow. GenRA assists researchers in identifying source analogues, evaluating their validity and making predictions of *in vivo* toxicity effects for a target substance. Predictions are presented as binary outcomes reflecting presence or absence of toxicity together with quantitative measures of uncertainty. The approach allows users to identify analogues in different ways, quickly assess the availability of relevant *in vivo* data for those analogues and visualize these in a data matrix to evaluate the consistency and concordance of the available experimental data for those analogues before making a GenRA prediction. Predictions can be exported into a tab-separated value (TSV) or Excel file for additional review and analysis (e.g., doses of analogues associated with production of toxic effects). GenRA offers a new capability of making reproducible read-across predictions in an easy-to use-interface.

GenRA – Next Steps

- Ongoing research:
- Summarising and aggregating the toxicity effect predictions to guide end users – what are the effects to be concerned about and which effect predictions are we most confident about
- Consideration of other information to define and refine the analogue selection – e.g. physicochemical similarity, metabolic similarity, reactivity similarity…
  - EPA New Chemical Categories
  - Quantifying the impact of physicochemical similarity on read-across performance

GenRA – Next Steps

- Dose response information to refine scope of prediction beyond binary outcomes
  - Transitioning from qualitative to quantitative predictions – how to apply and interpret GenRA in screening level hazard assessment
  - Starting with quantitative data – e.g. acute rat oral toxicity, ToxRefDB v2

GenRA & Physchem Similarity Context

- Important context of similarity in read-across
- Models "bioavailability"
- Properties selected: Lipinski Rule of 5 (LogP, MW, # HB donors/acceptors)
- Two approaches investigated as a means to identify source analogs and evaluate their predictive performance relative to GenRA:

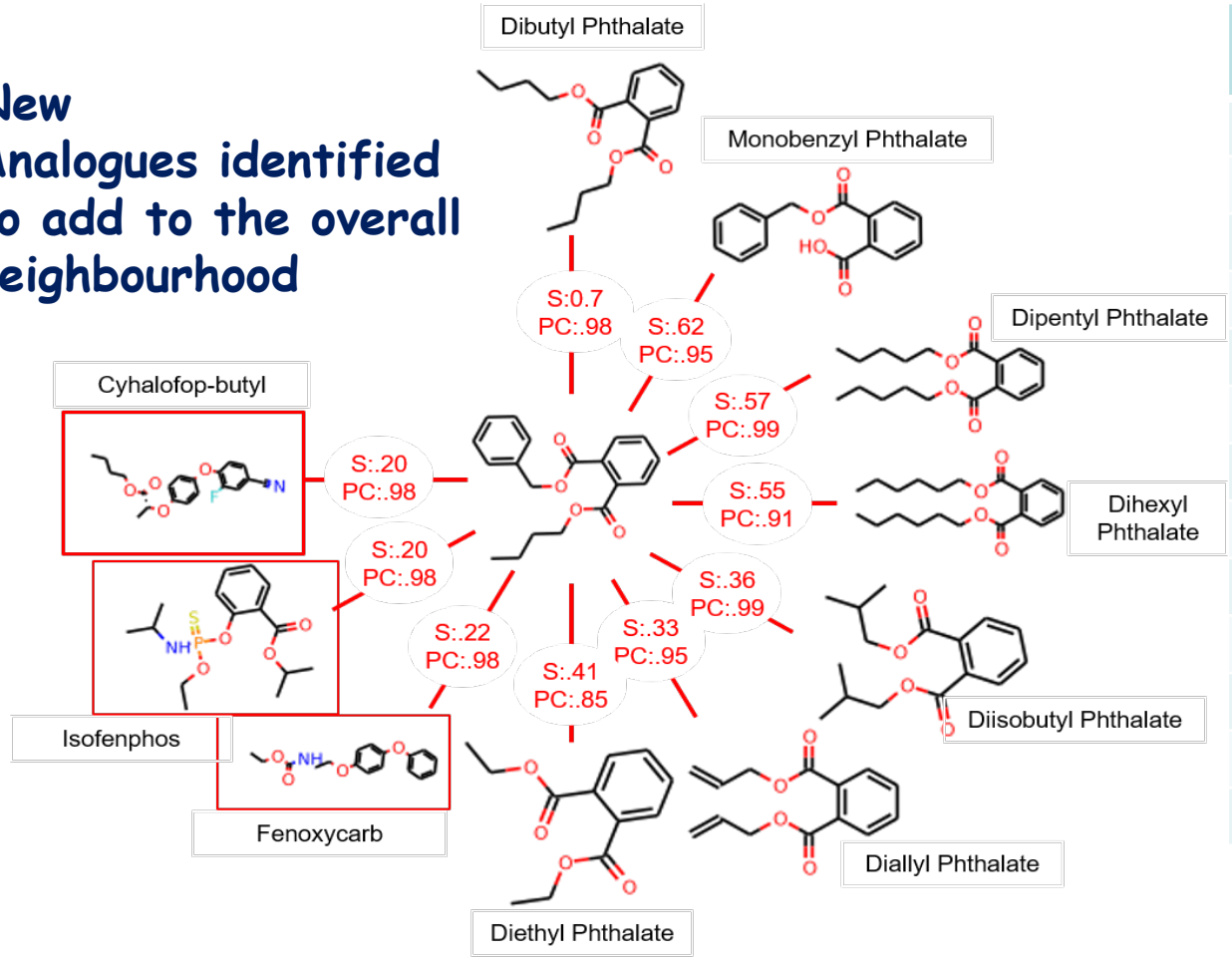
Approach 1: "Filter"	Approach 2: "Search Expansion"
Subcategorise from a set of analogues identified based on structural similarity	"Frontload" both structure and physchem into analogue identification
'Common' approach	'Novel' approach

Helman et al., 2018

Case Study: Butyl Benzyl Phthalate

Approach 2: Search Expansion

New Analogues identified to add to the overall neighbourhood

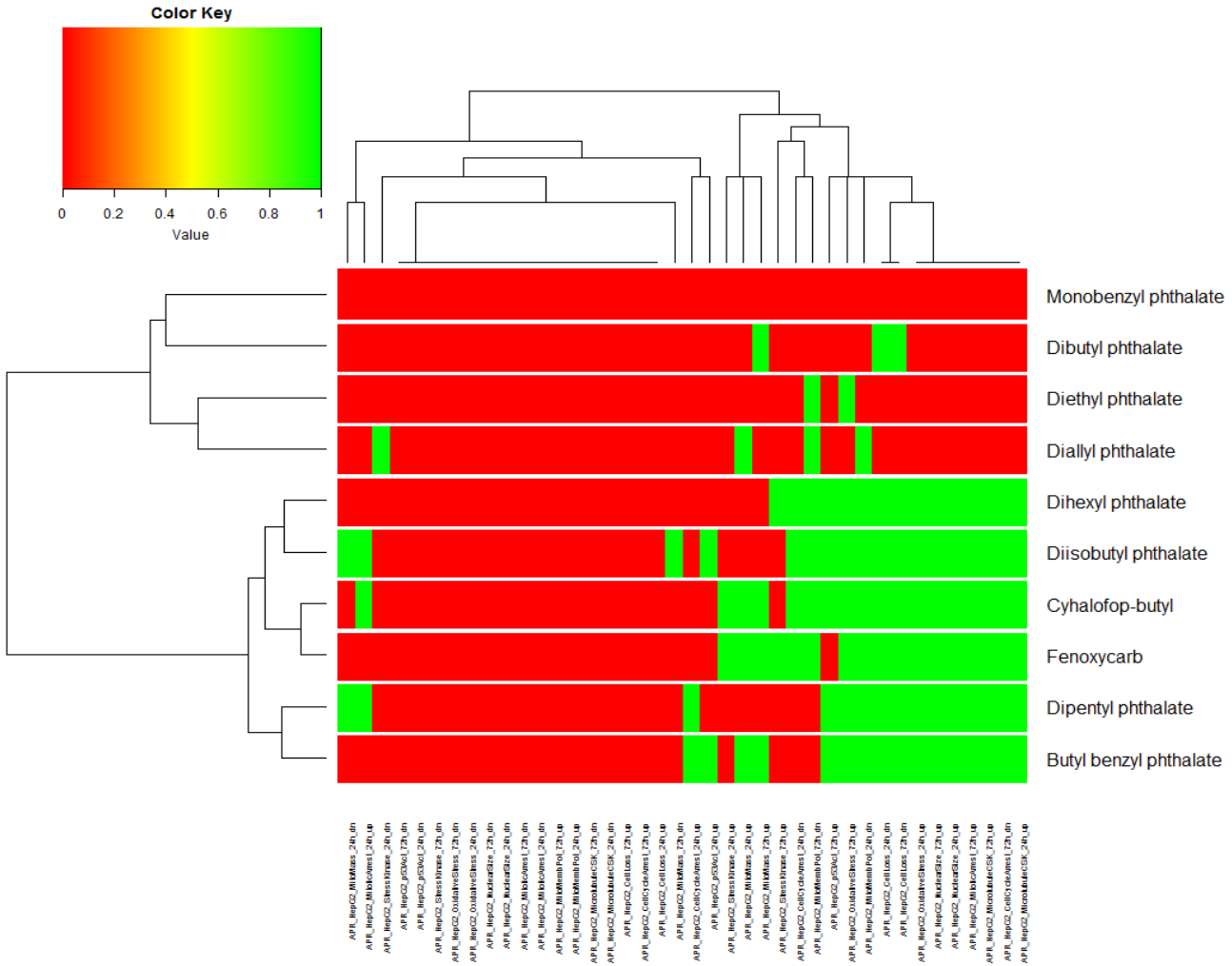


Endpoint	Baseline Prediction	Structure + Pchem Prediction
Body Weight	.78	.79
Clinical Chemistry	.27	.60
Food Consumption		
Hematology		
Kidney		
Liver		
Mortality		
Pancreas		
Prostate		
Skin	.27	.21
Spleen	0	.20
Tissue NOS	0	0
Urinary Bladder	0	0

- Adding phys-chem to similarity search overturns incorrect predictions for 2 endpoints
- Improves many others

Case Study: Butyl Benzyl Phthalate

Approach 2: Search Expansion



- Are the non phthalate analogues plausible from a biological similarity context?
- Heatmap of ToxCast bioactivity profiler from one (Apredica) technology
- From a qualitative perspective – these non phthalates exhibit similarity wrt their bioactivity profile to the target and other source phthalates

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Available online 23 July 2018
In Press, Corrected Proof

Extending the Generalised Read-Across approach (GenRA): A systematic analysis of the impact of physicochemical property information on read-across performance

George Helman [a, b], Imran Shah [b], Grace Patlewicz [b]
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https://doi.org/10.1016/j.comtox.2018.07.001

Highlights

- GenRA approach is summarised in the context of the category workflow.
- The impact of physicochemical information on read-across performance was assessed in 2 ways: filtering and search expansion.
- Search expansion resulted in an up to 9% improvement in read-across performance for 10 of the 50 data rich target organs.
- Results are summarised on a neighbourhood (chemical category) basis.
- A case study substance is used to compare and contrast the read-across performance using the 2 approaches.

Refinements to the GenRA approach

- Transitioning GenRA from binary predictions to quantitative predictions
- Investigated extending GenRA using the acute oral rat systemic toxicity data collected as part of the ICCVAM Acute toxicity workgroup
- NICEATM-NCCT effort to collate a large dataset of acute oral toxicity to evaluate the performance of existing predictive models and investigate the feasibility of developing new models

Refinements to the GenRA approach: Acute toxicity

Database Resource	Rows of Data (number of LD50 values)	Unique CAS
ECHA (ChemProp)	5533	2136
JRC AcutoxBase	637	138
NLM HSDB	4082	2238
OECD (eChemPortal)	10206	2314
PAI (NICEATM)	364	293
TEST (NLM ChemIDplus)	13689	13545

Rat oral LD50s:
16,297 chemicals total
34,508 LD50 values

Require unique LD50 values with mg/kg units

15,688 chemicals total
21,200 LD50 values

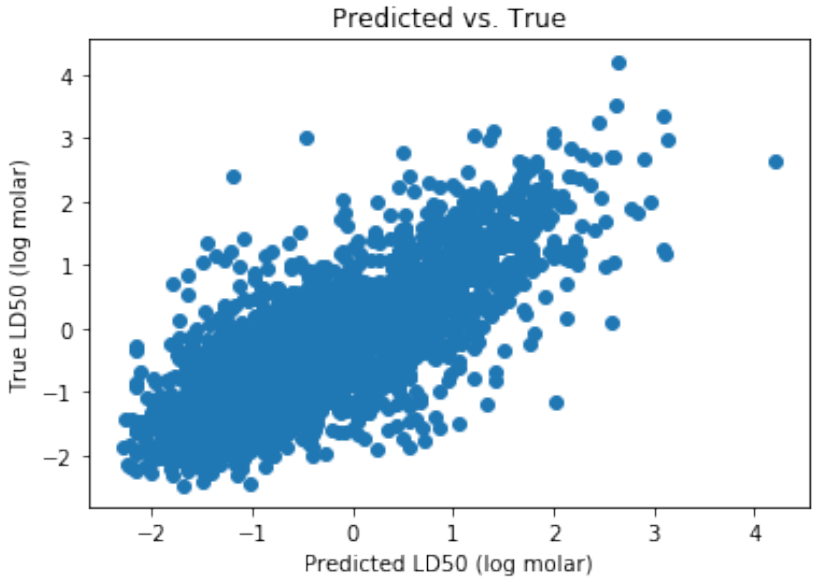
Preprocessing for modelling

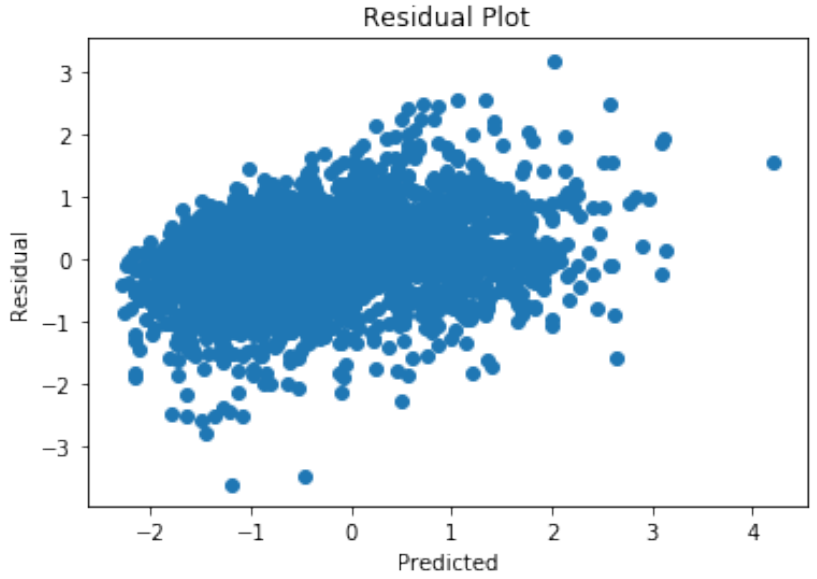
11,992 chemicals
16,209 LD50 values

Karmaus et al, 2018; Kleinstreuer et al., 2018

Refinements to the GenRA approach: Acute toxicity

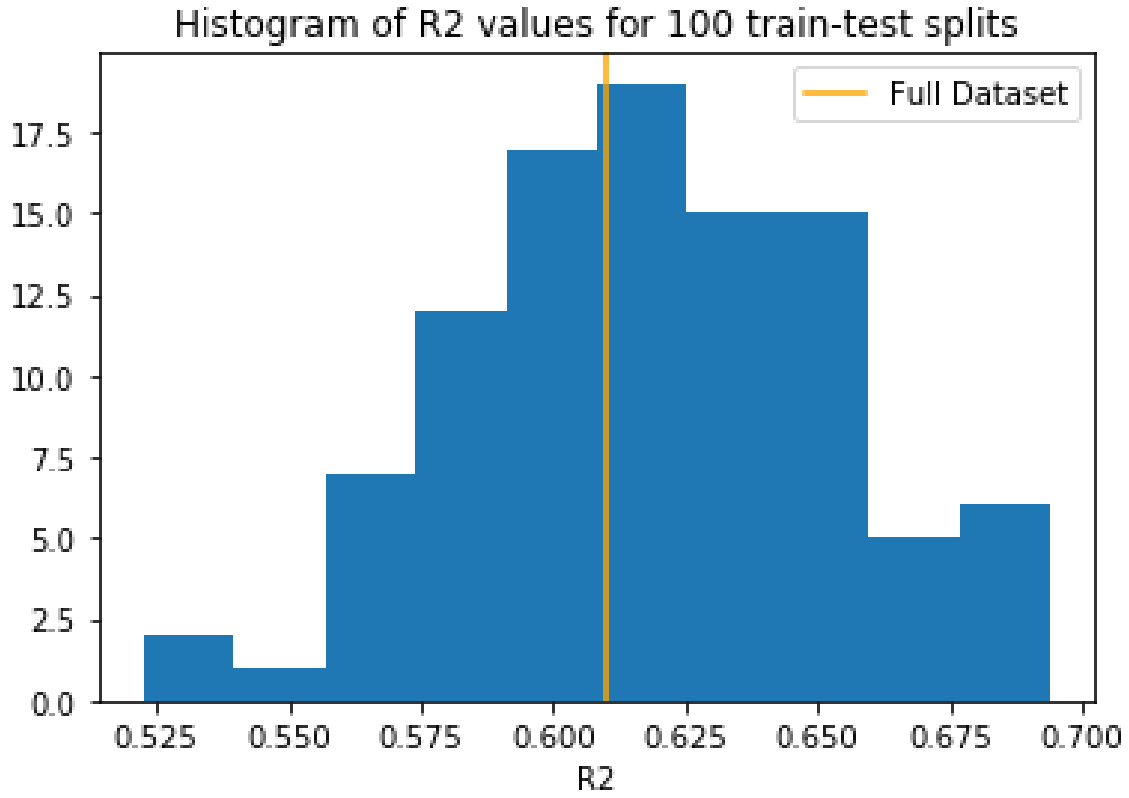
- Search for a maximum of 10 nearest neighbours on entire dataset
- Use a similarity threshold of 0.5





- R^2 = 0.61
- RMSE = 0.58
- A few outliers, but not too extreme
- Residuals clustered around zero with no obvious patterns

Refinements to the GenRA approach: Acute toxicity



- 75-25 train-test splits
- R^2 values range from 0.52 to 0.69
- GenRA performs strongly and robustly on this acute tox data set.

Helman et al., in preparation

Conclusions

- Current workflows for developing category/analogue approaches follows a series of steps
- There are many similarities between them – a harmonised version has been proposed
- There are many sources of uncertainty and proposals to address these for read-across to be more routinely accepted
- Many read-across tools exist that align to the workflow steps
- To move towards quantifying uncertainties we need to consider different approaches to structuring read-across – that will perform objective measures of performance to be determined
- GenRA has been used to illustrate some of the possibilities

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