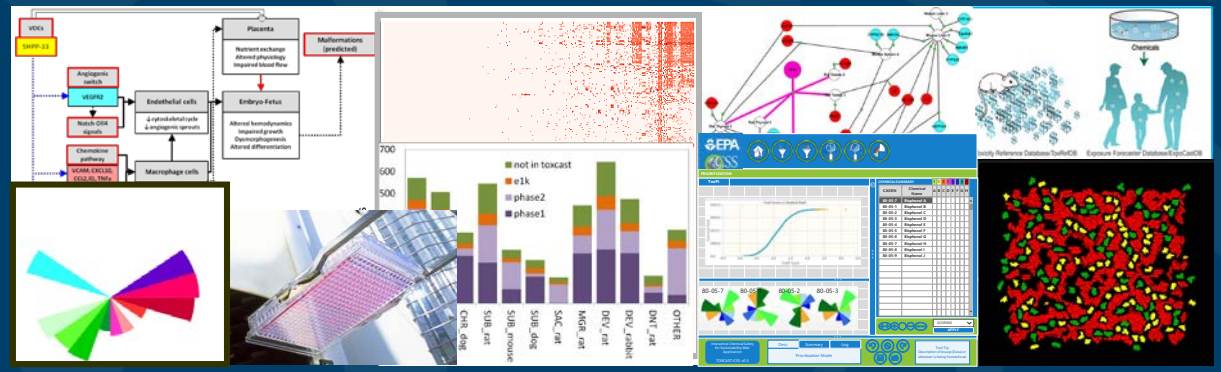
EPA United States Environmental Protection Agency

Building Scientific Confidence in the Development and Application of Tox21 Approaches



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- Rick Becker (ACC)
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Outline

- Acknowledgements
- Regulatory Drivers
- Computational (*in silico*) Toxicology
- Integrated Approaches to Testing and Assessment (IATA) – definitions and Adverse Outcome Pathway (AOP) informed
- Decision contexts and their impact on the approaches applied
- Risk-based prioritisation application
- Read-across approaches
- Summary remarks

Regulatory drivers

- Societal demands for safer and sustainable chemical products are stimulating changes in toxicity testing and assessment frameworks
- Chemical safety assessments are expected to be conducted faster and with fewer animals, yet the number of chemicals that require assessment is also rising with the number of different regulatory programmes worldwide.
- In the EU, the use of alternatives to animal testing is promoted.
- Animal testing is prohibited in some sectors e.g. cosmetics
- The European Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) legislation lays out specific information requirements, based on tonnage level triggers. However, the regulation explicitly expresses the need to use non-testing approaches to reduce the extent of experimental testing in animals.

Regulatory drivers

- REACH-like schemes also have been established in China, South Korea, and Turkey.
- In the US, the new Frank Lautenberg Chemical Safety for the 21st Century Act (LCSA) requires that a risk based prioritisation is conducted for all substances in commerce, some 80,000, many of which are lacking sufficient publicly available toxicity information.
- The LCSA also suggests developing alternative methods to reduce/refine animal testing.
- Risk based prioritisation is also an important aspect of regulatory frameworks in Canada (the Domestics Substance List), Australia and the EU.
- Non-testing approaches offer a means of facilitating the regulatory challenges in chemical safety assessment

Computational (*In Silico*) Toxicology

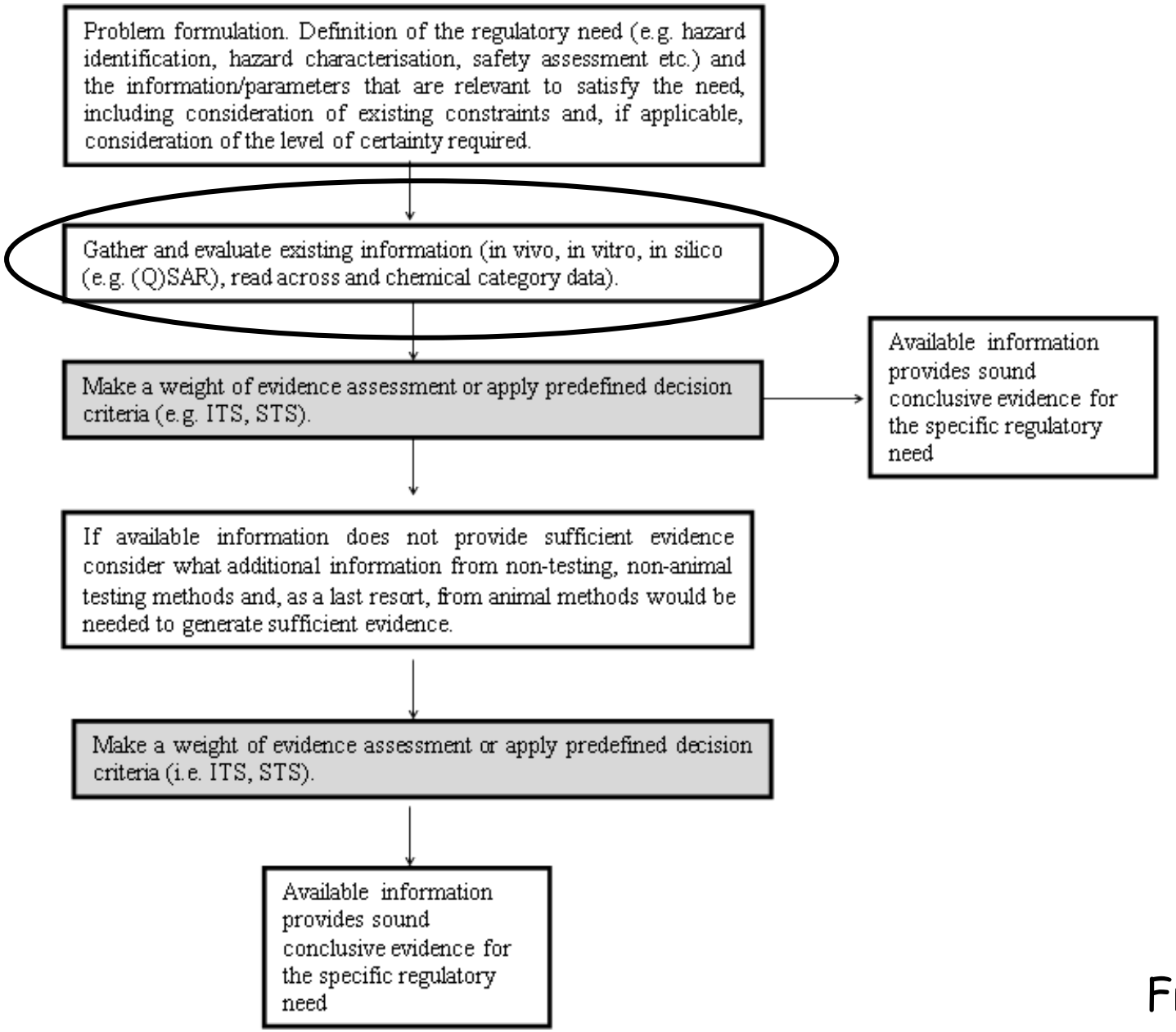
- Databases of existing information
- Structure-Ac
- Quantitative
- Expert Syste
- Category forn
- Bioinformatics
- Chemoinformatics
- Biokinetics (PBPK)

Integrated Approaches to Testing and Assessment (IATA)

- A means of integrating existing data and non-testing data together, determining what new information needs to be generated in order to make a decision with sufficient confidence for the purpose in mind

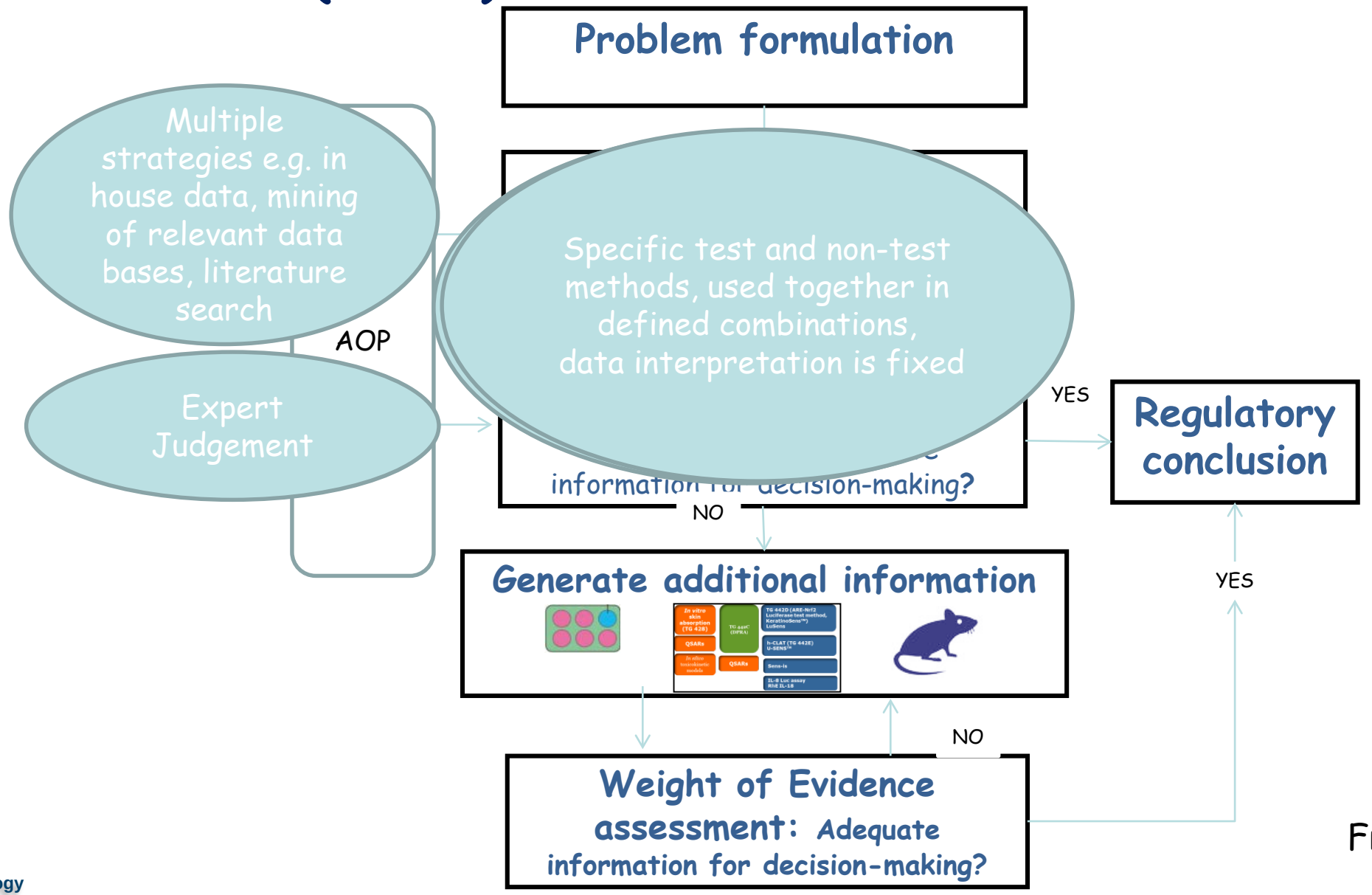
- IATA can be likened to workflows depicting the steps of gathering information for a substance and evaluate its fitness for purpose for the decision required

General framework of an IATA



From OECD

General workflow in Integrated Approaches to Testing and Assessment (IATA)



From OECD

Computational toxicology tools add value to most regulatory decisions

- Prioritisation
- Screening level hazard assessment
- Risk Assessment
- Exposure Assessment

Risk-Based prioritisation

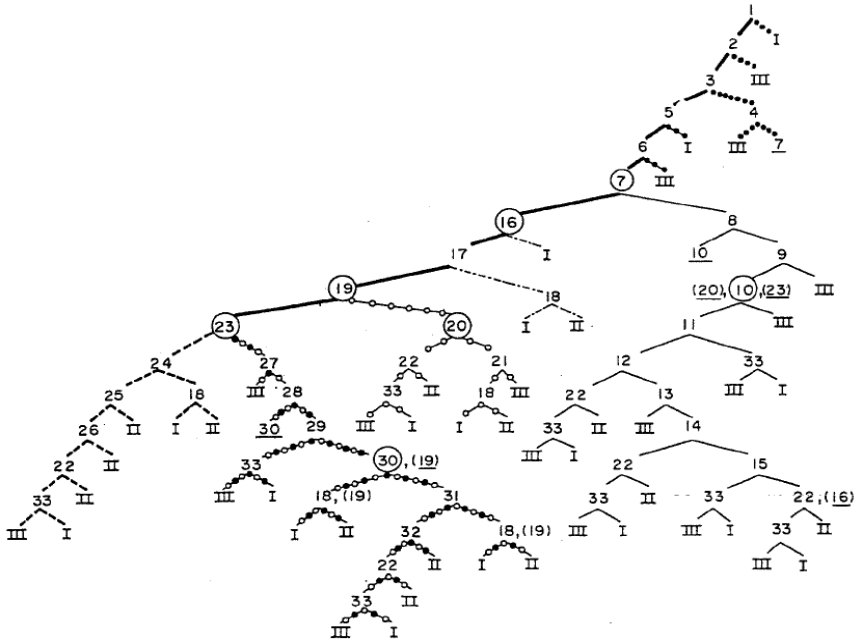
- Could involve a combination of available experimental data and new approach methods (NAMs) such as HTTR, HTS
- One approach considered involved coupling Threshold of Toxicological Concern (TTC) with High Throughput Exposure (HTE) modelling to rank order substances for further evaluation
- TTC is a principle that refers to the establishment of a human exposure threshold value for (groups of) chemicals below which there would be no appreciable risk to human health
- Relies on past accumulated knowledge regarding the distribution of potencies of relevant classes of chemicals for which good toxicity data do exist

TTC is based on a predicted tumour risk of 1 in a million, derived through an analysis of genotoxic chemicals

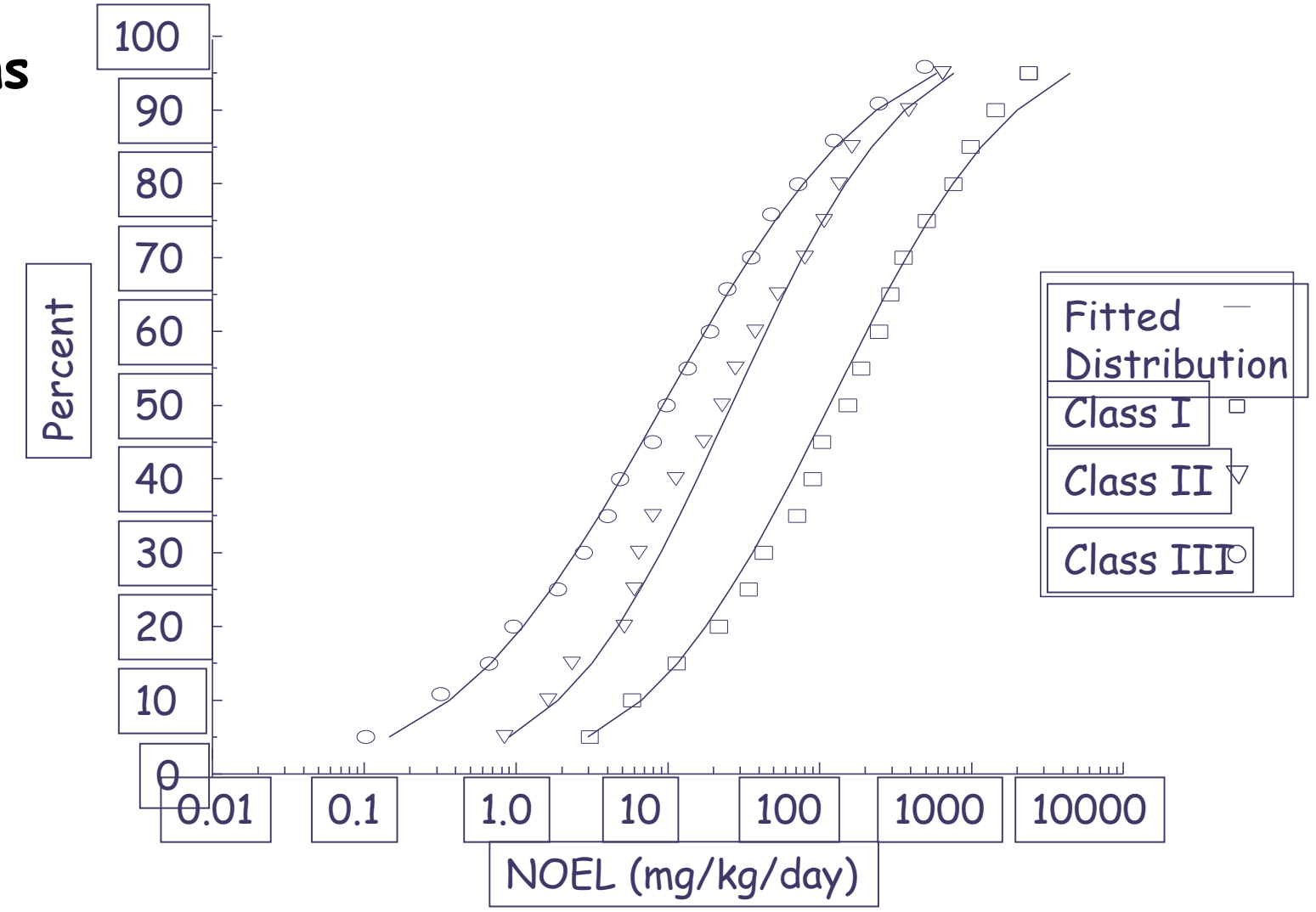
TTC is based on frequency distributions (5th percentile) of NO(A)ELs of non-genotoxic chemicals

Cumulative Distributions of chronic NOELs characterised by Cramer Structural Classes

- Decision tree of 33 questions



The 5th percentile NOEL was estimated for each structural class and this was in turn converted to the TTC limit by applying the conventional default safety/uncertainty factor of 100 (10X to account for extrapolation of animals to humans and 10X for human variability)



TTC values

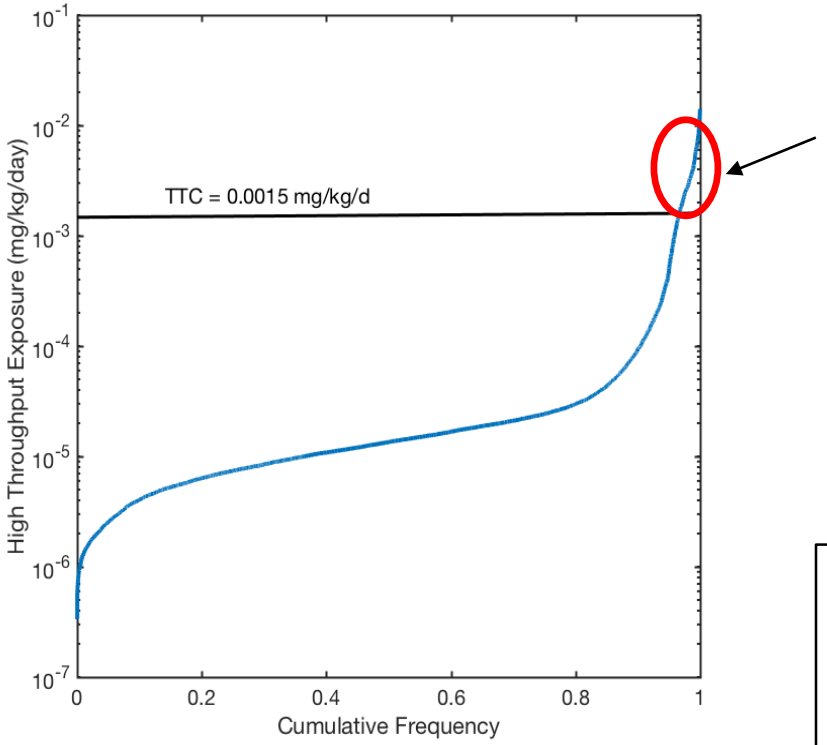
Type of substance	µg/person/day (µg/kg-day for 60 kg adult)
Alerts for potential genotoxic carcinogenicity	Kroes: 0.15 (0.0025 µg/kg-day) ICH: 1.5 (0.025 µg/kg-day)[a]
Acetylcholinesterase inhibitors (AChEI) Organophosphate/carbamate	18 (0.3 µg/kg-day)
Cramer Class III	90 (1.5 µg/kg-day)[b]
Cramer Class II	540 (9.0 µg/kg-day)
Cramer Class I	1800 (30 µg/kg-day)

Predicted HT exposures

- Wambaugh and colleagues (2014) developed a rapid heuristic high throughput exposure (HTE) model that enables prediction of potential human exposure to thousands of substances for which little or no empirical exposure data are available.
- The HTE model was calibrated by comparison to NHANES urinary data that reflects total exposure (all routes/sources)

Integrating TTC with predicted HT exposures

- Compared the conservative Cramer Class III TTC value of 1.5 µg/kg-day to the previously calculated median and upper 95% credible interval (UCI) of total daily median exposure rates for 7968 chemicals

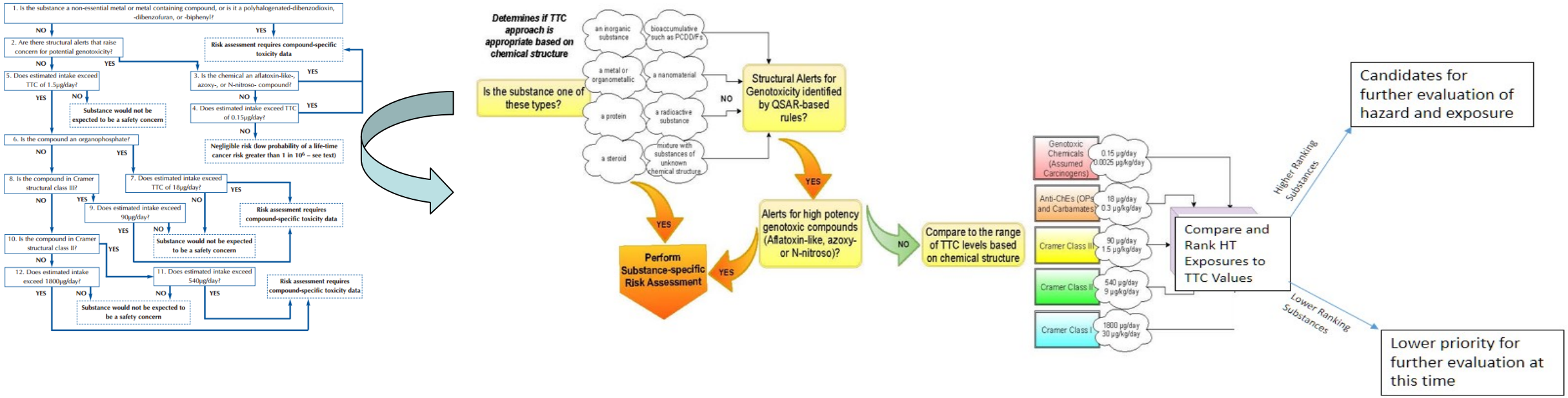


only 273 (fewer than 5%) were found to have UCI daily exposures estimates that exceeded the Cramer Class III TTC value of 1.5 µg/kg-day

Initial evaluation showed the approach of using the ratio of exposure to TTC (HTE: TTC) appeared promising for risk-based prioritisation

Risk-Based prioritisation

- Refined the approach using the Kroes et al structure-based workflow for TTC



- None of the substances categorised as Cramer Class I or Cramer Class II exceeded their respective TTC values.
- No more than 2% of substances categorised as Cramer Class III or acetylcholinesterase inhibitors exceeded their respective TTC values.
- Majority of chemicals with genotoxicity structural alerts did exceed the relevant TTC – recommendations were proposed for next steps

Presented at ASCCT 2017
Manuscript in clearance

Definitions: Chemical grouping approaches

- **Read-across** describes one of the techniques for filling data gaps in either the analogue or category approaches i.e. not to be confused with the "analogue approach"

- "**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).

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

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.

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

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
Grading scale	Each AE is scored by an	Low - High gradings which	None - possible strategies to	Low to High but no default

A harmonised read-across workflow

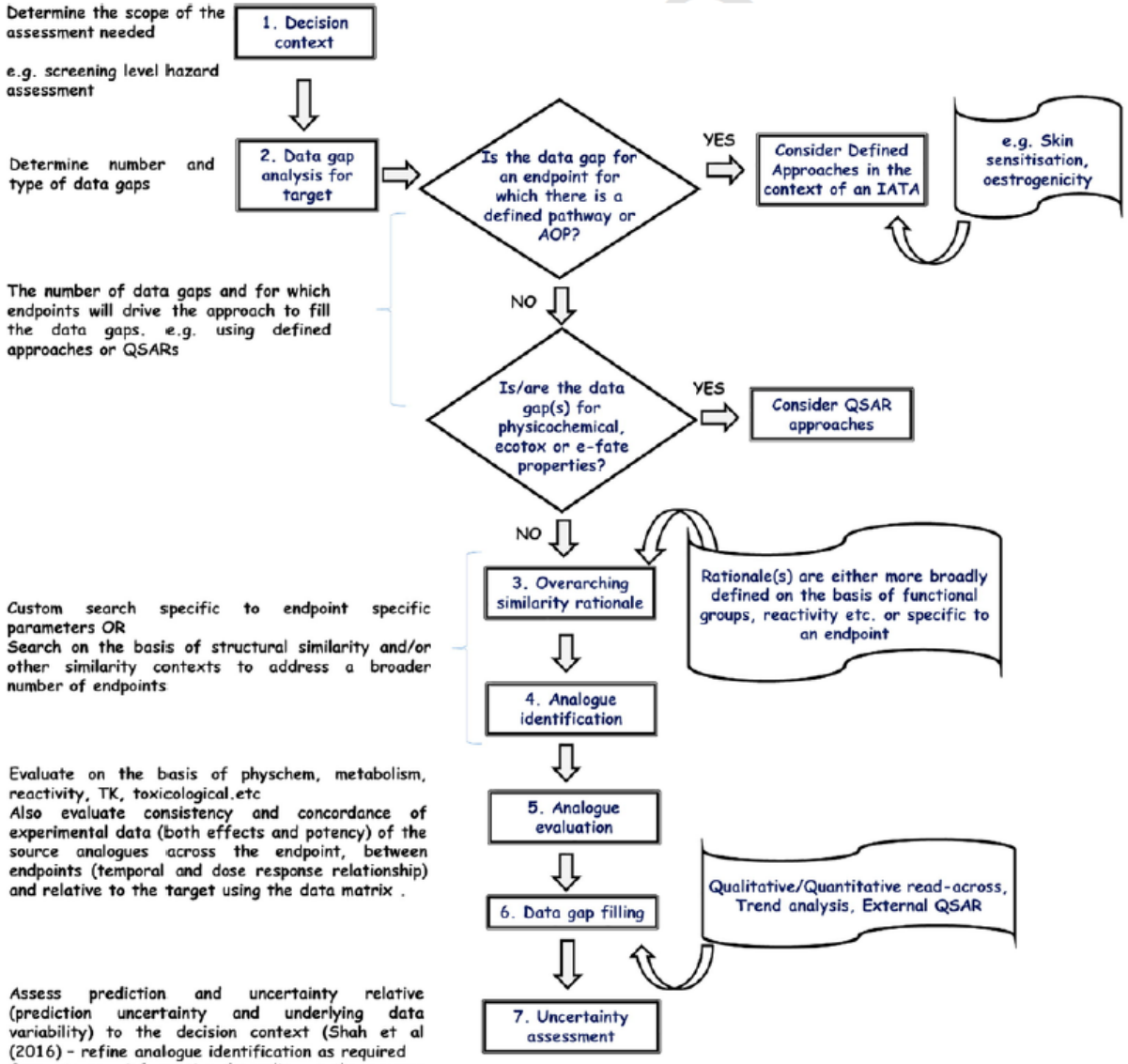


Fig. 9. A harmonised hybrid development and assessment framework.

Proposed in Patlewicz et al., 2018

EPA United States Environmental Protection

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Journal Cover Image

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
- One approach per Blackburn and Stuard (2014) is to use assessment factors
- Alternatively 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
- These examples rely on the qualitative use of NAM data and preferably in the context of an organising framework such as an AOP to ensure the appropriate biological context for interpretation

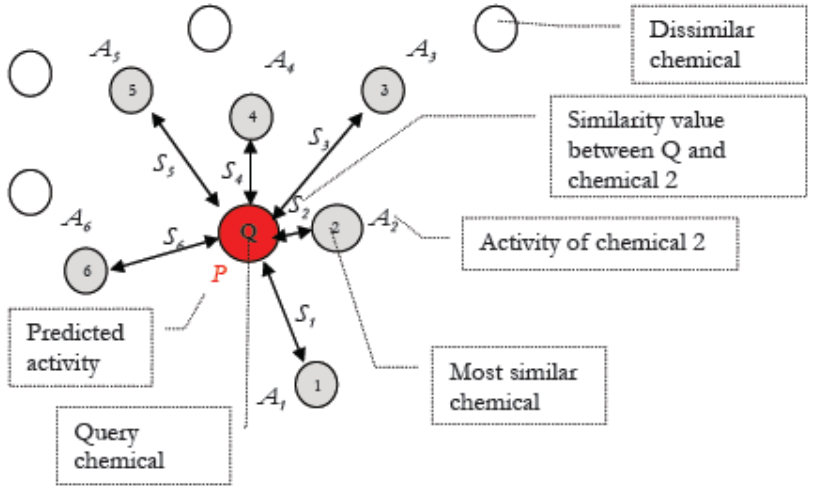
Ongoing issues with read-across

- Others such as Shah 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)

- Some of these efforts have been implemented into read-across tools

Quantifying Uncertainty & Assessing Performance of Read-Across

- **GenRA (Generalised Read-Across)**
- Predicting toxicity as a similarity-weighted activity of nearest neighbours based on chemistry and/or bioactivity descriptors
- Goal: to systematically evaluate read-across performance and uncertainty using available data
- The approach enabled a performance baseline for read-across predictions of toxicity effects within specific study outcomes to be established



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

Jaccard similarity:

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

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

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

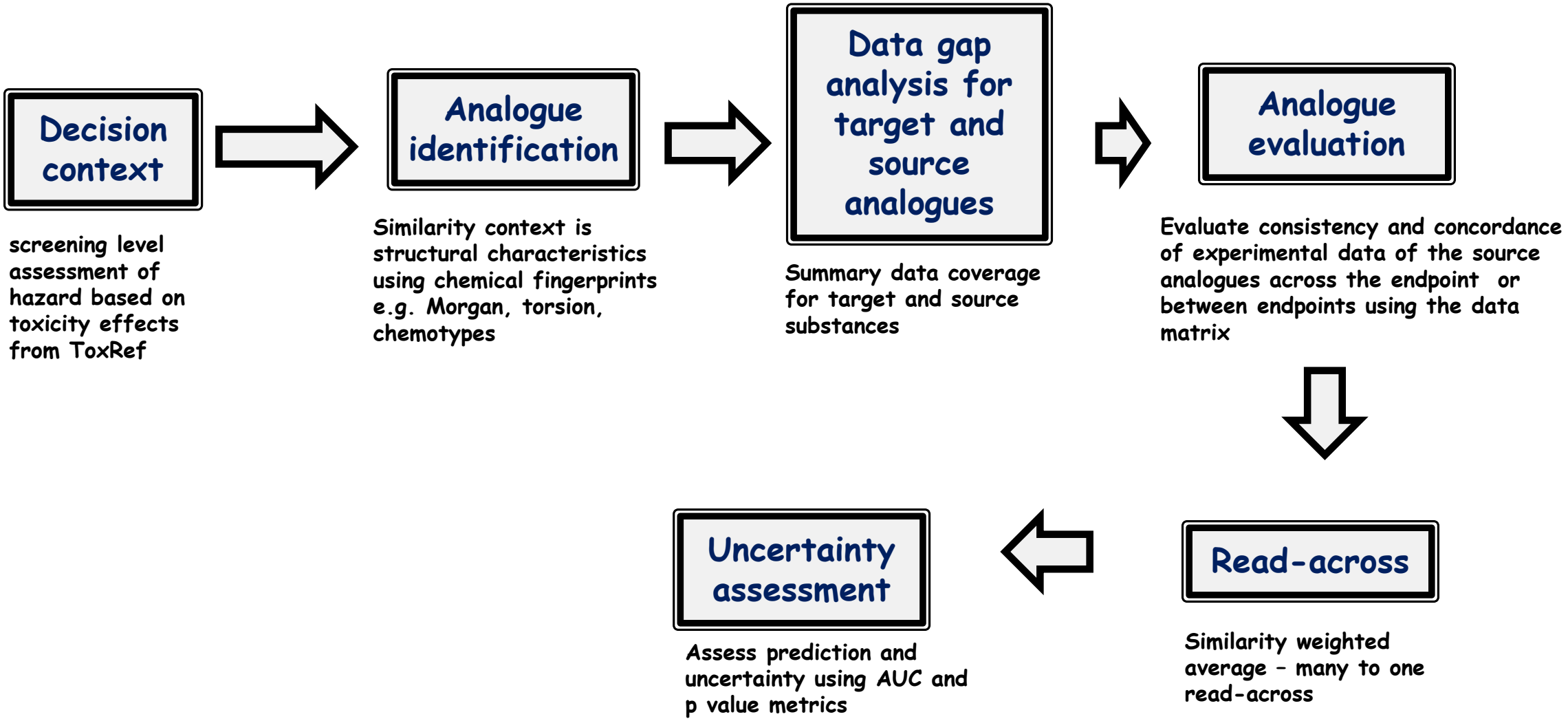
y_i = predicted activity of chemical (c_i)

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

Current Category Workflow in GenRA



Selected read-across tools

Tool	AIM	ToxMatch	AMBIT	OECD Toolbox	CBRA	ToxRead	GenRA
Analogue identification	X	X	X	X	X	X	X
Analogue Evaluation	NA	X	X by other tools available	X	X	X For Ames & BCF	NA
Data gap analysis	NA	X	X Data matrix can be exported	X Data matrix viewable	NA	NA	X Data matrix can be exported
Data gap filling	NA	X	User driven	X	X	X	X
Uncertainty assessment	NA	NA	NA	X	NA	NA	X
Availability	Free	Free	Free	Free	Free	Free	Beta for Internal testing

Selected read-across 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 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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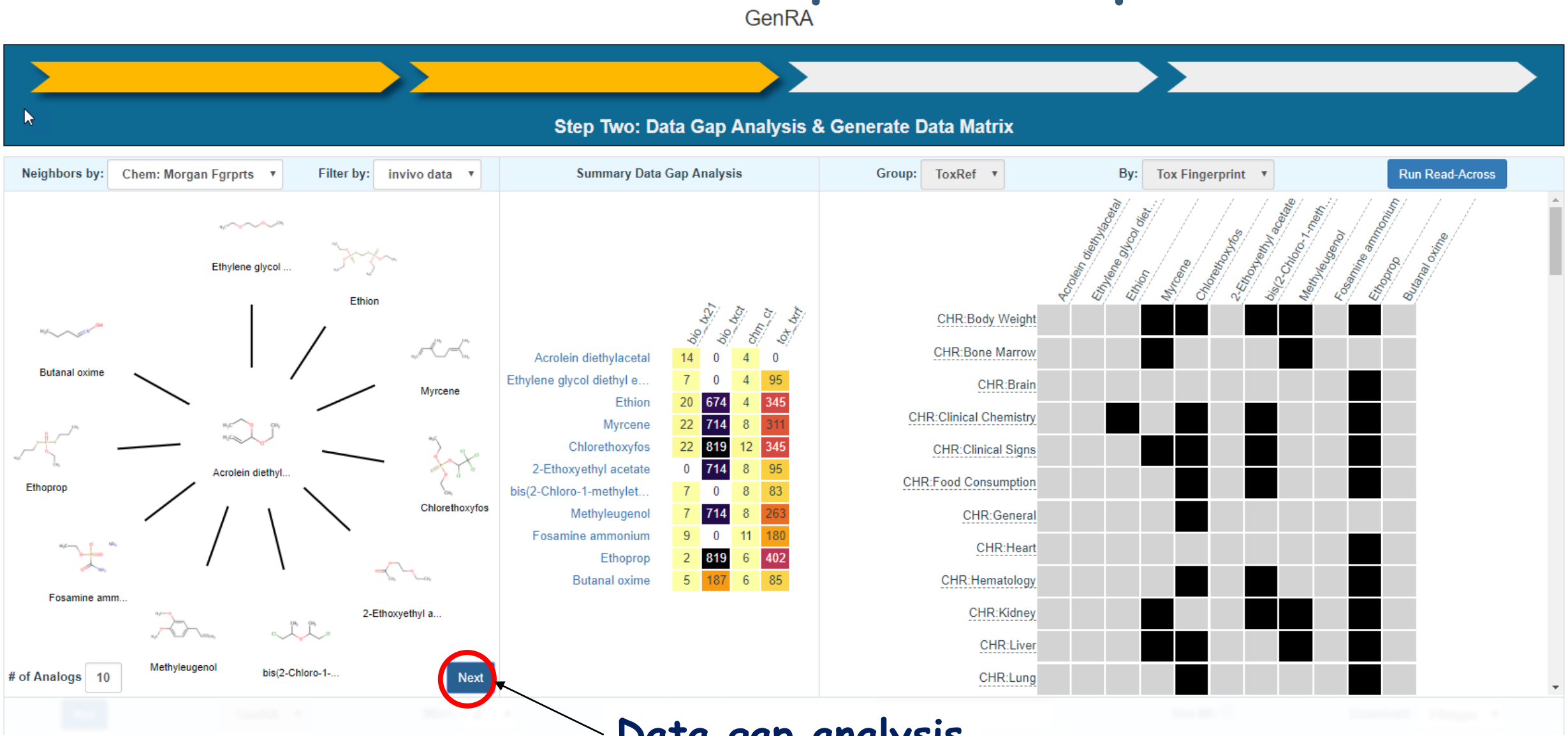
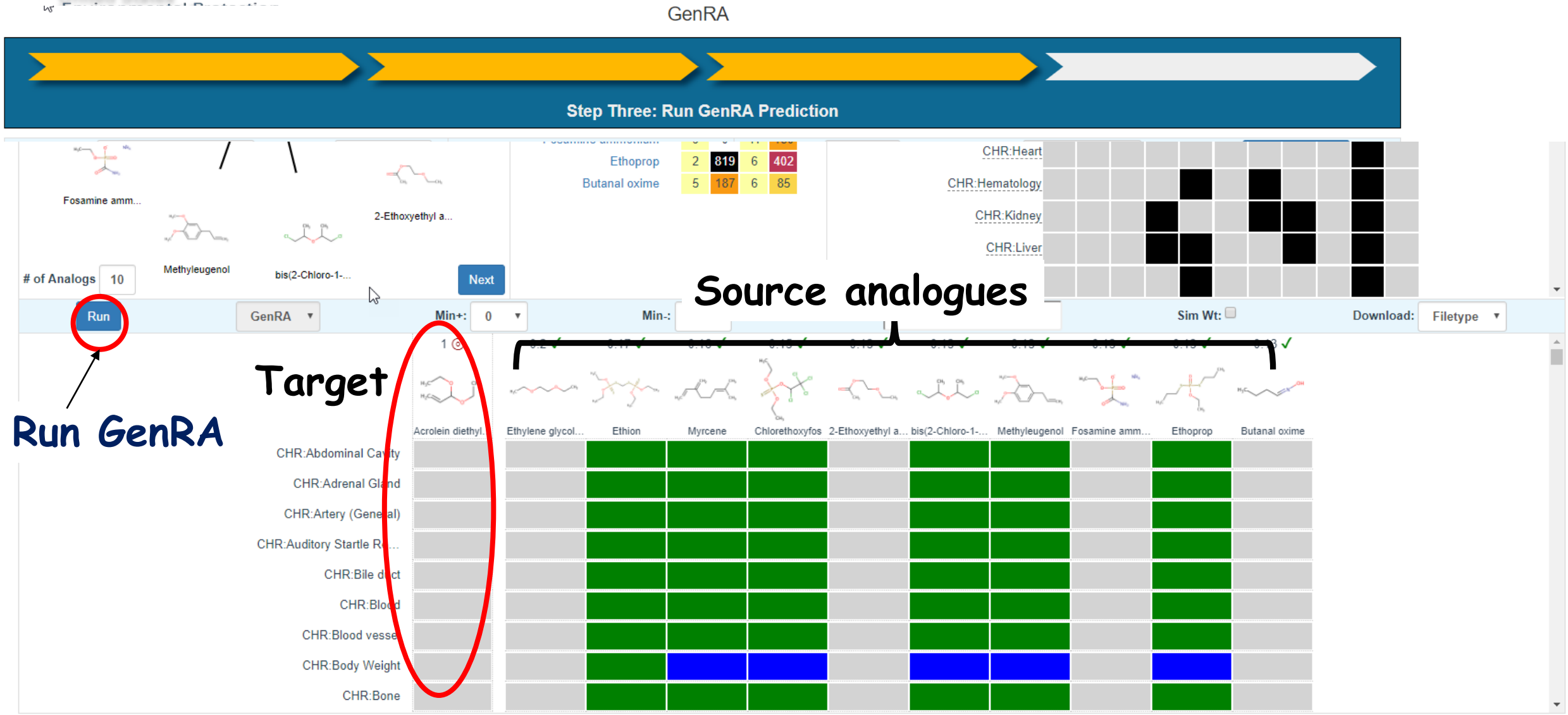
GenRA tool in development for public release

GenRA tool in development for public release



Data gap analysis

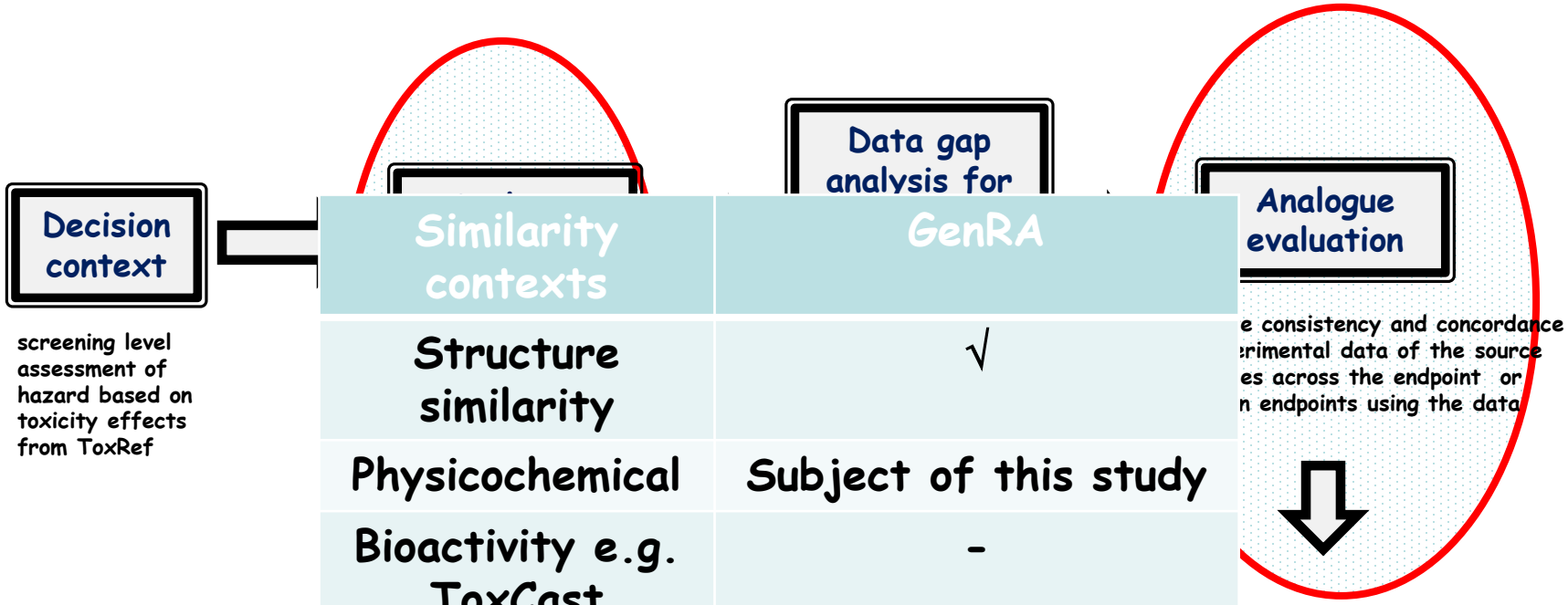
GenRA tool in development for public release



GenRA – Next Steps

- Ongoing analysis:
- Consideration of other information to refine the analogue selection – e.g. physicochemical similarity, TK similarity, metabolic similarity, reactivity similarity…
  - Quantifying the impact of physicochemical similarity on read-across performance
- 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

Refinements to the GenRA approach



Similarity contexts	GenRA
Structure similarity	√
Physicochemical	Subject of this study
Bioactivity e.g. ToxCast	-
Reactivity	-
Metabolic	-
Toxicokinetic	-

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"

Subcategorise from a set of analogues identified based on structural similarity

Common approach

Approach 2: "Search Expansion"

"Frontload" both structure and physchem into analogue identification

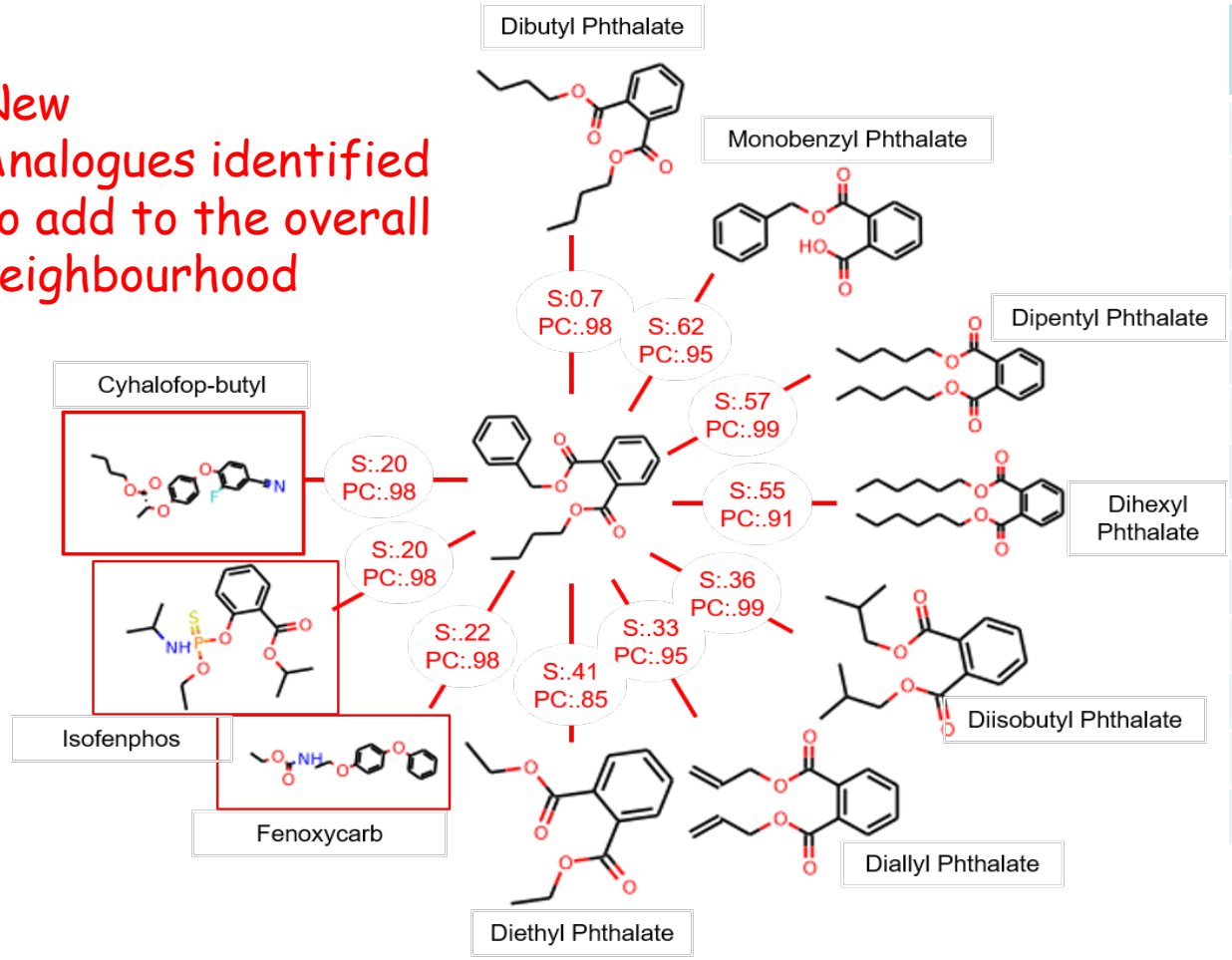
Novel approach

Presented at ACS 2018
Manuscript in clearance – Helman et al

Case Study: Butyl Benzyl Phthalate

Approach 2: Search Expansion

New
Analogues identified to add to the overall neighbourhood

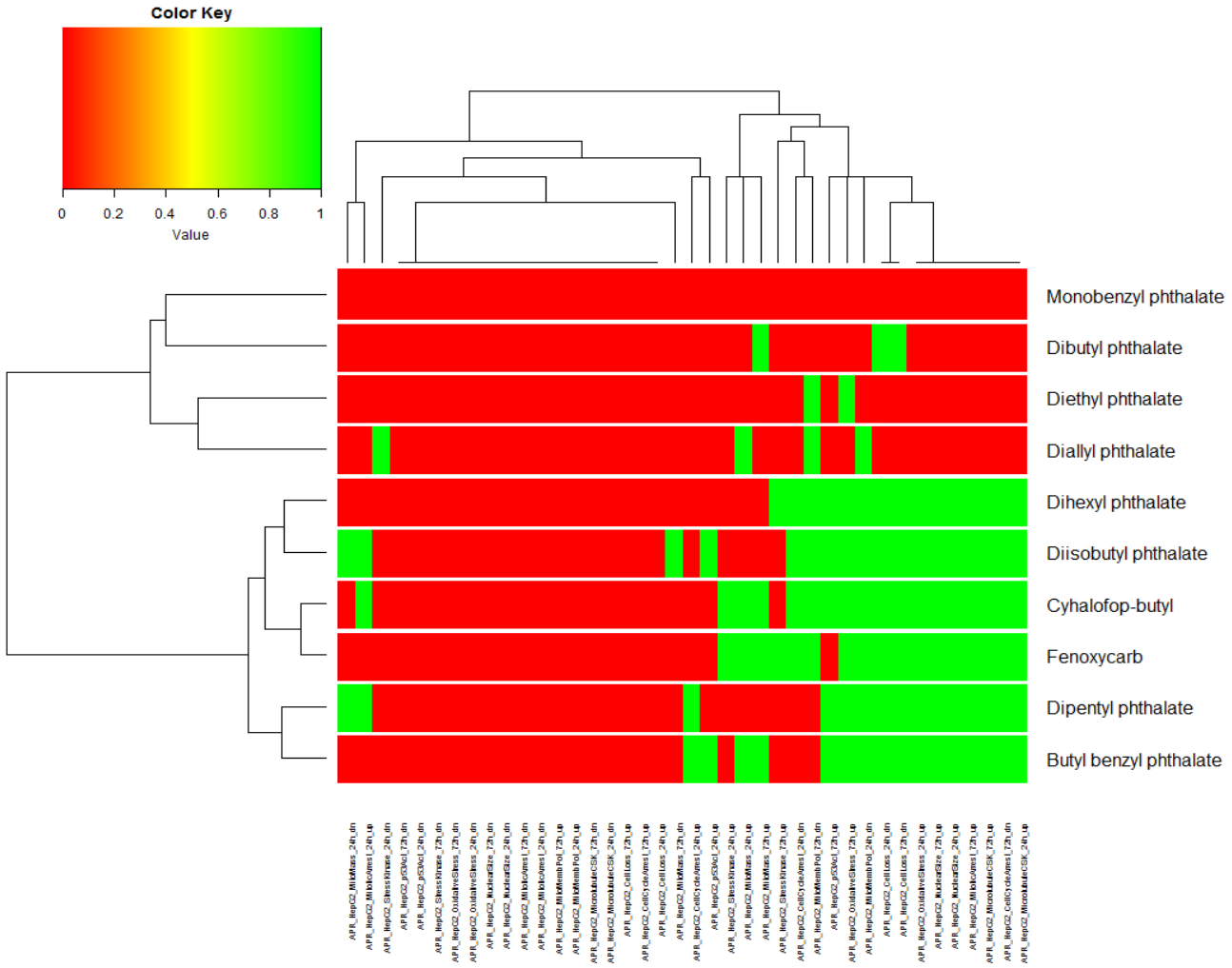


Endpoint	Baseline Prediction	Structure + Pchem Prediction
Body Weight	.78	.79
Clinical Chemistry	.27	.60
Food Consumption		
Hematology		
Kidney		
Liver		
Mortality		
Pancreas		
Prostate		
Skin		
Spleen		
Tissue NOS		
Urinary Bladder	0	0

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

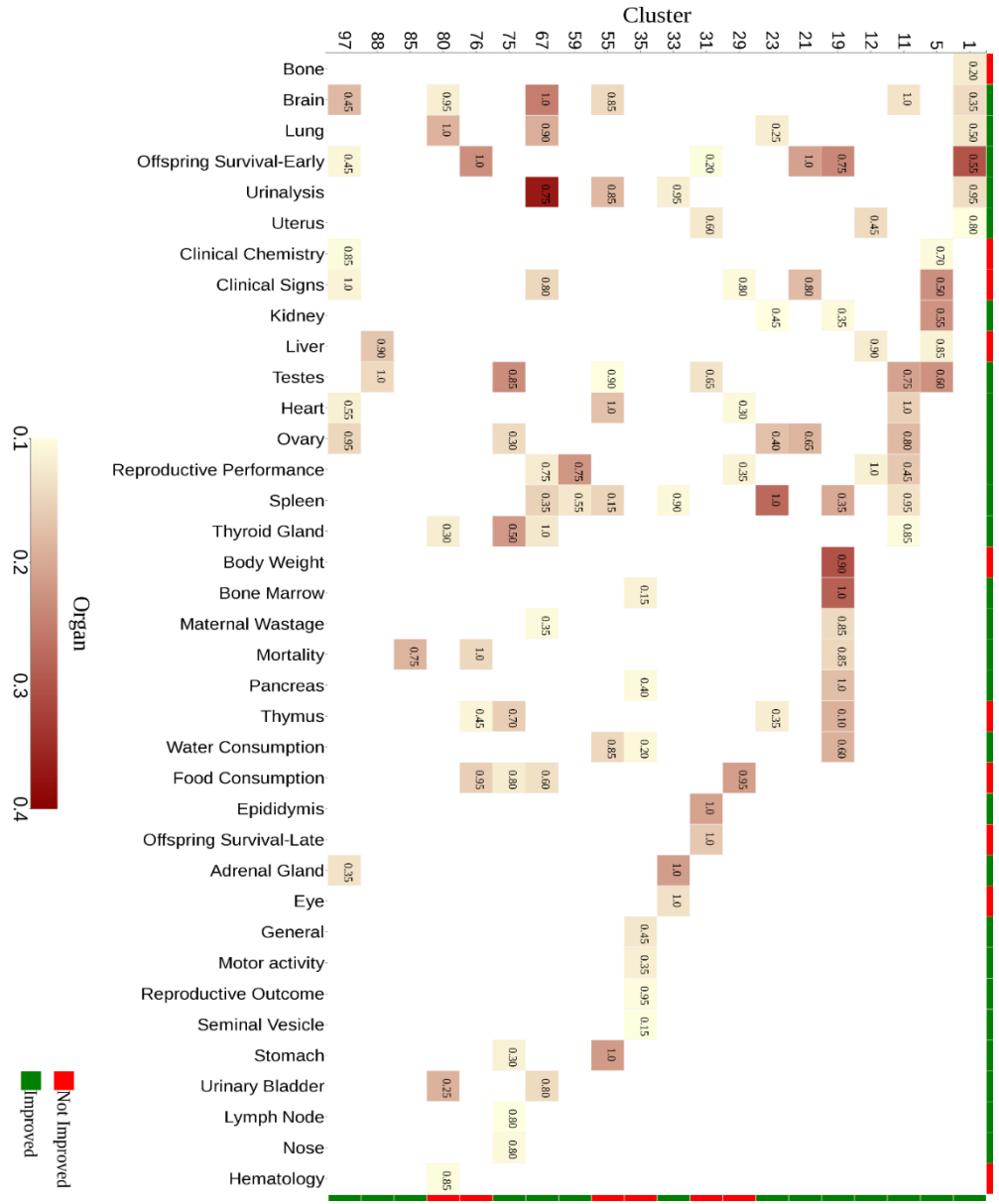
Case Study: Butyl Benzyl Phthalate

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

"Search expansion" in practice



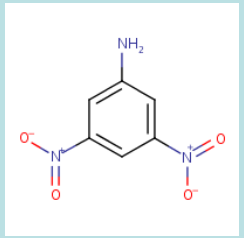
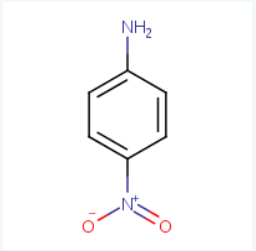
1) Identify target chemical

2) Perform Data gap analysis

3) Use cluster/organ key to guide selection of the optimal physicochemical threshold to use in source analogue identification for a specific toxicity effect of interest

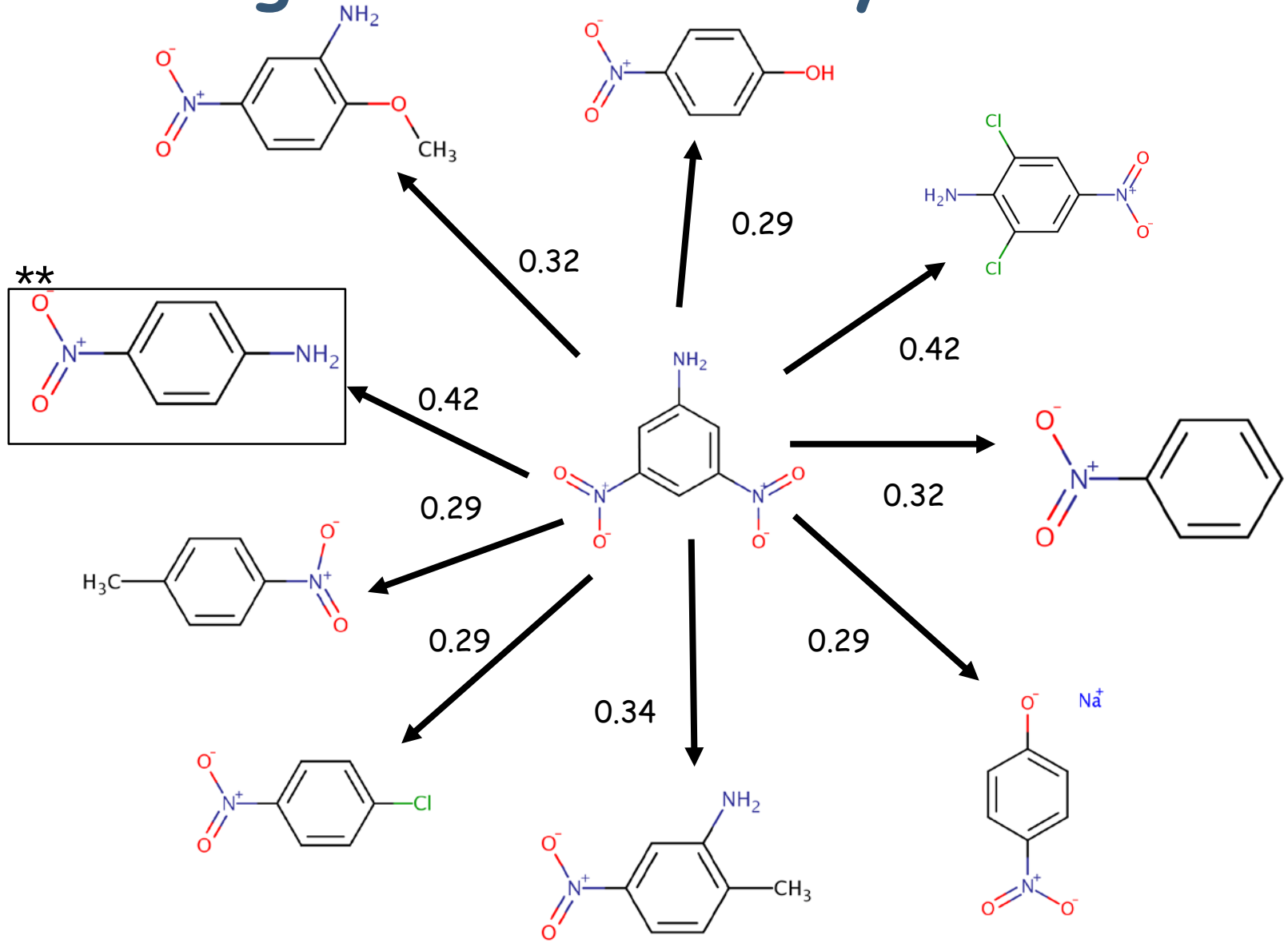
Presented at ACS 2018
Manuscript in clearance – Helman et al

Transitioning from qualitative to quantitative GenRA predictions

Target chemical	Proposed source analogue	Primary similarity rationale
Structural		
3,5-Dinitroaniline 	4-Nitroaniline 	Considerations for chemical class, structural moiety, reactivity, metabolism and toxicity were used to refine the pool of analogues. Selection of the source analogue is based on availability of toxicity values, duration of the principal study and health protectiveness of the adopted POD, given the commonalities in the toxicokinetic and toxicity profile for all the candidates.

Source analogues identified by GenRA

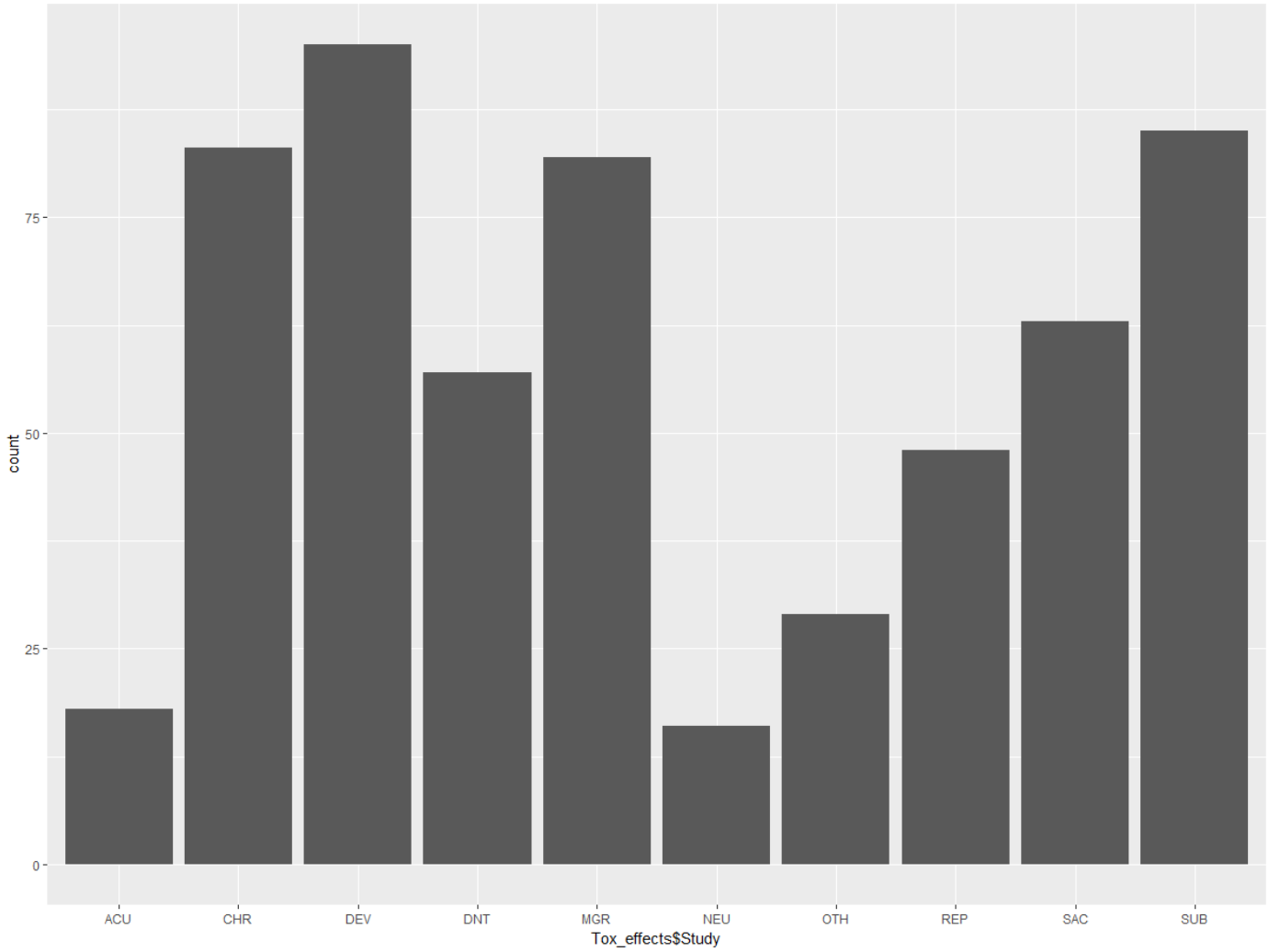
DTXSID	PREFERRED_NAME
DTXSID0020943	2-Methoxy-5-nitroaniline
DTXSID0021834	4-Nitrophenol
DTXSID0044151	**3,5-Dinitroaniline***
DTXSID2020426	Dicloran
DTXSID3020964	Nitrobenzene
DTXSID3027320	Sodium 4-nitrophenolate
DTXSID4020959	2-Methyl-5-nitroaniline
DTXSID5020281	1-Chloro-4-nitrobenzene
DTXSID5023792	4-Nitrotoluene
DTXSID8020961	**4-Nitroaniline****



*= Target ** = Proposed source by expert judgement

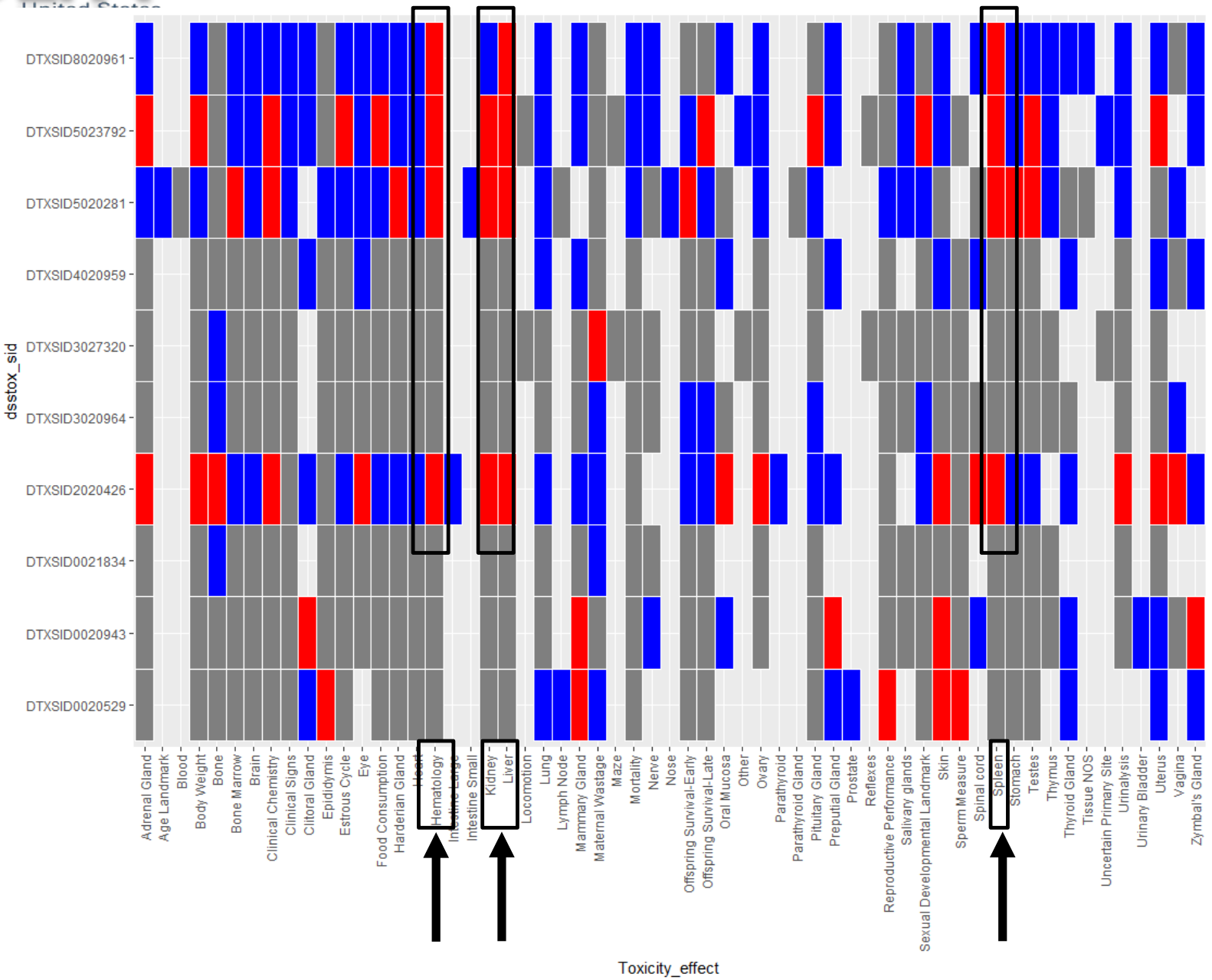
Analogues characterised by Morgan fingerprints

Available toxicity effects per study



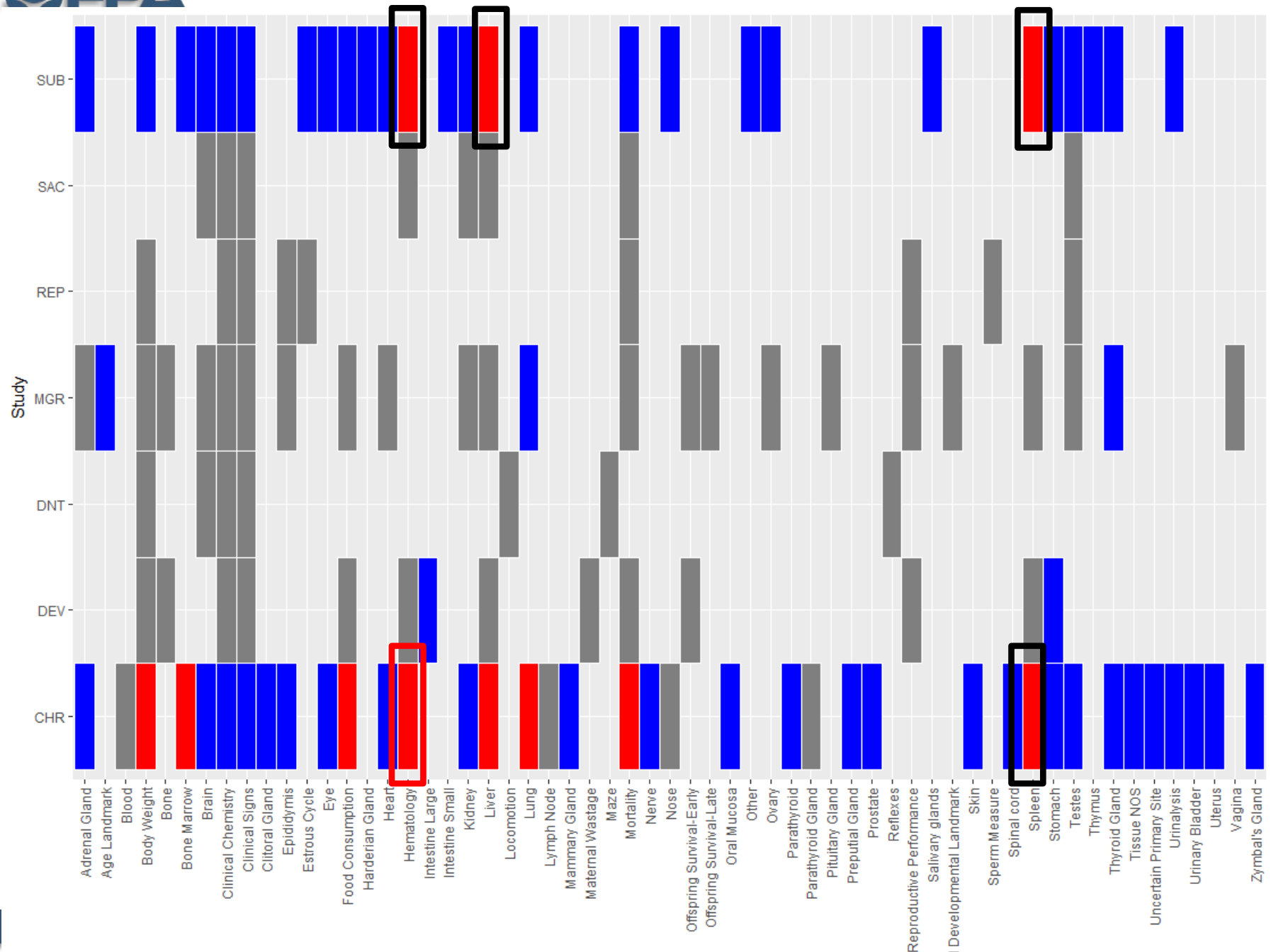
Available toxicity effects per study

Data matrix for source substances



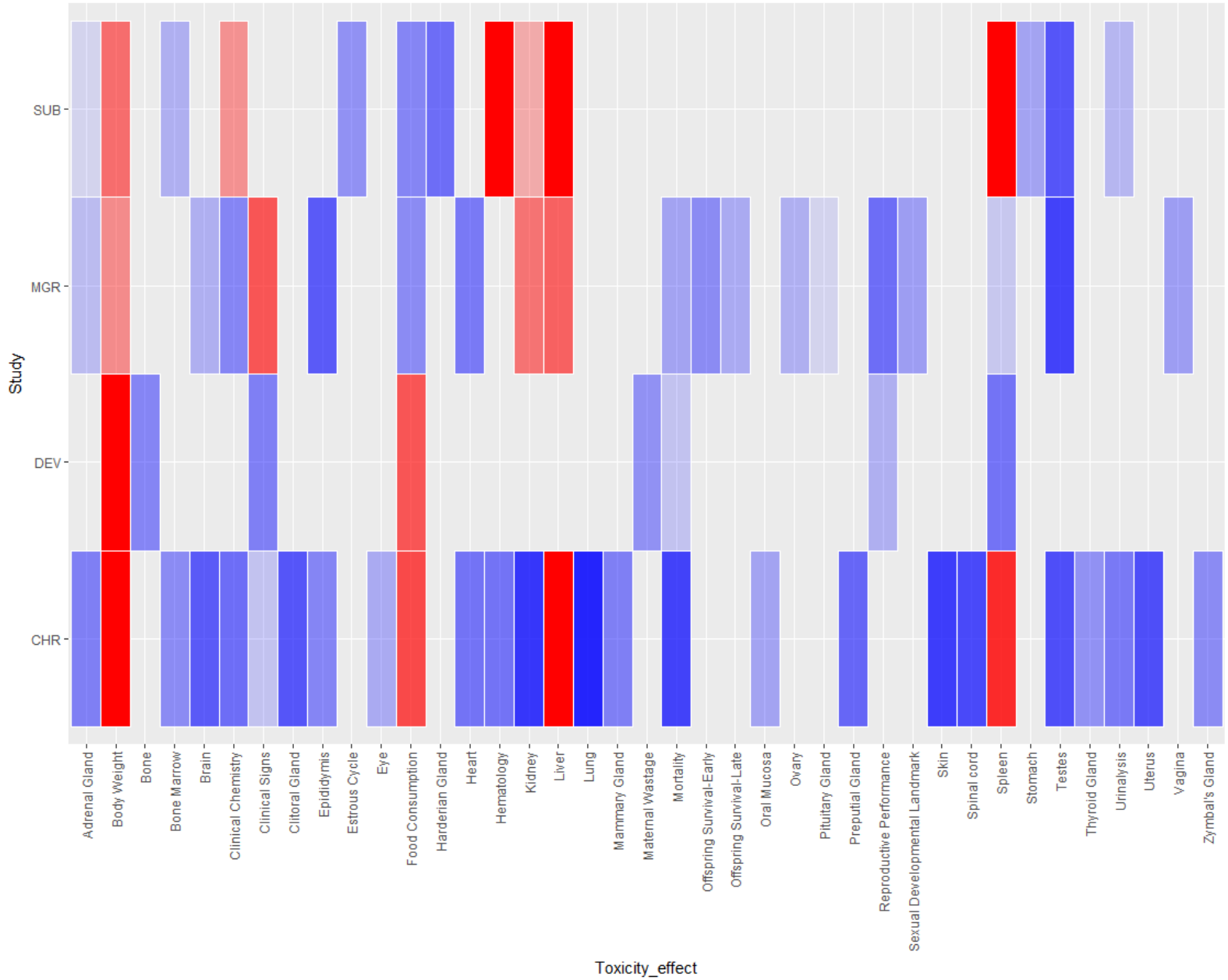
Identify the common 'positive' effects across analogues - haematology, liver, kidney and spleen effects

Data matrix for source substances



Haematology, liver, kidney and spleen effects most readily observed in Subchronic and chronic studies across the source analogues

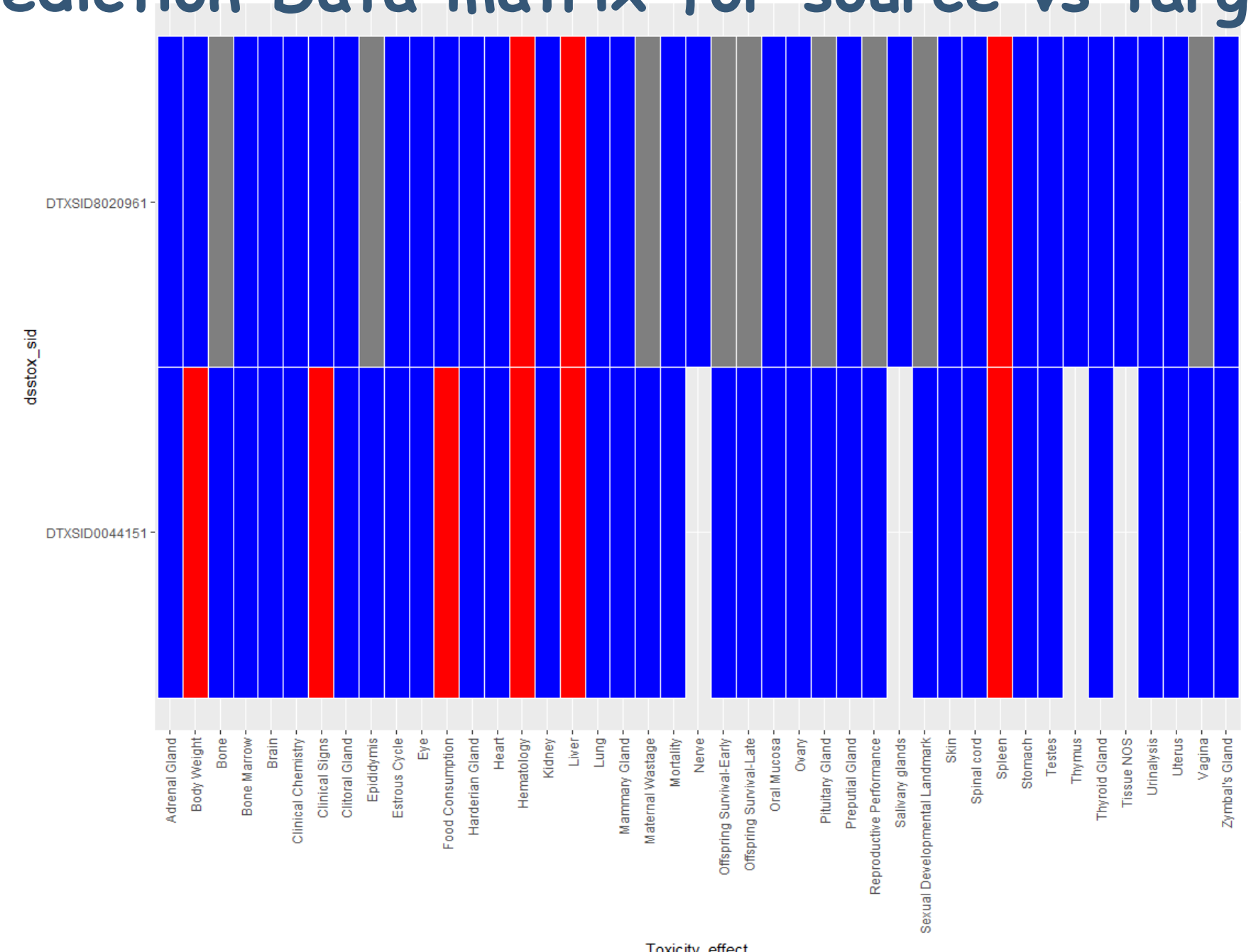
GenRA predictions for 3,5-dinitroaniline



Prediction Data matrix for source vs target

Proposed source analogue

3,5-dinitroaniline



From Qualitative to Quantitative predictions

- Suggestions:
- Approach 1:
- Focus on the positive effects observed in the experimental data from the source analogues
  - Assume hematology effects in a chronic study is the most sensitive effect based on confidence in prediction
  - No_effect – use a limit dose of 1000 mg/kg
  - Similarity weighted activity of the source analogue LEL data (converted into a –log(molar LEL)) for that toxicity effect => predicted LEL of 3,5-dinitroaniline would be ~134 mg/kg/day
- Approach 2:
- Use the lowest LEL from the source analogues i.e. 4-nitroaniline & 1.5 mg/kg/day
- Approach 3:
- Look at the range of LELs for all positive predictions across all studies irrespective of study type i.e. lowest LEL is for 4-nitroaniline & 1.5 mg/kg/day

Take home messages

- Computational toxicology approaches impact many aspects of regulatory contexts
- Outlined how computational approaches fit within an IATA
- Illustrated how we have explored coupling TTC & HTE for a risk-based prioritisation application
- Discussed read-across approaches & their frameworks
- Proposed a harmonised framework for read-across approaches

Take home messages

- Outlined GenRA, how it was developed and how it is aligned with this framework – public tool in development (*summer release slated*)
- Initial GenRA (baseline) considers structural similarity but current work has evaluated the quantitative impact of physicochemical similarity (as it relates to bioavailability)
- Ongoing work is considering dose predictions