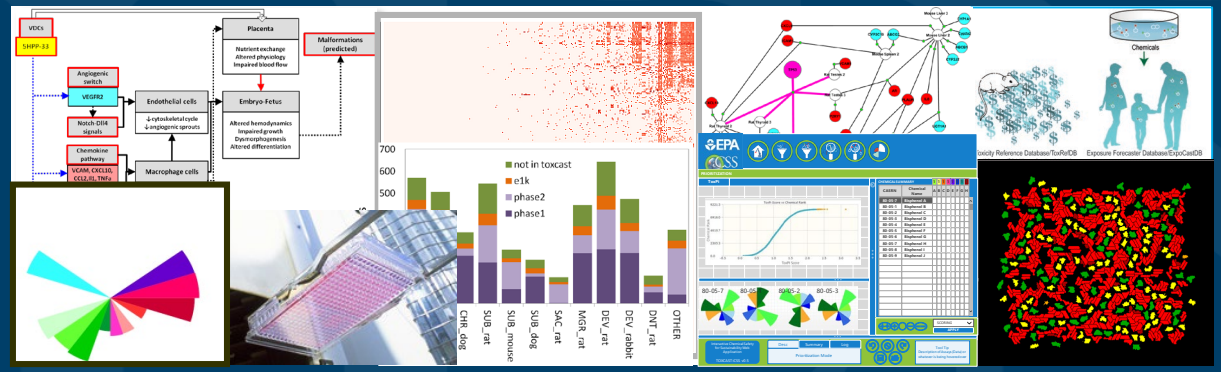
EPA United States Environmental Protection Agency

# Generalised read-across GenRA, research, implementation and practical application




ACS 2018: Evolving Chemical Hazard Evaluation Strategies to Address Compliance under the New Toxic Substances Control Act (TSCA)

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National Center for Computational Toxicology

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA

# Outline

- Definitions
- Frameworks for Development & Assessment of category/analogue approaches to facilitate read-across
- Proposal for a Harmonised framework for read-across
- GenRA approach – "Baseline" GenRA
- Recent refinements to GenRA
- Practical case study example
- Take home messages
- Acknowledgements

# 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).

# Frameworks for developing category/analogue approaches

OECD/ECHA

- ECETOC (2012) Patlewicz et al (2013; 2015)

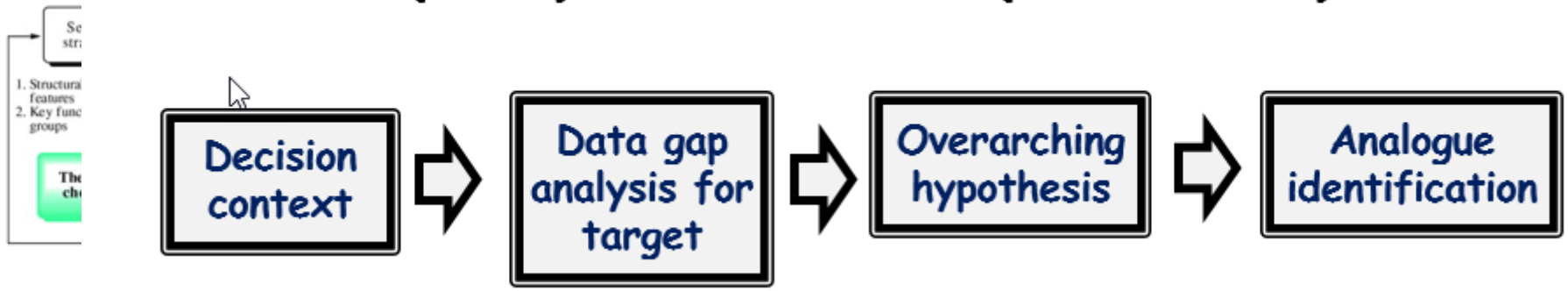
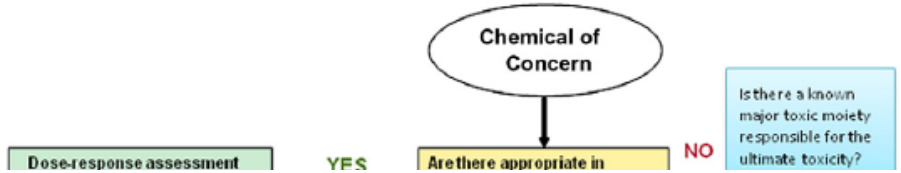
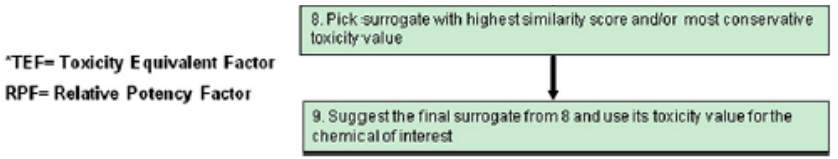



Figure 2 - Stepwise approach to an analogue approach




*TEF= Toxicity Equivalent Factor
RPF= Relative Potency Factor

Fig. 1. Tiered surrogate approach.

# 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 *under review***

# Frameworks for Assessing Read-across

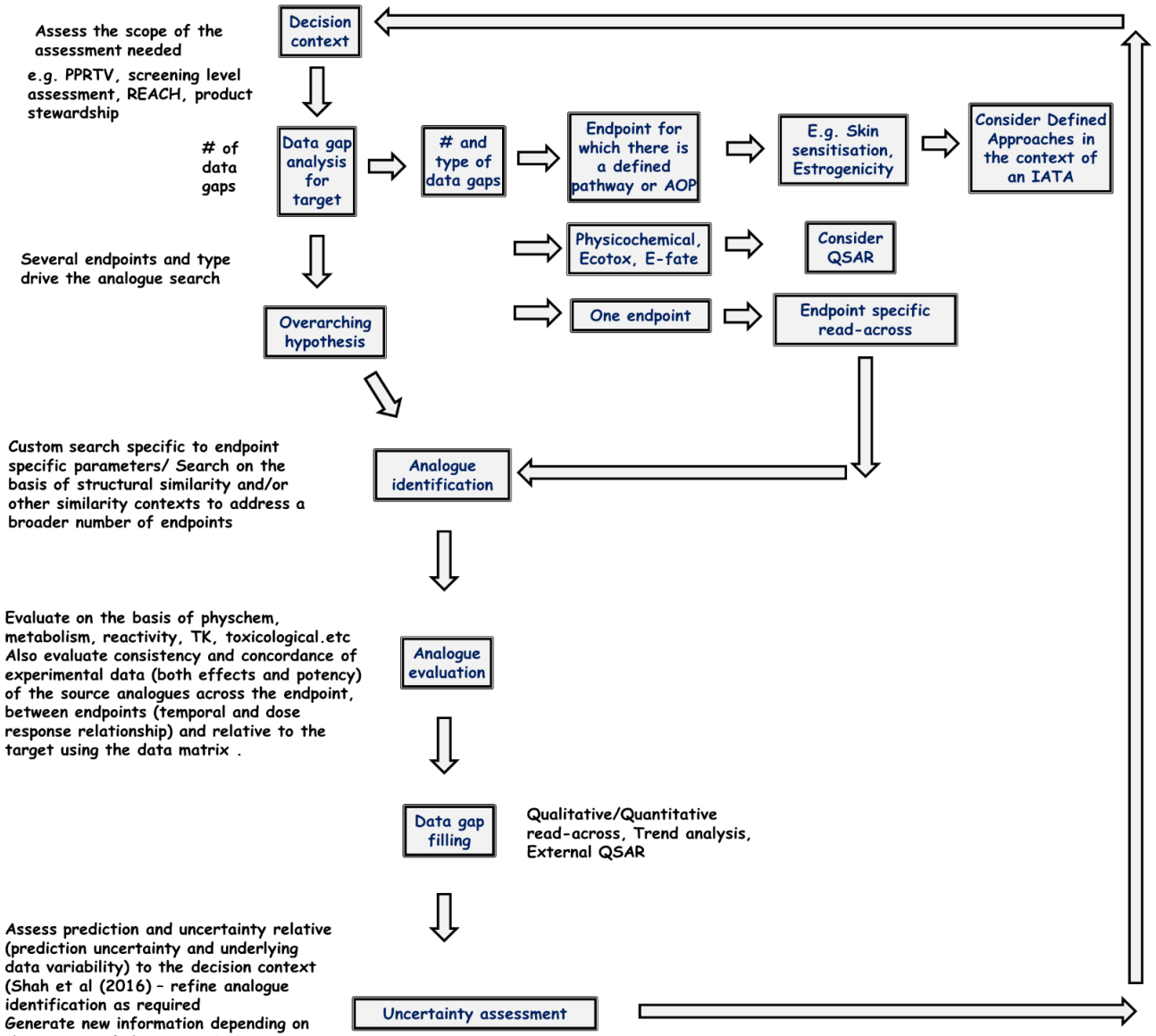
- Blackburn & Stuard
- Patlewicz et al (2015)
- Schultz et al (2015)
- ECHA RAAF (2015, 2017)

- 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<br>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 |

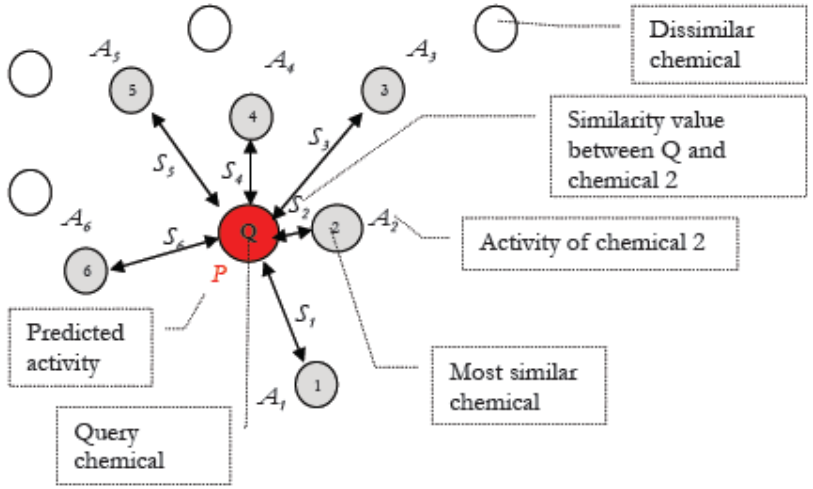
# A harmonised read-across workflow




**Proposed in Patlewicz et al., 2018 *under review***

# 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/or bioactivity descriptors
- Generalised version of Chemical-Biological Read-Across (CBRA) developed by Low et al (2013)
- Goal: to systematically evaluate read-across performance and uncertainty using available data




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

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 $(c_i)$

$x_j^{\beta}$ = activity of $c_j$ in $\beta$

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

$k$ = up to $k$ nearest neighbours

# GenRA - Approach

**I. Data**

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

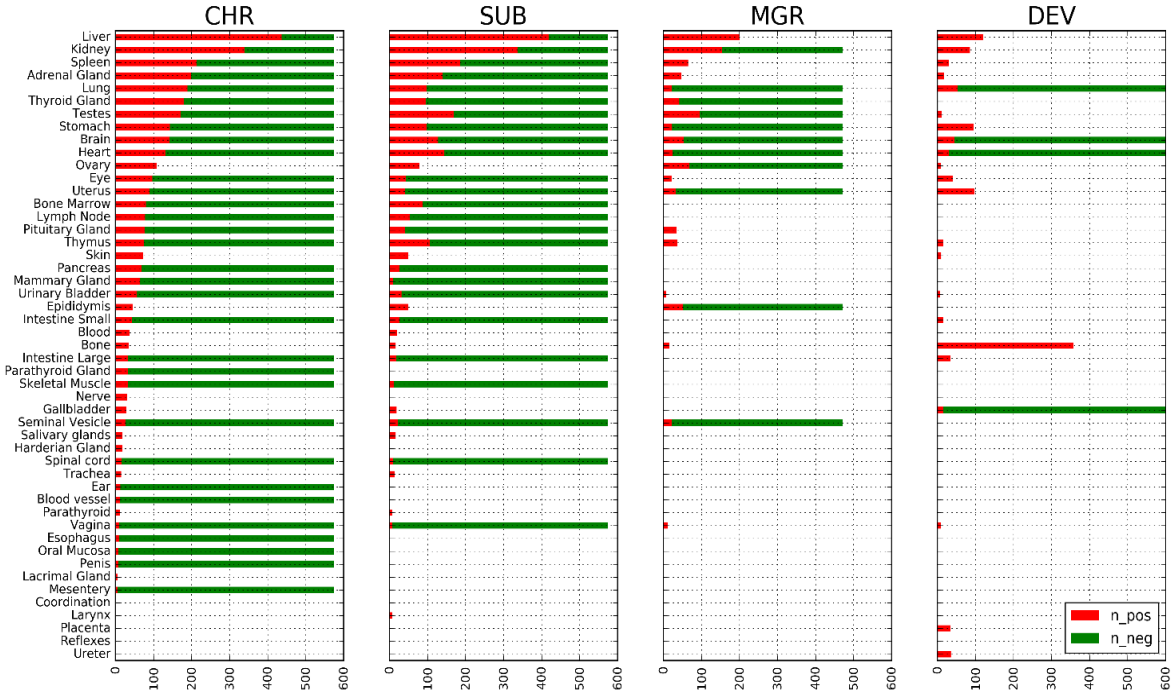
574 toxicity effects (tox) ToxRefDB

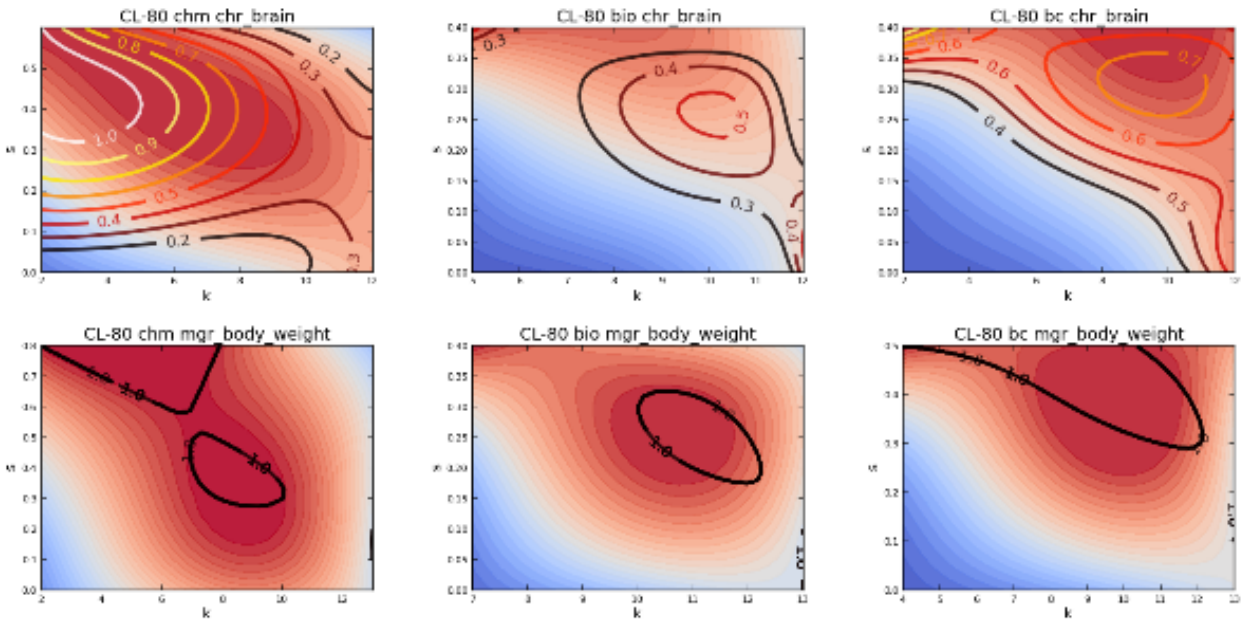
**II. Define Local neighbourhoods**

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

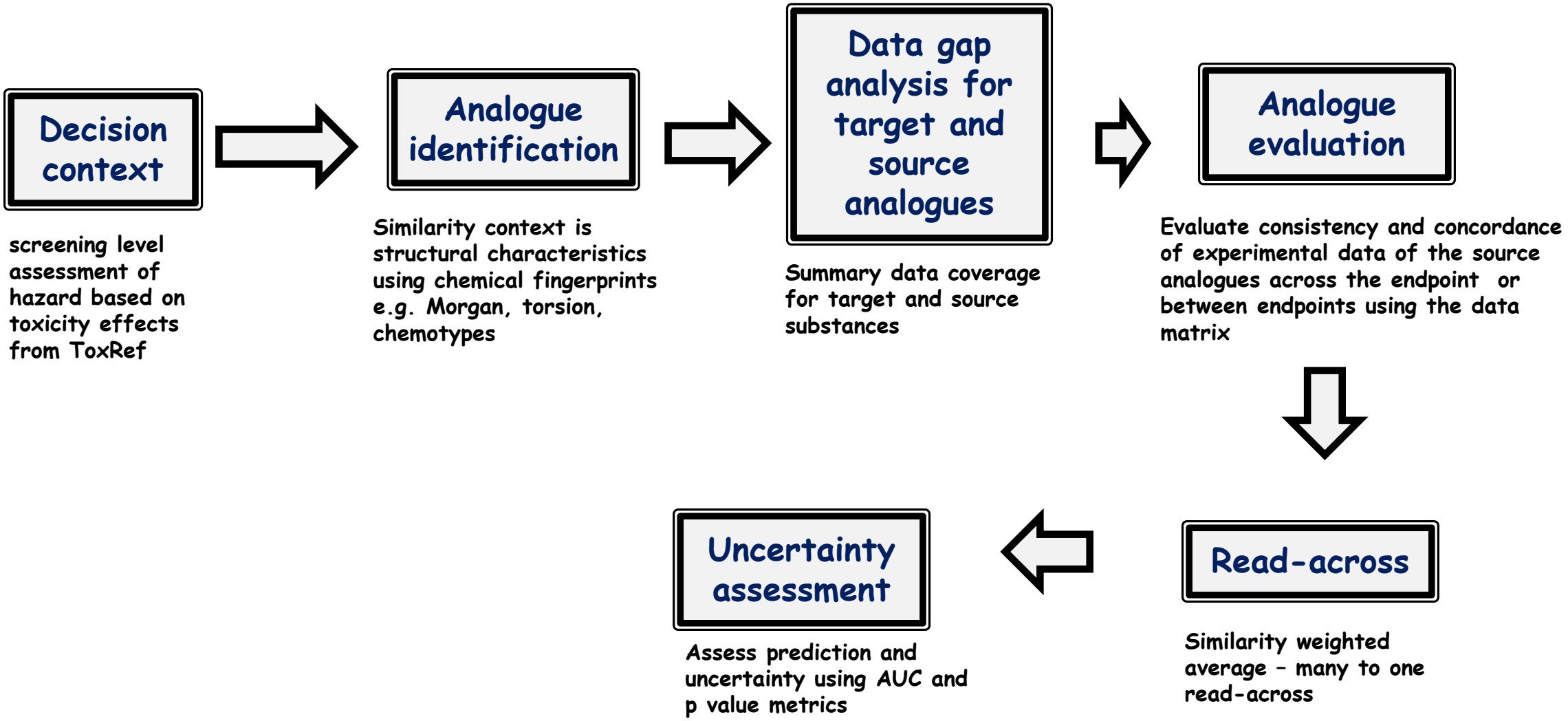
**III. GenRA**

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



# Current Category Workflow in GenRA



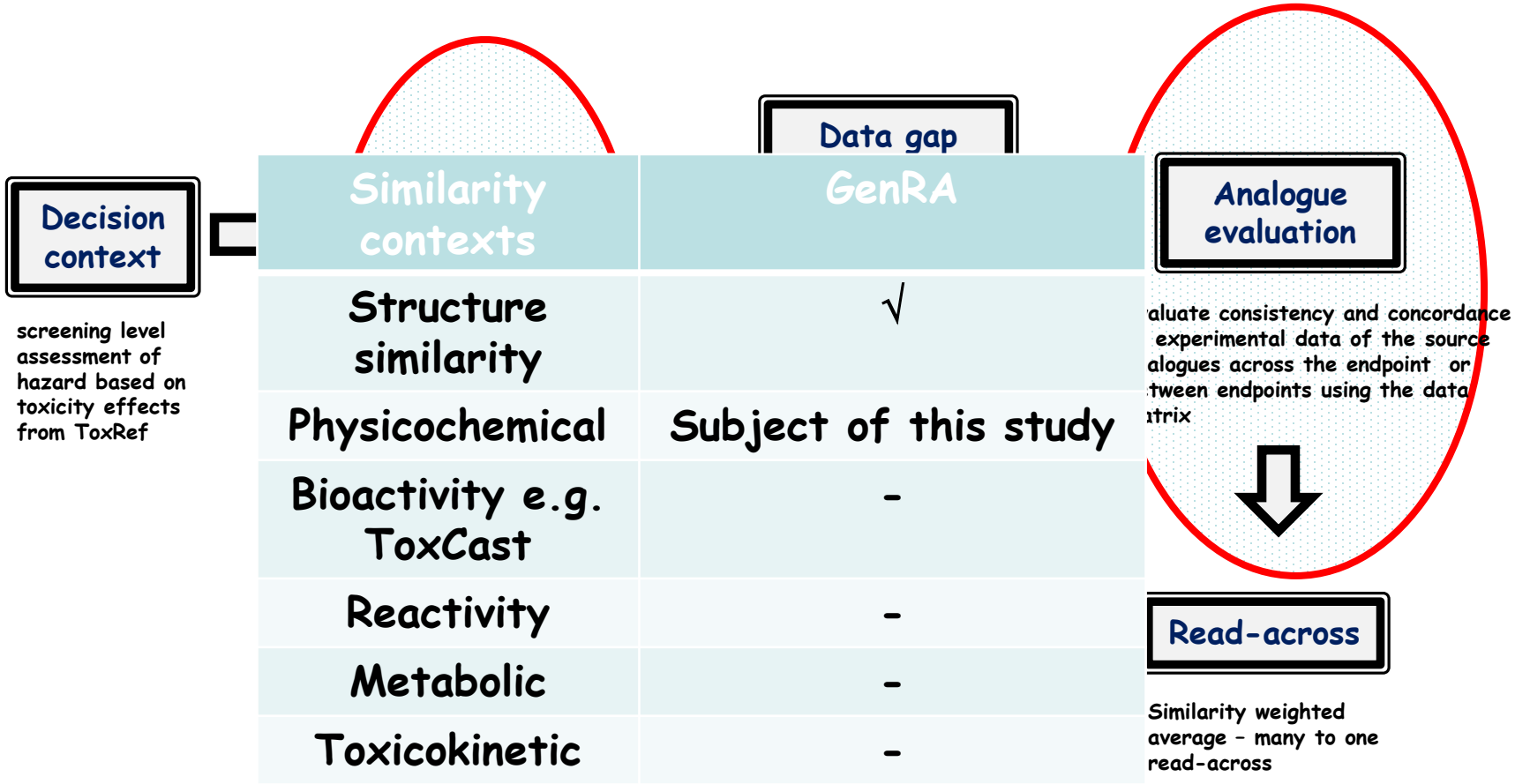
# GenRA tool in development for public release

# GenRA – Outcomes and Next Steps

- The approach enabled a performance baseline for read-across predictions of toxicity effects within specific study outcomes to be established but was still context dependent on the endpoint and the chemical

- Ongoing analysis:
- Consideration of other information to refine the analogue selection – e.g. physicochemical similarity, TK similarity, metabolic similarity, reactivity similarity…
- Dose response information to refine scope of prediction beyond binary outcomes..

# Refinements to the GenRA approach



# 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:

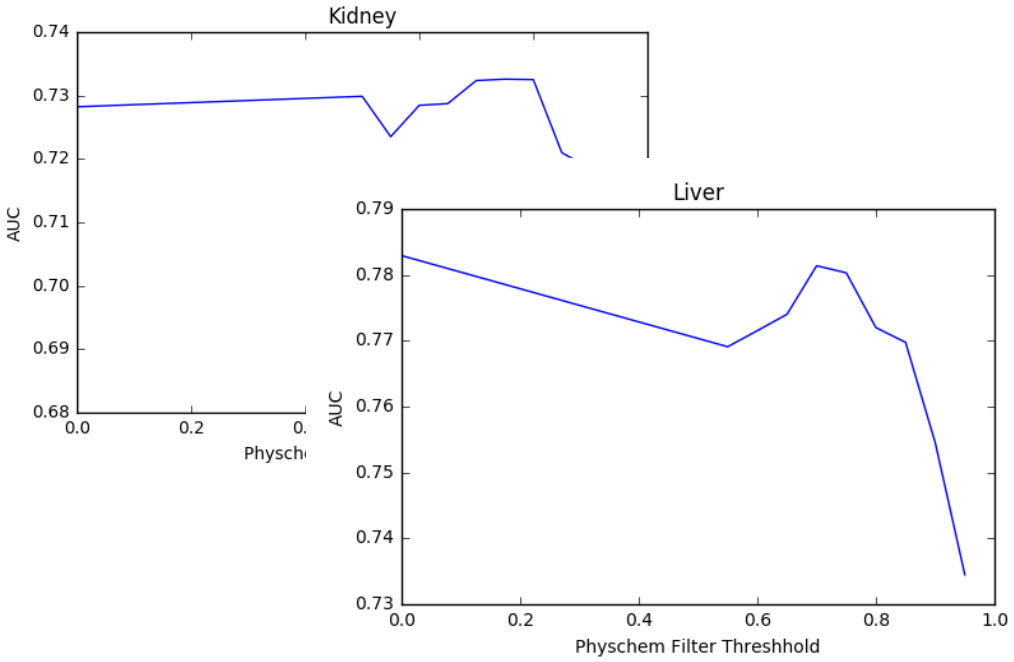
| Approach 1: "Filter" | Approach 2: "Search Expansion" |
| --- | --- |
| Subcategorise from a set of analogues identified based on structural similarity | "Frontload" both structure and physchem into analogue identification |
| Common approach | Novel approach |

# Approaches considered

United States Environmental Protection Agency

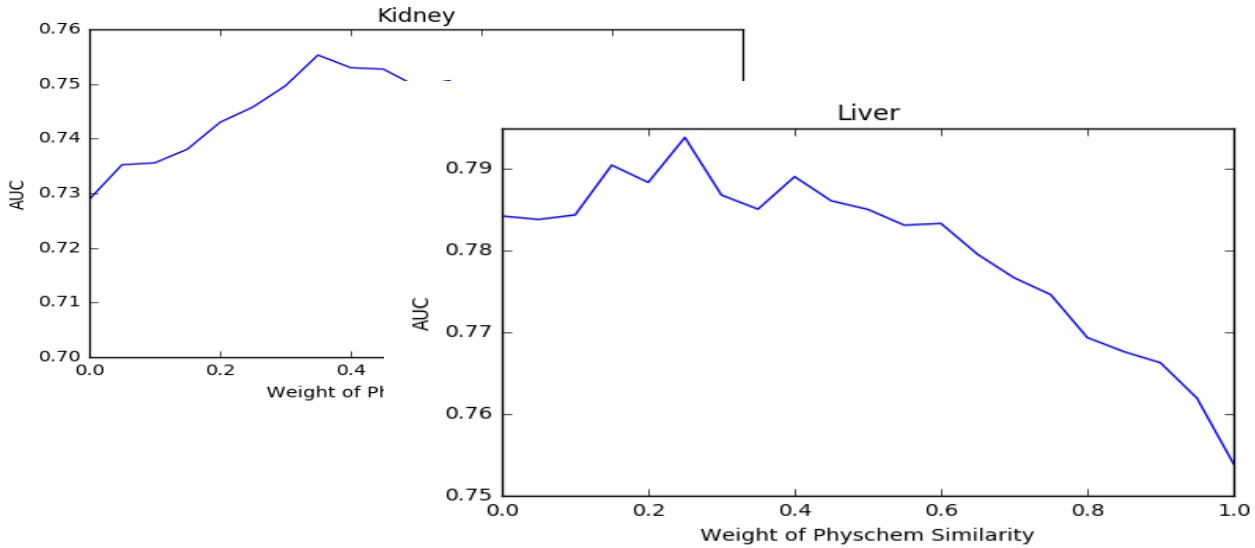
## Approach 1: Filter

- This approach did not perform as well as baseline GenRA for the entire dataset, nor did it significantly improve any target organ predictions.




## Approach 2: Search Expansion

- This approach showed a small improvement over baseline GenRA for entire dataset, but a larger improvement was observed for certain organs.
- Target organ predictions that were markedly improved: Intestine Large, Intestine Small, Mammary Gland, Pancreas, Ureter, Urinary Bladder

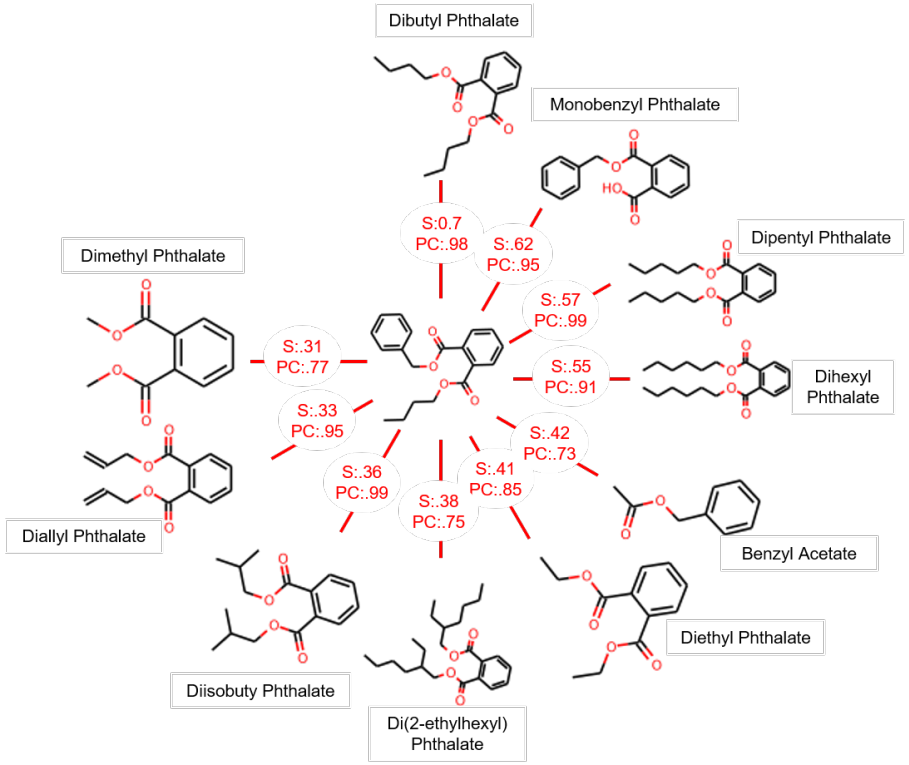





Target organ toxicity predictions aggregated over study type to organ level

# Case Study: Butyl Benzyl Phthalate

## GenRA: Baseline




| Endpoint | Baseline Prediction |
|---|---|
| Body Weight | .78 |
| Clinical Chemistry | .27 |
| Food Consumption | 0 |
| Hematology | 0 |
| Kidney | .27 |
| Liver | 1 |
| Mortality | .27 |
| Pancreas | .27 |
| Prostate | 0 |
| Skin | .27 |
| Spleen | 0 |
| Tissue NOS | 0 |
| Urinary Bladder | 0 |

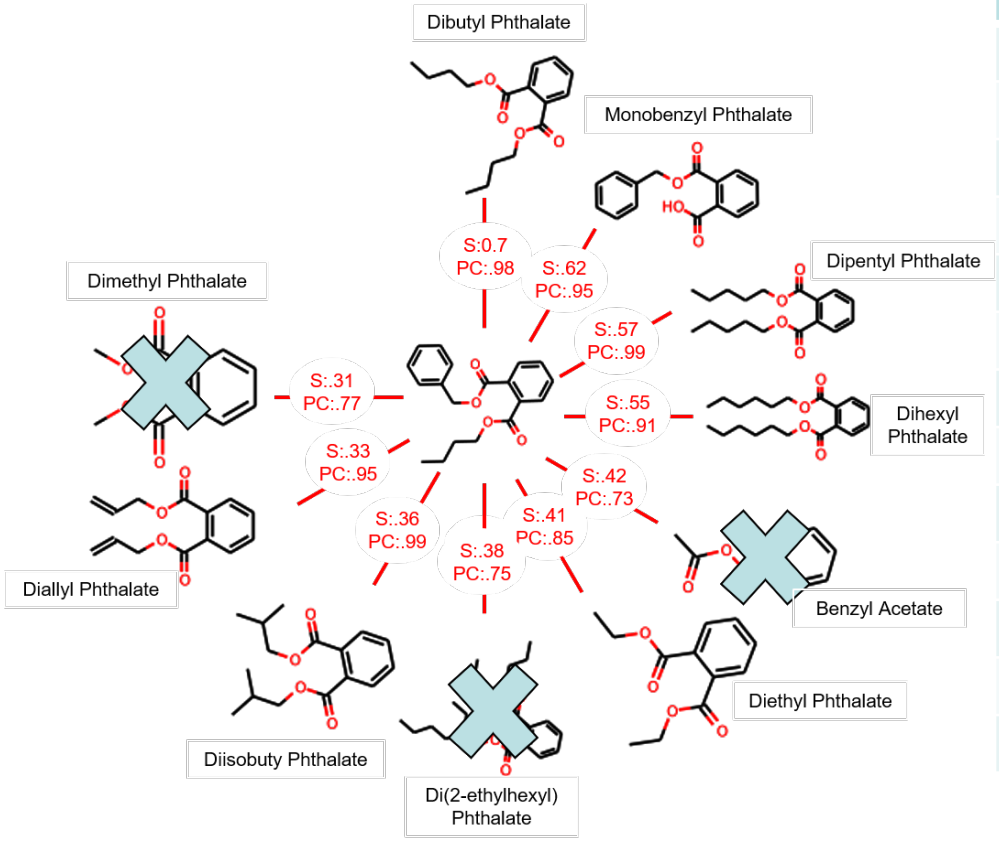
- Chronic studies
- All experimentally positive effects
- Predictions between 0 and 1
- Higher prediction indicates more and stronger positive neighbours

**0.5> indicates "positive" for toxicity effects**

# Case Study: Butyl Benzyl Phthalate

## Approach 1: Filter

**Filter out chemicals with physchem similarity <0.8**



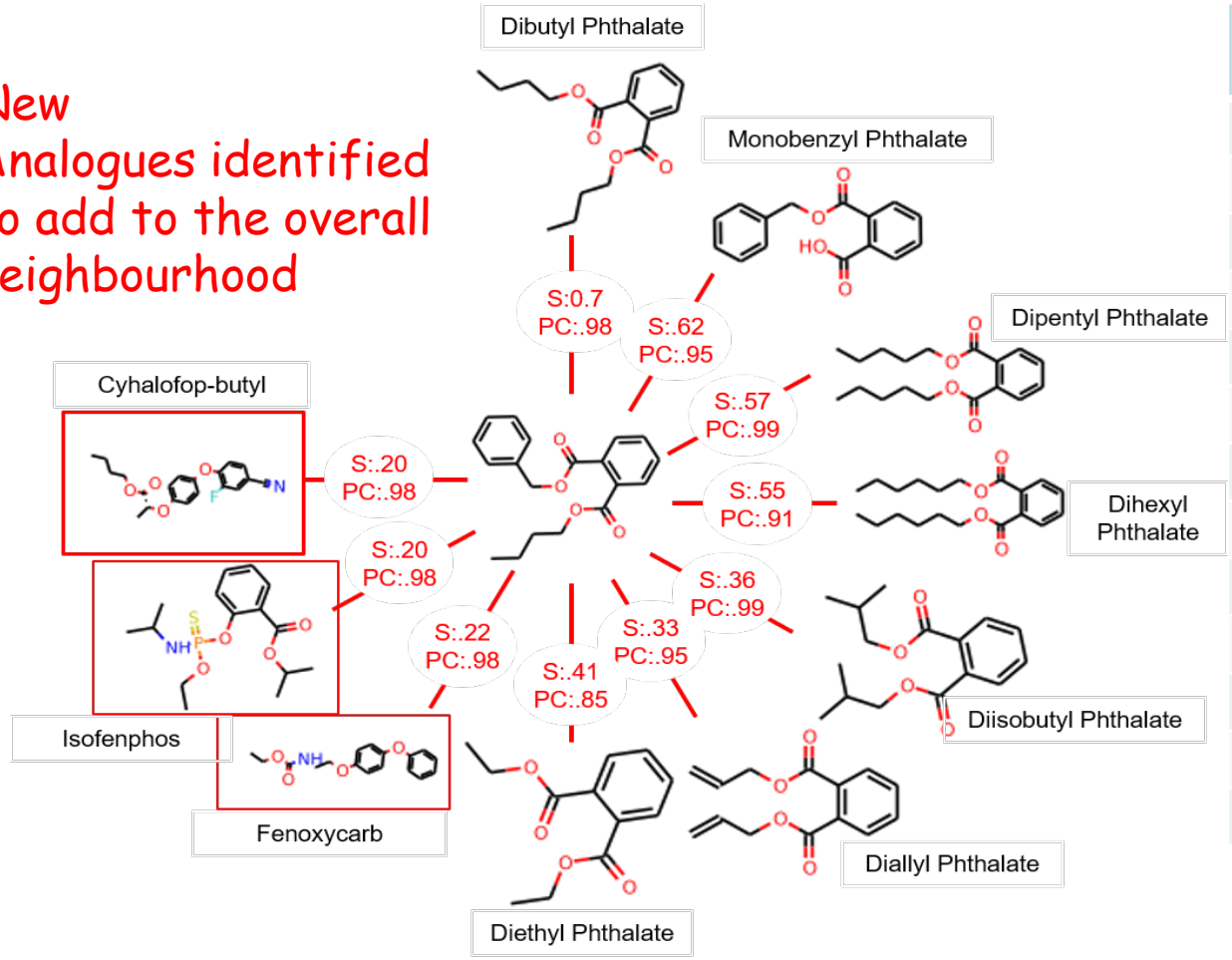

| Endpoint | Baseline | Filtered |
|---|---|---|
| Body Weight | | |
| Clinical Chemistry | | |
| Food Consumption | | |
| Hematology | | |
| Kidney | | |
| Liver | | |
| Mortality | | |
| Pancreas | | |
| Prostate | | |
| Skin | | |
| Spleen | | |
| Tissue NOS | | |
| Urinary Bladder | | |

- Filtering overturns incorrect predictions for 4 endpoints.
- BUT if filtering is too stringent, "significant" analogues are excluded resulting in a worse performance c.f original GenRA baseline

# Case Study: Butyl Benzyl Phthalate

## Approach 2: Search Expansion

New
Analogues identified to add to the overall neighbourhood

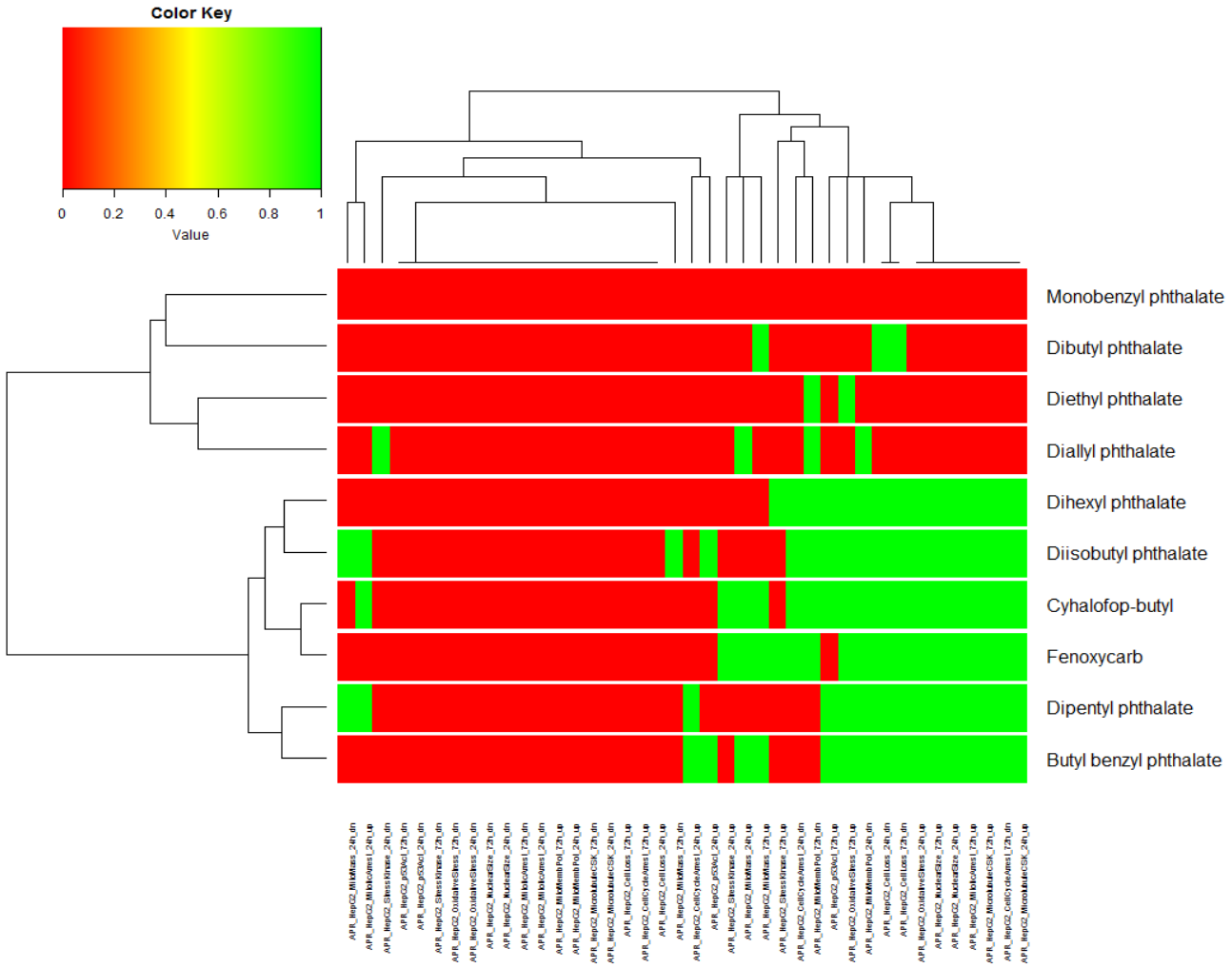



| Endpoint | Baseline Prediction | Structure + Pchem Prediction |
|---|---|---|
| Body Weight | .78 | .79 |
| Clinical Chemistry | .27 | .60 |
| Food Consumption | | |
| Hematology | | |
| Kidney | | |
| Liver | | |
| Mortality | | |
| Pancreas | | |
| Prostate | | |
| Skin | | |
| Spleen | | |
| Tissue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

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

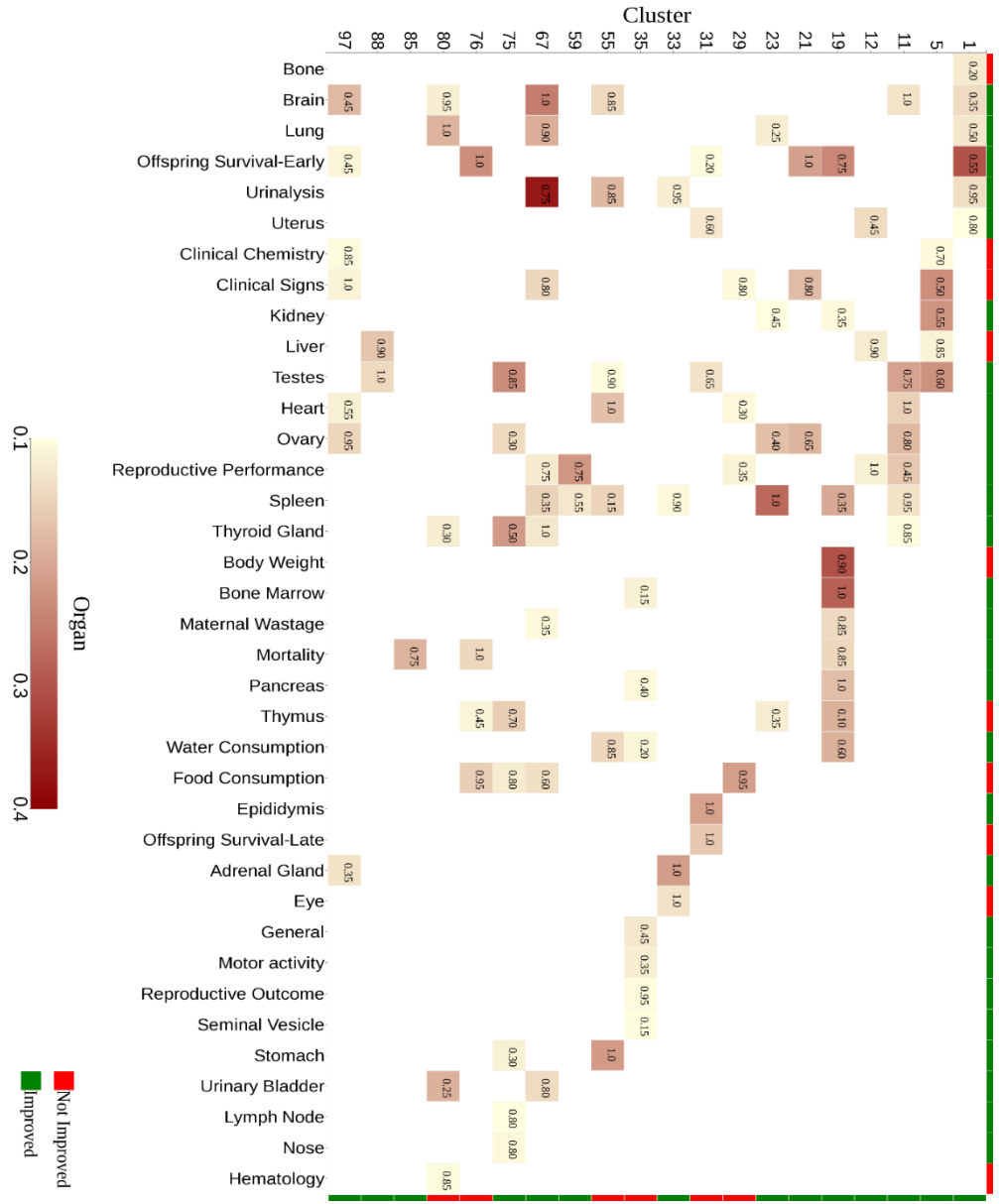
# Case Study: Butyl Benzyl Phthalate

## 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

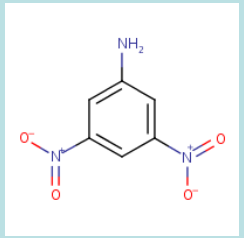
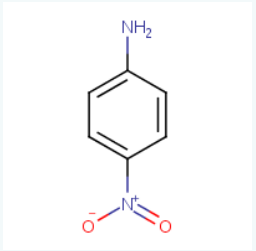



1) Identify target chemical

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

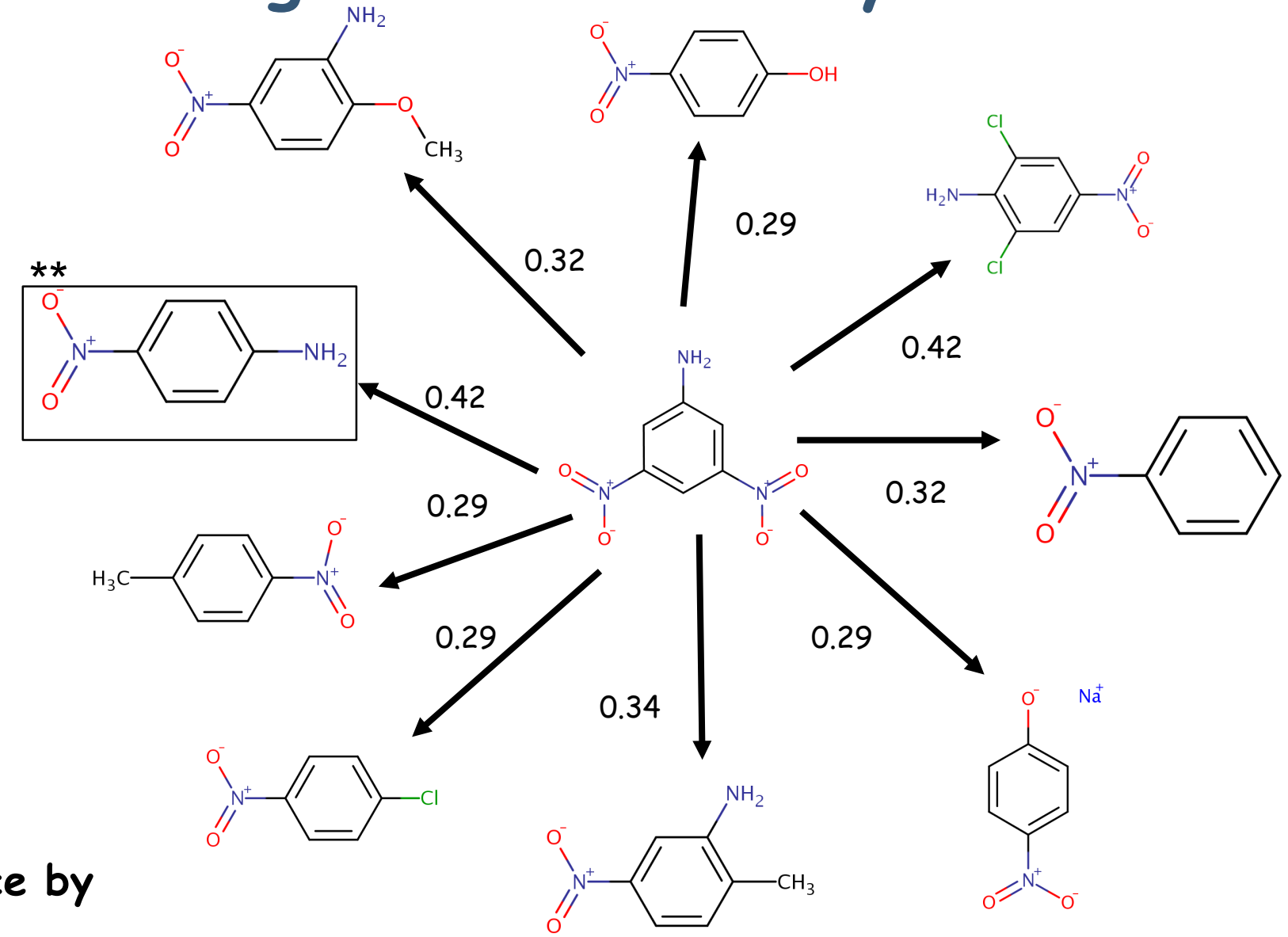
# GenRA in practice for a chemical of interest to Superfund: Case study 2

| 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. |

*Taken from SOT poster 2874/P399 from Lizarraga et al

# Source analogues identified by GenRA

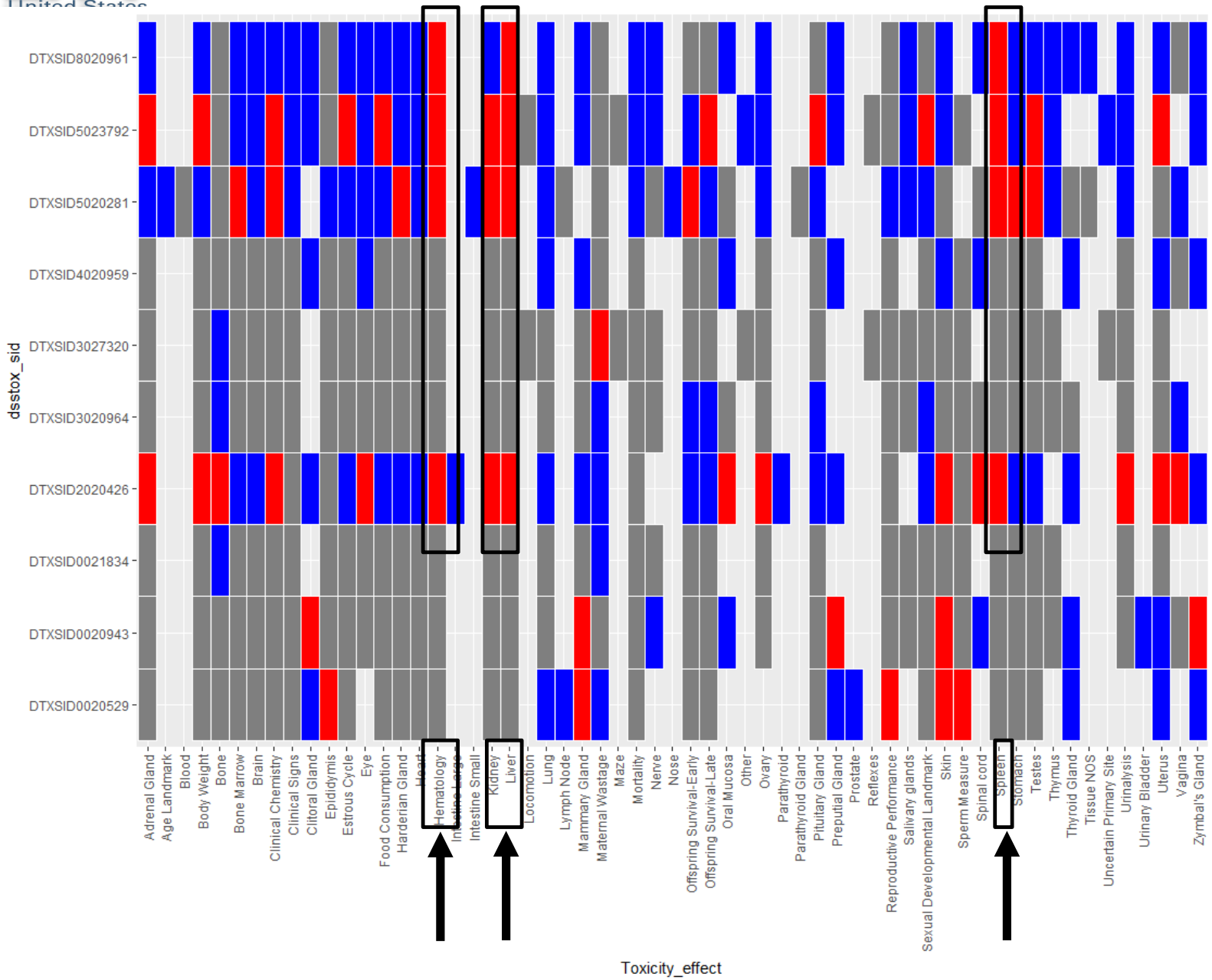
| DTXSID | PREFERRED_NAME |
|---|---|
| DTXSID0020943 | 2-Methoxy-5-nitroaniline |
| DTXSID0021834 | 4-Nitrophenol |
| **DTXSID0044151** | **3,5-Dinitroaniline*** |
| DTXSID2020426 | Dicloran |
| DTXSID3020964 | Nitrobenzene |
| DTXSID3027320 | Sodium 4-nitrophenolate |
| DTXSID4020959 | 2-Methyl-5-nitroaniline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




*= Target ** = Proposed source by expert judgement

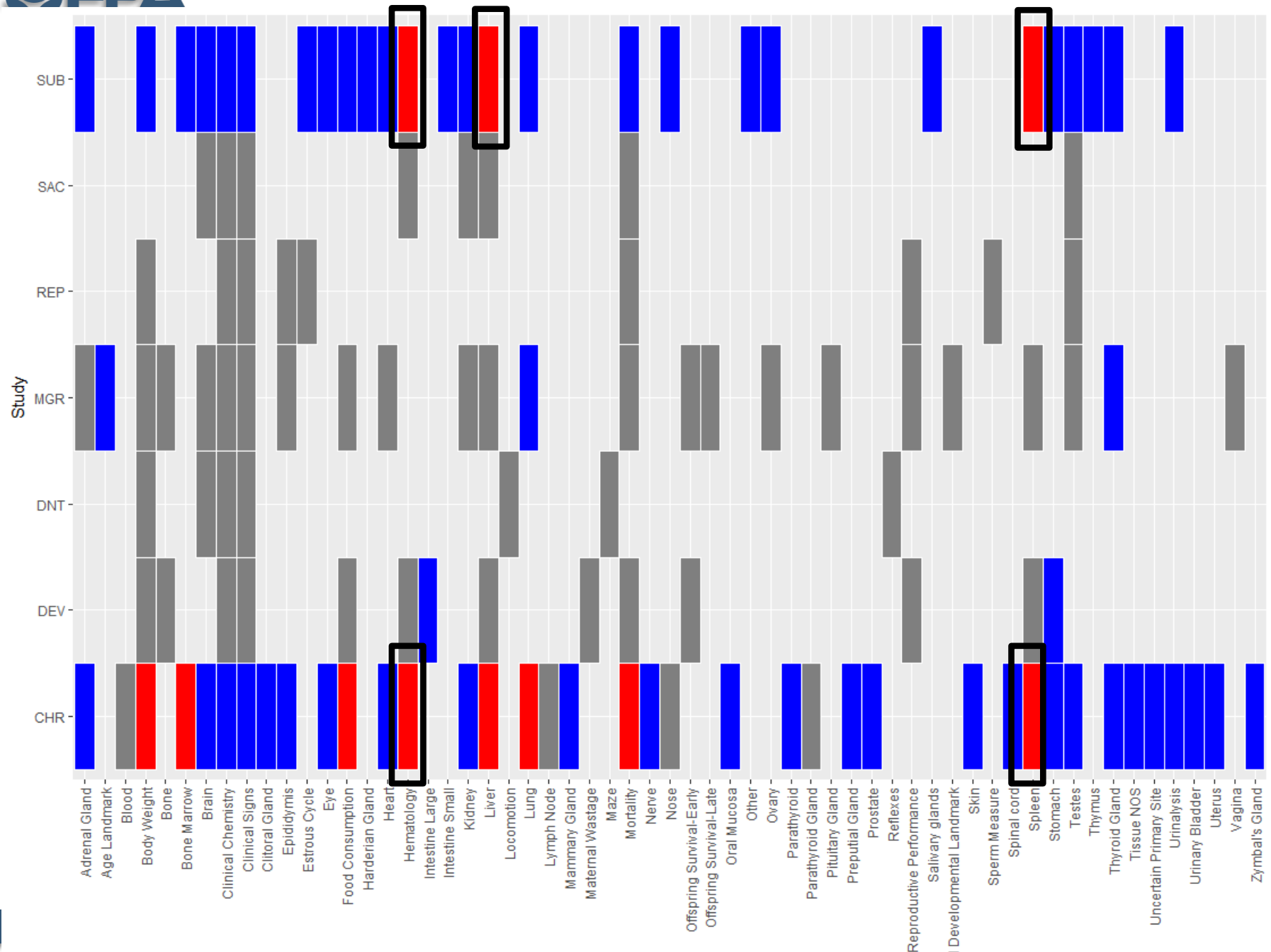
Analogues characterised by Morgan fingerprints

# Data matrix for source substances



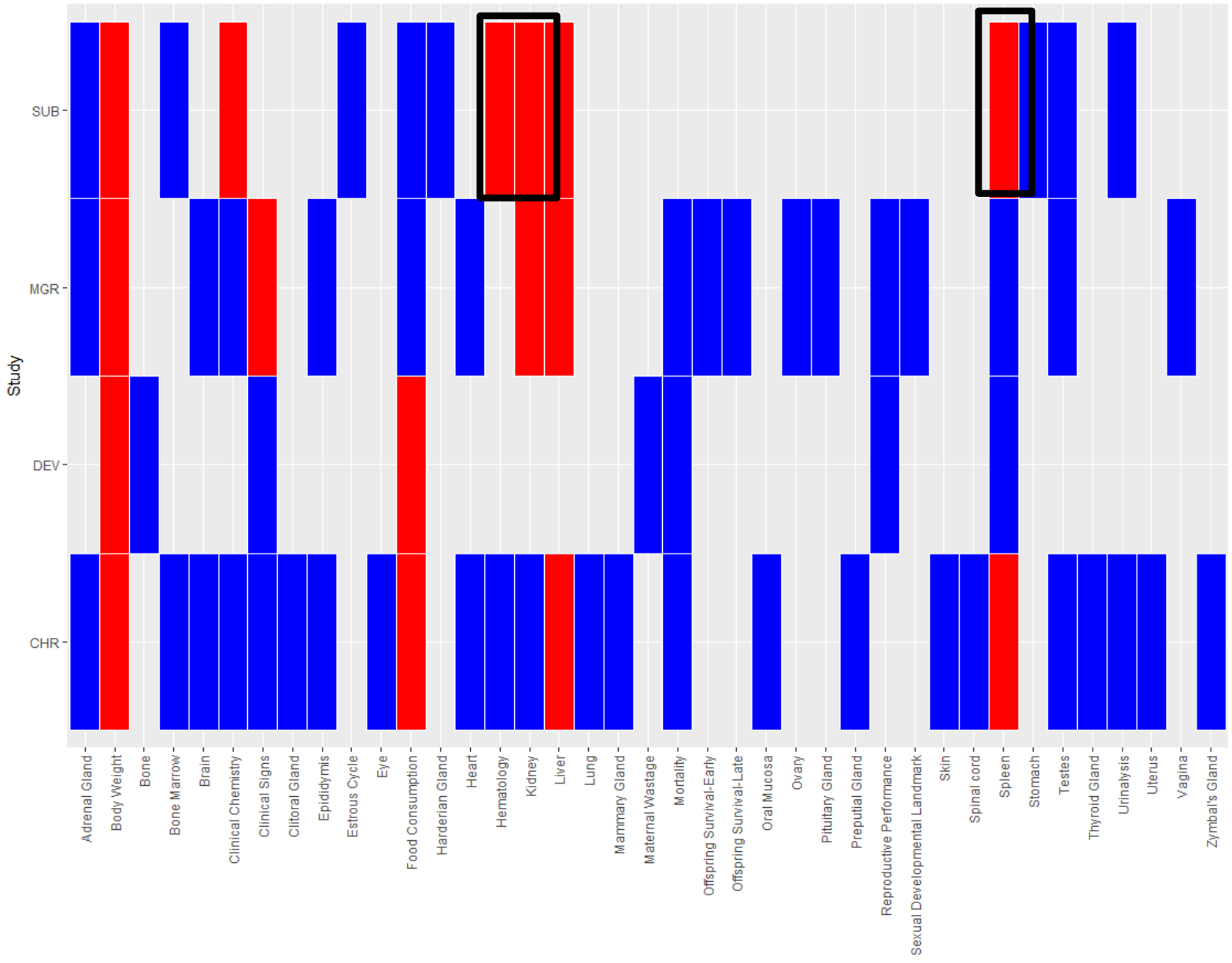

Common 'positive' effects across analogues include haematology, liver, kidney and spleen effects

# Data matrix for source substances




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

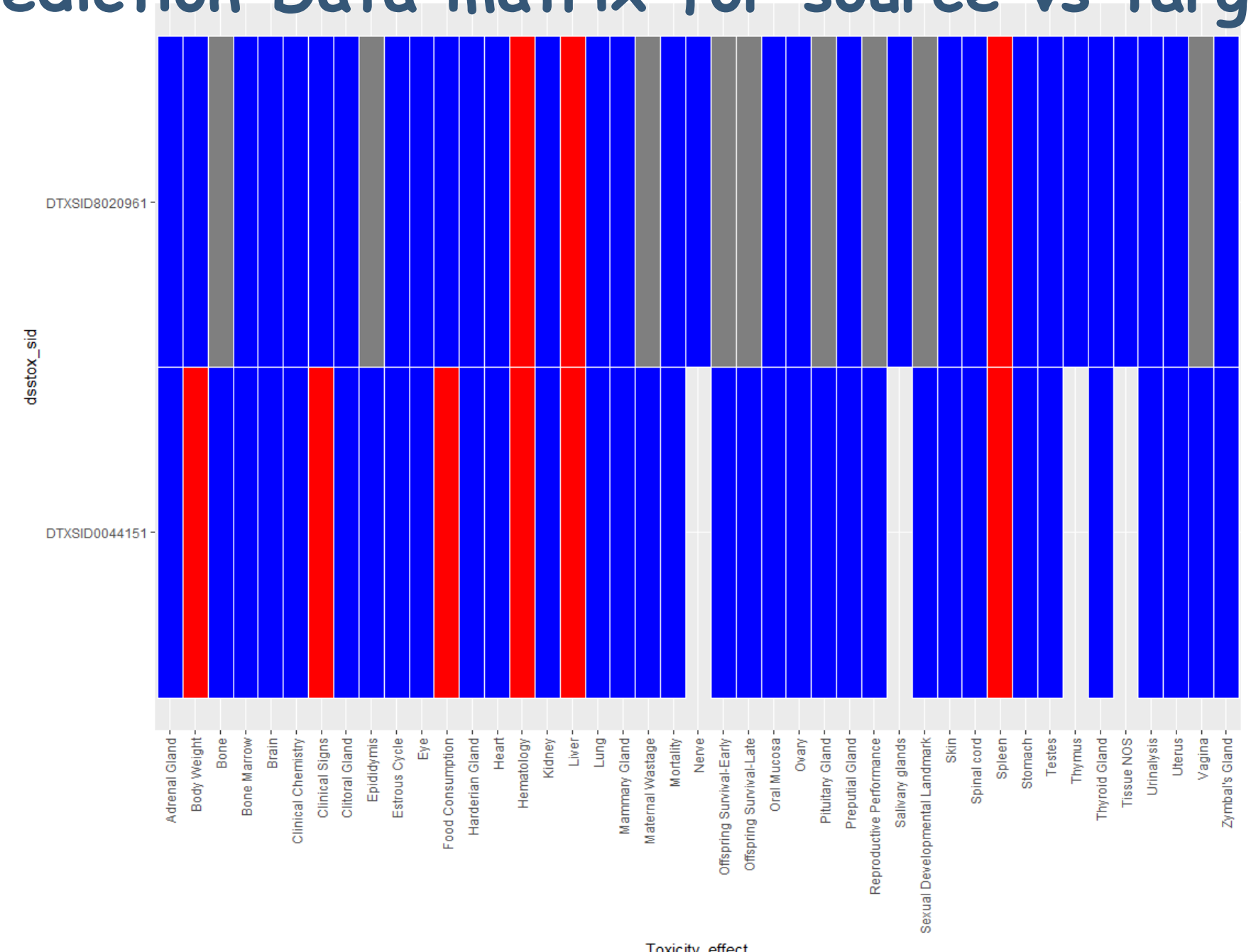
# GenRA predictions for 3,5-dinitroaniline



# Prediction Data matrix for source vs target

Proposed source analogue

3,5-dinitroaniline



# Take home messages

- Several read-across development & assessment frameworks – highlighted a handful. These are largely consistent with each other.
- Proposed a harmonised framework for read-across
- GenRA developed 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)
- Highlighted the practical application of physchem similarity using a case study chemical in practice
- Illustrated how GenRA baseline has been applied in concert with expert read-across approaches for a substance of interest to Superfund work

# Acknowledgements


- Many but in particular..
- NCCT
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- Jason Lambert
- Scott Wesselkamper