Transitioning towards objective Read-across approaches: landscape, research, and practical application

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Conflict of Interest Statement: NONE.
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 given ‘big data’ needs
- Generalised Read-across (GenRA) – an approach and an application
Talk Outline

• Definitions
• Frameworks for read-across development and assessment
• Harmonised hybrid read-across framework
• Selected tools for read-across
• Ongoing issues with read-across and its acceptance
• Current directions towards quantifying read-across performance and its associated uncertainties given ‘big data’ needs
• 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

## Read-across

<table>
<thead>
<tr>
<th>Property 1</th>
<th>Chemical 1</th>
<th>Chemical 2</th>
<th>Chemical 3</th>
<th>Chemical 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>•</td>
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<td>o</td>
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<table>
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<th>Chemical 2</th>
<th>Chemical 3</th>
<th>Chemical 4</th>
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<tbody>
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## Table

<table>
<thead>
<tr>
<th>Activity 1</th>
<th>Chemical 1</th>
<th>Chemical 2</th>
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</tbody>
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- **read-across**
- **interpolation**
- **extrapolation**

**Trend analysis or internal QSAR**

- **•** reliable data point
- **o** missing data point
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
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

<table>
<thead>
<tr>
<th>Framework</th>
<th>ECHA</th>
<th>OECD</th>
<th>Wu et al</th>
<th>Wang</th>
<th>Patlewicz</th>
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</thead>
<tbody>
<tr>
<td><strong>Context</strong></td>
<td>REACH</td>
<td>International regulatory purposes</td>
<td>Product Stewardship</td>
<td>Quantitative risk assessment</td>
<td>Regulatory purposes/Product stewardship</td>
</tr>
<tr>
<td><strong>Approach</strong></td>
<td>Analogue/Category - aim is to fill an endpoint specific study, focused on structural similarity as a starting point. Approach is more hypothesis driven.</td>
<td>Analogue/Category - a generalisation of the ECHA approach.</td>
<td>Analogue/Stepwise evaluation of analogue suitability based on structure, reactivity, p-chem and metabolism</td>
<td>Analogue/Approach is based on a WOE assessment from structure, ADME and toxicity considerations</td>
<td>Analogue/Stepwise approach considering general (pchem, reactivity, metabolism) and endpoint specific considerations</td>
</tr>
<tr>
<td><strong>Terms of reference</strong></td>
<td>Target/Source</td>
<td>Target/Source</td>
<td>Substance of interest/Analogue</td>
<td>Chemical of Concern/Surrogate</td>
<td>Analogue/Category</td>
</tr>
<tr>
<td><strong>Scope</strong></td>
<td>Endpoint specific</td>
<td>Endpoint specific</td>
<td>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</td>
<td>Approach is based on a WOE assessment from structure, ADME and toxicity considerations. &quot;Best&quot; surrogate is selected from a set of candidates based on most similar and most conservative toxicity value</td>
<td>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...</td>
</tr>
</tbody>
</table>

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

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

- 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

- 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

<table>
<thead>
<tr>
<th>Framework</th>
<th>ECHA</th>
<th>Blackburn and Stuard</th>
<th>Patlewicz et al</th>
<th>Schultz et al</th>
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</thead>
<tbody>
<tr>
<td>Context</td>
<td>REACH</td>
<td>Product Stewardship</td>
<td>Regulatory purposes &amp; Product stewardship</td>
<td>Regulatory purposes &amp; Product stewardship</td>
</tr>
<tr>
<td>Scope</td>
<td>Analogue/Category</td>
<td>Analogue/Category</td>
<td>Analogue/Category</td>
<td>Analogue/Category</td>
</tr>
<tr>
<td>Framework</td>
<td>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).</td>
<td>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.</td>
<td>Identifies the sources of uncertainty in relationship to the data and similarity context.</td>
<td>Different scenarios are articulated to frame up to 11 different similarity criteria. Factors proposed to evaluate mechanistic relevance and completeness of the read-across.</td>
</tr>
</tbody>
</table>
A harmonised hybrid read-across workflow

1. Decision context

2. Data gap analysis for target

3. Overarching similarity rationale

4. Analogue identification

5. Analogue evaluation

6. Data gap filling

7. Uncertainty assessment

YES

Consider Defined Approaches in the context of an IATA

e.g. Skin sensitisation, oestrogenicity

NO

Is the data gap for an endpoint for which there is a defined pathway or AOP?

YES

Consider QSAR approaches

NO

Is/are the data gap(s) for physicochemical, ecotox or e-fate properties?

Rationale(s) are either more broadly defined on the basis of functional groups, reactivity etc. or specific to an endpoint

Qualitative/Quantitative read-across, Trend analysis, External QSAR

Patlewicz et al., 2018
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 (e.g. big 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.
# Selected read-across tools

<table>
<thead>
<tr>
<th>Tool</th>
<th>AIM</th>
<th>ToxMatch</th>
<th>AMBIT Toolbox</th>
<th>OECD Toolbox</th>
<th>CBR A</th>
<th>ToxRead</th>
<th>GenRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analogue identification</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Analogue Evaluation</td>
<td>NA</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X For Ames &amp; BCF</td>
<td>NA</td>
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<tr>
<td>Data gap analysis</td>
<td>NA</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>NA</td>
<td>NA</td>
<td>X Data matrix can be exported</td>
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<tr>
<td>Data gap filling</td>
<td>NA</td>
<td>X</td>
<td>User driven</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Uncertainty assessment</td>
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<td>NA</td>
<td>NA</td>
<td>X</td>
<td>NA</td>
<td>NA</td>
<td>X</td>
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<tr>
<td>Availability</td>
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<td>Free</td>
<td>Free</td>
<td>Free</td>
<td>Free</td>
<td>Free</td>
<td>Free</td>
</tr>
</tbody>
</table>
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

**Decision Context**
Screening level assessment of hazard based on toxicity effects from ToxRefDB

**Analogue identification**
Similarity context is based on structural characteristics

**Data gap analysis for target and source analogues**

**Uncertainty assessment**
Assess prediction and uncertainty using AUC and p value metrics

**Read-across**
Similarity weighted average - many to one read-across

**Analogue evaluation**
Evaluate consistency and concordance of experimental data of source analogues across and between endpoints
GenRA tool in reality

- Integrated into the EPA CompTox Chemicals dashboard
GenRA tool in reality

- Structured as a workflow
GenRA tool in reality

Data gap analysis
GenRA tool in reality

Step Three: Run GenRA Prediction

Run GenRA

Target

Source analogues

Similarity index
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, bioactivity similarity, transcriptomics similarity...
  – Quantifying the impact of physicochemical similarity on read-across performance
  – Quantifying the impact of transcriptomic 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 first with quantitative classical toxicity data – e.g. acute rat oral toxicity, ToxRefDB v2
  – In the future, bringing in quantitative HTTr data
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”**
  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

Helman et al., 2018
Case Study: Butyl Benzyl Phthalate

Approach 2: Search Expansion

New Analogues identified to add to the overall neighbourhood

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Baseline Prediction</th>
<th>Structure + Pchem Prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight</td>
<td>.78</td>
<td>.79</td>
</tr>
<tr>
<td>Clinical Chemistry</td>
<td>.27</td>
<td>.60</td>
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<tr>
<td>Food Consumption</td>
<td></td>
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<tr>
<td>Hematology</td>
<td></td>
<td></td>
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<tr>
<td>Kidney</td>
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<td>.30</td>
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<tr>
<td>Mortality</td>
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<td>.21</td>
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<tr>
<td>Pancreas</td>
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<td>0</td>
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<td>Prostate</td>
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<tr>
<td>Skin</td>
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<td>.21</td>
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<tr>
<td>Spleen</td>
<td>0</td>
<td>.20</td>
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<tr>
<td>Tissue NOS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Urinary Bladder</td>
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<td>0</td>
</tr>
</tbody>
</table>

- 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 (Aprendica) 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

Weights for physchem (w1), structure (w2) differ dependent on toxicity effect of interest
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

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

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

- R² = 0.61
- RMSE = 0.58
- A few outliers, but not too extreme
- Residuals clustered around zero with no obvious patterns

- 75-25 train-test splits
- R² values range from 0.52 to 0.69
- GenRA performs strongly and robustly on this acute tox data set.
Conclusions

• Current workflows for developing category/analogue approaches follow 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
Future Directions

• Include..
• Capturing other contexts of similarity such as reactivity, metabolism information and quantifying the impact on read-across performance
• Moving from quantitative predictions using classical toxicity data such as LD50 acute rodent oral toxicity to bioactivity data from HTS assays such as those generated within ToxCast/Tox21 or HTTr (Benchmark Dose/Concentration)
Acknowledgements

Imran Shah – US EPA
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Rusty Thomas – US EPA
Jason Lambert – US EPA
Lucy Lizarraga – US EPA
Katie Paul Friedman – US EPA
Data Quality

References

Guidance and examples


References

Frameworks for identifying analogues:

References

Frameworks for assessing read-across:

• ECHA RAAF
New approaches in read-across

• Patlewicz et al 2017 Comp Toxicol
• Patlewicz et al 2018 Comp Toxicol
• Helman et al 2018 Comp Toxicol