

Building a compendium of expert driven read-across cases to facilitate an analysis of the contribution that different similarity contexts play in read-across performance

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Abstract

Read-across is a data-gap filling technique utilised to predict the toxicity of a target chemical using data from similar analogues. Read-across is predominately performed as part of an expert-driven assessment which can impede broad acceptance. Data-driven approaches such as *Generalised Read-Across (GenRA)* offer scope to generate reproducible read-across predictions where uncertainties and performance are quantified. A key issue is how to reconcile an expert-driven approach with a data-driven approach both in terms of how analogues are identified and evaluated as well as how the read-across prediction is derived. An important component of analogue identification and selection is in understanding the contribution that different similarity contexts play; i.e. does structural similarity play a larger role in analogue selection compared with metabolism similarity. This study aimed to explore some of these considerations through building a compendium of expert-driven read-across assessments that had been published for repeated dose toxicity endpoints.

Keywords: read-across, regulatory purposes, similarity context, read-across performance

1. Introduction

Read-across is an established data gap filling technique within analogue and category approaches that has been used for decades to address different regulatory purposes. Perhaps the earliest documented example of read-across was that published by Hanway and Evans in 2000, who illustrated how to substantiate an analogue approach using acute rodent oral toxicity and Ames mutagenicity as supporting data. The workflow for performing read-across has largely remained unchanged over the years though the introduction of data streams such as high throughput screening data, transcriptomics, metabolomics data has offered new possibilities for how source analogues can be identified and evaluated. The most definitive technical guidance which describes read-across remains that

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published by the OECD. Although this was last revised in 2014, a third edition of this guidance will be forthcoming later in 2024 which includes updated sections for how high throughput data can be used, how uncertainty can be characterised and documented as well as updates for specific types of analogue/category approaches including nanomaterials, UVCBs and metal containing substances. One limitation with the technical guidance notwithstanding this anticipated update is a lack of case studies to demonstrate what a successful and robust read-across assessment should resemble. The guidance has certainly benefited from the examples under the OECD IATA Case studies project but up until now there has not been a concerted effort to collect examples to showcase what a robust read-across justification looks like, what level of supporting information is warranted and how this might differ depending on the decision context and/or the regulatory jurisdiction.

The EU's REACH guidance offers additional indirect guidance for how ECHA assesses read-across cases based on its own read-across assessment framework (RAAF) but there are no published examples indicating which dossiers were successful. Read-across practitioners have only the RAAF for indirect guidance and access to the published ECHA decision letters that document shortcomings in dossiers. Identifying which dossiers have utilised read-across is not a trivial undertaking. Tracing back the dossier and cross referencing the associated decision letters tend to describe shortcomings in the dossier beyond read-across, e.g. poor substance characterisation. In Patlewicz et al (2024), an algorithmic approach was undertaken to help identify dossiers where read-across had been performed at least for oral repeated dose study outcomes but the study fell short of identifying how many of the dossiers had been ultimately successful in the application of read-across. Moreover even having identified the dossiers and the specific source analogue used in each case, the rationale behind the identification, evaluation and justification of those analogues remained opaque. The study record relying on a source substance was known such that an association between that and the target substance could be established but an assumption had to be made as far as whether those source substances arose from a category or analogue approach could not be readily made, moreover the process by which candidate analogues were identified and rationalised to make a determination that the source substance data was sufficient to address the data gap was not available. That said, the study did shine a light on some of the challenges with current read-across in terms of a disconnect between a regulation stipulating that structurally similar analogues should be identified yet finding that many of the source analogues ultimately used were often not particularly structurally similar or that in the majority of cases, a more similar analogue could have been selected. Most likely reasons for this disconnect are due to the absence of access to available toxicity data. Nonetheless the study was a useful exploration of the ways in which analogues could be characterised and compared relative to their target substances. Assuming the read-across inference itself was sufficient for purpose - in

many cases using a structure-based algorithmic approach gave rise to similar predictions of toxicity.

In this study, a concerted effort was made to perform a targeted literature search of primary and grey literature to identify read-across cases where the approach had been accepted either for the stated context of interest or at least where there had been more documentation to justify the entire workflow from the strategy of the candidate analogues, by which tools or expertise and how those were subsequently evaluated for the endpoint of interest. The study was limited to repeated dose toxicity outcomes and focused on the oral route of exposure to mitigate variability in the types of read-across scenarios under consideration. The aims of this study are several fold: 1) firstly conduct a targeted literature search to gather as many read-across examples as possible. 2) define a schema to capture relevant information in a harmonised and machine readable format and lastly 3) investigate different ML approaches to evaluate the similarities of the analogues identified as well as ways in which these could be codified for predictive profiling.

2. Materials and Methods

2.1. Identification of read-across examples

Initially read-across cases were compiled from the [EPA Provisional Peer Review Toxicity Values \(PPRTV\) assessments](#), [OECD IATA case studies](#) and [OECD High Production Volume \(HPV\) categories](#). A web query was created in python to query each of the ~400 PPRTVs (first accessed June 2021) and identify only those PPRTV assessments where an Appendix A was available in the final report as this was a crude proxy for whether a read-across assessment might have been performed. Each of the 37 cases initially identified were then manually checked and information extracted from the reports themselves. The HPV examples were reviewed to identify only those cases where there was a SIAR assessment for only categories containing members that were discrete organic substances. Inorganics were considered out of scope. All OECD IATA case studies tagged as read-across were identified and each was extracted if the endpoints of interest were repeated dose toxicity outcomes.

A preliminary literature search was conducted based on selected authors who were well known to have published on read-across such as Nicholas Ball, Karen Blackburn, Sharon Stuard, Terry Schultz, Mark Cronin, Grace Patlewicz and Sylvia Escher amongst others. A more comprehensive literature search was subsequently conducted against Pubmed using the Abstract Sifter Tool version 7 using the following search query term "Read-across [tiab] OR Read across [tuab] OR Chemical grouping [tiab] OR Category Approach [tiab] or category-approach [tiab] OR Analogue approach [tiab] OR Analog approach [tiab] OR Surrogate approach OR Grouping concept]". This netted 934 results as of 18th July 2023. Each of the abstracts were then manually

reviewed and tagged yes/no or maybe. Certain articles were potentially duplicative of cases already extracted based on selected authors or already captured as part of the IATA Case studies or HPV categories. These were tagged as far as possible. Approximately 100 articles were labelled as Yes with a further 76 as maybes for subsequent extraction. The full articles were then sourced and screened to determine whether they met minimal criteria of including a read-across assessment for a repeated oral toxicity endpoint.

2.2. Schema to capture the Information

A structured excel sheet was created to capture relevant information from each case. This included the target substance(s) being assessed, the candidate source analogues, the toxicity value information being read across, as well as the rationale used to identify and evaluate the analogues (so-named analogue evidence streams). A Standard Operating Protocol (SOP) was drafted to document the approach in an effort to ensure consistency in the extractions. Extractions were performed by one individual which were then checked for completeness and consistency by a second individual. The SOP was updated as necessary to capture revisions as the bread of case studies changed. This was inevitable given the HPVs, PPRTVs and IATA Case studies were structured per specific templates whereas there was more variability in terms of how literature studies described any read-across.

2.3. Chemical information

Since many of the evaluations to be explored were chemical specific, the read-across cases information needed to be augmented with structural information for both target and source analogues. Target and source analogue identities were queried using the EPA CompTox Chemicals Dashboard to retrieve CASRN identifiers and structures using SMILES as well as DSSTox Substance Identifier (DTXSID) information.

2.4. Landscape Evaluation

A initial summary overview of the read-across cases was performed to better understand the landscape of the chemicals under study, the use cases explored, whether a category or analogue approach had been employed, and what tools or approaches were used to identify analogues. The freely available web application ClassyFire was used to categorise all discrete organic structures into classes using its own chemistry ontology.

2.5. Similarity context evaluation

Similarity contexts evaluated included structure, structural alerts, physicochemical properties and predicted metabolism. Similarity in structure relied upon generation of Morgan chemical fingerprints using the open source python library RDKit, constructing a pairwise distance matrix using Jaccard as

the metric. Structural alerts were generated using the Derek Nexus version 2.5 by batch processing all substances using default settings. The union of all unique endpoint-toxicophore combinations was first extracted and a dataframe constructed with all substances as rows and all endpoint-toxicophore combinations as columns. If a substance presented an alert combination, the dataframe cells were populated by a 1 to denote presence of an alert and otherwise 0 to denote absence of an alert. Physicochemical properties relied upon using OPERA predictions of LogKow, calculated MW, number of Hydrogen Bond donors and Acceptors. A pairwise distance matrix using a normalised Euclidean distance was then calculated. The metabolism of the substances were simulated using the OASIS TIMES in vitro rat liver model. Using the tree report output based on default settings, metabolic similarity was then quantified in three ways: 1) evaluating the similarity in actual metabolites produced by each substance, 2) evaluating the pairwise similarity using the transformation pathways produced for each substance and 3) evaluating the similarity of the actual simulated metabolic graph using the graph kernel similarity metric, WL.

2.6. Quantifying the contribution of similarity contexts

A Bayesian logistic regression was created to investigate the contribution of the different similarity contexts and quantifying the distributions of their coefficients. Substances that were members of a read-across case were tagged as similar whereas substances between cases were tagged as dissimilar. This created a highly imbalanced dataset with only 2665 combinations of pairs as similar and 101,937 as dissimilar. To balance the dataset so that the number of pairs were evenly split, the dissimilar pairs were downsampled randomly so that a equal number of similar and dissimilar pairs set remained. This balanced set was then split 75:25 into a training: test set. The training set was subjected to a Bayesian logistic regression whereas the test set was reserved to evaluate overall performance in the predictions. The logistic regression was set up such that the target outcome (whether a pair of substances were similar) was assumed to follow a Bernoulli distribution with logit as the logistic function. To build the Bayesian logistic regression, normally distributed priors were set for each for the different similarity metrics (Structural similarity, Alert similarity, Physicochemical similarity, WL similarity, metabolites similarity and transformation similarity). Once priors were specified, posterior distributions were used to approximate the posterior distributions using Markov Chain Monte Carlo simulations. The PyMC library in python was used to draw samples from the posterior. The sampling algorithms used was NUTS, in which parameters are tuned automatically.

2.7. Bayesian Networks

Approaches to explore BBN for the analogue approaches? For consideration

2.8. Metric learning approaches

2.8.1. Deep learning approaches

A deep learning metric learning approach was attempted as follows: A pairwise matrix was constructed for all substances with SMILES. If a pair of substances were both members of the same read-across case, it was denoted by label 0, otherwise a label 1 was assigned. Since the number of dissimilar pairs was so much greater than the number of similar pairs, a random sampling was performed to downsample the number of dissimilar pairs to create a balanced dataset. A Graph Isomorphism Network (GIN) within the pytorch geometric library was then constructed using a Siamese network architecture such that a pair of substances could be fed into the network, the contrastive loss could be used as the loss function to learn the embeddings in which two similar substances had a low Euclidean distance and two dissimilar points had a large Euclidean distance. Target-analogue SMILES were used as inputs which were converted into pytorch-geometric graphs to extract embeddings representing both chemical structure information that could be predictive of whether an analogue pairs was part of the same read-across case or not. A Graph Convolutional Network was also constructed using a Siamese network architecture but in this case, 2 pytorch objects constructed based on the predicted metabolic graph using the same TIMES in vitro model where the nodes were the parent/metabolites and the edges the transformation pathway.

2.8.2. Shallow learning approaches

An Informational Theoretic Metric Learning (ITML) approach was also attempted using a paired learning approach. This relied on using a pair of similar or dissimilar substances with the aim of deriving an embedding that would discriminate dissimilar from similar pairs using Mahalanobis as the distance metric. ITML minimises the (differential) relative entropy, aka Kullback-Leibler divergence, between two multivariate Gaussians subject to constraints on the associated Mahalanobis distance, which can be formulated into a Bregman optimization problem by minimizing the LogDet divergence subject to linear constraints. The balanced dataset was split randomly into a test set and training set using a 80%:20% ratio. A 5-fold CV approach was undertaken to assess mean CV performance. A grid search was then undertaken to identify the best hyperparameters whilst generalising the performance. In the ITML approach, Morgan chemical fingerprint representations were used to characterise the analogue pairs.

2.8.3. UMAP

A different way of representing the input data was to take the difference between the analogue pairs and fit a UMAP to learn an embedding that would separate substance pairings that were similar from those that were dissimilar using Morgan chemical fingerprints as an input. A supervised UMAP

approach was undertaken using 5 neighbours and a Jaccard metric. The supervised embedding was then applied into a Support Vector Machine as classifier fit analogue pairs. A 10 fold cross validation strategy was used to evaluate the performance using balanced accuracy as a test metric. The model was refitted to the training set and applied to the 25% test set that had been left out during training and testing.

3. Results and Discussion

3.1. Schema for the Dataset

to be done

3.2. Dataset Summary

Currently, 82 read-across assessments were extracted from three main sources. There were 22 unique decision contexts which were then grouped by 3 main types - New Approach Methods (NAMs), technical guidance or regulatory purposes. For the 82 examples, 68 cases (82%) were developed to meet a regulatory purpose, the remainder were relatively evenly split between efforts to improve existing technical guidance (10%) or illustrate the utility of NAMs to substantiate read-across justifications (7%). The balance of decision contexts is not unsurprising given the origin of the case studies, ~25 (30%) of the cases were taken from the US EPA PPRTV effort, 38% were OECD SIDs examples, 15% were OECD IATA case studies, 10% from journal articles with the remainder comprising a couple of examples each from ECETOC or Health Canada. Of the approaches used, there was a bias towards category approaches with 55% of cases utilising a category approach and 36% being analogue approaches. All the EPA PPRTV cases relied on an analogue approach whereas in general over 90% of all other examples used a category approach.

3.3. Chemical Landscape

In terms of the chemical landscape, the read-across cases captured 498 unique substances of which structures could be mapped for 467 of them. For the substances without structures, these were all UVCBs arising from 11 different studies drawing from the OECD IATA and SID examples. For the 467 substances with SMILES, the ClassyFire service was used to tag each substance with a superclass, class annotation to summarise the types of functional classes captured by the read-across cases. ClassyFire superclasses Benzenes and substituted derivatives (25%), Fatty Acyls (14%), Carboxylic acids and derivatives (12%) as well as Organooxygen compounds (12%) captured ~63% of all the chemicals. A t-SNE plot Figure 1 based on Morgan chemical fingerprints colour coded by the most populist chemical superclasses illustrates the read-across landscape to provide some perspective of the makeup of the chemistry.

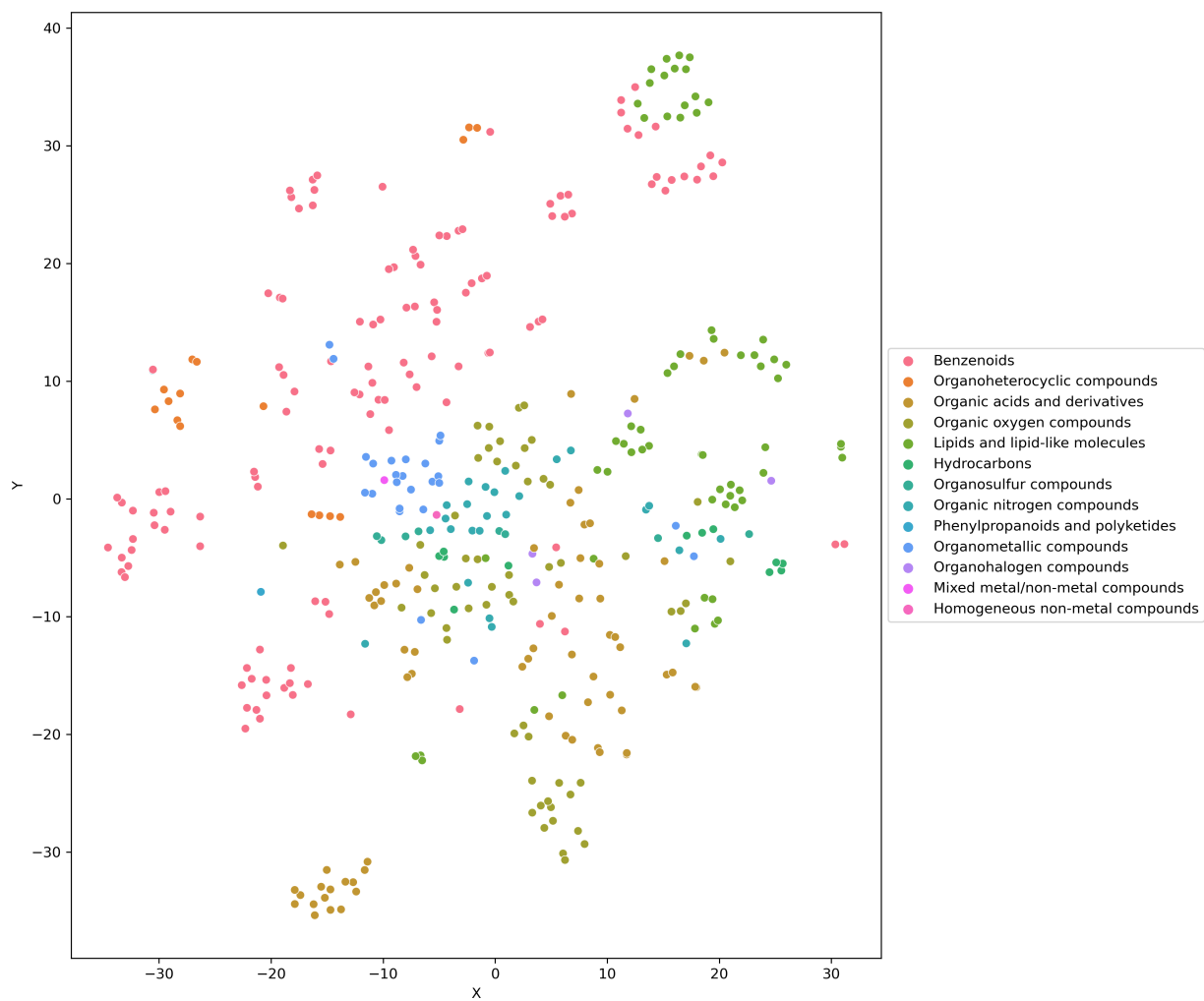


Figure 1: t-SNE plot colour coded by ClassyFire classes

3.4. Analogue Evidence Streams

In this study, the analogue identification strategy was recorded for each read-across case. This was captured in two ways in: 1) a narrative form and 2) more machine readable manner. The narrative provided a short description of the way in which analogues were identified and evaluated for their suitability for reading across the endpoint. The machine readable summary provided a long pipe-delimited string that captured all types of similarity rationales used to identify and evaluate analogues. This was referred to as an "analogue evidence stream". An example would be take the form of "Structural_description|Physchem_description|Metabolism_description|Mechanistic_description". An actual example is 'Structural_CHRIP_OECD-Toolbox_common-phenolic-group-at-same-position-on-benzotriazole | Physchem_similar-logKow-volatility | Metabolic_common-metabolite|Mechanistic_transcriptomic-profiles_similar-predicted-MOA |Toxicity_common-target-organ'.

To summarise the primary means of identifying candidate source analogues - the first component of the analogue evidence stream was extracted which revealed that in 72 cases, structure was the first means of identifying analogues. In contrast, metabolic similarity was the primary means of identifying analogues only in 4 cases. Across the 72 structure-based cases, it was possible to summarise the types of tools and approaches used to identify the candidate analogues. The OECD Toolbox (www.qsartoolbox.org), the [EPA CompTox Chemicals Dashboard](#) and [ChemIDPlus](#) or some combination were the main tools relied upon to identify candidate analogues. The barplot in Figure 2 highlights the main tools and approaches. Though structural similarity using a similarity metric is often used by these tools or their combinations, by far the most common means of identifying structural analogues within the examples was to look for common scaffolds based on functional groups.

3.5. Similarity contexts

The number of substances per case study varied considerably across the 82 cases with the median number of members being 5 and the maximum number of members being 42 as shown in Figure 3. The size of these neighbourhoods will have an impact of the range of variation expected in terms of structural similarity, physicochemical similarity etc.

3.5.1. Structural similarity Evaluation

Pairwise Jaccard structural similarity distributions Figure 4 were computed for all chemicals within each case finding a large variation in values. Although high Jaccard metrics were observed, the median of the distribution of median values for each case study was determined to be only 0.34 (see Figure 4a). Exploring the distribution of Jaccard structural similarities within each case also showed a large degree of variation (see Figure 4b).

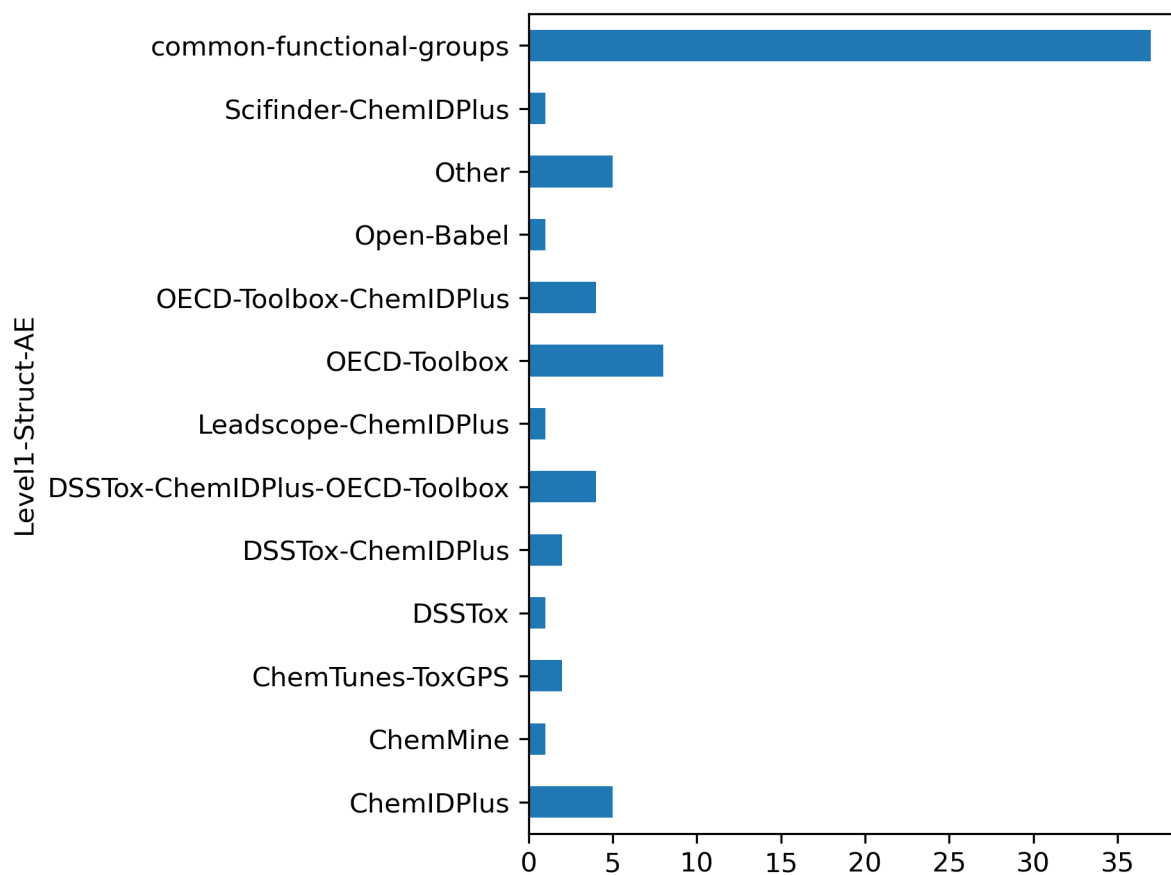


Figure 2: Main tools used in the structural analogue evidence streams

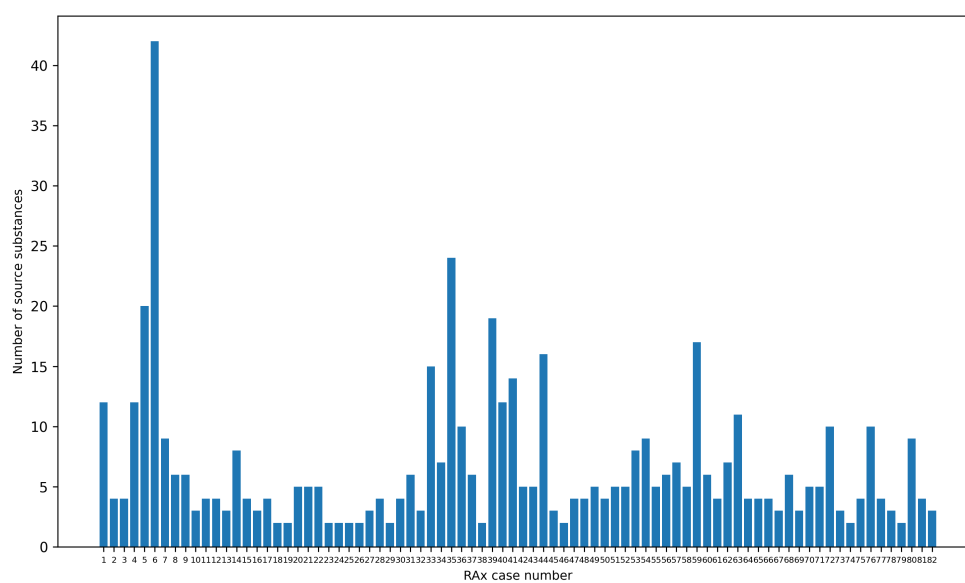
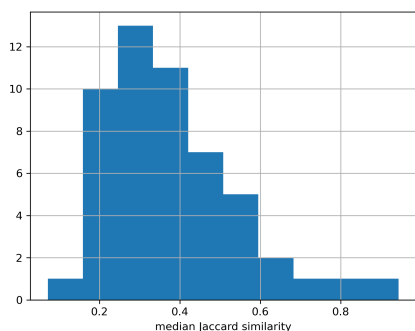
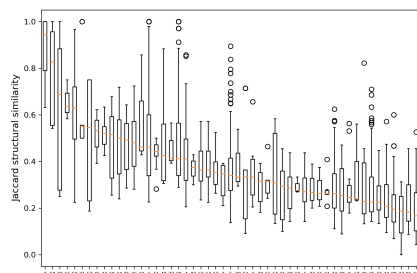


Figure 3: Membership count of substance per read-across case



(a) Distribution of pairwise Jaccard similarities



(b) Boxplot of pairwise Jaccard structural similarities within each read-across example

Figure 4: Structural similarity distributions

3.6. Alert Similarity Evaluation

There were 69 unique endpoint-toxicophore combinations across the substances included in the read-across cases. This captured 30 specific endpoints ranging from bone marrow toxicity, irritation to chromosomal damage and kidney function-related toxicity. A pairwise comparison of the profile across these endpoint-toxicophore combinations either was not particularly informative - substances either showed complete or no overlap, the same variation was noted within each read-across case as shown in Figure 5. A similar pattern of variation in physicochemical similarity within each read-across case was also seen in Figure 6.

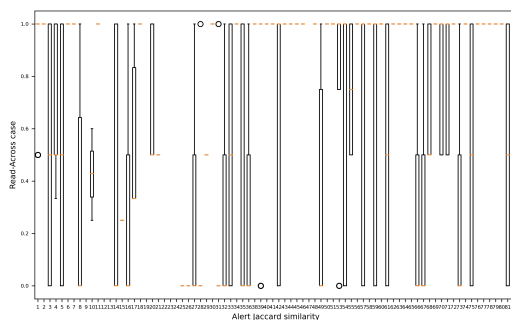


Figure 5: Boxplot of pairwise Jaccard alert similarities within each read-across example

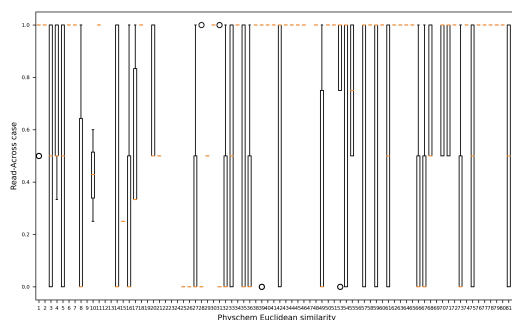


Figure 6: Boxplot of pairwise Euclidean similarities within each read-across example

3.6.1. Metabolic Similarity Evaluation

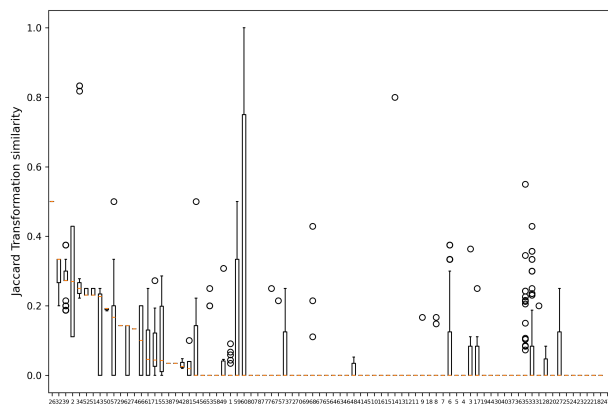
The pairwise similarities were computed within each read-across example to explore how metabolically similar the target and source analogues were amongst themselves with respect to their metabolic graph Figure 7c, transformation profile Figure 7b and metabolites Figure 7a as shown in Figure ?? There was a large degree of variation in pairwise similarities within each case study and the similarities were low overall.

3.7. Bayesian Logistic Regression of the balanced labelled analogue pairs

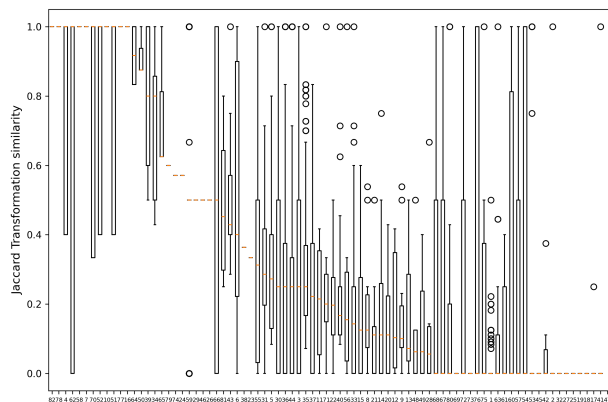
4 chains converged and the Rhat across the parameters all successfully converged. Based on the trace summary the similarity metric with the highest contribution for whether a pair of substances were similar was structural similarity, followed by similarity in the metabolism based on the graph kernel and transformation pathway. Figure 8 shows the posterior plot for the parameters estimated. The greatest uncertainty arises in the similarity of the metabolites. The balanced accuracy of the test set was determined to be 0.83. Figure 9 shows the confusion matrix for the test set predictions using the mean parameters. The profile of the parameters is well aligned to the features found to be most pertinent in the Patlewicz et al (2024) study even though the dataset was larger and more diverse.

3.8. Deep learning approach

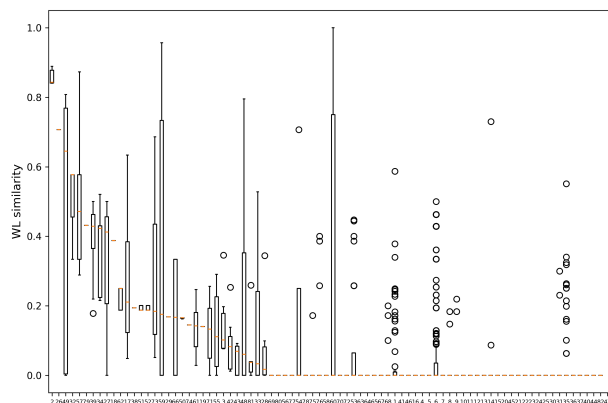
The deep learning approach using pytorch objects constructed from the SMILES themselves with the Graph Isomerism Approach network failed to create embeddings which could discriminate between similar and dissimilar analogue pairs. Whilst the accuracy was 0.67, the precision, recall and F1 scores on the test set were respectively 0.89, 0.40 0.55. Similarly the Graph Convolutional Network failed to create discriminating embeddings. The accuracy was only 0.6 with a precision, recall and F1 score of 0.68, 0.34 and 0.45 respectively.



(a) Boxplot of pairwise Jaccard metabolites similarities within each read-across example



(b) Boxplot of pairwise Jaccard transformation similarities within each read-across example



(c) Boxplot of pairwise WL graph kernel similarities within each read-across example

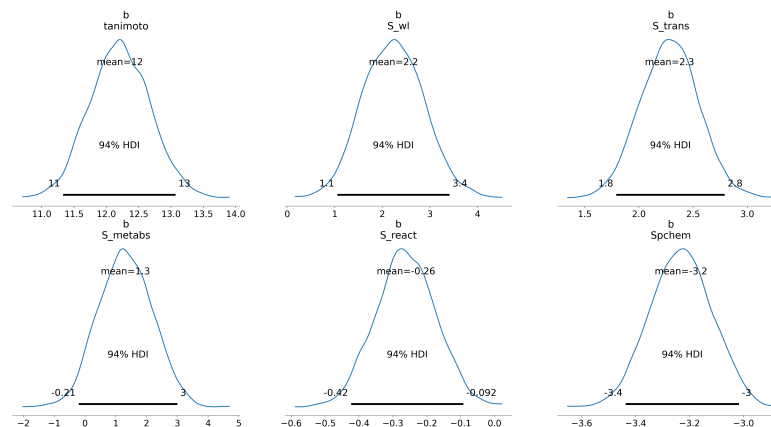


Figure 8: Posterior distribution of the parameters estimated

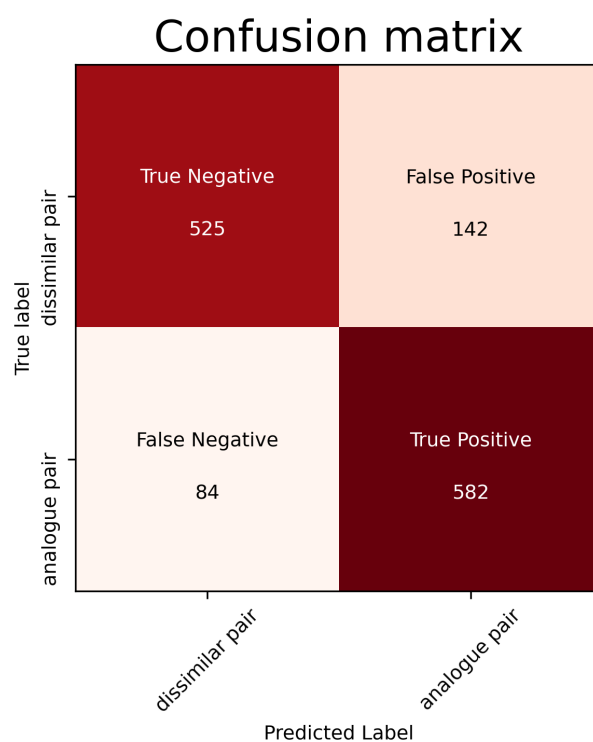


Figure 9: Confusion matrix for the test set not involved in the modelling process

3.9. Shallow learning approach

Using the same balanced dataset constructed, an ITML approach was performed as part of a stratified 5-fold CV workflow. The mean CV balanced accuracy was found to be 0.95 (sd 0.0058). A grid search for the hyperparameter gamma found that 0.75 gave rise to the highest mean CV performance. The ITML model was refit on the training set and predictions were made on the test set that had not been used during the training and testing loops. The accuracy of the test set was 0.95.

3.10. UMAP

Using the same balanced dataset constructed, a supervised UMAP was applied to learn an embedding that could discriminate similar pairs from dissimilar pairs. Different neighbours were used to preserve local structure in the embeddings though 5 neighbours proved to show the best separation in 2D for training and test sets (see Figure 10).

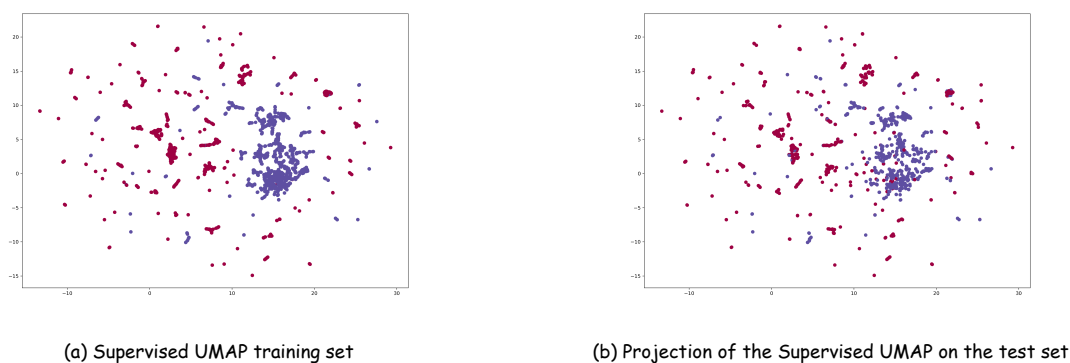


Figure 10: UMAP projections for the training and test sets.

Five neighbours and a Jaccard metric were then fed into a pipeline together with a support vector machine classifier. A 10 fold stratified cross validation approach was applied to assess performance. The mean (standard deviation) of the CV test set balanced accuracy was found to be 0.89 (0.017). The balanced accuracy of the test set that had been held out was 0.888. The performance was reasonable given that structure was expected to be a strong indicator of analogue suitability based the Bayesian logistic regression findings and the fact that there was a broad spread of Tanimoto indices across the similar and dissimilar analogue pairs showing that complete separation based on structural features alone was unlikely to be completely successful.

3.11. Conclusions

This compendium offers the potential to address a number of scientific questions related to how past read-across assessments have been performed and what future refinements are possible.

References