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Influence of Transcriptomic Descriptors on the Generalized Read-Across (GenRA) Performance

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ABSTRACT

Read-across is a data gap filling technique utilized to predict the toxicity of a target chemical using toxicity data from similar analogues. Recent efforts such as Generalized Read-Across (GenRA) (Shah et al., 2016) facilitate automated read-across predictions for untested chemicals. GenRA aims to make predictions of toxicity outcomes based on "neighboring" chemicals characterized by chemical and/or bioactivity fingerprints. Here we investigated the impact of biological similarities (based on targeted transcriptomic data) on neighborhood formation and read-across performance in predicting hazard classifications (based on repeat-dose testing outcomes from US EPA ToxRefDB v2.0) using the recently developed python package, *genra-py*. We treated HepaRG™ cells with 8 concentrations of 1,060 chemicals and measured the expression of 93 transcripts, which measure nuclear receptor activation, xenobiotic metabolism, cellular stress, cell cycle progression, and apoptosis. Transcriptomic similarity between chemicals was calculated using binary hit-calls from concentration-response data for each gene. We evaluated GenRA performance in predicting ToxRefDB v2.0 toxicity outcomes using area under the ROC curve (AUC) for the baseline approach (chemical fingerprints) versus transcriptomic fingerprints and a combination of both (hybrid). Overall, an increase in read-across performance was noted for various toxicity endpoints when using either transcriptomic or hybrid fingerprints over baseline. For example, for all liver endpoints, there was a 10% improvement in performance utilizing transcriptomic fingerprints and a 16% improvement with hybrid descriptors. We also saw improved predictive performance using a combination of various chemical fingerprints (Morgan, Torsion Topological, and ToxPrints). Thus, integration of diverse descriptors, either bioactivity combined with chemical information or combinations of various chemical fingerprints, offer significant benefit in predicting *in vivo* toxicity outcomes. *This abstract does not reflect U.S. EPA policy.*

OVERVIEW

- Given both national and international efforts to significantly reduce animal testing through developing new approach methodologies (NAMs) to inform chemical hazards and risks, our objective was to investigate the feasibility and performance of targeted high-throughput transcriptomics (HTTr) in assigning *in vivo* toxicity read-across predictions for untested chemicals using the Generalized Read Across (GenRA) approach (Shah et al., 2016).
- In subsequent analyses, we have focused on exploring enhancements to read-across; either through characterizing other similarity considerations, e.g., physicochemical properties as a surrogate for bioavailability (Helman et al., 2018) and quantifying their relative contribution to improving read-across performance or transitioning to predictions of potency (Helman et al., 2019a, b).
- Our current works utilizes the newly developed *genra-py* package to evaluate whether HTTr descriptors individually or in combination with chemical structure descriptors offer improvement and/or significant benefit in predicting *in vivo* toxicity outcomes.

METHODS

Data

1. 1060 Chemicals and Reference Chemicals

2. Chemical descriptors (chm):
- 2048 Morgan (mrgn)
- 2048 Topological Torsion (tptr)
- 729 ToxPrints (toxp)

3. Transcriptomic descriptors(bio):
- 95 Gene (ge)
- 189 Assay (asy)

4. 922 Toxicity outcomes (tox)

5. 86 Predefined Chemical Clusters

Find Best n-Neighbors and Similarity Metric

Utilize a 5-fold grid search cross validation and AUC scoring to determine appropriate number of neighbors and similarity metric for each descriptor type.

Generate Local Neighborhoods

Group chemicals using a similarity-weighted activity score of nearest neighbors.

Similarity calculated by:
- Jaccard distance
- Manhattan distance
- Euclidean distance

Performance Evaluation

Evaluate the impact of diverse descriptors (chm, bio, chm+bio) on GenRA global and local read-across performance.

Figure 1: Workflow for analysis of multiple descriptor types in GenRA.

RESULTS

Data Representation:
- 1060 test chemicals and reference chemicals from the ToxCast Phase I and Phase II libraries
- Each chemical was represented by binary chemical (chm), biological (bio), and toxicity (tox) vectors:
  - 729+ Chemical fingerprints descriptors were generated by the python library RDKit or the Chemotyper (Yang et al., 2015; chemotyper.org) (for ToxPrints)
  - 95 + Biological "hit call" descriptors from metabolically competent HepaRG™ cells LTEA assay of ToxCast HTS data set we derived for the tcpl package in R.
  - 922 Toxicity effects (derived from ToxRefDB v2.0) were aggregated by study types including: chronic toxicity (chr), subchronic toxicity (sub), subacute toxicity (sac), developmental toxicity (dev), multigenerational reproductive toxicity (mgr), reproductive toxicity (rep)), acute toxicity (acu), and neurological toxicity (neu).
  - Note: presence of chemical structure features , biological activity, and/or significant target effect (tox), was denoted as "1", else denoted as "0"
- A total of 13 chemical [mrgn, tptr, CA (all chemical fingerprints)], biological [ge, asy], and hybrid (chemical + gene) [ma, mg, tta, ttg, txa, txp, cb (all gene and chemical descriptors] were assessed for prediction of *in vivo* toxicity using *genra-py*.

Table 1: Determining appropriate metrics and number of nearest neighbors to assess the performance of various descriptor read-across prediction of chronic liver toxicity.

Descriptor Type	Descriptor Name	AUC	Metric	Number of Neighbors
Chm	tptr	0.6303	Euclidean	9
Chm	mrgn	0.64549	Jaccard	8
Chm	toxp	0.61379	Jaccard	7
Bio	ge	0.648847	Euclidean	14
Bio	asy	0.6632	Euclidean	11
Hybrid	mrgn + asy (ma)	0.6883	Jaccard	13
Hybrid	toxp + ge (txg)	0.7044	Jaccard	10
Hybrid	tptr + ge (ttg)	0.6818	Euclidean	6
Hybrid	(CB) all	0.6999	Jaccard	14
Chm	(CA) all	0.6702	Jaccard	10
Hybrid	**mrgn + ge (mg)**	**0.7049**	**Jaccard**	**10**
Hybrid	toxp + asy (txa)	0.6992	Jaccard	14
Hybrid	tptr + asy (tta)	0.6721	Manhattan	5

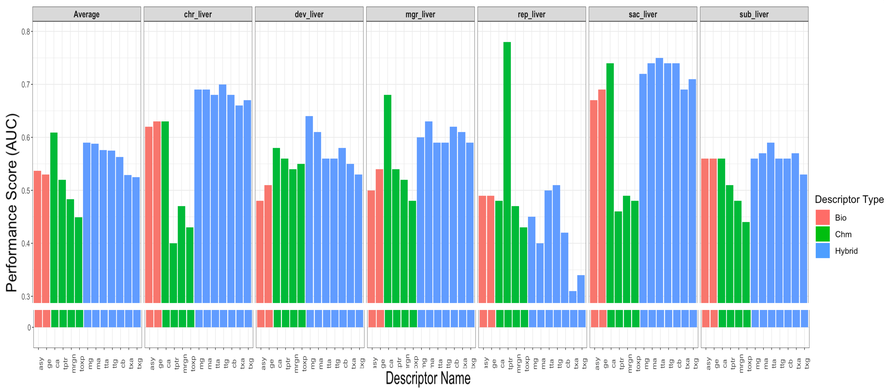
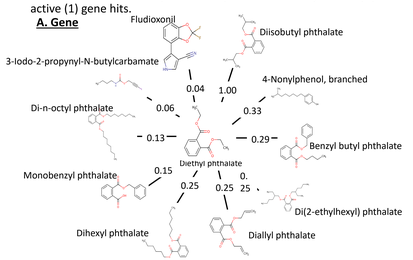
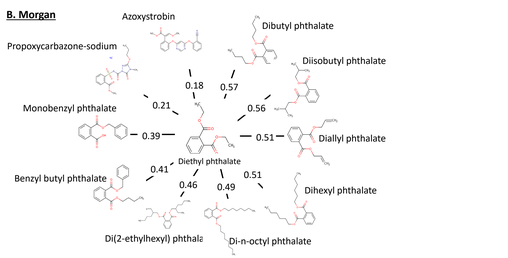
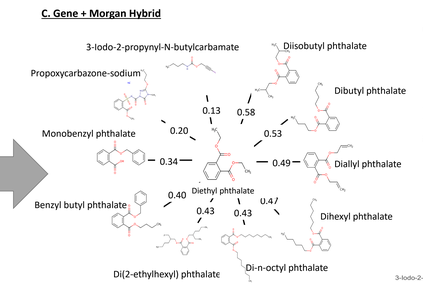


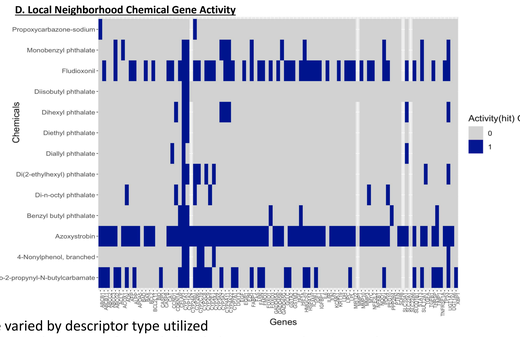
Figure 2: Global Prediction Performance (AUC) for All Liver Endpoints Using Various descriptor types (dt) including: Chemical Structure (C), Biological (B), and Hybrid (CB) Descriptors.

Figure 3: Gene (A), Morgan (B), and Hybrid (C) local neighborhoods for target chemical Diethyl phthalate derived from a predefined group of chemicals. Numbers in the center represent the pairwise similarity scores. **(D)** Heatmap of local neighborhood chemicals gene activity, 0 and 1 correspond to inactive (0) or active (1) gene hits.









- Chemical neighborhood order and pairwise score varied by descriptor type utilized
- While Gene descriptor produced a perfect pairwise similarity score for Diethyl phthalate Morgan descriptors the most consistent average scores for similar this group of chemicals.
- Note: Figure 3D confirms similar gene activity for various chemicals, particularly, diethyl phthalate and diisobutyl phthalate as expected by similarity score.

SUMMARY & FUTURE DIRECTIONS

In this study, using the newly developed stand-alone version of GenRA, *genra-py* (Shah et al., *submitted*) , we extended the GenRA approach to transcriptomic data comprising binary hit calls (activity calls) from concentration-response data for each gene. Our analysis estimated the global performance of GenRA (using diverse individual and combinations of transcriptomic binary hit call measures and chemical structure fingerprints) in predicting liver toxicity and other systemic toxicity effects.

The global read-across performance for all neighborhoods suggests that hybrid combinations of biological and chemical descriptors were effective for numerous toxicity endpoints. This was also the case for the combination of multiple chemical descriptors.

Next Steps in progress:
- Evaluation of HTTr in multiple cell types (beyond the liver) for screening thousands of chemical
- Expansion of scope of biological fingerprints to biological pathways
- Comparison of the GenRA approach to other machine learning approaches

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