Evaluation of USEPA's Generalized Read-Across (GenRA) Version 3 Using Example Case Studies

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This project will evaluate the most recent release of the USEPA's GenRA application (v3). Specifically, this new research project will review and analyze GenRAv3, highlight the updates that have occurred and discuss the strengths and limitations of GenRAv3. This information will be used to further guide discussions on potential research efforts to improve GenRAv3 and build confidence in its use by stakeholders. The project is comprised of 6 Tasks.

- Task 1. Evaluate and Document Changes in GenRA Since the Release of GenRAv1: An evaluation of any changes that have occurred in GenRAv3 since the release of GenRAv1 will be made and all updates will be documented.

- Task 2. Evaluate Similarity Metrics and Analogue Predictions: In ToxStrategies previous research, GenRAv1 was found to have significant issues in the on-line analogue prediction feature. These issues prevented using the on-line GenRA application to assess the similarity metrics and analogue predictions and necessitated the development of code by ToxStrategies to allow for critical evaluation of these functionalities. In this task, GenRAv3 analogue predictions will be evaluated using the online GenRAv3 application and the case studies identified in our previous work. The results of this analysis will be compared to the output from the ToxStrategies coded GenRA v1. For evaluation of new features e.g., Ketcher chemical structure editor, and those not evaluated in the initial project (due to GenRA coding issues), it will be assumed that there are no issues with the GenRAv3 code and no additional quality control of the GenRAv3 application will be performed. This will be confirmed. All features will be evaluated to determine their strengths and limitations in read-across.

- Task 3. Case Studies to Evaluate Read Across Toxicity Value Predictions: Read across case studies will be performed to assess toxicity value predictions using each of the similarity metrics available in GenRA v3. The predicted toxicity values will be compared to published toxicity and/or the results of the GenRA v1 case studies. Five similarity metrics are currently available: Chemical Similarity Metrics (Morgan fingerprints, Torsion fingerprints, ToxPrints) and Biological Similarity Metrics (ToxCast data, ToxRef data. Case studies will include two chemicals with published toxicity data (1,3- dichlorobenzene, 1,4-dichlorobenzene), one chemical with low analogue similarity predictions (spiroxamine) and one chemical with high analogue similarity predictions (dibutyl phthalate). If necessary, based on findings from these chemicals, other target chemicals may be included as needed to fully assess the strength and limitations of GenRA read across.

- Task 4. Evaluation of the GenRA Py Package for Batch Searching: GenRA v3 includes a stand-alone python library (genra-py package) that provides a programmatic batch means of using GenRA to enable user specific data to be analyzed. GenRA v1 did not provide a means of batch searching so this task will assess whether the read across predictions from batch searching are comparable to those from the GenRA online application as well as the usability of the python package.

- Task 5. Case Study: Chemical That is Metabolized to Toxic Substance: It is not apparent that there is a metabolism prediction within the GenRA tool that could evaluate similarity in metabolism between the target and source chemicals. A case study will be conducted of a target chemical known to be metabolized to a toxic substance. This case study will examine if the extent to which analog chemicals selected have similar chemical features and similar metabolites to the source chemical. ToxStrategies will identify potential criteria for selecting case examples to consider for this analysis.

- Task 6. Evaluation of Hybrid Similarity Metric: The hybrid similarity metric is an option to choose up to

three different criteria for defining similarity. ToxStrategies will investigate the different permutations of the three metrics to understand if any combination is better than the single metric at predicting toxicity endpoints. Multiple chemicals will be used in this evaluation.

Implications: Read-across is an important approach for inferring dynamic and kinetic properties for both new and existing chemicals. Read-across is considered a new approach methodology that does not require the use of laboratory animal toxicity testing. Therefore, research that improves read-across methods and establishes scientific confidence in such tools as EPA's GenRA v3 will contribute to improving the efficiency and scientific basis of chemical safety assessments.

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