A Benchmark Concentration Analysis Method for Zebrafish Larval Locomotor Response Data Using ToxCast Pipeline Software

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Background

- Zebrafish (danio rerio) larval locomotor response (LMR) assay is used to screen chemicals for potential hazard of developmental neurotoxicity (DNT).

Objective

- This work aims to develop a sensitive and reproducible benchmark concentration (BMC) analysis procedure for high-throughput LMR data that evaluates chemical effects on high-throughput Zebrafish LMR behavior in finer detail.

Problems Addressed

- BMC potency metrics are becoming the standard. BMC analysis of LMR data facilitates future comparison of assays.
- Typically, only Area Under the Speed by Time Curve (AUC)[1] or Average Speed[2], are used as LMR endpoints for BMC analysis resulting in a large loss of information.
- This work adds a set of LMR endpoints to be used for BMC analysis, reducing the information lost.

Set of Endpoints Analyzed

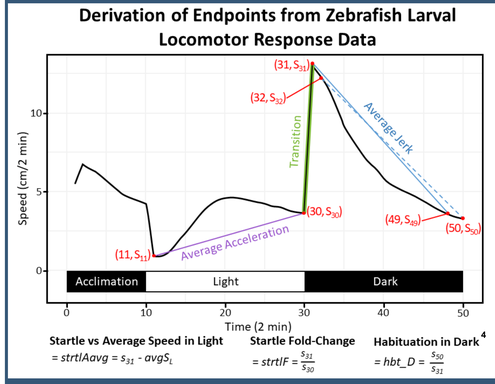

Fig 1: Endpoints for LMR overlayed on archetypical LMR data. Some endpoint formulas are shown below. Endpoints were gathered from literature or developed for this work.

- Added endpoints are designed to capture biologically relevant characteristics of the LMR.
- Most endpoints are calculated for Light, Dark, and Light+Dark separately.
- Average Acceleration is calculated as the slope of the purple line.
- Average Jerk is calculated as the difference in the slopes of the two blue lines.
- Startle Acceleration is the length of the green Transition line.

Benchmark Concentration Analysis with ToxCast Pipeline Software

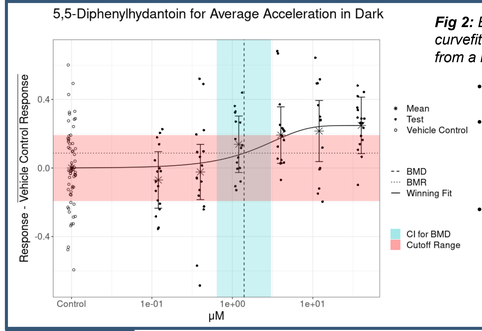

Fig 2: Example of ToxCast pipeline BMC analysis. tcplfit2 curvefitting of Average Acceleration in Dark endpoint data from a multi-concentration 5,5-Diphenylhydantoin exposure.

- Power transformed endpoint data is processed by tcplfit2.
- tcplfit2 fits 9 functions and identifies the best fit to the data.
  - **Functions Fit:** Hill, gain-loss, a constant function, 4 exponential functions, and 1st and 2nd degree polynomials.
- tcplfit2 outputs potency metrics and activity metrics.
  - BMR dependent on the variation of vehicle control response.
    *BMR = 1.349*SE (vehicle control response)*
  - Activity metrics are dependent on magnitude of response and quality of the curve-fit to the data.

Benchmark Concentration Analysis Applied to High-Throughput LMR Data with a Reproducible Workflow

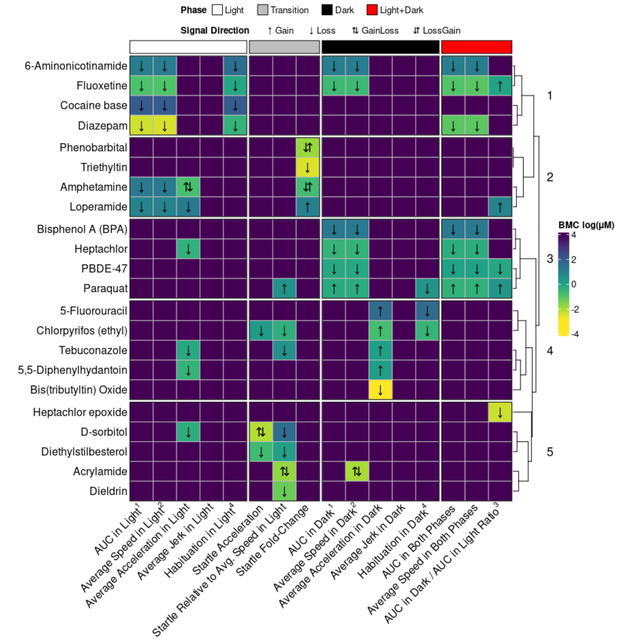

Fig 3: BMCs of active chemicals. Chemicals are considered active if tcplfit2 activity metric, hitcall, was greater than a threshold value. Rows correspond to active chemicals and columns correspond to endpoints. Colored annotation bar indicates phase that each endpoint describes. Arrows indicate directionality of response. Chemicals were clustered in a pairwise manner using Pearson's correlation coefficient.

- Analysis procedure was applied to data generated by multiple concentration LMR assay of 61 chemicals.
  - Zebrafish were exposed to chemicals during brain development.
  - 16 endpoints were calculated for each chemical.
- Chemical activity was seen across the supplemental endpoint set.
  - Only one endpoint, Average Jerk, did not detect chemical activity in this 61-chemical set.
- Clustering of chemicals by potency metrics appears to reveal general patterns in the data.
  - **Cluster 1** chemicals predominantly affect the Light, Dark, and Light+Dark phases.
  - **Cluster 2** chemicals predominantly affect the Light phase and Transition phase.
  - **Cluster 3** chemicals predominantly affect the Dark and Light+Dark phases.
  - **Cluster 4** chemicals affect rate of change metrics, most notably Average Acceleration in Dark.
  - **Cluster 5** chemicals predominantly affect the transition phase with one outlier, Heptachlor Epoxide.

Added Endpoints Double Number of Chemicals Found Active

A: Chemicals active in AUC or Average Speed.
B: Chemicals active in endpoints added by this work.

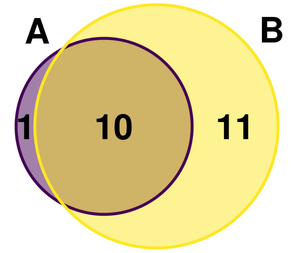

Fig 4: Activity detection added by analysis of endpoint set. Values indicate number of chemicals active per set.

A: Chemicals active in Average Speed or AUC in Dark.
B: Chemicals active in Average Acceleration in Dark.

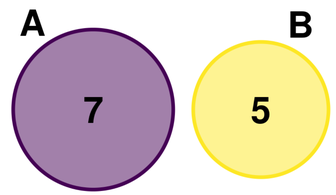

Fig 5: Information added by analysis of Average Acceleration endpoint in Dark phase. Values indicate the number of chemicals active in each set.

- Above left, supplemental endpoint set doubled the number of chemicals found active potentially indicating the information lost.
  - Future evaluation of the biological relevance of this endpoint set will be important in assessing the information added by this analysis procedure.
- Above right, inclusion of Average Acceleration in Dark endpoint identifies 5 chemicals that alter zebrafish behavior not detected by Average Speed in Dark.
  - Set of chemicals detected as active by Average Acceleration in Dark does not overlap with set of chemicals found active in AUC or Average Speed in Dark endpoint.

Conclusions

- Inclusion of the endpoints added by this work appears to capture chemical perturbations of behavior that are missed by commonly used endpoints, AUC and Average Speed, indicating the utility of these added endpoints in detecting potential DNT risk.
- Perturbation of Average Acceleration in Dark endpoint occurred separately from AUC in Dark and Average Speed in Dark and could be associated with symptoms of chemically induced neurodevelopmental effects missed by AUC and Average Speed endpoints.

Future Directions

- Application of the analysis procedure to a larger set of chemicals containing reference chemicals could elucidate activity profiles associated with known modes of action or neurological diseases.
- Comparison to phenotypic Zebrafish assay results will reveal chemicals that are developmentally toxic to Zebrafish at concentrations lower than initially observed.
- Application of analysis procedure to a set of chemicals with known positive and negative controls will allow for an evaluation of endpoint sensitivity and specificity.
- Application of this analysis procedure to LMR data generated by different labs using a common set of chemicals, derived from different could identify endpoints that produce the most reproducible results across data sources.
- An in vivo to in vitro assay comparison can be made to assess cross-assay ability to detect DNT.

References

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