Lack of Methodological Consistency Impedes Interpretation of Developmental Neurotoxicity Larval Zebrafish Behavioral Assays

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- Larval zebrafish behavior assays are utilized to screen for developmental neurotoxicity.
- An extensive literature review was conducted to evaluate the consistencies and transparency among protocols.

Abstract Sifter [1] review for zebrafish larval behavior studies.
Search terms “chemical name + CAS” and “zebrafish AND neurotox*,” “zebrafish and (behavior or locomotor) and develop*,” “zebrafish AND (swim OR swimming OR locomotion),” or “zebrafish”
(67 chemicals screened by our laboratory, n=3,570 abstracts)

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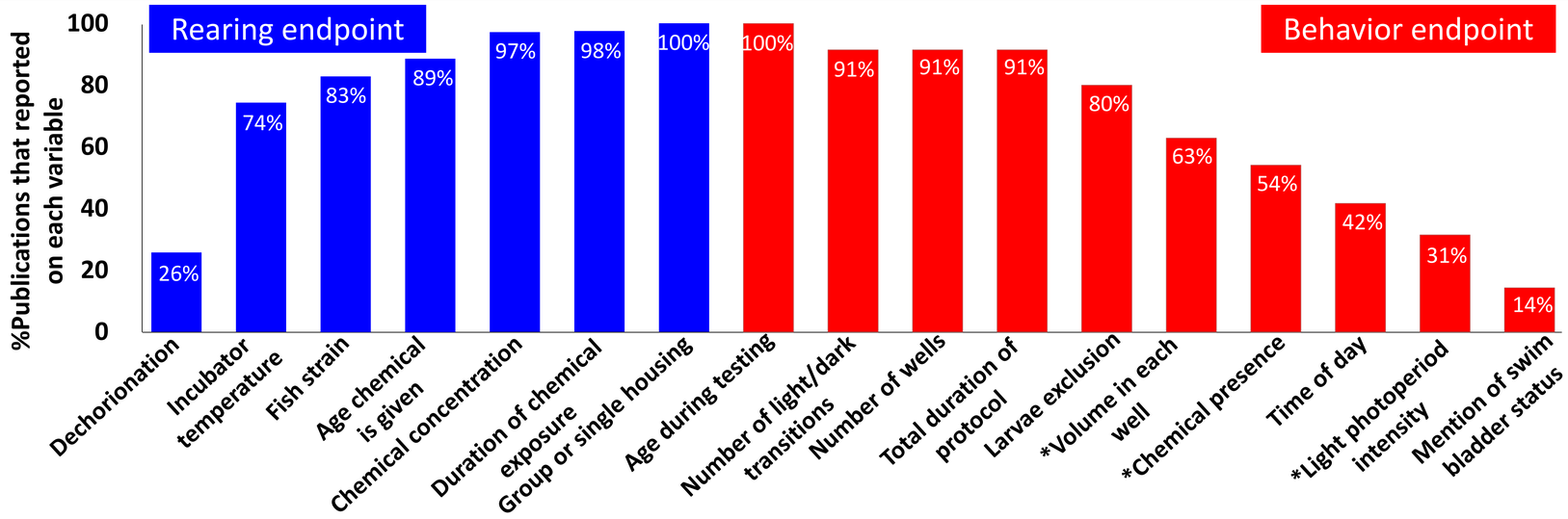
Methodological review for relevance. Focused on assays similar to our laboratory by asking the following questions:
(48 chemicals, n=105 publications)

- Were larvae tested for behavior between the ages of 5-6 days post fertilization (dpf)?
- Did the behavior protocol include at least one transition from light to dark?
- Was the chemical exposure during early development (0-3 dpf)?
- Did the chemical exposure last at least 24 hours?
- Was there an acclimation period prior to behavior testing?

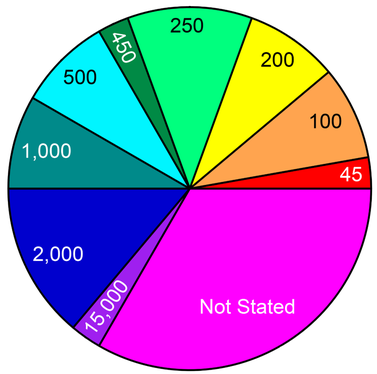
NO → Removed from review

YES or NOT STATED → Critiqued by two reviewers with 41 unique methodological variables extracted from publication
(39 chemicals, n=34 publications)

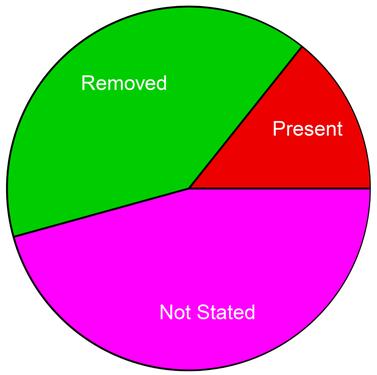
Focusing on variables known to influence baseline or chemically-induced changes in activity:
Many important methodological variables were not explicitly reported. Increased transparency is needed for replicability and data comparison.



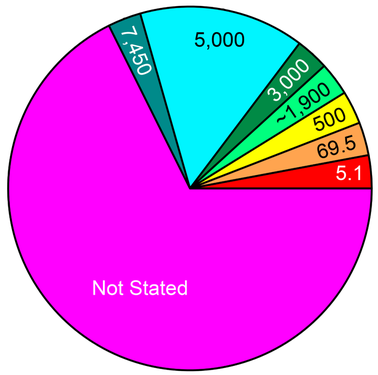
***Volume (µL) in each well during testing**



***Chemical presence during testing**



***Light (lux) photoperiod intensity during testing**



Highlighting some of these same variables, there a lack of consistency reported across publications with large proportions of these variables not stated. Protocol harmonization will increase consistency.

[1] Baker et al. Abstract Sifter: a comprehensive front-end system to PubMed. F1000Research 2017, 6(Chem Inf Sci):2164. We would like to thank Kimberly Jarema, Joan Hedge, Zach Rowson and Bridget Knapp for their insight and all animal care staff that maintain our zebrafish colony. *Disclaimer: The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.*