National Institute of Environmental Health Sciences
Division of Translational Toxicology

Understanding the Range of Phenotypic Responses for the Embryonic Zebrafish Developmental Toxicity Assay

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Background

- Embryonic zebrafish assays are used to screen chemicals for potential developmental toxicity.
- Different laboratories that screen chemicals using embryonic zebrafish employ different experimental protocols and often report toxicity as a single combined mortality and malformation metric.
- However, information on specific malformations is useful in chemical hazard assessments and can potentially inform on mechanism of action.
- The Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT) project explored how different experimental conditions can impact assessment of toxicological response to optimize zebrafish protocols (1).

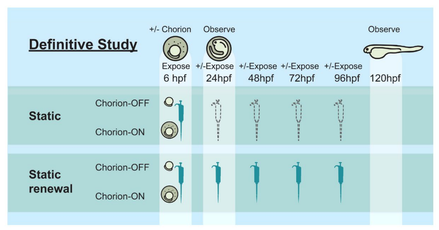
Interlaboratory Study

- SEAZIT coordinated an interlaboratory study that specifically focused on the effects of varying two protocol elements identified by zebrafish experts that may affect chemical potency:
  I. Experimental media renewal ("Static" vs. "Static Renewal")
  II. Chorion status of the embryo ("Chorionated" vs. "Dechorionated")
- This poster summarizes our findings on the variability of malformations observed by the vehicle or positive controls within three laboratories (Lab A, Lab B, Lab C) that used varying experimental protocol conditions.
- Data from all SEAZIT-coordinated studies are publicly available via the SEAZIT-DIVER resource (QR code below).

Explore SEAZIT-DIVER:

Protocol information, data, visualizations!
https://seazit.dtt.niehs.nih.gov/seazit/

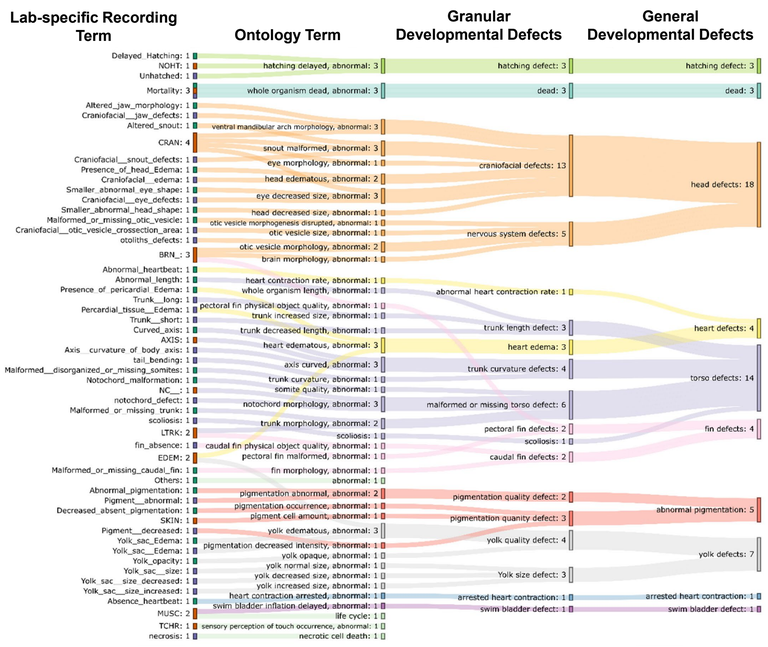
Experimental Design



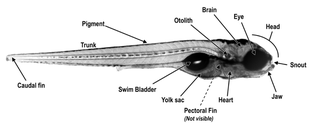
Labs participating in the study exposed embryos under four conditions: static exposure (-, dashed pipette), static renewal of exposure media every 24 hours (+, filled pipette), using both chorionated (ON) and dechorionated (OFF) embryos.

Phenotype Terminology Mapping

- Participating labs employed various terminologies to denote the same or similar phenotypes.
- The diagram below shows our harmonization approach for phenotype terms to enable comparisons among labs.
  - Lab-specific recording terms were first mapped to terms gathered from the Zebrafish Phenotype Ontology (2).
  - Terms were then grouped by granular and general developmental defects based on anatomical region.



Boxes next to each lab-specific recording term represent which of the three SEAZIT labs reported each term: Green = Lab A; Orange = Lab B; Purple = Lab C



Horizontal lines in this diagram, taken from Hsieh et al. (3), trace the term mappings from lab-specific recording terms (far left) to the general developmental defect grouping (far right), which are categorized by color.

Phenotype Responses Observed in Vehicle-Control Embryos

- The vehicle control was **0.5% dimethyl sulfoxide (DMSO).**
- All three labs observed head defects in vehicle-control embryos.
  - Lab-specific terms used for these included "Smaller_abnormal_head_shape" (Lab A), CRAN (craniofacial region, Lab B), and "Craniofacial_snout_defect", and "Craniofacial_jaw_defect" (Lab C).
- Labs A and C observed decreased survival of dechorionated embryos.
- There were few other observed alterations in the three labs particularly in Labs A and B.

general developmental defect	Lab A					Lab B					Lab C				
	lab-specific recording term	S-C	S-DC	SR-C	SR-DC	lab-specific recording term	S-C	S-DC	SR-C	SR-DC	lab-specific recording term	S-C	S-DC	SR-C	SR-DC
dead	**Alive with altered phenotype(s)** **Mortality**					**Alive with altered phenotype(s)** Mortality					**Alive with altered phenotype(s)** **Mortality**				
abnormal pigmentation	Abnormal_pigmentation † Decreased_absent_pigmentation					SKIN (pigmentation defects)									
fin defect	Malformed_or_missing_caudal_fin †					BRN_ (brain region) † LTRK (lower trunk region)					fin_absence †				
hatching defect	**Delayed_Hatching**					NOHT (non-hatched)					Unhatched †				
head defect	Altered_jaw_morphology Altered_snout † Malformed_or_missing_otic_vesicle † Presence_of_head_Edema † Smaller_abnormal_eye_shape Smaller_abnormal_head_shape					BRN_ (brain region) † CRAN (craniofacial region)					Craniofacial__edema **Craniofacial__jaw_defects** **Craniofacial__snout_defects**				
heart defect	Abnormal_heartbeat Absence_heartbeat † Presence_of_pericardial_Edema					EDEM (edema)									
swim bladder defect						MUSC (muscle and blood circulation) †									
torso defect	Abnormal_length Curved_axis † Malformed__disorganized_or_missing_somites † Malformed_or_missing_trunk † Notochord_malformation †					AXIS (axis) LTRK (lower trunk region) NC__ (notochord) †					**Axis__curvature_of_body_axis** notochord_defect † **scoliosis** **tail_bending**				
yolk defect	Yolk_opacity † Yolk_sac_Edema					EDEM (edema)					**Yolk_sac__Edema**				
not classified	Others					**TCHR (touch response in the caudal fin)**					**necrosis**				

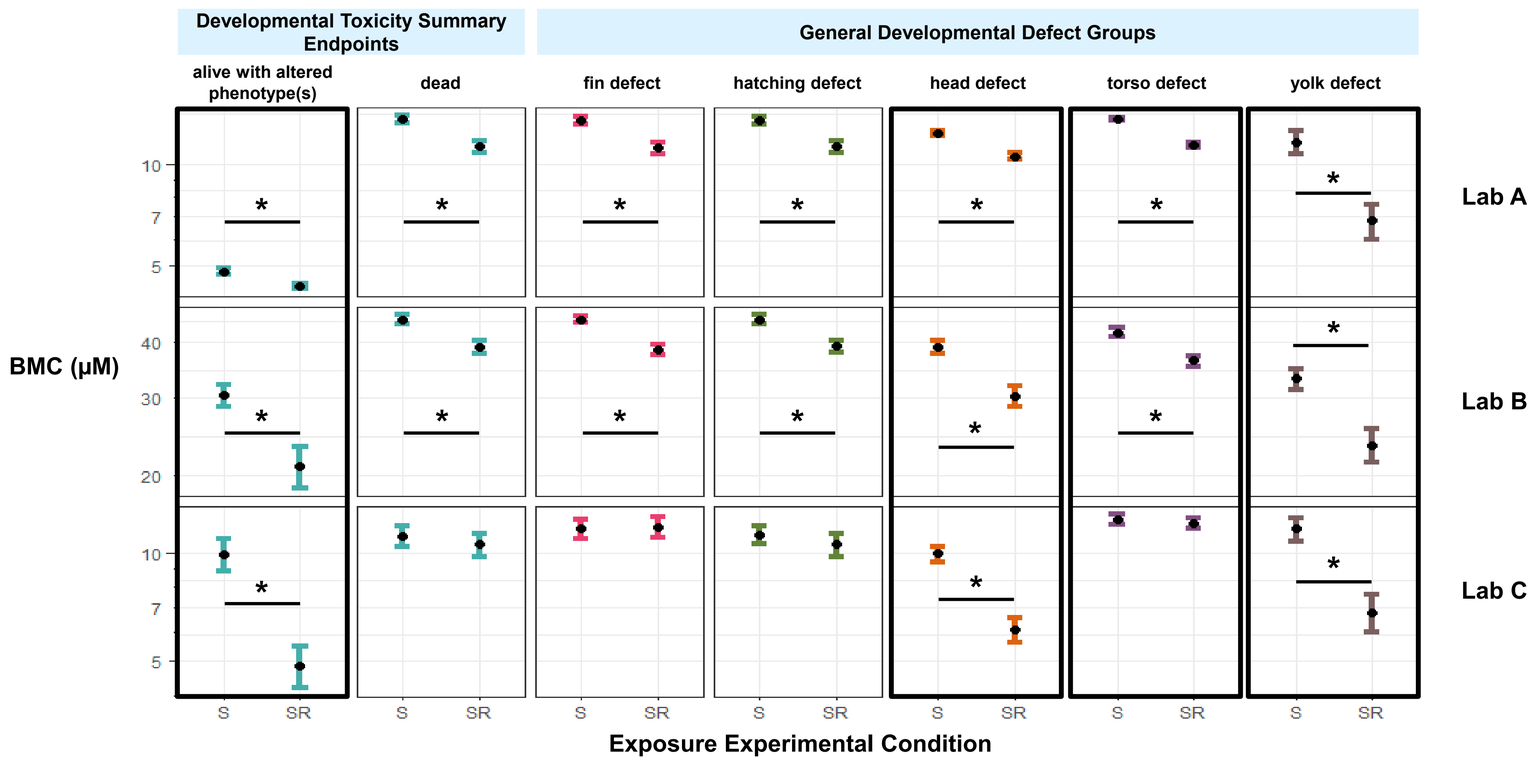
0-0.5% affected; >0.5-1% affected; >1-3% affected; >3-5% affected; >5-8% affected; >8-10% affected; >10-15% affected

Heatmap color represents the percent of larvae exhibiting lab-specific phenotypes, which have been further grouped into general developmental defects.
- Dark purple color indicates a lower percentage of affected larvae while light turquoise color indicates a higher percentage affected.
- Grey shading indicates either that a lab had no specific term representing a phenotype or that it did not include that phenotype in their assessments of development defects.
- Vertical lines indicate that the phenotype could not be assessed due to removal of the chorion.

Bolded and underlined terms indicate a significant difference ($p \leq 0.05$) between results observed under different experimental conditions within one lab (chi-squared test, with † indicating phenotypes that were not statistically evaluated due to small sample sizes).
S-C = Static-Chorionated, S-DC = Static-Dechorionated, SR-C = Static Renewal-Chorionated, SR-DC = Static Renewal-Dechorionated

Phenotype Responses Observed in Embryos Exposed to Positive Control

- The positive control **3,4-dichloroaniline (DCA)** was tested using a minimum of five concentrations in each lab.
- The experimental condition variable that produced the most differences among the altered phenotypes was media renewal.
  - Lower benchmark concentration (BMC) values were generally observed for static renewal protocols.
  - At least two of the three labs recorded significant differences in occurrence of altered phenotypes between exposure condition.
- While not shown, there was also an effect of chorion status for all aggregated general defect groups reported by Lab A and two aggregated defect groups reported by Lab C (head and torso).
- Notable differences in BMC values among labs across many developmental defect groups may have been due to experimental handling or genetic variation in the zebrafish strain.
- The degree of variability differed across labs but was generally consistent within a lab.



Plots above depict the average BMC values (µM) ± standard error of the mean between static (S) and static renewal (SR) exposure experimental conditions (regardless of chorion status) for each general developmental defect group that included at least one assessment term per lab.
- **Colors** indicate the defect groupings shown in the phenotype terminology mapping figure on the left.
- **Asterisks** indicate significant differences ($p \leq 0.05$) within a lab (two-way analysis of variance [ANOVA] adjusted for multiple comparisons).
- **Boxes** indicate that there was a significant ($p \leq 0.05$) difference between labs in a follow up nonparametric statistical test.

Key Takeaways and Next Steps

- These results help clarify the landscape of variability in phenotype assessments within and across laboratories that screen chemicals using embryonic zebrafish.
- The range of detail used by each laboratory to describe assessments indicates a need for phenotype term definition, refinement, and harmonization to ensure consistent reporting among laboratories that use zebrafish for developmental toxicity screening or research applications.
- The full interlaboratory study involved testing 42 chemicals in addition to the positive and vehicle controls reported here. Analyses of these data are in progress, which will enable between-lab comparisons, evaluations of phenotype alterations within this tested chemical set, and comparisons of variability among other models utilized for developmental toxicity screening approaches.

References and Acknowledgements

(1) Hamm et al. Toxics 2024, 12, 93. https://doi.org/10.3390/toxics12010093
(2) Zebrafish Phenotype Ontology: https://www.ebi.ac.uk/ols/ontologies/zp, accessed on 10 April 2023
(3) Hsieh et al. Toxics 2023, 11, 407. https://doi.org/10.3390/toxics11050407

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