

# Understanding the Range of Phenotypic Responses for the Embryonic Zebrafish Developmental Toxicity Assay <u>Bridgett Hill<sup>1</sup>, Jonathan Hamm<sup>1</sup>, Jui-Hua Hsieh<sup>2</sup>, Emily N. Reinke<sup>1</sup>, Helena T. Hogberg<sup>2</sup>, Kristen Ryan<sup>2</sup></u>

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## Background

**Explore SEAZIT-DIVER:** 

Protocol information, data, visualization

https://manticore.niehs.nih.gov/seazit

- Embryonic zebrafish assays are used to screen chemicals for potential developmental toxicity.
- Many research groups employ different experimental protocols and often report toxicity as a single combined mortality and malformation metric. However, specific information on 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) explored how different experimental conditions can impact assessment of toxicological response to optimize zebrafish protocols (1).
  - The study specifically focused on the effects of varying two protocol elements identified by zebrafish experts: experimental media renewal ("Static" vs. "Static Renewal") and chorion status of the embryo ("Chorionated" vs. "Dechorionated").
  - Data from SEAZIT is publicly available via the SEAZIT-DIVER resource (QR code below).
- This poster summarizes our findings on the variability of malformations induced by the positive or vehicle controls within three laboratories (Lab A, Lab B, Lab C) that used varying experimental protocol conditions.

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<u>Definitiv</u>	<u>e Study</u>	Chorion Expose 6 hpf	+/-	Observe	+/-Expose 48hpf	+/-Expose 72hpf	d +/-Expose 96hpf	Observe
Static	Chorion-OFF Chorion-ON			₩>====-	₩>====	94 4	94 V	
Static renewal	Chorion-OFF Chorion-ON			1	1	1	1	

#### SEAZIT Experimental Design for Definitive Study

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

#### **Ontology Mapping and Hierarchical Grouping of Laboratory-specific** Phenotype Recording Terms

This diagram, taken from Hsieh et al. (2), shows our harmonization approach for phenotype terms to enable comparisons between labs, since each of the labs utilized different terms to describe phenotypes. This process started with the lab-specific recording terms, which were mapped to ontology terms gathered from the Zebrafish Phenotype Ontology (3). Terms were then grouped by granular and general developmental defects based on anatomical region.

lab specific recording term	ontology term	granular developmental defects	general developmental defec
Delayed_Hatching: 1			
NOHT: 1 Unhatched: 1	hatching delayed, abnormal: 3	hatching defect: 3	hatching defect:
Mortality: 3	whole organism dead, abnormal: 3	dead: 3	dead:
Altered_jaw_morphology: 1		-	
Altered_snout: 1	ventral mandibular arch morphology, abnormal: 3		
CRAN: 4	snout malformed, abnormal: 3		
Craniofacialsnout_defects: 1	eye morphology, abnormal: 1	craniofacial defects: 13	
Craniofacialedema: 1	head edematous, abnormal: 2		boad defects: 1
Smaller_abnormal_eye_shape: 1	eye decreased size, abnormal: 3		field defects. I
Smaller_abnormal_head_shape: 1	head decreased size, abnormal: 1		
Malformed_or_missing_otic_vesicle: 1	otic vesicle morphogenesis disrupted, abnormal: 1		
Craniofacialotic_vesicle_crossection_area: 1	otic vesicle size, abnormal: 1	nervous system defects: 5	
otoliths_defects: 1	otic vesicle morphology, abnormal: 2		
BRN_: 3	brain morphology, abnormal: 1		
Abnormal_heartbeat: 1			
Abnormal_length: 1	heart contraction rate, abnormal: 1		
Presence_of_pericardial_Edema: 1	whole organism length, abnormal: 1	abnormal heart contraction rate: 1	
Percardial tissue Edema: 1	pectoral fin physical object quality, abnormal: 1		
Trunk short: 1	trunk increased size, abnormal: 1	1	
Curved_axis: 1	trunk decreased length, abnormal: 1 🛛	trunk length defect: 3	heart defects:
AXIS: 1	heart edematous, abnormal: 3	beart edema: 3	
Axis_curvature_of_body_axis: 1		near counter o	
alformeddisorganized_or_missing_somites: 1	axis curved, abnormal: 3	trunk curvature defects: 4	
Notochord_malformation: 1	trunk curvature, abnormal: 1		torso defects: :
NC: 1	somite quality, abnormal: 1		
notochord_defect: 1	notochord morphology, abnormal: 3	malformed or missing torso defect: 6	
scoliosis: 1	twink membels av shaemali 2		
	trunk morphology, abnormal: 2	pectoral fin defects: 2	fin defects:
fin absence: 1	scoliosis: 1	scoliosis: 1 🛛	
EDEM: 2	pectoral fin malformed, abnormal: 1	caudal fin defects: 2	
Malformed or missing caudal fin: 1	fin morphology apportal: 1		
Others: 1	abnormal: 1		
Abnormal_pigmentation: 1	pigmentation abnormal, abnormal: 2	and an an an and the second life of the second s	
Pigmentabnormal: 1	pigmentation occurrence, abnormal: 1	pigmentation quality defect: 2	
Decreased_absent_pigmentation: 1	pigment cell amount, abnormal: 1	pigmentation quanity defect: 3	abnormal pigmentation:
Pioment decreased: 1	volk edematous, abnormal: 3	pigmentation quarter b	
Yolk sac Edema: 1		yolk quality defect: 4	
Yolk_sacEdema: 1	pigmentation decreased intensity, abnormal: 1		volk defects:
Yolk_opacity: 1	volk normal size, abnormal: 1		for deread
Yolk_sacsize: 1	yolk decreased size, abnormal: 1	Yolk size defect: 3	
TOIK_SAC_SIZE_DECREASED: 1	yolk increased size, abnormal: 1		
Absence heartbeat: 1	heart contraction arrested, abnormal: 1	arrested heart contraction: 1	arrested heart contraction:
MUSC: 2	swim bladder inflation delayed, abnormal: 1	swim bladder defect: 1	swim bladder defect
	lire cycle: 1		<ul> <li></li> </ul>
TCHP+1	sensory perception of touch occurrence abnormal: 1 II		

lab-specific recording terms (far left) to the general developmental defect grouping (far right) which are categorized by color.



Boxes next to each lab-specific recording term represent which of the three SEAZIT labs reported each term: Lab A (green), Lab B (orange), Lab C (purple)

#### Vehicle and Positive Control Altered Phenotypes Differ Among Experimental Condition and Lab

In general, head defects were among the most sensitive endpoints across both positive and negative controls and all three labs, with some lab-specific terms such as "abnormal head shape" (Lab A), "snout defect", and "jaw defect" (Lab C) driving the head defect finding. There were also some notable differences in percent effect and benchmark concentration values among the labs, which may have been due to experimental handling or genetic variation in the zebrafish strain. Lab C reported a higher number of altered phenotypes in both the negative and positive control groups compared to the other two labs, and potency of the positive control was markedly lower in Lab B compared to the other labs.

The heatmaps below summarize the altered phenotypes observed by the three labs after treatment with vehicle and positive controls. Colors indicate severity of effects, as detailed below, per lab-specific recording term, which are furthered grouped by general developmental defect. Grey shading indicates that there was no additional lab-specific phenotype term or that a lab 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 between results observed under different experimental conditions within one lab (chi-squared test for vehicle control, with asterisks indicating phenotypes that were not statistically tested due to small sample sizes, and ANOVA test for the positive control endpoints alive with altered phenotype(s) and mortality).

general developmental defect	
dead	
abnormal pigmentation	
fin defect	I
hatching defect	
head defect	Μ
heart defect	
swim bladder defect	
torso defect	Malfor
yolk defect	
not classified	
Dark purple color indic	ates a

general developmental defect	
dead	
abnormal pigmentation	
fin defect	
hatching defect	
head defect	
heart defect	
swim bladder defect	
torso defect	Malfo
yolk defect	
not classified	

Green color indicates higher

S-C=Static-Chorionated, S-DC=Static-Dechorionated, SR-C=Static Renewal-Chorionated, SR-DC=Static Renewal-Dechorionated

Vehic	le Control, 0.5	% Dimethyl Sulfoxide	e, Percent Affe	cted	
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-D
Alive with altered phenotype(s)		Alive with altered phenotype(s)		Alive with altered phenotype(s)	
<b>Mortality</b>		Mortality		Mortality	
Abnormal_pigmentation*		SKIN (pigmentation defects)			
Decreased_absent_pigmentation					
Malformed_or_missing_caudal_fin*		BRN_ (brain region)*		fin_absence*	
		LTRK (lower trunk region)			
Delayed_Hatching		NOHT (non-hatched)		Unhatched*	
Altered_jaw_morphology		BRN_ (brain region)*		Craniofacialedema	
Altered_snout*		CRAN (craniofacial region)		Craniofacial_jaw_defects	
1alformed_or_missing_otic_vesicle*				Craniofacial_snout_defects	
Presence_of_head_Edema*					
Smaller_abnormal_eye_shape					
Smaller_abnormal_head_shape					
Abnormal_heartbeat		EDEM (edema)			
Absence_heartbeat*					
Presence_of_pericardial_Edema					
		MUSC (muscle and blood circulation)*			
Abnormal_length		AXIS (axis)		Axis_curvature_of_body_axis	
Curved_axis*		LTRK (lower trunk region)		notochord_defect*	
		NC (notochord)*			
meddisorganized_or_missing_somites*				scoliosis	
Malformed_or_missing_trunk*				tail_bending	
Notochord_malformation*					
Yolk_opacity*		EDEM (edema)		Yolk_sacEdema	
Yolk_sac_Edema					
Others		TCHR (touch response in the caudal fin		necrosis	
lower percentage of affected larvae while	light turquoise color indicate	es a higher percentage affected.			
0-0.5% affected >0.5-1% affect	ted >1-3% affected	>3-5% affected >5	-8% affected >8-1	.0% affected >10-15% affected	
Positive	Control, 3,4-D	ichloroaniline, Bench	mark Concent	trations	
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

lab-specific recording term       S-C       S-D       SR-C       SR-D       SR-C       SR-D	S-C S-DC SR-C S
Alive with altered phenotype(s)       Alive with altered phenotype(s)       Alive with altered phenotype(s)         Mortality       Mortality       Mortality       Mortality         Abnormal_pigmentation       SKIN (pigmentation defects)       Mortality         Decreased_absent_pigmentation       SKIN (pigmentation defects)       Mortality         Malformed or missing caudal fin       BRN_(brain region)       Image: Comparison of the phenotype(s)	
Mortality       Mortality       Mortality         Abnormal_pigmentation       SKIN (pigmentation defects)       Image: Comparise of the comparise of th	
Abnormal_pigmentation       SKIN (pigmentation defects)       Image: Comparising caudal fin the second seco	
Decreased_absent_pigmentation       Image: Constraint of the second	
Malformed_or_missing_caudal_fin BRN_(brain region) fin absence	
LTRK (lower trunk region)	
Delayed_Hatching Unhatched Unhatched Unhatched	
Altered_jaw_morphology   BRN_ (brain region)   Craniofacial_edema	
Altered_snout       CRAN (craniofacial region)       Craniofacial_jaw_defects	
Malformed_or_missing_otic_vesicle Craniofacial_snout_defects Craniofacial_snout_defects	
Presence_of_head_Edema	
Smaller_abnormal_eye_shape	
Smaller_abnormal_head_shape	
Abnormal_heartbeat EDEM (edema)	
Absence_heartbeat	
Presence_of_pericardial_Edema	
MUSC (muscle and blood circulation)	
Abnormal_length AXIS (axis) AXIS (axis) Axiscurvature_of_body_axis	
Curved_axis LTRK (lower trunk region) notochord_defect	
rmed_disorganized_or_missing_somites NC (notochord) scoliosis	
Malformed_or_missing_trunk tail_bending	
Notochord_malformation	
Yolk_opacity EDEM (edema) Yolk_sacEdema	
Yolk_sac_Edema	
Others TCHR (touch response in the caudal fin) necrosis	
benchmark concentration values (lower potency) while yellow colors indicate lower benchmark concentration values (higher potency). Benchmark concentration values for each phenoty	pe include mortality
0-5 uM \$5-10 uM \$10-15 uM \$15-20 uM \$20-25 uM \$25-30 uM \$30-25 uM \$25-40 uM \$40.45 uM	

## **Altered Phenotypes Varied After Exposure to Vehicle Control**

While Labs A and C observed decreased survival of dechorionated embryos, there were few other commonalities among altered phenotypes observed in the three labs. The bar charts below depict, for each lab: the percent mortality among larvae (number dead/total number tested; left chart) and the percent of the total number of altered phenotypes observed (number of larvae alive with altered phenotype(s)/total number alive; right chart).

S-C=Static-Chorionated, S-DC=Static-Dechorionated, SR-C=Static Renewal-Chorionated, SR-DC=Static Renewal-Dechorionated



#### **Co-occurrence of Phenotypes**

To demonstrate co-occurrence of phenotypes, we further analyzed data on live embryos according to how many altered phenotypes a larva was reported to have. In the bar graphs below, percent of larvae was calculated as the number of larvae with the number of observed altered phenotypes divided by the total number of live larvae with altered phenotypes. While the results varied among the different experimental conditions, for each lab on average 52-70% of larvae were observed to have 1 or 2 altered phenotypes among the vehicle control larvae, demonstrating that there was not a large co-occurrence of the reported altered phenotypes.



# Key Takeaways and Future Directions

- These results help clarify the landscape of variability within and across laboratories that implement unique zebrafish testing protocols.
- The range of phenotypes reported by each lab indicates that inclusion of refined phenotypes, such as those used to describe head defects in assessments, can enable sensitive findings.

S-C S-DC SR-C SR-DC

- Static-renewal exposure generally led to increased phenotypic effects after exposure to the positive control for all three labs, but some differences were noted between chorion status for the vehicle control.
- The SEAZIT definitive interlaboratory study included testing of a broader set of 42 chemicals. Future work will expand analyses to include 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. Interlaboratory Study on Zebrafish in Toxicology: Systematic Evaluation of the Application of Zebrafish in Toxicology's (SEAZIT's) Evaluation of Developmental Toxicity. Toxics 2024, 12, 93. https://doi.org/10.3390/toxics12010093 (2) Hsieh et al. Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT): Developing a Data Analysis Pipeline for the Assessment of Developmental Toxicity with an Interlaboratory Study. Toxics 2023, 11, 407. https://doi.org/10.3390/toxics11050407 (3) Zebrafish Phenotype Ontology: https://www.ebi.ac.uk/ols/ontologies/zp, accessed on 10 April 2023

#### Check out other SEAZIT related presentations:

Poster N673 Ryan et al. Evaluating the Impacts of Experimental Parameters on Chemical Potency in the Zebrafish Developmental Toxicity Assay: Findings from the SEAZIT Interlaboratory Study

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