

# The National Toxicology Program's Systematic Evaluation of the Application of Zebrafish In Toxicology: SEAZIT

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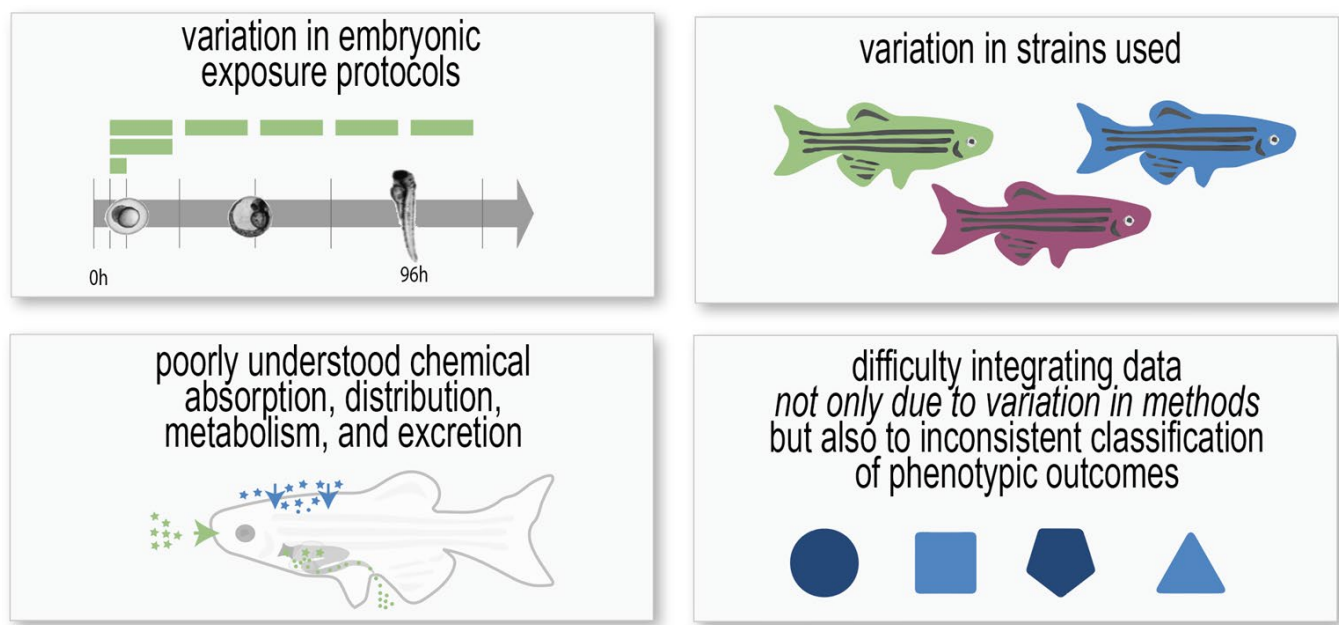
## BACKGROUND

### Zebrafish are a key animal model for toxicology, why?

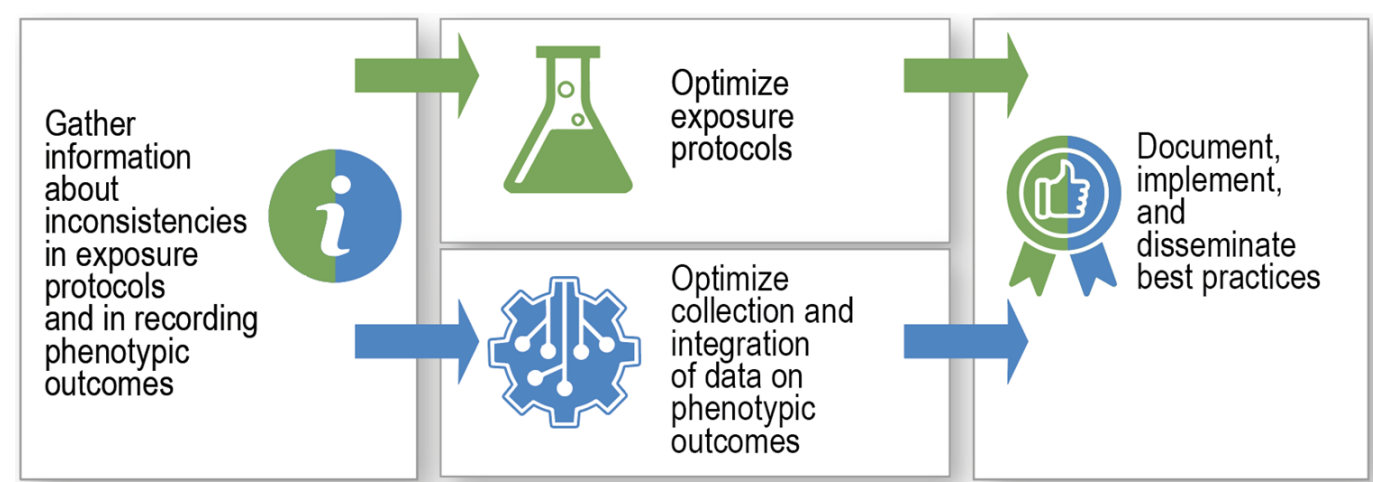
- Intact vertebrates with rapid development
- External development allows easy observation of chemical effects
- Easily maintained and bred in the laboratory
- Embryos are not subject to limitations of the Public Health Service Policy on Humane Care and Use of Laboratory Animals

Zebrafish embryos have been used for acute toxicity testing (OECD 2013), ToxCast™ testing (Padilla et al. 2012; Truong et al. 2014), and Tox21 testing (Tice et al. 2013). However, there are no standardized testing protocols that allow for easy comparison across assays. To address these deficits, the National Toxicology Program (NTP) initiated the Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT) program in 2015.

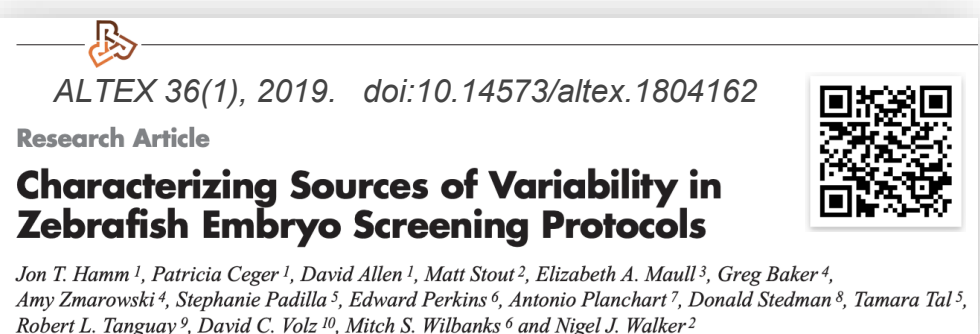
### The SEAZIT program aims to assess & address:



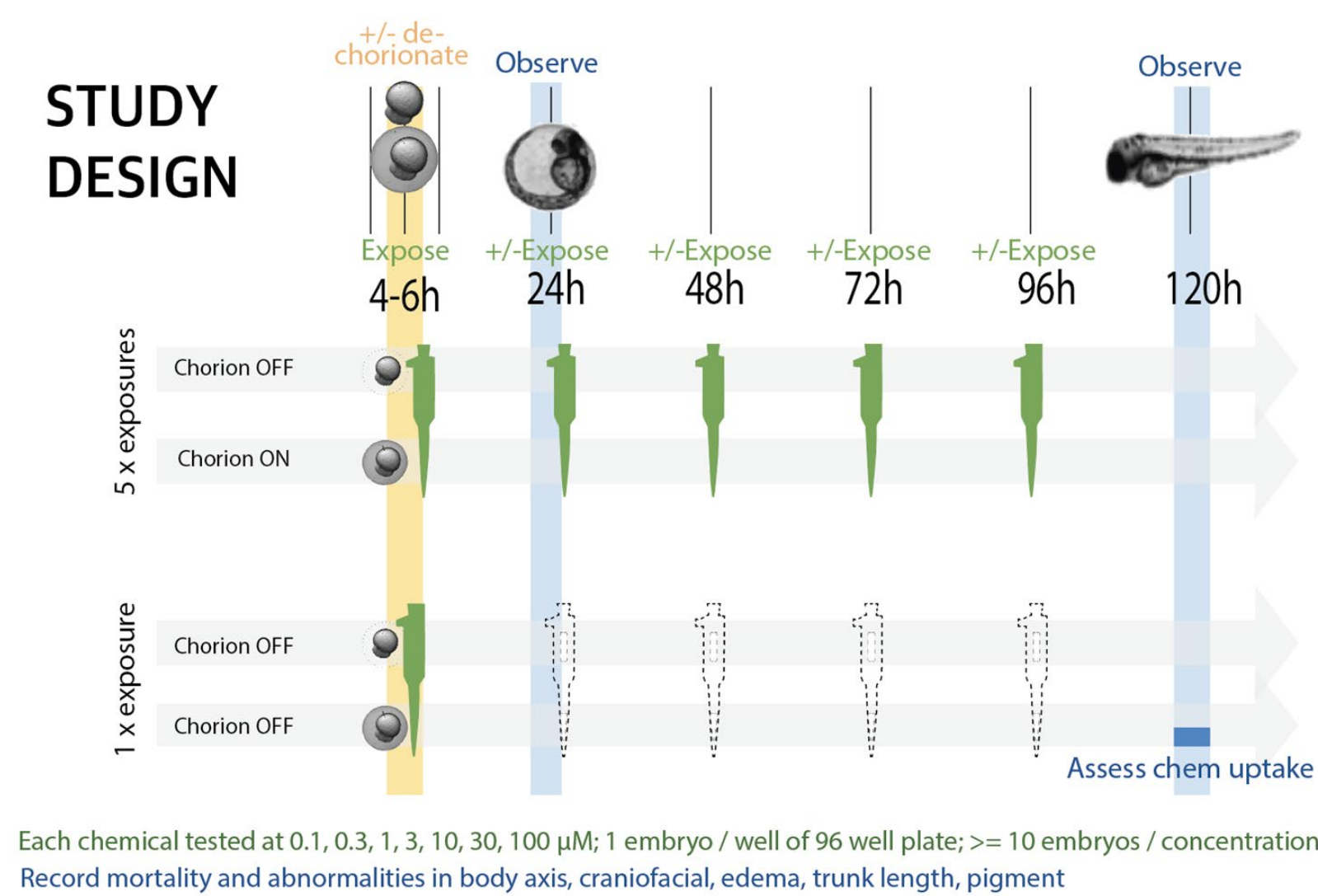
## SEAZIT OBJECTIVES



- Initial objective established an "Information Group" to obtain feedback on protocol elements and rationale.
- The SEAZIT team conducted interviews and gathered information on usage of zebrafish strains, types of feed, preparation of system water, disease surveillance practices, and embryo exposure conditions.
- Findings were published (Hamm et al. 2019) and results used in the interlaboratory study design.
  - Specifically, a better understanding of the role of the chorion, renewal of exposure media, and chemical uptake will advance this model system.



## INTERLABORATORY STUDY of impact of chorion removal and renewal of exposure solutions



**HYPOTHESIS:** Removal of the chorion and renewal of exposure solutions increase toxicity of chemicals in a zebrafish embryo screening assay regardless of other protocol differences.

**STATUS:** Four laboratories selected, study is ongoing

### Additional Experimental Conditions

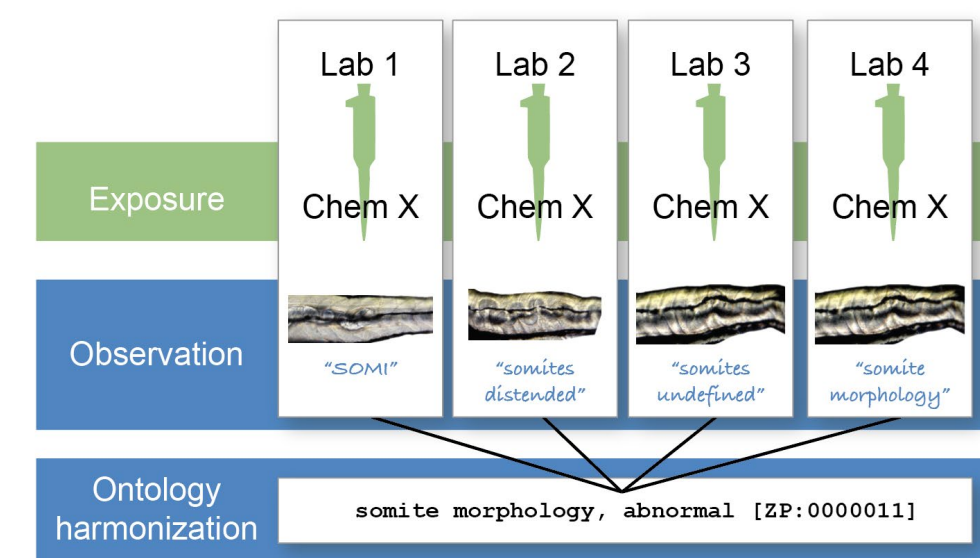
Condition	Interlaboratory Study Requirement
<b>Zebrafish Strain, source</b>	Varies by laboratory
<b>Plate type</b>	96-well, type varies by laboratory
<b>Exposure media</b>	E2 or E3 (100-200 µl per well)
<b>Dechoriation</b>	Mechanical or pronase treatment
<b>Positive control</b>	3,4 dichloroaniline; OECD TG236: Fish Embryo Acute Toxicity (FET) Test
<b>Solvent control</b>	~ 0.5% DMSO



## ONTOLOGY GROUP: assess and address nomenclature inconsistencies for phenotypic outcomes



**Challenge:** Labs differ in their conventions for how they record the same phenotype. These include differences of granularity (e.g., "abnormal caudal fin" vs. "curved caudal fin") or a differences in notation (e.g., "caudal fin" vs "CAUD").

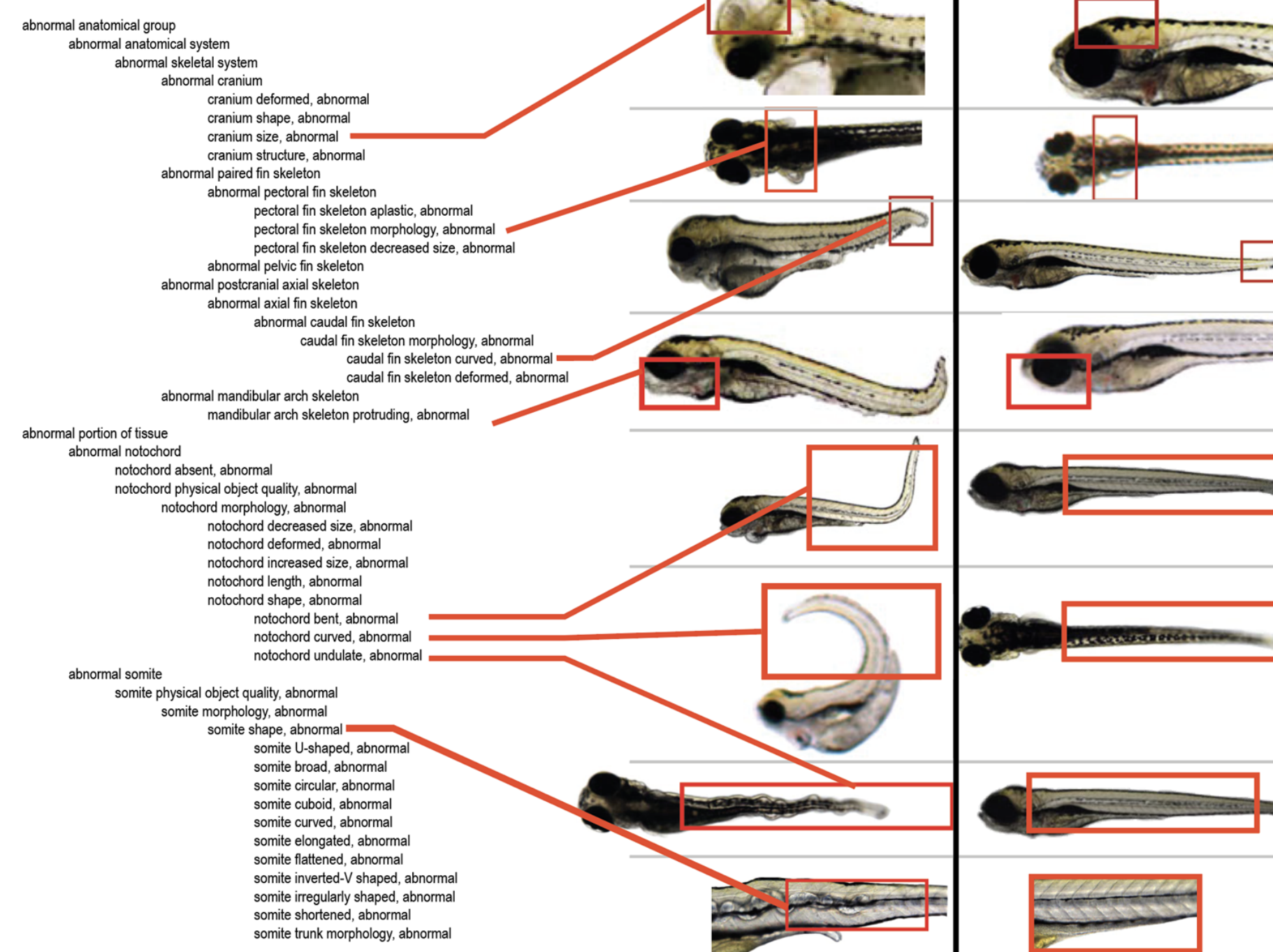


An **ontology** is a formal representation of a set of concepts and relationships between those concepts within a given domain. Ontologies are developed in many different domains, but share four common goals:

- Represent what is known
- Infer what is not otherwise obvious
- Promote the discovery of new insights from exploration and manipulation of complex data
- Provide context and intuitive navigation during the exploration process.

To address ontology needs for zebrafish screening, SEAZIT initiated collaborative projects involving four labs with similar protocols and the same chemical test set. The free-text results from each of the labs were shared and mapped to a standard ontology so that they could be compared.

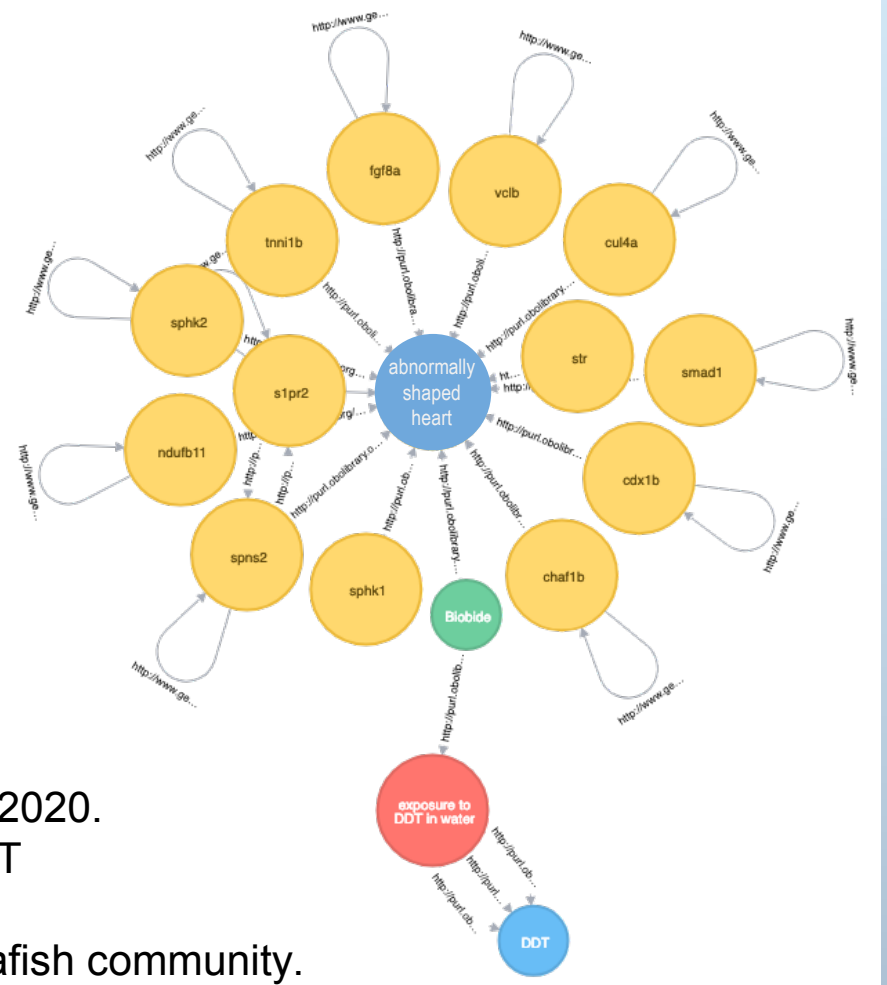
### Phenotypic outcome terms



## INTEGRATION AND BEST PRACTICES

Insights from the initial information gathering step informed the design for the interlaboratory exposure protocol study. In those ongoing studies, ontologies are being used to develop best practices for future data collection and processing. These practices will be applied to harmonize the existing results into a knowledge graph.

A Best Practices Workshop is tentatively scheduled for 2020. We will also discuss SEAZIT results with collaborators, stakeholders, and the zebrafish community.



## REFERENCES AND ACKNOWLEDGEMENTS

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- The views expressed above do not necessarily represent the official positions of any federal agency. Since the poster was written as part of the official duties of the authors, it can be freely copied.
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