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

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Introduction

- High throughput cell-based screens are currently used to screen and prioritize chemicals for further toxicological testing. There is an outstanding need for medium throughput models that can link *in vitro* data to molecular, cellular, or physiological effects in the whole animal.
- In 2014, a Collaborative Workshop on Aquatic Models and 21st Century Toxicology, organized by the U.S. National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), North Carolina State University (NCSU), Duke University, the U.S. Environmental Protection Agency, and the U.S. Food and Drug Administration, was held at NCSU.
- The 2014 workshop identified the lack of standardized protocols as an impediment to broader acceptance of aquatic models in toxicity screening (Planchart et al. 2016). Addressing these deficits could increase reproducibility and replicability of treatments, and in turn promote use of aquatic models to assess the potential human health impacts of chemicals in our environment.
- Specific issues identified were:
    - Lack of consistency of protocol elements, including:
        - Fish strains used
        - Exposure paradigms
        - Diet
        - Water quality and microbiome
        - Solvent used for chemical treatment
        - Use of chorionated vs. dechorionated eggs

  - Lack of understanding of mechanisms of chemical absorption, distribution, metabolism, and excretion (ADME) in aquatic models needed to support the extrapolation of chemical effects to other species
  - Lack of consistency of informatics approaches used for classification of outcomes

- Also in 2014, the NTP held the Toxicological Applications of Zebrafish workshop to focus discussion on zebrafish as the model species in toxicological screening.
- The zebrafish (*Danio rerio*), a small freshwater fish species widely used in developmental biology and toxicology studies, can be easily maintained and bred in the laboratory. In particular, zebrafish embryos can be exposed to waterborne test chemicals in a single well of a 96- or 384-well tissue culture plate.
- Protocols using zebrafish embryos allow for much greater throughput than traditional animal tests, making the embryonic zebrafish an ideal complement to in vitro tests.
- While the zebrafish embryo model has been used for acute toxicity testing (OECD 2013), pharmaceutical lead development (Barros et al. 2008; Olson et al. 2000), and in some ToxCast™ (Padilla et al. 2012; Truong et al. 2014) and Tox21 testing (Tice et al. 2013), there are currently no broadly adopted standard toxicological protocols to allow for easy comparison across assay results.
- With the goal of evaluating the utility of zebrafish for toxicity screening, the NTP initiated the **S**ystematic **E**valuation of the **A**pplication of **Z**ebrafish in **T**oxicology (SEAZIT) program. The information gathered by SEAZIT will provide the scientific basis on which to make a programmatic decision on the further routine use of zebrafish in screening of chemicals and fundamental knowledge on the use of zebrafish in toxicology, which will support further research endeavors by the broader research community.
    - The specific objectives and planned activities of SEAZIT are presented in **Figure 1** and **Table 1**.

Figure 1 Specific SEAZIT Objectives

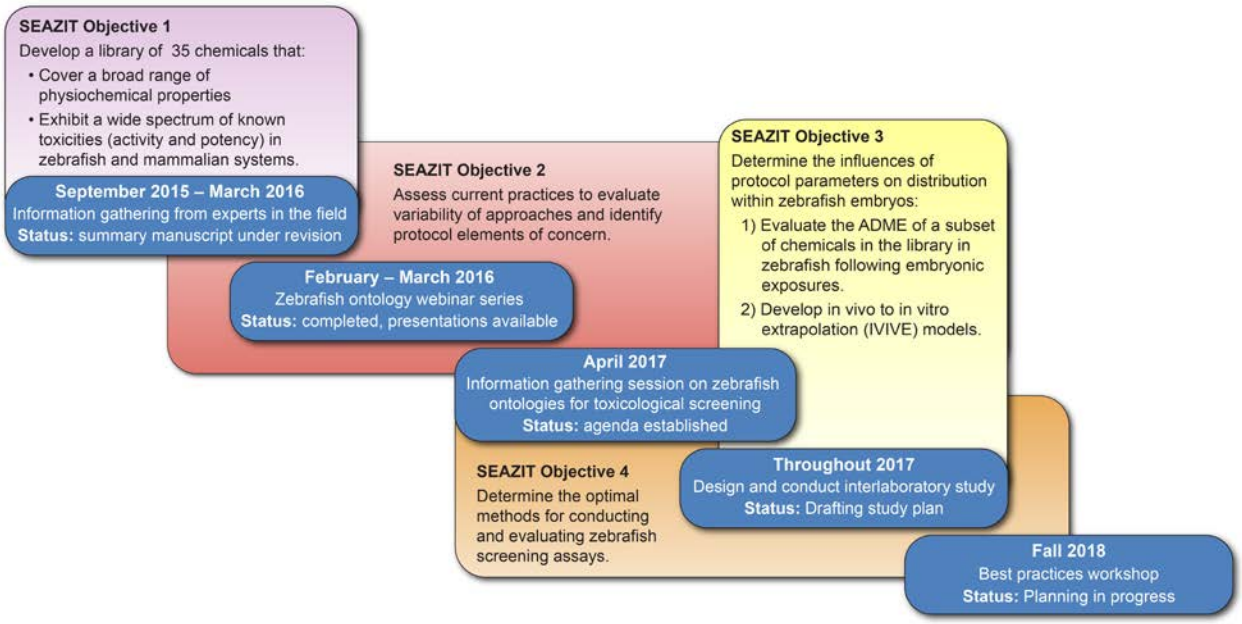


Table 1 SEAZIT Program Activities[1]

Activity	Scheduled	Status
Information gathering from experts in the field	September, 2015 – March, 2016	Summary manuscript drafted and under revision
Zebrafish Ontology webinar series	February 2, 2017 February 16, 2017 March 2, 2017	Completed – Archived presentation materials available[2]
Zebrafish Ontologies for Toxicological Screening information gathering session	April 4-5, 2017	Agenda established
Design and conduct of an interlaboratory study	2017	Drafting study plan
Best practices workshop	Fall 2018	Pending

[1]See also https://ntp.niehs.nih.gov/go/seazit
[2]Webinar materials at https://ntp.niehs.nih.gov/go/zfweb-2017

Information Gathering Phase

- The Collaborative Workshop on Aquatic Models and 21st Century Toxicology identified several protocol areas that appeared to differ significantly between laboratories.
- Subsequently, SEAZIT team members conducted a series of interviews with researchers identified at the workshop (**Table 2**).

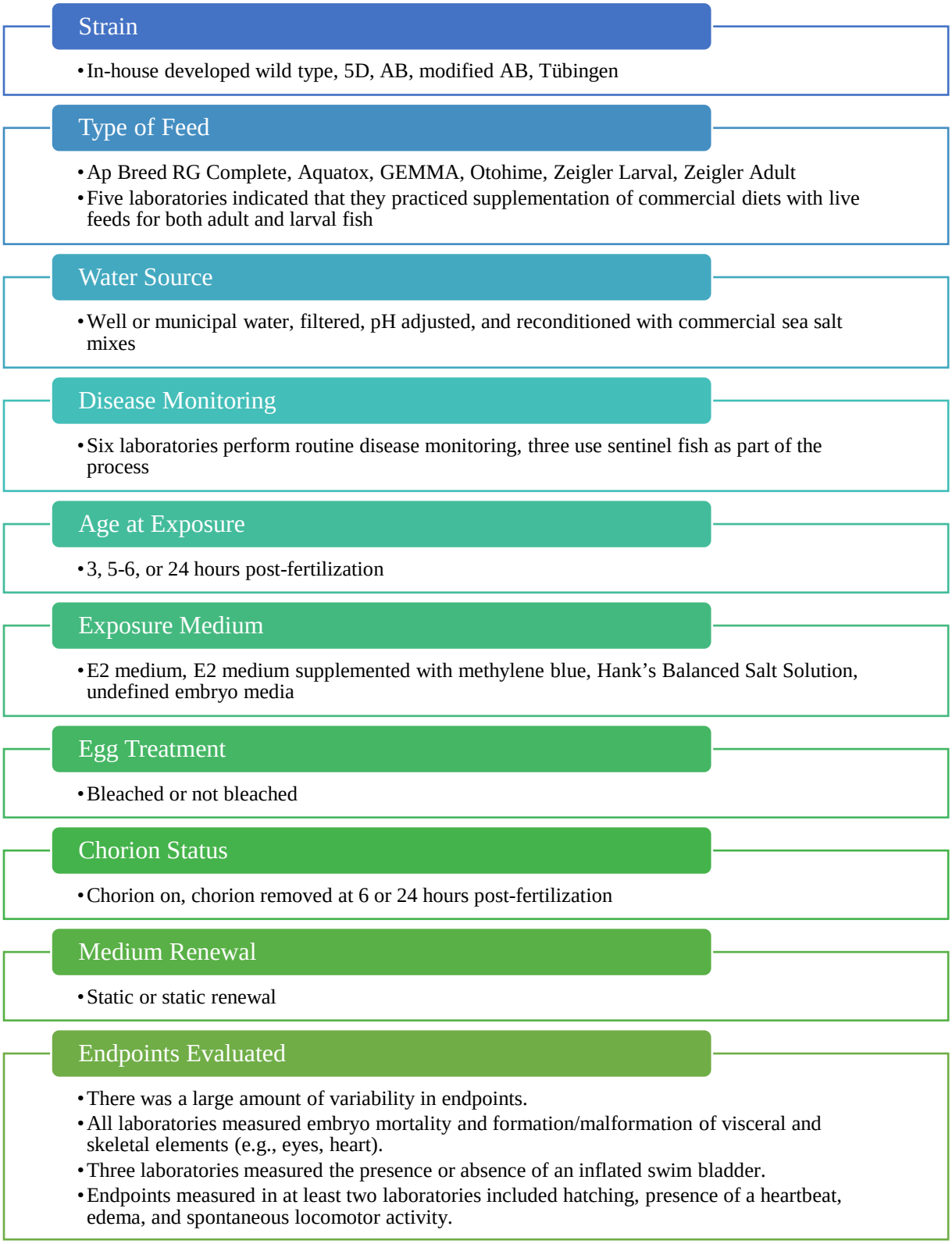
Table 2 Members of the SEAZIT Information Gathering Group

Member	Affiliation
Stephanie Padilla	Branch Chief, Genetics and Cellular Toxicology Branch and Research Toxicologist National Health and Environmental Effects Research Laboratory Office of Research and Development, U.S. Environmental Protection Agency
Ed Perkins	Senior Scientist Environmental Laboratory U.S. Army Engineer Research and Development Center
Antonio Planchart	Assistant Professor Department of Biological Sciences and Center for Human Health and the Environment North Carolina State University
Don Steadman	Senior Principal Scientist Pfizer Pharmaceuticals
Robert Tanguay	Distinguished Professor Department of Environmental & Molecular Toxicology Oregon State University
Tamara Tal	Biologist Integrated Systems Toxicology Division National Health and Environmental Effects Research Laboratory Office of Research and Development, U.S. Environmental Protection Agency
David Volz	Assistant Professor of Environmental Toxicology Department of Environmental Sciences College of Natural & Agricultural Sciences University of California, Riverside

Protocol Elements

- The SEAZIT information gathering group identified five areas key to development of a harmonized testing protocol for embryonic zebrafish studies: embryo exposure conditions, zebrafish strains, types and quality of feed, water parameters, and disease state (**Figure 2**).
- Interview and literature reviews revealed a large amount of variability among laboratories in
    - Endpoints measured and nomenclature used for endpoints
    - How endpoints are measured and severity scoring
    - Data analysis procedures to provide a toxicity estimate
- Two specific embryo exposure conditions, removal of the chorion and renewal of exposure solutions, were identified as being of particular interest due to the variability among laboratories and potential to influence toxicity estimates.

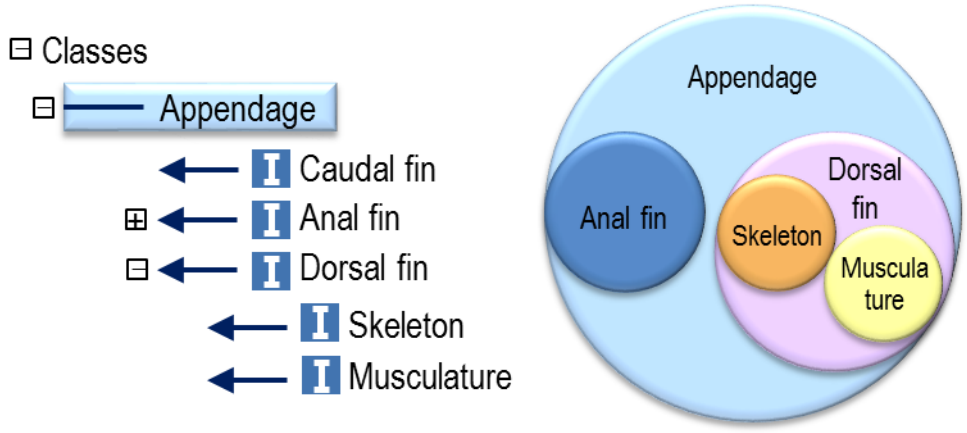
Figure 2 Summary of SEAZIT Information Gathering Group Findings



Ontologies

- The SEAZIT information gathering group discussions and literature review revealed high variability among laboratories in:
    - Which endpoints are measured
    - How endpoints are measured
    - Nomenclature used for endpoints
    - How the severity of alterations is scored
    - Data analysis procedures used to arrive at a toxicity estimate
- Establishment of more consistent zebrafish nomenclature and ontologies (**Figure 3)** would support harmonization of protocols.

Figure 3 An Ontology is a Classification



Adapted from: Melissa Haendel, An Introduction to Anatomy Ontologies. http://slideplayer.com/slide/3461901/

An ontology defines and formally describes terms, properties, and interrelationships between entities.

- To facilitate communication with zebrafish researchers, regulators, members of industry with an interest in the use of zebrafish-based toxicological screens, and data scientists, SEAZIT

presented three webinars (presentations available at https://ntp.niehs.nih.gov/go/zfweb-2017).

Webinar 1: Introduction to Zebrafish Screening

- Summarized the SEAZIT program and the variability found in zebrafish screening data
  - ***Nigel Walker, Ph.D.**, Deputy Division Director for Research, National Toxicology Program*
  - ***David Reif, Ph.D.**, Associate Professor, Bioinformatics Research Center and Center for Human Health and the Environment, Department of Biological Sciences, North Carolina State University*
  - ***Jon Hamm, Ph.D.**, Senior Staff Toxicologist, Integrated Laboratory Systems, Inc.*

Webinar 2: Ontologies 101

- Defined ontologies and described how they are employed to improve data analysis
  - ***Lyle Burgoon, Ph.D.,** Leader, Bioinformatics and Computational Toxicology Group, U.S. Army Engineer Research and Development Center*

Webinar 3: A Review of Relevant Ontologies and Application of Reasoners

- Provided information on relevant zebrafish, phenotype, and anatomy ontologies and examples of the application of ontologies and reasoners
  - ***Melissa Haendel,** Ph.D., Director, Ontology Development Group, Department of Medical Informatics and Clinical Epidemiology, Oregon Health and Science University*

- The webinars provided background information that will be used at an April 2017 Zebrafish Ontologies for Toxicological Screening information gathering session.
  - Participants will include SEAZIT team members, the information gathering group, and data scientists, who will review the state of the science for data analytics related to zebrafish screening studies.
  - Topics considered will include the utility of toxicity screening studies, need for a standardized ontology, and the advantages and limitations of available ontological approaches and software.
  - Specific use case scenarios with identified data sets will be discussed in breakout sessions.
  - The product of the meeting will be an outline with author assignments for a recommendations document. This document will:
    - Capture best practices for data production and analysis

    - Identify tools and other resources needed to advance the application of the zebrafish model in toxicology

Future Directions

- Following the April 2017 information gathering session, SEAZIT will initiate an **interlaboratory study**.
  - Participating laboratories will use many of their in-house protocol elements to test a defined chemical set while varying the protocol elements under investigation.
  - Study chemicals:
    - Overlap with other NTP studies
    - Include a range of physicochemical properties and developmental toxicity effects
    - Are backed by in vivo reference data available from rodent and other zebrafish studies
  - The interlaboratory study will include a pilot effort on chemical kinetics in support of future studies of ADME in zebrafish.
  - The data generated in this study will be made publicly available.
- A **best practices workshop** will serve as a public forum where experts from various fields can discuss continued development and standardization of assays, as well as practices for collecting, analyzing, and reporting of data.
  - The objectives of the workshop include:
    - Identify best practices for conducting zebrafish screening assays
    - Define the state of the science for data analysis of zebrafish screening assays and develop guidelines for analyzing and reporting data
    - Define minimum essential endpoints for zebrafish screening assays
    - Develop common terminology for endpoints and effect phenotypes in zebrafish screening assays
    - Define opportunities for improvement and greater utilization of the data generated in screening assays
  - The workshop will result in recommended guidelines for the conduct and reporting of zebrafish screening assays to be published in the peer-reviewed literature.
- SEAZIT team members and collaborators will develop **in vivo to in vitro extrapolation models**, which will help determine the influence of the various protocol parameters on distribution within the zebrafish during development and into adulthood.

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