An Overview of The National Toxicology Program's Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT)

Nigel Walker PhD
Deputy Division Director for Research
DNTPO

Webinar Series: Using Informatics to Improve Data Analysis of Chemical Screening Assays Conducted in Zebrafish

**Webinar 1-Introduction to Zebrafish Screening
February 2nd 2017**

What is the National Toxicology Program (NTP)?

- Interagency Federal Research Program

- Coordinated toxicology research

  – Thousands of agents evaluated in comprehensive rodent toxicology studies

  – Publicly available database of all data

- Literature-based hazard assessments

  – Report on Carcinogens (RoC)

  – Office of Health and Translation (OHAT)

- New approaches and methods

  – NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

  – Tox21 predictive toxicology partnership with USEPA, NIH/NCATS and FDA
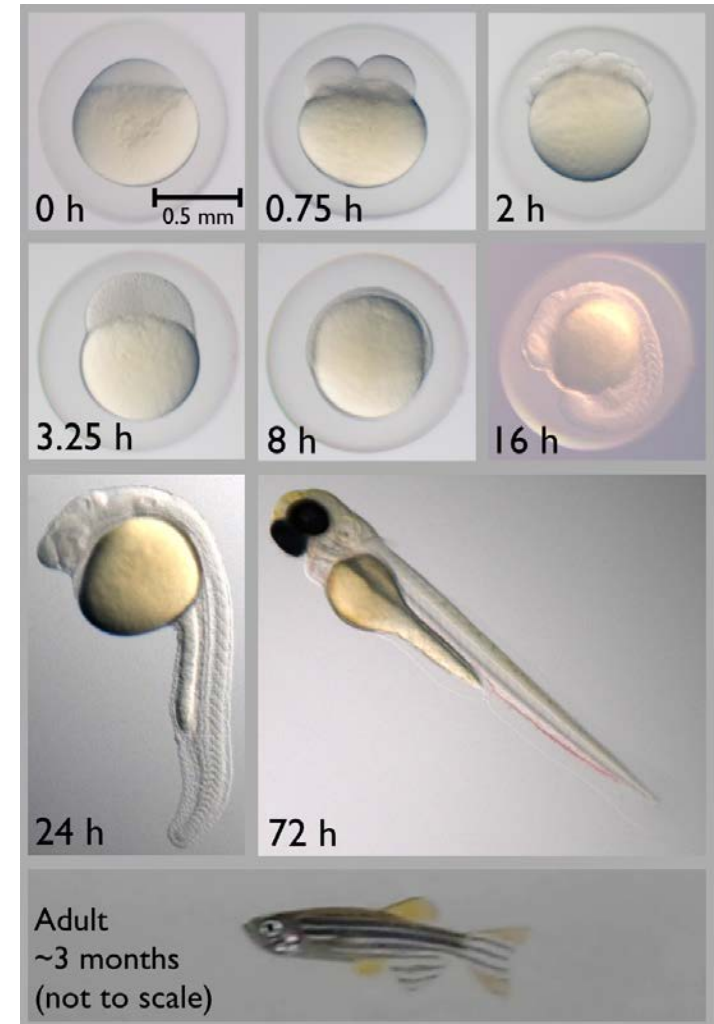
ntp.niehs.nih.gov
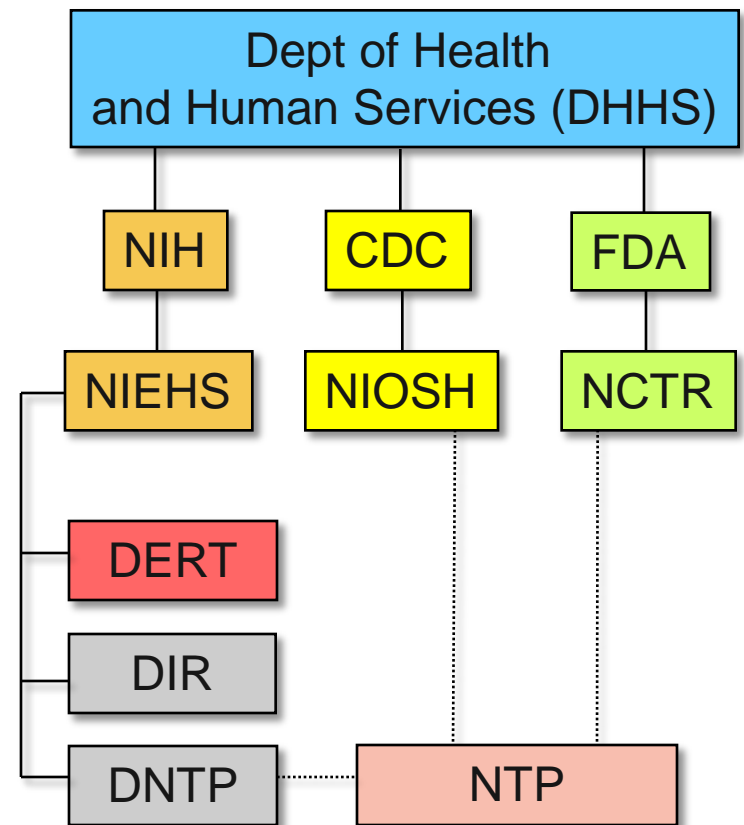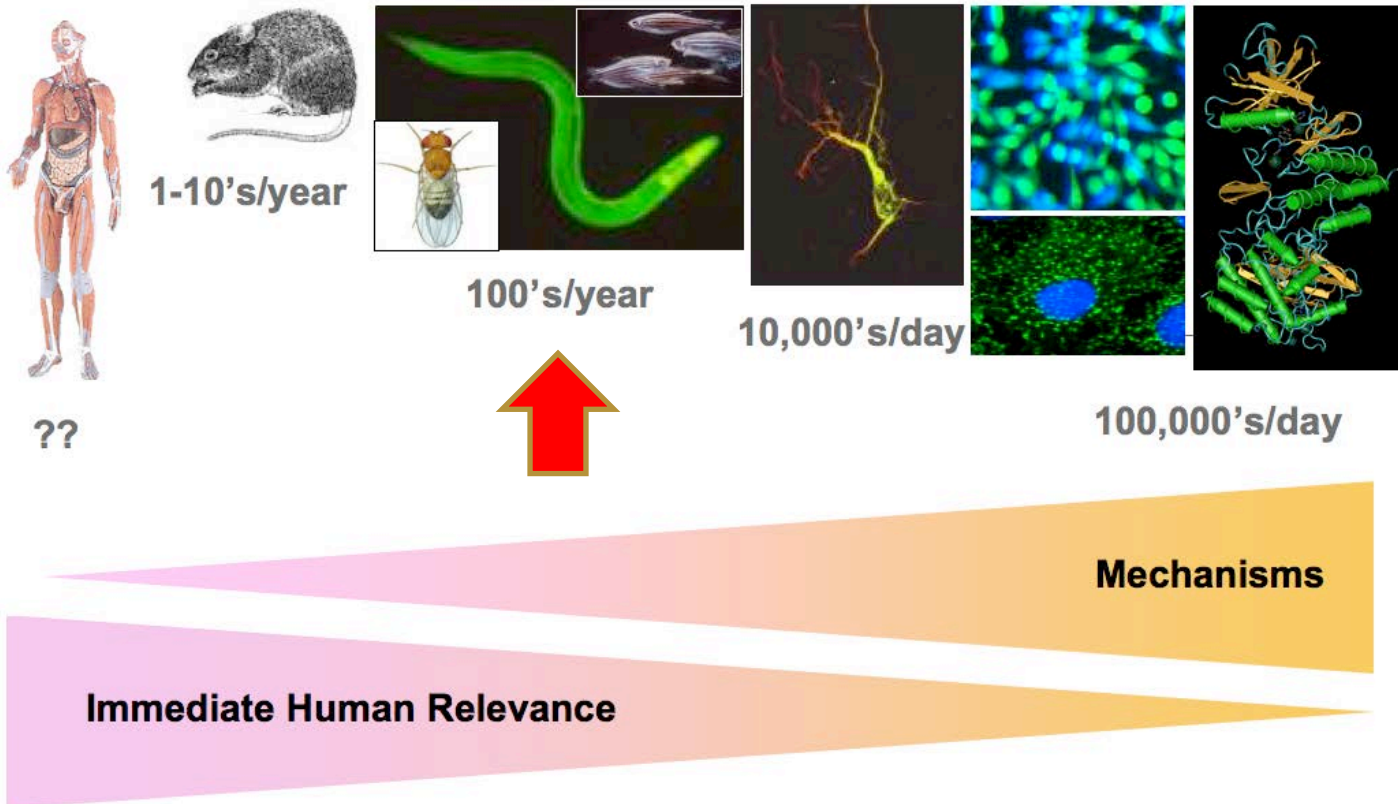
- "Alternate species" like zebrafish have been used to understand basic mechanisms in developmental biology and human disease

- Gaining wider interest as a toxicity screening tool including EPA's ToxCast and the interagency Tox21 consortia program
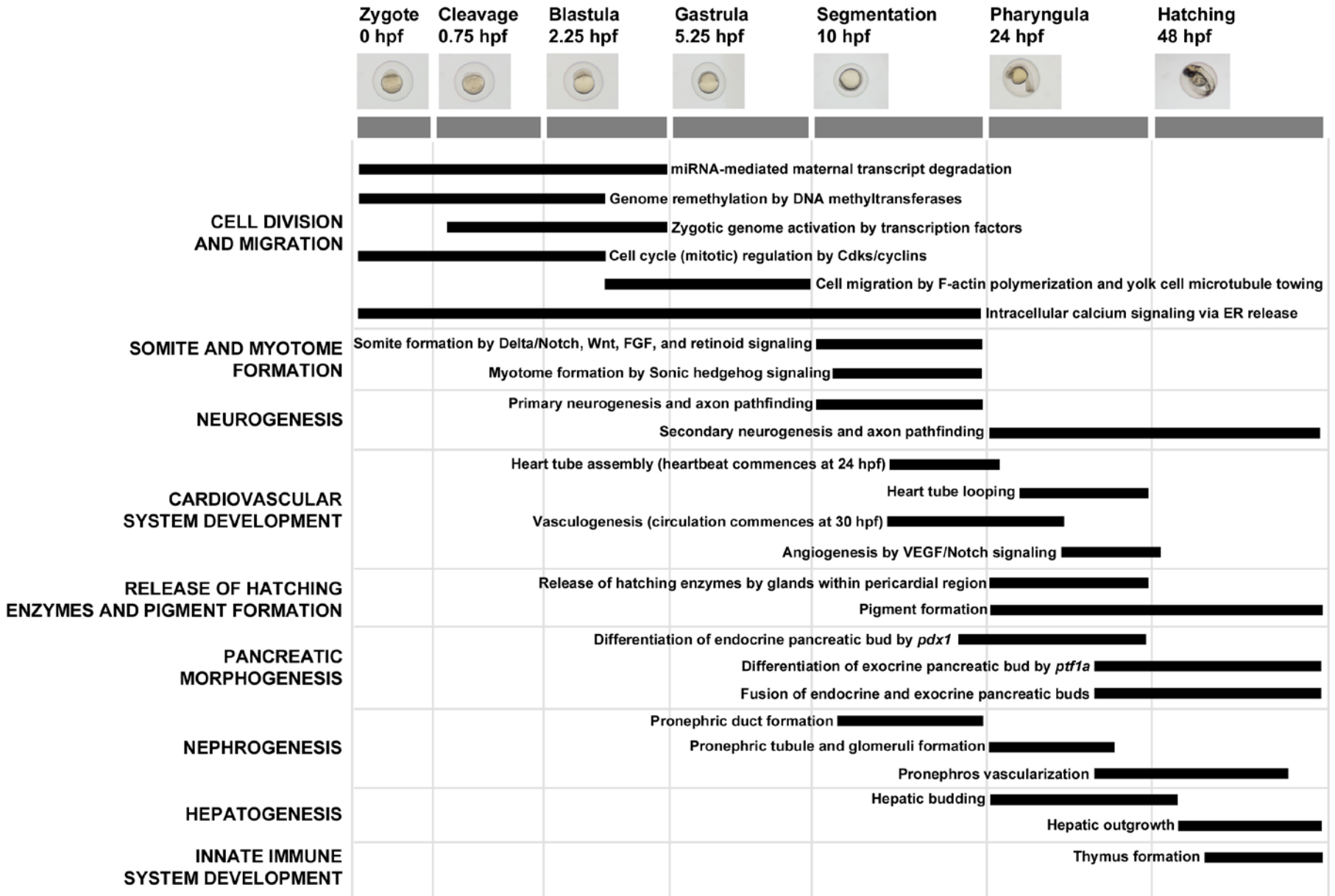
- Tropical freshwater fish native to the streams of the southeastern Himalaya

- Small size and rapid development

- Ability to assess impact of chemicals on development and the potential to adversely effect normal biological and physiological processes later in life.

- Mid to high-throughput assay systems

Major

	Zygote 0 hpf	Cleavage 0.75 hpf	Blastula 2.25 hpf	Gastrula 5.25 hpf	Segmentation 10 hpf	Pharyngula 24 hpf	Hatching 48 hpf

CELL DIVISION AND MIGRATION
- miRNA-mediated maternal transcript degradation
- Genome remethylation by DNA methyltransferases
- Zygotic genome activation by transcription factors
- Cell cycle (mitotic) regulation by Cdks/cyclins
- Cell migration by F-actin polymerization and yolk cell microtubule towing
- Intracellular calcium signaling via ER release

SOMITE AND MYOTOME FORMATION
- Somite formation by Delta/Notch, Wnt, FGF, and retinoid signaling
- Myotome formation by Sonic hedgehog signaling

NEUROGENESIS
- Primary neurogenesis and axon pathfinding
- Secondary neurogenesis and axon pathfinding

CARDIOVASCULAR SYSTEM DEVELOPMENT
- Heart tube assembly (heartbeat commences at 24 hpf)
- Heart tube looping
- Vasculogenesis (circulation commences at 30 hpf)
- Angiogenesis by VEGF/Notch signaling

RELEASE OF HATCHING ENZYMES AND PIGMENT FORMATION
- Release of hatching enzymes by glands within pericardial region
- Pigment formation

PANCREATIC MORPHOGENESIS
- Differentiation of endocrine pancreatic bud by *pdx1*
- Differentiation of exocrine pancreatic bud by *ptf1a*
- Fusion of endocrine and exocrine pancreatic buds

NEPHROGENESIS
- Pronephric duct formation
- Pronephric tubule and glomeruli formation
- Pronephros vascularization

HEPATOGENESIS
- Hepatic budding
- Hepatic outgrowth

INNATE IMMUNE SYSTEM DEVELOPMENT
- Thymus formation

Source: Villeneuve, Volz, et al. (2014)

NTP efforts

- DNTP is engaged in several efforts to evaluate the effects of various chemical sets

  - flame retardants

  - BPA-like compounds

  - endocrine disrupting compounds

  - immunotoxic compounds

  - polycyclic aromatic compounds

  - neurotoxic and developmental compounds

  - Elk river-spill chemicals

- Exposure primarily in developing zebrafish (<5dpf) embryos but also in adult animals.

- "Collaborative Workshop on Aquatic Models and 21st Century Toxicology"

  - May 5–6, 2014, North Carolina State University

  - Planchart et al 2016, ALTEX 33; 435-52

- "Toxicological Applications of Zebrafish" workshop

  - August 6th 2014, held at NIEHS

- Concerns about lack of standardized protocols as an impediment to broader acceptance of these models

- **S**ystematic **E**valuation of the **A**pplication of **Z**ebrafish **I**n **T**oxicology (SEAZIT) characterization studies by the NTP.

- Aims:

  - to provide the scientific basis on which to make a programmatic decision on the further routine use of zebrafish in toxicological evaluation of chemicals to which humans are exposed during development and into adulthood.

  - provide fundamental knowledge on the use of zebrafish in toxicology, which will support further research endeavors by the academic community.

Some barriers to broader adoption of zebrafish

- The lack of interrogation by diverse sets of compounds for concordance with known toxic effects observed in mammals

- The impact of different protocol elements on a broad range of chemically-induced phenotypes/endpoints, including but not limited to:

  – Zebrafish strain differences

  – Exposure paradigms, impact of chorion

  – Husbandry, diet, water quality, microbiome

  – Role of physical-chemical properties (e.g., log P, molecular weight)

- Assessment of impact of early life exposures on development through to adulthood.

- Understanding of chemical ADME to support the extrapolation of dose response of effects of concern to other species and humans

- NCATS and NIEHS were awarded funds redirected by NIH from cancellation of the National Children's Study to expand the Tox21 program to include developmental toxicology

- Goals:

  - Comprehensively test the 10,000 chemical collection on developmental pathways

    - (e.g., Sonic Hedgehog, Wnt, Notch, bone morphogenic proteins, TGF beta, MAP/ERK, Hippo, and HDACs) and cellular phenotypes

  - Create dynamic maps of the patterns of gene expression that drive normal differentiation of stem cells and study the potential for disruption by chemicals

  - Determine the critical parameters needed to standardize use of the zebrafish for developmental biology screening

- Develop a library of ~100 chemicals that cover a broad range of physiochemical properties and exhibit a wide spectrum of known toxicities (activity and potency) in mammalian systems.

- Evaluate the protocol elements of concern as well as any others deemed important/useful.

- Evaluate the ADME of the chemical library in zebrafish following embryonic exposures.

- Develop in vivo to in vitro extrapolation (IVIVE) models, as well as the influence of the various protocol parameters on distribution within the zebrafish during development and into adulthood.

- Assessment of the developmental origins of health and disease (DOHAD) in a zebrafish model.

- Evaluate and determine the optimal methods for evaluating the resulting zebrafish data and conduct informatics "challenges" using the resulting data from the SEAZIT program to best link the data to phenotypic outcomes in mammals.

Initial Approach

- Establish a program coordination team to refine specific proposals and objectives

- Identify specific partners for accomplishing the experimental work

- Assess current practices across a broad set of researchers

- Develop an initial harmonized approach/protocol

- Conduct inter-laboratory studies with milestones and deliverables

- Evaluate the resulting data for reliability and relevance.

End goal: Sufficient knowledge that by the end of 2018, make a go/no-go decision of routine use of zebrafish in toxicological characterizations by the NTP.

Phase 1-Information Group (FY16)

- Established "Information Group" to obtain feedback on protocol elements and rationale

  – Cataloged common current practices used in zebrafish assay protocols

  – Zebrafish strains, types of feed, preparation of system water, disease surveillance practices, and embryo exposure conditions.

- Conducted interviews and information gathering to identify commonalities and differences

  – Publication in progress

Information Group participants

	Affiliation
Dr. Stephanie Padilla	U.S. Environmental Protection Agency
Dr. Ed Perkins	U.S. Army Engineer Research and Development Center
Dr. Antonio Planchart	North Carolina State University
Dr. Don Stedman	Pfizer Pharmaceuticals
Dr. Robert Tanguay	Oregon State University
Dr. Tamara Tal	U.S. Environmental Protection Agency
Dr. David Volz	University of California, Riverside

Information Gathering summary outcomes

- Conducted a series of interviews with researchers considered to be experts in the use of zebrafish in toxicology studies

- Focused on five areas key to development of a harmonized testing protocol for embryonic zebrafish studies:

  - zebrafish strains, feed, water, disease, and embryo exposure conditions.

- Interview and literature reviews revealed a large amount of variability among laboratories in

  - endpoints measured and nomenclature used for endpoints,

  - how endpoints are measured, severity scoring,

  - data analysis procedures to provide a toxicity estimate.

- Removal of the chorion and renewal of exposure solutions were two protocol elements identified as particularly likely to influence study outcomes.

For updates on the SEAZIT project and other activities related to *in vitro* alternatives, subscribe to the NICEATM News email list.

– To subscribe to the NICEATM News email list, go to: https://tools.niehs.nih.gov/webforms/index.cfm/main/formViewer/form_id/361

– Check the NICEATM News box and click submit