

Leveraging Small Aquarium Fishes to Advance Understanding of Environmentally Influenced Human Disorders and Diseases

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Introduction

- The need to screen thousands of compounds for potentially deleterious effects in living organisms has propelled the use of high-throughput screens to the forefront of toxicology.
- To better understand toxicity in tissues and organs using these screens, model organisms are needed that recapitulate human development, physiology and disease processes.
- Zebrafish (*Danio rerio*), medaka (*Oryzias latipes*), and fathead minnow (*Pimephales promelas*) are excellent models in which to study the effects of environmental contaminants on heredity, development, and disease. Advantages to using these species include:
 - Modest husbandry cost
 - Short reproductive maturity and high fecundity rates
 - Life spans comparable to rodent models
 - External development and transparency during embryogenesis
 - Ability to perform high-throughput studies during embryogenesis
 - Availability and proven application of a plethora of genetic, molecular, and cellular techniques
- NICEATM and North Carolina State University (NC State) cosponsored the "Collaborative Workshop on Aquatic Models and 21st Century Toxicology" to highlight the scientific and economic benefits of these model organisms.
- Nearly 150 scientists from the U.S., Canada, Europe, and Asia met at the workshop to discuss how small fish or fish embryos could be used to:
 - Screen and prioritize compounds for further *in vivo* testing
 - Assess mechanisms of chemical toxicity and how this knowledge can impact environmental and human health
- The workshop included platform presentations organized around six themes followed by a concluding discussion session. A poster session included 23 presentations of ongoing research, development, and utilization of small aquarium fishes to assess mechanisms of chemical toxicity.

Workshop Outcomes and Next Steps

- Stakeholders from academia, industry, and government all displayed positive and enthusiastic support.
- Numerous advantages of fish over traditional mammalian models were shown, including small size, rapid development, high fecundity, and external development with similar physiology to that of traditional models.
- Workshop participants identified research initiatives to address information gaps for the use of aquatic models in risk and safety assessments for multi-organ toxicity, longitudinal studies to assess long-term consequences of chronic exposures, and the embryonic basis of adult disease.
- Regulatory acceptance is the greatest hurdle to broader acceptance and utilization of aquatic models.
- Current studies using aquatic models require development of standardized protocols and validation.
- Validation should include both formal validation studies and an examination of the value of adding aquatic models to existing mammalian model-based testing.
- Additional work is required to define the limitations of aquatic models and better understand absorption, distribution, metabolism, and excretion in these species.
- Widespread interest was evident in follow-up workshops to include regulators and industry representatives.
- A workshop report is in preparation and will be submitted for publication this fall.

Workshop Objectives

- Foster the establishment of networks between toxicologists and basic biomedical scientists using aquatic vertebrate animal models
- Raise awareness of the advantages of aquatic models within the toxicology field
- Explore the feasibility of a framework to integrate toxicology data from aquatic models with data from *in silico*, *in vitro*, and *in vivo* testing initiatives currently underway to enhance risk and safety assessments of chemicals and pharmaceuticals
- Explore the potential for studies using aquatic vertebrate organisms to aid in identifying genetic contributions to human exposure susceptibility and anchor phenotypic outcomes of exposure to molecular mechanisms of toxicity
- Identify and propose future research initiatives using aquatic vertebrate models to address current information gaps

Workshop Organizing Committee

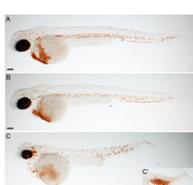
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Jon Hamm ILS/NICEATM	Tamara Tal Office of Research and Development, U.S. Environmental Protection Agency
David Hinton Nicholas School of the Environment, Duke University	Christopher Weis National Institutes of Health
Jyotshna Kanungo National Center for Toxicological Research, U.S. Food and Drug Administration	

Opening Session: Overview of Use of Aquarium Fish Models by Regulatory Agencies and Industry

- Dan Villeneuve (U.S. Environmental Protection Agency [EPA]): Use of aquatic models for regulatory toxicity testing at the EPA is evolving from observational ecological risk assessment towards a more mode-of-action approach.
- Jyotshna Kanungo (U.S. Food and Drug Administration [FDA]): The FDA is evaluating zebrafish models and is screening more chemicals to establish reliability and reproducibility.
- Matthew Winter (University of Exeter): Zebrafish models permit the pharmaceutical industry to economically maximize compound safety information early in drug development.

Session 1: Cardiovascular Toxicology

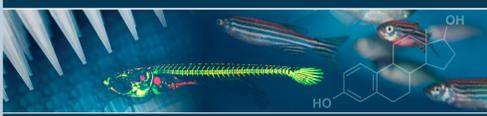
Aquarium fish are being used to study cardio- and vascular toxicants, and these assays can be adapted to high-throughput methods. The external development of small fishes allows for visual evaluation of the cardiovascular system throughout development.



- Maria Bondesson (University of Houston): Screening for vascular disruptor compounds *in vivo* and *in vitro*
- Warren Heideman (University of Wisconsin-Madison): TCDD and AHR in the zebrafish heart
- Dave Volz (University of South Carolina): High-content screening assay for identification of chemicals impacting cardiovascular function in zebrafish embryos
- Kenneth Poss (Duke University): Epicardial cells and heart regeneration

Arsenite exposure impedes blood circulation in zebrafish larvae. Lateral view brightfield representation of zebrafish embryos treated with control (A), 100 mg/L arsenite (B), or 400 mg/L arsenite (C) and stained with *o*-dianisidine. Blood accumulation observed in the posterior end (C). (Catherine McCollum, Maria Bondesson, University of Houston).

Collaborative Workshop on Aquatic Models and 21st Century Toxicology



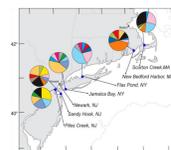
May 5-6, 2014

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Raleigh, North Carolina, USA

Presented by:
The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and
North Carolina State University

Session 2: Developmental Processes in Toxicology and Disease

Alterations in gene targets important to developmental processes often play a role in chemical toxicity and disease. Zebrafish are an appropriate model for studying development due to a high degree of gene homology with humans. In addition, their rapid reproduction and production of large numbers of offspring carrying mutations, knockouts, or other alterations greatly facilitates study of the role of specific genes in toxicity or disease.



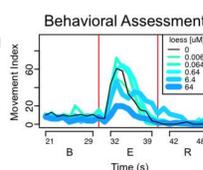
Distribution of AHR2 haplotypes in *Fundulus heteroclitus* (Sibel Karchner and Mark Hahn, Woods Hole Oceanographic Institution)

- Shawn Burgess (U.S. National Institutes of Health): Development of a rapid *in vivo* chemical screening method for the identification of antimetastatic compounds
- Mark Hahn (Woods Hole Oceanographic Institution): Diversity as opportunity—Using fish models to understand the role of conditional transcription factors in mechanisms of developmental toxicity
- Nancy Denslow (University of Florida): Growth of the mosquitofish anal fin in response to androgens and progestins

Session 3: Emerging Technologies

Technological advances are being applied to studies using fish, augmenting these systems.

- Keith Cheng (Penn State College of Medicine): Micron-scale synchrotron x-ray tomography as a tool for pancellular 3-D assessment of cellular and tissue architecture
- Matthew Harris (Children's Hospital Boston): Evolution's experiments: use of teleost diversity to mine the genetic regulation of development, physiology, and behavior
- David Reif (NC State): Rapid identification and characterization of neuromodulator chemicals using an embryonic zebrafish system
- Rodolphe Barrangou (NC State): CRISPR-Cas9 systems and genome editing applications



Chemical exposure affects movement of 24 hr postfertilization zebrafish embryos in response to light exposure. (David Reif, NCSU)

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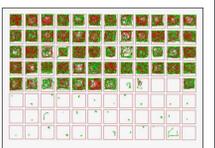
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A summary of NICEATM and ICCVAM activities at the Ninth World Congress is available on the National Toxicology Program website at <http://ntp.niehs.nih.gov/go/41583>.

Session 4: Models of Neurobehavior and Neurotoxicology

Fish have broad application to the study of neurobehavior and neurotoxicology, including studies to understand disease processes, screen compounds for neuroactivity, and elucidate mechanisms of neurotoxicity. Fish have the same neurotransmitters as humans, similar neurophysiology, and an intact blood-brain barrier.

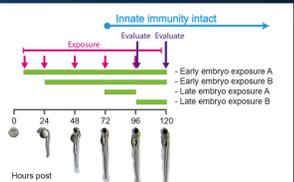


Heat map shows effects of chemical treatment on zebrafish embryo movement (Jeff Bronstein, UCLA)

- Michael Carvan (University of Wisconsin-Milwaukee): Assessing the subtle neurological effects of environmentally relevant methylmercury exposures in zebrafish
- Marc Ekker (University of Ottawa): Transgenic zebrafish models for the study of dopamine neuron development, loss, and regeneration
- Stephanie Padilla (EPA): Functional assays and alternative species: Using larval zebrafish in developmental neurotoxicity screening
- Andrew Rennekamp (Massachusetts General Hospital): Zebrafish as a tool for rapid, *in vivo* detection of small molecule effects on the vertebrate brain
- Jeff Bronstein (University of California, Los Angeles): Studying Parkinson's disease-related environmental toxins using zebrafish

Session 5: Predicting Alterations to the Immune System

Zebrafish are useful for immunotoxicology screening studies and investigations of the role of immune system dysfunction in cystic fibrosis due to the high degree of gene homology and presence of similar immune cell types between zebrafish and humans.



Experimental design to employ zebrafish embryos for evaluating the effects of chemical exposure on innate immunity (Jeff Yoder, NC State)

- Carol Kim (University of Maine): Gene-environment interactions: Effects of arsenic on the innate immune response
- Jeff Yoder (NC State): Strategies for *in vivo* immunotoxicology assays with zebrafish larvae

Session 6: Emerging Issues

Large numbers of both existing chemicals and chemicals under development require screening, and fish models could help achieve this goal. Specific presentations provided an overview of P450 systems in fish and how zebrafish are being used to study obesity.



Large-scale breeding of zebrafish (Robert Tanguay, Oregon State University)

- Robert Tanguay (Oregon State University): *In vivo* behavioral and morphological screening of a 1078-chemical library using zebrafish
- Jared Goldstone (Woods Hole Oceanographic Institution): Cytochrome P450 in fish
- John Rawls (Duke University): Zebrafish models for investigating environmental regulation of adiposity
- John Colbourne (University of Birmingham): Towards a science-driven solution for cooperative and effective management of chemical risks