NTP
National Toxicology Program

Updates:
National Toxicology Program

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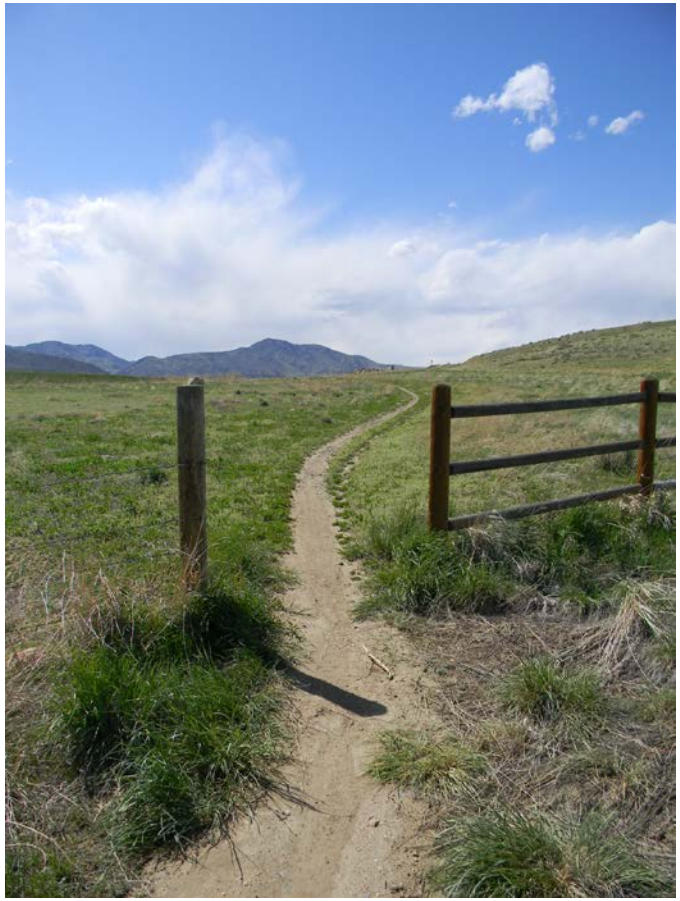
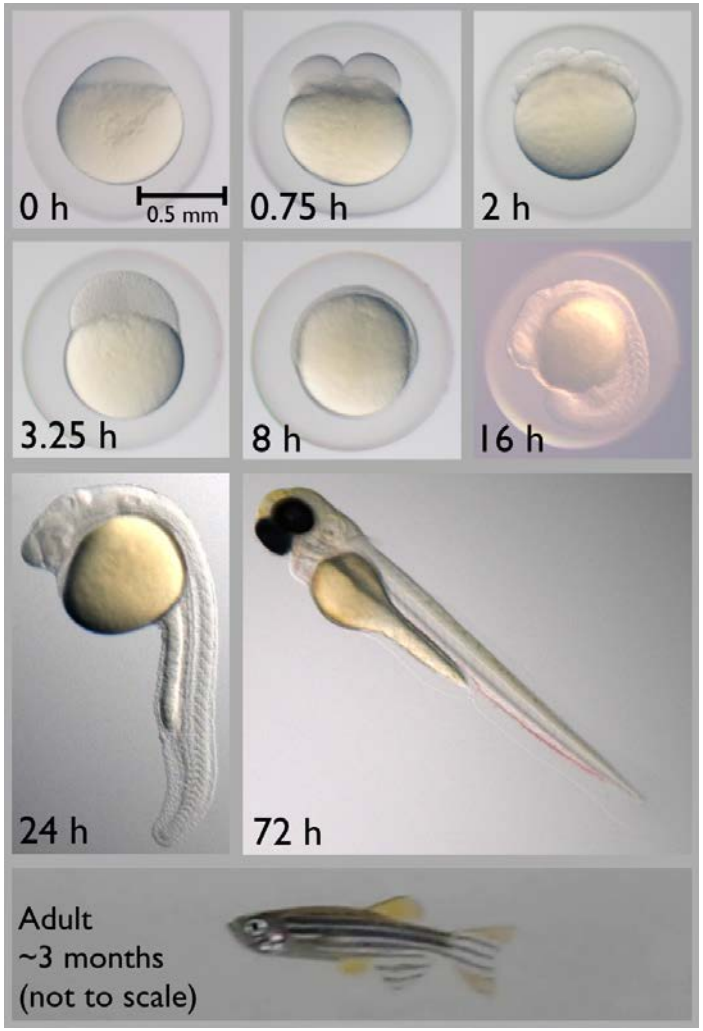
Outline

- NTP efforts in Zebrafish
  - SEAZIT
- Application of new approaches
  - Combined Exposures and Mixtures
  - Glyphosate



Zebrafish (Danio rerio)

- 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



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.

Background perspective

- “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

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

SEAZIT

- **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.

Key SEAZIT program activities

- Zebrafish information gathering (FY16)
  - identified areas key to development of a harmonized testing protocol for embryonic zebrafish studies and important sources of variability among laboratories.
- A webinar series (2017) focused on using informatics to improve data analysis for zebrafish screening studies
  - Capture best practices for data production and analysis
  - Identify tools to be developed and other needs to advance the application of the zebrafish model in toxicology
- An interlaboratory zebrafish study and data challenge
- A zebrafish best practices workshop (2018)

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

- Webinar 1: Introduction to Zebrafish Screening
  - overview of the SEAZIT program and reviewed the variability found in zebrafish screening data.
- Webinar 2: Ontologies 101
  - defined ontologies and described how they are employed to improve data analysis
- Webinar 3: A Review of Relevant Ontologies and Application of Reasoners
  - information on relevant zebrafish, phenotype, and anatomy ontologies and examples of the application of ontologies and reasoners.

Timeline

SEAZIT Objective 1

Develop a library of 35 chemicals that:

- Cover a broad range of physiochemical properties
- Exhibit a wide spectrum of known toxicities (activity and potency) in zebrafish and mammalian systems.

September 2015 – March 2016
Information gathering from experts in the field
Status: summary manuscript under revision

SEAZIT Objective 2

Assess current practices to evaluate variability of approaches and identify protocol elements of concern.

February – March 2016
Zebrafish ontology webinar series
Status: completed, presentations available

SEAZIT Objective 3

Determine the influences of protocol parameters on distribution within zebrafish embryos:

1) Evaluate the ADME of a subset of chemicals in the library in zebrafish following embryonic exposures.
2) Develop in vivo to in vitro extrapolation (IVIVE) models.

April 2017
Information gathering session on zebrafish ontologies for toxicological screening
Status: agenda established

SEAZIT Objective 4

Determine the optimal methods for conducting and evaluating zebrafish screening assays.

Throughout 2017
Design and conduct interlaboratory study
Status: Drafting study plan

Fall 2018
Best practices workshop
Status: Planning in progress

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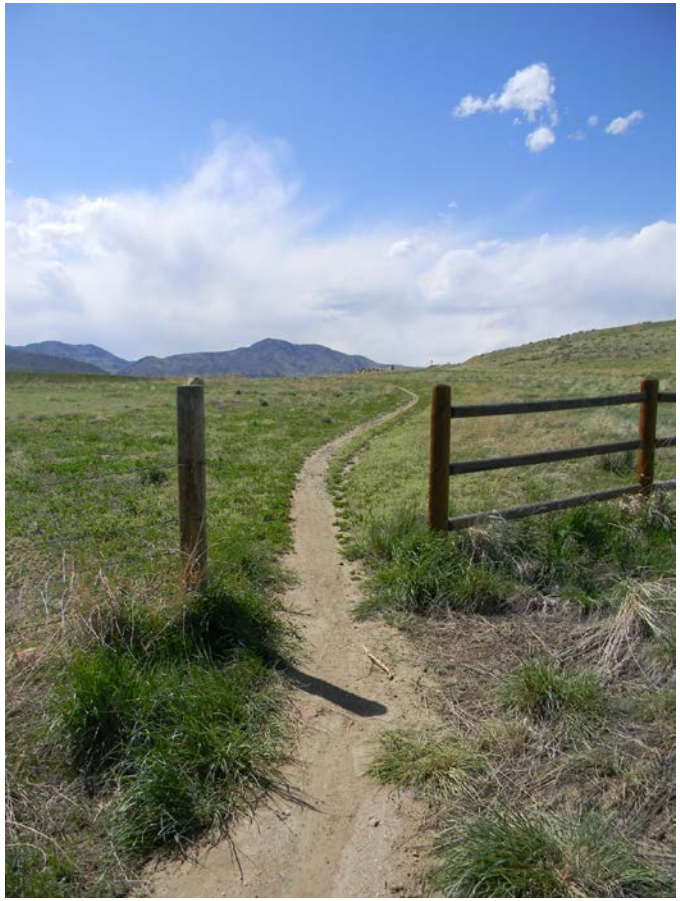


Application of “new approaches” to NTP research

- BSC meeting June 29th 2017
- Strategies for Studying Combined Exposures and Mixtures
  - Polycyclic Aromatic Compound Mixtures Assessment Program (PAC-MAP)
  - Sufficient Similarity and Botanical Dietary Supplements
  - Cancer Network and enVironmental Exposure Research Agenda (CNVERGE)
- Screening for Biological Activities of Concern in Consumer Products
  - Applying Tox21 techniques and approaches

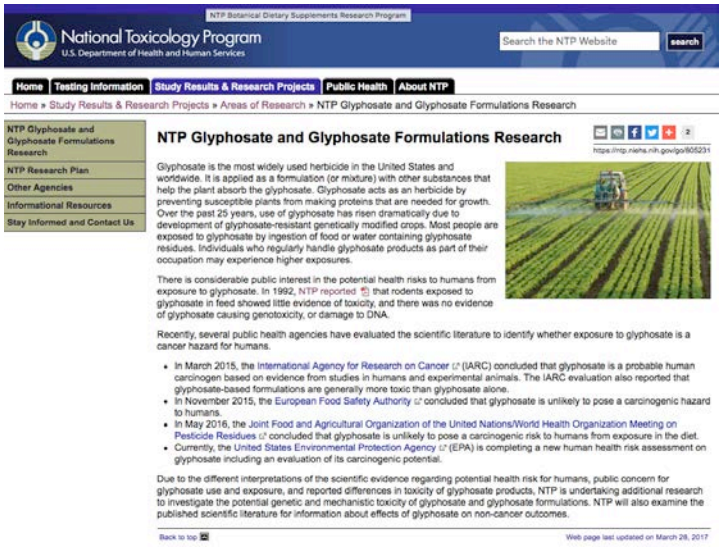
PAC-MAP

- PACs are widespread environmental contaminants.
- Exposure occurs to complex mixtures of PACs that differ depending on the source of the exposure
- Some PACs are known carcinogens. The vast majority of PACs have not been evaluated for potential effects on health
- NTP strategy combines a variety of in vitro approaches to assess the toxicity of individual PACs and select PAC mixtures

Studies	Description
High throughput screening	Assays to derive information about cellular and molecular targets and use for predicting potential biological effects
Cytotoxicity and gene expression in diverse cell lines	Cell-based assays to evaluate cytotoxicity and gene expression changes
Zebrafish developmental effects	Short-term study to evaluate developmental effects in a vertebrate model system
Rat immunotoxicity (28-day)	Assay to evaluate effects on the immune system
Rat toxicity and toxicogenomics (28-day)	Assay to evaluate general toxicity and genomic changes

Glyphosate

- Glyphosate is the most widely used herbicide in the United States
  - Applied as a formulation (or mixture) with other substances
  - IARC concluded that glyphosate is a probable human carcinogen
  - EFSA concluded that glyphosate is unlikely to pose a carcinogenic hazard to humans.



- NTP is undertaking additional research to investigate the potential genetic toxicity of glyphosate formulations.
- Battery of in vitro assays to evaluate glyphosate and formulations.
  - Focus on genotoxicity, or damage to DNA, and induction of oxidative stress

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