

Department of the Interior Alternative Approaches to Ecotoxicological Testing and Assessment



Jessica Leet, Barnett Rattner, Luke Iwanowicz, Clay Raines, Gavin Saari, Nadia Carmosini, Jon Amberg, Katy Klymus, Cathy Richter



Mission

Protect and manage the Nation's natural resources and cultural heritage

Provide scientific and other information about those resources

Honor trust responsibilities & commitments to American Indians, Alaska Natives and affiliated island communities





Some Applied Ecotoxicological Research Limited regulatory authority on "chemicals"

- 1. Research with direct application to natural resource management
- 2. Chemicals for invasive species control
- 3. Environmental contaminant biomonitoring
- 4. Natural Resource Damage Assessment
- 5. Alternatives to "lead shot" used in hunting

Embrace 3R's





In Silico and High Content Screening to Characterize Cyanotoxins



fli1-eqfp zebrafish

72 hours post fertilization

FITC image



- Harmful algal blooms (HABs) contain mixtures of hundreds of toxins with characterized toxicity
 - Individual toxins and mixtures
 - Tiered testing approach
 - Scale to field application

In silico approaches

- ICE
- Precepta
- Zebrafish assays
- Developmental cardiovascular toxicity assay
 - Length, pericardial area, heart rate, blood flow
- Behavioral screening
 - Swimming behavior
 - Optokinetic response





fli1-egfp zebrafish 72 hours post fertilization

Transmitted light image



Toxicant Prioritization and In Vitro Toxicity Screening

- In vitro to in vivo extrapolation
 - Target

Sea Lamprey, Bighead and Silver Carp

Non-target

Endangered Paddlefish, Rainbow Trout, Lake Sturgeon, Bluegill









Toxicant Prioritization and In Vitro Toxicity Screening

Prioritize new novel toxicants and predict susceptibility





Testing Environmental Samples for Endocrine Activity In Vitro

- Substrate-free bioluminescent yeast bioassays
 - Commercially available yeast strains
 - Estrogenicity
 - Androgenicity
 - Cytotoxicity
- Cost-effective screening of environmental water sample extracts
- 96-well plate format



Interagency Coordinating Committee on the Validation of Alternative Methods

UNITED STATES ICCVAM Advancing Alternatives to Animal Testing

Leveraging Citizen Science to Enhance Biosurveillance of Blotchy Bass Syndrome





BASS BONANZA

- Novel adomaviruses have recently been identified as the causative agent of blotchy bass syndrome
- USGS scientists are in the process of identifying where and when this condition this observed in North America
- In an attempt to minimize additional sampling efforts, citizen scientists have been recruited to contribute images of blotchy bass that they are catching during normal angling activity to contribute surveillance data to this effort
- This effort utilizes preexisting smartphone applications (Angler's Atlas, MyCatch) as mechanism for image documentation that include geospatial and time stamp metadata



Minimally invasive sampling

- Minimally invasive, non-lethal sampling of hyperpigmented lesions in being conducted for complete or partial viral genome sequencing to inform epidemiological efforts
- State fisheries managers conduct annual creel surveys and collect swab samples to minimize duplicative efforts





- Validating non-lethal gill sampling method
- Validating automated blood smear analysis tool





What is eDNA and How is it being used?

"The total pool of DNA isolated from environmental samples." Pawlowski et al. (2020)

A non-invasive genetic method for surveying biotic diversity



Sloughing of epithelial cells
 Released gametes



- 2. Ecological Questions
- 3. Estimate Population Location and Size
- 4. Contaminants

5. "Ecology of eDNA"
– what affects the physical state and detection of eDNA





Threshold Indicator Taxa ANalysis (TITAN): Mono-Nitrogen Oxides



Advancing Alternatives to Animal Testing

Registration of Non-toxic Shot





Lead shot replacements: iron (steel) iron-tungsten bismuth-tin copper-clad iron corrosion-inhibited copper tungsten-bronze tungsten-iron tungsten-matrix tungsten-nickel-iron tungsten-polymer tungsten-tin-bismuth tungsten-tin-iron tungsten-tin-iron-nickel

Bottom Line

- many shot types registered using existing information, risk assessment and no toxicity test
- harmonized with Canada, and interest expressed by European Chemicals Agency