Department of the Interior

Alternative Toxicological Test Method Activities

Barnett Rattner, Jennifer Brennan, Jessica Leet, Joel Putnam, Rachel Riegerix

Mission

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

Provides scientific and other information about those resources

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

Some Applied Toxicological Research

Limited regulatory authority on “chemicals”

1. Research with direct application to natural resource management
2. Environmental contaminant biomonitoring
3. Natural Resource Damage Assessment
4. Registration of chemicals used in aquaculture
5. Alternatives to “lead shot” used in hunting

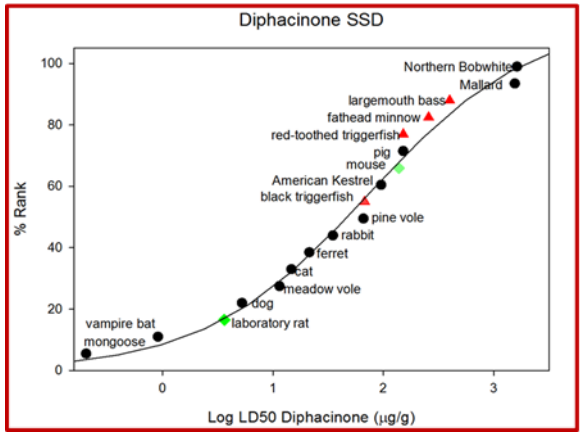
Embrace 3R’s

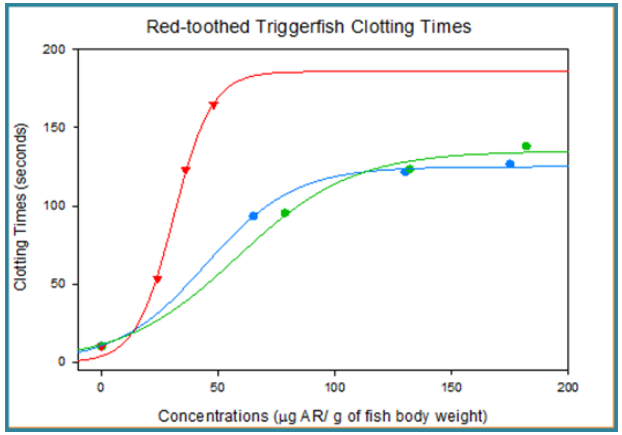
Anticoagulant Rodenticide Bait Application and Risk to Non-target Species

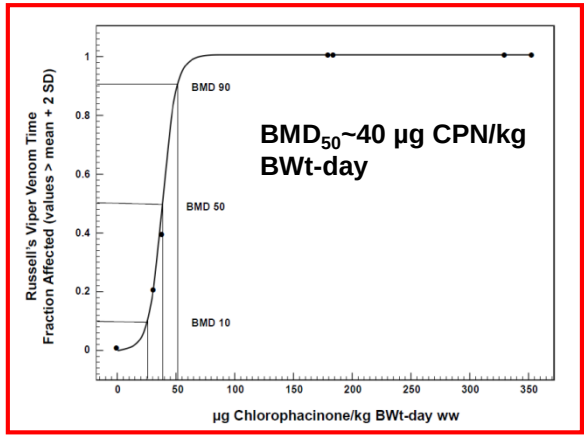
Global use - pest species eradication for ecosystem restoration on DOI properties

Toxicity and ADME studies to assess risk to non-target raptorial birds and fish

Up Down Test Procedure LC50 in fish
Sequential dosing procedure LD50 in American kestrel
Sublethal exposure thresholds (TRVs) for coagulopathy
Data for ecological risk assessments and EIS

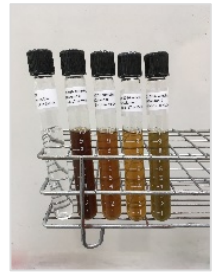


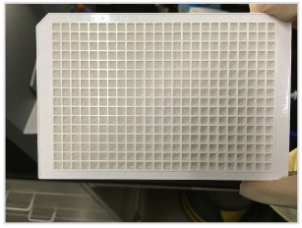


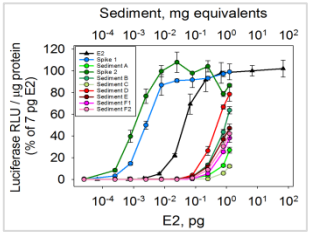


Testing Environmental Samples for Endocrine or Dioxin-like Activity in vitro

- Luciferase transactivation cell bioassays
  - 4 stably transfected human cell lines
    - Estrogen (ERα, ERα/ERβ) pathways
    - Androgen/Glucocorticoid/Progesterone pathways
    - Androgen/glucocorticoid pathways
  - 1 stably transfected rodent cell line
    - Aryl hydrocarbon pathways
- 384-well plate format
- Activity in cells normalized to protein content

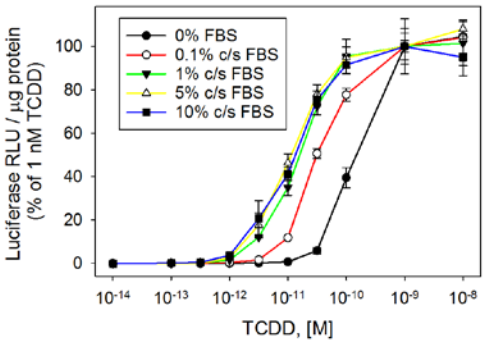


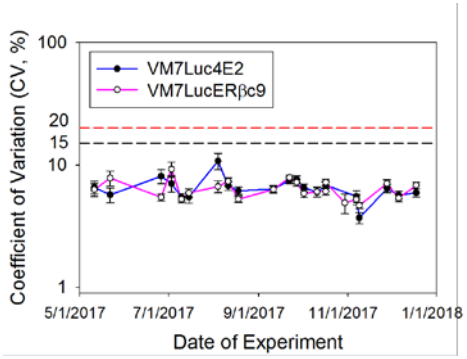


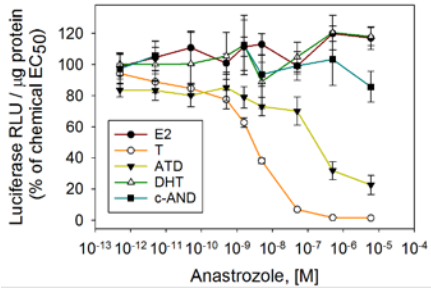


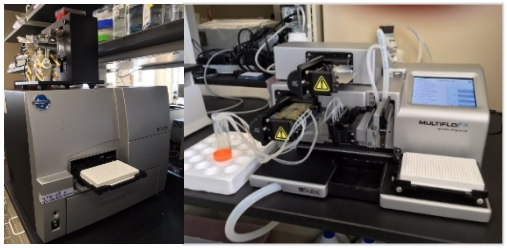
Behind the goal....

- Assay Optimization
  - Medium/serum selection
  - Treatment conditions
- Performance
  - Tracking assay parameters over time
- Characterization
  - Unexplored capabilities of existing assays
  - Selective agonists/antagonists in multiple-receptor cell lines
- Streamlining
  - Increasing automation on a modest budget
  - Increasing assay while maintaining integrity



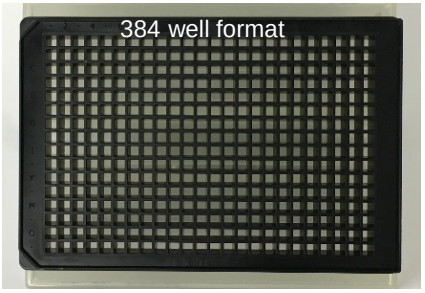


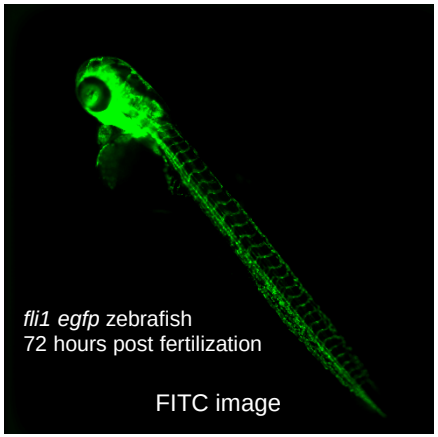


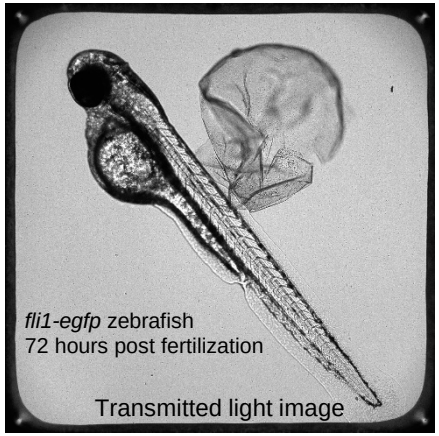


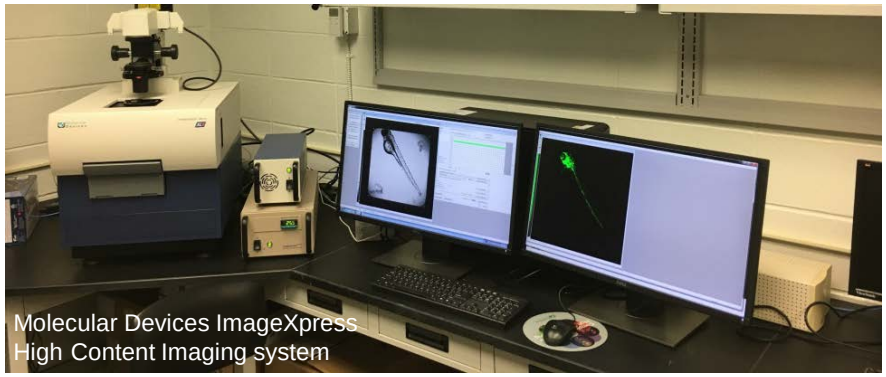
BioTek™ Synergy 4 with dual injector, BioTek™ MultiFlo FX with washer unit.

High-Content Tier I Screening









- Developmental cardiovascular toxicity assay at 72 hpf
  - Body length
  - Pericardial area
  - Intersegmental vessel area
  - Heart Rate
  - Circulation
- Targeted assessment of toxicity
  - LC50 and mode of action information
- Rapid image acquisition, data extraction, and analysis

High-Content Tier I Screening

- HCS to formulate hypotheses & prioritize compounds for further testing
- Utilizes pre-feeding fish embryos in microtiter plate format to **reduce**:
  - Animal use
  - Test compound needed
  - Waste
  - Labor
- Highly adjustable platform to incorporate a variety of endpoints
- HCS assays can provide evidence to justify larger-scale studies to determine actual risk versus perceived risk of contaminants.

Fish Toxicant Database

"modeling/screening compounds"

Species	Tetramethyl thiuram disulfide	Ziram
24 hour LC_{10} (ppb)		
Bluegill	610	850
Fathead minnow	170	380
Rainbow trout	310	470
24 hour LC_{99} (ppb)		
Bighead carp	510	614
Silver Carp	414	640
Grass Carp	>2000	390

- Database:

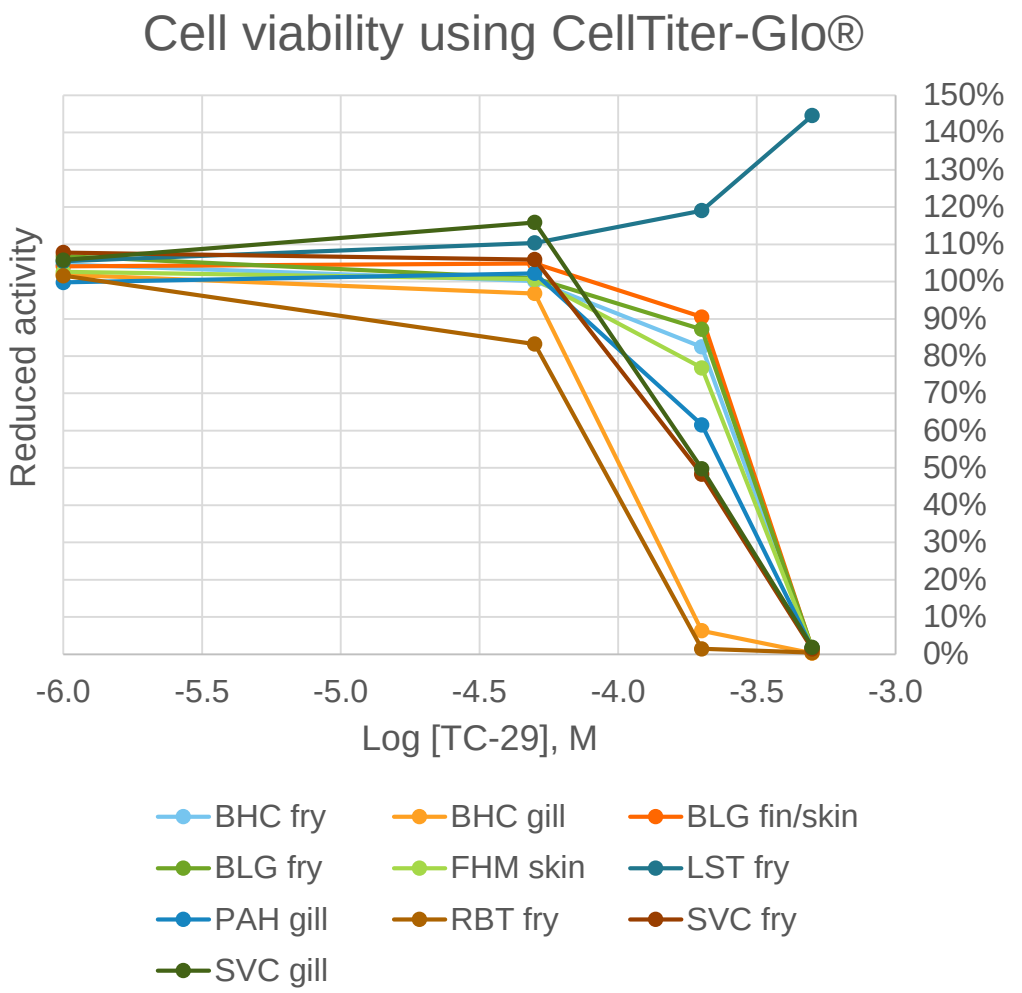
  >25 chemical parameters
  Many biological endpoints

  – Tetramethy thiuram disulfide would be selective for carp

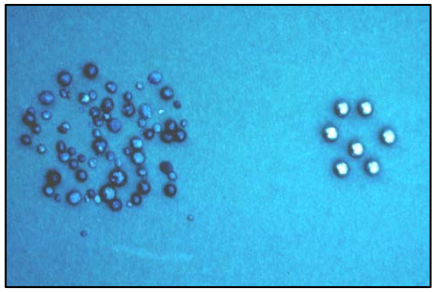
- Modeling indicated *in vivo* trials of Ziram would have similar effects
- Two compounds used to identify new invasive species toxicant
- No additional animals needed to initiate species selective cellular assays

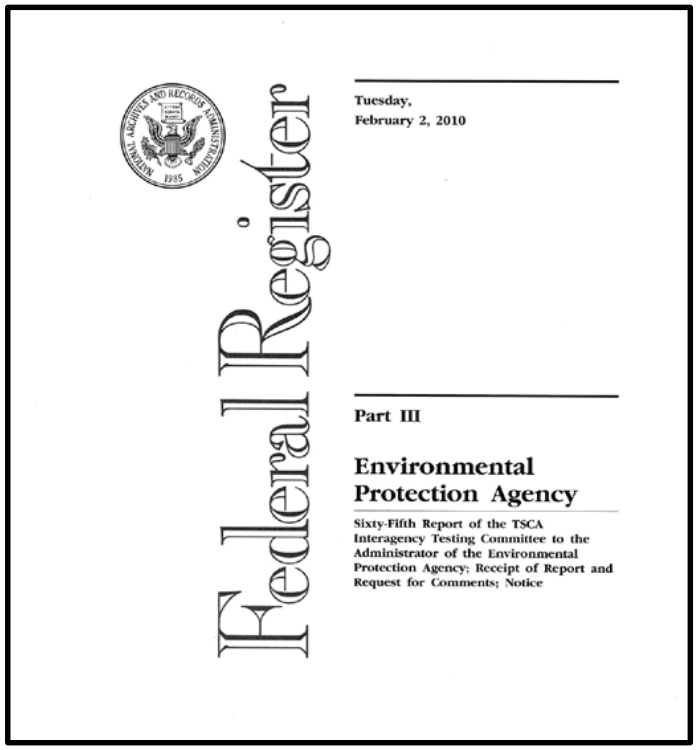
Rapid selectivity screening

- Fish cell lines
  - 7 fish species
  - Multiple organs from some species
  - Paddlefish and Sturgeon toxic response without using live animals
- TC-29 (new biocide)
  - Selected using toxicity models
- Results
  - Bighead carp and rainbow trout are similarly sensitive to TC-29
  - TC-29 stimulates the production of ATP in lake sturgeon
  - Traditional *in vivo* trials for both studies (TTS, Ziram, TC-29) would require more than 8,400 fish. We used 1,005 fish to validate the entire selection process



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)

Challenge…

extrapolating toxic/therapeutic effects among diverse groups of species

- Diverse exposure pathways
- Differences in life history and behavior
- Differences in ADME among species that can affect toxicity