

Department of the Interior

Alternative Toxicology Test Method Activities



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”

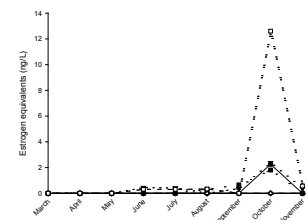
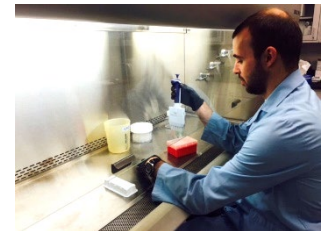
1. Research with direct application to natural resource management
2. Environmental contaminant biomonitoring
3. Natural Resource Damage Assessment
4. Registration of chemicals used in aquaculture
5. Alternatives to “lead shot” used in hunting

Embrace 3R's



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



Effects-directed analysis of endocrine-active chemicals



1) Sediment and water collected from locations with immunocompromised and/or intersex fish

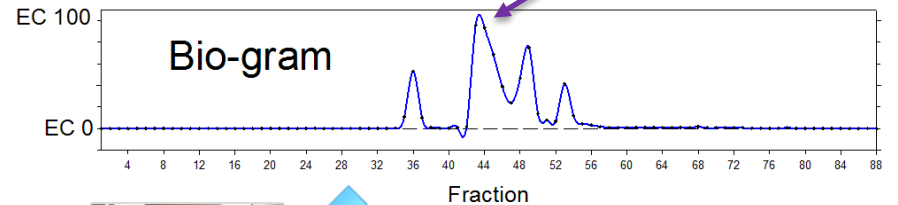


2) Sediment and water extracts are screened with reporter cell bioassays



3) Bioactive extracts fractionated and tested in fraction form on reporter cell bioassays

Bioactivity

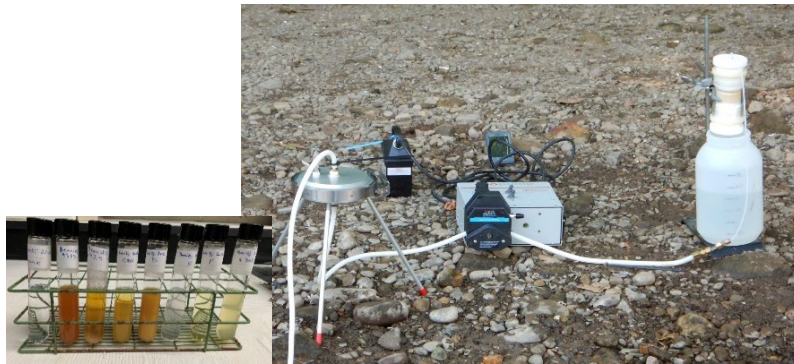


4) Bioactive fractions analyzed by UPLC-QTOF-MS for chemical suspects



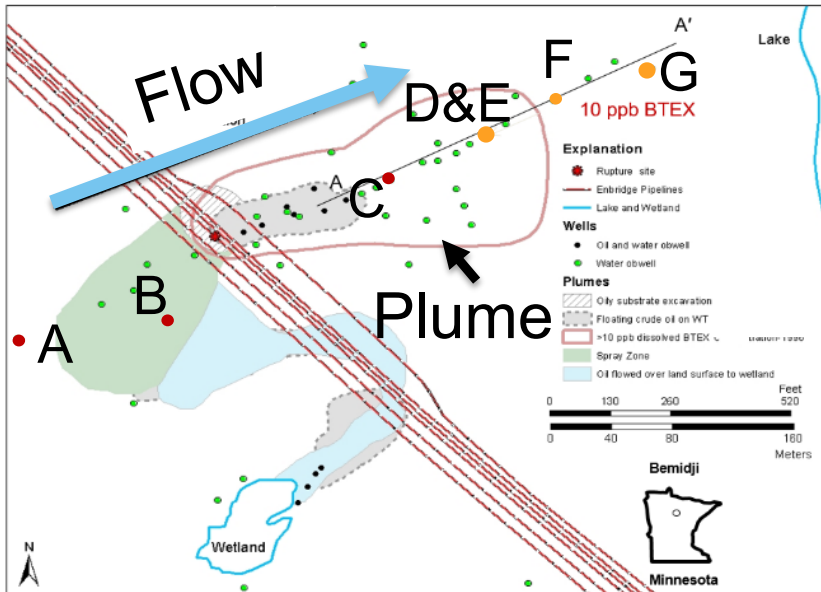
5) Further testing of the chemical suspects in higher Tier assays

In vitro testing for PAH metabolites in oil-contaminated groundwater



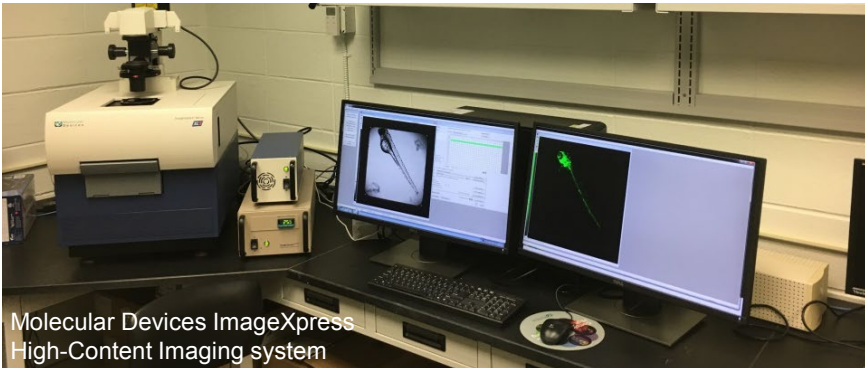
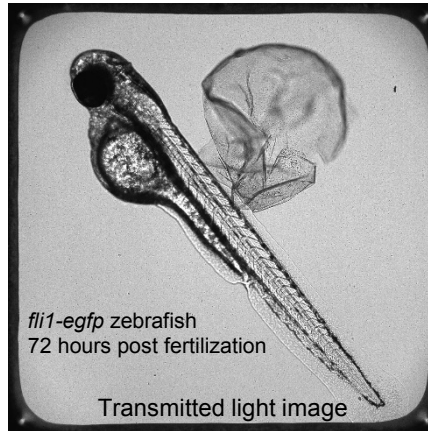
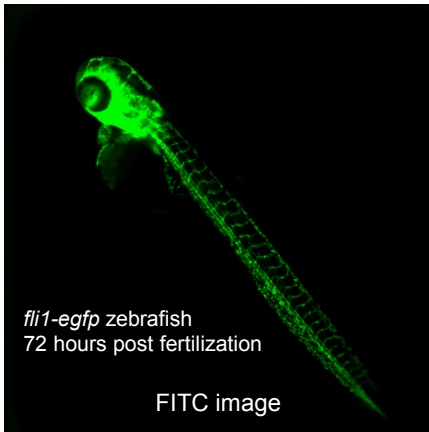
Large-volume water sampler provides enough sample for in vitro and in vivo testing

In vitro reporter assays track movement of PAH-type activity in groundwater plume



Sample Location	PAH-type activity, Human cells	PAH-type activity, Rat cells
A	/	/
B	/	/
C	+++	+
D	++	/
E	/	/
F	++	+
G	++	/

High-Content 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

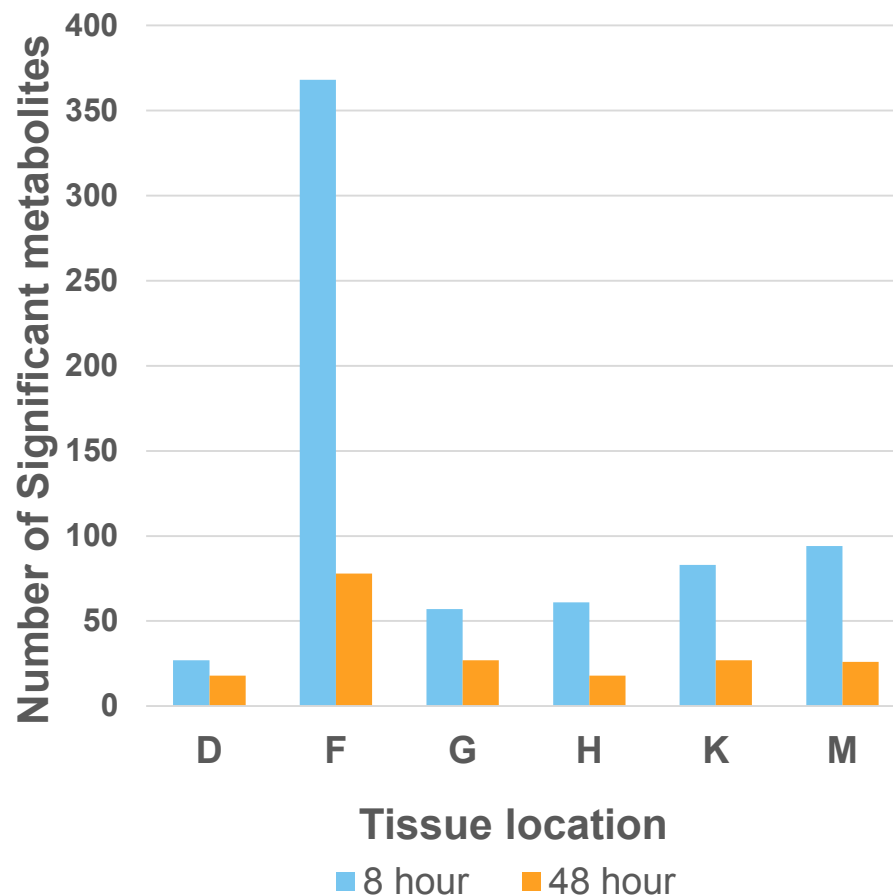
High-Content Screening

- 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
- HCS assays can provide evidence to justify larger-scale studies to determine actual risk versus perceived risk of contaminants.
- Currently: screening pesticides and pharmaceuticals of interest, as well as field samples to complement *in vitro* bioassays

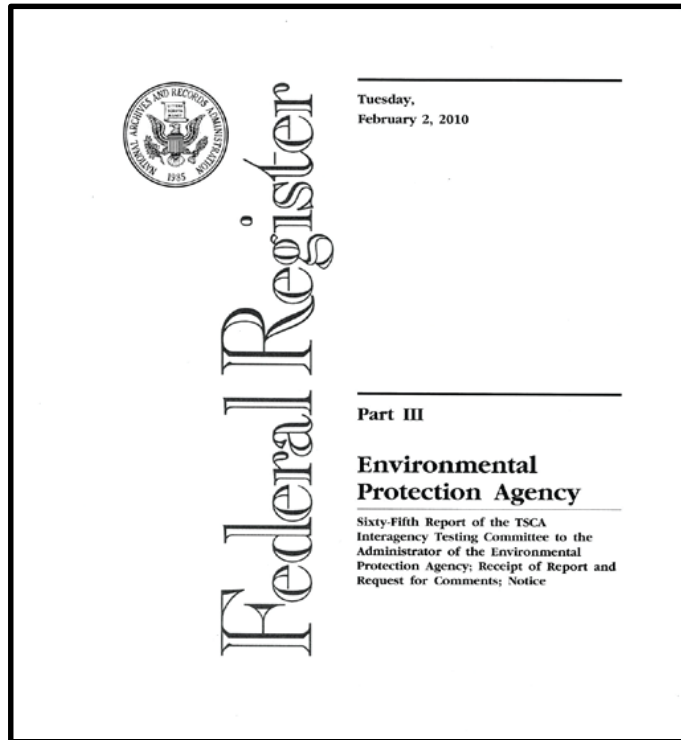
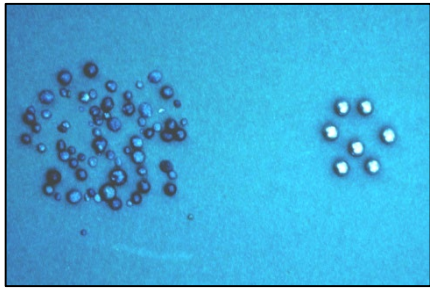
Non-lethal tissue sampling

- Diagnostic Testing
 - Massive mussel die-offs are occurring in the Clinch River, Virginia and the Embarrass River, Illinois
 - Metabolomic profiling can be used on individual animals to identify the underlying cause
 - Six tissues were selected from mussels exposed to niclosimide
- Tissue Response
 - The foot samples show the largest response to niclosamide and recovery is still occurring at 48 hours after exposure
 - This indicates that non-lethal sampling can be taken from this tissue

Exposure and Recovery in *Ligumia nasuta* exposed to Niclosamide



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

