

FUNCTIONAL BIOASSAY PLATFORM FOR ENVIRONMENTAL MONITORING

Remediation Technologies Symposium 2018

In Partnership with:







□ A new approach for a growing problem

□ Introduction to gene expression

□ Results from the field

□ Future applications





REGISTERED POLLUTANT EMITTING FACILITIES IN CANADA

Geographical locations and relative abundance of pollutant source facilities (NPRI)





AQUIFER VULNERABILITY INDEX FOR THE AGRICULTURE AREA OF ALBERTA







Areas of highest vulnerability also have the most intensive land usage





COMMERCIAL LAB SERVICE GAP

For Individual Contaminants

- 1. Limited by list of established guidelines.
- 2. Challenges with growing body of chemicals/contaminants.

For cumulative effects:

3. Evaluations made based on macroscopic appearance, behaviour and mortality. Do not allow for mechanistic insights required to improve process.





CUMULATIVE EFFECT



 $Hg^{+2} + H_2S \longrightarrow HgS$

Reduction in toxicity

Allowed Concentration



RESEARCH PROJECT

- Develop methodology to assess combinatorial effects of toxicants derived from complex water sources.
- Build on previous capacity developed at UofA.
- Bioassay platform that offers quantifiable outputs based on functional biological processes.
- Focus on combinatorial effects on immune competence and activation of metabolic detoxification systems.





EVALUATING GENE EXPRESSION

Polymerase Chain Reaction (PCR)





EVALUATING GENE EXPRESSION

- Exposure to toxicants can alter gene expression profiles through the regulation of the genes that are involved in cellular protection and damage repair mechanisms (Causton et al. 2001).
- Measuring gene expression profiles offers quantifiable endpoints for toxicity and can be utilized to evaluate effects on specific biological mechanisms (Pim de Voot. 2017).





GENE SELECTION

- Genes were selected from known list of immune response regulators.
- This includes proinflammatory cytokines which are important cell signaling agents.
- Cytochrome P450 (CYPs) is a family of proteins that play a crucial role for enzymatic processes. Markers for liver toxicity were selected form this group.



DOSE-RESPONSE EXPERIMENTS



Rainbow trout (Onchorhynchus mykiss) were exposed to an industrial effluent water

Treatment Concentration	Number of Fish	Mortality
Control (0%)	10	0%
50%	10	50%
25%	10	0%
12.50%	10	0%
6.25%	10	0%



ASSAY PREPARATION

The trout were euthanized and flash frozen in liquid nitrogen. Storage at - 80°C required for long term.



Internal organs (gills, kidney, gut, intestine, spleen and liver) were used to obtained RNA for analysis.



RESULTS

Gene expression analysis of Rainbow trout RNA following 72hour exposure to effluent water at concentrations of and 6.25%, 12.5%, 25%, 50%,.

Tumor Necrosis Factor alpha (TNF α) induces cell survival.

Interleukin one beta (IL-1 β), plays a key role in regulating the inflammatory process.





RESULTS

Cytochrome P450 1A1 (CYP1A); bioactivation and detoxication of polycyclic aromatic hydrocarbons (PAHs) and pharmaceutical drugs.

Granulocyte colony stimulating factor receptor (G_CFSR). maintaining a population of cells that take part in inflammatory responses.





GENE EXPRESSION: REFERENCE STANDARD

Trout were exposed to set concentrations of phenol solution to assess mortality against a standard with known toxicity effects.

The reference toxicity test must be performed by the lab after acclimation of each new batch of fish.

Treatment Concentration	Number of Fish	Mortality
Control (<0.1	10	00/
15 0 mg/L)	10	0% 100%
12.0 mg/L	10	80%
10.0 mg/L	10	20%
8.0 mg/L	10	0%
5.0 mg/L	10	0%





TYPICAL APPROACH







REAL WORLD SCENARIO: OIL & GAS SECTOR

- Release identified and environmental consultant called to site for exposure assessments. Source removal / control measures initiated.
- Typical testing for multi-phase pipeline release:
 - PAHs
 - VOCs/BTEX
 - F1-F4 hydrocarbons
 - Methanol/glycol
 - Trace Metals/Inorganic water chemistry
 - Emerging contaminants



- Analysis costs with rush surcharges can be > \$500 per sample.
- Monitoring time length determined by sufficient remediation of toxicants as a health risk to the environment.



REAL WORLD SCENARIO

- Risk assessment evaluates:
 - Potential toxic effects of contaminating substances on biological systems (receptors) in contact exposure routes (pathways).
 - Key question from Alberta Environment: "if exposure occurs, what is the likelihood that the substances will produce harmful effects?"
- Hard to show return to original state as regulated tests focus on the presence and amounts of individual contaminants only.
- Combinatorial effects of toxicants in whole body systems are not addressed.



SUMMARY

- Significantly expands the depth and relevance of regulated Rainbow Trout assays using gene expression analysis.
- Provides effective and reliable early markers for risk levels; contaminant stress is measured at non-lethal concentrations.
- Allows for on-site system process adjustments to prevent regulatory compliance failures.



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QUESTIONS?

Caring About Results... Obviously!

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