**ECOToxCHIP: A TOXICOGENOMICS TOOL FOR CHEMICAL PRIORITIZATION AND ENVIRONMENTAL MANAGEMENT**

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**CHALLENGE**

- Chemical contamination threatens the health of humans and wildlife globally
- Regulatory agencies and businesses are tasked to manage chemicals but are challenged:
  - many chemicals (e.g., 4,3K in Canada, 84K in USA, 101K in EU) need to be tested within short timeframes;
  - mandate to monitor many complex environmental samples (e.g., sediment, water, effluents);
  - testing is costly, time-consuming, and uses many animals; tests on limited model species poorly predict risk in native species of concern.

**PROJECT DELIVERABLES**

1. EcoToxChips for three key vertebrate model species used globally in ecological risk assessment (fathead minnow, Japanese quail, Xenoopus laevis; fish, bird, frog);
2. EcoToxChips for three naïve species of commercial, recreational, and Aboriginal concern (rainbow trout, double-crested cormorant, northern leporid frog);
3. EcoToxXplorer.ca is an online data evaluation tool that provides functions to allow users to upload EcoToxChip data and interpret results; and
4. Technical Guidance Document is informed by our GEiLS (social sciences) research and will be a government-verified resource to advising end users on how to adopt EcoToxChips.

**OPPORTUNITY**

Global paradigm shift in toxicity testing from historical whole-organism testing to mechanistic studies

**Human Health:**
- U.S. NRC’s “Toxicity Testing in the 21st Century-Vision and Strategy”
- New approach methods (e.g., toxicogenomics tools, alternative testing strategies) and stakeholder acceptance helping to transform the field

**Ecological Risk Assessment:**
- Avian ToxChip qPCR array developed by Environment and Climate Change Canada researchers via commercial partnership:
  - used to screen and prioritize chemicals; study varyably contaminated sites
  - embraced by Canadian regulators, environmental monitoring programs, and the scientific community

**PROJECT OBJECTIVES**

To develop, test, validate, and commercialize qPCR arrays (EcoToxChips) which consist of 384 genes covering key toxicity pathways of regulatory concern, and a data evaluation tool (EcoToxXplorer.ca) for the characterization, prioritization and management of environmental chemicals and complex mixtures of regulatory concern.

**ACTIVITIES & UPDATE (36 month)**

**PHASE I: Model Species (Y1-3)**

1. Exposure Studies (60%)
2. Omics + EcoToxChips (65%)
3. Dewarp EcoToxChips (25%)
4. User-Validates (15%)

**DELIVERABLE 1 (Y1-3)**

- Delivers 1:
  - The first EcoToxChips were “delivered” in October 2019
  - Officially launch the EcoToxChip product line at the 2019 SETAC meeting
  - Moving ahead, every 6-9 months a new species-specific EcoToxChip will be made commercially available

- Delivers 2:
  - Native species EcoToxChips will be released after the standard lab protocols are done

**PHASE II: Native Species (Y2-4)**

5. Exposure Studies (60%)
6. Omics + EcoToxChips (65%)
7. Dewarp EcoToxChips (25%)
8. Dewarp EcoToxChips (5%) (Y2-4)

9. Data Evaluation Pipeline (EcoToxXplorer.ca) (Y1-4, 65%)

**DELIVERABLE 2 (Y1-3)**

- Deliverables 1:
  - EcoToxChips Institutional Enterprise (Y1-4, 65%)

**ECOTOXCHIP VALIDATION**

**Tier 1 Technical validation study (inter- & intra-lab)**
- We tested 45 v0.1 JG EcoToxChip plates (3 test sites x 3 samples x 5 replicates of each sample)
- cDNA test samples derived from individual JG livers from control and chlorpyrifos-exposed; another cDNA sample was diluted
to various concentration lab ran samples using in-house approaches in total, data were available from 17,280 measurement wells including measures of 361 gene targets across 16,245 wells, 16 housekeeping genes (720 wells in total across all plates) and 315 control parameters (e.g., positive PCR controls, GDC)

Boxplot of CV variance of housekeeping genes from 3 test labs. Inter- and intra-lab variation is less than 2.5% (median values). Similar results for the gene targets. Variance is much less than the 20% criteria noted by the MAQC program (y-axis limit).

**Tier 2 Technical validation study (cross-platform)**
- EcoToxChip qPCR results (Ct values) were compared against RNAseq counts data for n=9 v0.1 JG EcoToxChip plates

Spearman plots demonstrate high concordance between EcoToxChip qPCR results and RNAseq counts. The overall R² is 0.81 (R²=0.8) which is above the MAQC reported value of R²=0.69

**ECOTOXCHIP DESIGN & UPDATES**

- EcoToxChip designed through design thinking steps (empathize, define, ideate, prototype, test) to maximize potential of end-user adoption of project deliverables

**ECOTOXCHIP DESIGN & UPDATES**

- v0.1 (alpha): internal Tier 1 tests in core project labs and Qiagen to assess technical performance
- v1.0: for the Tier 2 validation study with core study partners (e.g., ring test, cross-platform performance)
- v2.0 (gamma): tested by a larger community of users

**ECOTOXCHIP VALIDATION**

- Data upload from diverse sources (e.g., EcoToxChip, RNAseq, microarray)
- Enter key study details (e.g., exposure and apical outcomes) as per study reporting guidance documents
- Data QC options (e.g., control wells, housekeeping genes, normalization steps, cut-offs)
- Statistical analysis modules (e.g., benchmark dose-response, mode of action, tables)
- Visualizations (e.g., heat maps, volcano plots, radar and circle plots, EcoToxModules)
- Report generation
- Create account and save work

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Research Oversight Committee: Drs. Nancy Denilow (chair), Kevin Crofton, Daniel Scherrert, Roy Suddaby, and Carole Yauk

Core Partners: Government (Environment & Climate Change Canada, US Army Corps of Engineers, US EPA) & Business (SGS AXYS, Qiagen, Shell USA)