

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.

Urgent worldwide demand for improved testing tools that are more efficient, affordable, flexible, predictive, and less dependent on live animal studies

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., toxicogenomic 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:
- \rightarrow used to screen and prioritize chemicals; study variably contaminated sites
- \rightarrow 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.

ECOTOXCHIP: A TOXICOGENOMICS TOOL FOR CHEMICAL PRIORITIZATION AND ENVIRONMENTAL MANAGEMENT

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PROJECT DELIVERABLES

- EcoToxChips for three key vertebrate model species used globally in ecological risk assessment (fathead minnow, Japanese quail, *Xenopus laevis*; fish, bird, frog);
- 2. EcoToxChips for three <u>native species</u> of commercial, recreational, and Aboriginal concern (rainbow trout, doublecrested cormorant, northern leopard 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 GE³LS (social sciences) research and will be a government-vetted resource to advise end-users on how to adopt EcoToxChips.



ACTIVITIES & UPDATE (36 month)

PHASE I: Model Species (Y1-3)

- 1. Exposure Studies (80%)
- 2. Omics \rightarrow EcoToxChips (65%)
- 3. Develop EcoToxChips (25%)

DELIVERABLE 1 – YR3

4. End-Users Validate (15%)

PHASE II: Native Species (Y2-4)

- 5. Exposure Studies (60%)
- 6. Omics \rightarrow EcoToxChips (25%)
- 7. Develop EcoToxChips (5%)

DELIVERABLE 2 – YR4

8. Data Evaluation Pipeline (EcoToxXplorer.ca) (Y1-4; 65%)

DELIVERABLE 3 – YR4

9. GE³LS Institutional Entrepreneurship (Y1-4; 65%)

DELIVERABLE 4 – YR4

Deliverable 1:

- The first EcoToxChips were "delivered" in October 2019
- Officially launch the EcoToxChip product line at the 2019 SETAC meeting
- Moving ahead, every ~3 months a new species-specific EcoToxChip will be made commercially available

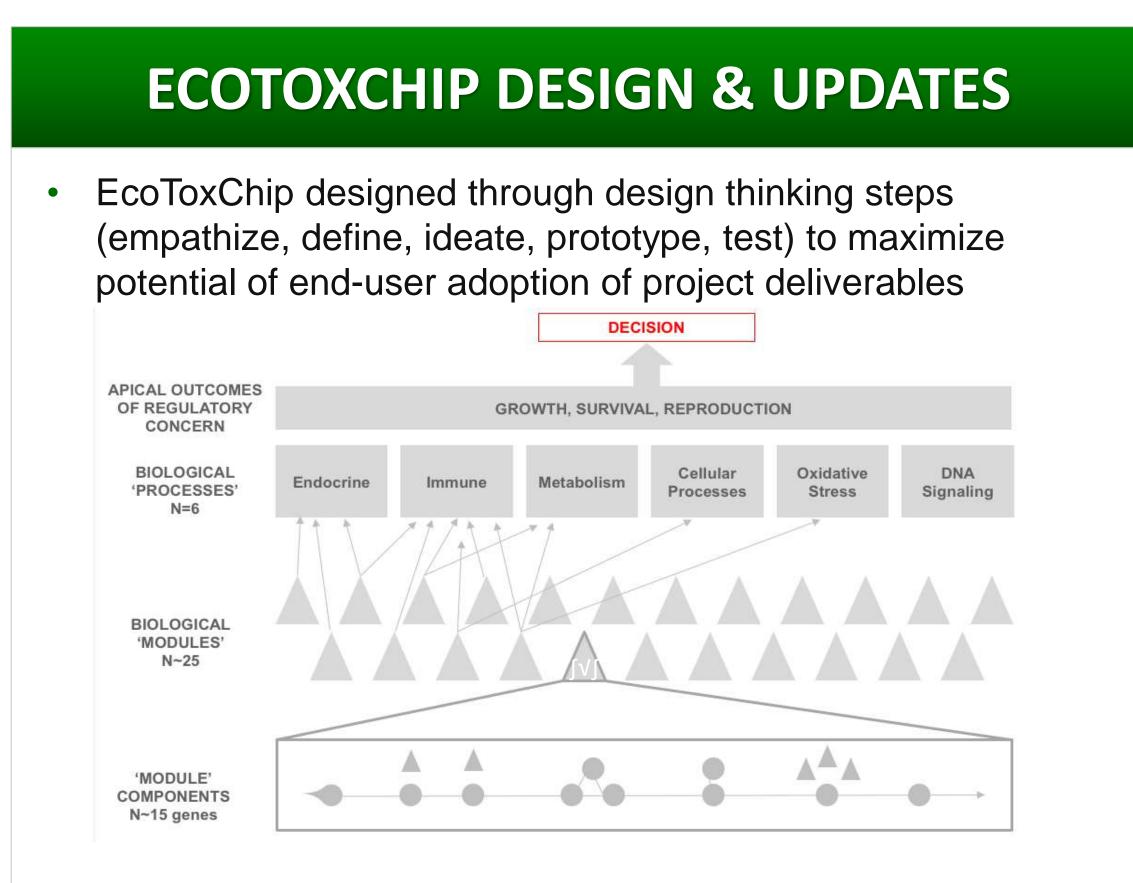
Deliverable 2:

• Native species EcoToxChips will be realized after the standard lab models are done **Deliverable 3:**

- Design and use of *EcoToxXplorer*.ca is underway
- Web interface for EcoToxXplorer.ca is online and operational
- ToxMine and ftp://ftp.ecotoxxplorer.ca as database sites for the project
- Refinement of algorithms that consider relevant approaches (POD, BMD, chemo-
- informatics, gene set signatures, etc.) to support interactive data mining and exploration • We are now evaluating different gene prioritization approaches based on this resource

Deliverable 4:

- To ensure that the work is user driven, we have adopted a design thinking
- philosophy, and thus prioritized 3 user journeys and 3 user groups
- A Registry (users, use cases, user journeys, desired features in all deliverables) has
- been helping us leverage end-user needs to inform the design of all deliverables • Validation plan: We have started to test EcoToxChips with key project partners (ECCC,
- US EPA, US Army Corps, Shell, USGS) • In addition to these users, the GE³LS team continues data gathering and analysis,
- outreach, and liaison activities • The information from the GE³LS research as well as users, along with prioritized partners at ECCC, will help us derive the Guidance Document in the final year of the project

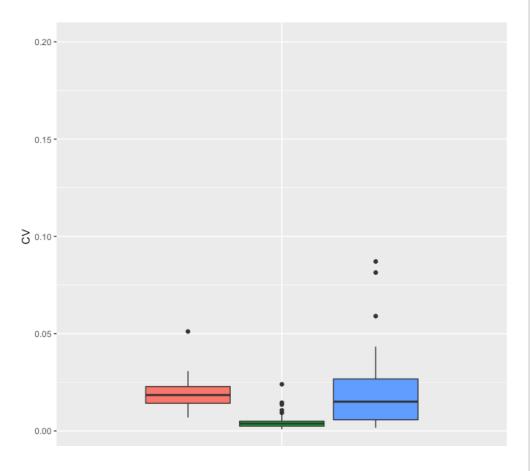


- v0.1 (alpha): internal Tier 1 tests in core project labs and Qiagen to assess technical performance
- v1.0 (beta): used in the Tier 2 validation study with core study partners (e.g., ring test, cross-platform performance)
- v2.0 (gamma): tested by a larger community of users

ECOTOXCHIP VALIDATION

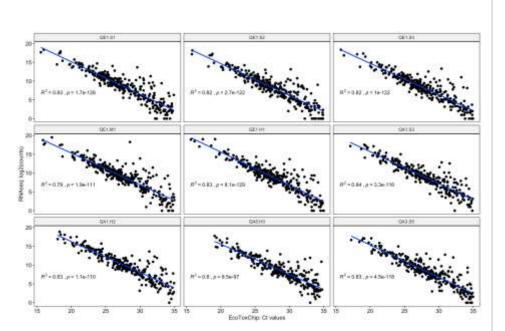
- Tier 1 Technical validation study (inter- & intra-lab)
- we tested 45 v0.1 JQ EcoToxChip plates (3 test sites x 3 samples x 5 replicates of each sample)
- cDNA test samples derived from individual JQ livers from control and chlorpyrifos-exposed; another cDNA sample was diluted
- each participating 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 QCPR results (CT values) were compared against RNAseq counts data for n=9 v0.1 JQ EcoToxChip plates

Scatterplots demonstrate high concordance between EcoToxChip QPCR results and RNAseq counts. The overall R^2 is 0.81 (R=0.9) which is above the MAQC reported value of R=0.69



EcoToxXplorer.ca Try it out! Go to <u>www.ecotoxxplorer.ca</u>		
Visua	EcoToxXplorer	ofiling
EcoToxChip	RNAseq	Microarray
Gene List	Cheminformatics	Other Utilities
	FEATURES	
ECOTOXCHIP ANALYSIS Perform analysis by mapping to the EcoToxChip panel set of 374 genes which are selected by experts to	INTERACTIVE EXPLORATION Generate well-known statistical plots (e.g. volcano plot or heatmaps) to analyze toxicogenomics data.	SEVERAL DATA INPUTS Start analysis using many types of inputs including RNAseq, microarray, gene list or chemicals.
characterize toxic exposures.		
EcoToxXplorer		PROJECT MANAGEMENT
Process raw RNA-seq files using our customized Galaxy server.	Summarize all executed steps of analysis into a report for documentation and further study.	Create an account for storing data and results and for resuming interactive analysis session.

- 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 doseresponse, mode of action, tables)
- Visualizations (e.g., heat maps, volcano plots, radar and circus plots, EcoToxModules)
- Report generation
- Create account and save work

ACKNOWLEDGEMENTS

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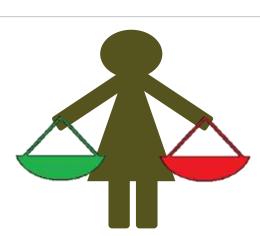
People: Pascal Poulin (Project Manager), Emily Boulanger, Jenny Eng, Anita Masse (Local Project Managers), several graduate students, postdoctoral fellows, and technicians, staff at Génome Québec, Genome Prairie, and Genome Canada

Research Oversight Committee: Drs. Nancy Denslow (chair), Kevin Crofton, Daniel Schlenk, 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)

Intro Paper: Basu N, Crump D, Head J, Hickey G, Hogan N, Maguire S, Xia J, Hecker M. 2019. EcoToxChip: A next-generation toxicogenomics tool for chemical prioritization and environmental management. Environ Toxicol Chem. 38(2):279-288.

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using toxicogenomics to nake decisions

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