# CHARACTERIZING EARLY CHANGES IN MOLECULAR TOXICITY PATHWAYS TO PREDICT ADVERSE OUTCOMES OF ETHINYL ESTRADIOL AND CHLORPYRIFOS IN AMPHIBIANS

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## INTRODUCTION

- Canada's Chemical Management Plan has named 4,300 chemicals as priorities for evaluation including ethinyl estradiol (EE2) and chlorpyrifos (CPY), which are known aquatic contaminants.
- Amphibians are of vital importance to ecosystems and are key receptors of concern with contaminant toxicity and ecosystem health.
- Exposure of amphibians to contaminants can have adverse outcomes, such as altered rate of metamorphosis, reproductive effects, immune suppression, and behavioural effects.
  - Such apical effects are often preceded by molecular changes, which can be used as early indicators of physiological changes
- Current methods of chemical testing are time consuming, expensive and use large numbers of animals.
- Identifying molecular toxicity pathways that are linked to adverse outcomes is a promising approach to screen chemicals for potential toxicity without the need for long term, animal intensive exposures.

## OBJECTIVES

- **To evaluate apical effects of chronic chemical exposure (embryo to metamorphosis) to EE2 and CPY in the amphibian *Xenopus laevis.***
- **To identify and validate key molecular toxicity pathways that are predictive of contaminant-induced apical responses.**

## RESULTS

### Ethinyl estradiol (EE2)

DMSO = 0.01%, Low = 0.04, Med = 0.2, High = 1 ug/L

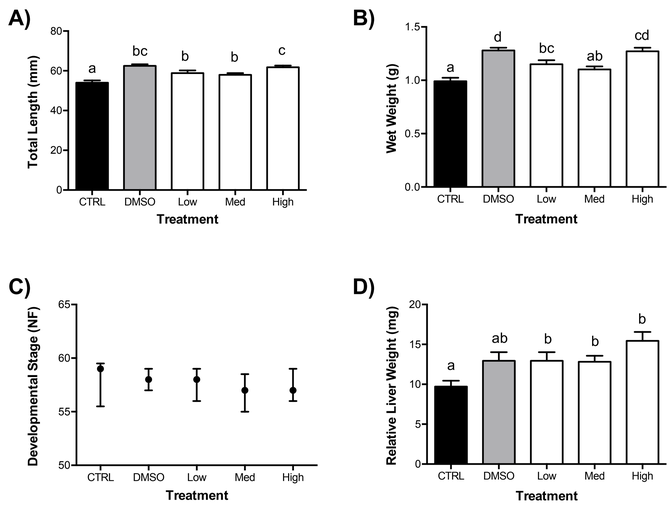



**Figure 2.** Effects of EE2 on (A) total length, (B) wet weight, (C) developmental stage (NF) and (D) relative liver weight of *X. laevis* tadpoles (NF stage 57-60) exposed for ~ 55 days in a flow-through system. Data for weights and length are presented as mean ± SEM, developmental stage is presented as median ± IQR. Letters indicate significant differences between treatments for total length (One way ANOVA: p<0.001, $F_{4,249}$=13.316 ), wet weight (One way ANOVA: p<0.001, $F_{4,249}$= 14.715), and relative liver weight (One way ANOVA: p=0.001, $F_{4,185}$=5.201). NF = Nieuwkoop Faber

### Chlorpyrifos (CPY)

DMSO = 0.01%, Low = 0.5, Med = 2, High = 8 ug/L

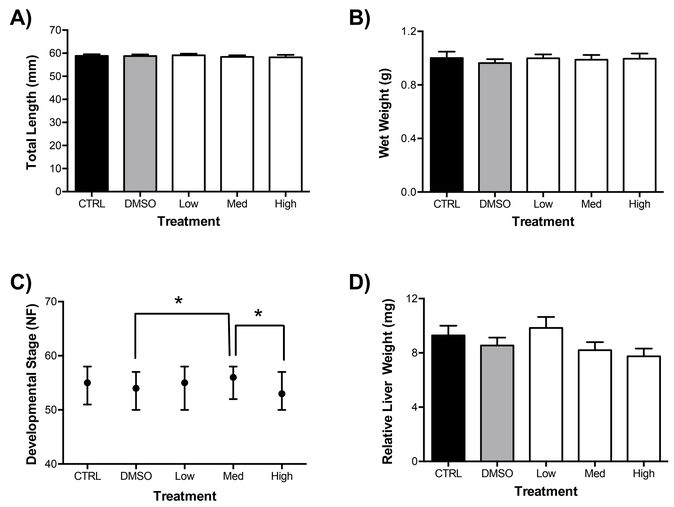



**Figure 3.** Effects of CPY on (A) total length, (B) wet weight, (C) developmental stage (NF) and (D) relative liver weight of *X. laevis* tadpoles (NF Stages 57-60) exposed for ~ 55 days in a flow-through system. Data for weights and length are presented as mean ± SEM while developmental stage is presented as median ± IQR. (*) indicates significant differences between treatments for developmental stage (one way ANOVA: p=0.013, $F_{4,533}$=3.188). NF = Nieuwkoop Faber

## METHODS

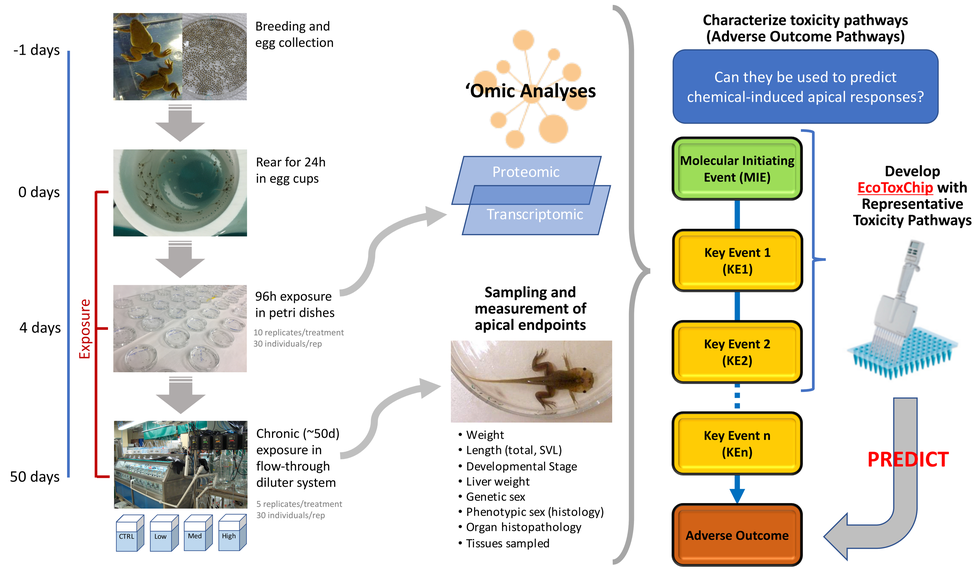
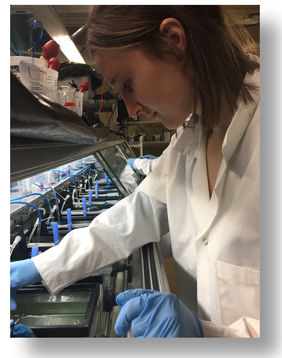



**Figure 1.** Schematic representation of the methodology for waterborne exposure of *X. laevis* to ethinyl estradiol (EE2) and chlorpyrifos (CPY). Sampling at 96 h (for transcriptomic and proteomic analyses) and after chronic exposure (~ 50 d, to assess apical effects) is indicated. Toxicity pathways identified at the transcriptomic and proteomic level after 96 h exposure will be correlated with effects on apical, histopathological, and biochemical endpoints at metamorphosis.

## DISCUSSION

- Preliminary results indicate that chronic exposure of *X. laevis* tadpoles, from embryo-larval stages through to metamorphosis, had significant effects on various apical endpoints.
  - Exposure to EE2 (1 ug/L) significantly increased total length, wet weight, relative liver weight as compared to water-only control group. There was no difference when compared to DMSO control. There were no significant effects on developmental stage.
  - There were no significant effects on total length, wet weight or relative liver weight with exposure to CPY. There were minor changes in developmental stage; however, not likely biologically relevant.
- Apical effects will be further assessed through histopathology of key organs (thyroid, gonads) along with evaluation of genetic/phenotypic sex ratios.
- Early-life stage individuals (sampled after 96 h exposure) will be evaluated for transcriptomic and proteomic responses using whole transcriptome (RNASeq) and mass-spectroscopy-based shotgun proteomics to characterize key molecular toxicity pathways.
- We will correlate molecular responses with apical outcomes in an effort to identify key genes that will predict adverse effects of ecological and regulatory relevance in amphibians.
- This data will feed into a multi-year and multi-species initiative to develop a screening tool – **the EcoToxChip and EcoToxXplorer.ca**– that can be used to assess and prioritize chemicals of concern while reducing cost, time and animal use.



## ACKNOWLEDGEMENTS

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