An ethical *in vitro* alternative model to screen for alterations to the epigenome in embryonic cells following exposure to environmental chemicals

SCAN ME

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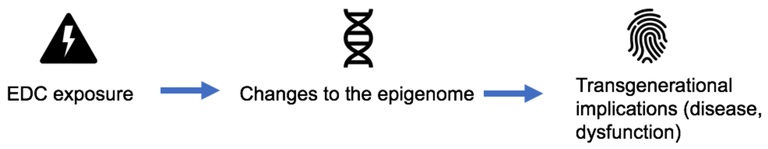
Background

Xenobiotic Exposures during Embryonic Development

- Environmental factors during development may result in disease states later in life or transgenerationally via epigenetic changes, which include exposures to drugs taken during pregnancy [1,2,3,4,5].
- The epigenome is particularly sensitive during embryonic development to external factors [6].



Epigenetic Toxicity Screening for Drugs

- Conventional drug safety assessments for genotoxicty and reproductive toxicities do not take into account epigenetic alterations - consequences of which may not manifest until later-in-life or future generations [7].
- Ideal criteria for a preliminary epigenetic toxicity test:
  - Reproducible
  - Relatively cheap and accessible
  - Rapid with potential for high-throughput applications
  - Sensitive
  - Avoid ethical complications for widespread use

Alternative Model Organisms for Embryonic Development

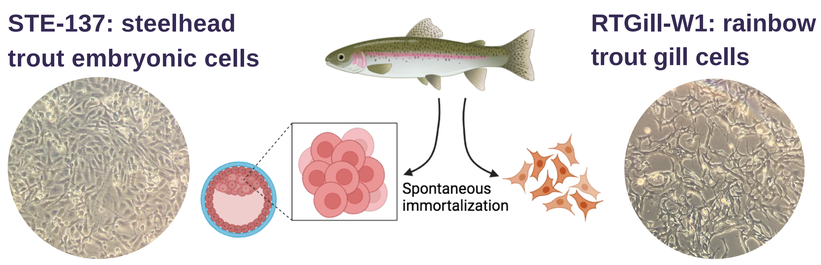
- Teleost fish such as rainbow trout (*Onchorhynchus mykiss*) and zebrafish (*Danio rerio*) exhibit remarkably high sensitivity to changes in their environment, which makes them important species in basic and translational research to explore the effects of environmental toxicants [8].
- The use of embryos derived from teleost fish such as rainbow trout can offer alternative models for studying the impacts of the environmental toxicant exposure on normal embryonic development.

<u>Project Aim</u>: Establish a screening tool for assessing epigenetic toxicity to embryonic cells following exposure to xenobiotics.

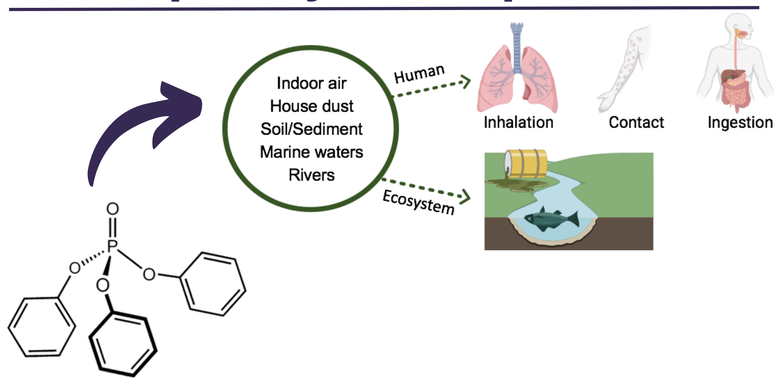
Aquatic *in vitro* Model

STE-137: steelhead trout embryonic cells

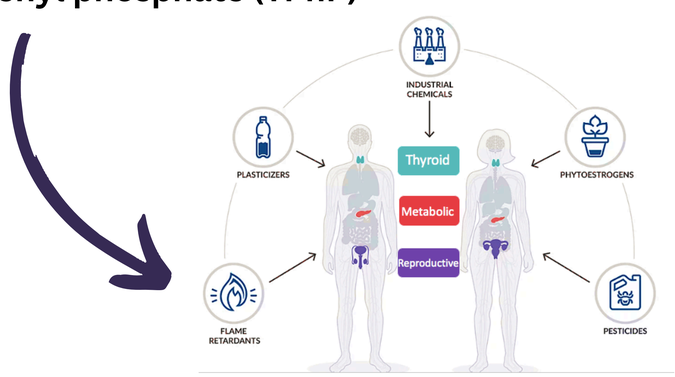
RTGill-W1: rainbow trout gill cells



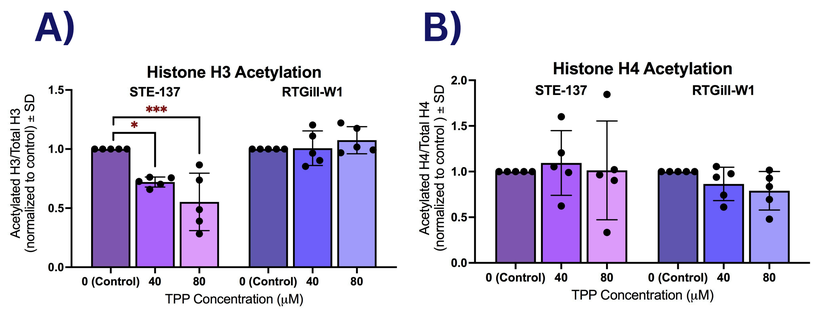
Triphenyl Phosphate

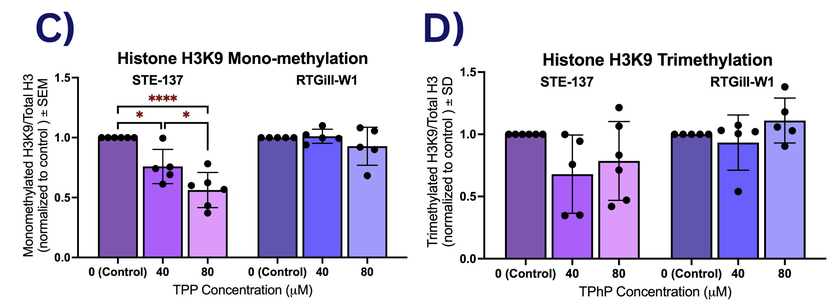


Triphenyl phosphate (TPhP)



Epigenetic Changes





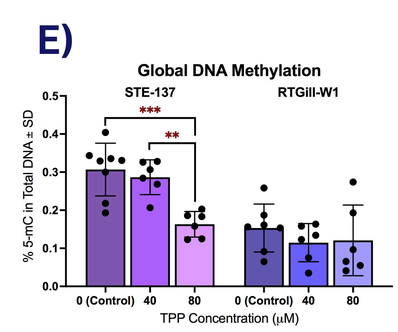
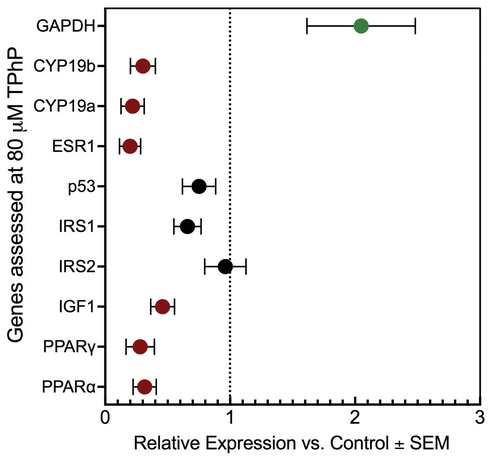


Figure 1. Histone modifications and DNA methylation profiles of STE-137 and RTgill-W1 cells exposed to 0 (control), 40 or 80 μM of TPhP for 24h. A-D) Histone modification expression of exposure groups was assessed via Western blot and is expressed relative to control. Histone H3 acetylation and mono-methylation were significantly reduced at 40 and 80 μM TPhP in STE-137 cells. E) Global DNA methylation was measured via ELISA and was signficantly reduced at 80 μM TPhP in STE-137 cells. ($p < 0.05$).

Current Conclusions

- TPhP exposure is altering the epigenome of embryonic cells derived from trout.
- TPhP is not yet on the Toxic Substances List in Canada, and its use/disposal is not yet regulated (though it has been under review since 2019) [9,10].
- TPhP is not considered to cause genotoxcity or reproductive toxicity *in vivo* based on conventional safety assessments [9,10], though changes to the epigenome were found at concentrations below acute toxicity to embryonic cells in this study.
- Similar epigenetic toxicity tests could be implemented in the screening process for drug safety.

Next Steps

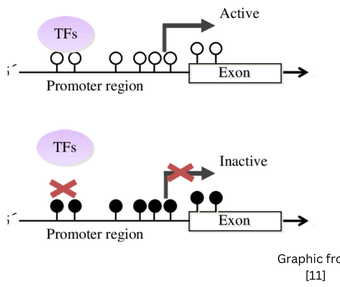


Green: significantly increased expression relative to control

Red: signficantly decreased expression relative to control

Figure 2. RT-qPCR analysis of estrogenic and metabolic genes in STE-137 cells following TPhP exposure. List of genes assessed at 80 μM TPhP exposure relative to control and normalized to the reference genes beta-actin and 18S. (* $p < 0.05$, $n = 6$ biological replicates).

Future Goal: Assess promoter-specific DNA methylation of altered genes shown above in STE-137 embryonic cells. This will demonstrate the applicability of this model to assess epigenetic mechanisms of altered gene expression caused by endocrine disrupting chemicals.



Graphic from [11]

Acknowledgements & References

Queen's University

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The Winn Lab

Scan for references!

REFERENCE LIST