Investigating the impact of triphenyl phosphate exposure on DNA methylation of estrogenic and metabolic pathways in embryonic cells derived from rainbow trout

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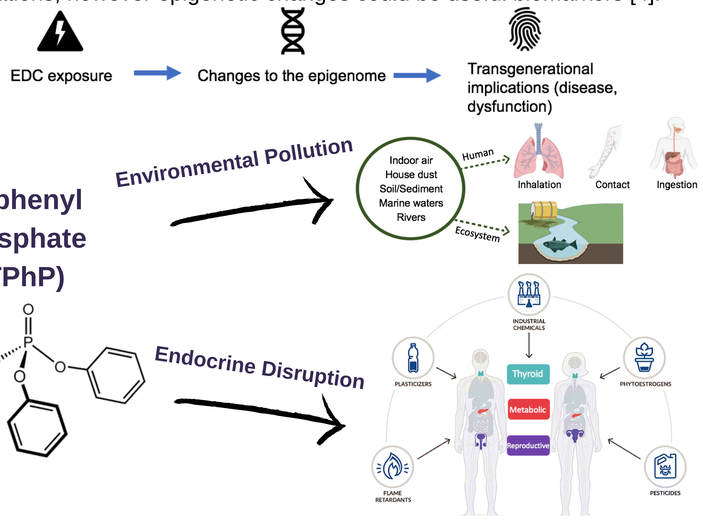
Background

Environmental Exposures during Embryonic Development

- Environmental factors during development may result in disease states later in life or transgenerationally via epigenetic changes [1,2].
- The epigenome is particularly sensitive during embryonic development to external factors [3].
- Chemical and drug safety assessments do not take into account epigenetic alterations, however epigenetic changes could be useful biomarkers [4].



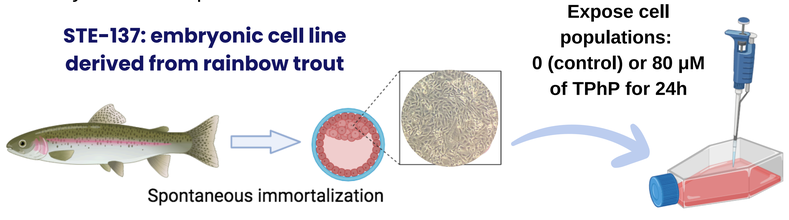
DNA Methylation

- DNA methylation profiles are a key mechanism that regulates gene expression during embryonic development [5].
- Chemical exposures can influence the DNA methylation landscape, leading to Differentially Methylated Regions (DMRs).
- DMRs can be used as biomarkers for endocrine/metabolic disruption [6].

Aquatic *in vitro* Model

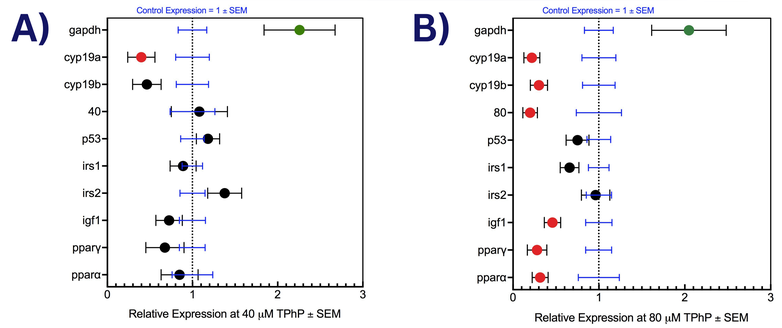
An Alternative Model for Embryonic Development

- Teleost fish such as rainbow trout and zebrafish exhibit high sensitivity to changes in their environment, which makes them useful models to explore the effects of environmental toxicants [7].
- The use of embryos derived from teleost fish can offer an alternative model for studying the impacts of the environmental toxicant exposure on normal embryonic development.



Hypothesis: TPhP exposure in STE-137 (embryonic cells) will result in Differentially Methylated Regions in metabolic and estrogenic pathway genes.

Gene Expression Changes



Green: significantly increased expression relative to control

Red: significantly decreased expression relative to control

Figure 1. RT-qPCR analysis of estrogenic and metabolic genes in STE-137 cells following A) 40 µM or B) 80 µM of TPhP exposure. List of genes assessed relative to control and normalized to the reference genes beta-actin and 18S rRNA. (* $p < 0.05$, n = 6 biological replicates).

Key Take Away #1: TPhP exposure in STE-137 is altering gene expression in estrogenic and metabolic pathways. Notably, estrogen receptor alpha, aromatase enzyme, insulin-like growth factor 1 and PPAR alpha and gamma gene expression are reduced, while glyceraldehyde-3-phosphate dehydrogenase gene expression is increased.

Estrogen Signalling

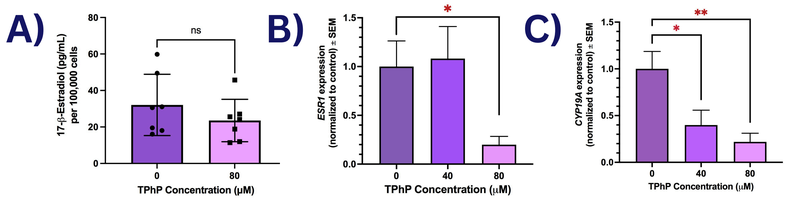
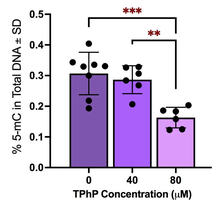


Figure 2. Analysis of A) 17-β-estradiol levels, B) *ESR1* and C) *CYP19A* gene expression STE-137 cells following 80 µM of TPhP exposure. The ELISA Estradiol Parameter Kit by R&D Systems was used to quantify serum levels of estradiol and RT-qPCR used to assess transcript levels relative to control and normalized to the reference genes beta-actin and 18S rRNA. (n = 7 biological replicates).

Key Take Away #2: Estradiol levels are not significantly altered following 80 µM of TPhP exposure in STE-137, however estrogen receptor alpha and aromatase gene expression are significantly reduced compared to control levels.

Global DNA Methylation

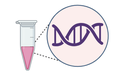


Key Take Away #3: TPhP exposure in STE-137 results in global hypomethylation, however this does not inform us about changes to gene-specific regulation.

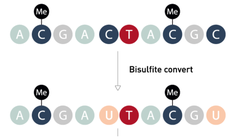
Figure 3. Global DNA methylation changes in STE-137 cells exposed to 0 (control), 40 or 80 µM of TPhP. Global DNA methylation was measured via ELISA and was significantly reduced at 80 µM TPhP in STE-137 cells. ($p < 0.05$, n = 6-8 biological replicates).

Whole Genome Bisulfite Sequencing

1. **DNA Extraction**

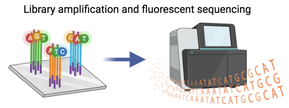


2. **DNA Bisulfite Conversion**



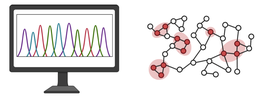
3. **Library Preparation**

4. **Illumina NGS**

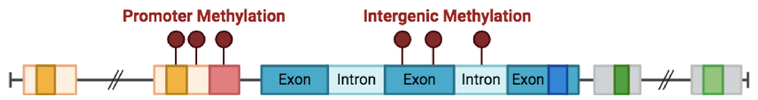


Illumina Next Generation Sequencing performed by the McGill Genome Centre

5. **Genome Alignment**



6. **Analysis of DMRs**



Key Take Away #4: Identification of potential DMRs following TPhP exposure in STE-137 will inform us of gene-specific changes to DNA methylation profiles.

Conclusions and Next Steps

- TPhP exposure is altering global DNA methylation and gene expression in endocrine and metabolic pathways of embryonic cells derived from trout.
- TPhP is not yet on the Toxic Substances List in Canada [8].
- TPhP does not appear to be altering serum estradiol levels in STE-137.
- Glycolysis will also be assessed in the near future.
- Illumina WGBS is currently being conducted by the McGill Genome Centre and analysis in the Winn Lab will inform us of gene-specific changes to the epigenome in STE-137, potentially relating to endocrine and metabolic disruption.

Acknowledgements & References

Scan for references!

The Winn Lab at Queen's

Queen's University