The Sublethal Effects and Bioaccumulation of 17α-Ethinyl Estradiol in *Lumbriculus variegatus*

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Introduction

- Ethinyl estradiol (EE) is a synthetic, steroidal estrogen used in birth control or contraception and is an endocrine disruptor [5]
- Endocrine disruptors are known to interfere with the endocrine system; EE affects behavioral patterns, enzymatic activity levels, natal development and brain structure [4]
- *Lumbriculus variegatus*, or blackworms, are freshwater invertebrates that resides in shallow ponds, lakes, and marshes
- Preceding data has found that EE exposure leads to an increase in mortality, a decrease in offspring, and changes in reproductive morphology among other freshwater invertebrates [1, 2, 3, 6]
- Organisms that feed on blackworms include Betta fish, Puffer fish, Black Knifefish, and African Dwarf frogs

Figure 1. Image of a blackworm

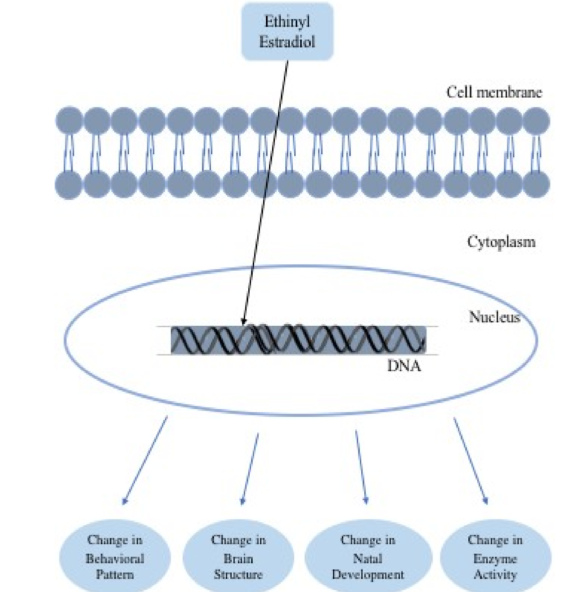

Figure 2. General concept of the signaling of EE, resulting in the amplification of certain genes and detrimental effects

Objective

- This experiment seeks to observe the sublethal effects and bioaccumulation of EE in *Lumbriculus variegatus*
- Experimental endpoints for sublethal effects include reproduction rate and segment regrowth
- Bioaccumulation of EE within *L. variegatus* will be observed through sediment tests and an ethinyl estradiol ELISA

Methods

- Sediment Reproduction Testing
  - 3 replicates of a vehicle control, 0.3 ng/mL, 3 ng/mL, and 30 ng/mL were run and in the future, 9,000 ng/mL and 18,000 ng/mL will be tested
  - 30 g of artificial sediment and 160 mL of spring water were used per concentration
  - 10 blackworms of roughly equal size were synchronized by cutting them in half and allowing for the head to regenerate before they were added to each jar
  - Worms were collected from the sediment after 30-31 days and total number of worms per jar was recorded
- Segment Regeneration Testing
  - Head and tail regeneration were observed in 3 replicates of a vehicle control, 0.3 ng/mL, 3 ng/mL, 30 ng/mL, and 300 ng/mL. Two trials were conducted.
  - Head and tail of worms roughly the same size were spliced and the middle portion of the worms were exposed to varying EE concentrations
  - Segment regrowth was observed under a microscope and length was recorded for 2 weeks

Results

- Sediment Reproduction Testing
  - The 2nd sample of the 30 ng/mL, the 2nd sample of the 0.3 ng/mL, and the 3rd sample of the 0.3 ng/mL concentrations had a higher amount of fragmentation
  - The vehicle control had the highest average of worms collected, however there was no statistically significant difference in numbers among the other concentrations

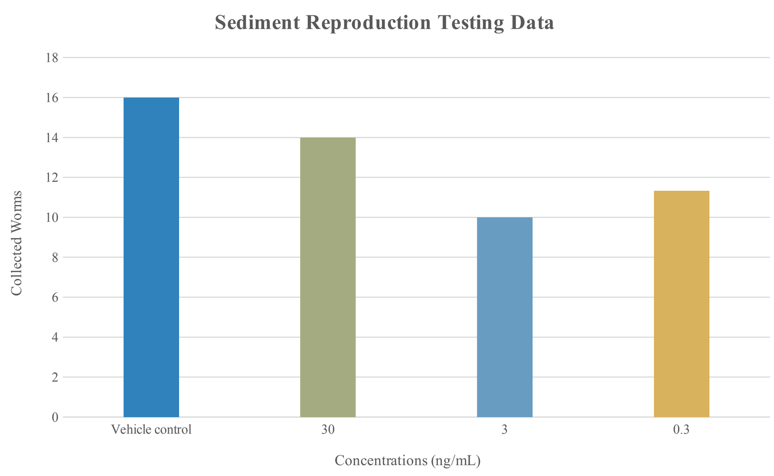

Figure 3. Number of worms per concentration after a 30-31 day trial period

- Segment Regeneration Testing
  - Head regeneration in both trials followed a logarithmic trend as the expected 8 segments within the head region regrow
  - Both head and tail regeneration in the 2nd trial had worm deaths and overall lower length results for 300 ng/mL and the vehicle control, possibly due to a higher concentration of ethanol in the concentrations
  - There did not appear to be a difference in lengths when comparing the vehicle control to the varying concentrations, however statistical analysis will be conducted

Figure 4. Progression of head regeneration (top left to bottom) and tail regeneration (top right to bottom) across the span of 2 weeks

Results (Continued)

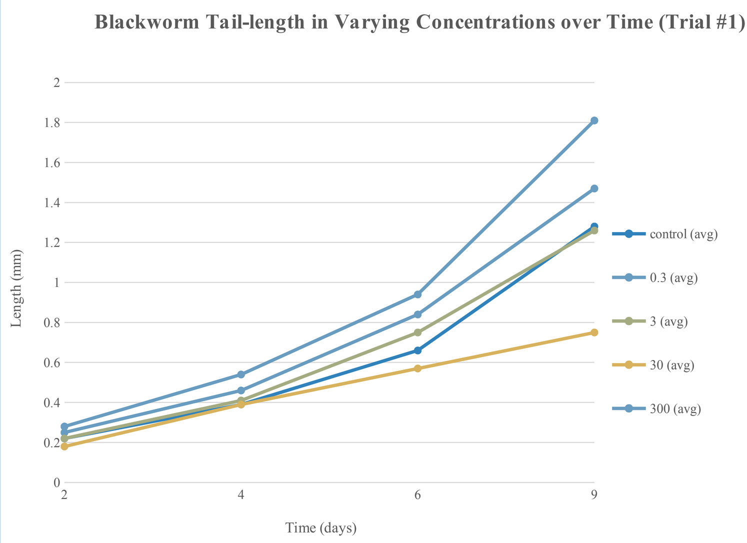

Figure 5. Average length of regenerated tails with concentrations ranging from 0 ng/mL to 300 ng/mL

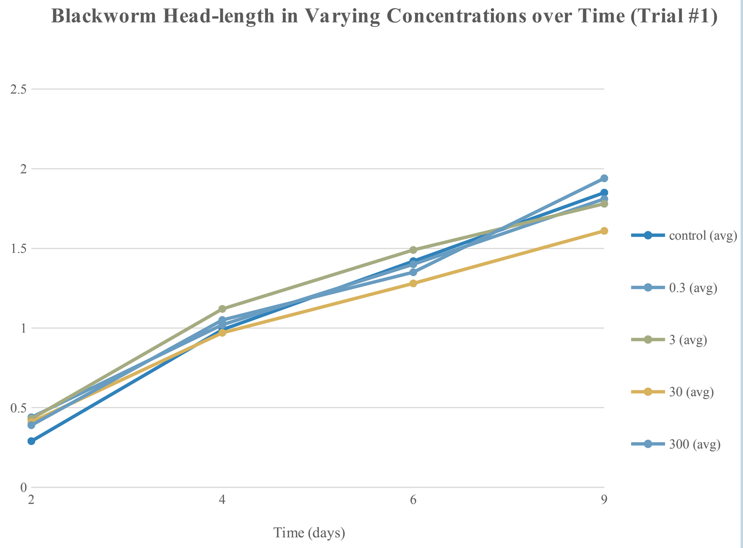

Figure 6. Average length of regenerated heads with concentrations ranging from 0 ng/mL to 300 ng/mL

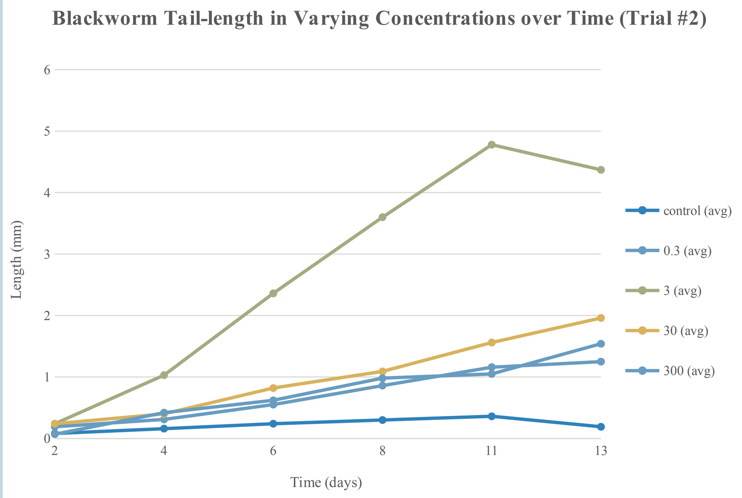

Figure 7. Average length of regenerated tails with concentrations ranging from 0 ng/mL to 300 ng/mL

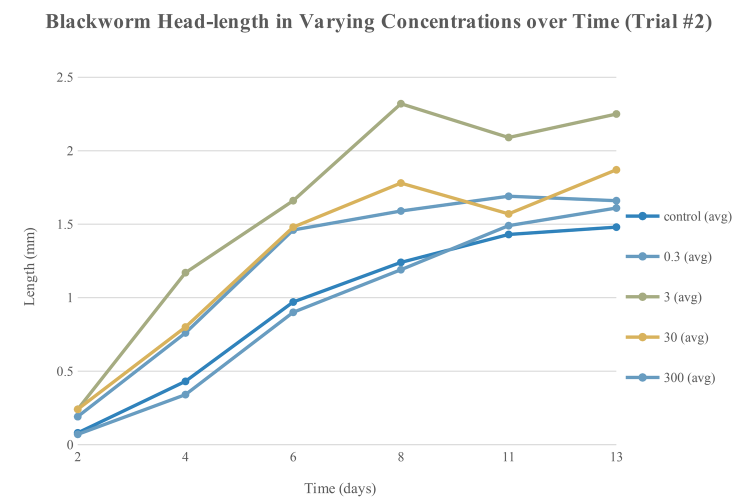

Figure 8. Average length of regenerated heads with concentrations ranging from 0 ng/mL to 300 ng/mL

Conclusion

- Both the results from the segment regeneration and the sediment reproduction trials show that there is little to no difference between the control and environmentally relevant concentrations of EE
- Deaths in the control and the 300 ng/mL concentrations within the 2nd segment regeneration trial were likely due to ethanol making up 3% of the solution
- Bioaccumulation data of EE within the tissue of the blackworms has yet to be collected for the final experimental endpoint

References

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Acknowledgements

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