TRY to keep everything in past tense

Experiment Run Down

1. **Zebrafish embryos were exposed to 0, 0.2 and 0.4 mg/mL triclosan** until they hatched.
2. Then, **RNA isolation** was performed to extract the RNA from zebrafish preserve tissues. After that, reverse transcriptase was used to generate cDNA from RNA template we made earlier in the RNA isolation.
3. Next, **qPCR** were performed using primers specific to CYP1A, B actin, and MMP9, to detect and quantitate the gene product.
    i. Beta actin is the normalized gene
    ii. CYP1A & MMP9 are genes may change with the treatment of triclosan

4. **S9 Fraction** was performed to obtain fraction that contains cytosol & microsomes . And the microsome from the fraction contains cytochrome P450 protein (produce by CYP1A).
    i. The fraction was prepared for the EROD assay (Activity Test)
    ii. Isolated concentrate Cytochrome P450 in zebrafish samples

5. **Lowry Assay**
    i. Use excel to plot out the standard curve of the BSA Standard
    ii. (BSA concentration(x) vs Absorbance (y))
    iii. Generate the Linear Equation y=mx+b
    iv. Insert the measured absorbance of Control and Testing group in the linear equation and calculate out the concentration of s9 fraction is needed for the control and Treatment groups for Western blot.
6. **EROD Assay (Activity test)**
    i. measure of cytochrome P-450 induction, specifically that of the hepaticCYP4501A (CYP1A) enzyme activity in response to environmental exposure to a variety of PAH pollutants
    ii. Measurement of ethoxyresorufin-*O*-deethylase (EROD) activity in fish to see any exposure to certain planar halogenated and polycyclic aromatic hydrocarbons (PHHs and PAHs) and other structurally similar compounds
7. **Western blot**
    i. Determine the presence of cytochrome P450 by size & levels of protein expression in the control and treatment groups

Introduction

I want you to write a little bit about the background of triclosan, and how it affects the zebrafish. Mainly focus how Triclosan relate to the AHR pathway to mediate the toxicity and how triclosan unregulated the transcription CYP1A RNA and translation of CYP1A enzyme (protein) in body.

Also, explain how each test (EROD, qPCR, Western blot) give what kind of result to help us to observed the effect caused by triclosan

EROD: Protein Activity (Protein) qPCR: RNA production (CYP1A)

Western blot: the band size (bp) help us to prove it is CYP1A protein, also can quantify the protein content in each samples

Methods & Materials

Also write about how each test will do the measurements and what statistics will perform on their results to determine the statistically significance.

EROD Assay

- Linear trendline (each samples have their own lines)
    - Each samples trendline points is the average of all samples during the 10mins reading
- 2 way Anova with replication
- Measure Fluorescence using fluorometer:
  Excitation/Emission: 544nm/590nm
  Read every 10 min for 70 min

Gene expression (qPCR)

- Bar graph
- 1 way Anova

Western blot

Results (all the figure and graph you need I already put them in separate word document, Also need to include the western blot pictures)

Keep all the Figure and tables at the end of the result section

Also add a footnote and short descriptive text below each figure and graphs

Always try to refer the result how does it affect the AHR pathway

EROD
I generate a linear trendline for the control, and sample groups. Based on the absorbance with time (10 mins)

Gene Expression (qPCR)

- Both CYP1A and MMP9 one way ANOVA test P value is < 0.05
- As a result there are significant difference in quantity of RNA (CYP1A MMP9) between the control groups and both treatment groups.
- CYP1A 0.4mg/mL has larger fold difference than 0.2mg/mL
  BUT MMP9 0.4mg/mL has lower fold difference than 0.2 mg/mL

Explain what makes the fold difference? How it affect the AHR pathway and change the quantity of CYP1A in the zebrafish

Western blot
The picture showed the control, 0.2mg/mL, 0.4 mg/mL zebrafish samples contains CYP1A enzyme (protein), However the picture is too blurry to determine the quantity of CYP1A protein exist in each samples. Explain it more how the western blot help for the assessment

Discussion
Add a paragraph about what future study can do with all the experiment data