



# 6th International Electronic Conference on Medicinal Chemistry

1-30 November 2020

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## Effect of cytostatic substances on zebrafish embryos

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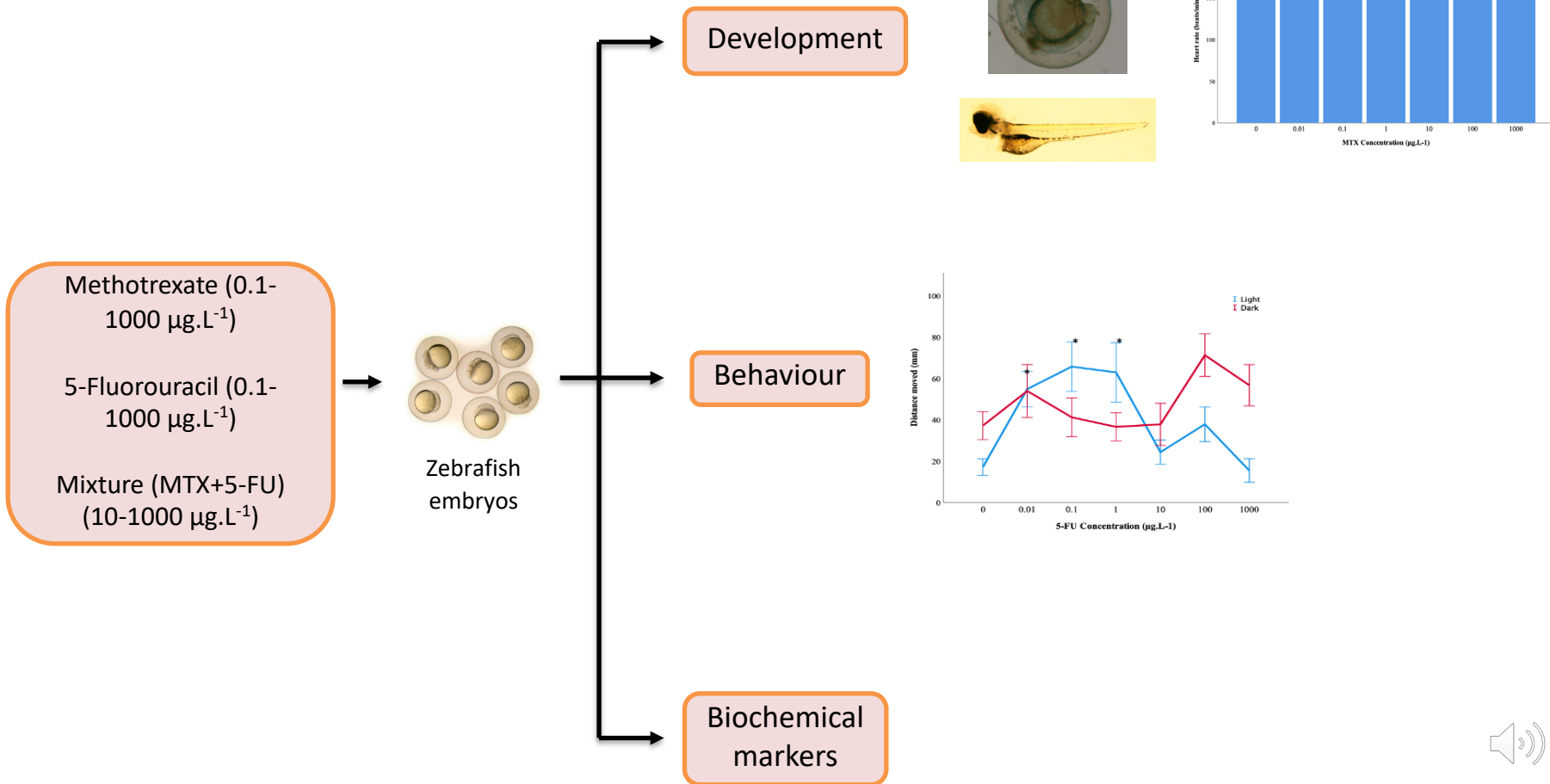
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# Effects of cytostatics substances on zebrafish embryos

## Graphical Abstract



## Abstract

Cancer is one of the top causes of death in humans. The currently available treatments, that depend on the type of cancer, may include cytostatic drugs. Methotrexate (MTX) and 5-Fluorouracil (5-FU) are cytostatic drugs and they have been used for chemotherapeutic treatment. Due to their nature, their presence in water systems, even in low concentrations, is of particular interest due to the potential risks to non target organisms and humans, through consumption of contaminated items like drinking water. However, knowledge of its impact to aquatic organisms is limited. Thus, this study aimed to evaluate the effects of MTX (0.01 up to 1000  $\mu\text{g.L}^{-1}$ ), 5-FU (0.01 up to 1000  $\mu\text{g.L}^{-1}$ ) and its mixture (10 up to 1000  $\mu\text{g.L}^{-1}$ ) on zebrafish (*Danio rerio*) focusing on different endpoints, such as fish development (Fish Embryo Toxicity (FET) assay and heartbeat rate), behaviour (light/dark stimulus) and biochemical endpoints associated with neurotransmission (AChE), antioxidant defences (CAT and GST) and metabolism pathways (LDH). Overall, zebrafish embryos demonstrated sensitivity in all tests. Exposure to MTX promoted increased heartbeat rate and swimming behaviour (between 0.01-100  $\mu\text{g.L}^{-1}$ ), however a decreased was observed at the highest concentration (1000  $\mu\text{g.L}^{-1}$ ). Our findings demonstrate that MTX and 5-FU use similar pathways both in humans and zebrafish, once MTX induced disruption in the heartbeat and behaviour and 5-FU induced disruption in behaviour. The mixture of compounds demonstrated an increase impact in the tested parameters.

**Keywords:** behaviour; biochemical endpoints, cytostatic, *Danio rerio*, development



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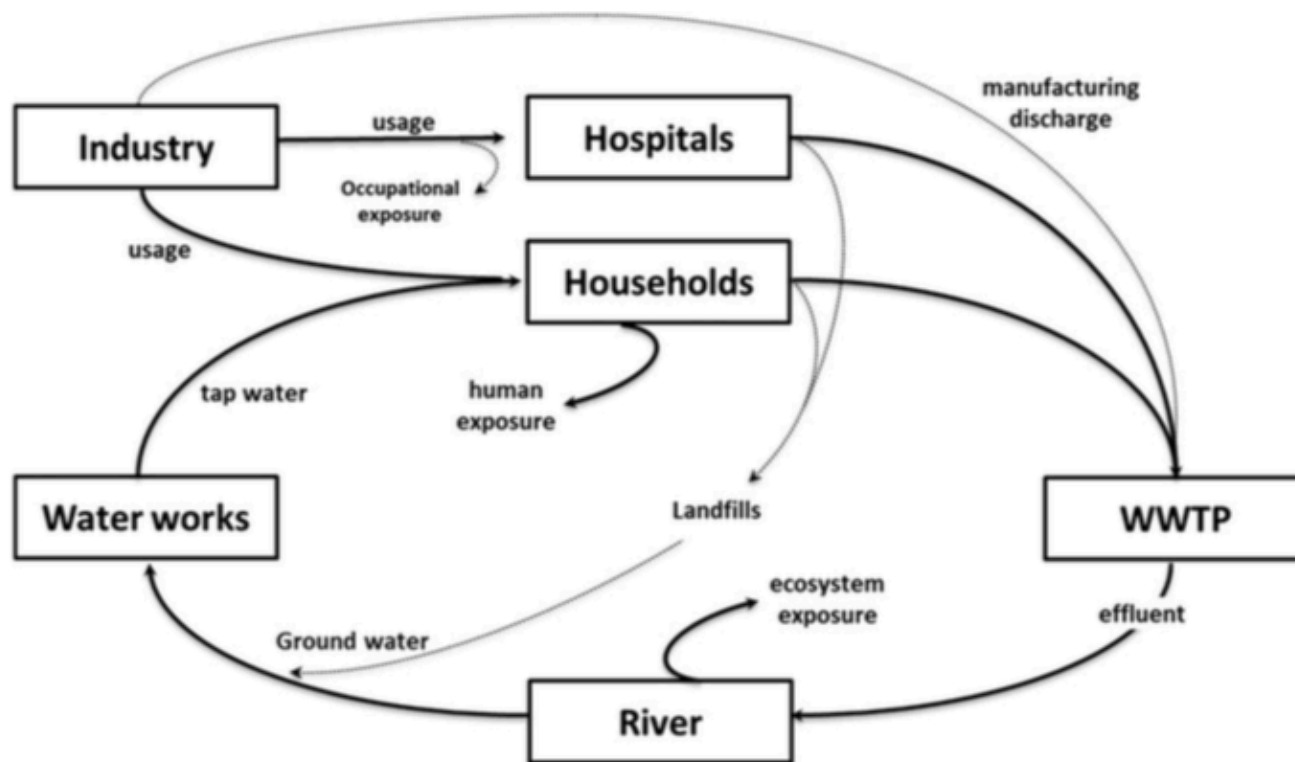
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# Cytostatic drugs

- Group of chemotherapy substances with mechanisms of action;
- Mechanism of action help to prevent tumor cell growth and division by interfering in the genetic material of the cells and cell signaling;
- The use of these drugs increased, being excreted by urine and feces from outpatients leading to its detection in the environment.

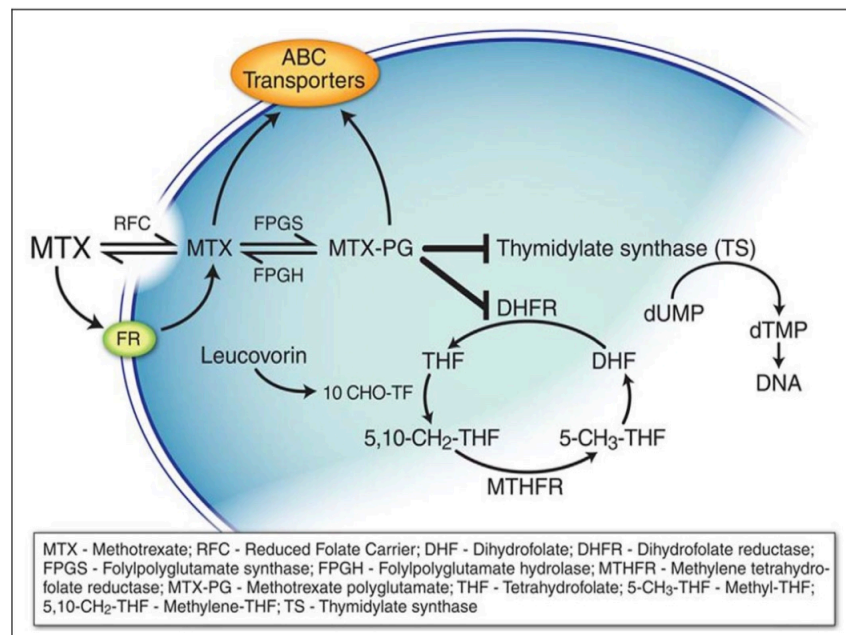


# Fate in the environment



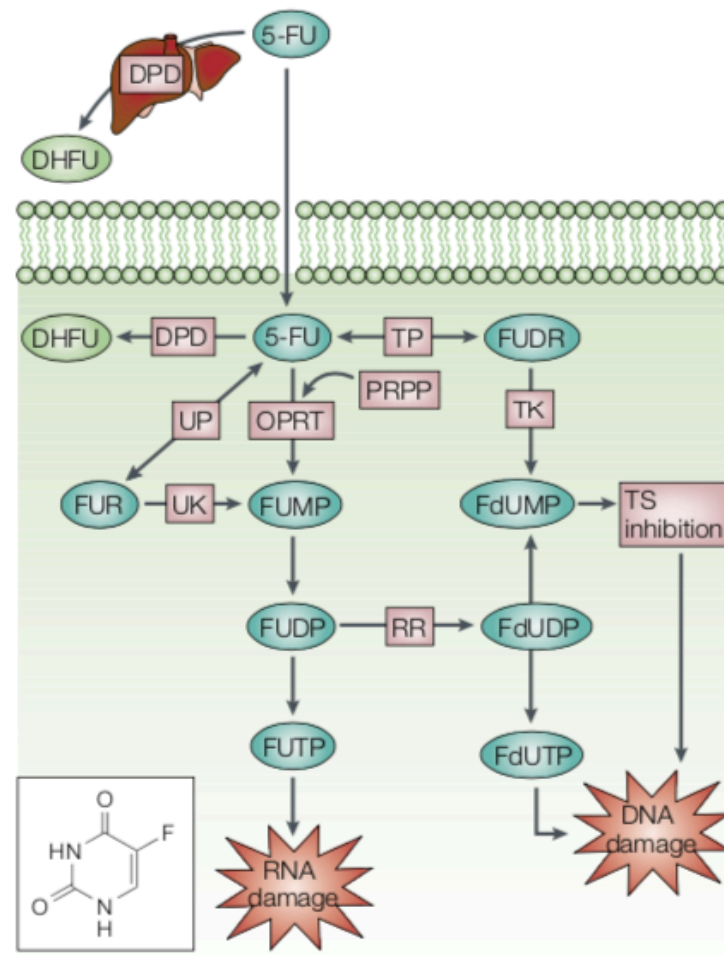
# Methotrexate

- It is the first antagonist of folic acid development for the treatment of malignancies;
- It is hydrophilic molecule and can only enter the cell through active transport process.
- It inhibits the synthesis of DNA, RNA, thymidylates and proteins.



# 5-Fluorouracil

- It is an active antineoplastic agent and is the most widely used chemotherapeutic agent in the world;
- In the environment water samples, 5-FU may be efficiently removed by photolysis and leading to a low concentration in environment.



# Tests



Biomarkers



FET and Heartbeat rate



Swimming behaviour



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# Biomarkers

- Biomarkers are molecular, physiological, biochemical and behavioral responses indicating modifications in the biological system due to a potential hazard.

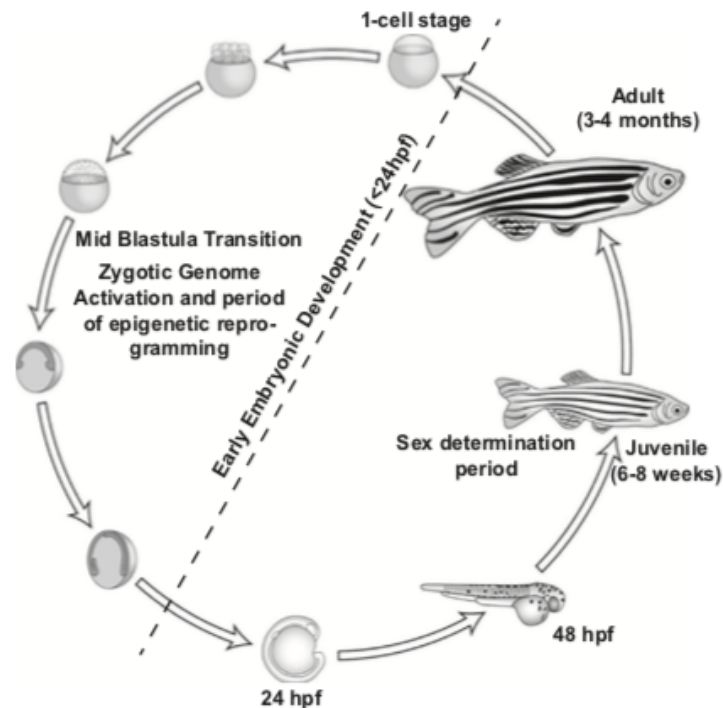
# FET

- FET consists in the exposure of newly fertilized zebrafish embryos to a test chemical for a total of 96h.



# Zebrafish

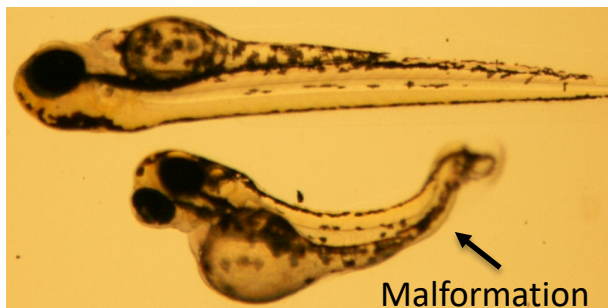
- It is a tropical freshwater fish belonging to the family Cyprinidae;
- It has been used as a model in development biology and molecular genetics;
- It is distinguished by numerous advantages.



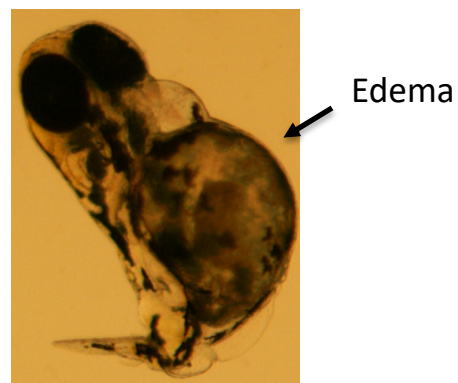
# Results: Methotrexate

## Fish Embryo Toxicity

[MTX]=1  $\mu\text{g}\cdot\text{L}^{-1}$



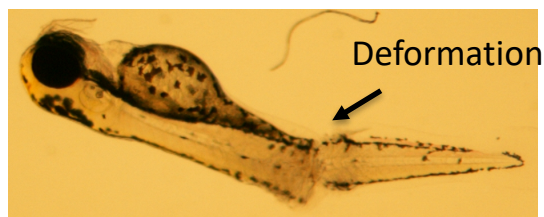
[MTX]=100  $\mu\text{g}\cdot\text{L}^{-1}$



[MTX]=10  $\mu\text{g}\cdot\text{L}^{-1}$

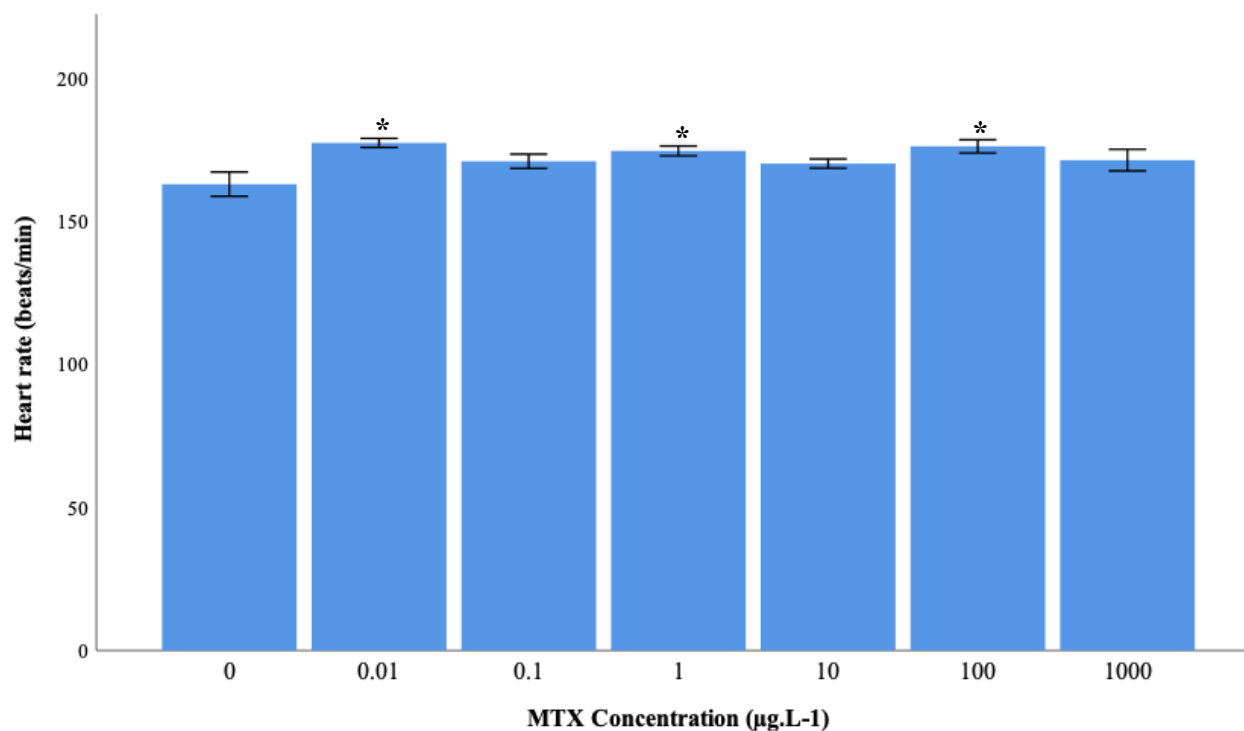


[MTX]=10  $\mu\text{g}\cdot\text{L}^{-1}$



# Results: Methotrexate

## Heartbeat rate



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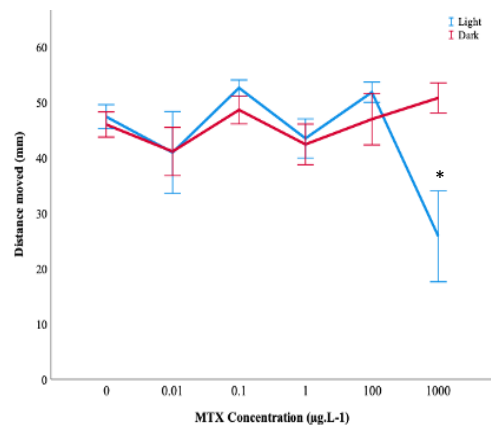
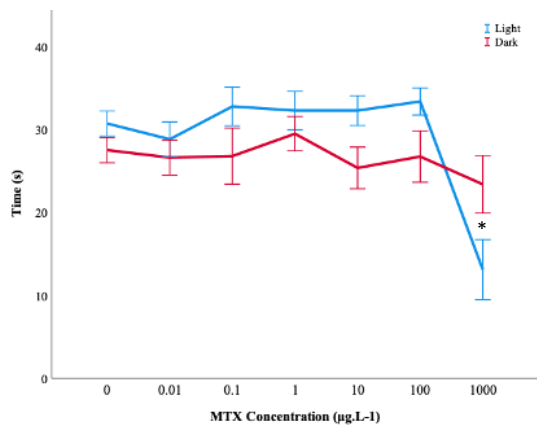


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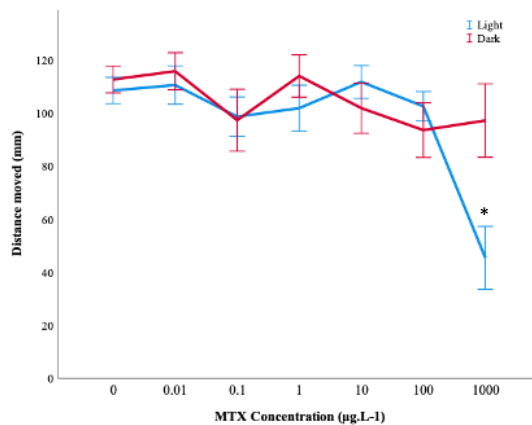
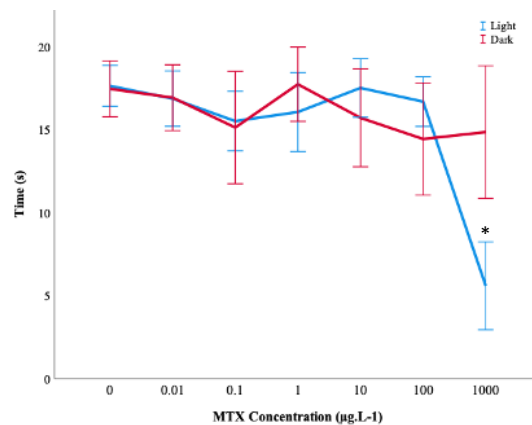
# Results: Methotrexate

## Swimming behaviour

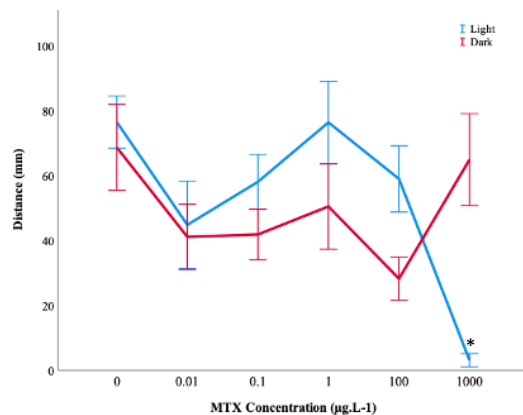
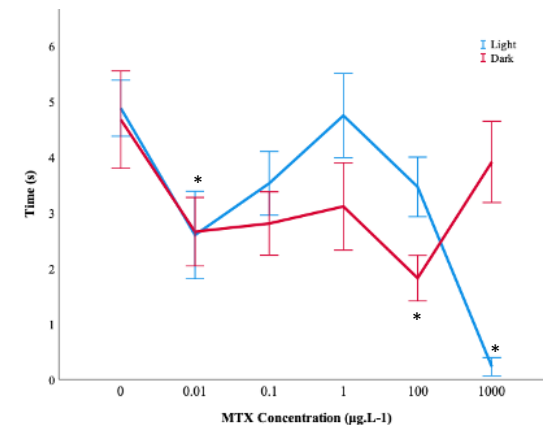
### Slow/inactivity movements



### Medium movements



### Rapid movements



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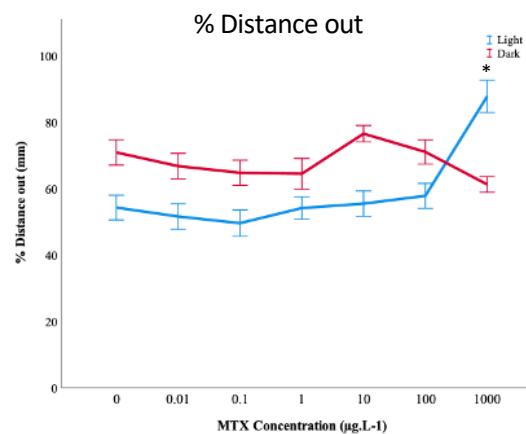
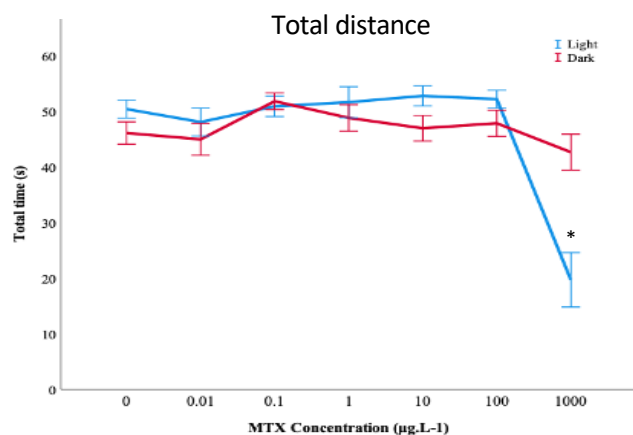
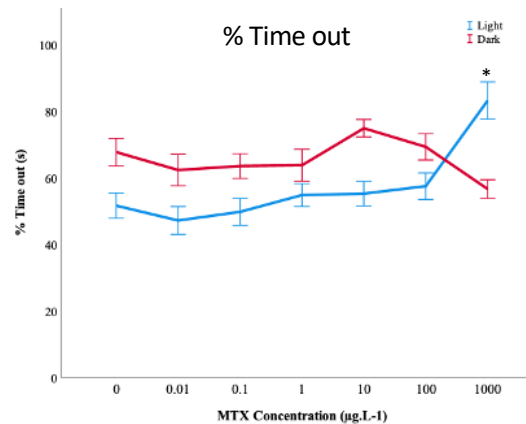
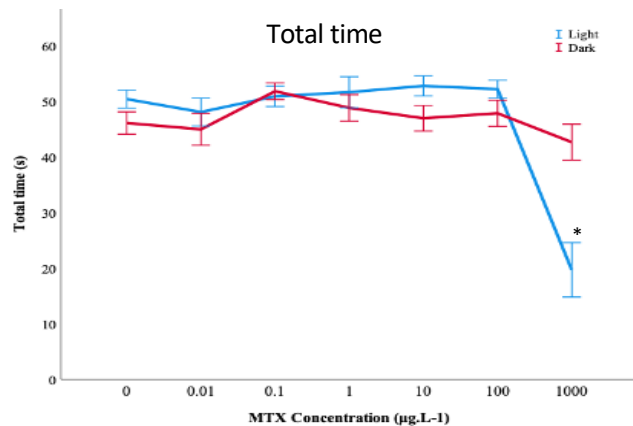
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# Results: Methotrexate

## Swimming behaviour



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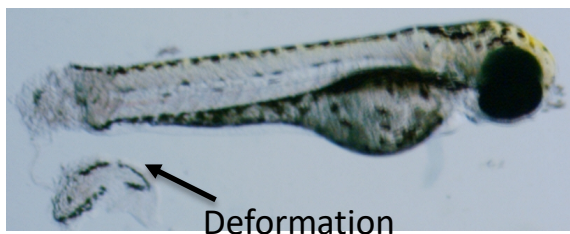


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# Results: 5-Fluorouracil

## Fish Embryo Toxicity

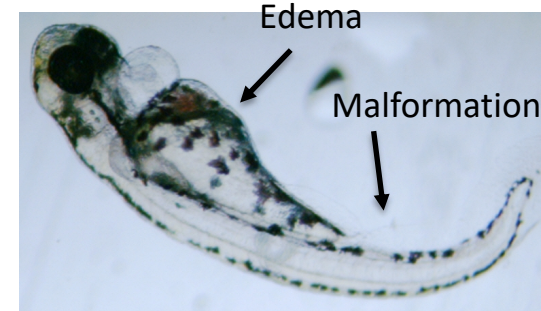
[5-FU]=0.1  $\mu\text{g.L}^{-1}$



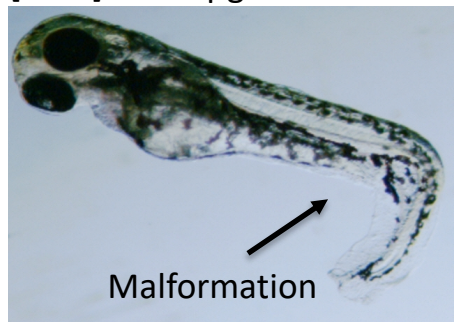
[5-FU]=0.01  $\mu\text{g.L}^{-1}$



[5-FU]=1  $\mu\text{g.L}^{-1}$



[5-FU]=1000  $\mu\text{g.L}^{-1}$



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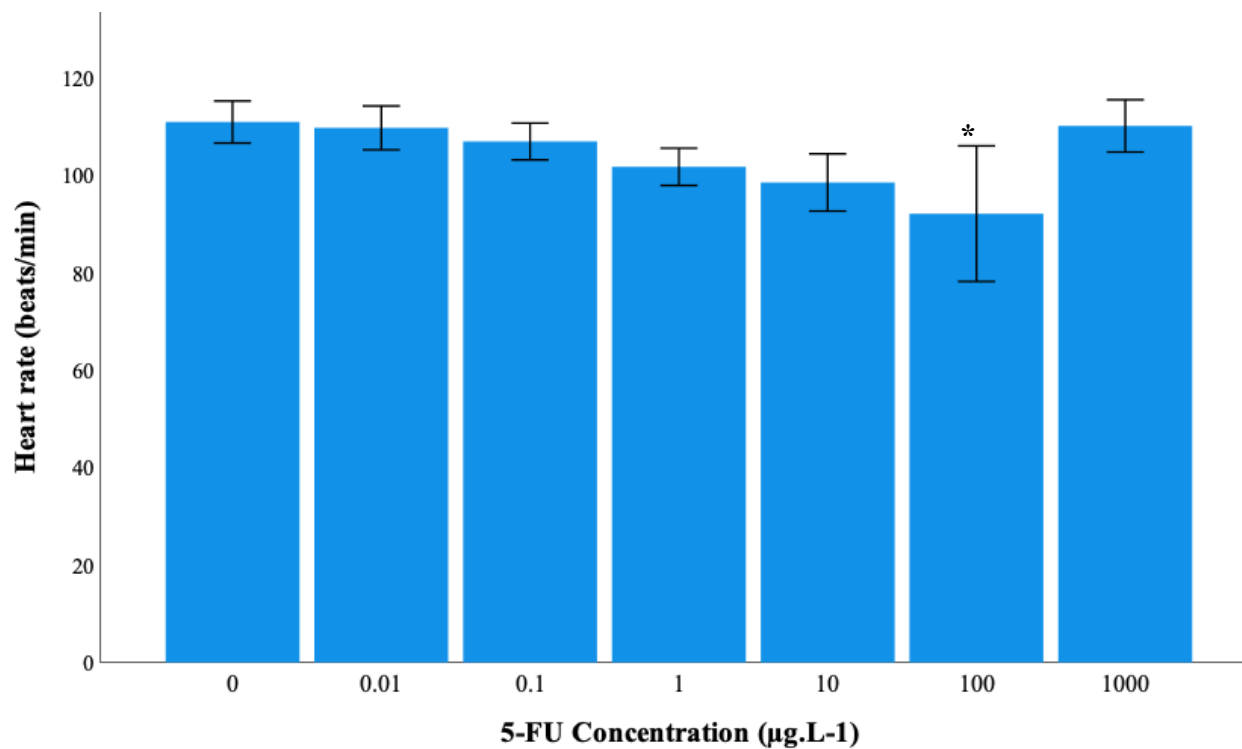
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# Results: 5-Fluorouracil

## Heartbeat rate

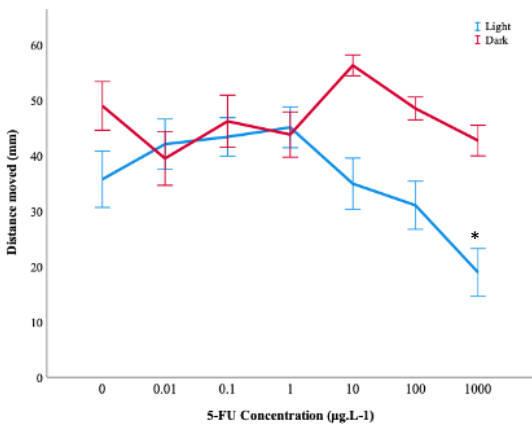
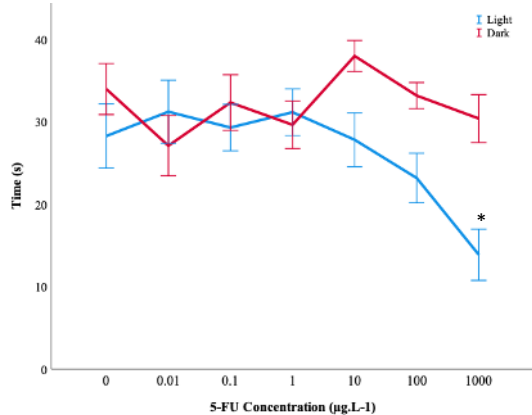




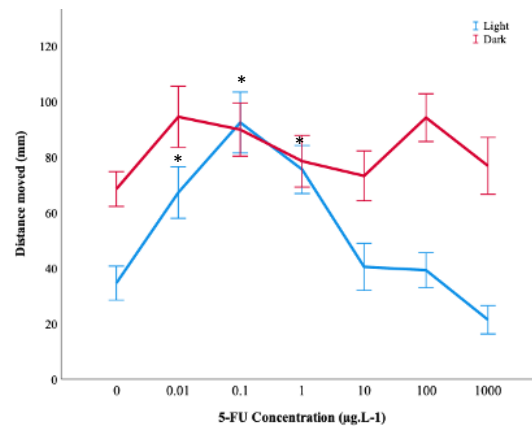
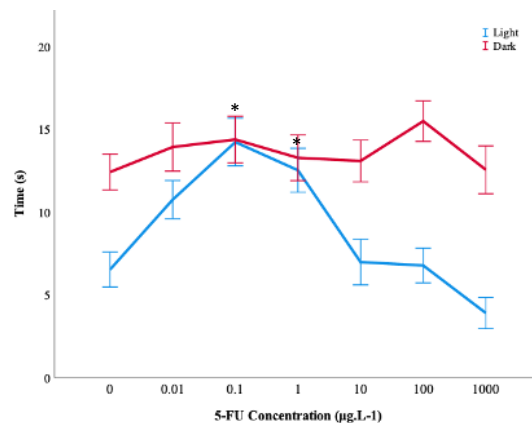
# Results: 5-Fluorouracil

## Swimming behaviour

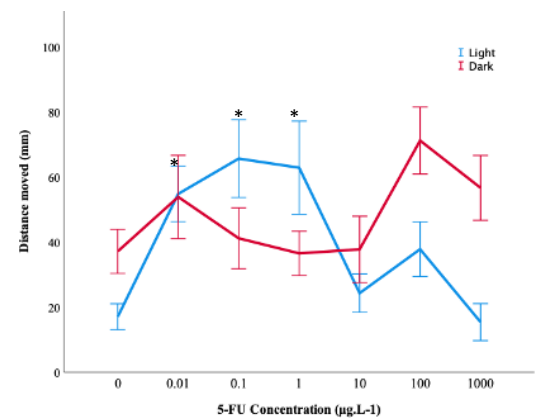
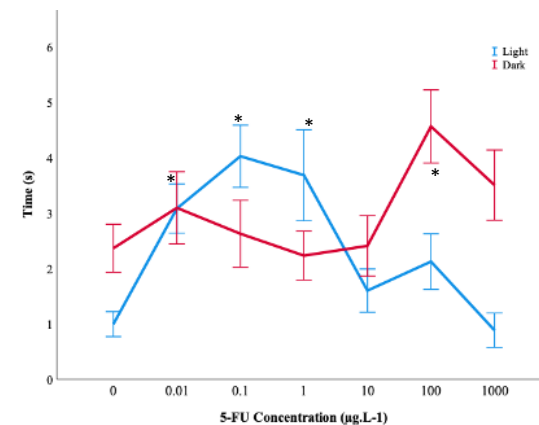
### Slow/inactivity movements



### Medium movements

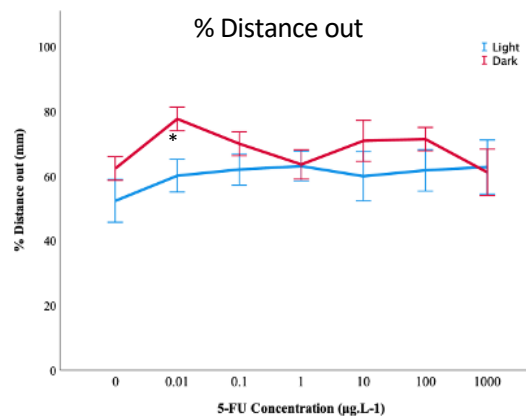
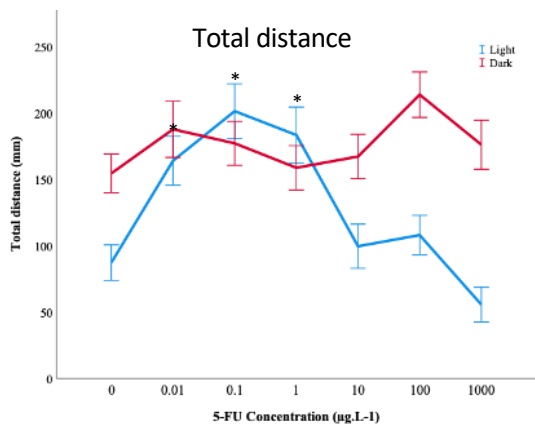
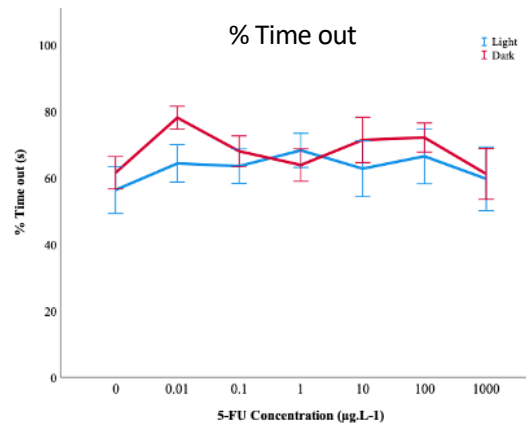
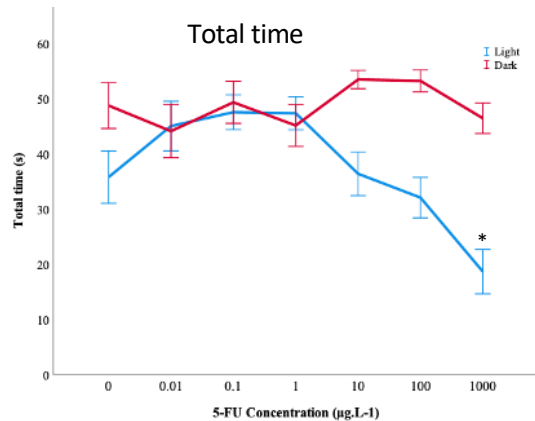


### Rapid movements



# Results: 5-Fluorouracil

## Swimming behaviour



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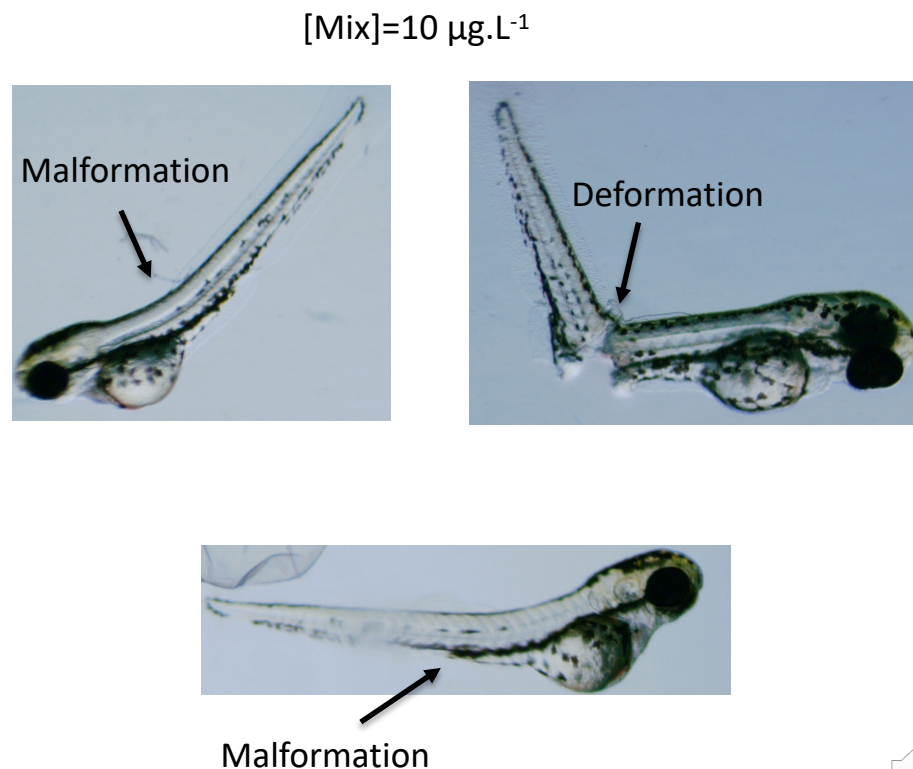
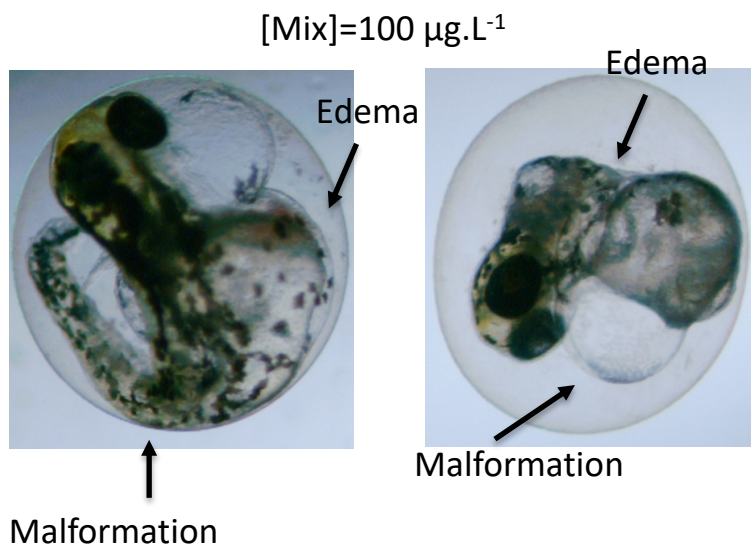
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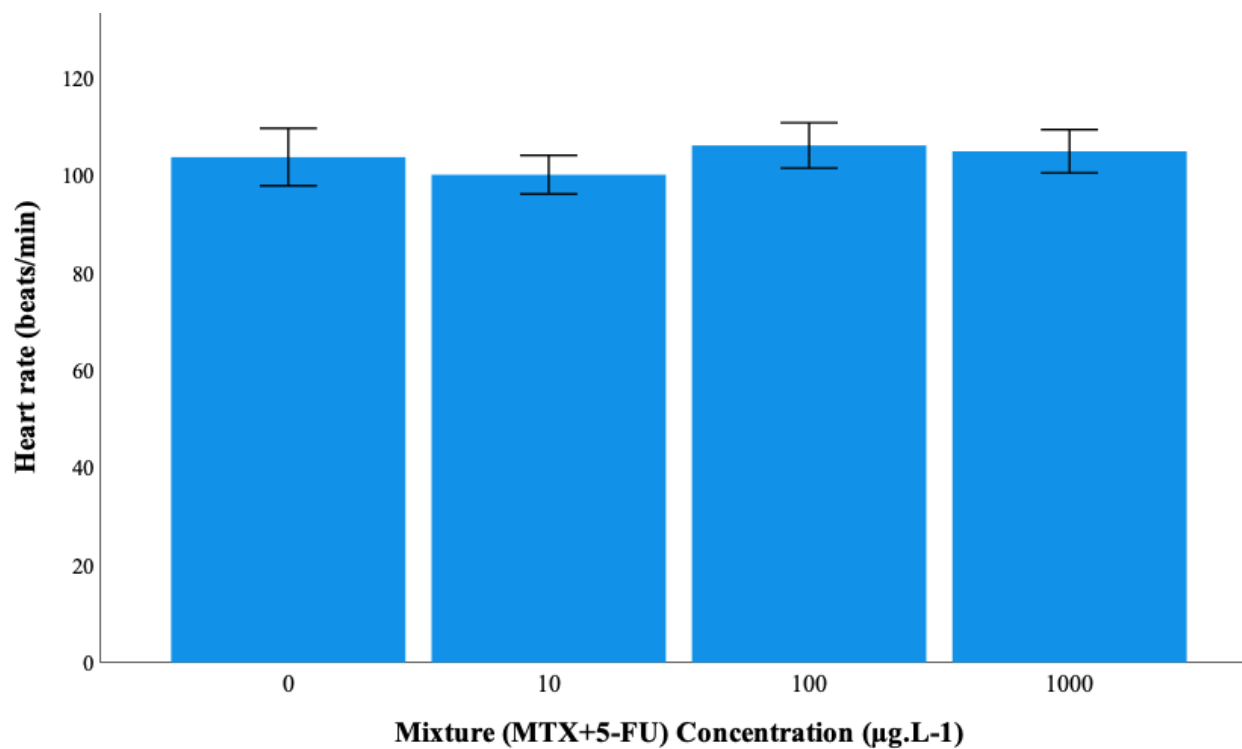
# Results: Mixture (MTX+5-FU)

## Fish Embryo Toxicity



# Results: Mixture (MTX+5-FU)

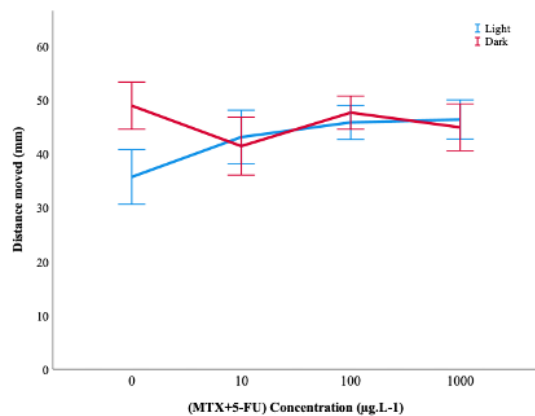
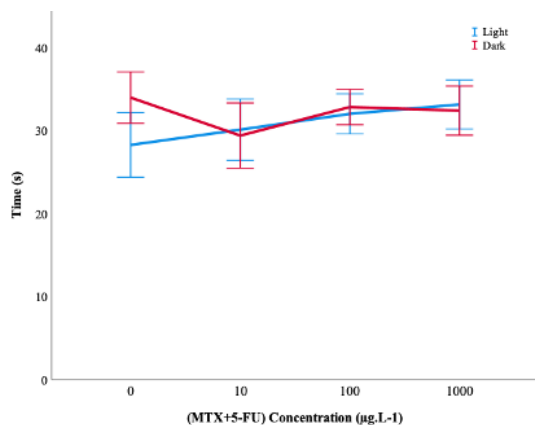
## Heartbeat rate



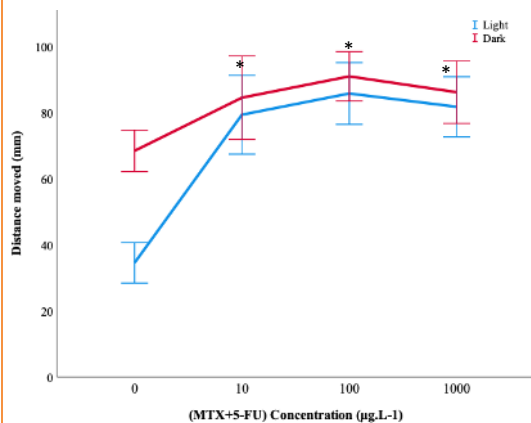
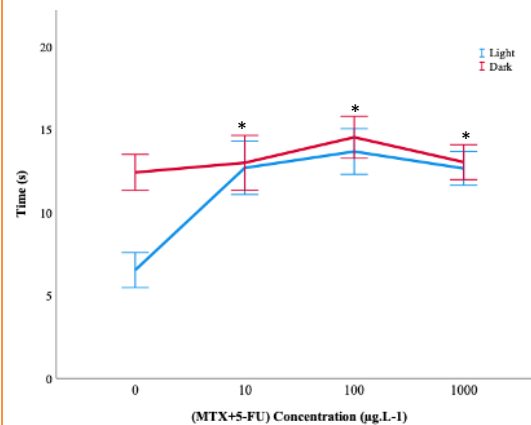
# Results: Mixture (MTX+5-FU)

## Swimming behaviour

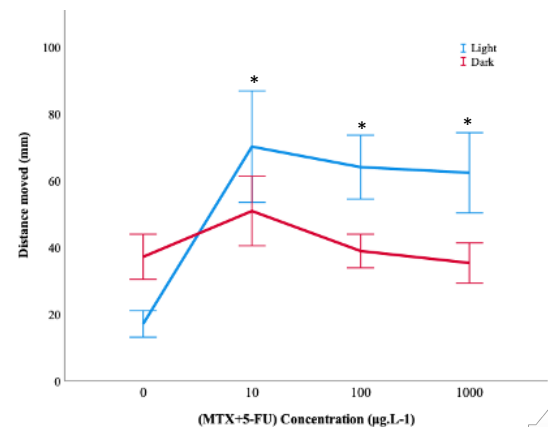
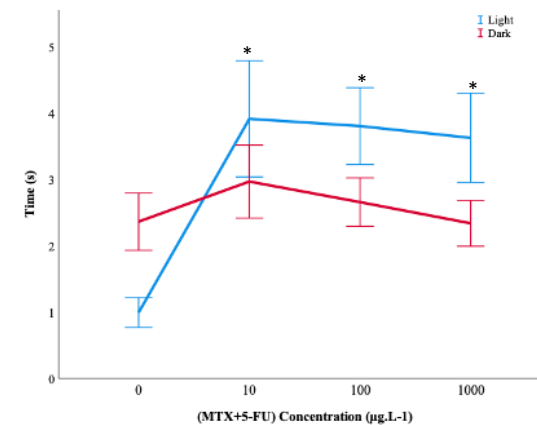
### Slow/inactivity movements



### Medium movements

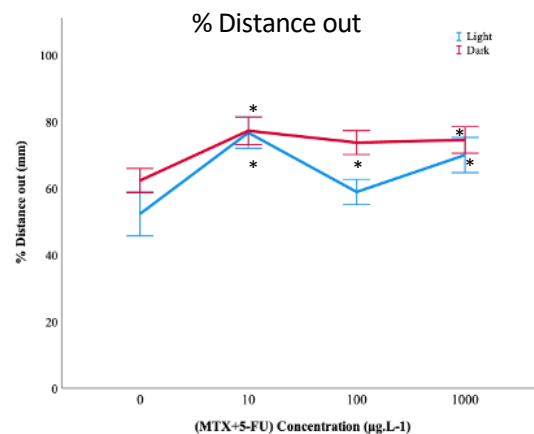
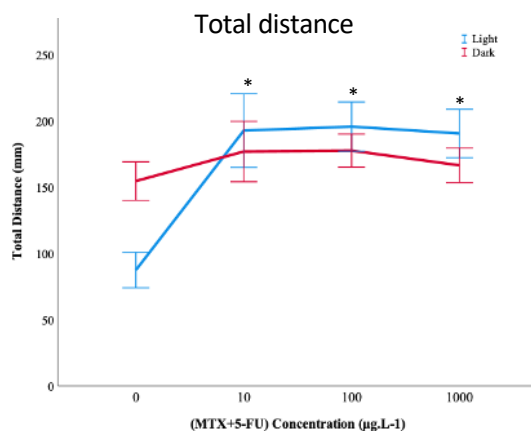
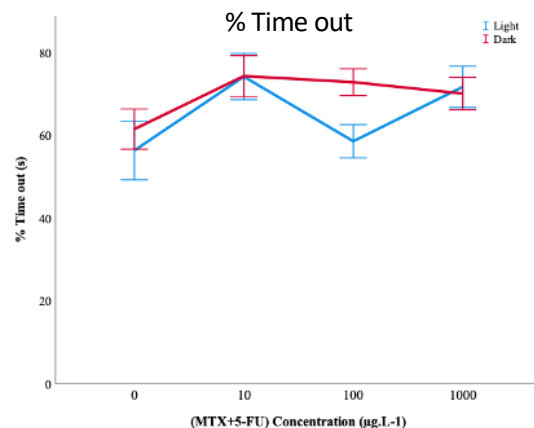
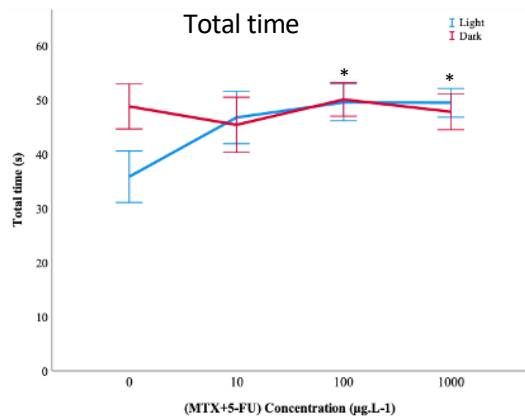


### Rapid movements



# Results: Mixture (MTX+5-FU)

## Swimming behaviour



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## Discussion

- The tested substances demonstrated ability to impact fish development, heartbeat and swimming performance.
- Comparing the obtained data, based on the assessed endpoints, 5-FU appears as more dangerous to fish, as effects were observed at lower concentrations.
- The combined exposure, in general induced less effects, as can be observed for example in heartbeat. Data suggests potential antagonistic effects.



# Conclusion

The obtained results in swimming behaviour and heartbeat rate have the same pattern demonstrating that MTX and 5-FU uses similar pathways in humans and zebrafish, once both induced disruption in the behaviour and heartbeat.

Data shows the need to perform long term studies to assess the effects of chronic exposure.





# Thank you for your attention!!!



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