Effects of Loperamide and Ibuprofen on *Danio rerio*

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STEM Webpage

For Supporting Documents

Phrase 1

Due to the ongoing increase in drug overdose deaths in the United States, it has become a greater priority to research various methods of mitigation and find alternatives to drugs that are less addictive.

Phrase 2

The overall aim of this project is to utilize over-the-counter medications (OTCs) named loperamide and ibuprofen to study the behavioral and physiological effects on larval samples of zebrafish (*Danio rerio*).

Background

67,300

Americans died from drug-involved overdose in 2018, including illicit drugs and prescription opioids (NIDA, 2020).

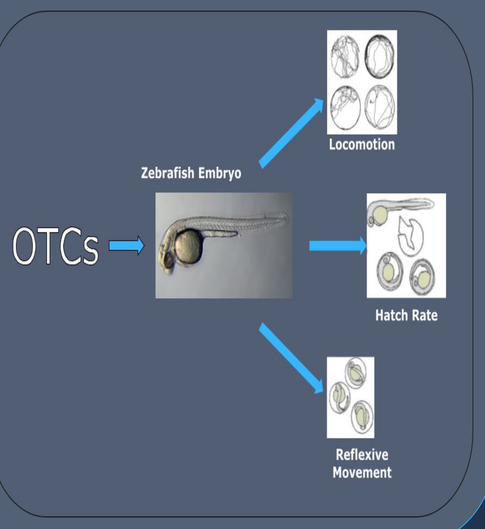
Drug overdose deaths involving opioids. Opioid-involved overdose deaths rose from 21,088 in 2010 to 46,802 in 2018 (NIDA, 2020).

46,802

13, 587

Increase in number of deaths involving prescription opioids. The deaths rose from 3,442 in 1999 to 17,029 in 2017 (NIDA, 2020).

Graphical Abstract



Background Cont.

WHAT IS ADDICTION?

Addiction is defined as a disease in which an individual has a compulsive urge to consume drugs or other substances, even though the individual might regard the harmful consequences.

MODEL ORGANISMS

Addiction models model the adverse effects of medications both in the long term and short term.

SCIENCE BEHIND ADDICTION

Prolonged usage of a drug creates a euphoria-dysphoria cycle, commonly known as a "reward circuit". This encourages the individual to continue the consumption of the drug.

ZEBRAFISH

Danio rerio, or D. rerio or commonly known as zebrafish has become one of the leading model organisms in the field of neuroscience which includes addiction studies.

OVER THE COUNTER DRUGS

OTC drugs are over-the-counter drugs and have been approved by the health agencies (such as the FDA) to be sold without any prescription or any necessary documentation. Since they are cheaper and more available they are subject to abuse.

WHY ZEBRAFISH?

The high percentage of homologous proteins in the endocannabinoid and opioid systems and the transparent eggs of larval zebrafish allows for easy examination of the development process.

LOPERAMIDE AND IBUPROFEN

Ibuprofen is commonly used by the American population for symptoms like pain, inflammation, and fever. Loperamide is a drug manufactured for anti-diarrheal effects. Both drugs have deadly consequences when abused.

Applications

This project allows for impact in various fields like:

- Modifying drugs to reduce drug overdose deaths in the years to come.
- Modifying drugs to make them more environmentally safe.

Statistical Tests

ANOVA

- Parametric
- Body Length and Locomotor Behavior

Cochran's Q Test

- Binary
- Hatch Rate and Reflexive Behavior

Methodology

CREATING THE SOLUTIONS

Create diluted solutions of 5 µg/L, 50 µg/L, and 500 µg/L for loperamide and ibuprofen using ethanol as the solvent.

DIVIDING THE GROUPS

Separate the zebrafish embryos into seven groups. It was grouped for loperamide vs. ibuprofen and each of the concentrations. One group will be the control group.

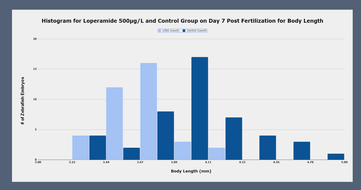
EXPOSURE TO OTC

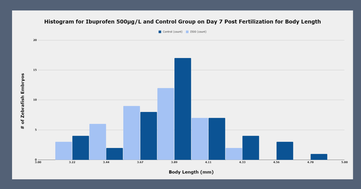
The samples were exposed to the different concentrations for a span of seven days. The water was changed regularly in addition to checking pH levels regularly.

OBSERVATIONS

Using the images and notes collected, ImageJ was used to analyze the data to provide numerical data. This was then run through the statistical tests.

Results





Body Length Distribution of All Groups

Found significant decrease in body length for both loperamide and ibuprofen groups. Loperamide's body length decrease when statistically tested display p-values < 0.01 for the higher concentrations. Similar results with ibuprofen

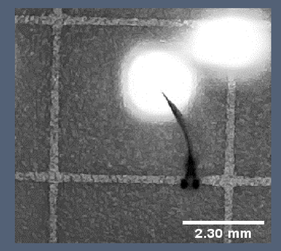
Hatch Rate for Days 2 and 3 Post Fertilization for All Groups

	Loperamide 5µg/L	Loperamide 50µg/L	Loperamide 500µg/L	Control Group	Ibuprofen 5µg/L	Ibuprofen 50µg/L	Ibuprofen 500µg/L
Day 2 (52h)	19	20	28	11	9	6	4
Day 3 (80h)	26	24	14	36	38	38	41
Total:	45	44	42	47	47	44	44

Hatch Rate Distribution of All Groups

Hatch rate was elevated for the loperamide groups on day 2 post fertilization. On the contrary ibuprofen groups had a dramatic decrease in hatch rate on day 2 post fertilization. The statistical significance of both events is high as the p-value was less than 0.05

Results Continued...



Abnormal Tail Shape.

Was found only in high concentrations of Ibuprofen (approximately 10% of population)

Reflexive Behavior for Day 4 Post Fertilization for All Groups

	Reflexive	Nonreflexive
Loperamide	98	30
Ibuprofen	54	78
Control	27	19

Reflexive Behavior

It was found that the reflexive behavior for loperamide groups were elevated while ibuprofen groups were reduced. The significance of this was supported by p-values less than 0.05

Locomotor Behavior

It was observed that for loperamide groups for day 6 and day 7 post fertilization showed elevated levels of activity. Both the control group and ibuprofen groups had reduced rates of locomotion. Specifically, the locomotion of the ibuprofen groups was dramatically decreased. This is all supported with the p-values of the tests being less than 0.05.

Future Extensions

This project allows for future extensions that include:

- Studying the specific receptors in larval zebrafish which could explain the effects observed in this experiment
- Studying more OTCs on larval zebrafish to understand the risk of other OTCS which are currently available.

Summary

The project can be summarized as the following:

- There is an increase in drug overdose deaths in recent years.
- *D. Rerio was* used as a model organism to study the adverse effects of loperamide and ibuprofen.
- Results included reduced locomotor/reflexive activity and delayed growth characteristics for ibuprofen groups, while elevated locomotor/reflexive behavior and delayed growth characteristics.
- Data collected was significant as all p-values < 0.05.

References

Abuse, NIDA. (2020, March 10). Overdose death rates. National Institute on Drug Abuse. https://www.drugabuse.gov/drug-topics/trends-statistics/overdose-death-rates