Project Notes:

Project Title: Effects of Loperamide and Ibuprofen on *D. rerio*

Name: Ansh Tripathi

Note Well: There are NO SHORT-cuts to reading journal articles and taking notes from them. Comprehension is paramount. You will most likely need to read it several times so set aside enough time in your schedule.

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<th>Page</th>
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<td>Effects of ibuprofen, diclofenac and paracetamol on hatch and motor behavior in developing zebrafish (Danio rerio)</td>
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<td>#16</td>
<td>Steps during the development of the zebrafish locomotor network</td>
<td>60</td>
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<td>#17</td>
<td>Effects of acetaminophen (paracetamol) in the embryonic development of zebrafish, Danio rerio</td>
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<td>Developmental exposure to acetaminophen does not induce hyperactivity in zebrafish larvae</td>
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<td>#19</td>
<td>Effects of embryonic exposure to ethanol on zebrafish visual function</td>
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<td>#20</td>
<td>Adverse effect of synthesized Naringenin derivatives investigate with Zebrafish (Danio rerio) embryos</td>
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<td>#1</td>
<td>Abuse-resistant hydrocodone compounds</td>
<td>100</td>
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<tr>
<td>#2</td>
<td>Pharmaceutical Compositions for the Deterrence and/or Prevention of Abuse</td>
<td>103</td>
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Knowledge Gaps:

This list provides a brief overview of the major knowledge gaps for this project, how they were resolved and where to find the information.

<table>
<thead>
<tr>
<th>Knowledge Gap</th>
<th>Resolved By</th>
<th>Information is located</th>
<th>Date resolved</th>
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<tbody>
<tr>
<td>Previous measures to resist abused drugs</td>
<td>Project Notes (Patents)</td>
<td>Project Notes (Patents)</td>
<td>09/21/2020</td>
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<tr>
<td>Common model organisms and their respective experimental niches (i.e. rodents are commonly used in addiction studies)</td>
<td>Project Notes (Articles)</td>
<td>Project Notes (Articles)</td>
<td>09/23/2020</td>
</tr>
<tr>
<td>Zebrafish and the common areas for experimentation done with them</td>
<td>Project Notes (Articles)</td>
<td>Project Notes (Articles)</td>
<td>09/29/2020</td>
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<td>Addictive OTC substances like loperamide which pose dangerous levels of toxicity for being an OTC</td>
<td>Project Notes (Articles)</td>
<td>Project Notes (Articles)</td>
<td>10/08/2020</td>
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<td>Measurable aspects of developmental success in zebrafish</td>
<td>Project Notes (Articles)</td>
<td>Project Notes (Articles)</td>
<td>11/16/2020</td>
</tr>
<tr>
<td>Locomotor structure of zebrafish and the aspects of study in zebrafish impacted by consumption of OTCs or other drugs</td>
<td>Project Notes (Articles)</td>
<td>Project Notes (Articles)</td>
<td>12/06/2020</td>
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</table>
**Literature Search Parameters:**

These searches were performed between 07/11/2020 and 10/13/2020. List of keywords and databases used during this project.

<table>
<thead>
<tr>
<th>Database/search engine</th>
<th>Keywords</th>
<th>Summary of search</th>
</tr>
</thead>
<tbody>
<tr>
<td>Google Patents</td>
<td>Pain Killers, Addiction</td>
<td>Patent references to common opioids, common procedures in making the opioids, and also various options for new opioids</td>
</tr>
<tr>
<td>Google Scholar</td>
<td>Addiction, <em>C. Elegans</em>, <em>Drosophila</em></td>
<td>Article references to studies that encompassed either <em>C.Elegans</em> or <em>Drosophila</em> in some study about addiction and the behavioral impacts or results found</td>
</tr>
<tr>
<td>Google Scholar</td>
<td>Model Organism, Addiction</td>
<td>Article references to studies that involved <em>C.Elegans</em>, <em>Drosophila</em>, and Zebrafish and their corresponding studies on addictive substances like morphine</td>
</tr>
<tr>
<td>Google Scholar</td>
<td>OTC, Abuse, Dangerous</td>
<td>Article references to studies which found common OTCs which were potentially life threatening and posed great danger to the public.</td>
</tr>
<tr>
<td>Google Scholar</td>
<td>Loperamide, Addiction</td>
<td>Article references to studies involving loperamide, an anti-diarrheal drug, and the side effects on the cardiac system.</td>
</tr>
<tr>
<td>Google Scholar</td>
<td>Zebrafish, OTCs, Adverse</td>
<td>Article references to studies that involved OTCs or other drugs on zebrafish and saw impairments and/or adverse effects. Moreover lots of references to the environmental aspect of these chemicals in water bodies.</td>
</tr>
<tr>
<td>Science Direct/Elsevier</td>
<td>Zebrafish, OTCs, Locomotor</td>
<td>Article references to impairment in the locomotor network or development of zebrafish after exposure to drugs or OTCs.</td>
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</table>
**Article #1 Notes: Title**

Article notes should be on separate sheets

**KEEP THIS BLANK AND USE AS A TEMPLATE**

<table>
<thead>
<tr>
<th>Source Title</th>
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<tr>
<td>Summary of key points (include methodology)</td>
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<tr>
<td>Research Question/Problem/Need</td>
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<tr>
<td>Important Figures</td>
<td></td>
</tr>
<tr>
<td>Notes</td>
<td></td>
</tr>
<tr>
<td>Cited references to follow up on</td>
<td></td>
</tr>
<tr>
<td>Follow up Questions</td>
<td></td>
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</table>
## Article #1 Notes: Immune Cell Assassins Reveal Their Nurturing Side

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Source type</td>
<td>Science Magazine Article (Website)</td>
</tr>
<tr>
<td>Keywords</td>
<td>cells, development, regeneration, stem cells, immune cells, cardiac</td>
</tr>
</tbody>
</table>
| Summary of key points (include methodology) | ● Research of macrophages, a type of immune cell, helps by healing cardiac cells and improving electrical impulses in the heart.  
● This research began after the effort to see the impact of stem cells as the cells used after heart attacks.  
● The effects of using stem cells, inflammatory drugs (like zymosan), and prompting an immune system attack were all equally as effective.  
  ○ This caused researchers to believe that immune cells, like macrophages, might have more function compared to just engulfing pathogens and foreign substances found in the bloodstream.  
● Researchers found out that different types of immune cells stay and live in certain parts and organs of mice.  
● Researchers then found out that if macrophages were reduced from the AV node of the heat the heart had weaker impulses and there was significant impairment of the electrical impulses traveling from the atria to the ventricles.  
● Different types of immune cells such as Natural Killer cells (NK cells) help in killing tumor cells while also helping during pregnancy where they reconstruct blood vessels depending on the requirement of oxygen of the baby.  
● Mast cells, the cells that are most commonly known to invoke allergic reactions were found in unborn children who have no exposure to the real world yet. |
Indicates that mast cells could also aid in the development and reconstruction of blood vessels.

What other functions does the human immune system host for the body in addition to the immunal side?

### Disease Fighters With Secret Lives

Some immune system cells do more than fight infections: They assist with processes that reshape and heal tissues or help them to function more efficiently.

<table>
<thead>
<tr>
<th>CELL</th>
<th>IMMUNOLOGICAL ROLE</th>
<th>NEUPLY DISCOVERED ROLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophage</td>
<td>Engulfs cellular debris, bacteria, fungi and other foreign objects in the body. Presents antigens that trigger further immune responses.</td>
<td>Repairs tissues, sculpts vasculature and improves electrical signaling in the heart. Removes unneeded synapses in the brain. Helps to regulate body heat and to recycle iron.</td>
</tr>
<tr>
<td>Natural Killer Cell</td>
<td>Quickly destroys cells tumor cells and virally infected cells.</td>
<td>Releases growth factors and other signals that direct the remodeling of blood vessels in the uterus during pregnancy. Regulates the migration of fetal cells into the uterus.</td>
</tr>
</tbody>
</table>

See summary of key Points


| Follow up Questions | 1) How can this research be furthered in my STEM Project?  
| | a) Can we use a similar idea and study what functions do other types of immune cells have? Maybe understand the correlation between the immune cells, and how they work with each other?  
| | 2) What was the method and procedure for this experiment?  
| | a) Is it feasible for me during COVID?  
| | 3) What were some of the restraints in this experiment?  
| | a) What was the sample size?  
| | b) Does it have any genetic influence? |
**Article #2 Notes: Males Are the Taller Sex. Estrogen, Not Fights for Mates, May Be Why.**

<table>
<thead>
<tr>
<th>Source Title</th>
<th>Males Are the Taller Sex. Estrogen, Not Fights for Mates, May Be Why.</th>
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</thead>
<tbody>
<tr>
<td>Source type</td>
<td>Science Magazine Article (Website)</td>
</tr>
<tr>
<td>Keywords</td>
<td>evolution, physiology, estrogen, testosterone</td>
</tr>
</tbody>
</table>

**Summary of key points (include methodology)**

- The comparison of the biological male body and female body physically.
  - How male bodies are stronger, more rugged, more taller due to sexual selection and male competition.
- Biological anthropologist Holly Dunsworth states that this theory can not be true.
  - She states that yes the theory might hold enough value and may impact to some extent but instead biologically men are taller due to a different factor: estrogen.
- Specifically she states that women are shorter than men because most of them have ovaries which secrete estrogen.
  - Ovaries help in the secretion of estrogen, like testes in the male anatomy but in larger amounts.
- Estrogen helps bone development.
  - As a result in the early ages of puberty, females are taller than males due to higher estrogen levels, and higher rates of bone development.
- This doesn't last long since the growth plates fuse and more so after estrogen levels start to decrease.
- Meanwhile men, with testes don't have the same barrier and continue to grow.
- As she proceeds with her evidence she talks about how hips of the
bodies differ too.
  ○ For many years, and actually still many scientists believe that female hips are wider to help in birth, compared to men (who do not have to give live birth).
  ● But Dunsworth disagrees and says that the wider hip is present in female bodies due to the fact that they have a larger, more complex organ system..
  ● She uses the example of chimpanzees who have wider hips even though their offspring are smaller than humans.

<table>
<thead>
<tr>
<th>Research Question/Problem/Need</th>
<th>Why are male individuals taller than female individuals? Is it evolutionary (genetic)? Due to the fights that occurred between males and not women?</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Important Figures</th>
<th>N/A</th>
</tr>
</thead>
</table>

| Notes | ● Dunsworth states that a lot of times in science, incorrect claims are made due to the fact that data is all over the place.
  ● Dunsworth also reiterates the fact that many biologists don't believe that physiology and endocrinology are not evolutionary, and asks why they aren't.
  ● She concludes her point by saying that science needs to change the way they perceive male and female bodies, by not representing female bodies as a derivation of male bodies because that isn't true.
  ● Moreover science needs a lot more tests and evidence before a claim should be regarded as accepted. |

|----------------------------------|----------------------------------------------------------------------------------------------------------------------------------|

| Follow up Questions | 1) Can we study the evolution of immunal cells as they experience more bacteria and viruses in the environment?  
2) Would evolution be a good topic to target too, since it doesn’t require labs?  
  a) Would creating an app which shows the evolutionary pathway of any organism be a possible STEM idea?  
3) What model organisms simulate human hormones? |
# Article #3 Notes: Hypoxia, Metabolism and Immune Cell Function

<table>
<thead>
<tr>
<th>Source Title</th>
<th>Hypoxia, Metabolism and Immune Cell Function</th>
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</thead>
<tbody>
<tr>
<td>Source type</td>
<td>Science Research Article (Website)</td>
</tr>
<tr>
<td>Keywords</td>
<td>oxygen, hypoxia, HIF signaling pathway, oxygen metabolism, immune cells, innate immune responses, adoptive immune responses, immunity, inflammation</td>
</tr>
</tbody>
</table>
| Summary of key points (include methodology) | ● Article discusses hypoxia, metabolism, and immune cell function.  
● Hypoxia is defined as an abnormal condition of the body where there is a decrease in oxygen supplied to or utilized usually by inflamed, infected, or damaged tissue.  
● Hypoxia is regulated by proteins named HIFs or hypoxia-inducible factors.  
● HIFs are also regulators of many immunological functions:  
  ○ production of cytokines and antimicrobial peptides  
  ○ phagocytosis  
  ○ cellular metabolic reprogramming  
  ○ antigen presentation.  
● This study focuses on the characteristic of immune cells in which they are able to infiltrate and operate in tissues with decreased levels of nutrients and oxygen (i.e. tissues in hypoxic conditions).  
● The main part of the study was to understand how different immune cells (i.e. neutrophils, mast cells, eosinophils, basophils, dendritic cells, macrophages, T lymphocytes, B lymphocytes, NK cells etc.) react to the presence or the absence of HIFs.  
  ○ Neutrophils, (or the most abundant type of white blood cell are phagocytic and tend to self-destruct as they destroy foreign pathogens) have found increased survival, glycolytic metabolism, and pro-inflammatory cytokine and antimicrobial peptide production in presence of HIFs.  
  ○ In contrast to this absence of HIFs for neutrophils seem to have disturbed ATP generation which is a negative impact |
on migration, invasion, motility, bacterial killing, and aggregation.

- Furthermore, Mast Cells, Eosinophils, and Basophils have been found to have increased survival, functionally, chemotaxis, and antimicrobial defense with presence of HIFs.
- Dendritic cells (antigen-presenting cells, located mainly in lymphatic tissues and skin) have found an impact on survival differentiation, maturation, migration, antigen presentation, regulated pro-inflammatory cytokine and chemokine production in presence of HIFs.
- For T-cells (T lymphocytes) there is a higher survival rate and for B-cells (B lymphocytes) an increased ion transfer and glycolytic metabolism when HIFs are present.
- B-cells when exposed to an absence of HIFs have found to have abnormal development, and autoimmunity.

- HIFs have been found to have an overall important role in virtually all immune cells via metabolic reprogramming and therefore have a bright future in the therapeutic industry.

| Research Question/Problem/Need | Analyze the role of hypoxia-inducible factors in the function of innate and adaptive immune cells in hypoxia. Then research how the hypoxic condition adjusts immune system response and metabolic pathways. |

**Important Figures**

*Figure 1. Regulation of HIF pathway. Under normoxia, PHDs (PHD1, PHD2 and PHD3) and factor inhibiting hypoxia-inducible factor (FIH) hydroxylate the HIF-1α and HIF-2α. This hydroxylation facilitates HIFα binding to the von Hippel-Lindau (VHL) E3 ubiquitin ligase complex, leading to fast ubiquitination and proteosomal degradation. During hypoxia, PHDs and FIH are inhibited by the absence of oxygen; in consequence, hypoxia reduces HIFα hydroxylation and leading to HIFα stabilization and activation. Once stabilized, HIFα subunit is translocated to the nucleus, where formed a complex with HIF-1β, then recruit coactivator p300/CPBP, and upon binding to the consensus hypoxia response elements (HRE) within target genes, involved in a large type of processes, as cellular metabolism, proliferation, differentiation, cell survival, migration, apoptosis or angiogenesis.*
Notes
See summary of key points

Cited references to follow up on


Follow up Questions
1) How does the immune system of the human body respond
<p>| | |</p>
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<thead>
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<tbody>
<tr>
<td>1)</td>
<td>differently in hyperoxia?</td>
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<tr>
<td>2)</td>
<td>Why does the body have built in advantages when hypoxic? Does this tell something evolutionary?</td>
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<tr>
<td>3)</td>
<td>What other factors other than HIFs boost immune system function?</td>
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## Article #4 Notes: Lifespan-regulating genes in *C. elegans*

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<tr>
<td>Source type</td>
<td>Science Research Article (Website)</td>
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<tr>
<td>Keywords</td>
<td>longevity, <em>C Elegans</em>, genes, IIS, lifespan</td>
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### Summary of key points (include methodology)
- The science behind aging has been researched for multiple years due to its association of making individuals more prone to type 2 diabetes, and cancer.
- After research found that the IIS (insulin/insulin-like growth factor-1 signaling) pathway, a metabolic pathway influenced by genes influenced the aging process, multiple other researchers have continued to research in the field of metabolic/signaling pathways and their effects on lifespan.
- Researchers in this study, studied *C.elegans* whose signaling has been found to be similar to humans
- Found that major signaling pathways have genetic influence and could lead to influencing animal lifespan.

### Research Question/Problem/Need
If aging is dependent on genes then what genes contribute to longevity by possibly decreasing rates of diabetes and cancer in those individuals?
This research question has value in society due to how universal aging is for all organisms, including humans, and is one of the major factors that inhibit us from living longer. Aging has long been found to host chronic diseases due to
weakened bodies and lower immune systems.
  ○ Examples of diseases that occur in the body due to old age range from type II diabetes to cancer.
● By finding mutant individuals in a population that has the ancestry of longevity, and then pinning down exactly what gene allows for this longevity, would be the method of study.
● The first example of this data trial was when in 1983 Klass, a fellow researcher in the same field, took eight longevity mutant C. elegans and found an increased life span (Klass, 1983).
● Further studies showed that the insulin/insulin-like growth factor-1 signaling (IIS) pathway is the first established lifespan-regulating signaling pathway.
● Some of the methods used to find this data was by using small animals like flies, mice, worms, and C.elegans.
● The basic gist of all these experiments was to host a lineage of generations that have this gene that allows for special signaling pathways compared to the individuals in the population who don’t have that.
● The analysis would be performed by the information collected from testing.
  ○ The researchers could extrapolate that if the mutant population was living longer the longevity gene has to exist in that mutant population’s genome.
  ○ Once spotted, methods could be adopted where the researchers stimulate that silenced gene in the non-mutant populations.
● The key reasons found for longevity in C.elegans waweres Insulin/insulin-like growth factor-1 signaling, TOR signaling, Sirtuin, AMP-activated protein kinase, Hypoxia-inducible factor-1 (HIF-1), and proteostasis.
● The researchers also found lifespan regulation by the interplay of different tissues.
● The analysis at the end told that aging is to a certain extent dependent on some genes, and more specifically the genes are expressed by more effective signaling pathways.
● But with all this research there lies a road ahead. Researchers are now convinced that genetic factors do play a role, but now the next step is to determine how these genes link to environmental factors.
● This answer tells us in the broader sense that we as individuals have the capability to possibly increase lifespan by decreasing rates of diabetes and cancer in populations more prone to these diseases.
● One more application towards this would be to improve people’s healthspan, the span of life where an individual has relatively low rates of diseases and health issues.
https://doi.org/10.1016/0531-5565(70)90023-9  
https://doi.org/10.1093/geronj/30.3.257  
https://doi.org/10.1016/0047-6374(83)90082-9 |
| Follow up Questions | 1) Would *C. Elegans* be a good model organism for my STEM project?  
a) Is it feasible? Can it be done at home?  
2) How could we further the studies of longevity in *C. Elegans*?  
3) Why were *C. Elegans* used in this experiment? What are the benefits? Drawbacks? |
# Article #5 Notes: Researchers warn of food-web threats from common insecticides

<table>
<thead>
<tr>
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<th>Researchers warn of food-web threats from common insecticides</th>
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<tbody>
<tr>
<td>Source type</td>
<td>Article (Webpage)</td>
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<tr>
<td>Keywords</td>
<td>Neonicotinoids, insecticides, environment</td>
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</table>
| Summary of key points (include methodology) | - Neonicotinoid insecticides only benefit a small niche of crops, but are still used widely. There seem to be some negative aspects of this insecticide  
  - Neonicotinoids have become the most widely used insecticides in the world (25%)  
  - New research indicates that neonicotinoids are transmitted through the herbivore to the environment as a carbohydrate that a lot of animals feed on  
  - Worried more about how it could spread through the entire food chain/web rather than only insects  
  - Would be better if we could refrain from using neonicotinoids as a common insecticide and just use it in the 5% of the time where it actually benefits.  
  - Researchers are worried about general environmental contamination. Also worried about how this could explain to some of the declines in insect/animal populations. |
| Research Question/Problem/Need | Neonicotinoids, a common insecticide, are being transmitted across the food web rapidly, and the amount of damage in the environment is unknown. |
Many insecticides are spreading through the environment, killing unintended species.

Researchers from North Carolina State University and Pennsylvania State University want limited use of neonicotinoids due to the environmental damage that have not been fully assessed.

The research that has assessed neonicotinoid transmission has forgotten many organisms.

Used in lawns, commercial landscapes and to protect trees (200,000 trees in Great Smoky Mountains).

A study uncovered that neonicotinoids can spread through the food chain through organisms (if the organism doesn’t die).

Neonicotinoids are transmitted by honeydew (excretion of aphids, mealybugs, and whiteflies). Honeydew is ingested by hoverflies, and parasitoid wasps.

Insects are eaten by many vertebrate organisms (birds, mice, lizards, frogs, and fish).

Due to this widespread reach, many organisms might be eating or transmitting toxins from nicotinoids.
| Follow up Questions | 1) How can transmission rates of neonicotinoids be reduced?  
2) How can we mitigate the damage that is on the horizon?  
3) How can we weaken the toxicity of neonicotinoids in animals and environments where it has already spread?  
4) Do other similar insecticides transmit similarly? |
# Article #6 Notes: How to Grow a New Model Organism

<table>
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<th>Source Title</th>
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<tbody>
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<tr>
<td>Keywords</td>
<td>Marine biology, cuttlefish, cephalopods, model organisms</td>
</tr>
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</table>
| Summary of key points (include methodology) | ● Researchers are hatching a plan to select and raise new varieties of model organisms  
● The go to choices for model organisms includes mice, fruit flies, Zebrafish, chickens and the roundworm C. elegans  
  ○ They are easy to grow, leading to higher rates for choosing them in experiments  
● Model organisms lack a lot of marine organisms.  
  ○ Ex: cuttlefish, which has a very unique camouflage characteristic and capability  
● Model organisms must be:  
  ○ animals that are easy to grow  
  ○ quick to reproduce  
  ○ can be cultivated over multiple generations  
● Researchers plan to include many cephalopods  
  ○ Ex: squid, octopus, cuttlefish and nautilus  
  ○ Cuttlefish do not grow as quick therefore isn’t the greatest model organism |
| Research Question/Problem/Need | How can more marine animals be part of the large list of common model organisms? |
| Important Figures | N/A |
| Notes | See summary of key points |
### Cited references to follow up on


### Follow up Questions

1. Are there any marine model organisms that could work for my experiment with drugs?
2. Out of C. Elegans, Drosophila, and Zebrafish which model organisms have been commonly used with drugs and addiction studies?
3. How similar are these model organisms to the human genome? Do they have enough similarity to draw conclusions by extrapolation for humans?
# Article #7 Notes: Exploring Hallucinogen Pharmacology and Psychedelic Medicine with Zebrafish Models

<table>
<thead>
<tr>
<th>Source Title</th>
<th>Exploring Hallucinogen Pharmacology and Psychedelic Medicine with Zebrafish Models</th>
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<td>Keywords</td>
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</tbody>
</table>

## Summary of key points (include methodology)

- **Abstract**
  - In this study, the researchers discuss the behavioral and toxicological effects of psychedelic medicine as well as hallucinogenic drugs. The model organism of choice is the *Danio rerio*, Zebrafish. Zebrafish is an ideal model organism that can both assess potential toxic and therapeutic effects of common drugs and hallucinogens.

- **Introduction**
  - After initial restrictions on hallucinogenic drugs the research about them slowed down. However, in the last ten years there has been a revival of hallucinogenic drug investigation that may show some promise in treating intractable psychiatric illnesses. But since hallucinogenic drugs do have adverse side effects at times and can lead to addiction the normal model organism (rodents) and in this case Zebrafish (of both infancy and adulthood) have to be studied for behavioral and cognitive change. This article will then get into how Zebrafish are ideal organisms for future research in the same field.

- **Psychedelic Medicine and Pharmacology**
  - See Important Figure #1
  - This figure summarizes this section of the article. It shows what the three types of hallucinogens are and their pharmacological and behavioral effects of each of these drugs.

- **Zebrafish Models of Hallucinogenic Drug Exposure**
This data table shows the behavioral side effect found on the Zebrafish models when exposed to the three different types of hallucinogens.

- Utility of Zebrafish Models in Psychedelic Medicine and Toxicology Research
  - Zebrafish’s genome is currently widely known and therefore there are multiple possibilities of using Zebrafish phenotypes which have a behavior. More specifically Zebrafish have been shown to give valuable and reliable data as a model organism for drug studies including this one. One extension to this experiment would be to test other drugs on Zebrafish and see their behavioral effects.

- Conclusion
  - In this study, the researchers began with the recent progress of psychedelic drug treatment for various psychiatric disorders, as well as the effects of hallucinogenic drugs on Zebrafish physiology and behavior. Lastly more Zebrafish models can be used to develop successful treatments for brain disorders, and drugs.

Research Question/Problem/Need

What can research on Zebrafish tell us on the safety of hallucinogens and psychedelic drugs, so that we can implement these drugs on mental health conditions of humans?

Important Figures

Figure #1:

Figure #2:

|   | 2) Are there any specific phenotype models of Zebrafish that are better used for drug models?  
|   | 3) How does the Zebrafish genome compare to mice genome, and what drawbacks are present with Zebrafish? |
### Article #8 Notes: Use of zebrafish as a model to understand mechanisms of addiction and complex neurobehavioral phenotypes

<table>
<thead>
<tr>
<th>Source Title</th>
<th>Use of zebrafish as a model to understand mechanisms of addiction and complex neurobehavioral phenotypes</th>
</tr>
</thead>
</table>
https://doi.org/10.1016/j.nbd.2010.05.016 |
| Source type | Science Research Article (Website) |
| Keywords | Zebrafish, addiction, model organism, anxiety, Schizophrenia, autism |
| Summary of key points (include methodology) | ● Massive efforts are put into exact pathogenesis and pathophysiology of addiction and neuropsychiatric disorders  
○ Examples of disorders:  
  ■ anxiety, schizophrenia and autism  
  ○ Pathogenesis: Process of development for a disease/disorder/condition  
  ○ Pathophysiology: The disrupted processes due to addiction or disorders  
● Model organisms can allow for the understanding of the etiology and pathogenesis of these disorders  
○ Etiology: the cause of a disease or condition  
● Zebrafish is one of the most relevant model organisms in this field  
○ allows for analysis of all developmental stages  
○ In addition allows for the imaging of pathological processes  
○ Lets researcher analyze automated behavioral quantification coupled with large scale screening and mutagenesis strategies |
| Research Question/Problem/Need | How to study addiction and neuropsychiatric disorders with model organisms like Zebrafish to produce relevant data for humans? |
Important Figures

Notes

- Article summarizes studies conducted over the last few years which:
  - demonstrate the relevance of the Zebrafish model to human diseases

### Table 1

<table>
<thead>
<tr>
<th>Neurobehavioral Disorder</th>
<th>Example of ZF model assay</th>
<th>Phenotype and/or studies of disease pathogenesis</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol dependence</td>
<td>Adult ZF brain neurotransmitter levels</td>
<td>Increased dopamine and serotonin levels</td>
<td>Getta et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>Microarray analysis of brain samples</td>
<td>Changes in gene expression following conditioning</td>
<td>Kiyi et al. (2008)</td>
</tr>
</tbody>
</table>
| Alcohol dependence      | Adult ZF saline
  Video recording of locomotion activity in adult ZF | Increased open-field exploratory behavior | Kis et al. (2007) |
|                        | Acute mutant
  Video recording of locomotion activity in acute ZF | Increased open-field exploratory behavior | Getta et al. (2006) |
| Alcohol withdrawal      | Novel tank swimming test | Reduced open-field exploratory behavior | Pang et al. (2008) |
|                        | Video recording of locomotion activity in adult ZF | Reduced open-field exploratory behavior | McMillan et al. (2006) |
| Cocaine dependence      | Non-exposed female rats
  Video recording of locomotion activity in adult ZF | Hypersensitivity | Lesch et al. (2006) |
|                        | Exposed female rats
  Video recording of locomotion activity in adult ZF | Decreased open-field exploratory behavior | Lesch et al. (2006) |
| Morpholine dependence   | Non-exposed female rats
  Video recording of locomotion activity in adult ZF | Hypersensitivity | Lesch et al. (2006) |
|                        | Exposed female rats
  Video recording of locomotion activity in adult ZF | Decreased open-field exploratory behavior | Lesch et al. (2006) |

### Table 2

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene</th>
<th>Technique used</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug dependency (ethanol)</td>
<td>fan</td>
<td>ENU mutagenesis, cloning</td>
<td>Peng et al. (2009)</td>
</tr>
<tr>
<td>Drug dependency (amphetamine)</td>
<td>calceinurin B</td>
<td>Microarray analysis</td>
<td>Kiyi et al. (2008)</td>
</tr>
<tr>
<td>Autism</td>
<td>SUSA1</td>
<td>MO knockdown</td>
<td>Tu et al. 2010</td>
</tr>
<tr>
<td></td>
<td>neurogin</td>
<td>Studied expression patterns</td>
<td>Rissone et al. (2010)</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>disc-1</td>
<td>MO knockdown</td>
<td>Burgess and Granato (2007)</td>
</tr>
<tr>
<td></td>
<td>nrd1a, nrd1b</td>
<td>studied expression patterns</td>
<td>Burgess and Granato (2007)</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>ophelia</td>
<td>Mutagenesis</td>
<td>Burgess and Granato (2007)</td>
</tr>
</tbody>
</table>

ZF = zebrafish, MO = morpholino, TILLING = targeted induced local lesions in genomes.
https://doi.org/10.3758/BF03192800  
https://doi.org/10.1016/j.pbb.2009.07.009 |
| Follow up Questions | 1) What are the key differences between adult and larval Zebrafish  
2) How will these differences contribute to our project (if any)?  
3) Have OTCs been used in previous Zebrafish study? Is there any erratic behavior that has been noted when studying OTCs? What differences? |
Article #9 Notes: CB1 and CB2 receptors play differential roles in early zebrafish locomotor development

<table>
<thead>
<tr>
<th>Source Title</th>
<th>CB1 and CB2 receptors play differential roles in early zebrafish locomotor development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original URL</td>
<td><a href="https://jeb.biologists.org/content/222/16/jeb206680">https://jeb.biologists.org/content/222/16/jeb206680</a></td>
</tr>
<tr>
<td>Source type</td>
<td>Science Research Article (Website)</td>
</tr>
<tr>
<td>Keywords</td>
<td>Zebrafish, locomotor, CNS, Endocannabinoids, CB1R, CB2R, AM251, AM630</td>
</tr>
</tbody>
</table>
| Summary of key points (include methodology) | ● When natural cannabinoid receptors of Zebrafish are disrupted then a detrimental effect on the development has been found.  
   ● The study encompasses the endocannabinoid system of the Zebrafish, which also correlates directly to humans.  
     ○ Regulates nervous, immune, and digestive systems using neurotransmitters as well as development and growth of the body.  
     ○ Is affected by levels of natural cannabis in the system (i.e. levels of cannabis for individuals that do not take it recreationally)  
   ● Many effects and differences were found when the receptors were disrupted.  
     ○ The effects were not significant individually, but as a whole there were some significant differences and changes found.  
     ○ There was an impaired growth of muscle fibers in motor neuron development  
     ○ Many physical deficits and behavioral abnormalities for Zebrafish who became adults  
     ○ Less locomotor activity for Zebrafish who had disrupted receptors. |
If cannabinoid receptors of Zebrafish are disrupted during early stages, does it affect the development of Zebrafish?

**Cited references to follow up on**


[https://doi.org/10.1089/zeb.2012.0785](https://doi.org/10.1089/zeb.2012.0785)

gastrulation. *Scientific Reports, 8*(1).

[https://doi.org/10.1038/s41598-018-28689-z](https://doi.org/10.1038/s41598-018-28689-z)

| Follow up Questions | 1) Are there any effects when the endocannabinoid receptors have a surplus of neurotransmitter flow?  
2) What does this mean to the human population? We as individuals require some level of natural cannabinoids?  
3) Can we use similar methods to implement our project? |
|---------------------|--------------------------------------------------------------------------------------------------|
### Article #10 Notes: Zebrafish models relevant to studying central opioid and endocannabinoid systems

<table>
<thead>
<tr>
<th>Source Title</th>
<th>Zebrafish models relevant to studying central opioid and endocannabinoid systems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source type</td>
<td>Science Research Article (Website)</td>
</tr>
<tr>
<td>Keywords</td>
<td>opioid system, endocannabinoid system, Zebrafish, withdrawal, addiction</td>
</tr>
<tr>
<td>Summary of key points (include methodology)</td>
<td>● The endocannabinoid and opioid systems in humans:  ○ Interplaying neurotransmitter systems  ○ Modulates drug abuse, anxiety, pain, cognition, neurogenesis and immune activity  ● Research in this field is limited therefore need of using a model organism to study further this field  ● Zebrafish are one of the best model organisms for this task:  ○ Extremely accurate translational models for:  ■ Neuroscience  ■ Biological psychiatry  ○ High physiological and genetic homology to humans  ■ Allowing for effective use in the study of endocannabinoid and opioid systems  ○ Zebrafish models are a promising tool to study the role of endocannabinoid and opioid systems</td>
</tr>
<tr>
<td>Research Question/Problem/Need</td>
<td>How does modifications to the endocannabinoid system in Zebrafish modulate Zebrafish’s CNS and brain functions?</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>---------------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>

**Important Figures**

**Fig. 3.** Venn diagrams showing average percentages of pairwise alignment homology scores for selected proteins of the endocannabinoid (A) and opioid (B) systems between humans, mice and zebrafish (based on Table 3, calculated using the HomoloGene database of homologous genes [https://www.ncbi.nlm.nih.gov/homologene](https://www.ncbi.nlm.nih.gov/homologene)).

**Fig. 2.** Central endocannabinoid and opioid systems are involved in withdrawal, conditioned place preference, anxiety-, pain- and cognition-related behaviors, and are highly amenable to genetic manipulations (SNPs) at different stages of zebrafish development (also see a brief summary of relevant behavioral tests in the Supplementary Table 2B).
### Notes


| Follow up Questions | 1) How are the receptors present in Zebrafish compared to humans? 2) What limitations do larval Zebrafish put forward in this study? 3) Could using similar methods described in the study work for my STEM project? |
Article #11 Notes: Chapter 3 - Embryonic and Larval Culture

Article notes should be on separate sheets

<table>
<thead>
<tr>
<th>Source Title</th>
<th>Chapter 3 - Embryonic and Larval culture Raising Larvae in a Nursery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original URL</td>
<td><a href="https://zfin.org/zf_info/zfbook/chapt3/3.2.html">https://zfin.org/zf_info/zfbook/chapt3/3.2.html</a></td>
</tr>
<tr>
<td>Source type</td>
<td>Science Article Instructions (website)</td>
</tr>
<tr>
<td>Keywords</td>
<td>Zebrafish, larval, adult, growth</td>
</tr>
</tbody>
</table>
| Summary of key points (include methodology) | - The daily duties can be divided into three categories:
  - cleaning the baby fish containers
  - feeding the baby fish
  - growing brine shrimp
  - Baby fish (4-9 days):
    - 250 ml beakers
    - 25-30 fish per beaker
    - Cleaned daily
    - Feed paramecium
  - 9 days plus:
    - Feed brine shrimp
    - Or feed paramecium
  - When the baby fish have reached 21 days of age, they are ready to be transferred out of the beaker/mouse cage into a full-sized tank |
| Research Question/Problem/Need | How to grow Zebrafish populations at home or any other facility? |
| Important Figures | No important figures |
| Notes | See summary of key points |
| Cited references to | No further references |
| Follow up Questions | 1) How will we be able to access Zebrafish populations for our experiment?  
2) How will our experiment differ for the fact that we need it in larval stages?  
3) What are safety procedures that must be completed while using Zebrafish |
# Article #12 Notes: Environmental concentrations of antibiotics impair zebrafish gut health

<table>
<thead>
<tr>
<th>Source Title</th>
<th>Environmental concentrations of antibiotics impair zebrafish gut health</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source type</td>
<td>Science Research Article (Website)</td>
</tr>
<tr>
<td>Keywords</td>
<td>antibiotic, intestinal microbiota, gut health, Zebrafish</td>
</tr>
</tbody>
</table>
| Summary of key points (include methodology) | ● Antibiotics are natural or synthetic drugs with the capacity to kill or inhibit the growth of bacteria  
  ○ Highly soluble properties  
● Antibiotic residuals are found in aquatic bodies  
● Prior research shows adverse effects of these antibiotics on the aquatic organisms  
● 600 Zebrafish used  
  ○ 3 Groups:  
    ■ Control  
    ■ Sulfamethoxazole  
    ■ Oxytetracycline  
● Methods of testing:  
  ○ Immunol attack after exposure to antibiotics  
  ○ Determination of oxygen consumption rate (metabolic test)  
  ○ Gut morphology  
  ○ Biochemical assays  
  ○ Bioinformatics & statistical analysis  
  ○ Bacterial genomic DNA extraction  
    ■ From symbionts in the gut  
  ○ Quantitative Real-time PCR  
● Results:  
  ○ Slower metabolic rate  
  ○ Weaker immune system |
<table>
<thead>
<tr>
<th>Research Question/Problem/Need</th>
<th>How do antibiotic levels in water affect gut health in Zebrafish and what does that tell us about our water source and potential toxicity for aquatic organisms?</th>
</tr>
</thead>
</table>
| **Important Figures** | ![Concept map for article:](image)

- Environmental trauma of antibiotics lead to impaired health or aquatic organisms
- Introduction
- Antibiotics are natural or synthetic drugs with the capacity to inhibit the growth of bacteria
- Highly blood properties
- Antibiotic residues are found in aquatic bodies
- Prior research shows adverse effects of these antibiotics on the aquatic organisms
- Materials & Methods
- Antibiotics: oxytetracycline (OTC), sulfamethoxazole (SMX)
- 60L Zebrafish
- 3 Groups: Control, Sulfamethoxazole, Oxytetracycline
- Experimental
- Immune attack after exposure to antibiotics
- Determination of oxygen consumption rate (metabolic test)
- Gut morphology
- Weaker immune system
- Weaker intestinal system
- Weaker expression of inflammation genes in the gut
- Environmental antibiotic concentrations can impair the gut health of Zebrafish
- Potential health risk of antibiotic residues in water should be evaluated now

| Cited references to follow up on | Yan, Z., Lu, G., Ye, Q., & Liu, J. (2016). Long-term effects of antibiotics, norfloxacin, and sulfamethoxazole, in a partial life-cycle study with zebrafish (Danio rerio): Effects on growth, development, and |
Tripathi 41


**Follow up Questions**

1) What does this study tell about possible physiological areas of study on Zebrafish with OTCs?
2) Can we create an experiment with 3 groups, each having varying levels of concentration of OTCs?
3) How would using larval Zebrafish change this method of testing?
Article #13 Notes: “Poor man’s methadone” can kill the poor man. Extra-medical uses of loperamide: a review

<table>
<thead>
<tr>
<th>Source Title</th>
<th>“Poor man’s methadone” can kill the poor man. Extra-medical uses of loperamide: a review</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original URL</td>
<td><a href="https://link.springer.com/article/10.1007/s11419-017-0365-x">https://link.springer.com/article/10.1007/s11419-017-0365-x</a></td>
</tr>
<tr>
<td>Source type</td>
<td>Science Research Article (Website)</td>
</tr>
<tr>
<td>Keywords</td>
<td>Loperamide, pharmacology, abuse, toxicity, cardiac effects</td>
</tr>
</tbody>
</table>
| Summary of key points (include methodology) | • Loperamide is a phenylpiperidine derivative and an opioid  
  ○ Phenylpiperidines are a chemical class of drugs with a phenyl moiety directly attached to piperidine  
• It was initially classified in the United States as a Schedule II drug  
  ○ Drugs classified as Schedule II are drugs with high potential of abuse  
  ○ Transferred to Schedule V in 1977  
  ○ Transferred to no control over substance in 1982  
• Loperamide is used for the symptomatic treatment of diarrhea and gastrointestinal inflammation  
• Low risks with CNS (central nervous system) when used in correct doses  
• When used in high doses, or used in a manner of abuse:  
  ○ High risk of cardiac effects  
  ○ Life-threatening at time  
• Has a specific metabolic pathway for desynthesis in the body  
• Recent years the FDA and other authorities have started to question the safety of this drug |
| Research Question/Problem/Need | What properties of loperamide, an anti-diarrheal OTC, allows for the usage of it as a drug for euphoria and abuse? |
It is an opioid-receptor agonist that acts on the mu-opioid receptors in the myenteric plexus of the large intestine.

Blocks calcium channels exhibiting calmodulin inhibitory activity and reducing paracellular permeability.

May cause serious heart problems such as QRS widening and QT interval prolongation, dysrhythmias, torsades de pointes, ventricular arrhythmias, syncope and cardiac arrest that can lead to death.

The use of loperamide as a medicine, a drug of abuse and a substitute for methadone.

Used extensively to control diarrhea.

Loperamide has the molecular formula C29H33ClN2O2.

Possibly useful to study receptors in Zebrafish compared to molecular structure.

Similar to morphine, loperamide decreases the activity of the myenteric plexus, which subsequently reduces the tone of the longitudinal and circular smooth muscles of the intestinal wall.

It inhibits the release of acetylcholine and prostaglandins, thereby reducing peristalsis and increasing intestinal transit time.

Loperamide is also a substrate for P-glycoprotein.

High doses of loperamide can in fact induce effects on the CNS and cause euphoria similar to that with morphine.

Acts as an inhibitor of transmembrane calcium flux in certain tissues.

In mice study:

- Development of tolerance to μ-opioid receptor agonist-induced constipation.
The results revealed that, unlike morphine, loperamide readily developed tolerance to the inhibitory effect on mouse gastrointestinal transit.

- After ingestion, loperamide is extensively metabolized in the liver through N-demethylation, oxidative N-dealkylation and hydroxylation of the α-phenyl group.
- The major metabolites of loperamide in urine were bis-desmethylated loperamide and 4-(4-chlorophenyl)-4-hydroxy-piperidine (Fig. 2), while in bile mono- and bis-desmethylated loperamide were present.
- Loperamide and its metabolites are ultimately excreted as glucuronide conjugates in urine.
- The peak concentrations in plasma occurred 5 h after the administration of a capsule and 2.5 h after intake of its liquid formulation.
- Given this low percentage of loperamide excreted in urine, therefore, forced diuresis is not expected to be effective in cases of loperamide overdose.
- The common route of administration is oral.
- In 1990, all formulations of loperamide for children were banned in Pakistan.
- Drugs that may interact with loperamide include cimetidine and ranitidine (H2 receptor antagonists that inhibit stomach acid production), clarithromycin and erythromycin (antibiotics), itraconazole and ketoconazole (antifungal CYP3A4 inhibitors), ritonavir (antiretroviral), gemfibrozil (agent that lowers lipid levels and a CYP2C8 inhibitor), quinidine (antiarrhythmic agent and P-glycoprotein inhibitor), and quinine (antimalarial agent).
  - Possibly use this to study what reactions occur between these drugs that results in this.
  - What change in molecular structure?
  - What change in effectiveness?
- Loperamide has been shown to cause mild physical dependence and a mild opiate withdrawal syndrome after abrupt discontinuation of long-term treatment.

<table>
<thead>
<tr>
<th>Cited references to follow up on</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="https://doi.org/10.1016/j.annemergmed.2016.03.047">https://doi.org/10.1016/j.annemergmed.2016.03.047</a></td>
</tr>
</tbody>
</table>
| Follow up Questions | 1) See if there are restrictions to buy Loperamide currently since this article was 3 years ago.  
2) Have previous studies with loperamide used Zebrafish?  
3) What would be the mode of administration for the Zebrafish? |

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[https://doi.org/10.3109/15563650.2015.1026971](https://doi.org/10.3109/15563650.2015.1026971)
Article #14 Notes: Establishing and maintaining a low-cost zebrafish breeding and behavioral research facility

<table>
<thead>
<tr>
<th>Source Title</th>
<th>Establishing and maintaining a low-cost zebrafish breeding and behavioral research facility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source type</td>
<td>Science Research Article (PDF)</td>
</tr>
<tr>
<td>Keywords</td>
<td>zebrafish, physical development, maintenance, reflexive behavior, locomotor activity, operant behavior</td>
</tr>
<tr>
<td>Summary of key points (include methodology)</td>
<td>● Why zebrafish?  ○ The importance of the zebrafish (<em>Danio Rerio</em>) to many fields of science-including genetics, biology, psychology, and neuroscience-is due to the fact that this vertebrate animal is able to reproduce prolifically and reach adulthood within 3 months.  ○ Zebrafish has transparent eggs, so its embryonic development can be examined without interfering with the process.  ○ They are extremely hardy animals and require minimum space to house.  ● Animal Husbandry Procedures  ○ General Maintenance  ● Physical Development  ○ Methods to measure  ● Locomotor Activity  ● Reflexive Behavior  ● Operant Behavior  ○ Appetitive  ○ Aversive</td>
</tr>
</tbody>
</table>
Research Question/Problem/Need

How could a low-cost zebrafish facility be made for research purposes?

Important Figures

![Graph showing mean response latency over training session]

Figure 1. Mean learning curve from four fish. Fish were conditioned to swim through an escape hole for food reinforcement. See text for details.

APPENDIX

Materials List for a Zebrafish Facility

<table>
<thead>
<tr>
<th>Equipment Set-Up (Required)</th>
<th>Cost for Each</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 ten-gal. glass aquarium tanks</td>
<td>$10 each</td>
</tr>
<tr>
<td>2 100-W heaters (10-in. Visi-Therm)</td>
<td>$12 each</td>
</tr>
<tr>
<td>2 aquarium thermometers</td>
<td>$2.50 each</td>
</tr>
<tr>
<td>2 corner filters (Penn Plax Clear Free)</td>
<td>$4 each</td>
</tr>
<tr>
<td>3 replacement filters (Penn Plax, 2 pack)</td>
<td>$2 each</td>
</tr>
<tr>
<td>1 super battery vacuum</td>
<td></td>
</tr>
<tr>
<td>1 air pump (Whisper 600)</td>
<td></td>
</tr>
<tr>
<td>1 air check valve (Aquametrics, 2 pack)</td>
<td></td>
</tr>
<tr>
<td>10-ft plastic tubing</td>
<td>$0.30/ft</td>
</tr>
<tr>
<td>2 fish nets (Whisper 3 in.)</td>
<td>$0.00 each</td>
</tr>
<tr>
<td>Instant Ocean Salt (25-gal. mix)</td>
<td></td>
</tr>
<tr>
<td>Flake food (Tetramin for Tropical Fish, 3.5 oz.)</td>
<td></td>
</tr>
<tr>
<td>12 adult zebrafish</td>
<td>$2.00 each</td>
</tr>
</tbody>
</table>

Total for set-up: $119.20

Water Conditioning (Optional)

<table>
<thead>
<tr>
<th>Equipment Set-Up (Aquarium Pharmaceuticals)</th>
<th>Cost for Each</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water deionizer</td>
<td></td>
</tr>
<tr>
<td>2 deionizer refill cartridges</td>
<td>$14.00 each</td>
</tr>
<tr>
<td>Chlorine neutralizer (Kedron NonAqua, 16 oz.)</td>
<td></td>
</tr>
<tr>
<td>pH stabilizer (Wardley Buffer, 7.0, 16 oz.)</td>
<td></td>
</tr>
<tr>
<td>pH increaser or decreaser (Jabico, 16 oz.)</td>
<td></td>
</tr>
<tr>
<td>Egg fungus treatment (Methylblue, 1 oz.)</td>
<td></td>
</tr>
</tbody>
</table>

Total for water conditioning: $77.00

Water Testing (Required)

<table>
<thead>
<tr>
<th>Equipment Set-Up (Aquarium Pharmaceuticals GH &amp; KH Kit)</th>
<th>Cost for Each</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH freshwater test (Aquarium Pharmaceuticals)</td>
<td>$5.00</td>
</tr>
<tr>
<td>Freshwater ammonia tester (Aquarium Pharmaceuticals)</td>
<td>$5.00</td>
</tr>
</tbody>
</table>

Total for water testing: $13.00

Breeding Set-Up (Required)

<table>
<thead>
<tr>
<th>Equipment Set-Up (Aquarium Pharmaceuticals)</th>
<th>Cost for Each</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minnow net (14-in. mesh)</td>
<td>$5.00</td>
</tr>
<tr>
<td>2 breeder aquariums</td>
<td>$15.00 each</td>
</tr>
<tr>
<td>Brine shrimp eggs (50L, 15 in.)</td>
<td></td>
</tr>
<tr>
<td>2 fry food solution (Liquidific for eggplants, 19.8 mL)</td>
<td>$2.00 each</td>
</tr>
<tr>
<td>Plastic freezer containers</td>
<td>$5.00 pack</td>
</tr>
</tbody>
</table>

Total for breeding set-up: $79.00

Developmental Staging (optional but suggested)

<table>
<thead>
<tr>
<th>Equipment Set-Up (Edmund Scientific, A52-365, A52-746)</th>
<th>Cost for Each</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stereoscope</td>
<td>$325–$600</td>
</tr>
</tbody>
</table>

Total Cost

- Required: $231.20
- Optional: 77.00
- Optional but suggested: 600.00

Total: $908.20

Notes

- Adult zebrafish range in body length from 2 to 5 cm
- The first procedure is to disinfect and clean the two 10-gal. tanks by placing about 3 in. of water in each tank with 65 ml of chlorine.
bleach
- Once they are disinfected, rinse the tanks well with water.
  - Never use detergents or cleaning chemicals that are not specifically designed for aquarium use
- The next step is to fill the tanks with fish water.
- Zebrafish will not breed if the water conditions are not right.
  - Tap water is generally not a good choice
  - There are several ways to obtain appropriate tank water.
    - One is to purchase distilled water; the other is to use a tap water deionizer for aquarium
    - Water should be soft (pH ~ 7.0)
    - You will need to add aquarium salts (e.g., Instant Ocean mix) to the water (about 7'/3 teaspoon per 10 gals. of water)
- An ideal temperature for both breeding and development of the embryos is 28 degrees Celsius.
- A light-dark cycle also should be established, because zebrafish breeding depends on the light onset.
  - A 14:10 hour light-on light-off cycle is the one used most commonly
- The next step is to create a biological filter in the tank, which consists of bacteria that will break down fish waste products into harmless material
- Put the fish into one tank (this will be called the housing tank) and feed them as much as they will eat in 5 min about three times a day.
  - At this point, feed the fish both flake food and live brine shrimp.
  - After several days, the females will develop a “belly”; males stay thin no matter how much they eat.
- Zebrafish lay their eggs every morning following the light onset
  - Eggs are then eaten by the adults.
  - To prevent this, a breeding net must be placed
    - The net is simply a 1/8th in. mesh minnow
- The number of embryos is determined primarily by the number and gender ratio of the adults.
  - For example, with six adults (three males and three females) expect to find about 150 fertilized eggs.
- The adults will lay and fertilize the eggs when the lights go on the next morning; this is referred to as Day 0 or 0 days postfertilization (dpf).
- To determine whether there are fertilized eggs, take a rigid, 1/4th in. diameter, plastic tube, and siphon a small portion of water from the tank bottom.
- Fertilized eggs should appear clear, with a small round yolk floating inside the shell. If the egg appears cloudy or covered with white particles, it is not viable
The fertilized eggs can be removed with a small eyedropper and placed into small storage containers.

It is very important to monitor the ammonia level of this tank following breeding.

During the first 3 days (i.e., 0-2 dpf), the embryos will feed off their yolks. They should begin to hatch at about 3 dpf.

At the age of 3dpf, they are too small for either flake food or brine shrimp.
  • An excellent food source for the fry is a commercially available live paramecium solution (Liquifry).

By 4-5 dpf, the fry will begin to swim around the tank. Begin preparing live brine shrimp around 6 dpf and feed the fry live brine shrimp along with the paramecium mixture at about 7 dpf.

By 9 dpf, if the fry are in the small containers, they should be placed into a larger one.

At about 16 dpf, flake food can be used, if the flakes are ground into a fine powder.

After 22 dpf, fish should be placed into the 10-gal. tank, if they are not there already.

Routine maintenance is required to keep the tanks in good condition. The best strategy is to do a little maintenance every few days, rather than once a month.

The most important job is to keep the tank free of debris and waste products.

Vacuum the tanks every couple of days.

A partial water change (about 1/3rd of the water) should be done every 3 to 4 weeks.

Aquarium salts are replaced accordingly.

Once a month, check the filter material in the corner filter and replace it when necessary.

Physical Development
  • These measures include body length, head, and eye diameter, and so forth.
  • The physical development measures can be used in psychopharmacology and behavioral teratology studies.
  • The same researchers are in conjunction with behavioral measures examining the effects of ethanol on prenatal development.
  • Viewing the embryos with a stereoscope while they are still in the egg (i.e., 0-3 dpf) is relatively easy to do because they have transparent eggs and remain relatively stable in the viewing field.
  • Once they have hatched and are capable of swimming, there are several ways to restrict their movement. One is to place them into a .16-mg/ml solution of tricaine methanesulfonate (MS-222; Sigma, A5040) for about 2 min.

  2 min of stable observation time will be achieved.
<table>
<thead>
<tr>
<th>Locomotor Activity</th>
<th>Reflexive Behavior</th>
<th>Operant Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperactivity is a locomotor behavior that can be measured</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The subject is placed into a small chamber with a grid on the chamber floor and the number of grid crossings recorded for a set period of time</td>
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<tr>
<td>The advantage of these behavioral measures is that they can be used immediately</td>
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<td></td>
</tr>
<tr>
<td>Unlike operant behaviors, they require no acquisition time</td>
<td></td>
<td></td>
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<tr>
<td>Zebrafish are schooling fish</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tend to follow other fish</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Example study in which: the stimulus that triggers schooling behavior for zebrafish was found</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refer to annotated PDF for more examples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Divided into appetitive and aversive conditioning techniques.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The subject's response latency for each training session was obtained by calculating the median latency on the basis of the total number of trials; using the median eliminated any extreme latencies that were due to the subject being distracted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The key is to find a behavior that matches zebrafish temperament.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For example, one should choose behavior that requires continuous movement or the opportunity to swim from a restrictive situation</td>
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<td></td>
</tr>
<tr>
<td>Zebrafish learned very quickly to swim through the opening for food reinforcement.</td>
<td></td>
<td></td>
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<tr>
<td>Operant conditioning procedures can be used with zebrafish, and the results are similar to those found in other species.</td>
<td></td>
<td></td>
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<tr>
<td>Aversive conditioning also has been used to train zebrafish.</td>
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<td></td>
</tr>
<tr>
<td>For example, researchers used an avoidance conditioning paradigm to examine the factors involved in the escape response to a predator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Or another unpublished study from Brothers (1996) is mentioned in which:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brothers (1996) used avoidance learning to determine the visual ability of the zebrafish under dark-adapted conditions. In this study, a two-chamber avoidance tank was used, in which the subjects avoided a shock when they escaped into the chamber after the presentation of a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
visual stimulus. By varying the intensity of the visual stimulus, Brothers was able to determine the visual thresholds of the zebrafish to stimuli of different wavelengths. Thus, these learning procedures can be used to examine response acquisition and extinction, stimulus generalization and discrimination.

- Zebrafish live about 2 to 3 years, so it is important to have a way of dealing with them within a small facility.
- Zebrafish are easy to breed and maintain, and there are a variety of procedures that can be used with the zebrafish in behavioral research.

https://doi.org/10.1016/j.jtherbio.2004.06.002

https://doi.org/10.1126/science.2218513


| Follow up Questions | 1) How can the procedures mentioned be used for larval zebrafish? What modifications will have to be made?  
2) What are some of the differences in reflexive behavior when in the larval stage?  
3) What can be collected and measured for my STEM project, and which behaviors and procedures would be most appropriate? |
# Article #15 Notes: Effects of ibuprofen, diclofenac and paracetamol on hatch and motor behavior in developing zebrafish (*Danio rerio*)

<table>
<thead>
<tr>
<th>Source Title</th>
<th>Effects of ibuprofen, diclofenac and paracetamol on hatch and motor behavior in developing zebrafish (<em>Danio rerio</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source type</td>
<td>Science Research Article (Website)</td>
</tr>
<tr>
<td>Keywords</td>
<td>Ibuprofen, Diclofenac, Paracetamol. zebrafish embryos, hatch rate, motor behavior</td>
</tr>
</tbody>
</table>
| Summary of key points (include methodology) | ● Studying behavioral effects of 3 NSAIDs: Ibuprofen, Diclofenac, Paracetamol on zebrafish embryos.  
● Effects on the hatch and motor ability of early-stage zebrafish  
● 2 of the NSAIDs, ibuprofen and diclofenac, significantly suppressed embryo motion and hatching.  
● However with paracetamol no significant effect was observed |
| Research Question/Problem/Need | NSAIDs are polluting and creating increased environmental concerns for teleost fishes, due to incomplete removal in wastewater treatment plants. What are the adverse effects of these NSAIDs on zebrafish? |
Fig. 1. Hatch rate of zebrafish embryos exposed to ibuprofen, diclofenac, and paracetamol (*P < 0.05, **P < 0.01).
Fig. 2. Spontaneous movement at 28 hpf affected by ibuprofen, diclofenac and paracetamol (**p < 0.01).

Fig. 3. Free swimming activity under dark condition at 120 hpf (**p < 0.05, ***p < 0.01). (A) Distance, (B) duration, (C) velocity, (D) track of embryo movement.
Fig. 4. Average swimming distance (A), duration (B) and track (C) under 10 min dark condition during alternating photoperiod stimulation tests at 120 hpf (*P < 0.05, **P < 0.01).

Fig. 5. Average swimming distance (A), duration (B) and track (C) in 10 min light condition during alternating photoperiod stimulation tests at 120 hpf (*P < 0.05, **P < 0.01).
NSAIDs which are widely used as pain relief medicines are of increased concern due to their incomplete removal in wastewater treatment plants which yields to potential toxicity on:
  ○ endocrine, kidney, and reproduction in teleost fish
Exposing embryos to the target chemicals at 5, 50 and 500 μg/L starting from 6 hpf.
A significant reduction in hatch rate at 55 hpf was caused by both ibuprofen (−63%) and diclofenac (−58%) at 500 μg/L.
Exposure to high concentration of ibuprofen significantly decreased the spontaneous movement by 25%, and reduced:
  ○ The free swimming distance: 41%
  ○ Duration: 29%
  ○ Speed under dark conditions: 30%
High concentration of diclofenac also caused 23% decrease in spontaneous movement, and reduced:
  ○ Swimming distance: 17%
  ○ Active duration under light stimulation
In comparison, the exposure to paracetamol did not cause any notable effect.
The expression of neurog1 was down-regulated from ibuprofen by 19% and diclofenac exposure 26%.
The expression of neurod1 was up-regulated only by ibuprofen by 31%.
These findings indicated that ibuprofen and diclofenac significantly affected embryo locomotivity.
Were potentially neurotoxic, thus posing threats to zebrafish development.

https://doi.org/10.1016/j.jphysparis.2003.10.009
https://doi.org/10.1016/j.aquatox.2006.11.002
<table>
<thead>
<tr>
<th>Follow up Questions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Were there any noticeable effects in the environment due to the staggered developmental and behavioral of teleost fish (like zebrafish)?</td>
</tr>
<tr>
<td>2) How does this information translate to humans? Do human embryos or even adults experience slower metabolic rates, staggered growth?</td>
</tr>
<tr>
<td>3) Is there a tolerance build up for the teleost fish that live in these water bodies? Over generations?</td>
</tr>
</tbody>
</table>


[https://doi.org/10.1016/j.chemosphere.2014.08.020](https://doi.org/10.1016/j.chemosphere.2014.08.020)
# Article #16 Notes: Steps during the development of the zebrafish locomotor network

<table>
<thead>
<tr>
<th>Source Title</th>
<th>Steps during the development of the zebrafish locomotor network</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source type</td>
<td>Science Research Article (Website)</td>
</tr>
<tr>
<td>Keywords</td>
<td>Zebrafish, locomotion, development, genetics, neural networks</td>
</tr>
</tbody>
</table>
| Summary of key points (include methodology) | ● The embryonic behaviors appear sequentially and include an early, transient period of spontaneous, alternating tail coilings, followed by responses to touch, and swimming.  
● Patch clamp recording, analysis of the synaptic drive, and other methods of analysis were performed to understand the locomotor development in zebrafish.  
● Ends with a discussion on the current state of genetic tools for study of the molecular causes in locomotor network development. |
| Research Question/Problem/Need | What are the main stages of development for zebrafish’s locomotor activity? |
Important Figures

Fig. 1. Identified neurons of the developing zebrafish locomotor regions. Upper, photomicrograph of a 28 h embryo indicating key anatomical structures (modified from [90]). Bottom left: an illustration of the reticulospinal neurons in a five-day old larva (from [46]). The Mauthner neuron (M) is localized in the fourth rhombomere. Each cell type has a homologue in its contralateral rhombomere, however for simplicity only cells with contralateral axons are indicated on the bottom and only cells with ipsilateral axons are shown on the top. Lower-right: Summary scheme of the identified neurons in an hemi-segment of the spinal cord, adapted from [9]. The grey and white shadings stand for ipsilateral and contralateral neurons, respectively. The abbreviated cell names are explained in Section 2.1.

Fig. 2. (A) The chronological sequence of the appearance of motility patterns during development of the zebrafish. Images emphasize key stages of zebrafish embryo development before and after hatching (~32 h). (B) Schematic diagrams of the possible neural building blocks underlying early motility patterns in the zebrafish. Left: the neural network active during spontaneous tail coiling (17 h) is limited to the spinal cord and includes only primary motoneurons (PMN) and a restricted number of interneurons (presented as D-descending interneuron and Co-commisural interneuron, for more details see Section 3.1). The network activity is based on electrical coupling. Center: the initial touch response (21 h) requires the activation of both hindbrain neurons (M=Mauthner; Tr=Trigeminal) and spinal neurons including the sensory Rohon-Beard (RB) neurons. Dashed lines represent hypothetical connectivity. At this time the synaptic response is mediated partly by glutamatergic synapses (see Section 3.2). Right: at 27 h the embryo can swim in response to touch. Swimming requires integration of both hindbrain reticulospinal neurons (RS) and spinal cord interneurons (In) and secondary motor neurons (SMN). The exact network interactions are not fully understood but the synaptic drive to motoneurons comprises rhythmic glutamatergic and tonic glycinegic components (see Section 3.3).

Notes

- Patch clamp recording in vivo revealed that an electrically coupled network of a subset of spinal neurons generates spontaneous tail
- Chemicals synaptic drives named glutamatergic and glycinergic underlie touch responses and swimming and requires input from the hindbrain.
- Swimming becomes sustained in larvae once serotonergic neuromodulatory effects are integrated.
- A brief overview of the genetic tools available for the study of the molecular determinants implicated in locomotor network development in the zebrafish.
- Combining genetic, behavioral and cellular experimental approaches will advance our understanding of the general principles of locomotor network assembly and function.

| Cited references to follow up on | Buss, R. R., & Drapeau, P. (2002). Activation of Embryonic Red and White Muscle Fibers During Fictive Swimming in the Developing Zebrafish. *Journal of Neurophysiology, 87*(3), 1244-1251. [https://doi.org/10.1152/jn.00659.2001](https://doi.org/10.1152/jn.00659.2001)  

| Follow up Questions | 1) What is the percentage of similarity between humans and zebrafish’s CNS systems?  
| | 2) What are some ways at home to measure CNS activity of zebrafish?  
| | 3) Could genetic studies be incorporated into the project? What benefits would this yield? |
Article #17 Notes: Effects of acetaminophen (paracetamol) in the embryonic development of zebrafish, *Danio rerio*

<table>
<thead>
<tr>
<th>Source Title</th>
<th>Effects of acetaminophen (paracetamol) in the embryonic development of zebrafish, <em>Danio rerio</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Source type</td>
<td>Science Research Article (Website)</td>
</tr>
<tr>
<td>Keywords</td>
<td>acetaminophen, zebrafish, larval development, larval growth, embryotoxic effects, AO (Acridine Orange)</td>
</tr>
</tbody>
</table>
| Summary of key points (include methodology) |  ● Acetaminophen is an OTC generally used by all groups of people including pregnant women for fever, pain or inflammation.  
  ● This study aims to investigate the effects of acetaminophen on the development of zebrafish  
  ○ One key point is in zebrafish embryogenesis is *ex utero*  
  ● The areas of investigation for the adverse effects of acetaminophen were:  
  ○ The early development  
  ○ Hatching  
  ○ Organogenesis (by altering the rate of apoptosis)  
  ○ Larval growth and morphometry  
  ○ Tail and tail-fin formation  
  ○ Pigmentation  
  ○ Larval behavior  
  ○ Survival rate  
  ● Acetaminophen interfered with the normal embryonic development, growth, behavior and survival of zebrafish larvae |
| Research | What are the embryotoxic effects of acetaminophen on the development |
Question/Problem/Need

of zebrafish embryos?

Important Figures

Table 1. Effects of acetaminophen on the total mortality, hatching and morphometry of Danio rerio larvae

<table>
<thead>
<tr>
<th>Treatment groups (n = 30)</th>
<th>DW</th>
<th>DW + V</th>
<th>1 μg L⁻¹</th>
<th>5 μg L⁻¹</th>
<th>10 μg L⁻¹</th>
<th>50 μg L⁻¹</th>
<th>100 μg L⁻¹</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total mortality</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Number of larvae</td>
<td>29</td>
<td>29</td>
<td>27</td>
<td>27</td>
<td>26</td>
<td>22</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>hatched</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>27.05</td>
</tr>
<tr>
<td>Body mass (mg)</td>
<td>1.2</td>
<td>1.2</td>
<td>1.0</td>
<td>0.6</td>
<td>0.5</td>
<td>0.3</td>
<td>0.1</td>
<td>63.74</td>
</tr>
<tr>
<td>Body length (mm)</td>
<td>3.1</td>
<td>4.0</td>
<td>2.5</td>
<td>2.2</td>
<td>2.1</td>
<td>2.0</td>
<td>1.8</td>
<td>20.04</td>
</tr>
</tbody>
</table>

Values are means ± standard errors. DW = distilled water (control group); DW + V = distilled water + 0.1% aqueous ethanol (carrier control group).

*Significant at 5%; df = 6, 14. **Significant (P < 0.05) compared with DW + V group. ***Significant (P < 0.05) compared with lower (1 and 5 μg L⁻¹) groups. ****Significant (P < 0.05) compared with 10 μg L⁻¹ group.

Table 2. Deformities caused by acetaminophen on developing zebrafish embryos

<table>
<thead>
<tr>
<th>Deformities</th>
<th>DW</th>
<th>DW + V</th>
<th>1 μg L⁻¹</th>
<th>5 μg L⁻¹</th>
<th>10 μg L⁻¹</th>
<th>50 μg L⁻¹</th>
<th>100 μg L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lethal deformities</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congenital aneuploidy at 4 hpf</td>
<td></td>
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<tr>
<td>Congenital aneuploidy at 8 hpf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sub-larval deformities</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of pigmentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Intragonad deformities</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Deformity of tail and fins</td>
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</tr>
</tbody>
</table>

Figure 1. (A–F) Effects of acetaminophen on zebrafish development at gastrulation stages. Control embryos showing intact blastomeres (A) at 20% epiboly (A) and germ ring (B) stages. Infiltration of blastomeres (arrows) into chorionic space and yolk sac regions at 30% epiboly and germ ring stages in embryos exposed to 100 μg L⁻¹ (C) and 50 μg L⁻¹ (D) acetaminophen. Acidine orange-stained embryos (E and F) showing AO-positive cells (arrows) in C and D stages. 40x. This figure is available in colour online at www.interscience.wiley.com/journal/jat
Figure 2. Shows number of larvae that hatched from day 3 to day 5 in control (DW), carrier control (DW + V) and different concentrations of acetaminophen.

Figure 3. (A–C) Effects of acetaminophen on pigmentation in newly hatched Danio rerio larvae. Arrows indicate abundant pigment granules in retinal cells and stellate melanophores (mp) in cephalic, yolk sac and trunk regions in control larva (A). Reduced pigmentation in corresponding larvae exposed to 50 μg L⁻¹ (B) and 100 μg L⁻¹ (C) drug doses. 40x.
These medications are generally known as COX inhibitors as they interfere with the synthesis or the activity of cyclooxygenase (COX-1 or COX-2 or COX-3) enzymes involved in the production of prostaglandins, the ubiquitous bioactive lipid molecules.

Use of NSAIDs such as aspirin, ibuprofen, diclofenac, piroxicam and tolfmetin, around the time of conception and implantation and during pregnancy is associated with increased risk of miscarriage.

Although paracetamol and propyphenazone (isopropylantipyrine) individually or in combination with caffeine are known to induce no serious teratogenic effects except fetal growth retardation and low birth weight.

The present study is an attempt to evaluate the embryotoxic effects of acetaminophen, if any, on the development of zebrafish, Danio rerio.

Studies have been conducted using in vivo, intrauterine development (mammalian) models in which observations at timely intervals, especially in early development, are not possible; therefore, the results are largely focused on end point only.

All tests were run in triplicate.

Eggs undergoing cleavage (two/four celled stages) were exposed to five different concentrations (1, 5, 10, 50 and 100 µg/L) of acetaminophen dissolved in 0.1% aqueous ethanol. Corresponding distilled water and carrier controls were maintained.

Mortality and deformities of embryos/larvae, if any, were recorded at every 24 h interval, for seven consecutive days.

Lethal, sub-lethal, teratogenic effects and behavioral parameters were recorded.

When larvae had survived to 120 h, the body mass (mg) and body length (mm) were recorded. The body length (snout to tip of the tail) of larvae was measured on the day of hatching to the nearest
0.01 mm under a stereo zoom fitted with an ocular micrometer

- Swimming pattern and behavioral responses were recorded from the day of hatching until day 7
- A gentle stimulus applied to the larva using a glass rod/needle, a focus of incident light of the microscope on the larva and tapping of the Petri plate using a glass rod served as stimuli to elicit tactile, photo and auditory responses respectively in experimental animals
- The experimental and control embryos/larvae were stained at the end of each hour for 6 consecutive hours and once after every 24 hours for 7 consecutive days with 5 µg/ml acridine orange dissolved in E3 medium (5 mM NaCl; 0.17 mM KCl; 0.33 mM CaCl2; 0.33 mM MgSO4) at room temperature (27 ± 1 °C) for 15 minutes followed by 3 washings in the medium
- The data were analyzed using two-way ANOVA followed by Dunnett’s multiple comparison test
- In the control and carrier control groups there was no significant change in the rate of mortality
- In the 1 µg/L acetaminophen exposed group, although not significant, there was a slight increase in the rate of mortality (Table 1).
- The rate of mortality of embryos increased (P < 0.05) in the 5 and 10 µg/L drug groups.
- In the 50 and 100 µg/L acetaminophen exposed group, the mortality of embryos increased to 40 and 50% respectively.
- Mortality in the higher (50 and 100 µg) drug groups was high during the early developmental stages
- The hatching process in the control group began on day 3 and completed on day 4
- In the 1 µg/L acetaminophen-exposed group hatching extended up to day 5
- The rate of hatching decreased significantly (P < 0.05) and the hatching process was extended up to day 5 in the 10, 50 and 100 µg L−1 drug exposed embryos
- In the control group the larvae were healthy and active with 1.2 ± 0.03 mg body mass and 3.1 ± 0.03 mm body length and they survived for 7 days without external feeding
- Fish embryos exposed to lower (1 and 5 µg/L) concentrations of acetaminophen exhibited a significant (P < 0.05) reduction in body length and mass
- The body mass (by 50%) and body length were significantly (P < 0.05) lower in the larvae exposed to 50 µg/L
- In the 100 µg/L group the larvae possessed the lowest body mass and lengths compared with all other drug exposed and control embryo
- Larvae in the control, carrier control and lower (1 and 5 µg/L) drug exposure group showed abundant pigmentation in the epithelial cells of the retina and in the stellate melanophores present near
the cephalic and yolk sac regions.

- In the 50 and 100 µg/L drug-exposed larvae, acetaminophen did not affect the formation of pigment cells, but there was a significant (P < 0.05) dose-dependent decrease in the distribution of pigment both in retinal cells and melanophores at the head and in the yolk sac region.
- Pigmentation pattern or concentration did not affect survival but resulted in ‘albinos’ owing to the absence of the characteristic longitudinal stripes along the length of the body.
- Formation of the tail and shaping of the tail fin were apparent in control, carrier control and in lower (1 and 5 µg/L) drug exposed embryo.
- Deformity in tail and tail fin, a teratogenic effect induced by drug exposure, was evident in embryos exposed to 10 µg/L acetaminophen and the size of the tail fin was reduced compared with controls and lower (1 and 5 µg/L) drug exposed embryos.
- The tail fin in the larvae exposed to 50 and 100 µg/L acetaminophen was deformed owing to cells that invaded from the tail into the fin region.
- Zebrafish larvae in the control and carrier control, 1 and 5 µg/L drug groups exhibited quick swimming behavior and showed a positive response to light, sound and tactile stimuli.
- Larvae exposed to higher (10, 50 and 100 µg/L) doses of acetaminophen concentrations showed altered behavior swimming behavior and lack of response to external stimuli.
- Impairment of swimming pattern and restricted responses such as vibratory/shivering, un-coordinated body movements to external stimuli were exhibited in larvae exposed to 10 and 50 µg/L acetaminophen and these larvae survived for 5–6 days without external feeding.
- Most larvae in the 100 µg/L drug exposure remained immobile but few showed vibratory or shivering body movements to external stimuli; they survived only for few hours.
- In the developing fish embryos, the effects of acetaminophen were manifested at different stages of development in a dose dependent manner, affecting/altering growth and survival, completion of development and hatching, rate of apoptosis during organogenesis, larval morphometry, tail and tail-fin formation, pigmentation and larval behavior.
- Larvae with retarded growth had a limited life span and were not viable beyond day 5, suggesting indirectly the effect of the drug on survival competence.
- Acetaminophen affected responses not only to touch but also to sound and light stimuli in newly hatched larvae exposed to high doses.
- Although acetaminophen is considered apparently safe in therapeutic doses for short-term use, there is evidence that the
drug induces fetal anomalies when ingested continuously in high daily doses
- The exact mechanism of action of acetaminophen is not understood precisely, it is believed to have highly targeted action blocking the enzyme involved in transmitting pain
- As COX-1 and COX-2 are already characterized in zebrafish whether COX-3 isomerase is also present in fish system is an interesting question to answer that provides a direct basis for explaining the findings of the present study

Cited references to follow up on


https://doi.org/10.1006/eesa.2002.2231


BMJ, 327(7411), 368-0. https://doi.org/10.1136/bmj.327.7411.368


Neurotoxicology and Teratology, 26(6), 719-723.
https://doi.org/10.1016/j.ntt.2004.06.013

Follow up Questions
1) There was a previous paper which said that paracetamol did not showcase negative effects, what was different in that?
2) What are the adverse effects for the zebrafish when they become adults?
3) Were there any genetic deficiencies in the population?

Article #18 Notes: Developmental exposure to acetaminophen does not induce hyperactivity in zebrafish larvae

Source Title
Developmental exposure to acetaminophen does not induce hyperactivity in zebrafish larvae
## Source citation (APA Format)

[https://doi.org/10.1007/s00702-016-1556-z](https://doi.org/10.1007/s00702-016-1556-z)

## Original URL


## Source type

Science Research Article (Website)

## Keywords

locomotion, behavior, impulsivity, *latrophilin3.1*, Acetaminophen, zebrafish

## Summary of key points (include methodology)

- A common pain relief medication during pregnancy is acetaminophen, as it is generally considered safe to use during gestation.
- Recent studies indicate a risk of developing ADHD-like symptoms in children if mothers use acetaminophen during pregnancy.
- Exposure to high doses of acetaminophen concentrations causes liver toxicity.
  - Previously investigated in different model organisms
- Common endophenotype for ADHD is hyperactivity.
- Zebrafish were used to investigate the potential impact of acetaminophen to compare locomotor activities of wild type zebrafish to a well-established ADHD zebrafish model *latrophilin3.1* (*Lphn3*).
- Neither acute nor chronic exposure to acetaminophen at sub-liver-toxic concentrations in wildtype or *lphn3.1* knock-downs increases locomotor activity levels.
- Findings show that embryonic or larval exposure to acetaminophen does not cause hyperactivity in zebrafish.
- No additive or synergistic effects of acetaminophen were found either.
- Research concludes that there is no direct link between embryonic acetaminophen exposure and hyperactivity.

## Research Question/Problem/Need

Does the exposure to acetaminophen during pregnancy for mothers increase the likelihood of ADHD in children?
**Important Figures**

![Images of zebrafish larvae and graphs](image)

**Fig. 1** Chronic exposure to APAP causes dose-dependent morphological defects and loss of the liver marker \( fslph \). a-f: 6 dpf live fish larvae chronically exposed to increasing concentrations of APAP between 0 and 6 dpf. g-i: 3 dpf zebrafish larvae processed for in situ hybridization to detect \( fslph \) transcripts after chronic exposure to increasing concentrations of APAP between 0 and 3 dpf. Note gradual loss of \( fslph \) transcripts (arrow head). a: 0 mg/l, b: 0.5 mg/l, c: 1.25 mg/l, d: 2.5 mg/l, e: 5 mg/l, f: 10 mg/l, g: 25 mg/l, h: 50 mg/l, i: 100 mg/l. Scale bar in a, 500 μm, and in G, 250 μm. a-qPCR showing down regulation of \( fslph \) after chronic exposure to indicated APAP concentrations between 0 and 3 dpf. Bars and error bars illustrate mean ± SD of three independent experiments expressed as fold change in relation to the untreated control (0 mg/l). Levels of \( fslph \) were normalized against \( gdpd \).

**Fig. 2** Chronic APAP exposure does not affect the locomotor activity in 6 dpf wild-types or \( \text{phn3.1} \) morphant zebrafish larvae. Analysis of total distance swim (cm) and number of entries into inactivity, slow or fast swimming (μ) during a 5 min interval in zebrafish larvae treated with indicated concentrations of APAP between 0 and 6 dpf. Uninjected control larvae are compared to \( \text{phn3.1} \) morphants in a. Numbers on bars indicate total number of analyzed individuals and are the same for a and b. Bars and error bars illustrate mean ± SD. *p < 0.05.
First line pain relief medication during pregnancy relies, especially in the third trimester, nearly entirely on the OTC analgesic acetaminophen.

- Also known as APAP

Children received more often a hyperkinetic disorder diagnosis, ADHD-medication prescription or having ADHD-like behavior at 7 years of age, if the mothers used APAP during pregnancy.

Suggests that the prenatal APAP intake in mothers might alter brain development, which then manifests in an ADHD-like condition.

ADHD is one of the most common childhood-onset behavioral disorders, with an overall prevalence of 7.2%.

In this condition, school-age patients display higher levels of motor activity, impulsivity and inattention.

- Genetic research has revealed several candidate genes/variants associated with the disorder including SLC6A3, SLC6A4, DRD4, DRD5, LPHN3

Several environmental or other factors during pregnancy have been identified that influence ADHD occurrence. These include low birth weight, delivery complications, food additives, toxin or drug exposure.

APAP might alter normal brain development and that APAP might...
interfere with endocannabinoid signaling and BDNF expression.

- Sub-liver-toxic APAP concentrations have not been experimentally investigated with respect to ADHD endophenotypes such as hyperactivity.
- Wild-type or *lphn3.1* morphant embryos were treated with indicated concentrations of APAP dissolved in Danieau’s solution during indicated time intervals.
- The APAP solution was made fresh and exchanged every day during the treatment period.
- To prevent pigmentation of specimens used for in situ hybridization, 0.2 mM N-phenyl-2-thiourea was added to the medium.
- RT-PCR Analysis
  - Locomotor activity was analyzed at 6 dpf under dark conditions by measuring the total distance swum.
  - The total distance swum for each individual was determined as the sum of distances reached during inactivity, slow and fast movements.
  - *p* values were calculated using two-tailed, unpaired or paired t-tests or one-way ANOVA followed Tukey’s post hoc method using Excel and R.
    - *p* values < 0.05 were considered significant.
- To investigate the effects of chronic exposure to APAP on the development of zebrafish larvae the researchers exposed late blastula stage embryos (4 hpf) to increasing APAP concentrations until 6 dpf when the living larvae were analyzed for morphological changes.
- APAP concentrations up to 100 mg/L did not cause any morphological defects visible with light microscopy. 250 and 500 mg/L resulted in dose-dependent developmental malformations.
  - reduced pigmentation, lack of/failure to inflate swim bladder, curved body axis, oedema, reduced brain and eye structures and pronounced cell death.
- 1000 mg/l was lethal prior to 6 dpf in most cases.
- Locomotor activity levels of 6 dpf larvae were measured using total distance swum during a period of 5 min.
- Impulsivity was estimated by counting the number of entries into inactivity, slow or fast swimming.
- Controls entered inactivity 343.0 ± 145.7 times during 5 min, slow swimming 512.9 ± 255.1 times and fast swimming 184.5 ± 122.7 times.
- After acute treatments, no impact on morphology was observed and therefore higher concentrations of APAP were included (up to 1000 mg/l) in the analyses.
- After exposure to 10, 100, 500 or 1000 mg/L APAP the total distance swum and the number of entries were significantly reduced as compared to before treatment.
The activity levels decreased after acute treatment with 10 mg/l or more APAP.

- Here, to test if APAP is influencing locomotor activity in a genetic susceptible background, *lphn3.1* was knocked down by morpholino injections.
  - Confirmed by RT-PCR.
- *lphn3.1* morphants reach a longer total distance by increasing durations of slow and fast swimming and increasing the speed.
- *lphn3.1* morphants switch more frequently between different types of activities compared to controls.
- Acute treatment with 0.5 or 500 mg/L APAP of *lphn3.1* morphans did not significantly change the total distance swum.
- Therefore, *lphn3.1* is not more sensitive to APAP exposure with respect to motor activity levels.
- The findings show that larval zebrafish do not exhibit hyperactivity traits after chronic or acute exposure to APAP.
- Our results confirm that zebrafish larvae are sensitive to chronic APAP exposure in a dose-dependent manner, similar to what has been reported previously.
- In zebrafish the metabolic pathways activated by APAP are similar to that of other common liver toxicity models in mammals.
- It has been reported that APAP affects the structure of the zebrafish larval nervous system.
- Such changes, i.e. reduction of motor axon lengths, are likely to rather reduce locomotor activity.
  - The researchers did observe reduced activity levels after acute APAP concentrations starting at 10 mg/L.
- Possible result of the study is that APAP is a risk factor only in humans.
  - It could be that due to human specific nervous system development or metabolism the phenotype cannot be provoked in animal models.
- Prompts for further mechanistic investigations of APAP impact on the developing brain to explain the correlation, seen in human epidemiological studies, between exposure to APAP during embryonic development and the increased risk of developing ADHD.

<table>
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<th>Cited references to follow up on</th>
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Follow up Questions

1) What were the long term effects for the zebrafish in this study? Was hyperactivity seen in later stages of the life-cycle?
2) Does decreased locomotor activity mean that the zebrafish will have weaker reflexive behaviors?
3) What are some steps that could be taken to indicate that APAP is not only a risk factor for humans? Are there any other model organisms who have shown adverse effects that relate to ADHD?
### Article #19 Notes: Effects of embryonic exposure to ethanol on zebrafish visual function

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<td>Source type</td>
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<tr>
<td>Keywords</td>
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<tr>
<td>Summary of key points (include methodology)</td>
<td>● The visual capability of the zebrafish were assessed:  ○ Physiologically  ○ ERGs  ○ Behaviorally (optomotor response)  ● Zebrafish larvae were exposed to 1.5% ethanol at various times during development  ○ Includes phases of maximum visual development  ● Results show that ethanol effects were most adverse and evident during eye development.</td>
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<tr>
<td>Research Question/Problem/Need</td>
<td>What are the effects of embryonic exposure to ethanol on visual cues for zebrafish?</td>
</tr>
</tbody>
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Important Figures

Fig. 1. Mean eye diameter of control subjects ($n=44$) and subjects exposed to 1.5% ethanol at 6–24 ($n=10$), 12–24 ($n=39$), 24–36 ($n=10$), 48–60 ($n=10$), and 60–72 ($n=15$) hpf. Subjects were examined at 3 dpf. Error bars represent ±1 S.E.M. Groups designated with a single asterisk (*) indicate that these groups are significantly different from the other groups; groups designated with double asterisks (**) indicate that the two groups are significantly different from one another. All significance levels are $P<.05$.

Fig. 2. Mean optomotor response score of control subjects ($n=23$) and subjects exposed to 1.5% ethanol at 6–24 ($n=17$), 12–24 ($n=7$), 24–36 ($n=16$), 48–60 ($n=23$), and 60–72 ($n=18$) hpf. Subjects were examined between 6 and 9 dpf. Mean values are estimated marginal means from the covariance analysis. The dotted line shows the average baseline response score. Error bars represent ±1 S.E.M. Groups designated with a single asterisk (*) indicate that these groups are significantly different from groups with a double asterisk (**); groups designated with the letter ‘a’ are significantly different from one another. All significance levels are $P<.05$. 
Embryos exposed to ethanol display eye abnormalities as well as deficiencies in visual physiology and behavior. Visual function was assessed physiologically, via electroretinogram (ERG) recordings, and behaviorally, by measuring visual acuity with the optomotor response. Zebrafish larvae were exposed to 1.5% ethanol at various times during development, including the period of maximal eye development. Results show that ethanol effects on visual function were most pronounced when exposure occurred during eye development. ERG recordings from ethanol-exposed larvae differed from normal.
subjects:
  - in shape of the response waveform
  - in visual thresholds under both light and dark adaptation
  - the differences were more pronounced under lower levels of adaptation
- Ethanol-exposed larvae displayed lower visual acuity as determined from the optomotor response.
- Results indicate embryonic ethanol exposure affects visual function particularly when exposure occurs during eye development.
- Thus zebrafish are a viable option for studying Fetal Alcohol Syndrome (FAS).

| --- | --- |

| Follow up Questions | 1) Are there adverse effects on optomotor response for zebrafish when exposed to drugs and OTCs?  
2) How does the visual response of ethanol translate to human populations?  
3) Do different concentrations display different effects or does high concentration as a whole display a singular effect? Were there any subdued effects in lower concentrations? |
Article #20 Notes: Adverse effect of synthesized Naringenin derivatives investigate with Zebrafish (*Danio rerio*) embryos

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<th>Source Title</th>
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<td>Source type</td>
<td>Science Research Article (Website)</td>
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<tr>
<td>Keywords</td>
<td>zebrafish, <em>Danio rerio</em>, Naringenin, toxicity, fatty acid</td>
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</tbody>
</table>
| Summary of key points (include methodology) | ● Naringenin derivatives was synthesized through esterification of acid chlorides with Naringenin under ambient reaction conditions with good yields.  
● Zebrafish embryos were exposed to the synthesized compounds to find the toxicity of the compounds.  
● Adverse drug effect assessment in zebrafish embryos showed less toxicity towards 3a, more than the other compounds. |
| Research Question/Problem/Need | What are the adverse side effects of newly synthesized Naringenin on zebrafish embryos? |
| Important Figures | ![Scheme 1. Synthesis of Naringenin derivatives (3a-e).](image) |
Table 1: Synthesis of Naringenin derivatives (3a–e).

<table>
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<th>R</th>
<th>t (h)</th>
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<td>5</td>
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![Image of embryos showing different phenotypic deformities](image_url)

**Fig. 1.** Phenotypic deformities in embryos using Naringenin synthesized compounds. A) Control embryo-1 (b), B) Control embryo-2, C) 3b causes pericardial bulging at 10 µg/ml, D) 3e causes hemorrhage at 200 µg/ml, E) 3d causes ophthalmic damage at 500 µg/ml and F) 3b causes cardiac malformation at 50 µg/ml.

![Heart rate analysis chart](chart_url)

**Fig. 2.** Adverse drug effect studies using Heart beat rate analysis for Naringenin derivatives.
[Image 200x605 to 536x712]

Notes

- Naringenin is a common compound found in citrus fruits:
  - High levels found in grapefruit (43.5 mg/100 mL)
  - Lower levels found in orange juice (2.13 mg/100 mL)
  - Significantly lower in lemon juice (0.38 mg/100 mL)
- Naringenin has been studied for its pharmacological effects:
  - Antioxidant, anti-inflammatory, immunomodulatory, hepatoprotective, nephroprotective, neuroprotective, anti-cancer, anti-atherosclerotic, and anti-diabetic properties
- Naringenin could alleviate LPS-induced neuroinflammation, as was evident from attenuation of oxidative stress and modulation of Nrf2
- Every compound has unique properties and a mode of action for its nature.
- For chemically synthesized compounds there is a set of unique properties.
  - These unique properties include: broad spectrum activity, toxicity, binding site and dosage level.
  - This is both target and dose dependent, since overdose may cause adverse effects in the heart, brain, head and liver cells.
- Zebrafish assays calibrate the dysfunction of heart and lethal toxicity of compounds at low or high dosage.
- Five chemically synthesized compounds from one compound were used in the present study.
- Toxicity and adverse drug effects were assessed based on the dose dependent cardiotoxicity, dose dependent lethality and phenotypic deformities of each antibiotic in zebrafish embryos.
- The embryos were treated with varying concentrations of compounds at 32 (μg/ml), 100 (μg/ml), 250 (μg/ml), 500 (μg/ml) and the Heart Beat Rates (HBR) was examined under a light microscope and analyzed.
- Treated and control embryos were monitored for 24 h for its dose dependent lethality.
- Each day embryos were monitored under the microscope for any abnormalities in the organ development of the brain, eye, heart, ear, somite, notochord, trunk, tail and fins.
- Heart beat rate assessment was performed to find the off target
effects in 2 dpf zebrafish embryos

- The cardiac assay revealed that the increasing concentrations of drug dose showed variations in HBR either increase or decrease.
- 3a was found to be least toxic upto 1000 μg/ml
  - Effects included eye deformity and cardiac chamber bulging
- 3b displayed fast & slow heart beats, mouth damage, eye damage and thrombosis.
- 3c displayed brain damages.
- 3d displayed intestine damage, eye damage and reduces the heart beat.
- 3e exhibited more toxic than the other compounds. 3e shows thrombosis.
- All the compounds displayed damages towards the brain, eye, mouth and intestine.
  - Some compounds displayed worse effects like thrombosis, pericardial edema and cardiac malformation.

| Follow up Questions | 1) Why was Naringenin used in this experiment? Were there cases of |


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<table>
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<tbody>
<tr>
<td></td>
<td>death related to this compound?</td>
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<tr>
<td>2)</td>
<td>Do other flavonoids create similar adverse effects for organisms?</td>
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<td></td>
<td>Zebrafish?</td>
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<td>3)</td>
<td>What structural component made 3e the most lethal?</td>
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# Article #21 Notes: Molecular and behavioral responses of zebrafish embryos/larvae after sertraline exposure

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<th>Molecular and behavioral responses of zebrafish embryos/larvae after sertraline exposure</th>
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<tr>
<td>Source type</td>
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<tr>
<td>Keywords</td>
<td>antidepressant drugs, accelerated hatching, behavioral alterations, Serotonin signaling, zebrafish</td>
</tr>
</tbody>
</table>
| Summary of key points (include methodology) | ● Embryo exposure to sertraline accelerated the hatching rate.  
● Sertraline reduced larval locomotor behaviors.  
● Low concentrations of sertraline increased dark-avoidance behavior of larval zebrafish.  
● Sertraline up-regulated the expression of *serta* and *5-ht2c*. |
| Research Question/Problem/Need     | What are the behavioral and molecular responses in larval zebrafish following Sertraline exposure with a niche in mode of action? |
| Important Figures                  | ![Important Figures](https://www.sciencedirect.com/science/article/pii/S0147651320315372) |
Fig. 1. Sertraline effects on the development of zebrafish larvae after 6 d exposure (mean ± SEM). (A) Early spontaneous movement rate at 28–29 hpf (n = 10); (B) Hatching rate at 48–62 hpf (n = 30); (C) Heart rate at 72 hpf (n = 12); (D) Body length at 96 hpf (n = 10). Asterisk denote significant differences (One-way ANOVA followed by Holm-Sidak’s multiple comparisons test; **p < 0.01) between treatments and controls.

Fig. 2. Sertraline effects on the behavior of zebrafish larvae after 6 d exposure over 50 min during the dark phototaxis behavioral assay (mean ± SEM). (A) Group mean of the distance moved (mm) per minute intervals; (B) Total distance moved (mm) per 10 min intervals of the light and dark cycles; (C) Group mean of the mobile cumulative duration (s) per minute intervals; (D) Total mobile cumulative duration (s) per 10 min intervals of the light and dark cycles. Bars with different letters denote significant differences from each other (One-way ANOVA followed by Holm-Sidak’s multiple comparisons test; n = 24; p < 0.05).
Fig. 3. Sertraline effects on the anxiety-like behavior and locomotor behavior of zebrafish larvae after 6 d exposure over 60 min in the dark-light preference assay (mean ± SEM). (A) Group mean of the cumulative duration per minute in dark zone per intervals; (B) Cumulative duration (s) of larval zebrafish in dark zone per 15 min intervals; (C) Group mean of the frequency of visiting the dark zone per minute intervals; (D) Frequency of visitation in the dark zone per 15 min intervals; (E) Total distance moved (mm) in whole arena, dark zone and light zone over 1 h; (F) Mean velocity (mm/s) in whole arena, dark zone or light zone over 1 h. Bars with different letters denote significant differences from each other (One-way ANOVA followed by Holm-Sidak’s multiple comparisons test; n = 24; p < 0.05).
Sertraline (SER) is one of the most frequently detected antidepressant drugs in aquatic environments.

SER has been found to have adverse effects on fish populations, yet the data and research is insufficient.

Zebrafish embryos were exposed from 6hpf to 6dpf with three separate concentrations of SER (1, 10, and 100 μg/L).

The following aspects were measured:
  - Development
○ Behavior
○ Transcripts related to serotonin signaling
○ Serotonin levels
○ Acetylcholinesterase activity

● Accelerated hatching rate was observed for the population exposed to 100 μg/L SER (measured at 54 hpf).
● Locomotor activity was significantly reduced in larval zebrafish following exposure to 10 and 100 μg/L SER.
● Increased dark-avoidance after exposure to 1–100 μg/L SER.
● Only serotonin transporter (serta) and serotonin receptor 2c (5-ht2c) mRNA levels were increased in fish in response to 10 μg/L SER treatment.
  ○ serotonin levels were unaltered in larvae exposed to SER.
● No differences among groups in AChE activity at any concentration tested.
● Exposure to SER alters behavioral responses in early-staged zebrafish, which may be related to the abnormal expression of 5-ht2c.
● Molecular responses to SER and characterizes targets that may be sensitive to antidepressant pharmaceuticals in larval fish.

Cited references to follow up on


[https://doi.org/10.1016/j.aquatox.2014.01.007](https://doi.org/10.1016/j.aquatox.2014.01.007)


### Follow up Questions

1) What are other molecules similar in structure to SER?
2) Will these adverse effects translate to human populations in any shape or form?
3) What is the methodology to confirm that zebrafish are avoiding dark hours? Increased death rate during dark hours? Reduced locomotor activity?
Article #22 Notes: Zebrafish embryos as an alternative model for screening of drug-induced organ toxicity

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<td>Keywords</td>
<td>zebrafish, model, toxicity, hepatotoxicity, RNA-seq</td>
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| Summary of key points (include methodology) | ● A major reason for the attractiveness of this small teleost is associated with the suitability of its embryonic life stage.  
● Zebrafish embryos have the capability of high-throughput analysis and can be used in screenings for low toxicity of drug candidates.  
● Hepatotoxicity is one of the major concerns of organ toxicity in drug development.  
● Identification of hepatotoxicity as early as possible in the drug development process would reduce the costs of development.  
● Zebrafish embryos can be used to identify hepatotoxicity.  
● However histopathological effects and individual gene responses may differ among vertebrates. Therefore it is very important to not translate zebrafish embryos data exactly on human populations.  
● Identification of key events and conserved mechanisms in zebrafish can be supported by toxicogenomic analysis.  
● Transcriptional profiling of embryos enables organ-specific profile analysis.  
● Next generation sequencing (NGS) and methods like transcriptome profiling by RNA-sequencing (RNA-seq) are provided also for the zebrafish embryo model. Allows for deeper analysis.  
● Using RNA-seq novel transcripts from annotated or non-annotated regions not available in predefined probe sets, alternative splicing forms and rare transcripts can be detected.  
  ○ This could be important for the comparative analysis and identification of conserved key toxicity pathways in vertebrates.  
● RNA-seq is limited due to expensive methodology associated with
- RNA-seq may also be applied for an organ-specific analysis, by using transgenic strains, in which specific organs are labelled by the expression of a reporter gene or protein.
- RNA-seq will be complementary to microarrays since they have limitations.
  - Important signals detected by microarrays may not be sufficiently read due to coverage limitations
- A major issue for the extrapolations from zebrafish embryos to humans is also how the effect concentrations in embryos can translate to appropriate effect levels in mammalian models.
- For mammalian organisms drugs are often administered by oral doses leading to specific time courses of plasma concentrations depending on the adsorption, distribution, metabolization and excretion rates of the drug.
- In fish embryos, exposure is static and internal concentrations are established by partition equilibrium.
- Physiologically based kinetic models could provide a link between fish embryo and mammalian toxicity, but such models have not been developed for zebrafish yet.
- An alternative approach could be the comparison of concentration–response curves for drug targets (therapeutic targets) with the appropriate curves for adverse effects such as hepatotoxicity.
  - This requires, however, also an appropriate concentration–response analysis that is lacking in most of zebrafish embryo studies with molecular endpoints or toxicogenomic analysis

<table>
<thead>
<tr>
<th>Research Question/Problem/Need</th>
<th>How do zebrafish embryos model organ toxicity and what are the current methods to perform these studies? What are the current drawbacks with the results?</th>
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<tr>
<td>Important Figures</td>
<td>N/A</td>
</tr>
<tr>
<td>Notes</td>
<td>See summary of key points</td>
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Ecotoxicology and Environmental Safety, 76, 11-22.

https://doi.org/10.1016/j.ecoenv.2011.10.010


https://doi.org/10.1016/j.cbpc.2008.11.006


https://doi.org/10.1016/j.aquatox.2009.12.008

Follow up Questions

1) How can RNA-seq be applied to my study? What are the basic requirements to incorporate this into my study?
2) What are the advantages of RNA-seq? What are similar methods which are cheaper, and provide an equal amount of data?
3) Does zebrafish genome similarity allow for more translatable results? If not, how do other studies incorporate and make connections between humans and zebrafish?

Article #23 Notes: Time course of the development of motor behaviors in the zebrafish embryo

| Source Title | Time course of the development of motor behaviors in the zebrafish embryo |
https://doi.org/10.1002/(SICI)1097-4695(199812)37:4<622::AID-NEU10>3.0.CO;2-S |
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<td>zebrafish, embryo, contractions, touch response, lesion, hindbrain, spinal cord, swimming</td>
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<tr>
<td>Summary of key points (include methodology)</td>
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• The development and properties of locomotor behaviors in zebrafish embryos were tested.  
• Different methods like video analysis, deliberate probing, lesions to different sections of the brain, and analysis spontaneous contractions were implemented.  
• Results suggested the spontaneous contractions result from activation of a primitive spinal circuit, while touch and swimming require additional hindbrain inputs to elicit mature locomotor behavior. |
| Research Question/Problem/Need | What are the stages of development of the locomotor system of zebrafish, and what are the basic prerequisites for each stage? |
Important Figures

**Figure 1** Example of one cycle of spontaneous contractions of an agarose-restrained embryo at 19 hpf. Images were captured every 15 frames (250 ms). The embryo was dorsal side up with the head facing the top of the image. The behavior consisted of a slow contraction to the left followed by a contraction to the right. The length of the embryo was 1.4 mm.

**Figure 2** Development of spontaneous contractions. Each data point represents the average frequency of contractions and standard errors for all freely moving embryos examined at each time point. Measurements were performed on four to eight embryos per batch, and two to six batches were used per time point. Average n per time interval = 10; range, 8–44.
Figure 3. Example of a touch response at 25 hpf in a freely moving embryo. Each successive tail image is separated by 4 ms. For clarity, arrowheads point to the tip of the tail. In this example, the embryo is dorsal side up with the rostral end facing the top of the image. Note that the embryo held its contracted position during the last three frames. The total length of the embryo was 1.9 mm.

Figure 4. Onset of the touch response. The onset of the touch response was evaluated for each agarose-restrained embryo tested by comparing the contraction frequency during repetitive stimulation (at 1 or 2 Hz) to the frequency during control periods (without repetitive stimulation) at different times of development, as described in the text. The open circles are the results for prestimulation controls, the closed circles are poststimulation controls, the closed triangles are 1-Hz stimulation and the open triangles are 2-Hz stimulation. Error bars represent standard error. Measurements were performed on three to five embryos per batch, and one to three batches were used per time point. Average n per time interval = 8, range 3–13.
Zebrafish embryos were raised at 28.5°C.

3 stages of locomotor activity were identified when freed from the chorion (sequentially):
- A transient period of alternating, coiling contractions
- Followed by touch-evoked rapid coils
- Finishing off with organized swimming

The three different behaviors were recorded by video microscopy.

Spontaneous, alternating contractions of the trunk appeared at 17 hpf, with a frequency of 0.57 Hz, peaked at 19 hpf at 0.96 Hz, and gradually decreased to <0.1 Hz by 27 hpf.

Starting at 21 hpf, touching either the head or the tail of the embryos resulted in vigorous coils.
- The coils accelerated with development, reaching a maximum speed of contraction before 48 hpf, which is near the time of hatching.

At 27 hpf touching the embryos, particularly on the tail, could induce partial coils.

At 27 hpf embryos started to swim in response to a touch, preferentially to the tail.

The swim cycle frequency gradually increased with age from 7 Hz at 27 hpf to 28 Hz at 36 hpf.

Lesions of the central nervous system rostral to the hindbrain had no effect on the three behaviors.

Lesioning the hindbrain eliminated swimming and touch responses, but not the spontaneous contractions.
| Follow up Questions | 1) Are the stages of locomotor activity similar to other mammals, and specifically humans?  
2) Are there any differences in the hindbrain between zebrafish and humans?  
3) Would there be an impact if there was a group of zebrafish which were exposed to drugs or OTCs? Delayed developmental stages? |
<table>
<thead>
<tr>
<th>Source Title</th>
<th>Abuse-resistant hydrocodone compounds</th>
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<tbody>
<tr>
<td>Source type</td>
<td>Patent</td>
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<tr>
<td>Keywords</td>
<td>Painkillers, hydrocodone, addiction</td>
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| Summary of key points (include methodology) | ● This patent decreases the potential for abuse of hydrocodone by covalent modification.  
● The invention provides methods of delivering hydrocodone as conjugates  
● Hydrocodone compositions of the invention are resistant to oral abuse  
● Some alternates like ribo-hydrocodones or galacto-hydrocodone have been tested |
| Research Question/Problem/Need | How to manipulate hydrocodones, a very common addictive painkillers for, by methods like covalent modification so that there is a decreased risk in abuse and addiction? |
Important Figures

Figure 1: The summarizing of chemical reactions made to produce Galacto-hydrocodone, one of the alternates

Figure 3: The summarizing of chemical reactions required for production of ribo-hydrocodone.

Notes

- The new medication method releases the hydrocodone following oral administration
- Resistant to abuse by injection and sniffing.
- Release of the hydrocodone at the prescribed doses reaches saturation after proper administration

Cited references to follow up on

N/A
| Follow up Questions | 1) How could covalent modification be used in my project to change the molecular structure of common OTC painkillers?  
2) Are there any other methods like covalent modification which could be implemented in my project?  
3) What is the time duration required for covalent modification and what resources do I need for that? |
# Patent #2 Notes: Pharmaceutical Compositions for the Deterrence and/or Prevention of Abuse

Article notes should be on separate sheets

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<thead>
<tr>
<th>Source Title</th>
<th>Pharmaceutical Compositions for the Deterrence and/or Prevention of Abuse</th>
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<tr>
<td>Source type</td>
<td>Patent</td>
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<tr>
<td>Keywords</td>
<td>Painkillers, addiction, polymer, antagonist, agonist</td>
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**Summary of key points (include methodology)**

- Invention includes possible methods for creating medication less prone to addiction where there is an antagonist, agonist, seal coat, and at least one polymer unit.
- The antagonist and agonist do not have direct contact
- Delivered to the patient in a regular way, no special mode of delivery
- There is a second method where the release the composition in vivo at 37 degrees Celsius but this compromises incubation and overall effectiveness.

**Research Question/Problem/Need**

- Although opioids are effective pain management medications, there has been an increase in abuse by individuals who are psychologically dependent and by individuals who do not use it for the intended purpose.
Study more about the different types of polymers.

Go more in depth when you get time about the different methods presented by the authors and try to see if anything can be used for our STEM project.

1) How prevalent are these processes mentioned by the authors used in the world today?
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<td>2)</td>
<td>What is the statistical data that shows the benefit to the processes mentioned?</td>
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<td>3)</td>
<td>How expensive and time efficient are these methods?</td>
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