The Negative Effects of Sodium Benzoate on Zebrafish Embryos

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Abstract

The consumption of sodium benzoate has led people to believe it negatively affects the human body. When sodium benzoate is consumed by a pregnant woman, it affects not only herself but her child as well. To observe these effects, zebrafish embryos were exposed to sodium benzoate concentrations and observed for the following 96 hours post-fertilization. Two groups of 40 zebrafish embryos were exposed to sodium benzoate at concentrations of 750 ppm and 1000 ppm. Another group of 40 embryos was a part of the control in an instant ocean solution. The embryos exposed to sodium benzoate were in a concentration that was at an intensity comparable to the maximum daily intake the FDA approved. None of the zebrafish survived past 48 hours of living in sodium benzoate. Comparing the intoxicated embryos to the control, deformities, and delay in the survival rate all showed to be significant. By observing this experiment, connections can be made between the development of the human body and zebrafish. A relationship can be made with how the structure of the body changes and the rate at which development occurs when sodium benzoate is consumed. This experiment is important because all it takes is a can of soda every day to leave neurological effects on children. The findings from this experiment will help awaken people about the risk they take when they consume large quantities of sodium benzoate regularly.

Introduction

Sodium Benzoate is a common preservative found in many different foods, beverages, and personal care products. It is often used to prevent spoilage and prolong shelf life in acidic foods such as jams and soda. Sodium benzoate is a man-made compound from benzoic acid and sodium hydroxide. Benzoic acid is a natural preservative by itself and naturally occurs in foods like apples and cinnamon. Sodium Benzoate is the

first preservative approved by the Food and Drug Administration (FDA) and classified as safe when used for the correct intentions. It is used in medications to allow tablets to break down rapidly after swallowing and can be found in household items such as baby wipes and toothpaste (McCulloch, 2019).

Sodium Benzoate is believed to trigger hyperactivity in children with attention-deficit hyperactivity disorder (ADHD). It also can form benzene which is a chemical linked to increased risk for leukemia and other cancers. Benzene is found in soft drinks between two and 20 parts per billion when the safe level is about five parts per billion (Mischel, 2020). Animal studies have suggested sodium benzoate can inflame pathways in the body promoting cancer development (McCulloch, 2019).

Zebrafish studies have concluded that there were morphology changes in muscle fibers and defects in the motor neurons present. Gut abnormalities and pericardial sac edema were also present after treatment with Sodium Benzoate (Tsay et al 2007). Sodium Benzoate exposure also showed a significant decrease in the survival rates of zebrafish embryos and neurological deficits (Chen et al 2009).

Groundbreaking research has been conducted with the use of zebrafish embryos. Zebrafish embryos are prime candidates for research studies because the females can regenerate eggs every two to three days. They lay hundreds of eggs making hundreds of test subjects. For being a vertebra, zebrafish have a short gestation period lasting three to four months. After fertilization has occurred, zebrafish start to exhibit food-seeking behaviors within 72 hours (Shapiro, 2012). Even though rodents such as rats have advantages when it comes to showing similarities in their nucleotide sequences with humans, they are costly when it comes to placing them in a controlled environment. Zebrafish are a cost-efficient model due to their capability to survive in highly populated environments (Tran et al., 2017).

In this experiment, zebrafish embryos will be treated with different concentrations of Sodium Benzoate. If zebrafish embryos are exposed to sodium benzoate over an extended period post-fertilization, they will experience neurological defects, physical deformities, and an elevated death rate when compared to non exposed embryos.

Materials

Materials and Equipment	
Quantity	**Item**
1 bottle each	Stock Solutions of Sodium Benzoate (750 ppm,1000 ppm)
1	Beaker for dead embryos and liquid disposal
1	Tape roll for labeling
1	Sharpie
1	Bottle of Instant Ocean/ Embryo Media Solution
5	Disposable pipette, minimum bore, 1.5 mm for transferring eggs
5	Disposable pipette 1mL
1	Sterile well plate with cover
1	28.5℃ incubator
1	Compound Microscope
1	Dissecting Microscope

Procedure

Day 1:

1. Receive petri dish, holding embryos from the teacher.
2. Obtain a sterile well plate and label with a name, hour, control, and toxicant.
3. Use a 1mL disposable pipette to separate 10 embryos into one well. Repeat this step 3 more times until there are 4 wells, each filled with 10 embryos. This is the control.
4. Place the plate under the microscope and observe. Replace the dead embryos with living embryos.
5. Remove old instant ocean solution from the wells. Use a 1 mL disposable pipette to transfer 2 mL of clean instant ocean solution into the wells.
6. Repeat steps 3 and 4.

7. Remove old instant ocean solution from the wells. Use a 1 mL disposable pipette to transfer 2 mL of Sodium Benzoate concentrated to 750 ppm.
8. Repeat steps again for the second concentration of Sodium Benzoate at 1000 ppm.
9. Place the plate into the 28.5℃ incubator overnight.

Day 2:

1. Remove the plate from the incubator.
2. Place the plate under the microscope and observe the control. Record the observations in the data table and note any abnormalities.
3. Remove dead embryos with a disposable pipette. Next, place all of the dead embryos in a waste beaker.
4. Count the remaining embryos, hatched fish, and record in the data table.
5. Remove instant ocean solutions from each well.
6. Place 1-2 embryos on a depression slide and observe and record observations. Next, return the embryos to the correct wells.
7. Use a 1mL disposable pipette to transfer 2 mL of clean instant ocean solution into the wells.
8. Repeat steps 2-6 for the embryos exposed to Sodium Benzoate at 750 ppm and 1000 ppm.
9. Remove Sodium Benzoate solutions from the wells and use a 1mL disposable pipette to transfer Sodium Benzoate concentrated to 750 ppm and 1000 ppm.
10. Return the plate to the 28.5℃ incubator.

Day 3:

1. Repeat Day 2 work and observations and record all data.

Day 4:

1. Repeat day 2 work and observations and record all data.

Day 5:

1. Repeat day 2 work and observations and record all data.
2. Place all embryos and fish in the waste container to be properly disposed of.

Above procedure was taken from SEPA Program- UW- Milwaukee

Data

Data Table 1: Number of Living Embryos

Number of Living Embryos				
Concentration	# alive 24 hpf	# alive 48 hpf	# alive 72 hpf	# alive 96 hpf
Control	31	31	29	29
750 ppm Sodium Benzoate	27	27	0	0
1000 ppm Sodium Benzoate	30	29	0	0

Present in Data Table 1 above are the number of living embryos when exposed to the instant ocean, 750 ppm Sodium Benzoate, and 1000 ppm Sodium Benzoate solutions over 96 hours. Data were recorded every 24 hours post-fertilization.

Figure 1: Survival Rate in Fertilized Zebrafish Embryos

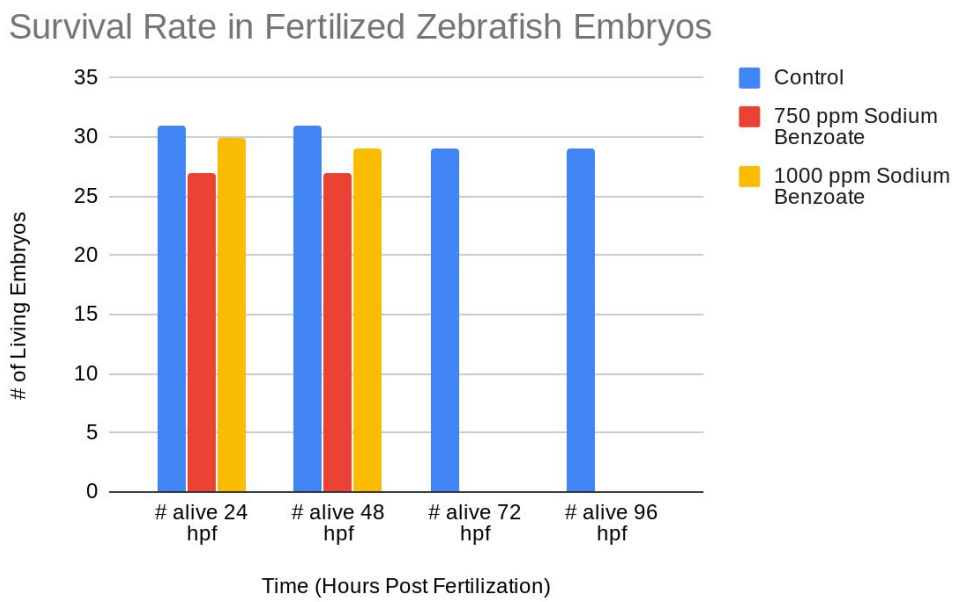


Figure 1 compares the survival rate of zebrafish embryos living in the instant ocean, 750 ppm Sodium Benzoate, and 1000 ppm Sodium Benzoate solutions over 96 hours. Data were recorded every 24 hours post-fertilization.

Sodium Benzoate- Survival Rate

Data table 2:

P-Values and Significance of Unpaired t-test Comparisons

Significance of Survival Rate			
HPF	Comparison	P-Value	Significance
24	Control vs 750ppm	0.0161	Statistically Significant
	Control vs 1000ppm	0.3739	Not Statistically Significant
	750ppm vs 1000ppm	0.3739	Not Statistically Significant
48	Control vs 750ppm	0.0161	Statistically Significant
	Control vs 1000ppm	0.2302	Not Statistically Significant
	750ppm vs 1000ppm	0.5614	Not Statistically Significant
72	Control vs 750ppm	0.0001	Extremely Statistically Significant
	Control vs 1000ppm	0.0001	Extremely Statistically Significant
	750ppm vs 1000ppm		
96	Control vs 750ppm	0.0001	Extremely Statistically Significant
	Control vs 1000ppm	0.0001	Extremely Statistically Significant
	750ppm vs 1000ppm		

Data Table 2 shows the p-values and significance of unpaired t-test comparisons. This table focuses on the survival rate of the zebrafish embryos. The survival rate showed extremely significant at 72 hours post-fertilization. The zebrafish embryos living in the Sodium Benzoate concentrations lived longer than anticipated, however, they experienced a high fatality.

Figure 2: Control 48HPF **Figure 3: 750 ppm 48HPF** **Figure 4: 1000 ppm 48HPF**

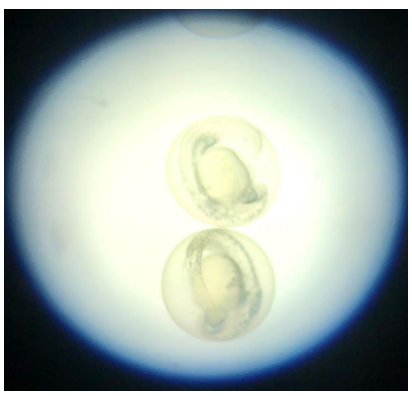
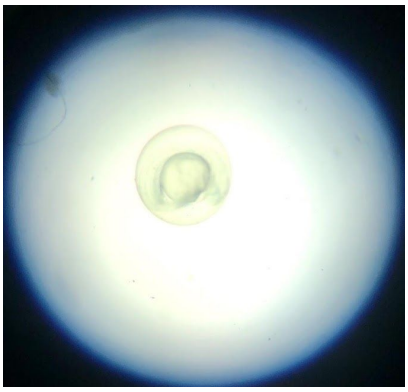
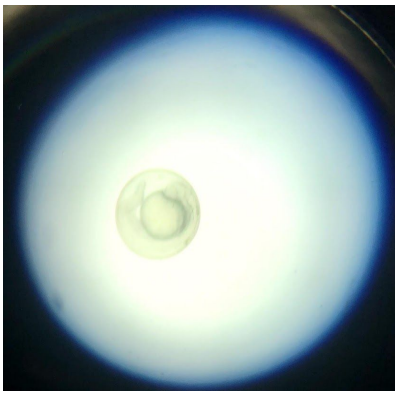
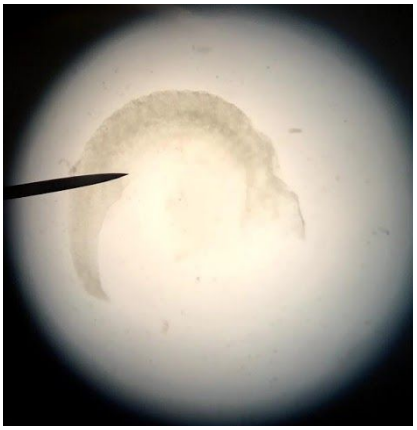
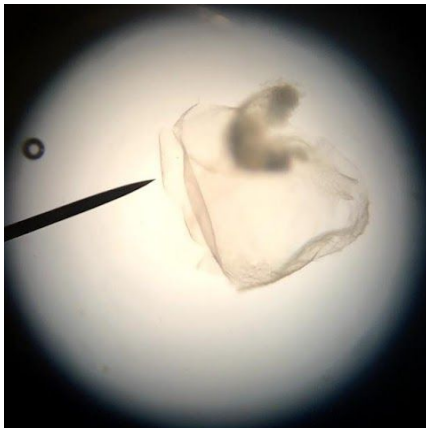
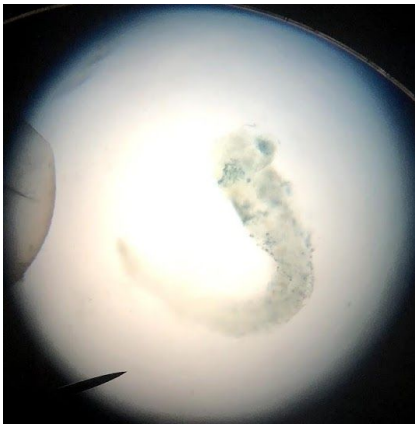




Figure 2 shows what developing zebrafish embryos should look like at the 48 HPF mark. Embryos pictured in Figures 3 and 4 were exposed to Sodium Benzoate concentrations. The embryos are far less developed than the control at the same stage in development.

Figure 5: 750 ppm 72HPF **Figure 6: 1000 ppm 72HPF** **Figure 7: 1000 ppm 72HPF**

At the 72 HPF mark, all embryos in the sodium benzoate solutions were dead. Figures 5, 6, and 7 are pictures of embryos that were developing but never fit to survive. All of these zebrafish embryos look as if they were developing with major defects.

Results

During this experiment, fertilized zebrafish embryos were exposed to sodium benzoate concentrations. The experiment was designed to note the effects of sodium benzoate by focusing on survival rates and development. The control in this experiment was the zebrafish developing in the instant ocean solution. Forty embryos were living in instant ocean solution while two separate sets were exposed to 750 ppm and 1000 ppm sodium benzoate solutions. The independent variable in this experiment was the different solutions as they affected the dependent variable. The dependent variables were the survival rate and hatching rates. Due to this experiment, it is clear that the sodium benzoate solution negatively affected the survival rates when compared to those living in instant ocean.

Discussion

The data found supports the hypothesis for this experiment. The zebrafish embryos exposed to sodium benzoate over an extended period experienced a significantly larger amount of physical deformities and casualties than non exposed embryos. Throughout the experiment, neurological defects were unable to be observed due to none of the affected embryos hatching before dying.

Significant data was found when observing the survival rates. Significance within the data was observed in the survival rate between the 48-72 hour mark. Once the zebrafish embryos exposed to sodium benzoate concentrations hit 72 hours post-fertilization, data showed extremely significant. 29 out of the 40 control zebrafish were still thriving after 72 hours, but 100% of the exposed zebrafish to sodium benzoate died within the same time frame.

Significance was also present in deformities. The sodium benzoate exposed embryos were never able to hatch, however, microscopic images of the embryos during development do not compare to control embryos. The development of the exposed embryos was significantly behind the control at the 48 HPF mark. Once HPF hit 72, no identifying zebrafish features were present. The heart's location was not visible, and the number of eyes varied from embryo to embryo. The severity of the deformities caused the embryos to be unrecognizable when compared to the control.

Some limitations of this experiment were how much class time was available. The amount of class time affects how careful and precise work can be completed. With having a longer amount of class time more observations would be able to be recorded and more concentrations could be tested. Cross-contamination is another factor that could lead to errors in the results.

The data collected from this experiment is comparable to other results that are statistically significant when researching the effects of sodium benzoate on zebrafish. According to research, the FDA allows up to a 0.1% concentration of sodium benzoate by weight in food and beverages (McCulloch, 2019). After converting a 0.1% concentration to parts per million, it came out to be comparable to 10,000 ppm. The WHO has declared an acceptable daily intake of sodium benzoate to not exceed 2.27 mg/lb or 4.994 ppm/lb (McCulloch, 2019). If a 150-pound person were to consume the safe limit of sodium benzoate, she would consume nearly 749.1 ppm of sodium benzoate. The 750 ppm concentration tested on the zebrafish embryos in this experiment killed and showed major defects, so anyone over 150 pounds would be consuming even more than the concentrations in this experiment showed. More information needs to be discovered before the FDA can 100% prove sodium benzoate does not have effects on human behavior.

Works Cited

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