The Effect of Juul on the Development, Hatch Rate, and Survivorship of *Danio Rerio*

Ismail Ademi, Connor Loeffler, Ryan Mroczenski

Greendale High School

I. Abstract

This experiment examines the effect of Juul juice on the development, hatch rate, and survivorship of *Danio Rerio* (zebrafish). Juul is a popular type of e-cigarette that is known to cause birth defects in newborns if pregnant women use the product (Small, 2017). Juul is advertised as a healthier alternative to smoking cigarettes, but usage among teenagers has risen in the recent years, with a 78% increase in the number of high schoolers using Juul this past year (Fox, 2018; Juul, 2018). Four flavors of Juul were tested in order to compare results between different flavors popular among teenagers. The experiment found that Juul flavors had a slight effect on the survivorship of the zebrafish. 87.88% of the zebrafish in the Instant Ocean solution were alive after 96 hours post fertilization (hpf), while an average of 83.72% of the fish were alive in the Juul solutions. While there was no significance in the hatch rate after 96 hpf, the Virginia Tobacco Juul solution and the Mango Juul solution had a significant effect on the high rate of early hatchlings in their respective solutions. The development of the zebrafish was slightly affected by all four flavors, but only one of those flavors was statistically significant. None of the fish in the control solution had deformities, while 12.90% of the fish exposed to the Virginia Tobacco flavor of Juul had deformities. Affected hatchlings had a curvature of the spine and appeared shorter than hatchlings in the control solution. Further study should be conducted, but it is clear that Juul juice had a significantly negative effect on the development of Zebrafish in terms of potential deformities.

II. Background Information

Why Zebrafish were used in this experiment?

Zebrafish were used in this experiment because as vertebrates, they share some characteristics with mammals (Brennen, 2014). They have many of the same organs and tissues

as humans, and 70% of the genes found in humans can also be found in Zebrafish (Burke, 2016). Zebrafish reproduce externally and thus fertilized embryos develop outside their mother, making the eggs easy to access and manipulate (Brennen, 2014). The embryos and larvae are transparent which allows for easy observation of defects present in the fish. Zebrafish offspring also develop quickly which allows for quick experiments (Brennan, 2014).

Why Juul juice was tested?

Because the e-cigarette device, Juul, is the most popular form of vaping among teens, the purpose of the experiment was to find the effects of Juul juice on the zebrafish embryos as it could determine whether Juul exposure might have an effect on developing human fetuses (Fraga, 2018). The Virginia Tobacco flavor, Mango flavor, Cool Mint flavor, and Crème brûlée flavors of Juul were tested to see if effects varied with different flavors (see figure 1).

The rise of Juul and other easy access vaping devices among teens is a recent development that has raised alarms in the National Institute on Drug Abuse (NIDA). NIDA not only reports that vaping devices are more popular than cigarettes among teens, but 37.3% of high school seniors in America reported having vaped in the last year (NIDA, 2018). Most teenagers are fully aware of the dangers of cigarettes due the social stigma of it, and the overwhelming data showing the detrimental effects of smoking tobacco, but not with vaping (IARC 2004; Raven 2018). About 66% of teenagers believe flavoring is the only ingredient in vape juice, but Juul contains 59 mg/mL of Nicotine per Juul pod, along with glycerol, propylene glycol, benzoic acid, and artificial flavors (Belluz, 2018; NIDA, 2018).

The ingredients of Juul are not known to be dangerous on their own. Glycerol is commonly found in toothpaste, propylene glycol in asthma nebulizers, and benzoic acid is a common food additive (Tolentino, 2018). However, when glycerol and propylene glycol are combined with benzoic acid and vaporized through a dehydration and cyclization reaction, the result is the human carcinogen Benzene, and water vapor (Pankow et. al, 2017). Benzene is the

largest single known cancer-risk air toxin in the U.S. (Pankow et. al, 2017). While the risks of Benzene are lower in Juuls than they are in cigarettes, the carcinogen can have an effect on non-smokers who are chronically exposed to the chemical compound (Pankow et. al, 2017). Though Juul smokers are exposed to Benzene, the embryos tested on would not be as they would be exposed to un-vaporized Juul juice.

Previous experiments concerning vaping

The effects of nicotine on zebrafish Embryos are well known, but not too much is known on the effects of vape juice (Connaughton and Parker, 2007). Previous studies on the effects of vape juice on the number of living zebrafish embryos showed that a concentration of 27.8 mM E-cigarette nicotine had a higher death rate in the embryos than an Instant Ocean solution; however, the experiment was not significant ($p = 0.0972$) (Peterson, 2016). A study concerning the correlation between vaping and respiratory issues found that e-cigarettes have possible links to a higher prevalence of asthma in adolescents (Clapp and Jaspers, 2017). The study found that two common ingredients of vape juice, propylene glycerol and vegetable glycerin, produce respiratory toxicities when vaporized which can affect the respiratory system of developing teenagers. Another study regarding the additives of Juul found that the flavors of e-cigarette liquids impair respiratory innate immune cell function (Clapp et. al, 2017). Using nicotine-free e-cig juice, they found that artificial flavors containing cinnamaldehyde have the potential to impair respiratory immune cell function.

Hypotheses tested

This experiment aimed to answer how Juul would affect the survivorship, hatch rate, and development of zebrafish? Thus, the hypotheses were as follows:

1) Embryos exposed to a Juul solution would experience lower levels of survival compared to the Instant Ocean solution's embryos because the nicotine in Juul is known to cause deformities, which can lead to death (Juul Labs, 2018).
2) Embryos exposed to a Juul solution would experience a lower hatch rate compared to the embryos exposed to an equal nicotine concentration, because the embryos in Juul were not only exposed to nicotine, but other ingredients which are potentially harmful.
3) Embryos exposed to any type of Juul solution would experience a higher rate of abnormalities than the Instant Ocean solution, because the nicotine and benzoic acid that is found in tobacco and some vaping products are known to cause deformities in human fetuses (CDC, 2018).

III. Materials & Methods

Materials & Variables

Variables

The only variable manipulated in this experiment was the type of solution. The number of zebrafish alive, hatched, and with curved spines was measured.

Materials

The materials listed were based off of the investigation, effect of nicotine on embryo development, from Zebrafish as Models made by the University of Wisconsin-Milwaukee (Petering et. al, 2018).

- Stock solutions of **Juul Juice** (0.2 mg/mL Juul juice)
  : Virginia Tobacco variant
  : Crème brûlée variant
  : Cool Mint variant
  : Mango variant
- Stock solutions of Nicotine
  : 1 mg/mL Nicotine stock solution (given)
  : 0.2 mg/mL Nicotine solution (made)
  : 0.01 mg/mL Nicotine solution (made)
- Beaker for dead embryos and liquid disposal
- Graduated Cylinder used to mix solutions

- Two pipettes each day (one big and one small)
- 6 glass bottles to store solutions
- Sharpie used to label multi-well plates and solution containers
- Instant Ocean/Embryo Media Solution
- Large bore transfer pipette, minimum bore, 1.5 mm for transferring eggs to observation container and manipulating them in the container)
- Transfer pipette used to mix solutions
- Multi-well plates
- 28.5 Celsius Incubator
- Dissecting and compound microscope to record observations

Sample Size- 33 in the negative control of Instant Ocean solution, 32 in the positive control of 0.2 mg/mL, 29 in the 0.01 mg/mL nicotine solution, 35 in the Virginia Tobacco Juul solution, 27 in the Mango Juul solution, 27 in the Cool Mint Juul solution, and 30 in the Crème brûlée solution = 213 zebrafish embryos

Procedure

The experiment began when fertilized zebrafish embryos were obtained and about 10 live, fertilized embryos were placed into 3 wells of 3 rows of one multi-well plate. About 10 live, fertilized embryos were also put into each well of 4 rows of a second multi-well plate. The multi-well plates with embryos were filled about halfway with deionized water. The embryos were then stored overnight in a 28.5 degrees Celsius incubator. The next day, about 24 hpf, the embryos were removed from the incubator.

A negative control of Instant Ocean solution was used to compare the effects of Juul exposed fish to normal developing fish, and a positive control of a 0.2 mg/mL nicotine solution was used to ensure the credibility of the materials given, as that concentration of nicotine has effects on zebrafish (Connaughton and Parker, 2007). Juul is 5% nicotine by weight (Juul, 2018). This experiment used a 0.2 mg/mL Juul concentration so that the concentration would include 0.01 mg/mL of nicotine. 5% of 0.2 mg/mL is 0.01 mg/mL. The experiment also sought to see the effects of the additives of Juul juice compared to nicotine to see whether nicotine was the only ingredient that would have effects on the embryos. To single out the

additives of Juul juice, the amount of nicotine in the Juul stock solutions (0.01 mg/mL) were set equal to a nicotine concentration (0.01 mg/mL) in a nicotine stock solution.

To create the solutions, a certain amount of stock solution was diluted in Embryo Media (Instant Ocean). To create a 0.2 mg/mL nicotine solution, 20 mL of a given 1 mg/mL nicotine solution was mixed with 80 mL of Embryo Media. To create a 0.01 mg/mL nicotine solution, 1 mL of the given 1 mg/mL nicotine solution was mixed with 99 mL of Embryo Media. To create the Juul stock solutions, 20 µl of the Virginia Tobacco Juul (VTJ) flavor was mixed with 20 mL of Embryo Media to generate a working stock solution of 1 mg/mL VTJ. This working stock solution was further diluted by mixing 20 mL of the 1 mg/mL VTJ with 80 mL of Embryo Media, creating a final VTJ concentration of 0.2 mg/mL. The process used to create the VTJ solution was used for the other 3 Juul flavors, creating a 0.2 mg/mL Cool Mint Juul (CMJ) solution, a 0.2 mg/mL Crème brûlée Juul (CBJ) solution, and a 0.2 mg/mL Mango Juul (MJ) solution. Each solution was stored in a capped glass bottle.

With 6 stock solutions created, the wells of the multi-well plates were filled with their intended solutions. Row A of plate 1 had its solution replaced by Instant Ocean and any dead embryos were disposed of in a waste beaker, and the number of embryos alive, hatched, and with curved spines was recorded. The process of disposing of dead embryos and recording the number alive, hatched, and with curved spines was repeated in every other row except different solutions were given to each row. Row B of plate 1 had its solution replaced by a 0.2 mg/mL nicotine solution. Row C of plate 1 had its solution replaced by a 0.01 mg/mL nicotine solution. Row A of plate 2 had its solution replaced by a 0.2 mg/mL VTJ solution. Row B of plate 2 had its solution replaced by a 0.2 mg/mL MJ solution. Row C of plate 2 had its solution replaced by a 0.2 mg/mL CMJ solution. Row D of plate 2 had its solution replaced by a 0.2 mg/mL CBJ

solution. After each well of the two plates had its appropriate solution, the plates were put back into the incubator.

The process of recording the amount live, hatched, and the amount with deformities in each row was repeated at 48 hpf, 72 hpf, and 96 hpf. A Microscope was used to observe deformities and to see whether an embryo or fry was dead. Along with recording data, at 48 hpf, 72 hpf, and 96 hpf, the wells of both plates were refilled with their appropriate solutions and the multi-well plates were placed into an incubator at each time interval.

Adequate safety measures were taken in this experiment by wearing gloves and goggles whenever making, or handling any of the solutions. Pipettes were used to take out the dead embryos and transfer pipettes were used to mix various solutions, thus ensuring no direct human contact with anything that could potentially raise health concerns. The procedure was based off of the investigation, effect of nicotine on embryo development, from Zebrafish as Models made by the University of Wisconsin-Milwaukee (Petering et. al, 2018).

Figure 1: The front of a Juul package showing the flavors tested. Bought with approval of instructor and administration.

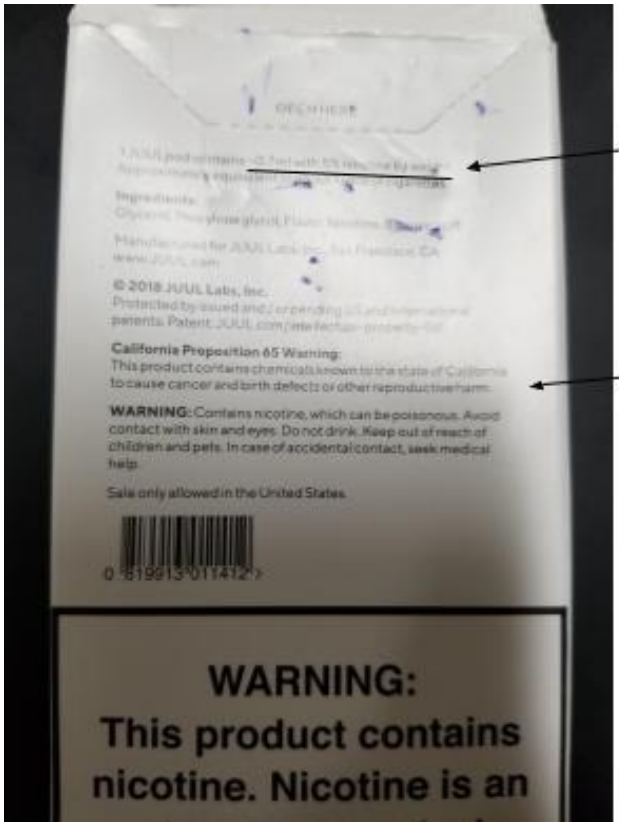


Figure 2 : The back of the Juul Package showing the warning that this product contains nicotine.
Note : The Pen marks aren't originally part of the package.

Results

As the hours past fertilization passed, the percent of living embryos/fry decreased. Table 1 and Graph 1 show this trend. At 96 hpf: the living rate of the negative control of Instant Ocean solution was 87.88%, the positive control of 0.2 mg/mL nicotine was 46.88%, the 0.01 mg/mL nicotine solution was 68.97%, the 0.2 mg/mL VTJ solution was 88.57%, the 0.2 mg/mL MJ solution was 88.49%, the 0.2 mg/mL CMJ solution was 74.07%, and the 0.2 mg/mL CBJ solution was 83.33%. Living rate was calculated by dividing the amount alive by the starting amount. Two of four of the Juul solutions had a lower living rate than the Instant Ocean solution, and all 4 Juul solutions had a higher living rate than the 0.01 mg/mL nicotine solution. Though the CMJ solution and the CBJ solution had lower living rates than the Instant Ocean solution, this was not significant ($p = 0.3712$ CMJ, $p = 0.4766$ CBJ).

Table 2 and Graph 2 show the effect of time on the percent of embryos hatched. Hatch rates increased as time increased. Hatch rate was calculated by dividing the total hatched by the number of starting embryos. The living embryos of every solution were hatched by 96 hpf except the positive control solution and the nicotine solution. There were still 6 embryos living in the 0.2 mg/mL nicotine solution and 3 embryos living in the 0.01 mg/mL nicotine solution by 96 hpf. Every Juul solution had a higher hatch rate than the nicotine solution of 0.01 mg/mL nicotine. There was no significance in this ($p = 0.1268$ VTJ, $p = 0.4100$ MJ, $p = 0.7994$ CMJ, $p = 0.3869$ CBJ). Two of the four Juul solutions barely had a higher hatch rate than the Instant Ocean solution, while the CMJ solution and the CBJ solution had lower hatch rates. There was no statistical significance in these differences.

Table 3 and Graph 3 show the effect of time past fertilization on the amount of alive fry that had curved spines. As time increased, the amount with curved spines increased. A normal zebrafish fry should have a straight tail as shown in Figure 3. There were no abnormalities in the Instant Ocean solution. All the other solutions had fry that had a deformity in the spine. The deformity rate was calculated by dividing the amount of alive fry with curved spines over the number of hatched embryos at that time interval. The Juul solutions deformity rates were as followed: VTJ solution at 12.90%, MJ solution at 8.33%, CMJ solution at 5%, and the CBJ solution at 12%. There was statistical significance for the VTJ solution ($p = 0.0161$, 1.33 ± 0.33). Therefore, there is a 98.39% probability that VTJ solution was the reason for the discrepancy in the results. The other Juul solutions were not statistically significant ($p = 0.1161$ MJ, $p = 0.3739$ CMJ, $p = 0.1583$ CBJ).

Table 1: The table shows the effect of time on the number of living fish. 0.2 mg/mL nicotine solution and the 0.01 mg/mL nicotine solution ended up having the lowest living rates. The highest living rate was the MJ solution. There was a decrease in the amount living as time passed.

The Effect of Time on the Number and Percent of Embryo/Fry Alive

Treatment	Row	Number of starting embryos	24 hpf - Live / Percent Alive	48 hpf - Live / Percent Alive	72 hpf - Live / Percent Live	96 hpf - Live / Percent Alive
Negative Control (Instant Ocean)	1A	33	33 / 100%	33 / 100%	32 / 96.67%	29 / 87.88%
Positive Control (0.2 mg/mL nicotine)	1B	32	30 / 93.75%	29 / 90.63%	25 / 78.13%	15 / 46.88%
Nicotine Solution (0.01 mg/mL)	1C	29	29 / 100%	29 / 100%	26 / 89.66%	20 / 68.97%
VTJ solution (0.2 mg/mL Juul)	2A	35	35 / 100%	35 / 100%	33 / 91.43%	31 / 88.57%
MJ solution (0.2 mg/mL Juul)	2B	27	27 / 100%	25 / 92.59%	25 / 92.59%	24 / 88.89%
CMJ solution (0.2 mg/mL Juul)	2C	27	25 / 92.59%	23 / 85.19%	20 / 74.07%	20 / 74.07%
CBJ solution (0.2 mg/mL Juul)	2D	30	30 / 100%	28 / 93.33%	26 / 86.67%	25 / 83.33%

Graph 1 : The line graph shows the relationship described in Table 1. There is a decrease in the percent living as time passes.

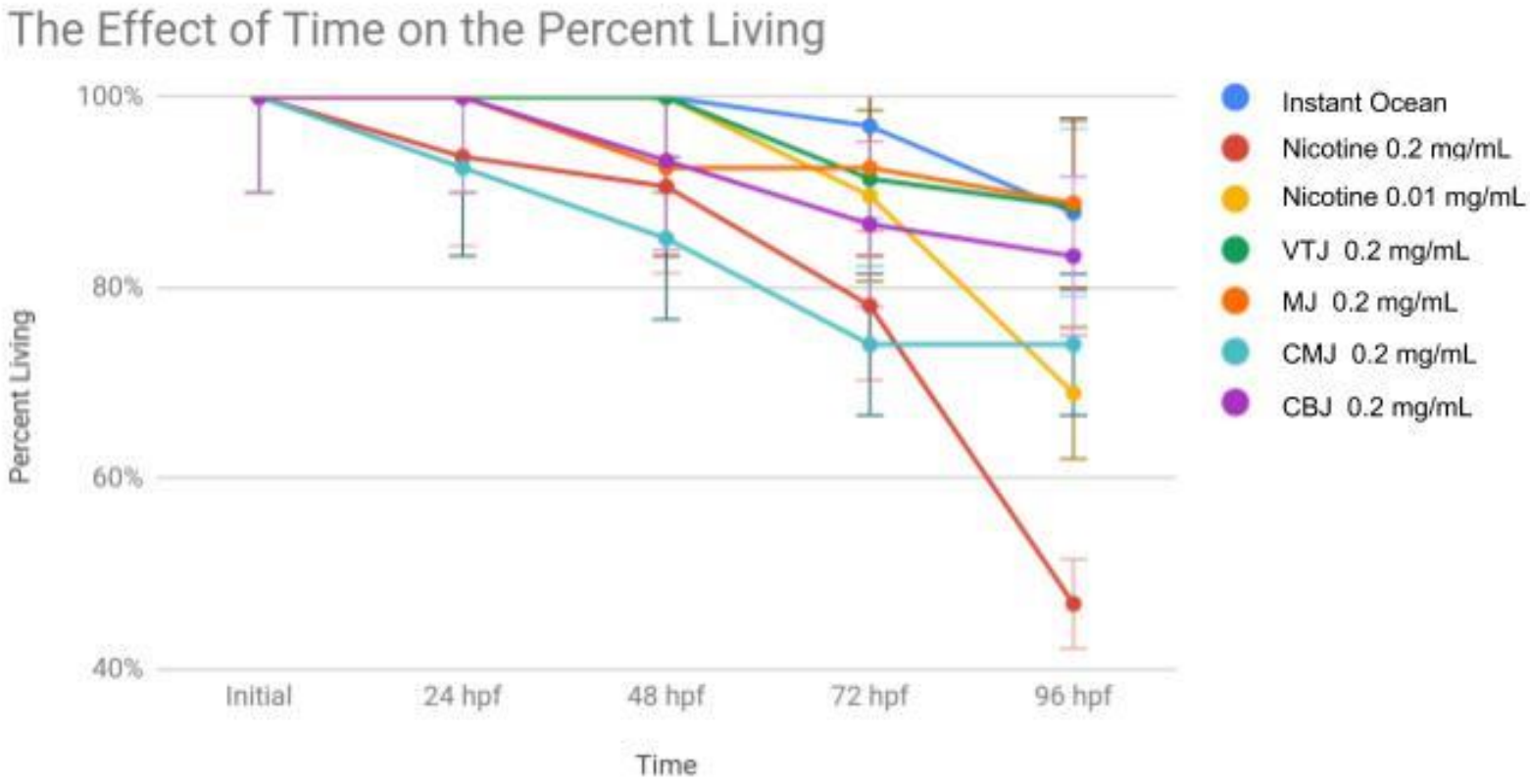


Table 2: The table shows the effects of time on the number of hatched embryos. Hatch rates increased as time increased, but three solutions hatch rates dropped after 72 hpf. The 0.02 mg/mL nicotine solution had the lowest hatching percentage while the Instant Ocean solution, VTJ solution, and the MJ solution had higher percentages at about 88.5%

The Effect of Time on the Number and Percent of Embryos Hatched

Treatment	Row	Number of starting embryos	24 hpf - Hatched / Percent Hatched	48 hpf - Hatched / Percent Hatched	72 hpf - Hatched / Percent Hatched	96 hpf - Hatched / Percent Hatched
Negative Control (Instant Ocean)	1A	33	0 / 0%	8 / 24.24%	30 / 90.91%	29 / 87.88%
Positive Control (0.2 mg/mL nicotine)	1B	32	0 / 0%	0 / 0%	12 / 37.50%	9 / 28.13%
Nicotine Solution (0.01 mg/mL)	1C	29	0 / 0%	8 / 27.59%	19 / 65.52%	17 / 58.62%
VTJ solution (0.2 mg/mL Juul)	2A	35	0 / 0%	19 / 54.29%	32 / 91.43%	31 / 88.57%
MJ solution (0.2 mg/mL Juul)	2B	27	0 / 0%	16 / 59.26%	24 / 88.89%	24 / 88.89%
CMJ solution (0.2 mg/mL Juul)	2C	27	0 / 0%	4 / 14.81%	19 / 70.37%	20 / 74.07%
CBJ solution (0.2 mg/mL Juul)	2D	30	0 / 0%	12 / 40.00%	23 / 76.67%	25 / 83.33%

Graph 2: The line graph shows the relationship described in Table 2. As the first 72 hpf passes, the number of hatched increased, but after 72 hpf, there was a decrease in the number hatched. There were still living embryos at 96 hpf.

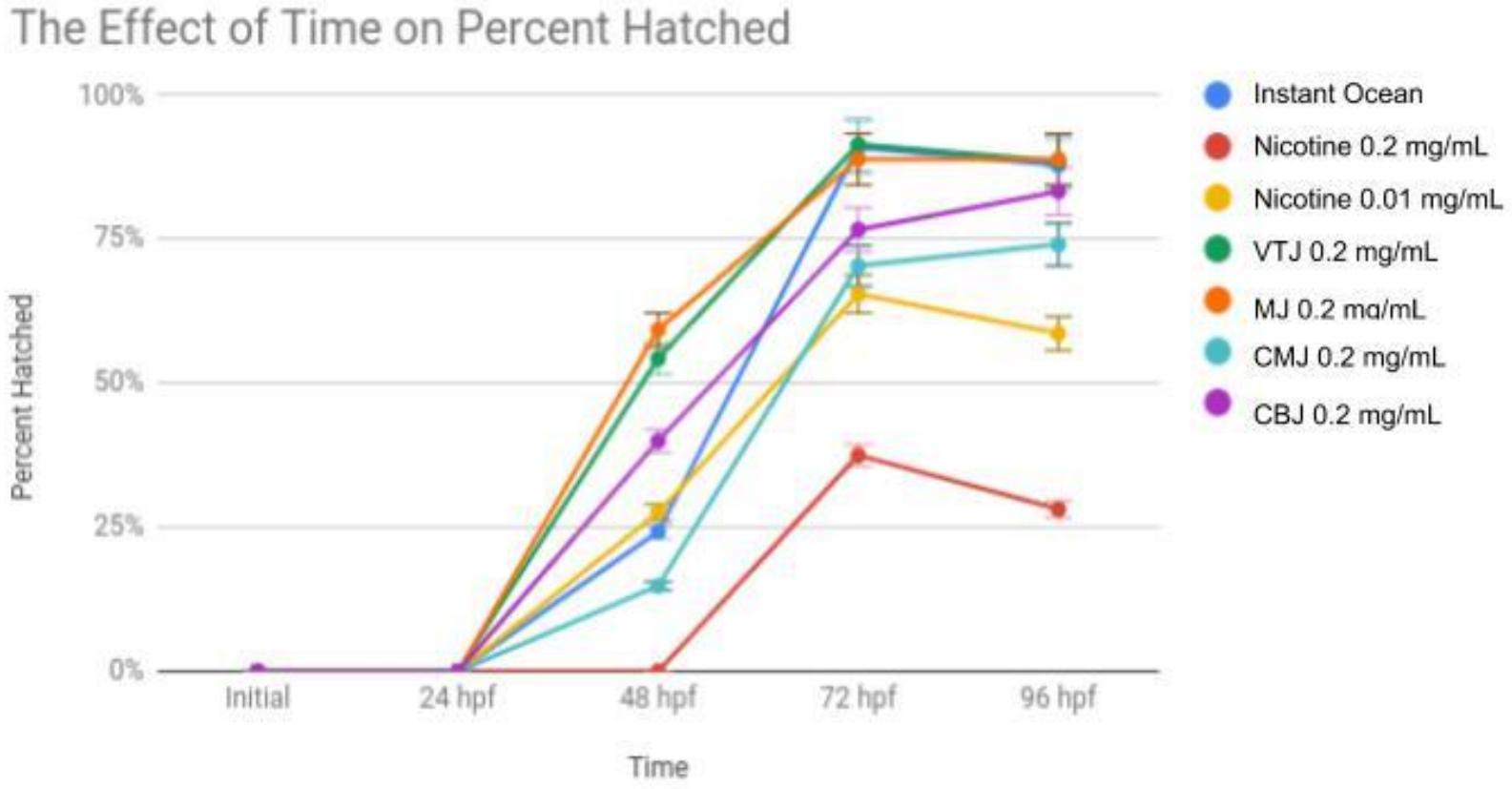
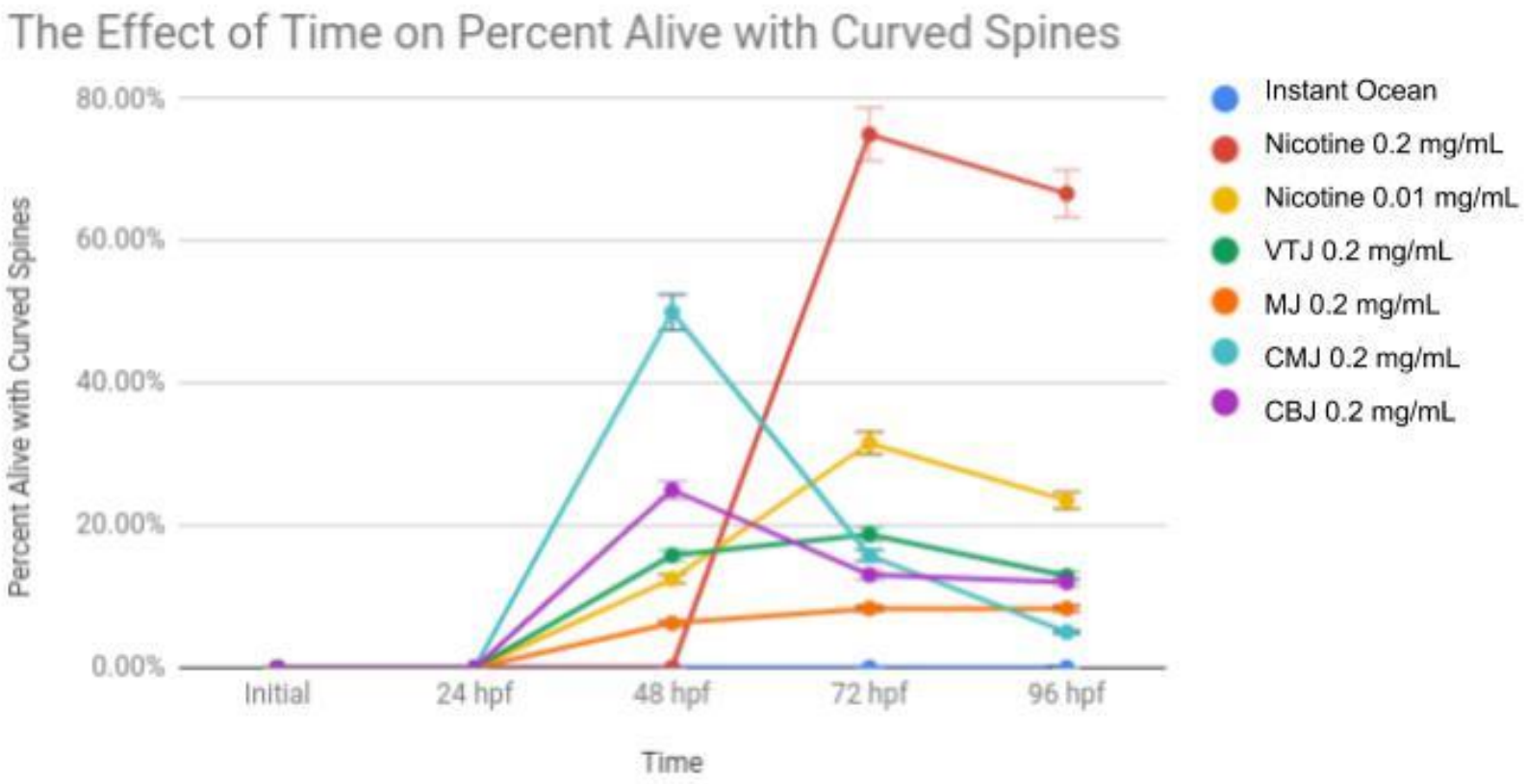


Table 3: The table shows the relationship between the amount of fish hatched with curved spines and time. As time increases, the number of fish with curved spines increased in all solutions except the Instant Ocean solution, but after 72 hpf, the number of fish with curved spines decreased due to abnormal fish dying off in high numbers.

The Effect of Time on the Number and Percent Alive with Curved Spines

Treatment	Row	Number of starting embryos	24 hpf - Alive Curved Spines / Percent with Curved Spines	48 hpf - Alive Curved Spines / Percent with Curved Spines	72 hpf - Alive Curved Spines / Percent with Curved Spines	96 hpf - Alive Curved Spines / Percent with Curved Spines
Negative Control (Instant Ocean)	1A	33	0 / 0%	0 / 0%	0 / 0%	0 / 0%
Positive Control (0.2 mg/mL nicotine)	1B	32	0 / 0%	0 / 0%	9 / 75.00%	6 / 66.67%
Nicotine Solution (0.01 mg/mL)	1C	29	0 / 0%	1 / 12.50%	6 / 31.58%	4 / 23.53%
VTJ solution (0.2 mg/mL Juul)	2A	35	0 / 0%	3 / 19	6 / 18.75%	4 / 12.90%
MJ solution (0.2 mg/mL Juul)	2B	27	0 / 0%	1 / 6.25%	2 / 8.33%	2 / 8.33%
CMJ solution (0.2 mg/mL Juul)	2C	27	0 / 0%	2 / 50.00%	3 / 15.79%	1 / 5.00%
CBJ solution (0.2 mg/mL Juul)	2D	30	0 / 0%	3 / 25.00%	3 / 13.04%	3 / 12.00%

Graph 3: As time increases, the amount of fish with curved spines increased in all solutions except the Instant Ocean solution, but after 72 hpf, the number of fish with curved spines decreases. At 48 hpf, there was statistical significance.



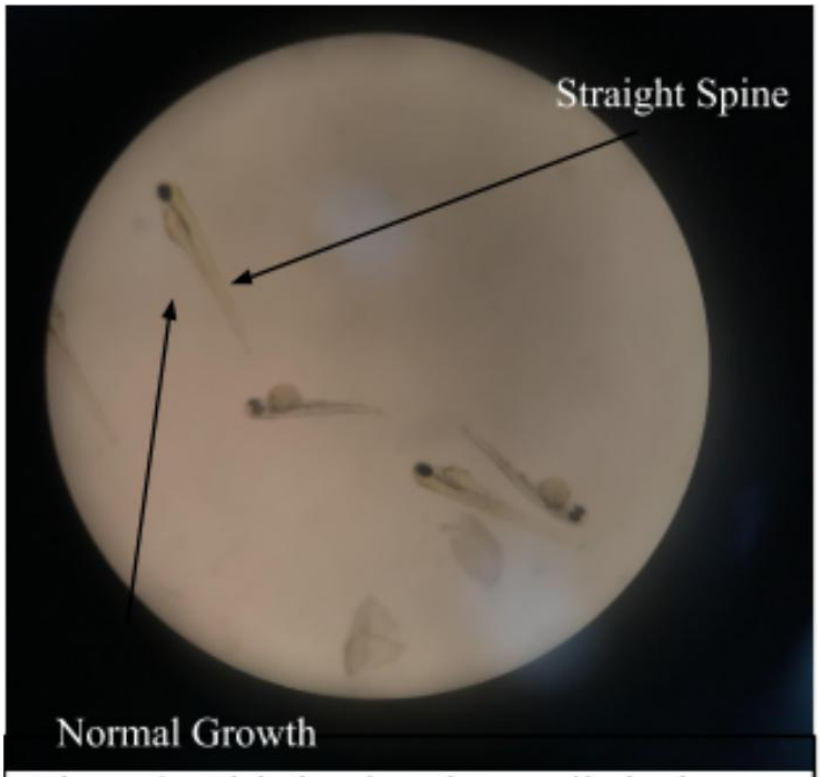


Figure 3 : Fish developed normally in the Instant Ocean Solution

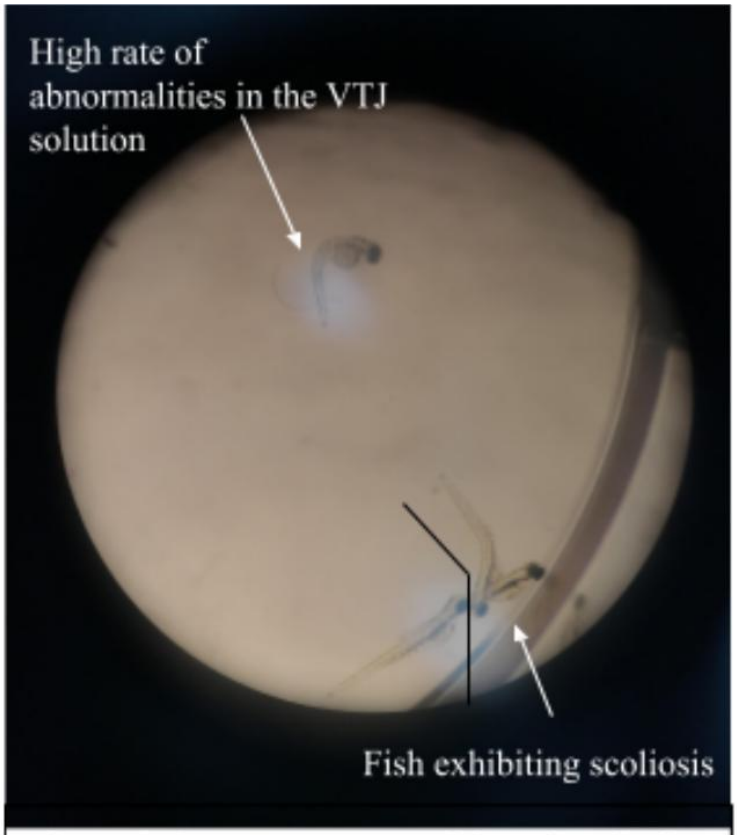


Figure 4: Severe curvature of the spines in the VTJ solution

Figure 5 : Fish in the .2 mg/mL nicotine solution experienced high rates of dysfunctional spines and mortality

Figure 6 : Fish in the CBJ solution exhibiting a deformity seen in all Juul solutions

V. Discussion

This experiment found that Juul juice had a slight effect on the living rate, hatch rate, and deformity rate of the embryos at 96 hpf. 87.88% of the zebrafish in the Instant Ocean solution were alive at 96 hpf, while an average of 83.72% of the fish in the Juul solutions were alive at 96 hpf. There was no significance in this slight difference. For the hatch rate, an average of 83.72% of Juul exposed fish were hatched by 96 hpf, while 58.62% of the fish in the 0.01 mg/mL nicotine solution were hatched by 96 hpf. There was no significance in the differences in hatch rate at 96 hpf, but there was statistical significance in the rate of early hatchlings. For the deformity rate, an average of 9.56% of Juul exposed fish displayed deformities, while 0% of the fish in the instant ocean solution had deformities. Only the VTJ solution showed statistical significance for the amount with curved spines when compared to Instant Ocean.

Hypothesis 1 stated that if embryos were exposed to Juul juice, then those embryos would experience a lower living rate than fish in the Instant Ocean solution because the nicotine in Juul is known to cause deformities (Juul Labs, 2018), which would lead to death. The data doesn't support hypothesis 1. Though deformities did happen at a higher rate in the Juul solution, there was still a high living rate for the fish in these solutions. The VTJ solution and the MJ solution had slightly higher living rates than the Instant Ocean solution, while the CBJ solution and the CMJ solution had lower living rates than the negative control solution. As stated previously, this was not significant.

Possible errors for the experiment include a delay in embryo exposure to their intended solutions, and a lack of a device which would allow the smoke from Juul to be liquidized. In regards to the former point, the embryos could not be exposed to their intended solutions on the first day of the experiment due to time constraints, and as such, were not exposed to the

chemicals of Juul and nicotine during a vital part of their development. In regards to a vaporizer device, there is a device that liquidizes the smoke from a Juul called a Vaping Aerosol Collection Device, but such a device was not acquired (Petering and Tomasiewicz, 2018). Because of that, the Benzoic acid from the Juul juice that turns into Benzene once vaporized, was not turned into Benzene (Rubinstein et al., 2018). Benzene is a human carcinogen, which is a substance capable of causing cancer (Rubinstein et al., 2018). If Benzene coupled with nicotine were exposed to the fish, the living rate likely would have been lower. Other ingredients in the Juul like propylene glycol and glycerol didn't seem to have an effect on the living rate of the fish.

Hypothesis 2 stated that if embryos were exposed to a Juul solution, then they would experience a lower hatch rate than the embryos exposed to an equal nicotine concentration, because the embryos in Juul were not only exposed to nicotine, but other ingredients which are potentially harmful. The data did not support hypothesis 2. Every Juul solution had a higher hatch rate than the 0.01 mg/mL nicotine solution, though there was no statistical significance in that. A possible reason for the higher hatch rates was time constraints. The experiment asked that the solutions of each well in each row be replaced every day, but due to time constraints, the wells containing the Juul solutions in multi-plate 2 were prioritized, and their solutions were replaced first, causing a lack of diligence with the nicotine solutions in multi-plate 1.

Three Juul solutions, VTJ, MJ, and CBJ had very high hatch rates by 48 hpf. Their hatch rates were 54.29%, 59.26%, and 40.00% respectively. The VTJ and MJ solutions were over twice the hatch rate of the Instant Ocean solution which was at 24.24%. The discrepancy between the CBJ solution's hatch rate and the Instant Ocean solution's hatch rate was not significant ($p = 0.2051$), but there was significance in the discrepancy in both the VTJ solution and MJ solution ($p = 0.0295$, 6.33 ± 0.88 VTJ, $p = 0.0474$, 7.25 ± 0.85 MJ). There was a 97.05%

chance that the VTJ solution was the reason for the high hatch rates, and there was a 95.26% chance that the MJ solution was the reason for the high hatch rate. Nicotine is correlated to premature birth, and there is a warning on the Juul box that says that this product contains chemicals that causes reproductive problems and/or birth defects (CDC, 2018 and figure 2). Though the fish weren't measured in length, observations of the Juul exposed fish appeared to be shorter. A logical explanation for the short length of the Juul exposed fish would be that they were not fully developed when they exited the embryo; however, because each fish wasn't measured, this conclusion cannot be reached.

Hypothesis 3 stated that if embryos were exposed to a Juul solution then those hatched fry would experience a higher rate of abnormalities than the negative control solution, because the nicotine and benzoic acid that is found in tobacco and some vaping products are shown to cause deformities in human fetuses (CDC, 2018). All Juul solutions supported this data as all four solutions displayed deformities in growth, and in the spine. Although all four Juul solutions supported the hypothesis, only one of the solutions had statistical significance ($p = 0.0161$, 1.33 ± 0.33 VTJ). The VTJ solution was significant likely due to an increased amount of benzoic acid compared to other Juul solutions. Benzoic acid is found in tobacco, and is most likely used to a greater extent in the Virginia Tobacco flavor, than in the Mango, Cool Mint, or Crème brûlée flavors (Juul Facts, 2018). Though benzoic acid is not dangerous in small doses, it is possible that because the VTJ flavor had an increased amount of benzoic acid that the embryos exposed to the VTJ solution were exposed to too much benzoic acid. The artificial flavor of the tobacco flavor most likely includes some of the same chemicals found in tobacco plants to simulate the taste of tobacco, and tobacco smoke is bad for one's health (CDC, 2018).

To improve the experiment, the number of fish with curved spines, dead or alive, should have been counted. Fry that developed abnormalities often died within a day or two, so by 96

hpf, a lot of fish with curved spines had died. If all fish with curved spines were accounted for, there would have been a high chance of statistical significance once the data was analyzed. Another improvement to this experiment would be the measuring of the fish. Though this would require that the microscope was at a constant focus so each fish would be proportional upon observation. Measuring every fish or measuring a small sample of 10 fish from each row would be needed to draw a conclusion on whether there was a discrepancy in length between the fish in the various solutions because a sample size is needed for an experiment.

Conclusion

Although two hypotheses were not supported by the data, and a third hypothesis was partially supported, this experiment is still relevant because it shows that Juul is not safe. Pregnant women should keep away from vaping Juul. Not only because this experiment showed that Juul causes scoliosis and possible growth stunts in the fish, but because the VTJ and MJ solutions were shown to cause an early hatching in the embryos. Zebrafish young and human young are not identical, but the early stages of fetal development for both organisms are very similar (Howe et. al, 2013). High schoolers are vaping and getting addicted to Juul at an alarming rate. There were 1.3 million more high school vapers in 2018, and 37.3% of 12th graders said they had vaped in the past year (Hoffman, 2018).

In 1998, 22.4% of high school seniors smoked cigarettes daily; in 2018, cigarette use among teenagers is at an all-time low, with 3.6% of high school seniors smoking daily (NIDA, 2018). Though the effort to reduce the amount of kids smoking has been very successful, the recent rise of teens vaping has replaced smoking among adolescents. The reasons why kids use Juul and other e-cigarette products is because of its addictiveness, and because of the social climate of the current teenage generation (Reitmeyer, 2018). There is a Juul culture with kids today. It is a very social device that is used in part by teens to show others that they are carefree,

and fun (Tolentino, 2018). Teens are constantly wanting validation from their peers because they have a natural tendency to want to feel accepted (Sparks, 2013). Using Juuls is seen by many as validation and appearing carefree and rebellious. Because Juuling is a such a social tool that appears at social gatherings, non-vapers are being exposed to second-hand vape. The Benzene that is created from Juuling goes into the air and affects the respiratory system of developing teens (Pankow et. al, 2017). It is important to stop the niche of Juul so teens stop experimenting and getting addicted to Juul.

It is one thing for an older generation to get addicted to vaping, but this is the future of America. Many of these young Americans will be in the workforce in 10 years, starting families and having kids. To have expecting mothers addicted to Juul would not be beneficial to their prospective children, or to society. The total cost of cigarettes to society is estimated to be more than $300 billion every year, including $170 billion for health care, and $156 billion in lost productivity due to premature death and exposure to secondhand smoke (Howe et al., 2013). Though Juul is advertised to be a healthier alternative to cigarettes, it still contains nicotine and a known carcinogen in benzene along with possible repository toxicities. Juul would have a detrimental impact on society and to future babies. It is important that future studies be done on Juul because most teens are naive and do not truly understand the effects of Juul (Raven, 2018). Future studies will help future generations know the dangers of Juul.

VI. References

Benzene formation in e-cigarettes https://www.ncbi.nlm.nih.gov/pubmed/ - PubMed - NCBI. (n.d.). Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/?term=Benzene formation in e-cigarettes https://www.ncbi.nlm.nih.gov/pubmed/28983782

Doran, C. Hall, W. (2016, May 10) How Much Can the USA Reduce Health Care Costs by Reducing Smoking? Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4862676/

Fox, Maggie. Do teens need help kicking the vape habit? The FDA thinks so. (n.d.). Retrieved from https://www.nbcnews.com/health/health-news/do-teens-need-help-kicking-vape-habit-fda-thinks-so-n949046

Hoffman, J. (2018, December 17). Study Shows Big Rise in Teen Vaping This Year. Retrieved from https://www.nytimes.com/2018/12/17/health/ecigarettes-teens-nicotine-.html

IARC (2004) *IARC Monographs on the Evaluation of Carcinogenic Risks on Humans* Volume 83 Retrieved from https://monographs.iarc.fr/wp-content/uploads/2018/06/mono83.pdf

Inside Juul Culture: A Campus Epidemic. (2018, February 20). Retrieved from https://www.34st.com/article/2018/02/inside-juul-culture-a-campus-epidemic

JUUL Basics | (n.d). Retrieved from https://support.juul.com/home/learn/faqs/juulpod-basics

JUUL Facts | If You Don't Smoke Or Vape, Don't Start. (n.d.). Retrieved from https://www.juulfacts.com/

National Institute on Drug Abuse. (2018, December 17). Teens using vaping devices in record numbers. Retrieved from https://www.drugabuse.gov/news-events/news-releases/2018/12/teens-using-vaping-devices-in-record-numbers

NC3Rs. (n.d.). Retrieved from https://www.nc3rs.org.uk/news/five-reasons-why-zebrafish-make-excellent-research-models

Parker, B., & Connaughton, V. P. (2007). Effects of nicotine on growth and development in larval zebrafish. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/18041943

Petering, David & Tomasiewicz, Henry. SEPA retreat E-cigarettes final. Retrieved from https://docs.google.com/presentation/d/1iCwS8cch_xh4txuoklov9FCzEuwJf46rjc72rxdO5XY/edit#slide=id.p1

Peterson, C. (n.d.). *The Effects of E-Cigarette Nicotine on Zebrafish Development* (Rep.). doi:https://cpb-us-w2.wpmucdn.com/people.uwm.edu/dist/8/202/files/2016/06/Peterson_zf_Pardeeville-1i7kd9u.pdf

Raven, K. (n.d.). Your Teen Is Underestimating the Health Risks of Vaping. Retrieved from https://www.yalemedicine.org/stories/teen-vaping/

Reproductive Health. (2018, June 28). Retrieved from https://www.cdc.gov/reproductivehealth/maternalinfanthealth/tobaccousepregnancy/index.htm

Small, L. (2017, November 16). Vaping while pregnant could cause craniofacial birth defects, study shows. Retrieved from https://medicalxpress.com/news/2017-11-vaping-pregnant-craniofacial-birth-defects.html

Smoking & Tobacco Use. (2017, May 15). Retrieved from https://www.cdc.gov/tobacco/data_statistics/fact_sheets/health_effects/effects_cig_smoking/index.htm

Sparks, S. D. (2019, February 21). Teenagers Are Wired for Peer Approval, Study Says. Retrieved from https://www.edweek.org/ew/articles/2013/05/22/32peers.h32.html

Teens using vaping devices in record numbers. (2018, December 17). Retrieved from https://www.nih.gov/news-events/news-releases/teens-using-vaping-devices-record-numbers

The Dangers of Juuling. (2018, December 21). Retrieved from http://www.center4research.org/the-dangers-of-juuling/

The Smoking Alternative, unlike any E-Cigarette or Vape | JUUL. (n.d.). Retrieved from https://www.juul.com/

Tips From Former Smokers ®. (2018, April 23). Retrieved from https://www.cdc.gov/tobacco/campaign/tips/diseases/pregnancy.html

Tolentino, J., & Tolentino, J. (2018, May 31). The Promise of Vaping and the Rise of Juul. Retrieved from https://www.newyorker.com/magazine/2018/05/14/the-promise-of-vaping-and-the-rise-of-juul

Wellcome Trust Sanger Institute (2013, April 25). The Zebrafish Reference Genome Sequence and its Relationship to the Human Genome. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23594743

Why Use Zebrafish to Study Human Diseases? (2016, October 14). Retrieved from https://irp.nih.gov/blog/post/2016/08/why-use-zebrafish-to-study-human-diseases