TITLE: Cherry Electronic Cigarette Flavoring with Nicotine and Its Effects on Zebrafish Embryo Development

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ABSTRACT: *Zebrafish are important to research studies because they share a lot of the same major organs as humans, including the pancreas, brain and heart. In fact, 70% of human genes are found in zebrafish which make them useful in this experiment to see the possible short-term effects of different chemicals on a model organism. This experiment used wells that were filled with different concentrations of cherry electronic cigarette liquid containing nicotine. All of the embryos and hatched fry were killed by the liquid. This shows that the vaping chemicals are not good for embryos.*

INTRODUCTION:

A zebrafish is a freshwater fish belonging to the minnow family. Zebrafish are native to the Himalayan region and are a popular aquarium fish. They are often sold under the trade name “Zebra Danio”. Zebrafish are found in the southern region of the Himalayan region in parts of India, Bangladesh, Pakistan, Nepal, and Myanmar(https://www.sciencedaily.com/terms/danio_rerio.htm) . Zebrafish are important to research studies because they share a lot of the same major organs as humans, including the pancreas, brain and heart. In fact, 70% of human genes are found in zebrafish which make them useful in this experiment to see the possible long-term and short-term effects of different chemicals on humans. Zebrafish also breed rapidly, their embryos are laid and fertilized externally, and embryos are also clear making them useful in research (https://en.wikipedia.org/wiki/Zebrafish) .

This experiment involved exposing zebrafish embryos to different concentrations of cherry vape flavoring with nicotine. Vape liquid is made from vegetable glycerin, propylene glycol, different flavoring chemicals, and nicotine. Humans are exposed to it by inhaling the chemicals into their body and then exhaling to produce the vape clouds. What is known from previous studies is that vape juice also contains nicotine, which is found in tobacco and can make young humans and older humans addicted to the vape juice.

The hypothesis of this experiment is that the cherry e-cigarette liquid, which contains flavoring and nicotine, will increase the mortality rate of the zebrafish embryos. This will happen because nicotine is a very harmful chemical and so might be the chemicals in the flavoring. The independent variable will be the two concentrations (0.1 mg/mL and 0.2 mg/mL of nicotine) of cherry e-cigarette liquid and the dependent variable will be the hatch rate and mortality rate.

MATERIALS AND METHODS:

List of materials:

- 1 bottle of 0.1 mg/mL of cherry electronic cigarette liquid
- 1 bottle of 0.2 mg/mL of cherry e-cigarette liquid
- Beaker for dead embryos and liquid disposal
- Beaker for clean embryo media solution
- Dry erase marker
- 12 wells containing 10 zebrafish embryos from UW-Milwaukee’s Science Education Partnership Award (SEPA) Program
- 5 large bore pipettes

- 12 small bore pipettes
- Latex gloves and safety goggles

To conduct this experiment, twelve wells were filled with ten zebrafish embryos. Four wells were filled with an embryo media solution, another four wells were filled with 0.1 mg/mL of cherry electronic cigarette liquid containing nicotine, and the last four wells were filled with 0.2 mg/mL of the same cherry nicotine e-cigarette liquid. Data was obtained daily, recording hatch rate, and mortality rate of each well. The solutions were refilled daily, and the dead embryos were removed from each well. While handling the cherry e-cigarette concentrations, latex gloves and safety goggles were used.
To determine if the data was statistically significant, a T-test was used.

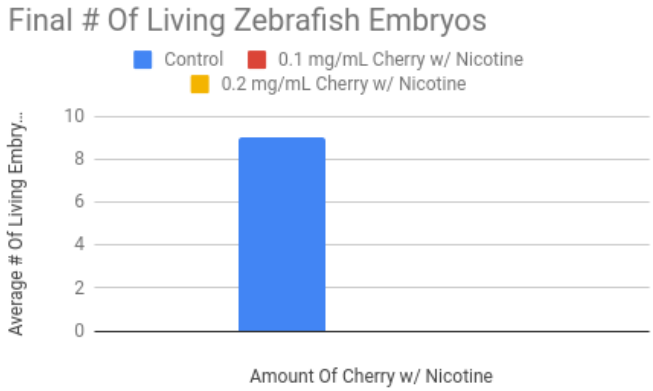
Results: The reason that this experiment was conducted was to test how cherry e-cigarette liquid containing nicotine acts on embryos. When the concentrations of cherry electronic cigarette liquid with nicotine were added into the wells, the fish embryos moved around a lot. All of the embryos and hatched fry were killed by the liquid. This supports the hypothesis that adding these concentrations would increase mortality rates within the wells. If the amount of cherry e-cigarette concentration increases in the wells containing zebrafish embryos, then the mortality rate of the zebrafish embryos also increases and happens over a faster period of time. Additionally, one of the fish in the 0.1 mg/mL concentration hatched without any eyes.

Treatment	Well 1	Well 2	Well 3	Well 4	Average	Probability	Result
Control	8	9	10	10	9		
0.1 mg/mL Cherry w/ Nicotine	0	0	0	0	0	p =0.0001	extremely statistically significant.
0.2 mg/mL Cherry w/ Nicotine	0	0	0	0	0	p =0.0001	extremely statistically significant.

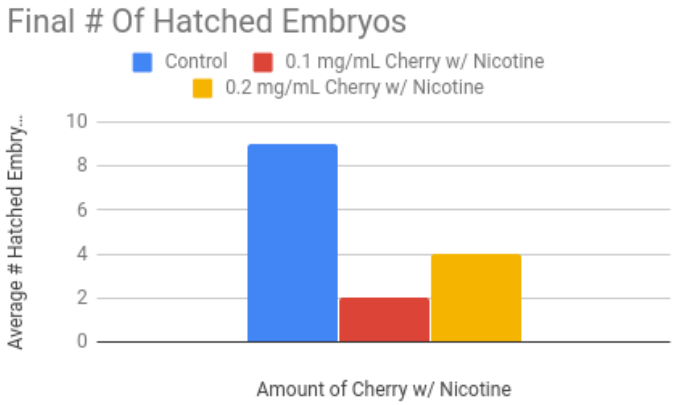
This data shows that the zebrafish affected in this test proved to be extremely statistically significant. The chemical that was being used to test on the zebrafish was Cherry flavoring with Nicotine.

Treatment	Well 1	Well 2	Well 3	Well 4	Average	Probability	Result
Control	8	9	10	9	9	-	-
0.1 mg/mL Cherry w/ Nicotine	0	2	3	3	2	p = 0.0022	very statistically significant.
0.2 mg/mL Cherry w/ Nicotine	4	7	0	3	4	p = 0.0647	not quite statistically significant.

This data shows that the zebrafish affected in this test proved to be very statistically significant. But, this is flavoring with Nicotine and not just plain Nicotine.



Fish hatched in the concentration but, all of them died and most of them lived in the control.



In this data some of the zebrafish in the concentration hatched, and most of them hatched in the control.

This photo shows a hatched zebrafish interacting with an unhatched zebrafish in our control well.

This shows a dead zebrafish in one of the wells with our chemical.

DISCUSSION: In conclusion, the data supports the hypothesis that cherry electronic cigarette liquid will increase mortality rate of zebrafish. No zebrafish survived in the wells containing concentrations of e-cigarette liquid. There was also an increase of fish deformities in these wells. Condensation found on the lid of the well plate could suggest that some of the cherry nicotine e-liquid was evaporated and then ended up in the control group wells. The control wells experienced little unnatural deformities, low mortality rates, and high hatch rates. These results relate to human ingestion of e-liquids because zebrafish and humans are similar. This type of research is a growing field, and much more research needs to be conducted, ideally in a long-term, professional setting.

REFERENCES:

Becker, Rachel. "Vape Flavors Can Make Nasty New Chemicals in Your e-Liquid." *The Verge*, The Verge, 18 Oct. 2018, www.theverge.com/2018/10/18/17996706/vape-juice-flavoring-electronic-cigarettes-chemicals-ingredients-irritating-e-liquid.

Clark, Karl. "How Zebrafish Genetics Informs Human Biology." *Nature News*, Nature Publishing Group, 2015, www.nature.com/scitable/topicpage/how-zebrafish-genetics-informs-human-biology-132740496.