# The Effects of Benzaldehyde on *Daphnia magna*

Alexandra Zajko

Anna Maria College

Author's Note
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**Abstract**

In recent years there has been a rise of young people using e-cigarettes. Typically, these young people have never smoked regular cigarettes before, this is surprising because the purpose of e-cigarettes is to be a safer alternative to smoking traditional cigarettes, which contain tobacco. Also, they serve as a safer way to smoke in public spaces and in front of people because the second-hand smoke from e-cigarettes doesn't compare to the second-hand smoke of traditional cigarettes (Home, n.d.). Opponents and critics of e-cigarettes will say that it is a gateway for newer generations to have a nicotine addiction and that this can have the effect of leading people into smoking traditional cigarettes. There isn't complete knowledge about the chemicals that replace the tobacco in these e-cigarettes. The present study investigates the impacts of benzaldehyde, a chemical found in e-cigarette solution, on growth and development in a small freshwater crustacean, *Daphnia magna.* Our results suggested that *D. magna* that were exposed to benzaldehyde from the one cell stage of their development grew faster than those who weren't exposed.

*Keywords: benzaldehyde, Daphnia magna (D. magna), e-cigarettes*

# The Effects of Benzaldehyde on *Daphnia magna*

## Introduction

A little over a decade ago, China had a single company that sold e-cigarettes as a tobacco-free alternative to traditional cigarettes. Since then, it has become a multibillion dollar industry worldwide. There is a big split in the public health community about whether or not e-cigarettes are beneficial, or not, to society. Nicotine is a stimulant found in tobacco cigarettes and frequently used in combination with chemicals for e-cigarettes (UW, n.d.). Although nicotine is found in solutions of liquid it is not a liquid itself and is mostly insoluble in water. Nicotine in its vaporized form has been a prevalent addiction in today's society to a degree that has been unmatched in world history. There has been a lot of controversy over the use of e-cigarettes (also called vaping) that contain nicotine.

The e-cigarette idea is very popular among the high school and college aged students. It can lead to nicotine addiction from something that, ironically, is supposed to be an alternative for someone trying to quit smoking traditional cigarettes (The Smoking Alternative, 2018). Evidence of nicotine addiction can be seen as people developing irritability and headaches from lack of having nicotine in their system. A common misconception, due to good marketing by manufactures of e-cigarettes, is that there are no adverse effects from smoking an e-cigarette because there is a lack of tobacco and no offense odor.. Although e-cigarettes don't contain tobacco, they contain many other chemicals. Current research aims to determine what risk is associated with the chemicals found in different brands of e-cigarettes.

Some e-cigarette chemicals are used to keep the nicotine in a semi-liquid form, to preserve or flavor it, or to help it burn so you can inhale the vapor. This raises some questions. What exactly are the chemicals? What are in these chemicals? What are the long-term effects of

using e-cigarettes? What are the by-products? Are they more addicting or harmful than the by-products? Are they more addicting or harmful than the chemicals found in normal cigarettes? These are the questions people are wondering. These are the questions that major research facilities are trying to answer. The research that will be discussed in this paper will be about the e-cigarette chemical, benzaldehyde, and its effects on *D. magna* growth and development. This research does not aim to answer all of the aforementioned questions, but this research instead aims to open the door into a conversation about e-cigarettes. There is a lot to be known about these manufactures and what they are putting into e-cigarettes. The high demand for products and goods makes the market place a highly competitive one. To stop the manufactures from stealing formulas from one another, they can label certain things as "flavorings" and they don't have to be specified in the ingredients section. Manufacturers can exploit the fact that the general public doesn't know about e-cigarette manufacturing and use this as a motive to make the flavored nicotine more addictive. Consumers may be unaware of this because manufactures are not required to provide chemical details. The research presented here will go into a chemical the public knows little about, benzaldehyde, which forms after the vape juice in e-cigarettes gets heated by a coil in order to become vaporized.

This research will investigate how benzaldehyde affects a small freshwater invertebrate, *D. magna*, specifically we observed how the growth and development of the species was impacted. Benzene is a well-known human carcinogen, but there is still much to learn about the impacts this chemical has at the cellular level. The carcinogenic properties of the similar chemical, benzaldehyde, is poorly understood. Although it is said that benzaldehyde is "probably not a human carcinogen" according to the United States Environmental Protection Agency's

Classification of Evidence of Cancer Causation, there has not been enough evidence to say with certainty that it is or isn't.

**Statement of the Problem**

Benzaldehyde is the simplest aromatic aldehyde. Aldehydes are organic compounds that tend to smell good. Benzaldehyde, in particular, is an industrial useful material because of its almond-like flavor. It is a colorless liquid. It is regarded as a safe food additive by the Generally Recognized as Safe (GRAS) foundation. One interesting feature is that in the body, it gets metabolized as benzoic acid. If it is deemed as safe for a food additive then it should be fine being added to e-cigarettes, right? This is where the critical question lies. In previous studies, the inhalation of vaporized benzaldehyde effected rabbits by, causing irritation to their eyes, edema, erythema, pain and allergic reaction. Vaporized benzaldehyde also caused death to the rabbits (Final Report, 2006).

As of right now, our previous experience from working with *D. magna* has allowed us to look at their development and see how this aldehyde may affect normal growth and development. As most know, a cell is made up of many different components. The structure of a cell is kept in place by three types of filaments. There are microtubules, microfilaments called actin-filaments (f-actin) and intermediate filaments. Together, these filaments make up the cytoskeleton that give structure to the cell just how the skeleton gives structure to the human body. The cytoskeleton also helps with movement and duplication of the cell. It would be the hypothesis that if there was going to be a cellular change in the *D. magna* cells it would take place in the filaments.

**Hypotheses**

1. Benzaldehyde will impair the ability of *D. magna* to develop correctly.
2. Benzaldehyde will have cytotoxic effects on *D. magna*
3. Benzaldehyde will have mutagenic effects on *D. magna*

**Questions**

How will benzaldehyde affect living *D. magna*?

How will benzaldehyde affect the cells of the carapace?

**Purpose of Study**

The goal with currently studying e-cigarettes is to see how it effects biological, social and behavioral perspectives in our culture. E-cigarettes were invented to be a way to stop people from smoking traditional cigarettes which are known to not only have many negative effects on the human body but also the second and third hand smoke they give. The main purpose for bigger researchers is to see how the chemical components of e-cigarettes affect areas of the brain and the body's. This research focuses mainly on the potential development changes that could be seen in *D. magna.* There is also room in this study to see the toxicity of benzaldehyde. The significance of this study could reveal the impacts of benzaldehyde on the early stages of the *D. magna.* Benzaldehyde has shown mutagenic and cytotoxic effects in other species of animals at different concentrations so, the interest is to see if benzaldehyde disrupts the normal growth pattern of the *D. magna*.

Recent studies have suggested that there is a high risk among young Americans to use flavored e-cigarettes because, the numbers of usage are increasing. This means exposure to

chemicals like benzaldehyde are also increasing. E-cigarettes is supposed to be seen as a way to stop people who are addicted to smoking cigarettes, but now adolescents are smoking e-cigarettes first, then leading to cigarettes. The Food and Drug Administration (FDA) recently decided that big commercial companies like JUUL brand vapes cannot sell flavored nicotine because they did not show progress in efforts to stop selling their products to adolescence. Communities like Holden and Paxton, Massachusetts, and in other places around the nation had the ban on flavored JUUL products before this ban was implicated.

**Significance to Field/Significance of the Study**

This sort of research is very important to the science field. In order to educate individuals about their personal health and choices they can make. The increase consumption of vaporized benzaldehyde could become concerning especially because there are no long-term effects recorded by humans. The public not knowing about the effects of e-cigarettes is something that is so predominate in our culture, it should be a concern to everyone. The chemicals in e-cigarettes has been plaguing many cultures for only a short time, it is not too late to change course. This research came at a very interesting time because there is so much being done with e-cigarettes right now (Cheng, 2014). This research can get students at every level to start talking about the dangers of unknown substances we put in our bodies. This research can be so groundbreaking that it can start a larger, more important conversation with young adults and parents everywhere in our culture.

**Definitions**

Benzoic acid- A simple aromatic carboxylic acid, occurs naturally

Carapace- Shell of a crustacean (In *D. magna*: the carapace is transparent)

*Daphnia magna (D. magna)- Planktonic freshwater crustacean,* commonly called water fleas because they swim in a jumping motion

*Drosophila melanogaster*- Common fruit fly

Exposure Time- The length of time when the subjects are exposed to the benzaldehyde

Genotoxicity- Disruptive effects on DNA (cell's genetic material)

Morphology- In biology, this is a concept that examines the forms of an organism and the relationship between its structures

Paraformaldehyde- A product used for fixation. It is the polymerization product of formaldehyde

**Time Restrictions**

During this semester, one to two weeks were required to isolate female *D. magna* and raise them in a controlled environment that is similar to their natural pond water, with the 400 mL beaker filled with synthetic pond water and *Ankistrodesmus* algae. The females were raised at a constant temperature (20 ˚C) and artificial lighting (16-hour light: 8-hour dark) periods, to ensure that the environmental conditions were controlled. This way, the study may be done again, by us, or others. Next, a specific amount of benzaldehyde 5 µg/mL was added. This amount was decided upon based on previous research that was reviewed. In this trial, there were four groups. Two groups of juveniles and two groups of early exposure. One group from the juveniles and one group from the early exposure were counted as the negative control group

would not be exposed to benzaldehyde. The other two groups would be exposed. Next, the cultures were monitored daily to observe and check on each female and the neonates that are produced. If there were neonates produced then the mother will be removed to further analyze the growing neonates. While analyzing the data, it will be important to note any cell difference from the exposed population versus the control group. If there aren't any differences, that is also very important to note. What the result yields will depend on how reliable the experiment is.

It will take many weeks to read previous research that has been done on *D. magna* and benzaldehyde. This is needed to understand what others have done and how this research could avoid practices that didn't work and implement practices that do work. There will have to be several weeks planned out to plan and organize the experiment. Lastly, a few weeks will be needed to do one exposure and then some more time to analyze the results. This is a time restriction that has to stay in mind. One semester will shorten the exposure and observing times significantly than if there were two semesters to complete this research project. Dr. Christensen from Anna Maria College will supervise and help assist this experiment.

**Ethical Consideration**

In order to test benzaldehyde effects on a living species, the test subject will be *Daphnia magna*, which are a common model system for aquatic research. There is not thought to be any significant moral consideration for this species, as an arthropod it is in the same taxonomic grouping as a mosquito. For federal research funding, the U.S. government only starts to require ethical justification once the test subject has vertebrae like a zebra fish, for example. *Daphnia magna* are inexpensive to obtain, so there won't be financial limitations to maintaining the cultures. They produce asexually, which means that the test subject should be exact clones of the

parent; this is important for testing reliability. Species like *D. magna* only take a few days to mature from neonates to adults. This is beneficial because the effects that the benzaldehyde has on the species will be able to see as they grow up. It is observable to see the whole lifespan of the animal unlike in humans. Due to the fact, e-cigarettes haven't been around for long, there haven't been any lifelong effects recorded. Maybe something that happens to the *Daphnia magna* throughout early development will be mirrored in humans. There is no other ethical consideration for the study population and there is no need to complete the IRB consent or proposal form.

## Chapter Two: Literature Review

E-cigarettes is prevalent in today's culture and has been a spotlight topic in the media and in many social circles. The spike in the use of e-cigarettes especially among youth has grabbed the concern of the United States FDA. There are reports that go into this epidemic and they all express the same thing; the rate of using e-cigarettes is dramatically increasing. The consensus is that no child should want to be using nicotine products nor they should feel the need to, due to the danger of addiction. Although there are many campaigns and social groups that all agree with the idea that children shouldn't use tobacco products, most people recognize that children shouldn't be hooked on any addictive substances, including nicotine products. Benzaldehyde is a chemical that is present in combusted e-cigarette juice solutions. The purpose for conducting this study is to determine the effects of benzaldehyde's growth and development. We exposed *Daphnia magna* juveniles and neonates still developing in the mother to benzaldehyde to investigate potential effects of benzaldehyde on normal growth and development. The defects

that might be shown in the D. magna growth and development may highlight some over looked health risks for e-cigarette juice.

**Other Works**

In order to complete this experiment there had to be prior research into other similar works. For example, how a single by-product produced by the vaporized coil was chosen was by first reviewing what chemical were most prevalent in common e-cigarettes. Once the list was done, there were a few options, but benzaldehyde made the most sense for this experiment. Once a single chemical was chosen there was extensive viewing done to see what other kinds of experiments have been done with benzaldehyde and *D. magna.* We did not identify any existing studies conducted that test the effects of benzaldehyde on *D. magna* growth and development. Surprisingly, there were a lot of studies claiming that benzaldehyde was actually shown to have beneficial effects (Cheng, T). When researching this chemical, it was interesting to see that it was seen as a carcinogen for rats but not mice (Final Report, 2006). Furthermore, it was seen as a dermal irritant in rabbits. When looking up the effects benzaldehyde had on humans however there was little said. Eventually, there were two articles that were chosen. Both articles were with benzaldehyde and *Drosophila melanogaster*.

This was seen as useful because both *Daphnia magna* and *Drosophila melanogaster* are invertebrate experimental models. One article went into detail about the visual flight orientation of *Drosophila melanogaster* when exposed to benzaldehyde (Demir, 2008). The other article also focused on the relationship between *Drosophila melanogaster* and benzaldehyde but focused on the genotoxicity in a wing spot test (Liu, 1998). Reading through both articles and viewing the concentrations that proved to have adverse effects versus the ones that did not, it was decided

that the concentration we would use would be 50 μg/mL. Eventually, this got ruled out which is explained in the discussion section.

**Consumer Use**

It seems that every day new and exciting products come out for consumer use. There are many chemical combinations that get mixed together in order to make products better. There are many advancements in science that lead to products like e-cigarettes to be pushed into today's markets. The idea of fast production is good for consumers and the economy, but it might not be good for the environment, animal or human health. Big researchers are just now looking at the long-term effects of e-cigarettes and their chemicals. The chemical that people are starting to pay attention to are the ones that produce once the juice is vaporized into a smoke that the consumer can than inhale and then exhale. The areas of study will include research on benzaldehyde, research on the species of *Daphnia magna,* and finally the connections that can be made on a cellular between the aquatic organism and humans.

**Environmental Concerns: Water and Aquatic Life**

The environment is what allows all life to exist on Earth. Water is a vital and gigantic part of the ecosystem. It provides life needed substances and habitats for many species. For humans, it is important for transportation and fluid support, but this need for humans comes from big bodies of water. The small streams, lakes, and ponds don't provide instant need for humans. These smaller bodies often fall victim to waste disposal by companies and human personal disposal. Although benzaldehyde isn't fully soluble in water, it can still be dumped into waterways by accident or on purpose. E-cigarette companies might use waterways as a cheap

disposal method to bypass laws and regulations. Aquatic life will be the research's major focus. There is a lot of evidence to support the idea that humans impact the water and its species. In previous years, there has been a lot of intentional dumping of all sorts of different waste products such as run-off chemicals (Learn, 2018).

These chemicals can disrupt species by, reduce the number of offspring, cause mutations and cause species to go extinct. Some of the chemicals effect won't be able to be seen by the visible eye but on a cellular level. That is why observing the carapace shell of our species after the exposure time is the best solution to see the data. *D. magna* is a great model system for all aquatic life (Ebert,1970). There are no vertebrae so there is no ethical or moral concern. This is a cheap alternative to fish and other larger systems. *Daphnia magna* are a great alternative, but it is both a weakness and a limitation that we are using them. Society's ultimate goal is to see the effects of e-cigarette chemical effects on humans. It would be more beneficial to work with a species that have a similar genome and genetic makeup as a human, but these tend to be expensive, and would have many ethical and moral concentrations that might not yield results that are useful.

**Humans**

As states previously, humans are the primary goal with this kind of research because the vaporized chemicals effect humans directly. It is very hard to do research on humans for a variety of reasons. There are many guidelines and regulations that go into using human subjects especially when you are trying to prove something harmful which is what this research aims to prove. Luckily, animals across most species have very similar cells and the function of those cells are pretty similar. Comparing *D. magna* and human cells, they are in fact structurally close

to the same. If there is a major defect in the cell formation, division or replication then it could be linked to humans immediately or in other research in the future.

With the rapid increase of e-cigarettes, both users of the products and non-users are exposed to aerosol and chemicals of the cigarettes. The lack of tobacco in these products are by far the most attractive feature allowing consumers to continuously say that e-cigarettes are the heathier alternative to traditional cigarettes. E-cigarettes have the ability to be used in a smoke-free environment. As a society, we are allowing e-cigarettes to get a free pass without knowing all of the risks and long-term effects of all of the chemicals and their byproducts. The exposure that the environment is coming into contact with isn't fully known due to the harmful chemicals and their short and long-term effects. The chemical that was chosen was benzaldehyde because of the dominate nature of the chemical being in so many consumer products due to its almond odor. The research in this paper aims to answer what harmful effects could happen to the vulnerable aquatic organisms, *Daphnia magna.* This will give indication on how these chemicals could affect other water living organisms as well. These are the primary concerns of this research. Although we can't make full assumptions about what the chemicals do to humans, we can still see the cellular response given to us by *Daphnia magna* and relate that to humans.

## Chapter Three: Methods

We are able to make these comparisons because of how similar the structures of both species' cells are. For this experiment it is very important to have complete control of the experimental design. In order to do this, a female *Daphnia magna* had to be isolated from the stock environment from the US Environmental Protection Agency (EPA) where they were allowed to grow in a controlled environment. Due to the fact that *D. magna* are asexual when

conditions are fair means that isolating one female will allow for her to produce neonates by herself. The female got fed a specific amount of 10 ml of *Ankistrodesmus* algae every day. There were specific light and dark periods or 16 hours of light and 8 hours of dark, and the temperature regulated at 20 degrees Celsius or 68 degrees Fahrenheit. Allowing the female to have several batches of neonates was key to make sure that the mother was only producing neonates with the controlled environment and not the free stock one. Once the female produced her second batch of neonates, they are subject to the exposure of benzaldehyde. Using a very specific concentration of benzaldehyde 5 μg/mL we will expose the *Daphnia magna*. By doing this, it will be clear to see the observable effects that the chemical had on the *Daphnia magna.* At the completion of the study subjects were preserved in a paraformaldehyde solution, and we subsequently look for developmental defects and measure the size of each animal. Measuring mortality during the experiment and making observations at the conclusion at the end of the study will be important in seeing how benzaldehyde actually effects the species.

The setting of this research is through Anna Maria College located in Paxton, Massachusetts. The experiment took place in the small lab located on the left side lab of the basement of St. Joseph's Science Building. The participants are *Daphnia magna* were selected because of how much information that is known about them already and the fact that they are a good model system to compare to other aquatic life which was previously stated. This species of *Daphnia* lives in brackish waters. They live in rocky pools along the Atlantic coastlines. There have been smaller populations that have been seen in the Northern Americas (Elenbaas, n.d.). In order to obtain this specific species, they were provided by the EPA. An adult female can grow up to about 0.5 cm while the males are much smaller only growing to about 0.2 cm. The independent variable will be the benzaldehyde concentration in the water, because that is being

controlled and *Daphnia magna* is being tested and observed. Measurements of growth and developmental defects will be the dependent variables. In order to complete this experiment there are a lot of marterials that are needed.

**Prep Materials:**

- 400mL beaker filled with synthetic pond water, *Ankistrodesmus* algae, and stock population of *D. magna*
- 2 400mL beakers filled with synthetic pond water with 10mL Ankistrodesmus algae and with two females in each
- Countertop VWR refrigerator that is temperature controlled with the degrees digitally displayed
- Artificial light: lamp: 16 hours during the day and is turned off for 8 hours during the night
- Journal kept for monitoring progress
- Feed females 20 mL of Ankistrodesmus algae a day for three-week period

- **Experiment Materials:**
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- - benzaldehyde (final concentration to 5 μg/mL)
- - Paraformaldehyde in a 1.5 mL Eppendorf tube
- - Fisherbrand Falcon conical tubes 50 mL
- -Plastic transfer pipettes
- -Microscope with camera
- -Glass slides and slip covers for viewing

After the *Daphnia manga* were in the prep stage for three weeks in a controlled environment and were allowed to produce neonates then, it was time for the exposure. The females were picked based on how far they are into the neonate producing process. The goal is to get four sets of females that match with one other female. For this particular experiment, there were two females that had no signs of neonates at all and two females that were in about the same stage of pregnancy. Then there were two other females from each group were chosen to be the control and another two were chosen as the benzaldehyde reciprocates.

The most important material will be the benzaldehyde. This material is the key substance that will make the difference in the experiment. The next chemical of importance would be paraformaldehyde. This will be used to suspend the *Daphnia magna* after we initially observe them in their tubes we will fix them using paraformaldehyde, to better observe them under the microscope. This is needed to determine if there are morphology differences within the two-test groups. Transfer pipettes are the third most important instrument because they cannot be contaminated when it comes to moving the *Daphnia magna* from different beakers. This way nothing can be contaminated or used for any other procedure. These pipettes are reliable, but the tips have to be cut off in order to ensure that the *Daphnia magna* are allowed to be transferred without getting stuck in the tube. The next instrument that is very important is the assistant electronic pipette that ensures that the *Daphnia* get exactly 10 mL of food every day. Then, the stereo microscope will be the next important because that is what will be using to view the *Daphnia magna* once they have been suspended in the solution to view the shells to see the benzaldehyde effects on the species. The microscope camera allows us to see the details of the carapace structure.

The study was done by again isolating females and for several weeks observing them every weekday. The temperature, light, food intake, number of adults, and number of offspring were all kept in a journal. Once a female had a round of neonates they were removed until the third batch so that the neonates were definitely being produced and being born in the controlled environment. After this was completed, the exposure was ready to begin. The first step in this process is to pick two sets of females that look to be at the same point of developing. Originally, there were two chosen that were in the middle stage of giving birth and two that showed no signs of pregnancy but eventually had to substitute a different female that did show signs of early pregnancy, explanation for this shown in the results section. After, we put one female in each group in regular media and the other one with 5 μg/mL of benzaldehyde. Letting them be exposed for exactly one week, there were observations made. In order to give better and more detailed observations, the *D. magna* were then subjected to paraformaldehyde in order for the *D. magna* to be suspended in their state. Once this was done the *D. magna* could then be reviewed under a microscope and be photographed. After photographing, the measurements of the carapace were taken. The measurements were put into excel in order to get viable data. The charts and tables can be seen on page 20 and 21.

## Chapter 4: Results

At the end of the study, the animals were preserved in paraformaldehyde. Transfer pipettes were used to move preserved *Daphnia magna.* The microscope camera showed the cellular responses and impacts on *D. magna's* Development from the exposure of benzaldehyde. The initial idea of using 50 μg/mL benzaldehyde into 50 mL of synthetic pond water proved to be too toxic for the crustaceans. Within an hour the isolated females died. With this is mind, the concentration went down to a tenth of the original concentration so that the *Daphnia magna* would be affected and

not die before the exposure was completed. At 5 µg/mL it appeared that the growing rate of the Daphnia magna was more consistent than compared to the controlled group. Under the microscope that seemed consistent and with the added discovery that the *D. magna* looked like they grew very constant with each other. The groups that weren't exposed to benzaldehyde grew at slightly different rates from each other and also were smaller than the benzaldehyde exposed groups. Results with this can be seen in graph 1. Other possibilities for results could have been a change in the carapace, digestive tract, eyes etc., but there was no evidence of these changes. The P-value is 4.7 X 10 -7 (0.00000047) which is a lot lower than the standard of 0.05. This means that there is a rejection of the null hypothesis because there is a significance. There is a significant difference between the four groups sizes. The standard average error was a range between 0.074 and 0.019 for the exposed groups and 0.11 and 0.12 from the controlled group.

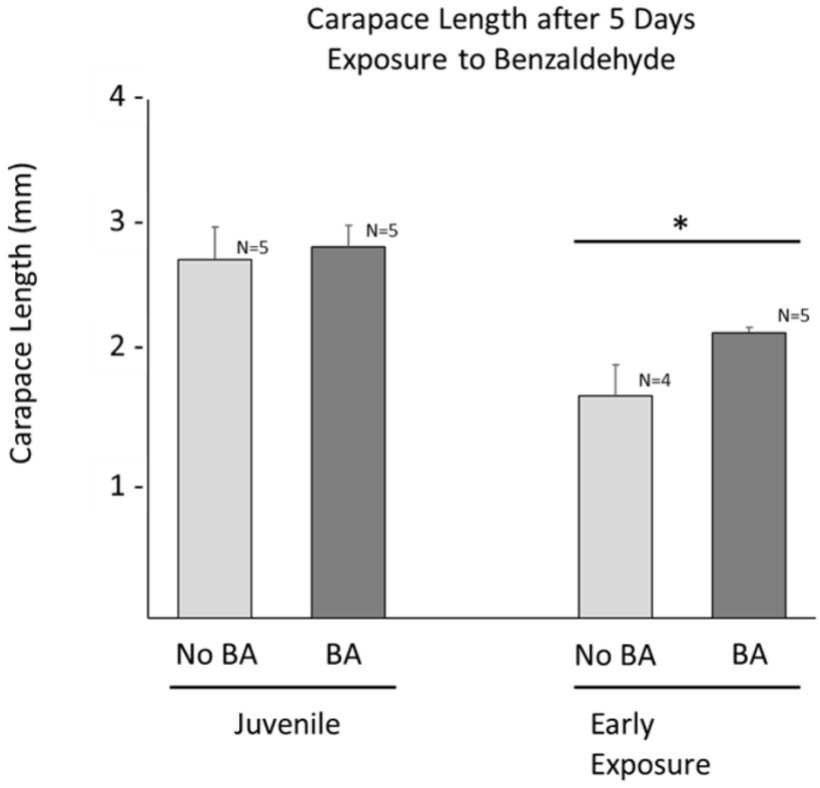


*Figure 1:* Carapace length after exposure to benzaldehyde

## Chapter 5: Discussion

There are obvious reasons why traditional smoking is a negative activity to participate in. There are many smoking related problems like cardiovascular, reproductive, and respiratory problems. Most people know of the harmful consequences to smoking. With the newer form of smoking through e-cigarettes, there are a lot of unknown chemicals and hazards that can affect not only humans but other animals and the environment as well. To battle the up-and-coming trends that are seen in e-cigarettes like flavor additives. Seeing the clear increase in e-cigarette usage especially, with children starting at a very young age, was the first idea about doing research surrounding the e-cigarettes. After further research there were clear chemicals that were abundant in many of the more popular brands and flavors of e-cigarettes. This chemical was benzaldehyde. Although it is regulated as a safe food additive there is no regulations for when this is a chemical of e-cigarettes.

The results surrounding the experiments were much different to the expected results. At first, it was hypothesized that 50 μg/mL would be a good amount for the *Daphnia magna* to be exposed too. This was because of other research and articles that were looked into before the experiment took place. It was quickly ruled as a too intense concentration with the 50 mL of water. The two groups that were exposed to this concentration died only after about an hour of being exposed. It is believed that because benzaldehyde isn't fully soluble in water, this caused there to a be a pocket of the chemical. Deciding to drop the concentration to a tenth of the original, 5 μg/mL, was done in order to have adverse effects but without killing off of the population and so that the chemical would be more soluble in water compared to the original concentration.

After reviewing the *Daphnia magna* before they were suspended in paraformaldehyde was interesting. From a visible eye perspective, it seemed that the *D. magna* that were exposed to benzaldehyde grew at a faster rate than the *D. magna* that weren't exposed. Under the microscope that seemed to be consistent as well. The *D. magna* that were exposed also grew more consistent than the groups without benzaldehyde. The entire bodies and digestive guts of the *D. magna* in group 3 *(*weren't exposed to benzaldehyde) looked smaller than any other group but that could be for reasons explained later in the limitations and weaknesses section. There was no significance between group 1 and 2 of the unexposed and exposed juvenile group. There was however, significant differences were shown between group 3 and 4 of the early exposed neonates. This might be because the early exposed neonates were exposed from the one cell stage and were certainly more vulnerable than the juveniles that were farther along in their development process.

**Limitations and Weaknesses**

The biggest limitation/weakness of this study would have to be the time frame. One semester might be enough time for academic based thesis, but most experiments take more time to complete leaving the results feeling rushed. Being able to do several different trials so that there were comparison trials or be able to fix problems in the previous trail. This allows to limit human error as much as possible and validate any results that are produced. The next limitation would be the school resources. Although it is nice that there are opportunities for undergraduate students to work with smaller invertebrate organisms, there is a lot to be said for larger institutions and organizations that put more money into their science programs. Working with other organisms that are closer related to humans would give more of a hint towards what the

long-term effects of benzaldehyde have on humans. The final limitation was with the viability of the results. Between sample group 3 and 4. These neonates were off within a few days of each other and although there was an effort to correct this by waiting to expose group 3 to paraformaldehyde. This way, group 3 had a chance to catch up with group 4. By having to do this could have disrupted or altered the results.

**Recommendations for Future Research**

In addition to this research, there could be a lot of changes made in order to get more reliable results. Having the experiment over a longer period of time would allow for multiple trials of the same experiment and more modifications along the way. Doing a slightly higher concentration of benzaldehyde would have allowed for a more obvious difference in the *D. magna.* Also, being able to have a longer period of time would allow for a longer exposure time. Comparing exposure times would be interesting because it is possible that the time the *D. magna* spent in the benzaldehyde would make a difference.

Finally, Benzaldehyde did not make as a significant difference as was hypothesized. However, there is significant purpose in doing this sort of research. E-cigarettes are at every gas station, smoke shop and convenient store around America. There are constant modifications being added to e-cigarettes, a major one is the flavorings that are offered. It is hard to preform valid research when the new trends are coming and going so quickly in today's culture. The long-term effects of the chemicals and by products from e-cigarettes are going to have to be looked at very carefully in the future because there is so little known about the substances.

Weather it is the vaporized nicotine, the chemical vape juice, the vaporized by-products or a combination of all three, there is a lot unknown about these substances that young teens are putting into their bodies.

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**Appendix A: Catholic Church on Scientific Research**

Many people assume that religion in general and science repeal each other. There is a stigma that if you have a religious standing, you cannot have a scientific understanding and if you have a scientific standing you cannot have a religious understanding. There is also a misunderstanding that religion doesn't seek movement or change and science has to stand alone while making contributions to society. Both science and religion aim to seek truth. Both can work together to discover truth and to correct the wrongs/evil of the world. The Catholic Church does touch upon scientific research in its teachings about human dignity. A main lesson of the Catholic Church says that human life is sacred and above all that the life of a human being

should be respected and protected. Although the Church says that human life can be wrongly comprised under practices such as abortion and euthanasia, the idea of research can be a lot different. Current human research can include embryonic cell research, stem cell research and gene editing. This appendix will go into each of these ideas about human research slightly and will overall express what the Catholic Church says about human research.

A recent and popular type of research is embryonic and stem cell research. They are usually thought of as very similar as far as their uses, but the Catholic Church separates them clearly. The Catholic Church actually supports ethically responsible research of stem cells because stem cells have the ability to be directed into almost any differentiated cell type. Using stem cells in order to replace damaged or mutated cells can help lead to treatment of a wide variety of diseases like spinal cord injuries, heart disease, muscular denegation and arthritis. Stem cells do not have the ability to grow into another person and that is why it is why the Catholic Church actually supports this as a research option. The Catholic Church only opposes embryotic stem cell research because embryonic cells have the gift ability to grow into a human and therefore are sacred and deserve just as much dignity as anyone else. Even though the government allows certain labs to use embryotic cells in their research, it is under tight restrictions. Even with these restrictions set in place, there has been people who have illegally kept embryotic cells alive for longer than the allowance in order to practice gene editing (Larson, 2018). The practice of gene editing in itself does have some support coming from the Catholic Church.

There are so many diseases that come from genetics. Gene therapy aims to provide a way to correct these abnormalities without allowing for over compensation to make the individual better than average and without passing any complications to future generations. The scientific

community is working hard to find a way to fix the genetic mutations and pre-dispositions that humans face every day. The Catholic Church also seeks to end the suffering brought upon families and communities by genetic diseases and conditions. Where the Catholic faith stops supporting is when the therapy of the gene edits turns into enhancements. The idea of making superhumans for the military and being able to pick and change physical appearances has been in movies and television shows for a very long time. With the rapid speed of technology and where it is heading these supernatural ideas could become a scary reality. These enhancements could have long term effects and lead to permanent damage of the human species. Man was made in God's likeness and genetically enhancing humans can be seen as a way of disrupting that. With this being said, science and Catholic faith should be only focusing on gene therapy can do to fix the mutations with genes, not enhance them.

Bio-ethics is extremely important because it is a part of the Catholic Tradition that isn't static in time. Bio-ethics moves and expands with healthcare, scientific research and technology advancements. The Catholic faith tries to be a guide to developing concerns and create the dividing line between yes and no. For the most part, despite what some might think, the Catholic Church supports a big part of the scientist community. The Church ultimately wants people to live a more comfortable and balanced life and through scientific research, this is possible. At first, being a Health Science major at a Catholic college I assumed that I was going to have to constantly modify my beliefs in order to make sense of any course I took. Throughout my four years here, I have found that this was not the case. Religion and science do go hand in hand and together they can provide reality, comfort, compassion, mercy, and overall truth to a lot of problems that the world has been and continues to face today.