

The Effect of Antiperspirants Containing Aluminum Compounds on the Growth and  
Development of Zebrafish Embryos

Jelena Vorkapich

Greendale High School

### Abstract

Antiperspirants and deodorants are widely used by most teenagers and adults. However, recent concerns about the safety of aluminum compounds found in antiperspirants has challenged the popular use of them. This experiment tested the effects of two antiperspirants containing aluminum compounds on zebrafish embryos, to see if antiperspirants negatively affect the growth and development of zebrafish embryos. Zebrafish embryos were exposed to wells coated with either *Degree* or *Secret* antiperspirant (both of which contain aluminum compounds). Additionally, *Tom's* natural deodorant (which lacks aluminum compounds) was tested on the zebrafish embryos, and Instant Ocean solution served as a negative control for the experiment. Zebrafish embryos exposed to both antiperspirant and *Tom's* natural deodorant had a lower amount of alive zebrafish embryos at 72 hours, compared to the amount of alive zebrafish embryos exposed to Instant Ocean solution. Additionally, zebrafish embryos exposed to both antiperspirants had undefined body plans. Overall, because the antiperspirants and deodorant tested contained multiple ingredients, this created multiple confounding variables within the experiment. Further studies should be tested which isolate aluminum compounds; then, more definitive conclusions could be made.

### Introduction

To the majority of Americans, antiperspirants aren't seen as a major threat; in fact, about 90% of Americans use some sort of cosmetic underarm product (McCoy, 2014). Antiperspirants differ from deodorants (Unilever, n.d.). While deodorants contain antimicrobial ingredients that work to reduce one's body odor, the majority of antiperspirants contain aluminum compounds which dissolve in one's sweat to form a gel that temporarily "plugs" one's sweat gland, decreasing sweat and odor (Unilever, n.d.). However, the majority of antiperspirants also contain ingredients that work to reduce one's body odor as well (Unilever, n.d.). Today, many are questioning the safety of the aluminum compounds that are active ingredients in both antiperspirants and deodorants, as several studies have proven that a positive correlation exists between aluminum compounds and breast cancer (Croager, 2004).

In a study performed by Kris McGrath from Northwestern University, 437 women with breast cancer answered a survey regarding deodorant and underarm shaving habits (Croager, 2004). Out of that sample, women who applied deodorant weekly and shaved twice a week were diagnosed with breast cancer about 15 years earlier than women who did not use deodorant or shave (Croager, 2004, 174). Additionally, in a study which aimed to observe the amount of aluminum in breast tissue, biopsies of four different regions of the breast tissue from 17 breast cancer patients who had undergone mastectomies were observed through graphite furnace atomic absorption spectrometry (Exley, Charles, Barr, Martin, Polwart, & Darbre, 2007, 1344). Not only was there a significantly higher concentration of aluminum found in the outer regions of the breast across all patients, but there was an overall higher concentration of aluminum found in the patient's' breast tissue compared to the concentrations of aluminum in normal breast tissue

(Exley et al., 2007). Because one typically applies antiperspirant/deodorant near the outer regions of the breast/armpit, it is concerning that a higher amount of aluminum would be found in that location.

*Danio rerio*, commonly known as the zebrafish, are freshwater fish originally found in the tropics (Badman, Dougan, Greenberg, Heindel, Matthews, & Trevarrow, n.d.). Because zebrafish are vertebrates, they are more similar to humans compared to other organisms (Badman et al., n.d.). This allows one to easily apply the results of an experiment on zebrafish embryos to everyday life, and served as the reason behind testing zebrafish embryos specifically in this experiment. Additionally, zebrafish embryos are transparent, allowing one to better observe any developmental changes that occur throughout the experiment (Badman et al., n.d.). The purpose of this experiment is to see how antiperspirants containing aluminum compounds affects the growth and development of zebrafish embryos.

The hypothesis for this experiment states that if zebrafish embryos are exposed to antiperspirants containing aluminum compounds, then they may have abnormal structural changes, because antiperspirants containing aluminum compounds have been associated with breast cancer, showing that they may have adverse effects on organisms. The results of this experiment can benefit all consumers of antiperspirants and natural deodorants. Not only will consumers be more informed about the safety of antiperspirants containing aluminum compounds when making purchases, but they will also be able to make more cost-effective purchases (as natural deodorants are typically more expensive).

In this experiment, two types of antiperspirant, *Degree: Invisible Solid (Shower Clean)* and *Secret: Invisible Solid (Powder Fresh)*, were used. Although there are various brands of antiperspirant, both *Degree* and *Secret* are widely known brands, allowing the results of the experiment to be applied to a broader audience. These specific types of antiperspirant were also chosen in order to minimize the amount of confounding variables in the experiment. Both types were composed of a similar amount of aluminum compounds. 18.2 % of the *Degree* antiperspirant tested was composed of aluminum zirconium tetrachlorohydrate, while 18% of the *Secret* antiperspirant tested was composed of aluminum zirconium trichlorohydrate. Furthermore, the majority of the ingredients were the same; both antiperspirants tested contained cyclopentasiloxane, stearyl alcohol, hydrogenated castor oil, PPG-14 butyl ether, and some fragrance. Additionally, *Tom's* natural deodorant was tested; because this type of deodorant lacks aluminum compounds, exposing zebrafish embryos to *Tom's* natural deodorant would serve to test whether aluminum compounds (or other ingredients in the deodorant) produced the results of the experiment. Instant Ocean solution served as the negative control in this experiment as well. The wells holding the zebrafish embryos were originally coated with either *Degree* or *Secret* antiperspirant, *Tom's* natural deodorant, or nothing. The wells were then filled with Instant Ocean solution, with the idea that the antiperspirants/deodorant would leech into the solution, and affect the embryos. Overall, the average amount of alive zebrafish embryos that were exposed to both *Degree* antiperspirant, *Secret* antiperspirant, and *Tom's* natural deodorant was

significantly lower than the average amount of alive zebrafish exposed to Instant Ocean solution. Although the results of this experiment indicate that antiperspirants and deodorants have potentially harmful effects, the confounding variables present in this experiment require that further studies to be conducted.

### **Materials and Methods**

- 1 roll of masking tape
- 1 sharpie
- 60-80 Zebrafish embryos
- 1 bottle of Instant Ocean solution
- 1 stick of *Secret: Invisible Solid (Powder Fresh)* antiperspirant
- 1 stick of *Degree: Invisible Solid (Shower Clean)* antiperspirant
- 1 stick of *Tom's: Natural, Long Lasting* deodorant
- 2 3x4 well plates
- 8 pipettes
- 3 thin paintbrushes
- 1 Beaker
- Camera (for observations)

### **Safety**

One should wear goggles when dealing with glassware. Additionally, one should wash their hands after dealing with the zebrafish embryos, so as to avoid irritating them.

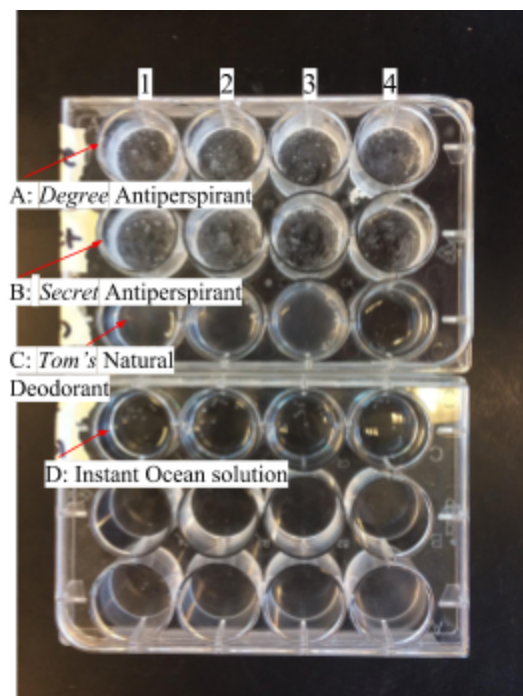
### **Method**

Gather all materials. Using masking tape and a sharpie, label one row on the well plate as follows: Degree, Secret, Tom's Natural (Aluminum Free), and Instant Ocean control. Designate two pipettes to each row. Label one pipette according to the row's label, and label the other pipette "transfer pipette". Evenly coat the bottom of the first row of wells with *Degree* antiperspirant using a clean paintbrush. Using the "transfer" pipette, insert 5-10 zebrafish embryos in the each well for the first row. After this is complete, use the same pipette to remove any fluid in each well. Use the pipette labeled according to the row to insert Instant Ocean solution into each well. Fill each well halfway with Instant Ocean solution. Repeat this process when using the *Secret* antiperspirant and *Tom's* natural deodorant. For the row of wells containing solely Instant Ocean solution, insert 5-10 zebrafish embryos in each well using the "transfer" pipette, remove the embryos with the same pipette, and then insert Instant Ocean solution into the wells using the pipette labeled, "Instant Ocean control." For each day of the experiment, remove any dead embryos from each well (using the pipette labeled according to the row), refill the wells with Instant Ocean solution, and record the amount of dead and alive

zebrafish for each well. Additionally, record the appearances of the zebrafish embryos using a camera. Following this process, place the wells in an incubator at 28.5°C.

### Variables

The independent variable in this experiment is antiperspirant; specifically, antiperspirants containing aluminum compounds. Two popular brands of antiperspirant, *Degree* and *Secret*, were tested on the zebrafish embryos. The dependent variable is the amount of zebrafish embryo dead/alive. Although it is difficult to maintain all of the same ingredients across the antiperspirants, both brands have similar amounts of aluminum compounds (18% of *Secret* is composed of aluminum compounds, while 18.2% of *Degree* is composed of aluminum compounds). As stated above, both brands contain a majority of identical ingredients as well. The Instant Ocean solution is the negative control in the experiment. Although *Tom's* natural deodorant was intended to be another negative control (because it lacks aluminum compounds), the results of the experiment showed that *Tom's* natural deodorant affected the zebrafish embryos, which contradicts *Tom's* deodorant being a negative control. Constant variables include the temperature of the room and temperature in which the incubator is maintained, the amount of deodorant coating the well, and the amount of zebrafish embryos in each well. The experimental groups are the wells that contain either *Degree* or *Secret* antiperspirant, while the control group is the Instant Ocean solution (although intended to be both *Tom's* natural deodorant and Instant Ocean solution).



The setup of the experiment.

### Results

Compared to the zebrafish embryos exposed to Instant Ocean solution, there were a lower amount of alive zebrafish embryos that were exposed to *Degree*, *Secret* and *Tom's* natural deodorant at 72 hours post-fertilization. Additionally, zebrafish embryos exposed to *Degree* and *Secret* antiperspirant had undefined body plans.

#### The Effect of Instant Ocean Solution (Negative Control) on the Number of Alive Zebrafish After 72 Hours:

Treatment:	Well:	Number Alive at the Start:	Number Alive After 72 Hours:
Instant Ocean Solution (Control)	D1	10	10
	D2	10	9
	D3	11	11
	D4	10	9

#### The Effect of *Degree* Antiperspirant on the Number of Alive Zebrafish After 72 Hours:

Treatment:	Well:	Number Alive at the Start:	Number Alive After 72 Hours:
<i>Degree</i> Antiperspirant	A1	10	6
	A2	10	7
	A3	10	2
	A4	10	2

Overall, the number of zebrafish embryos exposed to *Degree* antiperspirant that were alive after 72 hours was lower than the amount of zebrafish embryos exposed to “Instant Ocean” solution. When a t-test was performed to check for statistical significance, the results proved to be statistically significant, with a p-value of 0.0077.

**The Effect of *Secret* Antiperspirant on the Number of Alive Zebrafish After 72 Hours:**

Treatment:	Well:	Number Alive at the Start:	Number Alive After 72 Hours:
<i>Secret</i> Antiperspirant	B1	14	2
	B2	8	2
	B3	10	1
	B4	8	1

The amount of zebrafish embryos exposed to *Secret* antiperspirant that were alive after 72 hours was lower than the amount of alive zebrafish embryos exposed to Instant Ocean solution. When a t-test was performed to check for statistical significance, the results proved to be statistically significant, with a p-value of 0.0001.

**The Effect of *Tom's* Natural Deodorant on the Number of Alive Zebrafish After 72 Hours:**

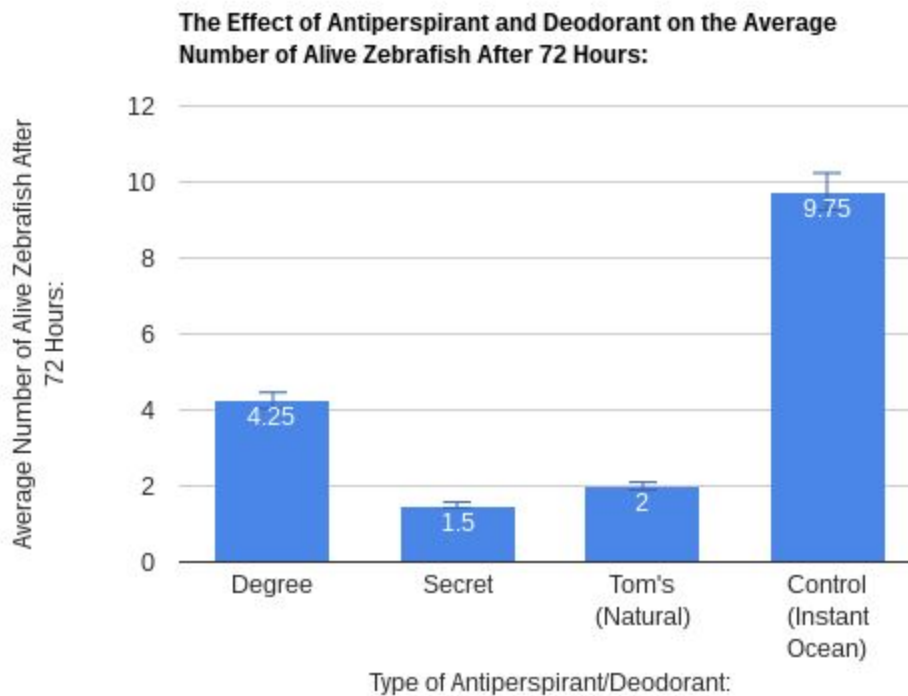
Treatment:	Well:	Number Alive at the Start:	Number Alive After 72 Hours:
<i>Tom's</i> Natural Deodorant	C1	10	0
	C2	10	0
	C3	6	0
	C4	10	8

Although the data shows that all of the zebrafish exposed to *Tom's* natural deodorant were dead after 72 hours in three out of four of the wells, 80% of the zebrafish were alive in the fourth well after 72 hours, making it an outlier in the data set. When a t-test was performed to check for statistical significance, the results proved to be statistically significant, with a p-value of 0.0093.

**The Effect of Antiperspirant and Deodorant on the Average Number and Standard Deviation of Zebrafish Alive after 72 Hours:**

Type of Experimental/Control Group:	Average Number of Alive Zebrafish after 72 hours:	Standard Deviation:
<i>Degree</i> Antiperspirant	4.25	2.63
<i>Secret</i> Antiperspirant	1.25	0.58
<i>Tom's</i> Natural Deodorant	2.00	4.00
Instant Ocean Solution	9.75	0.96

**Graph:**



**Trend Statement:** The average amount of alive zebrafish at 72 hours was highest when the zebrafish were exposed to Instant Ocean solution (the control). When the zebrafish embryos were exposed to antiperspirant/deodorant, the average amount of alive zebrafish at 72 hours decreased.

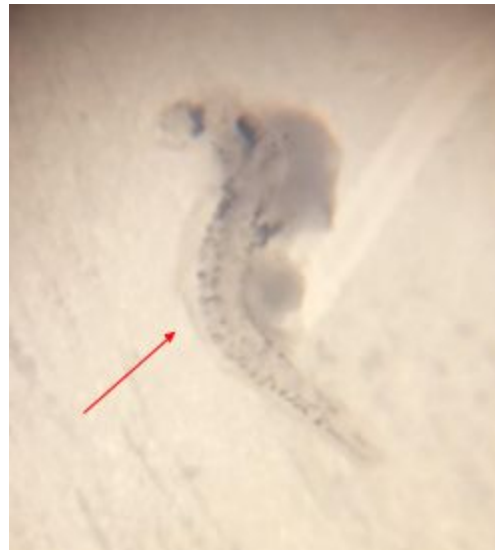


**The Effect of Antiperspirant and Deodorant on the Development of Zebrafish Embryos  
After 72 Hours:**

Experimental Group 1: *Degree* Antiperspirant



Image of zebrafish from well A1. All of the zebrafish seem to have undefined body plans, which means that the embryos appear to be cloudy. The opaque solid surrounding the embryos is the antiperspirant, which coated the bottom of each well.



One zebrafish embryo that appeared to have hatched in well A4, but was found to be dead, with a curved spine as well.

Experimental Group 2: *Secret* Antiperspirant

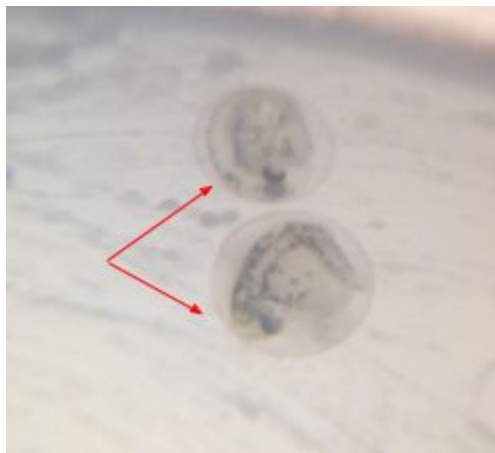
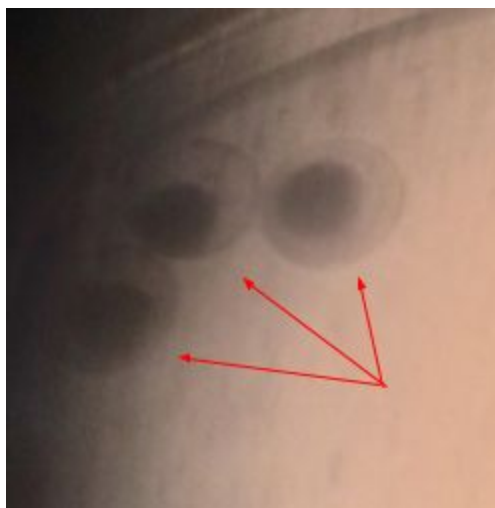


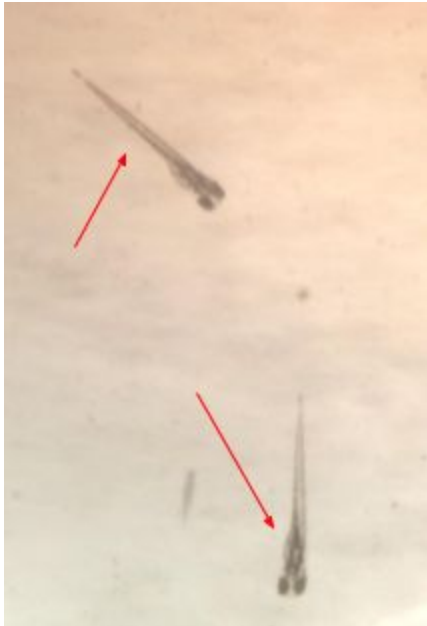
Image of zebrafish embryos in well B2. The body plans of the zebrafish embryos appear to be undefined.

Experimental Group 3: *Tom's Natural* Deodorant  
(Intended to be a negative control)



After 72 hours, all of the zebrafish embryos in wells C1, C2 and C4 had died, and resembled the embryos pictured above.



	<p>Out of the original 10 zebrafish embryos in well C4, 8 had hatched after 72 hours, and resembled the zebrafish above.</p>
<p>Negative Control Group: Instant Ocean</p>	 <p>Hatched zebrafish from well D3 72 hours post-fertilization. All of the zebrafish embryos and zebrafish that hatched by 72 hours exhibited no structural abnormalities, and resembled the zebrafish pictured above.</p>

### Discussion

Overall, a lower amount of zebrafish embryos that were exposed to *Degree* or *Secret* antiperspirant, in addition to *Tom*'s natural deodorant, were alive at 72 hours post-fertilization. All of the zebrafish embryos in three out of four of the wells exposed to *Tom*'s natural deodorant were dead at 72 hours post-fertilization. Although zebrafish embryos that were exposed to *Degree* or *Secret* had a lower amount of zebrafish embryos that were alive compared to the control group, zebrafish embryos exposed to either antiperspirant were also observed to have undefined body plans. This observation supports the hypothesis, which predicted that if zebrafish embryos were exposed to antiperspirants, then they would have abnormal structural changes throughout development. However, the hypothesis was false in that it did not predict *Tom*'s natural deodorant having a lower amount of alive zebrafish embryos compared to the control, and also did not predict that there would be a lower amount of alive zebrafish embryos exposed to antiperspirants compared to the control group at 72 hours.

Several errors may have contributed to the results of the experiment. Because substances containing various ingredients were tested on the zebrafish embryos, it is impossible to determine what substance specifically affected the zebrafish embryos. Although the method aimed to focus on the aluminum compounds within antiperspirants, it is equally as probable that another ingredient caused the results of the experiment. Additionally, although one attempted to coat all of the wells with equal amounts of antiperspirant or deodorant, it is probable that all of the wells were not coated with equal amounts of a specific substance. As a result, slight variances in the amount of antiperspirant/deodorant coating the wells likely affected how the zebrafish embryos reacted to the material. Furthermore, although all of the zebrafish embryos in three of the wells exposed to *Tom*'s natural deodorant were dead by 72 hours post-fertilization, 8 out of the initial 10 zebrafish embryos in one well exposed to *Tom*'s natural deodorant were alive by 72 hours, and resembled the control group (in that many had hatched, and didn't exhibit any structural changes). As a result of this outlier, the average amount of alive zebrafish embryos at 72 hours is skewed; the average amount of alive zebrafish exposed to *Tom*'s natural deodorant appears to be higher.

Although the confounding variables within this experiment make it difficult to determine what specific ingredient caused the lower amount of alive zebrafish at 72 hours, aluminum compounds are likely still harmful. Genotoxicity is the ability for a substance to cause mutations and damage to DNA, which can possibly lead to cancer and other diseases (Shah, 2012, 43). A study which aimed to investigate the genotoxicity of aluminum revealed that zebrafish embryos exposed to aluminum had high amounts of double strand breaks (referred to as DBS) in DNA (Pereira, Cavalie, Camilleri, Gillan, & Guillermin, 2012). This was indicated by high amounts of micronuclei, which are separate nuclei that can develop when damaged DNA is left unrepaired (Pereira et al., 2012). As a result, this study determined that aluminum acts as a genotoxin towards zebrafish embryos (Pereira et al., 2012). It's possible that the aluminum compounds (which were exposed to the zebrafish embryos in this experiment) acted on the zebrafish in a

similar way. If the aluminum compounds contained in both *Degree* and *Secret* antiperspirants caused mutations in the DNA of the zebrafish embryos, then this could account for the undefined body plans and low amount of alive zebrafish at 72 hours. Further studies should be conducted to further test whether aluminum compounds cause genetic mutations in zebrafish embryos.

However, *Tom's* natural deodorant did not contain any aluminum compounds. Despite this, all of the zebrafish in three out of four of the wells were dead by 72 hours. Because of this, *Tom's* deodorant did not act as a negative control in the experiment (which is what originally was the purpose of exposing the zebrafish embryos to *Tom's* deodorant). Although the outlier in the data (the fact that 8 out of 10 zebrafish embryos in one of the wells exposed to *Tom's* natural deodorant were alive at 72 hours) indicates an error in the experiment, the fact that all of the zebrafish embryos in three of the wells exposed to *Tom's* natural deodorant died contradicts the claim that aluminum compounds are the sole reason for the low amount of alive zebrafish at 72 hours. As a result, it is possible that other ingredients contained in *Tom's* natural deodorant caused the adverse effects. Glycerol, an ingredient that is both soluble in water and a preservative, is the main ingredient in *Tom's* natural deodorant (Maes, Verlooy, Buenale, Witte, Esquerra, Crawford, 2012). A study which tested the effect of glycerol on zebrafish embryos revealed that embryos exposed to glycerol displayed no extended tail, in addition to "brain segmentation defects," which means that the zebrafish embryos had less developed brains (Maes et. al, 2012, 7). Although the zebrafish embryos in this experiment died, the study above reveals that glycerol has the potential to negatively affect zebrafish embryos. As a result, further studies should be completed to determine whether or not the exposure of glycerol to zebrafish embryos can cause death.

When a t-test was performed on all of the data sets to check for statistical significance, all of the data proved to be statistically significant ( $p_{Degree} = .0077$ ,  $p_{Secret} = .0001$ ,  $p_{Tom's} = .0093$ ). Because all of the p values are relatively low, this shows that there is a low chance the data could have resulted from chance. Despite this, the confounding variables in the experiment (such as the fact that multiple ingredients make up each antiperspirant/deodorant) make the results inconclusive. Although zebrafish embryos exposed to antiperspirants (containing aluminum compounds) experienced negative effects, zebrafish embryos exposed to deodorant which lacked aluminum compounds also experienced negative effects. While it is possible that aluminum compounds are harmful, it is also possible that other ingredients caused the results of the experiment. Because of this, further studies should be conducted to determine the specific effects of aluminum compounds on zebrafish embryos. Regardless of what caused the negative effects on the zebrafish embryos, this experiment highlights the possible risk in using antiperspirants or deodorants. In the future, one should carefully examine the ingredients within certain cosmetic products for one's underarm area. Through becoming more aware about the possible effects of using antiperspirants and deodorants, consumers will be able to make more conscientious decisions about the products they purchase.

### References

- Badman, D., Dougan, S., Greenberg, J., Heindel, J., Matthews, J., Trevarrow, B. (n.d.). Zebrafish faqs. *Zebrafish K-12*. Retrieved from <http://www.uoneuro.uoregon.edu/k12/FAQs.html>
- Croager, E. (2004). In the news: deodorant debate rages on. *Nature Reviews Cancer*, 4(3), 174. doi: 10.1038/nrc1311
- Exley, C., Charles, L., Barr, L., Martin, C., Polwart, A., Darbre, P. (2007). Aluminum in human breast tissue. *Journal of Inorganic Biochemistry*. 1344-1346. doi: 10.1016/j.jinorgbio.2007.06.005
- Maes, J., Verlooy, L., Buenale, O., Witte, P., Esguerra, C., Crawford, A. (2012). Evaluation of 14 organic solvents and carriers for screening applications in zebrafish embryos and larvae. *PLoS One*, 7(10). doi: 10.1371/journal.pone.0043850
- McCoy, T. (2014). Antiperspirants may actually make you smell worse. *The Washington Post*. Retrieved from <https://www.washingtonpost.com/news/morning-mix/wp/2014/08/13/antiperspirants-may-actually-make-you-smell-worse/>
- Pereira, S., Cavalie, I., Camilleri, V., Gilbin, R., Guillermin, C. (2012). Comparative genotoxicity of aluminum and cadmium in embryonic zebrafish cells. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 19-24. doi: 10.1016/j.mrgentox.2012.07.007
- Shah, S. (2012). Importance of genotoxicity & s2a guidelines for genotoxicity testing for pharmaceuticals. *IOSR Journal of Pharmacy and Biological Sciences*, 1(2). 43-54.
- Unilever. (n.d.). Antiperspirants and deodorants. *Antiperspirant Info*. Retrieved from <http://www.antiperspirantsinfo.com/en/antiperspirants-and-deodorants/about-antiperspirants-and-deodorants.aspx>