The Effects of Fast Food Packaging Chemicals on Developing Zebrafish Embryos

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ABSTRACT:

This research chose to answer how the independent variable, PFAS, extracted from the wrappers of fast food packaging, might impact the following dependent variables: the development of the somites in zebrafish embryos, their hatch rate, and mortality rate. The dangers of PFAS are that it's made up of thousands (approximately 9,000) of man-made, forever chemicals which are harmful to almost every human organ and the immune system (Stoiber, 2019). After receiving the embryos from UW Milwaukee, the living and deceased embryos were separated under the microscope. The living embryos were then arranged into the 12-well plate with a pipette into their selected solutions. The first column held the control zebrafish in embryo media solution, the second column held the zebrafish exposed to Subway packaging chemicals, the third column held zebrafish exposed to Culver's packaging chemicals, and the fourth column held the zebrafish exposed to McDonald's packaging chemicals. Based on prior research that confirms the amount of PFAS does indeed affect the spine; it is reasonable to conclude that McDonald's likely had the most amount of per-polyfluorinated chemicals. Therefore, it was concluded that Culver's likely had the second-highest concentration of PFAS. Notably, the effects of PFAS on the zebrafish were more long-term than they were fatal.

INTRODUCTION:

Ever since the 1940s, the whole globe has been pretty much engulfed by PFAS. PFAS is a man-made chemical toxicant that has been used in the industry and consumer products (Perfluoroalkyl and Polyfluorinated Substances). It's everywhere. In the air, water, soil, food, non-stick cookware, and some of the packaging of fast food items. When people consume fast food products wrapped in grease-resistant waxy paper, they are putting all of the thousands of chemicals known to be in PFAS into their bodies and blood. With more and more information being put out on the dangers of PFAS, the question of how prominent this issue was, was raised, especially in developing human fetuses. During this experiment, that question was put to the test. Zebrafish are tropical freshwater fish with white stripes and a blue body that resembles a zebra. They are very similar to humans in a structural sense and share 70% of the same DNA as humans (Manner, 2018). They are very ideal to use when doing laboratory experiments because they are very cheap to house and maintain. The hypothesis in this experiment was that PFAS from certain fast food packaging would cause malformations in the somites of the zebrafish. The independent variable PFAS, extracted from the wrappers of fast food packaging, might impact the following dependent variables: the development of the somites in zebrafish embryos, their hatch rate, and mortality rate. The control will not be exposed to the PFAS chemicals and the variables held constant are the temperature of fish, room temperature, light, and time exposed to the PFAS for some of the experimental groups.

MATERIALS AND METHODS:

After receiving the embryos from UW Milwaukee, the living and deceased embryos were separated under the microscope. The living embryos were then arranged into the 12-well plate with a pipette into their selected solutions. The 12-well plates were divided into four different columns of treatment, each containing three wells. The first column held the control zebrafish in embryo media solution, the second column held the zebrafish exposed to Subway packaging chemicals, the third column held zebrafish exposed to the Culver's packaging chemicals, and the fourth column held the zebrafish exposed to McDonald's packaging chemicals. The types of solutions used were from various wrappers such as a Subway sub wrapper, a Culver's cheese curd wrapper, and a McDonald's fry wrapper. The solutions were made by extracting the chemicals from the wrappers. Each wrapper was separately submerged in water and boiled in graduated cylinders for twenty minutes. Then, once cool, the wrappers were taken out of their respective containers and the remaining water left over was filtered through a coffee filter into their solution bottles. From Day 1 (Wednesday) to Day 3 (Friday) the embryos were analyzed with the microscope. The living and deceased embryos were counted and separated each day. After data was collected the embryos were placed back into the well plate after changing the solution out. As the solution was changed it was kept at 1 mL from Day 1 (Wednesday) to Day 3 (Friday). However, the only variable not kept constant in the procedure was setting up the experiment for the weekend. The difference in doing this was that Day 4 (Monday) had to be prepared at the end of Day 3 (Friday). The well plate was prepared for the weekend by adding 3 mL instead of 1 mL to prevent the embryos from drying. The solutions were also not changed over the weekend like the previous 3 days.

RESULTS:

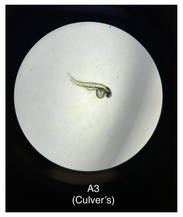

Figure 1: Taken on Day 2 and has a 'S-Shaped' spine.

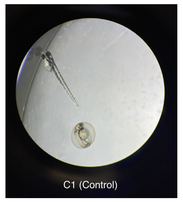

Figure 2: Day 2 control with a straight spine.

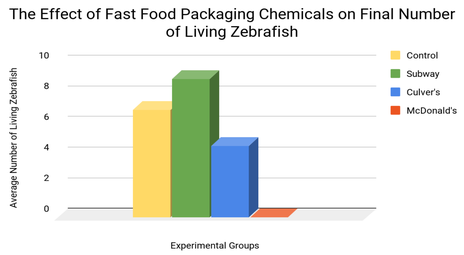

Figure 3: This shows the final number of living zebrafish

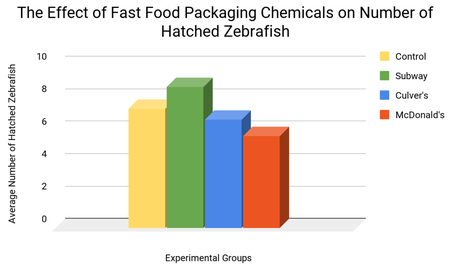

Figure 4: This shows the number of final hatched zebrafish.

Final Number of Living Fry						
Treatment	Well 1	Well 2	Well 3	Average	Probability	Result
Control	4	9	8	7	-	-
Fast Food Packaging Chemicals Subway	10	7	10	9	p = 0.3349	not statistically significant.
Fast Food Packaging Chemicals Culver's	4	3	7	5	p = 0.2962	not statistically significant.
Fast Food Packaging Chemicals Mcdonald	0	0	0	0	p = 0.0102	statistically significant.

Figure 5: This shows the average of final living zebrafish and probability by a t-test

Final Number Hatched						
Treatment	Well 1	Well 2	Well 3	Average	Probability	Result
Control	6	9	8	8	-	-
Fast Food Packaging Chemicals Subway	9	7	10	9	p = 0.4676	not statistically significant.
Fast Food Packaging Chemicals Culver's	8	4	9	7	p = 1.0000	not statistically significant
Fast Food Packaging Chemicals McDonalds	5	3	9	6	p = 0.3679	not statistically significant

Figure 6. This shows the average of final hatched zebrafish and probability by a t-test.

DISCUSSION:

The zebrafish in the McDonald's chemical solution had the most urgent deformities in their spine every day, and concluded on the additional day, the death of all the once healthy embryos. Based on prior research that confirms the amount of PFAS does indeed affect the spine; it is reasonable to conclude that McDonald's likely had the most amount of per-polyfluorinated chemicals. With this, it is safe conclude that Culver's chemical solution likely had the second highest concentration of PFAS. The Culver's solution was always behind the McDonald's solution when measuring the drastic difference between the data in the control and the experimental groups. However, the zebrafish in the Subway were fairly successful in development, and only had few deaths throughout the duration of the experiment. Also, notably the Subway exposed fish did display differences in mortality from McDonalds exposed fish, the difference was McDonalds' 0 final living fish vs. Subway's 7 final living fish. The claim made prior to the experiment was that the PFAS from certain fast food packaging calls malformations in the somites of the zebrafish. The data in this experiment supported the hypothesis because of the significant difference between the control and experimental groups. The differences were not fully represented in the fish in the Subway chemical solution, but were in the 2 other groups. Any possible errors in the experiment would have most likely taken place in preparing for Day 4. The error that was most likely to occur would've been not being able to change out the solutions in the well-plate over the weekend. It is possible that dead tissue from passed zebrafish could've affected the living environment of the fry.

Comparing the zebrafish embryos to actual human development suggests a problem that is present in Juneau County, and many others. The embryos supplied eye opening information that supports the claim that many people, including mothers and citizens, who do not have proper access to fresh food, may rely on fast food wrapped in potentially dangerous chemicals. To see how negatively these chemicals impacted the embryos was a wake-up call. It's time to start mending this ongoing problem, or at least spread awareness about it, because if it's not addressed, then the health and well-being of future generations is put at risk.

References:

Manner, J. A. (2018). Zebrafish: Practically People. ZEBRAFISHFILM.ORG - Home. https://www.zebrafishfilm.org/

PFAS chemical exposure | ATSDR. (2022, November 1). Agency for Toxic Substances and Disease Registry. https://www.atsdr.cdc.gov/pfas/health-effects/exposure.html

Stoiber, T. (2019, June 21). PFAS Chemicals Harm the Immune System, Decrease Response to Vaccines, New EWG Review Finds. EWG. https://www.ewg.org/news-insights/news/pfas-chemicals-harm-immune-system-decrease-response-vaccines-new-ewg-review

Wiley-Blackwell. (2021). Perfluorooctanesulfonic Acid–Induced Toxicity on Zebrafish Embryos in the Presence or Absence of the Chorion. https://orbit.texthelp.com/?file=https://drive.google.com/uc?id=1LVjxlusYbNUzpHy1mS70cYOlMk8MYYo9&export=download&filename=Attachment:%20PDF.%20Enviro%20Toxic%20and%20Chemistry%20-%202020%20-%20Mylroie%20-%20Perfluorooctanesulfonic%20Acid%20Induced%20Toxic

Toxic-Free Future & Schade, M. (2022). Retailers committing to phase out PFAS as a class in food packaging and products. Toxic-Free Future. https://toxicfreefuture.org/mind-the-store/retailers-committing-to-phase-out-pfas-as-a-class-in-food-packaging-and-products/