The Long Term Effects of Tartrazine (FD&C Yellow No. 5) on Learning, Cognitive Flexibility, and Memory of Zebrafish (*Danio rerio*) Embryos into Adulthood

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Abstract

On average, children will consume about 100 mg to 200 mg of food dyes everyday. This can be concerning as some food dyes have been known for their toxicity to humans, especially Yellow 5. These concerns include anxiety, stress, depression, memory loss, and lack of cognitive flexibility. This experiment focuses on testing zebrafish exposed to Yellow 5 at different stages of the zebrafish life. The first treatment group tested zebrafish exposed to 22 μM of Yellow 5 from 24 hpf until the day of the T-maze trials. The second treatment group tested zebrafish exposed to 22 μM of Yellow 5 from 24 hpf until 7 dpf. At that time, the zebrafish in this group were transferred into a control tank until the day of the T-maze trials. 22 μM of Yellow 5 was chosen as the concentration level due to other studies showing that this concentration had no effect on zebrafish but the exposure time was limited to one day prior to testing. This experiment was conducted by using a T-maze to teach the zebrafish the "correct arm" to go into. For the T-maze tests, the zebrafish were expected to reach the 1st and 2nd criterion for each day of the trials. On day 1, day 2, day 3, and day 8 of the trials, the zebrafish were tested for their learning, cognitive flexibility, and memory, the zebrafish exposed to 22 μM of Yellow 5 from 24 hpf until the day of the trials experienced numerous difficulties in learning, memory and cognitive flexibility. Although this was not exactly the same case for zebrafish exposed to 22 μM of Yellow 5 from 24 hpf until 7 dpf, humans should try to cut back on their daily consumptions of Yellow 5 as it can be detrimental to cognitive flexibility in reference to these findings.

1. Introduction

Color additives have been used in almost every food produced since around 1500 BC.[6] After being introduced by the Egyptians, different cultures and societies have continued using food dyes to increase consumer appeal and artistic attraction.[3] This also includes certain pharmaceutical medication and other non-food applications.[4] These kind of dyes that are used for aesthetic appeal are known as azo dyes and some of them are very toxic.[9] The most popular azo dyes used in food coloring include Blue 1, Red 40, and Yellow 5 as these are all used under permission of the FDA.[7] Although there has been health issues cited by different research groups for one dye in particular, Yellow 5 or Tartrazine, the FDA has refused to follow suit with rest of the countries in banning this azo dye.[10]

With Yellow 5 being one of the most widely used food dye in American culture, it can be hard to pinpoint the health causes of this dye as there is only a very small portion of a control group that has consumed Yellow 5 before.[16] Although research on this dye has been limited, studies have shown that Yellow 5 can take effect on both the reproductive and neurobehavior of different animals but not with the same effects on humans.[18] One of the more serious effects of the dye is the possibility of it affecting the behavior and learning of humans, especially children.[2,11] Other studies have also linked this dye to possibly causing anxiety and depression as these can be caused by the increased stress level also possibly induced by Yellow 5.[14,19] These effects can also include ADHD symptoms after consuming the average amount of Yellow 5.[1]

Yellow 5 or the Tartrazine formula is $C_{16}H_9N_4Na_3O_9S_2$ as it usually is formed as a solid yellow powder at room temperature.[13] It can be estimated that children consume somewhere between 100 mg and 200 mg a day of dye powders.[5] When eating eating foods that contain Yellow 5, one's body can lose zinc which is essential for cognitive function.[8] This could mean that zinc would have a detrimental effect if the body is taking in too much Yellow 5 but other studies have shown that the effects of Yellow 5 could also possibly be deferred if also taking a sufficient amount of Vitamin E with the dye yet these studies are very limited.[17]

Due to the consideration of ethics, this experiment will be conducted using zebrafish instead of humans. Zebrafish have been used increasingly in various experiments due to their developmental similarities when compared to humans.[12] Zebrafish are generally cheaper to maintain compared to the alternates (i.e. mice etc) and have internal organs that are easy to monitor due to the chorions transparency.[15,20] The biggest reason why zebrafish are used in more experiments, is because of their genetic similarities to humans. Around 70 percent of the genetic makeup in a human is shared with zebrafish.[20]

How does Yellow 5 affect the cognitive learning of the zebrafish? Will this also affect the memory of the zebrafish? How long of an exposure does it take for the effects of Yellow 5 to be exhibited by the zebrafish choices and behavior. It is hypothesized that if zebrafish are exposed to Yellow 5 throughout their entire lives then the zebrafish will have cognitive difficulties, memory problems, and limited learning abilities because of the known toxicity of the dye.

2. Materials and Procedure

2.1. Equipment

Materials and equipment used in this experiment included three 8 liter tanks, three tank heaters, three oxygen pumps, a compact mesh fish net, a 2 milliliter pipet, a 6 liter T-maze, a size three rubber stopper, two clear gates, a glass stirring rod, a regular sized fish net, a petri dish, a circular mesh strainer, a 5 liter jug with nozzle, a 5 gallon bucket, a clean classroom thermometer, nine 1 liter holding containers, Aqueon Tropical Flakes, a well plate with 12 wells, a dissecting microscope, a compound microscope, a 50 mL beaker, an incubator, a microscope slide, three breeding tanks with wire mesh bottoms, about 50 marbles, three small plastic fish tanks, and two 4 liter jugs.

2.2. Chemicals

Although tartrazine (yellow No. 5) was the subject of this experiment, other chemicals were also used to assist the fish in well-being before the trials. These chemicals include methylene blue, instant ocean, and API stress coat Calculations to determine how much tartrazine needed to be added to the zebrafish media in order to reach a certain concentration were calculated after the addition of the chemicals previously mentioned. Methylene blue was primarily used in the embryonic state of the zebrafish as this chemical prevented the growth of unhealthy bacteria in the the zebrafish embryos tank. This chemical was used very generously as it was not harmful at any concentration to the zebrafish embryos. Instant ocean media was also used for the zebrafish embryos as this is used to treat the water to make it healthy for zebrafish embryos to grow in.. Lastly, API stress coat was used to maintain the health of the zebrafish once they became adults. The stress coat and distilled water solution was a 1 mL/L concentration.

2.3. Animal treatment

Due to genetic and intestinal similarities to humans, nine zebrafish in total were used as test subjects for this experiment. Six of the nine zebrafish had to be grown in the lab tank space for six months to a year. This required thousands of zebrafish embryos at or before 24 hpf to be exposed to the tartrazine concentration of 22 μM to make sure there would be enough zebrafish embryos that would be able to survive into adulthood. To accomplish this, it first had to be determined if zebrafish embryos could live in a tartrazine concentration of 22 μM. After several tests proved no correlation between exposed zebrafish embryos and the control group in regards to survival rates and embryonic defects, the only concern was being able to continue regular maintenance and monitoring as required.

The exposure process started with harvesting fertilized and unfertilized zebrafish embryos at about 2 hpf from the science lab's breeding tank using a circular, mesh strainer. Excess food debris was kept in the petri dish along with the embryonic media due to the noticeable increase in survival rate of the zebrafish embryos within approximately 7 dpf due to a previous test. A pipet was then used to discard the dead and unfertilized eggs into a separate beaker. Once it was presumed that all of the dead and unfertilized eggs in the petri dish were indeed ejected into the beaker, the contents of the beaker were then disposed of properly.

After the disposing of the dead and unfertilized zebrafish embryos, the living zebrafish embryos and food debris left over in the petri dish, were then immediately strained and dispersed into an eight liter tank containing 16 liters of the 22 μM tartrazine solution and a small tank heater. The tank heater was set at 28 °C and checked daily using a clean classroom thermometer. Starting at 14 dpf, approximately 2-4 medium size flakes of Aqueon Tropical Flakes were ground into a fine powder and sprinkled into the fish tank every other day. About two to three months after the zebrafish embryos were introduced to the tank, an oxygen pump was added to improve survival rate and the amount of food given to the zebrafish was doubled and increased to daily feedings. The same process was repeated at approximately the same time but the zebrafish embryos were removed out of the 22 μM tartrazine solution using a compact mesh fish net and were carefully placed into a 20 liter tank containing 16 liters of the control solution at approximately 7 dpf. Although a significant amount of concern was witnessed through this process along with quite a bit of trial and error, ultimately seven zebrafish in total were able to live into adulthood while being raised in a high school science lab.

Once the zebrafish were about six months to a year old, they were ready to be tested with the T-maze. The zebrafish in the constant control solution were the first to be prepared and tested. The constant control zebrafish were randomly selected from the breeding tanks approximately one to two days before the test. These zebrafish were kept in 1 liter holding containers throughout the tests and were feed 2 flakes of Aqueon Tropical Flakes daily throughout the experiments. Once all of the constant control zebrafish were tested, each one was then netted back into the original tank. This process was repeated with the 22 μM tartrazine solution zebrafish and the 22 μM tartrazine to control solution zebrafish.

2.4. T-maze tests

The following procedure is suggested by the Science Education Partnership Award (SEPA) through the University of Wisconsin- Milwaukee. The process started by randomly selecting a control fish from the breeding tank and placing it in a one liter holding tank for 24 hours. After 24 hours was passed, the control fish was placed in a T-maze filled with 6 liters of water as seen below with all of the gates left open. The fish was left in the T-maze to swim freely for five minutes. Arm preference was determined during this time as the “correct arm” was whichever arm the fish did not prefer to swim into whereas the “incorrect arm” was the other arm that the fish preferred to swim into. This observation was only made on the first day of testing. After five minutes passed, the fish was then gently guided back to the starting block as the front gate was closed behind it. The fish was allowed to swim freely within the gate of the starting block for 15 seconds. After 15 seconds passed, the gate was opened and the fish was allowed to swim out of the starting block and into the straight away. If the fish did not exit the starting block after 15 seconds, then the fish was gently guided into the straight away with a net. The fish would swim up to the bisector of the two arms and make a choice of which arm to swim into. If the fish swam into the “correct arm”, then the gate to the “incorrect arm” was closed immediately and the fish was allowed to swim freely for 45 seconds. The gate to the incorrect arm was not closed until the fish was completely in the “correct arm”. If the fish swam into the “incorrect arm”, then the gate to the “incorrect arm” was closed immediately after the fish. A stirring rod was then used to gently stir the fish for 2 seconds with excessive caution to not accidently hit the fish. After the allotted times were up, the fish was then gently netted and put back into the starting block with the gate closed. This trial was repeated until the fish choose the “correct arm” 5 out of 6 trials in a row. This was considered reaching 1st criterion. After the 1st criterion was reached, the “correct arm” became the “incorrect arm” and the “incorrect arm” became the “correct arm” also known as the reverse. Achieving the 2nd criterion happened when the fish choose the “correct arm” 5 out of 6 trials in a row after the rverse. These trials continued until the 2nd criterion was reached or until the fish reached 20 trials for the day. The fish was then gently netted and placed in the one liter holding container until the next testing day. This process was repeated for day 2, day 3, and day 8 as Each day started with the same direction of the “correct arm”. After all the testing days were done for the 1st control fish, a new control fish was randomly chosen and the complete test was repeated with the new fish. These trials continued until all of the fish required to be tested were tested and recorded into a Google Spreadsheet.

2.5. Statistical analysis

Each exposure level was determined to be significant or not significant by using several unpaired T-tests. GraphPad Software was used to conduct the statistical analysis by determining the significance of a set of data for each set of data and comparison recorded. The significance of each comparison was also used to differentiate a significant result from random chance. The significance was determined by calculating the p-value of each comparison. A comparison was considered to be significant if the p-value was at or below 0.05.

3. Results and Data

Data was collected through the choices of the zebrafish as they proceeded through the T-maze tests. The choices of the zebrafish were recorded on a separate document and then averaged out into six different categories: Number of Trials to Complete Task 1, Number of Trials to Complete Task 2, Longest String Correct, Longest String Incorrect, Number of Fish to Complete Task 1, and Number of Fish to Complete Task. These data tables are all recorded below.

3.1. Number of Trials to Complete Task 1

Trials to Complete Task 1 for Day and Fish

Fish	Day 1	Day 2	Day 3	Day 8
C1	20*	10	6	16
C2	20*	9	14	14
C3	20	8	8	10
Y1	20*	6	20*	20*
Y2	20*	20*	20*	20*
Y3	20*	20*	8	20*
Y-C1	20*	10	7	20*
Y-C2	7	6	7	12
Y-C3	20*	11	7	20*
Average				
Fish	Day 1	Day 2	Day 3	Day 8
Control	20	9	9.33	13.33
Yellow 5	20	15.33	16	20
Yellow 5-Control	15.67	9	7	17.33

Data Table 1

Data Table 1 shows the number of trials it took each fish in each exposure level to complete task 1 for each day then the average for each exposure level for every day.

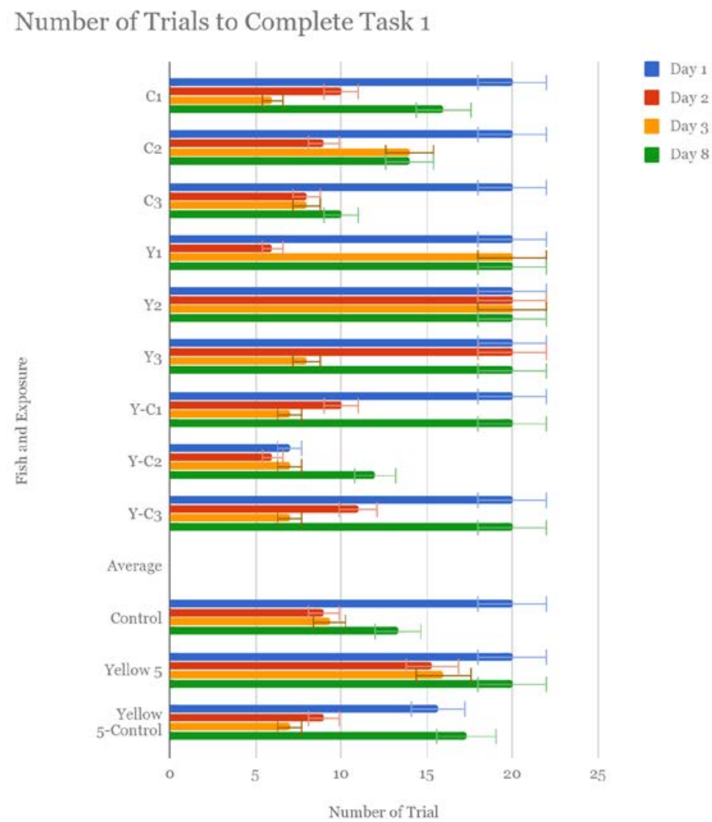

Figure 1

Figure 1 shows a graph for the number of trials it took each fish in each exposure level to complete task 1 for each day then the average for each exposure level for every day.

*Denotes a fish not completing Task 1

3.2. Number of Trials to Complete Task 2

Trials to Complete Task 2 for Day and Fish

Fish	Day 1	Day 2	Day 3	Day 8
C1	20*	8	10	20*
C2	20*	8	6	6
C3	20*	6	6	7
Y1	20*	20*	20*	20*
Y2	20*	20*	20*	20*
Y3	20*	20*	20*	20*
Y-C1	20*	20*	11	20*
Y-C2	11	20*	8	20*
Y-C3	20*	20*	10	20*
Average				
Fish	Day 1	Day 2	Day 3	Day 8
Control	20	7.33	7.33	11
Yellow 5	20	20	20	20
Yellow 5-Control	17	20	9.67	20

Data Table 2

Data Table 2 shows the number of trials it took each fish in each exposure level to complete task 2 for each day then the average for each exposure level for every day.

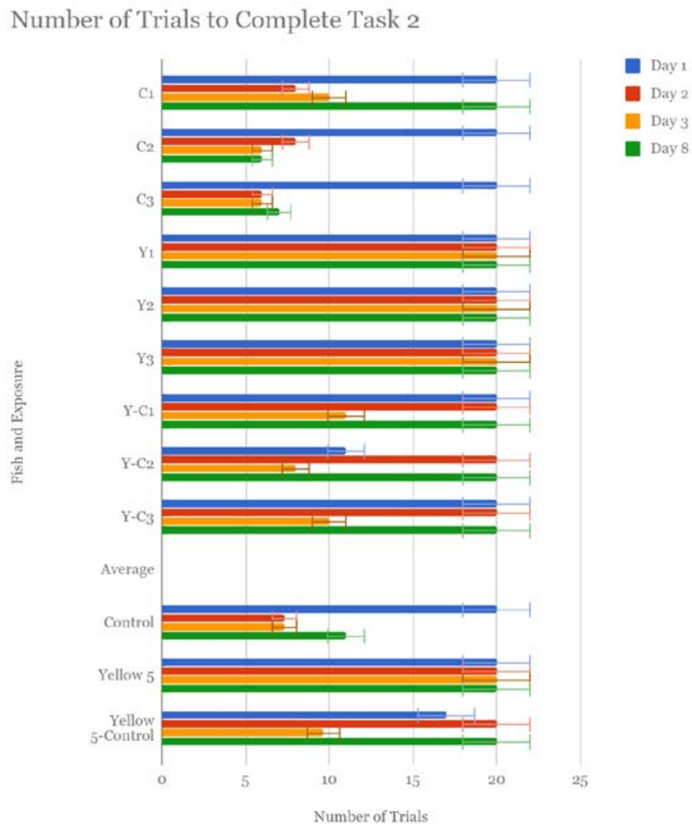

Figure 2

Figure 2 shows a graph for the number of trials it took each fish in each exposure level to complete task 2 for each day then the average for each exposure level for every day.

*Denotes a fish not completing Task 2

3.3. Longest String Correct

Average Longest String Correct for Task 1

Fish	C	Y	Y-C
Day 1	3	2.67	2.33
Day 2	4	3	3.67
Day 3	3.33	2.67	3.67
Day 8	3.67	1.33	3.33

Data Table 3

Data Table 3 shows the average longest string of correct choices when completing task 1 from each exposure level and for everyday the numbers were recorded.

Average Longest String Correct for Task 2

Fish	C	Y	Y-C
Day 1	0	0	1
Day 2	4.33	1	2
Day 3	4.67	0.33	4.67
Day 8	3	0	0.33

Data Table 4

Data Table 4 shows the average longest string of correct choices when completing task 2 from each exposure level and for everyday the numbers were recorded.

3.4. Longest String Incorrect

Average Longest String Incorrect for Task 1

Fish	C	Y	Y-C
Day 1	3	4.67	5.33
Day 2	2	3.33	1.33
Day 3	1.67	7.33	1
Day 8	4	11.33	4.33

Data Table 5

Data Table 5 shows the average longest string of incorrect choices when completing task 1 from each exposure level and for everyday the numbers were recorded.

Average Longest String Incorrect for Task 2

Fish	C	Y	Y-C
Day 1	20	20	14
Day 2	2.33	16.33	3.33
Day 3	2	14.67	2
Day 8	1.33	20	14.33

Data Table 6

Data Table 6 shows the average longest string of incorrect choices when completing task 2 from each exposure level and for everyday the numbers were recorded.

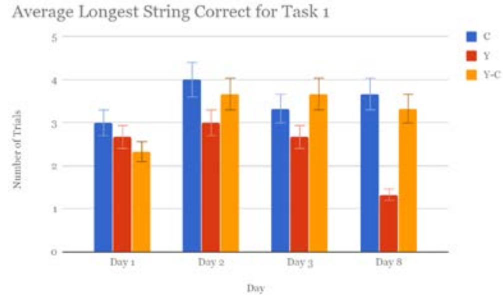

Figure 3

Figure 3 shows a graph of the average longest string of correct choices when completing task 1 from each exposure level and for everyday the numbers were recorded.

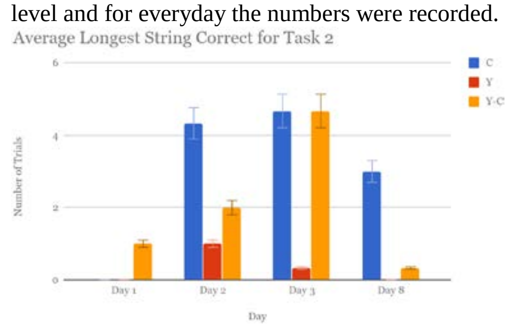

Figure 4

Figure 4 shows a graph of the average longest string of correct choices when completing task 2 from each exposure level and for everyday the numbers were recorded.

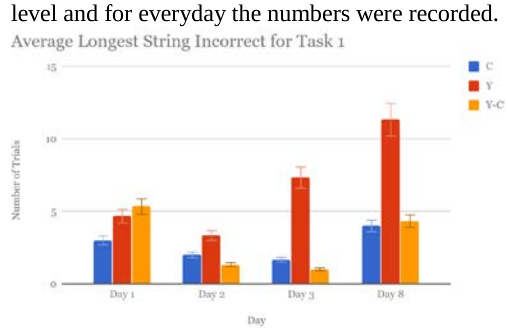

Figure 5

Figure 5 shows a graph of the average longest string of incorrect choices when completing task 1 from each exposure level and for everyday numbers were recorded.

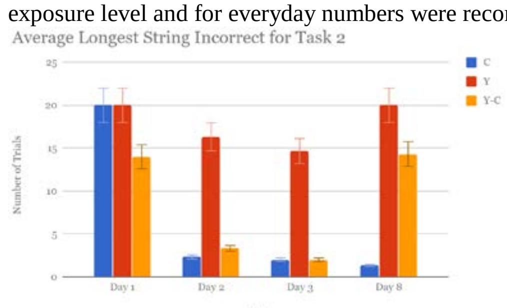

Figure 6

Figure 6 shows a graph of the average longest string of incorrect choices when completing task 2 from each exposure level and for everyday numbers were recorded.

3.5. Overall Success for Task 1

Number of Fish to Complete Task 1

Fish	C	Y	Y-C
Day 1	1	0	1
Day 2	3	1	3
Day 3	3	1	3
Day 8	3	0	1

Data Table 7

Data Table 7 show the number of fish from each exposure level that completed task 1 for everyday.

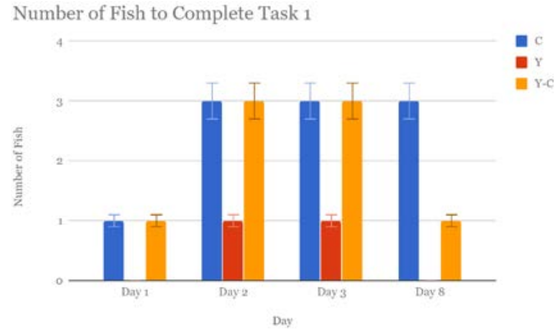

Figure 7

Figure 7 shows the graph of number of fish from each exposure level that completed task 1 for everyday.

3.6. Overall Success for Task 2

Number of Fish to Complete Task 2

Fish	C	Y	Y-C
Day 1	0	0	1
Day 2	3	0	0
Day 3	3	0	3
Day 8	2	0	0

Data Table 8

Data Table 8 show the number of fish from each exposure level that completed task 2 for everyday.

Figure 8

Figure 8 shows the graph of number of fish from each exposure level that completed task 2 for everyday.

3.7. Significance

Comparison		P-Value	Significance
Trials to Complete Task 1 (Average)	Control vs Yellow 5	0.0650	Almost Significant
	Control vs Embryonic Yellow 5	0.9493	Not Significant
Trials to Complete Task 2 (Average)	Control vs Yellow 5	0.0289	Significant
	Control vs Embryonic Yellow 5	0.3312	Not Significant
Longest String Correct Task 1	Control vs Yellow 5	0.0450	Significant
	Control vs Embryonic Yellow 5	0.5385	Not Significant
Longest String Correct Task 2	Control vs Yellow 5	0.0498	Significant
	Control vs Embryonic Yellow 5	0.5100	Not Significant
Longest String Incorrect Task 1	Control vs Yellow 5	0.0728	Almost Significant
	Control vs Embryonic Yellow 5	0.7927	Not Significant
Longest String Incorrect Task 2	Control vs Yellow 5	0.0535	Almost Significant
	Control vs Embryonic Yellow 5	0.2995	Not Significant
Fish to Complete Task 1	Control vs Yellow 5	0.0134	Very Significant
	Control vs Embryonic Yellow 5	0.5370	Not Significant
Fish to Complete Task 2	Control vs Yellow 5	0.0300	Significant
	Control vs Embryonic Yellow 5	0.3559	Not Significant

Data Table 9

Data Table 9 shows the t-test comparison of each concentration to the control.

The significance of each comparison was also used to differentiate a significant result from random chance. The significance was determined by calculating the p-value of each comparison. A comparison was considered to be significant if the p-value was at or below 0.05.

4. Data Analysis

This test was designed to test the effects of different exposure times of tartrazine on the learning, cognitive flexibility, and memory of adult zebrafish. The results from these tests were recorded and separated into different categories to analyze the overall effect on the zebrafish. These categories included reaching criterion as seen in ***Data Table 1***, ***Figure 1***, ***Data Table 2***, and ***Figure 2***, consistency in choices as seen in ***Data Table 3***, ***Figure 3***, ***Data Table 4***, and ***Figure 4***, perseverance as seen in ***Data Table 5***, ***Figure 5***, ***Data Table 6***, and ***Figure 6***, and group success as seen in ***Data Table 7***, ***Figure 7***, ***Data Table 8***, and ***Figure 8***. The significance of these results were recorded in p-values as seen in ***Data Table 9***.

Reaching criterion was the first dependent variable tested as this was the mean number of trials to reach task 1 and the mean number of trials to reach task 2. When looking at ***Data Table 1*** and ***Data Table 2***, the first day appeared to be the hardest for almost all of the fish as this was training day. After the first day was the two learning days and although when looking at ***Figure 1*** all the fish appeared to be learning, it is important to note that the yellow 5 group took much longer to complete task 1. This was also true for the next day and when the fish were trying to complete task 2 as seen in ***Figure 2***. On the last day which showed the memory of the zebrafish, the zebrafish in the yellow 5 could not remember how to complete task 1 or the reversal. Even though the zebrafish in the yellow 5- control group appeared to be trained better in completing task 1 on the first day, this was only true for one of the fish. The zebrafish Y-C2 completed both task 1 and task 2 on the first day but since none of the other fish could complete task 1 or task 2 on the first day, this fish was considered an outlier. Although this happened on the first day, this skill seemed to disappear on the second day as the zebrafish in the yellow 5- control group could complete task 1 but not the reversal as seen in ***Figure 2*** and ***Data Table 2***. The memory of the yellow 5- control group was also deterred as some of the fish could complete task 1 but none of the fish could complete task 2 on the last day of testing.

The next dependent variable tested was consistency in the choices the zebrafish made as this was recorded under the average longest string of correct choices for task 1 and the average longest string of correct choices for task 2. For these tests it, it was important for the zebrafish being tested to maintain the correct choice instead of the string being cut off by a wrong choice. In ***Data Table 3*** and ***Figure 3***, it can be seen in the training the correct choices string for each group tested. However, this changed in the second day as the average longest string of correct choices while doing task 1 for the yellow 5 group did increase slightly but not as much as the increase in the other treatment groups. For the last day, the consistency definitely dropped for the yellow 5 group as the memory was not very strong and it seemed as though their choices were very random and had no direction to them. The consistency in choices for the yellow 5- control group also seemed more random than consistent. When looking at ***Figure 3*** it can be seen that the average longest string of correct choices on day 1 for the yellow 5- control group is slightly lower when compared to the control group. Another interesting statistic is that the yellow 5- control group did not really seem to have a problem with understanding task 1 but struggled on half of the days when trying to understand and complete task 2. This can be seen in ***Figure 4*** and ***Data Table 4*** as the zebrafish in the yellow 5- control group did increase in the consistency of their choices for task 2 but not as quickly as the zebrafish in the control group. This was also evident on day 8 as the choices for the zebrafish in the control group were very consistent in their choices of which arm to swim into but the yellow 5- control group appeared more lost and uncertain as too which way was the correct arm.

Perseverance was the next dependent variable tested as this was displayed in the average longest string of incorrect choices for task 1 and the average longest string of incorrect choices for task 2. As seen in ***Data Table 5*** and ***Figure 5*** the strings of incorrect choices for the yellow 5 group were very long. This was true for all of the days of testing but the longest strings of incorrect choices increased once it became day 3 and day 8 of testing. ***Data Table 6*** and ***Figure 6*** also showed the lack of perseverance in the correct choices made by zebrafish in the yellow 5 group as even the fish that did reach the reversal could not understand what to do from then on. This lack of perseverance in the choices was also seen in the yellow 5- control group as they seemed to struggle more on the first day and last day of testing. In reference to ***Figure 5***, it can be seen on the first day when trying to understand and complete task 1 that the yellow 5- control group had on average longer incorrect strings than the other treatment groups. Although this data would not be considered significant when comparing the yellow 5- control group to the yellow 5 group, it does look significant when comparing the error bars to the control group. This was also similar when the zebrafish were trying to understand and complete task 2 as the zebrafish in the yellow 5- control group could not remember what to do after completing task 1 on the memory day as seen in ***Figure 6***.

The last dependent variable tested was the overall group success as this is shown in the number of fish to complete task 1 and the number of fish to complete task 2. As seen in ***Data Table 7*** and ***Figure 7*** the number of zebrafish from the yellow 5 group struggled to complete task 1 throughout all the days. Two of the fish in the yellow 5 group were able to complete task 1 on at least one of the learning days but none of them could remember how to complete task 1 on the last day. These fish were also not able to learn the reversal for any of the days as seen in ***Data Table 8*** and ***Figure 8***. This set of data definitely showed the lack of understanding and cognitive flexibility in the yellow 5 group of zebrafish overall. Although this was not exactly the case for the yellow 5- control group, their data was slightly similar. It can be seen in ***Figure 7*** on the last day that there was an extreme decrease in the zebrafish ability to complete task 1 for the yellow 5- control group. All of the zebrafish in the control group could complete task 1 on the last day but only one of the zebrafish in the yellow 5-control group could do the same. This was also similar for when they were trying to complete trial 2 as seen in ***Figure 8*** as none of the fish in the yellow 5- control group could do the reversal on day 2 and day 8.

Once all of the data was recorded for all the exposure level groups, the significance of each data sample was determined using an unpaired t-test. This unpaired t-test can be seen in ***Data Table 9*** and is categorized by the different data samples on the left side column. For almost all of the comparisons of the control group to the yellow 5 group, the p-values turned out to be less than 0.05 meaning that these data samples were considered significant. The two comparisons that did not have a p-value lower than 0.05 were still low enough to be considered almost significant. Although all of the comparisons of the control group to the yellow 5- control group were not considered significant, this could be due to the small sample size.

5. Discussion

After collecting and examining all of the data, a significant amount of conclusions could be drawn about the effects of tartrazine on the learning, cognitive flexibility, and memory of zebrafish. A lot of the more obvious effects of the toxin can be seen in the memory of the zebrafish affected by the toxin as the memory day was the day that they struggled with the most. However the first day appeared more equal for all the zebrafish as this was just the training day and none of the zebrafish were expected to be able to know what to do right away.

The zebrafish in the yellow 5 group were exposed to tartrazine since 24 hpf and were affected the most by this toxin. Overall, it can be seen in the data provided that the zebrafish in the yellow 5 group struggled the most on the learning and memory days. However, they did also struggle on the training day, but so did the zebrafish in the other treatment groups. None of the zebrafish in the yellow 5 group could reach the reversal and only one of them could not complete task 1 on the second and third days. For being able to reach the first criterion, the zebrafish in the yellow 5 group were not able to understand the task as well as the zebrafish in the other treatment groups. This was also the case for the number of trials for them to complete the reversal as none of them were able to complete task 2. It should be noted that these fish were highly incapable of understanding the reversal let alone the first task. This group of zebrafish were also very inconsistent with their choices. Even if the zebrafish did decide on the correct arm initially, it would still go to the wrong arm in the next trial as it could not properly learn which arm is correct and which is not correct. This is also true for the zebrafish in the yellow 5 group that made it to reversal as they were even more clueless on what they were supposed to achieve. The yellow 5 group of zebrafish also struggled with perseverance as they often chose the wrong arm many times over the correct arm. This can be seen as the zebrafish are trying to complete task 1 and the zebrafish in the yellow 5 group are consistently choosing the incorrect arm. These results are also the same when this group was trying to complete task 2 if they had even completed task 1 initially for that day. If the zebrafish did not complete task 1 for that day, then the longest string incorrect for task 2 would be 20 for that day. Overall group success was also not very strong for the zebrafish in the yellow 5 group as none of them could complete task 2. Even for seeing if the zebrafish could complete task 1, the yellow 5 group did not do very well. Once all this data was organized, each category was compared to the control group through a t-test. Almost all of the p-values for these tests turned out to be statistically significant and it can be assumed through these tests that daily intake of tartrazine at 22 μM does affect the learning, cognitive flexibility, and memory of zebrafish.

The zebrafish in the yellow 5- control group were exposed to tartrazine at 24 hpf and then moved out of the yellow 5 substance at 7 dpf and into a control tank. Although the effects were not as severe as the effects this toxin took on the yellow 5 zebrafish group, these zebrafish had troubles in completing task 1 and task 2 especially on the memory days. When looking at the zebrafish in the yellow 5- control group's ability to reach the 1st criterion, it can be seen that struggled the most on the memory day. This was also the case when the zebrafish were trying to complete task 2 but the zebrafish in the yellow 5- control group were affected the most by the toxin on all the days except on the training day. For consistency, the zebrafish in this group appeared to know what they were doing for completing task 1 but this was not the case for completing task 2. The only day that the yellow 5- control group was as consistent in their choices in completing task 2 as the control, was on the third day. Once it got to the memory day, the zebrafish in this group's consistency when completing task 2 dropped significantly. When looking at the perseverance of the zebrafish in the yellow 5- control group's choices when completing task 1, it can be seen that they struggled more on the training day but evened out with the control group for the rest of the testing days. However, for perseverance when completing task 2, the zebrafish did better on the first day but struggled on the memory day. Overall group success when completing task 1 appeared equal with the zebrafish in the control group except on the memory day. This was also true when completing task 2 but the zebrafish in the yellow 5- control group also struggled on the second day showing that they were learning slower than the control group. When looking at the t-tests of the yellow 5- control group compared to the control group, none of the p-values turned out to be statistically significant. This could be due to many reasons with the main one being the small sample size. It is also important to note that one of the zebrafish in the yellow 5- control group could complete task 1 and task 2 on the first day but could not do the same for the learning days or the memory day. This would be why a lot of the p-values were recorded as not significant and why some of the data for the first day is skewed as this fish only affected the data for the first day.

This data supports the idea that constant exposure to tartrazine will affect the ability to learn, remember, and reduce the cognitive flexibility of the organism. This data also suggests that exposure at an embryonic level, with no future exposure, will still significantly affect the organism's ability to remember and have cognitive flexibility. In the future and for further research into the effects of tartrazine on learning, cognitive flexibility, and memory of zebrafish, it would be optimal to test more zebrafish and do more trials with even lower concentrations to be able to better capture the true effects of the toxin. In about a year, it would also be interesting to test the yellow 5- control group again to see if their cognitive flexibility and memory becomes better with more exposure to yellow 5 free water. The data collected in this experiment supports the conclusion that it is advisable to try to cut back on the consumption of tartrazine as it can possibly affect the learning, cognitive flexibility, and memory of humans. Although the zebrafish in the yellow 5 group could not complete either of the tasks consistently, it can still be cautioned from the yellow 5- control group that pregnant women should also cut back on their daily consumptions of tartrazine.

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