Effects of Atrazine on Zebrafish Learning

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Abstract: *The experiment will determine how atrazine, a common water pollutant, affects the learning of zebrafish. A T-maze was used to help test the memory of adult zebrafish. The fish exposed to no atrazine averaged 1.4 reversals while the fish exposed to 4 ppb of atrazine only averaged 0.2 reversals. Due to harmful toxicants, Atrazine decreases fish learning.*

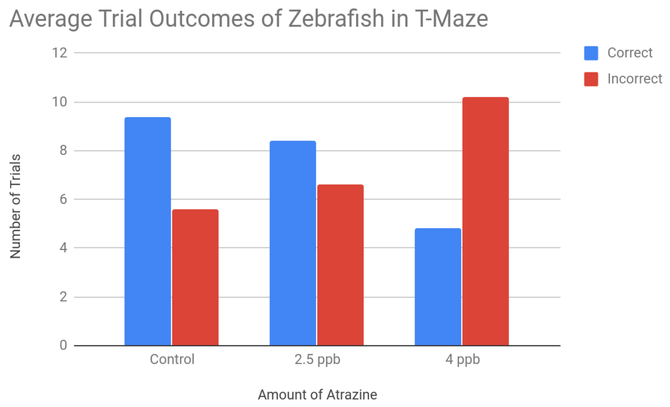
Introduction:

Water pollution is a widespread problem. Water pollutants can include things such as oil, sewage, or even pesticides (nrdc.org). Because of farming over the last century, herbicides have become a common method to increase agricultural growth. One of the most common herbicides is atrazine, which is found to be one of the top water pollutants as well. Some recent findings have shown the effects atrazine can have on frogs, rats, and zebrafish. The legal amount of atrazine in water is 3 ppb; a study done with frogs has shown 2.5 ppb could affect the health of amphibians (panna.org). It has also been proved that rats who digested atrazine developed kidney and liver problems (CDC, 2015). Not only does atrazine lead to liver and kidney problems in rats, but it can lead to reproduction issues and delayed development in zebrafish (CDC, 2015). Atrazine has only been around for half a century, therefore not all long term effects are known (panna.org). For example, scientists know zebrafish embryos are affected by water pollution, but what about other processes of life such as learning. This experiment will test the effect of atrazine on zebrafish learning and memory in order to see how water pollution is harming aquatic life. If a T-maze is used to test the effect of atrazine on zebrafish learning, then the atrazine will have a negative effect on fish learning because atrazine has already been proven to be harmful to other life processes of the fish such as reproduction.

Control Day 2 | 2.5 ppb Day 2 | 4 ppb Day 2

Control Day 4 | 2.5 ppb Day 4 | 4 ppb Day 4

Image Results: An additional study was done with zebrafish embryos to see how atrazine effected development. Each image shows little to no physical deformities over the 4 day period.



- **Figure 1 Results:** The graph indicates how many incorrect and correct trials each group of fish had. The group in 0 ppb (control) had the highest average of 9.4 correct trials, while the group of fish put in 4 ppb had the lowest correct trials with an average of 4.8.

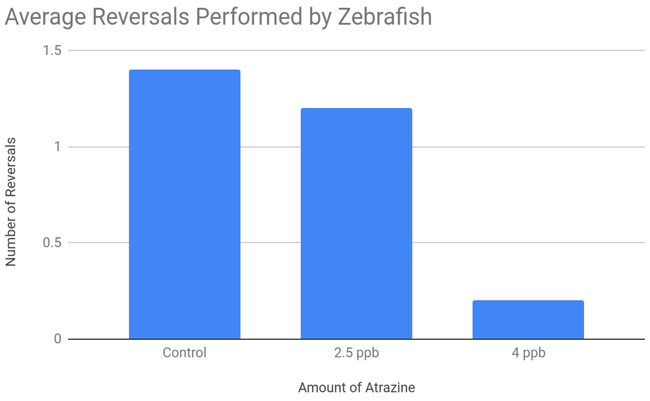


Figure 2 Results: As the amount of atrazine was increased, the amount of reversals the fish could perform decreased. The fish exposed to no atrazine were able to average 1.4 reversals while the fish exposed to 4 ppb only averaged 0.2 reversals. The chi square test was significant at 1.057, meaning that atrazine effected memory and learning of the zebrafish.

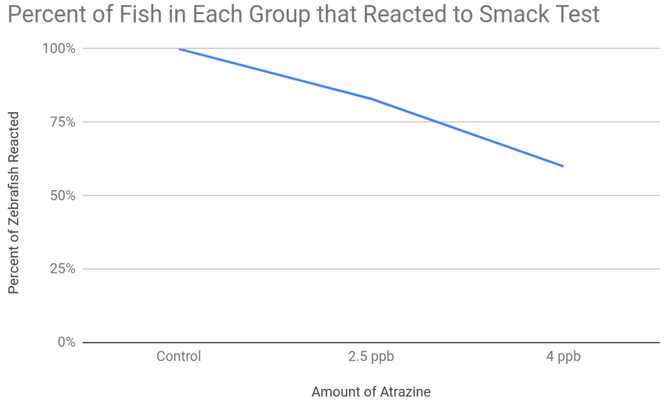


Figure 3 Results: As the amount of atrazine increased, the number of fish that reacted to sound and vibrations decreased. When exposed to no atrazine, all fish reacted to the smack test, showing good responsiveness. When exposed to 4 ppb of atrazine only 60% of the fish reacted, showing weakened responsiveness. The chi square test was significant at 0.967, meaning that atrazine effected the responsiveness of zebrafish.

Procedure:

1. Set up 3 tanks (0 ppb,2.5 ppb, and 4 ppb), and place 5 fish in each tank. Expose fish for 3 weeks.
2. Fill T-maze with water that is safely prepared for fish.
3. Place fish in T-maze and allow fish to acclimate for 5 minutes.
4. Choose correct direction for fish (right or left).
5. Place fish at starting position (bottom of the T) then wait 15 seconds.
6. Release fish and watch it choose direction.
7. If fish chooses correct direction, close off other side and reward fish by letting it swim for 45 seconds. If fish chooses incorrect direction, agitate water using the net for 10 seconds.
8. Record which side fish went to, then get fish back to starting position by scooping it with the net or coaxing the fish with the bottom of the net.
9. Repeat steps 5-8 for each trial. Perform 15 trials unless fish has gotten 5 out of 6 attempts choosing the correct side. If fish gets 5 out of 6 attempts before the 15 trials, perform a reversal.
10. To perform reversal, switch previously correct side to become incorrect side and then repeat steps 5-8. Record reversals.
11. Repeat process of steps 3-10 when switching fish.

Discussion:

The data supported the hypothesis that atrazine has an effect on zebrafish learning and memory. As seen in figure 1, the control group had the highest correct and lowest incorrect trials with 9.4 correct and 5.6 incorrect. On the other hand the highest dose of atrazine had the least amount of correct trials, but the greatest amount of incorrect trials with 4.8 correct and 10.2 incorrect. The big difference in the amount of incorrect and correct trials for each dose supports the hypothesis that atrazine causes a fish to not learn as quickly. Figure 2 showed how increasing atrazine caused fish to not perform as many reversals. The chi square test resulted in a significant number (1.057), thus further supporting the hypothesis fish were not able to perform as many reversals due to the atrazine affecting their learning and memory. Figure 3 was part of an supplemental study on hatched zebrafish embryos, showing how embryos placed in 4 ppb of atrazine were not as responsive as embryos placed in no atrazine. This data suggests atrazine is affecting the brain of zebrafish in some way. More fish should be tested in order to get more accurate results. This research suggests that water pollution should be monitored, as it is affecting the lives of fish. Agricultural use of herbicides should also be monitored as it is one of the leading water pollutants that cause these defects in fish. In addition, because Zebra fish have similar embryonic development to humans, further research should be done to investigate the affect of Atrazine on human development and learning as herbicides are found on food products.

References

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