The Effect of Herbicides on Zebrafish Development

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Abstract

Zebrafish were observed over the course of four days. Ten embryos were placed among two well plates and then solution was added to each well. The wells were split into four groups: one control group and three experimental groups. The wells in the control group were filled with instant ocean solution and the in the wells in the experimental group were filled with a solution of 0.1, 0.2, or 0.4 mg of herbicides mixed with 100 mL of instant ocean. After observing embryonic development for four days, it was observed that there were the most embryos alive in the control group and the least amount of embryos alive in the experimental group with 0.4 mg of herbicides at day 1. Likewise, there were the most hatched zebrafish the control group and the least amount of hatched zebrafish in the experimental group with 0.4 mg of herbicides by day 4. The results for the other two experimental groups followed this pattern. These results are significant because it shows that herbicides do have a harmful effect on embryos suggesting that women who are pregnant should not be largely exposed or able to inhale significant amounts of herbicides(while they garden, for example) because the herbicides can have a damaging effect.

Background Information

•Pesticides are used by farmers to control weeds, insect infestation, and disease.(EPA, 2016)
- Could have toxins that could be potentially harmful to embryo development.
- Zebrafish negatively affected by insecticides. (DeMicco, Cooper, Richardson, and White, 2010)
  - Consistent with mammalian studies
- Herbicides have glyphosate and isoprophylamine salt
  - May cause harm to zebrafish development (EPA, 2016)

•Using zebrafish to test
- Show higher levels of thinking (Petering, 2015, p. 12)

Methods

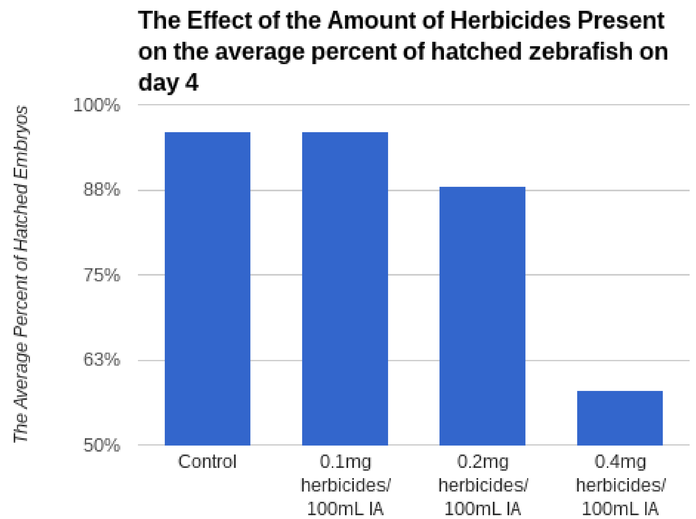
1.Solution of 0.1, 0.2, and 0.4 mg Eliminator herbicides/ 100 mL instant ocean (IA) were made and labeled in bottles. Pure instant ocean was the control group.
2.10 embryos were pipetted into each well. 2 plates were used with 12 wells each.
3.About 3 mL of the control solution was pipetted into column 1 in each well plate.
4.Step 3 was repeated with the 0.1, 0.2, and 0.4mg solutions into columns 2, 3, and 4 respectively in each well plate.
5.In 24 hours embryos were observed for how many were alive and dead. Dead ones were removed. Solutions were replaced.
6.In 24 hours embryos were observed under the microscope for how many were hatched and not. These values were recorded and solutions were replaced. Pictures were taken of the embryos.
7.In 24 hours they were observed again for hatched and not hatched. Pictures were taken.
8.Zebrafish disposed of properly.

Well Plate Example:

1A	2A	3A	4A
1B	2B	3B	4B
1C	2C	3C	4C

Results

The trends of our results were that as the concentrations of the Eliminator herbicides increased the amount alive and amount hatched decreased. Additionally. On the last two days of observation the control group zebrafish appeared to be normal looking with straight spines and normal looking heads. As the concentrations got greater there appeared to be some cranial abnormalities like bumps by the eyes and there also appeared to be more bent spines.



As the amount of herbicides present increased, the average percent of alive embryos decreased.

This picture was taken on day 3 of observations and was taken of a zebrafish under control conditions. It shows a straight spine and no apparent cranial abnormalities.

This picture was taken on day 4 of observations and the zebrafish was in 0.4 mg/100 mL. It shows large curvature of the spine.

Statistics

The t-tests were calculated between the consecutive increasing concentration of herbicides and also between the control group and 0.4 mg of herbicides/100 mL.

Comparing the Control Group(1) and 0.4 mg herbicides/100 mL IA (2)

P-value: 0.0220

This difference is considered to be statistically different.

Group	Group One	Group Two
Mean	96.17	57.83
SD	6.15	34.12
SEM	2.51	13.93
N	6	6

- The other t-tests calculated the differences to be considered not significant

Discussion

•Expected: as the amount of herbicide concentration increased, the number of dead embryos and abnormalities in the developmental process of the zebrafish would increase
•Results
  •Higher percentage of dead embryos and lower percentage of hatched and alive zebrafish in the higher concentration of herbicides than there was in the lower concentration
  •More abnormalities in the higher concentrations shown by pictures
    •Control: straight spines and normal looking heads
    •Higher weed killer concentration: More spine curvature and cranial abnormalities
  •Experiment did not show much statistical significance, some errors are:
    •Hard to pick up solutions without removing zebrafish
      •Many were sucked up with the pipette which could harm them
        •Could cause abnormalities not caused by the herbicides
    •Bacteria or fungus could have entered the wells
      •Could cause zebrafish to die but not because of the herbicide
  •One t-test showed to be significantly different
    •Control group and the 0.4 mg of herbicides/100 mL on day 4
      •With more time and higher concentrations of herbicides there could've been more of a harmful effect shown
•Suggested that expecting mothers do not do gardening with weed killers in order to eliminate this risk
  •Weed killer killed embryonic cells in previous study (Gammon, 2009)

References

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