The Development and Risk of Deformities/Disabilities the Chemicals in Hair Dye have on Developing Zebrafish Embryos

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Abstract

If permanent hair dye is used during the first trimester of pregnancy for an extended period of time it could result in many negative effects such as allergic reactions, and disabilities/disorders. Hair dye can also cause non-Hodgkin's lymphoma and multiple myeloma. All of these side effects could cause life threatening circumstances for both the woman and the developing fetus. This is important to bring to the attention of expecting mothers and the general public in an attempt to reduce the rate of disabilities and disorders in the development of the fetus. The purpose of the experiment performed was to further examine the risks of women using hair dyes while pregnant and the potential negative effects it has on their child. Zebrafish were placed in increasing amounts of hair dye and the rate of development and hatch rate was recorded along with any other meaningful observations over the course of 72 hours. The data collected during the experiment brought forth increasing evidence towards the hypothesis of, if zebrafish embryos are exposed to hair dye, then development will be slower and will have a higher risk of developing deformities and disorders because of the toxic chemicals most hair dyes contain. It was observed that the tails of the zebrafish embryos in the higher concentrations of hair dye were slightly deformed. This observation proves that the chemicals found in most hair dyes could have negative effects such as deformities and disorders on a developing fetus.

Introduction

According to Ayed H. Ziadat (2010), chemicals used in any hair dye, but primarily permanent hair dye such as ammonia, peroxide, p-phenylenediamine (PPD), diaminobenzene, and mercury agents can cause many disabilities and disorders when women are pregnant specifically during the first trimester. “The first trimester of pregnancy is the most sensitive period where the toxic liquids of hair dye use could negatively affect the formulation of the kidneys, liver, brain and nervous system of the developing fetus” (Ayed H. Ziadat , 2010). The chemicals in hair dye can also cause

severe allergic reactions (The Editors of E-Magazine, 2004). Which may result in swelling/itching of the face and scalp, blisters or warts, itching of the feet/hands, swelling of the throat and neck which can be fatal to the 5% that are allergic to the chemicals in hair dye. It was also found that by The Editors of E-Magazine (2004) “In 1994, the Journal of the National Cancer Institute announced that deep-colored dyes (like dark brown and black), when used over a prolonged period of time, seemed to increase the risk of non-Hodgkin’s lymphoma and multiple myeloma.” Both of these potential effects of hair dye could result in a life threatening circumstance which could hinder the development of the embryo. This experiment was done to further investigate the effects of women using hair dye while pregnant and the effects it has on their children. If zebrafish embryos are exposed to hair dye, then development will be slower and will have a higher risk of developing deformities and disorders because of the toxic chemicals most hair dyes contain.

Materials & Methods

- One box of L’Oréal forever rouge hair dye
- Four disposable pipettes, 1 mm
- One 28.5 °C incubator
- One plate with wells
- One Dissecting Microscope
- One sharpie
- One beaker (for disposal of dead embryos and liquid)
- Four 100mL beakers (necessary for the mixing of the stock solution of hair dye 0 µl, 50 µl, 100 µl, 200 µl, 300 µl)
- One 50mL (for disposing of the dead embryos and liquid)
- One bottle of instant ocean/embryo media solution
- One 100 mL graduated cylinder
- 4 pair of gloves per person (one for each day)

Note: When performing this experiment safety precautions should be taken when mixing and changing the solutions to ensure that no live embryos were discarded. Gloves should be worn and hands should be washed after each day to make sure there is no chemicals are left on the hands/skin.

Day 1:
The plate was labeled with three pieces of tape, the first piece had the hair dye concentration of each well, another piece had the group members names and the last piece of tape had the class hour. Ten zebrafish embryos were placed in each of the five wells used. Each well was filled with the appropriate stock solution of hair dye 0.0 µl in

the first well in the second row, 50 µl in the first well in the first row, 100 µl in the second well in the first row, 200 µl in the third well in the first row, and 300 µl in the fourth row in the first row. Pictures of the embryos were taken using the dissecting microscope and were used for reference during the other days. Data was recorded and the plate was placed in a 28.5 °C incubator for 24 hours.

Day 2-3:
The zebrafish embryos observed through a dissecting microscope and pictures were taken.The embryos that were cloudy and dark were considered dead and were taken out of the wells along with the old solutions and were disposed. New solutions for the 0.0 µl, 50 µl, 100 µl, 200 µl, and 300 µl hair dye solutions were placed in the appropriate well. The wells were set aside and the amount of hatched, and live embryos were recorded. The plate was returned to the incubator for 24 hours.

Day 4:

The embryos were observed under a dissecting microscope to see if any of them were dead. The developmental rate, movement, and deformities were recorded as well as the final number of living embryos. The remaining, alive embryos were placed in a tank where they would live and develop fully in a normal environment.

A Chi Square analysis was completed on the data to ensure statistical significance.

Results

The hypothesis used for this experiment was if zebrafish embryos are exposed to hair dye, then development will be slower, and there will be a higher risk for deformities and disorders, due to the toxic chemicals found in most hair dyes. The experiment was set up to investigate the effects of hair dye on zebrafish embryos and use that data to correlate to women using hair dye while pregnant and the effects it has on their children.

In this experiment, the dependent variables were the speed of development, hatch rate, and the disorders/deformities. The independent variables were the concentrations of the hair dye which were 0 µl, 50 µl, 100 µl, 200 µl, and 300 µl. The control variable was the solution that contained no hair dye which is where the zebrafish embryos developed without the risk of potential negative effects hair dye may have on the embryos. The controlled variables during this experiment were also the temperature of the incubator, size of the wells, and the amount of solution in each well. During this experiment, it was found that when the concentration of the hair dye increased, the amount of embryos that hatched and survived for more than 24 hours were few to none. This was seen as a difference when it was observed that in the lower concentrations (50

µl and 100 µl) the embryos survived for between 24-48 hours after they had hatched and in the control group the embryos lived for over 72 hours after they hatched. It can be seen in table 1 as well as in figure 1, that once 48 hours had passed the embryos no longer hatched in any of the wells. It was also found during the experiment that as the concentration of the hair dye increased, the embryos had a few apparent deformities. One of the deformities can be seen in figure 2 which shows the difference between the tails in the control group (0 µl) and in the 100 µl well.

The chi-square analysis performed on the data collected shows that the results observed could not have happened by chance and that the hatched/unhatched rates were not a coincidence. The chi-square statistic for data table one which only had the data for the hatched embryos and the expected value (5) was 13.6. The second chi-square table contained the expected value (5) and the number of unhatched embryos data came out with the same chi-square statistic which was 13.6. The final chi square value was 27.2 which was found by finding the sum of both chi square statistics. The degree of freedom used was 4, due to this, the critical value used was 9.49. This proves that the results from the experiment performed were not by chance due to the fact that the final chi square value (27.2) was higher than the critical value that was used (9.49). This was performed to test the independence of hair dye solution and the development of zebrafish embryos. This experiment rejected the null hypothesis, this means that there was a correlation between the hair dye solution and the development of zebrafish embryos proving that the results observed/collected were not by chance.

Table 1: # of hatched embryos in each concentration for each day

The table below shows the amount of hatched embryos per concentration for each day. As the time goes on past 48 hours the amount of hatched embryos stays the same.

Treatment (hair dye)	# of starting fish	# hatched 24 hours post fertilization	# hatched 48 hours post fertilization	# hatched 72 hours post fertilization
Control (0.0 µl)	10	0	10	10
50 µl	10	0	8	8
100 µl	10	0	5	5
200 µl	10	0	0	0
300 µl	10	0	2	2

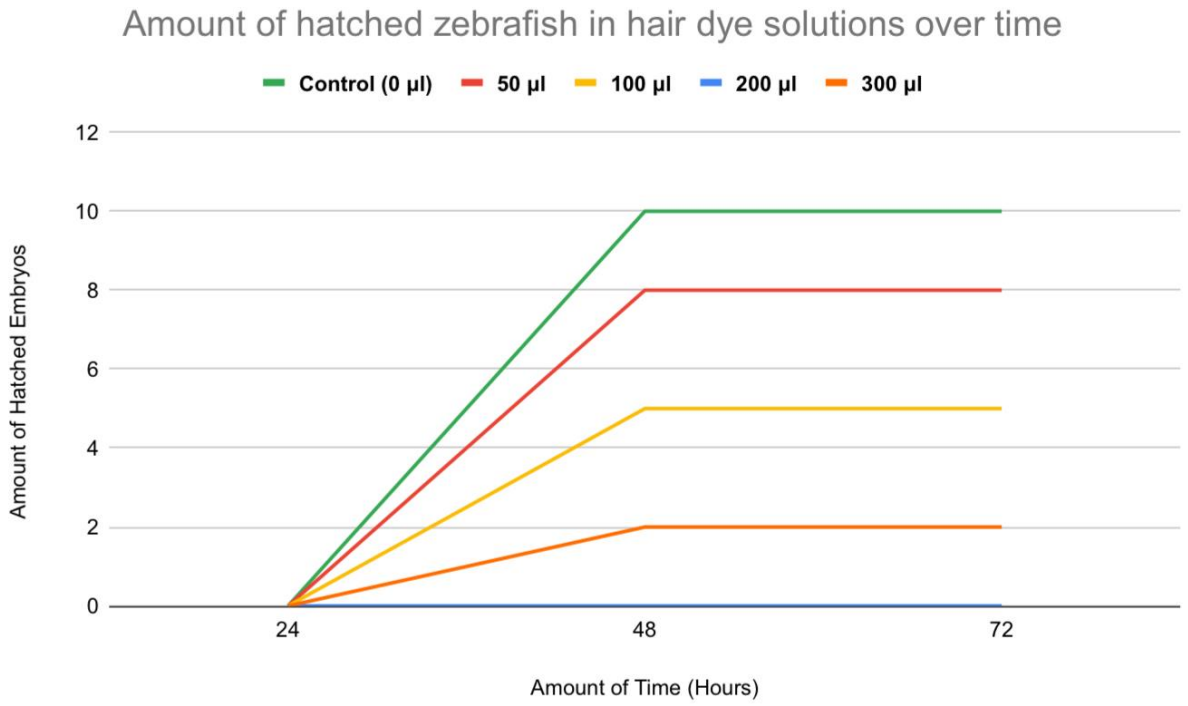

Figure 1: The line graph above displays the amount of hatched zebrafish embryos overtime while they were immersed in increasing amount of hair dye solutions. As the concentration of the hair dye increased the amount of hatched embryos generally decreased.

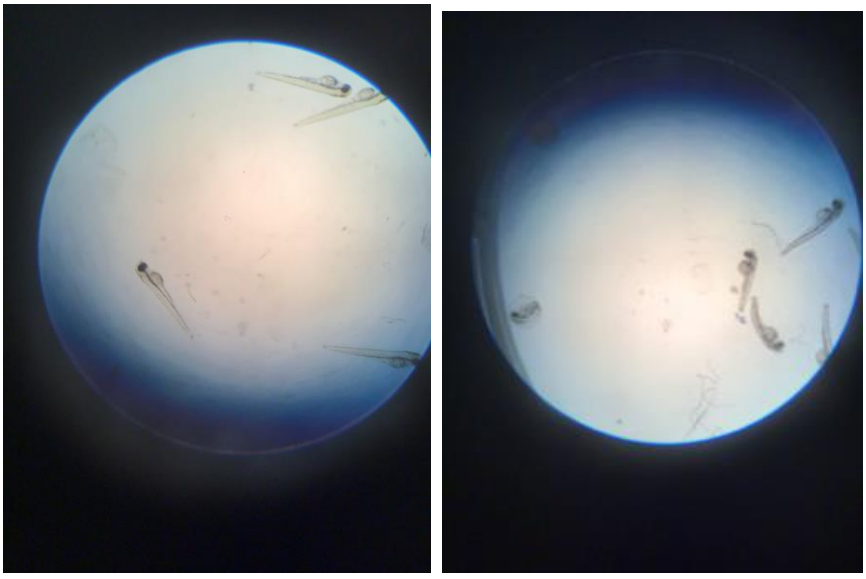

Figure 2: The pictures above show the difference in the sharpness of the tails between the control group 0 µl (left) and the 100 µl well (right).

Discussion

The hypothesis used for the experiment was correct, the embryos developed noticeably slower and had some deformities such as the tip of their tail, which was not as sharp as the control groups tails. It was also observed that as the concentration of the hair dye increased the development of the embryos got increasingly slower and some didn't hatch. When researching, it was found that hair dye can affect the developing fetus and cause disabilities and disorders if used during the pregnancy especially in 1st trimester. During the first trimester, the embryo's organs are the most vulnerable and the toxins in hair dye can negatively affect the formation of organs including the liver, nervous system, kidneys, and brain.

Although the results observed were not a coincidence, it may have been better for future results and experiments to have less hair dye in each solution. If less hair dye was added into each solution, the results may have been more accurate. If more of the embryos survived, the long term effects of the toxic hair dyes could have been observed. Adding less hair dye may also be beneficial for future experiments due to the fact that in the 50 µl and the 100 µl wells the results were impacted by protozoa. The protozoa impacted the results in this experiment. The protozoa ate the remaining alive embryos in those wells causing further results and observations that may have appeared over time to not be recorded. A larger sample size would also be beneficial to getting better results when performing this experiment. A larger sample size would be beneficial due to the fact that there would then be a better chance for more embryos to hatch and then more accurate results could have been seen. If more of the embryos in the higher concentrations hatched, the experimental results would be more accurate and the effect of the toxins in hair dye can cause such as slower development, and a higher risk for deformities/disorders have been more clear. With a larger sample size a trend related to the experiment may have also occurred, unlike during the experiment performed. During the experiment there was a trend in the 200 µl however, it was not was not an overall trend related to the hypothesis, making it hard to come to a concise conclusion on whether or not hair dye has a significant effect on developing embryos.

This experiment provided an opportunity to observe the effects of many chemicals such as p-phenylenediamine (PPD), which can be found in many commonly used permanent hair dyes. The observations and results of this experiment can be used to recognize the effects most hair dyes have on developing embryos.

References and Literature cited

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