

Developmental Deformities and Deaths in Zebrafish

Embryos Exposed to Hair Dye

Isabella Moeller

ABSTRACT

Scientists often use zebrafish to examine how certain variables may affect humans. Hair dye is a popular cosmetic item that contains various chemicals, yet there has been little research on the effects these chemicals may have on the fetuses whose mothers use hair dye. We performed an experiment analyzing the death rate, hatch rate, and abnormalities in zebrafish embryos placed in a control solution and three solutions with different concentrations of hair dye. After forty-eight hours of exposure to various levels of hair dye, the results displayed the zebrafish exposed to higher concentrations of hair dye exhibited a higher death rate, delayed growth, cardiac edema, and curved spines and tails. As hair dye becomes an increasingly popular beauty product, it's important to understand how these results in zebrafish reflect on developing children and fetuses exposed to hair dye.

INTRODUCTION

Hair dye is a popular cosmetic among teens and adults that changes the appearance of one's natural hair color. Because of its popularity, it's important to understand how exposure to chemicals from hair dye may affect developing fetuses whose mothers use hair dye pre-pregnancy or while pregnant. P-Phenylenediamine (PPD) is a major component of hair dye, and it is estimated that, under the use of permanent dyes, 0.1% to 0.5% of PPD may be absorbed into the skin (Bolt, Golka). Once absorbed, a small amount may target amino acids that cause sensitization (Pot, Scheitza, et al.). According to the U.S. Environmental Protection Agency,

“acute exposure to high levels of p-phenylenediamine may cause severe dermatitis, eye irritation and tearing, asthma, gastritis (stomach inflammation), renal failure, vertigo, tremors, convulsions, and coma in humans.” Additionally, pre-pregnancy hair dye use increased the risk of infants having a lower birth weight. Abnormalities at birth can affect an infant later in life; for instance, delay in cognitive and physical development and a lower functioning immune system (Jiang, Hou, Huang, et al.).

Further tests must be performed to analyze the effects of hair dye on developing fetuses. Scientists often use zebrafish for biomedical research because of their fully sequenced genome, the large number of offspring produced, and rapid external fertilization, which makes them efficient to observe. Additionally, zebrafish have a complete organ system that is similar to humans (Teame, Zhang, Ren, et al.). A study was performed to test the effects of henna hair dye on zebrafish embryos; the zebrafish were exposed to different levels of henna hair dye at 100, 200, and 275 micrometers. The embryos experienced abnormalities at each concentration, such as mortality, hatching delay, slow blood circulation, edema, body deformities, weak heartbeat, and delay in growth (Manjunatha, Wei-bing, Ke-chun, et al.). Because of the popularity of hair dye (42% of beauty consumers used at-home dye in 2019) (Sandler), further investigations should be performed on how common hair dye products affect consumers. It was hypothesized that if zebrafish embryos are exposed to different levels of an at-home hair dye, those exposed to the highest levels would experience more lethal and sub-lethal effects, such as mortality, delayed growth, and deformities.

MATERIALS

- One box of Nutrisse Dark Brown 30 (Sweet Cola) at-home hair dye kit (contains PPD)
- Gloves

- Zebrafish embryos supplied from SEPA UW-Milwaukee
- 4 beakers
- 1 waste-beaker for disposal of dead embryos and liquid
- 2 Falcon dishes
- Pipettes
- Micropipette
- Graduated cylinder
- Instant Ocean Solution (200 mg/L)
- One dissecting microscope
- One incubator (set to 28°C)
- Camera

METHODS

Steps were followed according to the box directions to mix the hair dye. Four beakers were collected and labeled: one for the control and three for varying hair dye solutions. The Instant Ocean solution was measured using a graduated cylinder, and the hair dye was measured using a micropipette. The control beaker (green) was filled with 100 mL of Instant Ocean solution (0% concentration of hair dye). The pink beaker was filled with 200 mL of Instant Ocean solution and 50 microliters of hair dye (0.025% concentration of hair dye). The purple beaker was filled with 300 mL of Instant Ocean solution and 50 microliters of hair dye (0.017% concentration of hair dye). The blue beaker was filled with 800 mL of Instant Ocean solution and 100 microliters of hair dye (0.0125% concentration of hair dye). All solutions were mixed with separate, clean pipettes. Using a clean pipette, 3 mL of the control solution was put into three separate wells of the Falcon dish. This step was repeated for the other three solutions (12 wells in

total). Five zebrafish embryos were placed into each well using a pipette to the best of our abilities (there were six or seven embryos in a few wells). The Falcon dishes with the embryos were placed in the incubator at 28°C for twenty-four hours.

Each day, the embryos were removed from the incubator and examined under a dissecting microscope. A data table tracked the total number of embryos that were alive, dead, and hatched for each solution. Using the microscope, additional qualitative data was evaluated: stages of development the embryos were in and any deformities present. Pictures were taken to note the stages of development and any abnormalities. At the end of each day, dead embryos were removed from the wells and more of each solution was added to the wells to replace the lost liquid. The embryos were placed back in the incubator after each observation. After three days, all embryos and liquids were appropriately disposed of by the instructor.

Note: When removing dead embryos and changing the solutions, precautions were taken to avoid the removal of live embryos, and new pipettes were used for each solution to avoid cross-contamination. Gloves were worn when handling the hair dye, and hands were washed after to avoid contact with chemicals.

RESULTS

We performed an experiment to test the effects of hair dye on zebrafish embryos in order to correlate whether pre-pregnancy or pregnancy use of hair dye affects developing human fetuses. Independent variables of the experiment included the four levels of hair dye concentrations: the control (no hair dye), 0.025%, 0.017%, 0.0125%. Dependent variables included the number of deaths, number of hatched embryos, and any deformities that arose. Controlled variables included putting the same amount of each hair dye concentration (3 mL)

into three wells (12 wells total), the temperature of the incubator (28°C), analyzing the embryos at the same time each day, and putting, approximately, the same number of embryos in each well.

Initially, the concentrations of the hair dye were higher with one control solution, a 0.025% concentrated solution, a 0.05% concentrated solution, and a 0.1% concentrated solution; however, all the embryos in the solutions with hair dye died after day one, so the solutions were diluted and the experiment was restarted.

The embryos placed in the control solution developed normally with straight spines and normal-sized eyes, yolks, and tails (figure 1). At forty-eight hours post-exposure, one death was recorded, and thirteen out of eighteen embryos hatched with zero deformities. The embryos placed in the 0.0125% concentrated solution also fared well with zero deaths; however, there was a delay in growth with five hatchlings out of sixteen after forty-eight hours, and those that hatched had a shortened body (figure 2). The embryos placed in the 0.017% concentrated solution had a higher death rate with six out of seventeen dead forty-eight hours post-exposure. Additionally, only two out of seventeen embryos hatched (one survived), and the surviving hatchling had a curved spine (figure 3). Last, the embryos placed in the 0.025% concentrated solution had the most lethal effects. Twenty-four hours post-exposure, one embryo displayed an enlarged yolk and curved tail, and another displayed a curved tail and cardiac edema (figure 4). After forty-eight hours, all seventeen of the embryos were dead (one had hatched but was tiny and dead) (figure 5).

Overall, we found that the zebrafish embryos placed in higher concentrations of hair dye experienced more lethal and sub-lethal effects, as expected. With zebrafish embryos' close relationship to human embryos, we can infer from these results that exposure of human fetuses to chemicals in hair dye can delay growth and cause deformities.

Two Fisher Tests were performed to determine the significance of the results between zebrafish embryos exposed to hair dye and not exposed. The first test examined the relationship between the number of dead embryos in hair dye solutions compared to the number of dead embryos in the control solution. The P-value equaled 0.0016 and was very statistically significant. This supported the hypothesis that embryos exposed to hair dye would experience more deaths than those not exposed. The second Fisher test examined the relationship between the number of hatched embryos in hair dye solutions compared to the number of hatched embryos in the control solution. The P-value equaled less than 0.0001 and was extremely statistically significant. This supported the hypothesis that embryos exposed to hair dye would experience a slower growth rate than those not exposed. The P values observed rejected the null hypothesis, meaning that there was a significant difference between the development of zebrafish in hair dye solutions and the solution with no hair dye.

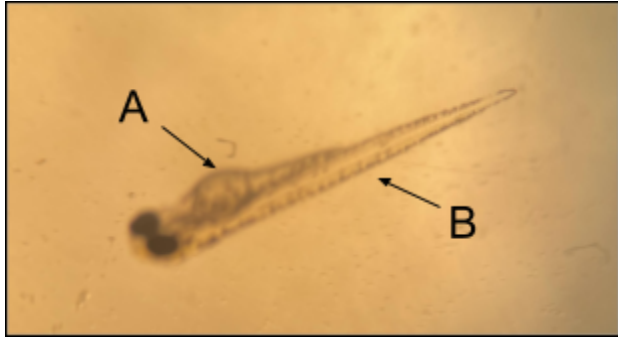


Figure 1

Image of one hatched zebrafish 72 hours after fertilization. This zebrafish served as the control. Point A displays how the embryonic sac should look, and Point B shows a straight spine.

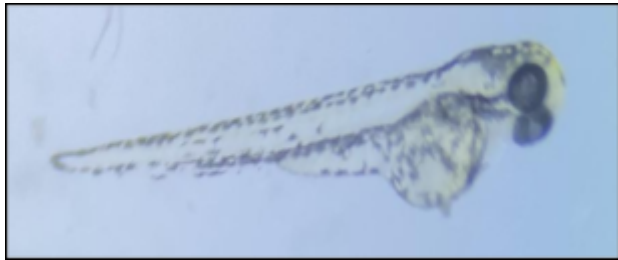


Figure 2

Image of a hatched zebrafish 48 hours post-exposure to a 0.0125% concentrated solution of hair dye. The hatched embryo exhibits a delay in growth with a reduced body length.

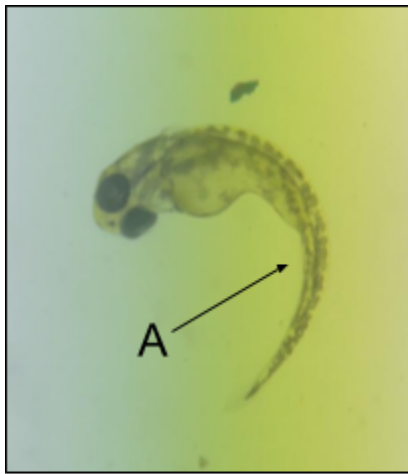


Figure 3

Image of a hatched zebrafish 48 hours post-exposure. The embryo was placed in a solution of 0.017% hair dye. Point A displays a curved spine.



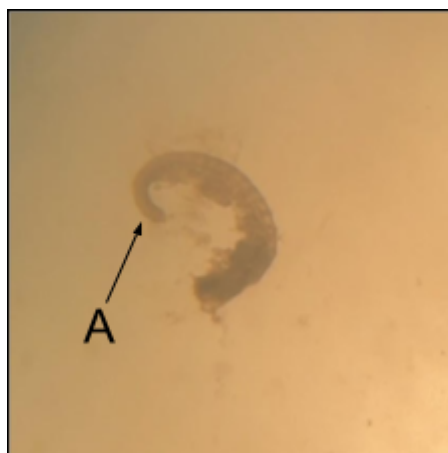
Figure 4

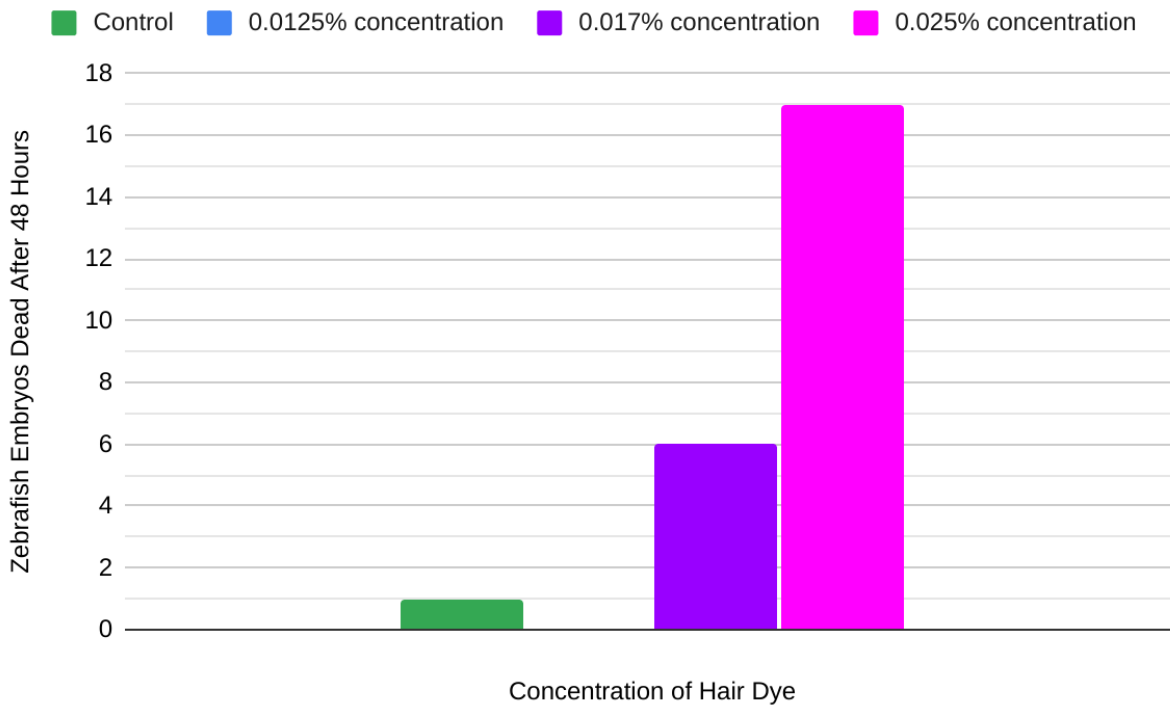
Image of two zebrafish in a solution of 0.025% hair dye 48 hours post-exposure. Point A shows an enlarged yolk. Point C shows cardiac edema. Points B and D show curved tails.



Figure 5

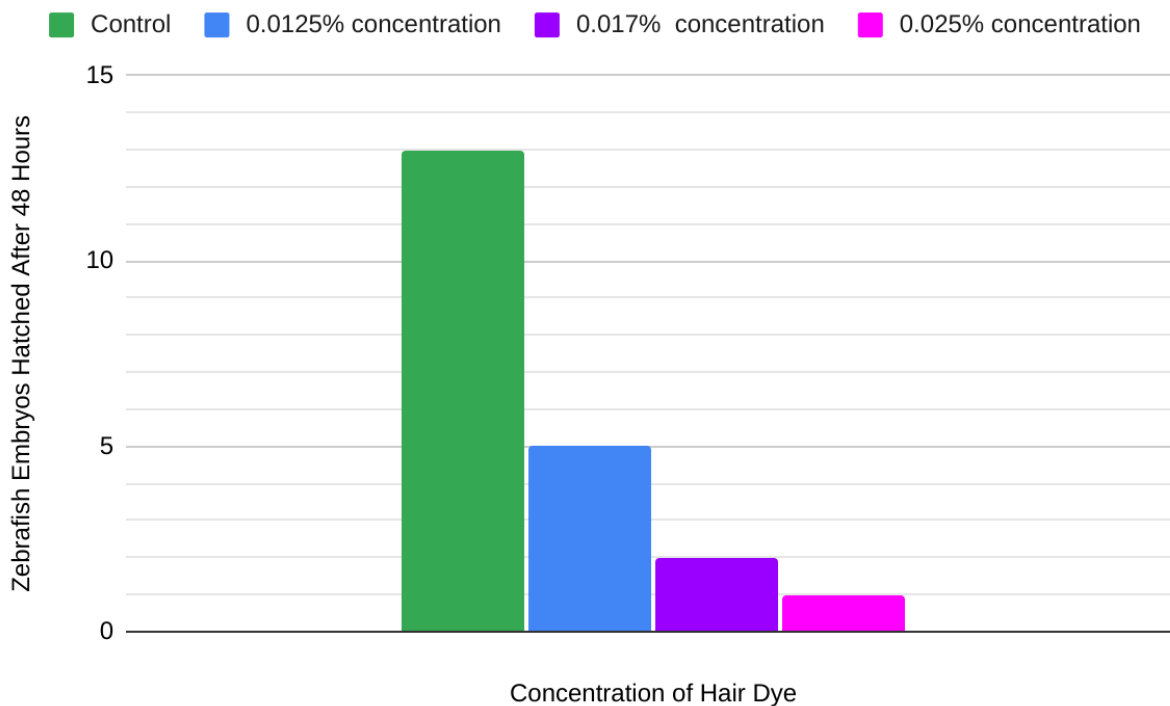
Image of a hatched zebrafish that died 48 hours post-exposure. The embryo was placed in a solution of 0.025% hair dye. Point A shows a curved tail.





Graph 1

This graph exhibits the effects of different levels of hair dye solution on the death rate of zebrafish embryos. Those exposed to the lowest concentration of hair dye at 0.0125% showed the lowest death rate (0 deaths), whereas the embryos exposed to the highest concentration at 0.025% showed the highest death rate (17 deaths).



Graph 2

This graph exhibits the effects of different levels of hair dye solution on the hatch rate of zebrafish embryos. Those not exposed to hair dye (control) had the highest number of hatched embryos (13), whereas those exposed to the highest concentration of hair dye at 0.025% had one hatched embryo.

DISCUSSION

The results supported the hypothesis, exhibiting a significant difference in the number of deaths and abnormalities between embryos exposed to hair dye and those not exposed. A trend observed was that the zebrafish embryos exposed to higher concentrations of hair dye experienced more abnormalities than those exposed to lower concentrations. The zebrafish exposed to the 0.025% concentrated solution died after forty-eight hours, had a very slow growth

rate, curved tails, one deformed yolk, and one cardiac edema. Contrastingly, the zebrafish exposed to the lowest concentration of 0.0125% all survived and had multiple hatchlings, although they were slightly underdeveloped.

Errors were made during the experiment that could have compromised the results. Because we had to restart the experiment after twenty-four hours due to all the embryos dying, the second group was exposed to the hair dye later in development than was planned, so a critical time period may have been missed that could have affected the survival rates and abnormalities observed. Additionally, larger sample sizes could have been useful in obtaining more data, and it could have been beneficial to test how different hair dyes influenced the results.

The results confirm what was discussed in the introduction. Similar to the study that tested the effects of henna hair dye on the embryonic development of zebrafish (Manjunatha, Wei-bing, Ke-chun, et al.), the zebrafish exposed to hair dye in this experiment exhibited mortality, delayed hatching and growth, edemas, and body deformities. As mentioned in the introduction, a study performed by *BMC Pregnancy and Childbirth* found that human fetuses were at an increased risk of having a lower birth weight when the mother used hair dye pre-pregnancy. In addition, studies have found that hair dye use may be a cause of bladder cancer, non-Hodgkin's lymphoma, multiple myeloma, and acute leukemia; however, more research has to be performed to confirm these results (Rollison, Helzlsouer).

As hair dye increases in popularity, especially among children and young teens, more experiments have to be conducted to evaluate how chemicals in hair dye, such as p-phenylenediamine, affect developing youth and fetuses exposed to hair dye. Because the chemicals in hair dye vary between different colors and permanent and temporary dyes, it's also important to experiment with how different forms of hair dye may have various effects.

Sources

Bolt HM, Golka K. “The debate on carcinogenicity of permanent hair dyes: new insights.”

PubMed, 2007; <https://pubmed.ncbi.nlm.nih.gov/17661215/>.

Jiang, Hou, Huang, et al. “The effect of pre-pregnancy hair dye exposure on infant birth weight:

a nested case-control study.” *SpringerLink*, BMC Pregnancy Childbirth, 09 May 2018;

<https://link.springer.com/article/10.1186/s12884-018-1782-5#citeas>.

Manjunatha B, Wei-bing P, Ke-chun L, Marigoudar SR, Xi-qiang C, Xi-min W, Xue W. “The effects of henna (hair dye) on the embryonic development of zebrafish (*Danio rerio*).”

PubMed, Environ Sci Pollut Res Int, 27 May 2014;

<https://pubmed.ncbi.nlm.nih.gov/24859694/>.

Pot, Shcheitza, Coenraads, Blomeke. “Penetration and hapteneration of p-phenylenediamine.”

Wiley Online Library, 20 March 2013;

<https://onlinelibrary.wiley.com/doi/full/10.1111/cod.12032>.

“p-Phenylenediamine.” EPA, Jan. 2000;

<https://www.epa.gov/sites/default/files/2016-09/documents/p-phenylenediamine.pdf>.

Rollison, Helzlsouer. “Personal Hair Dye Use and Cancer: A Systematic Literature Review and Evaluation of Exposure Assessment in Studies Published Since 1992.” *Journal of*

Toxicology and Environmental Health, vol. 9, 15 Dec. 2006;

<https://www.tandfonline.com/doi/abs/10.1080/10937400600681455>.

Sandler. “As at home hair dye surges brands are strategizing to keep new customers.” *Glossy*, 30 April 2020;

<https://www.glossy.co/beauty/as-at-home-hair-dye-surges-brands-are-strategizing-to-keep-new-customers/>.

Tsegay Teame, Zhen Zhang, Chao Ran, Hongling Zhang, Yalin Yang, Qianwen Ding, Minxu

Xie, Chenchen Gao, Yongan Ye, Ming Duan, Zhigang Zhou, The use of zebrafish (*Danio rerio*) as biomedical models, *Animal Frontiers*, Volume 9, Issue 3, July 2019, Pages 68–77; <https://doi.org/10.1093/af/vfz020>.