The effect of lead concentration on Artemia hatch rate

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Lead is a heavy metal that is known to cause serious health effects, such as lead poisoning. As a result, the World Health Organization (WHO), in 2017, set a safe lead exposure level at 0.02mg per 200mL. However, recent research indicates that lead is still toxic at this level [1]. This study sought to determine whether lead is safe at the level set by the WHO, and if not, determine a safer lead exposure level. To determine this, six different concentrations of lead were used, with the concentrations being less than, equal to, and greater than the lead exposure level set by the WHO. The different lead concentrations were then added to artemia eggs and the amount of hatched artemia was measured. The research results indicate that lead is still toxic at and below the lead concentration set by the WHO. Finally, it was found that the toxicity of lead significantly increases after the lead concentration of 0.02mg per 200mL. Follow-up data analyses confirm that the data were significant, with a p-value of less than 0.05. From our data, it can be concluded that the "safe" lead exposure level set by the WHO is not actually safe. Additionally, lead is still toxic at concentrations less than the level set by the WHO.

Keywords: Artemia, Lead, Neurotoxin

1 Introduction

More than 20% of all Americans are exposed to lead through pipes or paint in a given year [2] . Lead is a deadly neurotoxin that affects the human body. As a result, the World Health Organization set the minimum safe exposure level to lead to a concentration of 0.02mg per 200mL in 2017 [3]. However, many people suspect that although this exposure level is deemed "safe," it is dangerous with previous research suggesting that there is no safe exposure lead level [1] . It must be noted, however, that this study did not actually test different lead concentrations to see its effects. This study, instead, actually tested different lead levels aiming to determine whether the recommended WHO safe exposure level is actually "safe," and if it is not, to determine a lead exposure level that is safe.

The primary sources of lead are usually found in paints, pipes, and petrol which have the potential to leak into our environment. Today, lead is found in household dust, residential soil, drinking waters, and our atmosphere [3] . For the health of society, it is vital to confirm which levels of lead are nontoxic because Americans who are exposed to toxic lead concentration levels can develop a multitude of health concerns [3] . In young children, lead toxicity can lead to anxiety, hyperactivity, attention deficit hyperactivity disorder (ADHD), and severe damage to the central nervous system [4]. In adults, those who are exposed to lead could develop lead poisoning, high blood pressure, and kidney damage [4] . Additionally, research suggests that lead negatively impacts children's behavioral and intellectual development. A recent study discovered that lead can alter the intellectual and neurobehavioral capabilities of children [5] . In the study, they found that blood lead levels were negatively correlated with the development of children's behavior, motor performance, language development, and social behavior ($P<0.01$). They also discovered that children with lead poisoning had abnormal behaviors including social withdrawal, depression, aggravation, and destructive behaviors [5]. It is clear that lead can be extremely dangerous to young children. These detrimental effects could completely destroy the next generation. Therefore, it is of high importance that exposure to lead in people's daily lives is limited.

Some level of lead exposure prevention has already been instituted. In 1992, the Toxic Sub[1] stances Control Act set the recommended levels of lead in paint, household dust, and soil [6]. Furthermore, in 1991, the Lead and Copper rule enforced lead testing in drinking waters as an attempt to regulate lead levels throughout the nation [6]. However, lead concentrations still exceed recommended levels in many parts of the United States. In 2018, the state Department of Health and the New York City Department of Education found that 58% of schools in New York tested above the recommended safe levels by over 50% [7] . However, there are few studies that verified whether the safe lead exposure level set by the WHO is accurate and whether the fact that schools are over the recommended limits is meaningful. To that end, this research sought to verify whether the WHO's recommendations of lead exposure are safe and whether lead levels above that deemed safe by the WHO is actually dangerous.

To conduct this study, it was decided to work with artemia, a type of brine shrimp. A study done in the past has shown artemia larvae were very sensitive to their environment and the different

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compounds [8]. This means that very low concentration levels can be used, and the effects of the different concentrations can still be seen [9]. As a result, artemia egg and larvae development would be ideal for testing the toxicity of lead, as lead can be toxic in minuscule amounts. Furthermore, artemia eggs hatch quickly, and can be raised fairly easily. Hundreds or even thousands of artemia eggs could be hatched in a short time span, allowing this study to be completed in a reasonable time frame while still having a large sample size. Based on our literature review, we hypothesized that (1) an increase in lead concentration leads to a decrease in artemia egg hatch rates and (2) lead would not become toxic at concentrations lower than 0.02mg of lead per 200mL of water. Based on our literature review, we hypothesized that (1) an increase in lead concentration leads to a decrease in artemia egg hatch rates and (2) lead would not become toxic at concentrations lower than 0.02mg of lead per 200mL of water.

2 Method

Clean water free of impurities was required in order to begin the study. Essentially, the water should not have any lead or other heavy metals as the study aimed to solely measure lead concentrations, and other water impurities would confound the results. Therefore, tap water was run through a RODI filter, also called Reverse Osmosis Deionized water. This specific filter was used because it is able to remove sediments, heavy metals, and other impurities from water. To do this, a four-stage water filter, which consists of a micron sediment filter, carbon filter, DI resin filter, and a membrane filter was used. Then tap water was run through the filters, which removed all impurities within the water. Eventually, there were 1.5 liters of RODI water created.

After the RODI water was created, it was tested for the amount of total dissolved solids (TDS) using a TDS meter. Results from the TDS meter show that there were still dissolved solids in the water. These dissolved solids might include heavy metals, so to be safe, ROX 0.8 Activated Carbon was added. ROX 0.8 Activated Carbon is carbon pellets that contain small and large pores, allowing it to capture dissolved solids. Adding this carbon filters the water and removes any remaining lead in the water, ensuring the water will have no lead in it at all. Two grams of ROX 0.8 Activated Carbon were then added two the water and the water was mixed thoroughly with a magnetic stirrer for 20 minutes. Afterward, the TDS was re-tested, and the TDS meter reads 0, meaning all dissolved solids were removed from the water.

After creating this ultra-purified water, Instant-Ocean salt was added to the RODI water to create saltwater. According to previous research done on artemia, the recommended salinity, or salt concentration for artemia was 1.026. This salinity level is closest to what artemia in the wild lives in. Therefore, when adding Instant Ocean Sea Salt, the salinity (or salt concentration) level of the water was measured using a refractometer, and salt was continuously added until the water had a salinity of 1.026. A magnetic stirrer was then used to thoroughly mix the salt for 30 minutes. Finally, the RODI water was poured into 6 beakers with each beaker having 200mL of RODI saltwater in it.

Adding Lead Nitrate

Different amounts of lead nitrate were added to the 6 beakers to create 6 different concentrations of lead nitrate. The concentrations used were based on the recommended levels of lead set by the World Health Organization. The 6 concentrations were: 0mg/200mL, 0.005mg/200mL, 0.01mg/200mL, 0.02mg/200mL, 0.1mg/200mL and 0.5mg/200mL. The control had a lead-nitrate concentration level of 0mg/200mL. Two concentrations levels were less than the set level set by the WHO, which meant that the lead should have no effect on organisms. These levels were 0.005mg/200mL and 0.01mg/200mL. One concentration was the level set by the WHO, which was 0.02mg/200mL. The last two concentrations were levels above the recommended levels set by the WHO. These concentrations in theory should have major harmful effects on organisms. These concentrations were 0.1mg/200mL and 0.5mg/200mL.

Each beaker was mixed using a magnetic stirrer for 5 minutes. In a 4 by 6 well plate, the concentrations of lead nitrate and saltwater were added to each well. In columns 1-6, 2mL of the 0/200, 0.005/200, 0.01/200, 0.02/200, 0.1/200 and 0.5/200 concentrations of lead-nitrate saltwater were put in, respectively. Therefore, every 4 wells contained a different lead-nitrate saltwater concentration.

Adding Artemia and Using Moticam

After the different concentrations of lead nitrate were created and added into the 4 by 6 well plate, artemia eggs were added into each well. The artemia eggs were from San Francisco and ordered through Brineshrimpdirect.com. 6 containers and 6 toothpicks were used to add the artemia eggs into each well. This procedure made sure the eggs did not cross-contaminate each well with different concentrations of lead nitrate. A sample size of 20-50 artemia eggs was used per well. In total, it is estimated that approximately 600 to 800 artemia eggs were used. The artemia eggs were then exposed to light for two hours to stimulate artemia egg hatching. The 24 well plates were then stored at a constant temperature and light exposure level for three days.

After three days, a microscope and a Moticam were set up. A Moticam is essentially a tiny camera that was built to take pictures through the lens of a microscope. The Moticam was used to take pictures of each. Afterward, the total amount of eggs, the number of eggs that were not hatched, the number of eggs that hatched, and the amount of live artemia were counted. To determine if an egg was hatched, the shape and color of each egg were analyzed. If an egg was a dark brown color and very circular, it meant that the egg was not hatched. However, if an egg was clear to a very light brown color and was misshapen, then the egg was determined to be hatched. The number of eggs hatched was divided by the total amount of eggs to find the hatch rate of artemia eggs.

3 Results and Discussion

In Figure 1, the hatch rate of artemia at the various lead concentrations are shown. At 0mg of lead nitrate per 200ml of the RODI saltwater, the hatch rate was 100%. The hatch rate of artemia in .005 mg of lead nitrate per 200 mL of water was 97.76%. The hatch rate of artemia in 0.01 mg of lead nitrate per 200 mL of water was 96.15%. The hatch rate of artemia in 0.02 mg of lead per 200 mL of water was 92.31%. The hatch rate of artemia in 0.1 mg of lead per 200 mL of water was 61.63%. And the hatch rate of artemia in 0.5 mg of lead per 200 mL of water was 41.88%.

The data was input into SPSS and statistical tests were performed. A T-test was used to

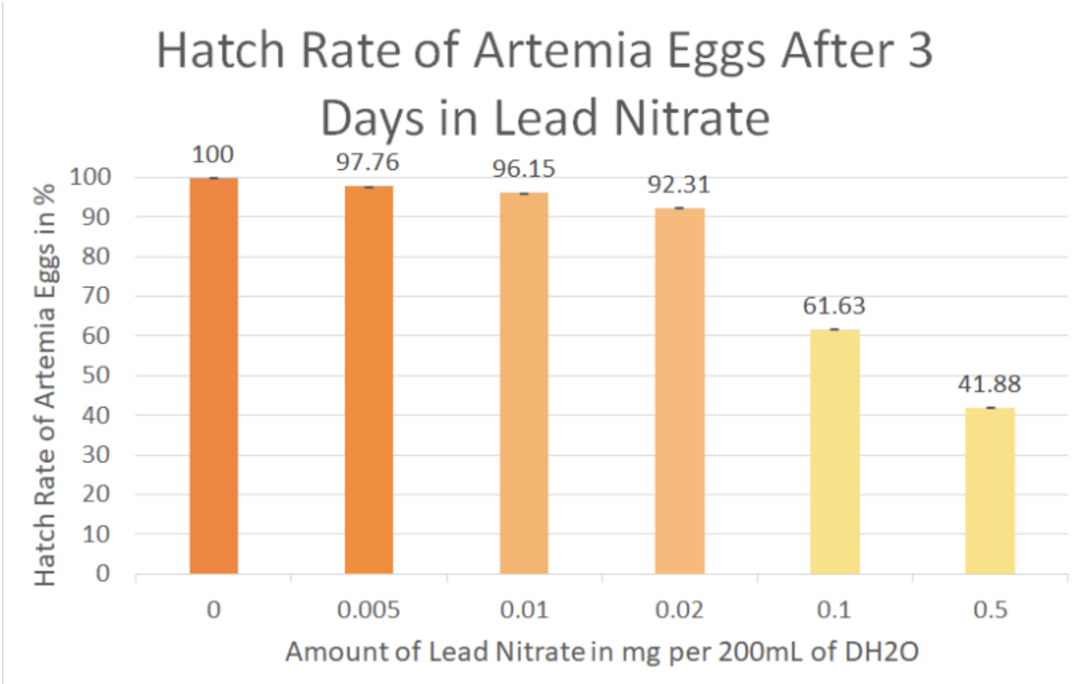

Figure 1: The percent of artemia eggs which haatchatchedhed in each of the 6 different conditions over the span of 3 days.

compare the experimental to the control group. All of the results were found to be significant with a p value less than 0.05. This means that the findings were significant and were not likely due to chance.

When there was no lead nitrate present in the water, the hatch rate of artemia was 100%. This meant that every single egg hatched. This was not unexpected, as they should all hatch when placed under regular or perfect conditions. While for the other concentrations, none had a hatch rate of 100% thus proving the primary hypothesis of the study correct as it was hypothesized that compared to artemia in lead nitrate, artemia in clean water would have a higher hatch rate.

Additionally, from the results, it is clear that at the lead-nitrate concentrations of 0.005, 0.01, and 0.02 mg of lead nitrate per 200 mL of water the hatch rate of the artemia gradually decreased. These results went against the second hypothesis as it was hypothesized that lead nitrate concentrations of less than 0.02mg of lead nitrate per 200mL of water would have no effect on artemia egg hatch rates. The results refuted the hypothesis as it was expected there would be a 100% artemia hatch rate, just as the "perfect conditions" had. However, this did not happen. This result clearly indicates that lead is still toxic at levels equal to or lower than the World Health Organization's safe exposure level, and lead was still toxic at concentrations as low as 0.005mg per 200mL.

From figure 1, one can see that lead concentrations lower than or equal to the recommended levels set by the WHO had decreasing artemia egg hatch rates by an average of only 2.14% per concentration. However, when the lead nitrate concentrations were higher than the recommended level set by the WHO, the artemia egg hatch rates decreased by an average of 22.22% per concentration. This is a significant jump from the 2.14% decrease in artemia egg hatch rates. Clearly, lead increases in its potency as a toxic substance once the concentration of lead surpasses the threshold of safe exposure.

4 Limitations and Further Study

This study encountered many limitations in the study. Firstly, humans are not the same as artemia. Humans could not be used for this study as it would be dangerous and unethical. However, artemia are different biological organisms that might react differently to lead levels. Therefore, the comparative ability of this study is questionable. Additionally, to determine whether an artemia egg was hatched, only qualitative analysis could be used. Only by examining the color and appearance of the artemia egg could it be determined whether the egg hatched which may lead to misjudgments and inaccuracies harming the reliability of the hatch rate calculations.

In a future study, it is recommended that one utilizes many different lead concentrations below 0.005 mg/mL. The hope is that by focusing on the interval of 0mg/mL and 0.005 mg/mL, a truly safe lead exposure level can be found. Another recommendation for further study would be to test other dangerous toxins commonly used in materials that are abundantly found in society. For example, cadmium is another dangerous metal that is useful in creating batteries but can harm the central nervous system resulting in decreased memory in humans.

5 Conclusions

Overall, lead is a dangerous neurotoxin that should be limited in use no matter the concentration it is used in. The results from this study evidently point to the idea that no level of lead is truly safe for living organisms. This is very dangerous because, as previously mentioned, the state Department of Health and the New York City Department of Education found that 58% of schools in New York tested above the recommended safe levels by over 50% in 2018 [7] . Based on the results of this study, any level of lead is dangerous and is very dangerous above the recommended World Health Organization exposure level, meaning schools are endangering children on a daily basis.

Ultimately, from this study, it must be concluded that active campaigns and calls for the reduction of lead usage are needed.

1. Vorvolakos, T., Arseniou, S. & Samakouri, M. There is no safe threshold for lead exposure: A literature review. *National Library of Medicine* **27**, 204–214 (2016). URL 10.22365/jpsych.2016.273.204;https://pubmed.ncbi.nlm.nih.gov/27837574/.

2. Philip, A., Sims, E., Houston, J. & Konieczny, R. (2017). URL https://www.usatoday.com/story/news/2017/08/14/63-million-americans-exposed-unsafe-drinking-water/564278001/.

3. Lead poisoning and health (2019). URL https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health.

4. Lead: Health problems caused by lead - NIOSH workplace safety and health topic. *Center for Disease Control and Prevention* (2018). URL http://www.cdc.gov/niosh/topics/lead/health.html.

5. Hou, S. *et al.* A clinical study of the effects of lead poisoning on the intelligence and neurobehavioral abilities of childre. *A clinical study of the effects of lead poisoning on the intelligence and neurobehavioral abilities of children* **10** (2013). URL https://dx.doi.org/10.1186/1742-4682-10-13;https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3598508/.

6. United States Environmental Protection Agency. Basic information about lead in drinking water (2019). URL https://www.epa.gov/ground-water-and-drinking-water/basic-information-about-lead-drinking-water.

7. Glenza, J. & Milman, O. A hidden scandal: America's school students exposed to water tainted by toxic lead (2019). URL https://www.theguardian.com/environment/ng-interactive/2019/mar/06/america-schools-water-lead-crisis.

8. Bryan, G. The effects of heavy metals (other than mercury) on marine and estuarine organisms. *Biological Society* **177**, 389–410 (1971). URL https://doi.org/10.1098/rspb.1971.0037;https://royalsocietypublishing.org/doi/10.1098/rspb.1971.0037.

9. Sorgeloos, P., Wielen, C., Persoone, G. & G. The use of Artemia nauplii for toxicity tests-A critical analysis. *National Library of Medicine* 249–255 (2005). URL https://doi.org/10.1016/s0147-6513(78)80003-7;https://www.sciencedirect.com/science/article/abs/pii/S0147651378800037.