The Effects of Methanol, Propylene Glycol and Phenoxyethanol on Zebrafish Embryonic Development

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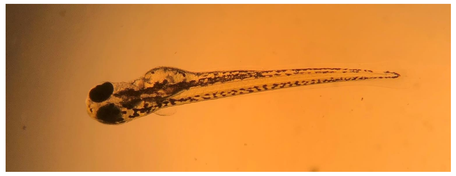
Abstract

Humans working with the WardSafe Concentrate Preservative are likely to unintentionally inhale or ingest some of the chemical compound. To study the possible outcomes, zebrafish embryos were subject to different concentrations of the preservative over four consecutive days. Data on survival rate and developmental changes were recorded. Physical defects such as spinal curvature and cardiac embolisms were detected. In addition, a greater number of embryo deaths were noted in the higher preservative concentrations. Because humans and zebrafish share similar characteristics, the data collected on zebrafish embryos may indicate probable human consequences.

Introduction

Ward's Science uses a water, methanol, and propylene glycol solution — a common high school lab preservative — to maintain their dissection specimens. While this preservative isn't considered a carcinogen, inhalation could "cause irritation of the respiratory tract, nausea, shortness of breath and headaches". Ingestion of the solution "may cause headache, dizziness, weakness, euphoria, drowsiness, shortness of breath, vomiting and incoordination" (1). The methanol component of this solution is known to have adverse effects on embryo development. An experiment on zebrafish found changes in genetic structural components when exposed to methanolic coal dust (2). Furthermore, research of methanol on rat embryos found higher concentrations of MeOH showed a "significant decrease in developmental score and crown-rump length" and 80% embryolethality (3). This experiment could be used to predict changes in pregnant humans working with the solution. Based on previously known information, it was predicted that increasing WardSafe Concentrate Preservative concentrations over a prolonged time will decrease zebrafish embryo survival and increase deformities.

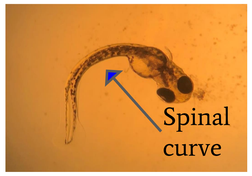
* The independent variable is the percentage of Wardsafe Concentrate Preservative liquid, while the dependant variable is the number of embryo deaths and deformities. The control in this experiment is the embryo media, or instant ocean solution.



This is a hatched zebrafish on day 3 in a control solution.

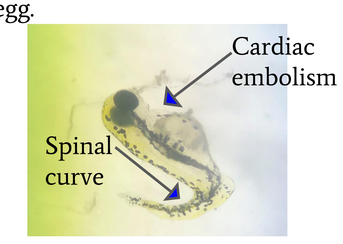
This zebrafish is in .05% solution on day 3. As seen, it has a slightly lighter color than the control.

This zebrafish is in .75% solution. It is noticeably less pigmented than the control and there is a cardiac embolism.



This is a hatched zebrafish on day 3 in 1.5% solution. It can clearly be seen that spinal deformities are present, and have created a u shaped arch, along with a cardiac embolism present.

This zebrafish in 5% solution on day 3 is developmentally delayed, with less pigmentation than the control and still in its egg.



This is a hatched zebrafish in .75% solution on day 4. There is a lack of pigmentation compared to the control, a large cardiac embolism and a spinal deformity in the shape of a v.

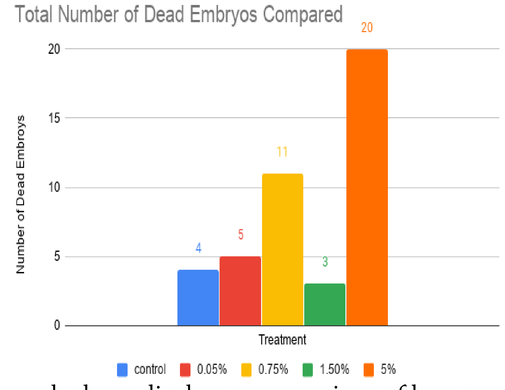
Methods

Five small jars were filled with a combination of embryo media (instant ocean solution) and warsdafe science preserving liquid at different concentrations. The solutions were placed in five labelled falcon dishes and 25-26 zebrafish embryos were placed in each. They were incubated overnight at 28 degrees celsius for four days. Each day, different concentrations were looked at under a microscope and data was collected based on the embryo development. Dead embryos were removed each day using disposable pipettes and the solution was replaced each day. The Chi Squared analysis test was done in order to determine whether or not there was a significant difference in the survival rate of the embryos in the different concentrations.

	P Value	Accept/Reject Null Hypothesis
Control vs. 0.05% Concentration	$0.25 < p < 0.50$	We fail to reject the null hypothesis.
Control vs. 0.75% Concentration	$p > 0.9$	We reject the null hypothesis.
Control vs. 1.50% Concentration	$0.1 < p < 0.25$	We fail to reject the null hypothesis.
Control vs. 5.00% Concentration	$p > 0.9$	We reject the null hypothesis.

Discussion

The results of this experiment resulted in partial support for the hypothesis. It was hypothesized originally that as the concentrations increased zebrafish die in greater numbers. It was found that in the 0.05% and 1.50% concentrations, the preservative did not have an impact on the hatching or death of the zebrafish embryos. Conversely, in the 0.75% and 5.00% concentrations preservative had a significant impact on the number of embryos that died. It should be noted that the majority of the deaths occurred within the first day of observation. However, throughout the experiment the zebrafish that survived all had a variety of cardial embolisms and an increased heart rate which could be seen inside the embolism. As concentrations increased, the hatchlings began to show spinal developmental issues resulting in a rainbow-like shape. Based on this experiment, prolonged exposure or ingestion of the chemicals in this lab preservative can result in harmful conditions. Pregnant biology teachers, and others that are work extensively in labs, are at risk when interacting in an environment in which they are exposed to to this chemical. Through this experiment, multiple developmental issues have been discovered, and could occur in the early stages of human embryo development due to the similarity between zebrafish and human embryos. However, there were also some limitations to this experiment—time, sample size, and the concentrations. The experiment was limited in time as it was allotted only four days to yield results, while a longer time frame had the potential to yield more deformities. Similarly, had there been more fish to work with, perhaps more deformities could have been seen and the results been more consistent. Concentrations chosen for the experiment were a limitation as well, despite the approximate toxicity limit being touched, the results were inconsistent with the hypothesis presented. With a greater variety in concentration levels, the result could have proven more consistent with the hypothesis, and the toxicity limit could have been more accurate.



The graph above displays a comparison of how many embryos died throughout the experiment. A total of 20 embryos introduced to 5% solution died, leaving only about 5 that hatched. The second largest amount of fatalities occurred in the .75%, where just under half died.

References

1. *Wards Safety Data Sheet* . 18 Apr. 2018, wardsci.com/assetsvc/asset/en_US/id/25466796/contents/sds_wardsafe-concentrate_ww0095.pdf.
2. Guerrero-Castilla, Angélica, et al. "Toxic Effects of a Methanolic Coal Dust Extract on Fish Early Life Stage." *Chemosphere*, U.S. National Library of Medicine, July 2019, www.ncbi.nlm.nih.gov/pubmed/30986591.
3. Andrews, J E, et al. "Developmental Toxicity of Methanol in Whole Embryo Culture: a Comparative Study with Mouse and Rat Embryos." *Toxicology*, U.S. National Library of Medicine, 27 Aug. 1993, www.ncbi.nlm.nih.gov/pubmed/8212026.