The Toxicological Effects of P-Phenylenediamine (PPD) on Aquatic Insects Using Bean Beetles (*Callosobruchus maculatus*) as a Proxy

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ABSTRACT: P-phenylenediamine (PPD) is an aromatic compound, that when H_2O_2 oxidized, is used in cosmetics such as hair dyes and black henna. Exposure to PPD can lead to a range of side effects in humans, from skin sensitization and muscle tenderness and is potentially carcinogenic. PPD can pass through wastewater treatments, and thus aquatic life can be exposed to it. As a result, it is important to understand the toxicological effects PPD can have on key linkages in the food web, such as aquatic insects. Bean beetle larvae (Callosobruchus maculatus) were used as a proxy for aquatic insects. They are a good model organism that are relatively easy to culture. A trypan assay was used to determine the percentage of viable cells in samples tested at different toxin concentrations. We observed a significant difference in percent mortality between different toxin and the control. Repeated trials were run at differing toxin levels to determine the LD50 of PPD. Because cosmetics tend to utilize H_2O_2 oxidized PPD, which is present because of the addition of hydrogen peroxide, trials were conducted to test the toxicity of H_2O_2 oxidized PPD. Percent mortality was significantly higher than that of heat oxidized PPD and increased with the concentration of the toxin. Further trials were then conducted to determine toxicity of PPD over time; it was shown that toxicity increased linearly as the sample incubated over time. This work has identified that PPD can have a significant endotoxic effect on larvae and the use may have negative ecological ramifications.

BACKGROUND:

- Raw materials found in household products could go on to pollute bodies of water, even after passing through wastewater treatment.
- P-phenylenediamine (PPD) is found in several cosmetic products and is used in dark hair dyes and in black henna and can wash off into wastewater during bathing.
- Several studies link PPD to be a possible genotoxin to mammals that can induce toxic effects to the liver, kidney, heart, and pancreas in mammals after repeated doses of dermal application.
- Several studies on PPD's toxic effects on mammals, however other types of animals, such as aquatic species, which are directly effected, have not been studied in depth.
- Bean beetles, Callosobruchus maculatus, are used as a proxy for aquatic insects and are excellent experimental subject due to their short lifespan (1-2 weeks), minimalistic necessities for culture maintenance, and ability to survive in relatively high-density populations.

OBJECTIVE AND HYPOTHESIS:

This study intended to determine the LD50 of PPD in various conditions. First, toxicity of heat oxidized and H_2O_2 oxidized PPD was measured a various concentration over a fixed incubation time. It was predicted that toxicity would increase logarithmically as concentration increased. While a specific LD50 was not previously established for insects, it was predicted that the LD50 of H_2O_2 oxidized PPD would be greater than 50ppt, which is the maximum concentration allowed in cosmetics. We also predicted toxicity of PPD at a fixed concentration would increase logarithmically over a period of time.

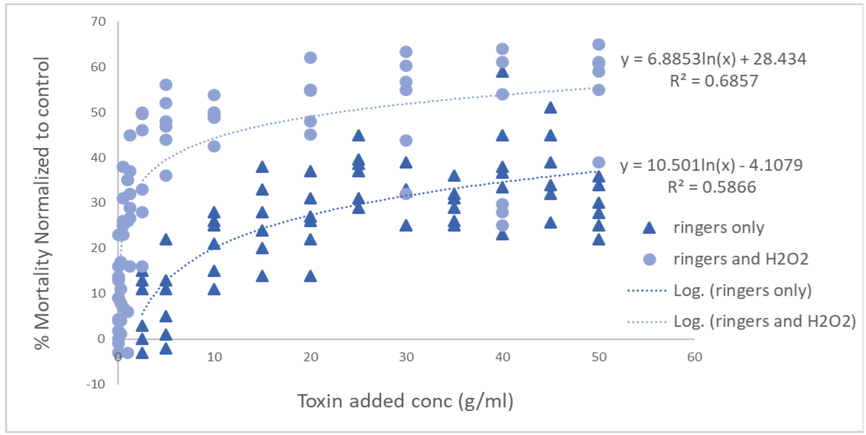
SOLUTION PREPARTION:

- Bean beetles were cultivated on mung beans for tissue sample.
- Toxicity of two solution types were tested- those with PPD dissolved in Ringer's buffer solution and those with PPD dissolved in 90% buffer solution and 10% concentrated hydrogen peroxide to enhance oxidation.
- Solutions were prepared by determining a set stock concentration, partially dissolving PPD in solution, and heating the solution to 70-75C for 2 minutes to fully dissolve the PPD.
- A trypan assay was used to stain samples and distinguish viable and nonviable cells (see determining percent mortality).
- Samples contained equal proportions of PPD solution, trypan assay, and Ringer's buffer as well as one larva as a source of cells.
- Samples were allowed to incubate for 15 minutes at room temperature before percent mortality was determined.

RESULTS:

- The toxicity of heat oxidized PPD was measured over several toxin concentrations ranging from 2.5 to 50 ppt and the toxicity of H_2O_2 oxidized PPD was measured over concentrations ranging from 0.5 to 50 ppt with controls containing no PPD were alongside every trial.
- Toxicity increased logarithmically as concentration increased at fixed incubation time.
- The toxicity of heat oxidized PPD and H_2O_2 oxidized PPD were measured at a fixed concentration of 5ppt over the course of approximately an hour. Toxicity at a fixed concertation increased linearly over time.
- Toxicity of H_2O_2 oxidized PPD was significantly greater than heat oxidized PPD. There was no significant difference between the percent mortality of controls with or without concentrated H_2O_2.

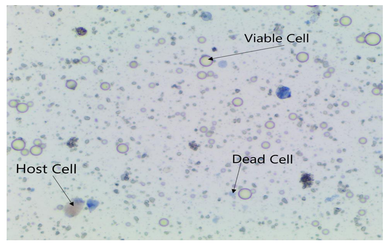
TOXICITY OF PPD AT VARIOUS CONCENTRATIONS:



The normalized percent mortality of samples with and without hydrogen peroxide at concentrations ranging from 0 to 50 ppt were collected. Percent mortality of the samples was determined through a count of at least 50 cells. The data was normalized by subtracting the average percent mortality of the controls (17%).

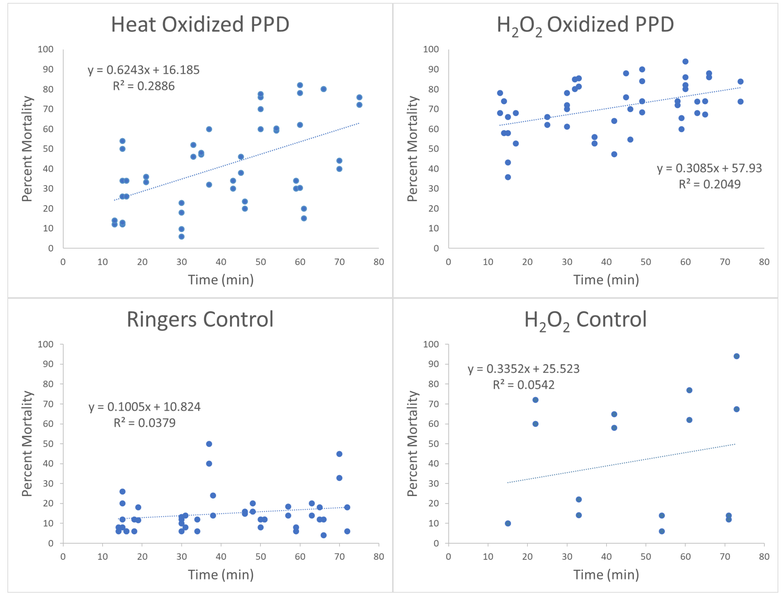
DETERMINING PERCENT MORTALITY:

In the trypan assay, viable cells exclude the dye and remain clear while non-viable cells absorb the dye and appear solid blue. These cells were counted, and percent mortality was determined by a count of at least 50 cells. Plant host cells were excluded from this count and are identified by the slight brown tint in the cell.



Bean beetle tissue sample at 100x, treated with 40 µL of 15% WV PPD solution and incubated for 15 minutes.

TOXICITY OF PPD AT 5PPT OVER TIME:



The toxicity of PPD at a fixed concentration of 5 ppt over time was measured with a trypan assay. Both H_2O_2 oxidized and heat oxidized species were studied and controls of buffer solution and H_2O_2 at 3% in Ringer's buffer solution were also compared as a standard. Percent mortality of the samples was determined by a count of at least 50 cells, and each slide prepared provided two samples at that time point.

CONCLUSIONS:

- Toxicity varied significantly between varying concentrations of PPD.
- The addition of hydrogen peroxide as an oxidizing agent for PPD did not significantly effect percent mortality in the controls.
- Oxidizing PPD with hydrogen peroxide increased percent mortality significantly compared to heat oxidized PPD.
- Using the logarithmic models, the LD50 for heat oxidized and H_2O_2 oxidized PPD was predicted to be 173 ppt and 23 ppt, respectively.

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