Cardiovascular and Respiratory Effect of 6PPD in *Daphnia magna*

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Background

Significance

A recent study identifying the emerging contaminant, 6PPD, has been closely related to an increased mortality rate in Coho salmon in minimal concentrations at acute exposures (Zhenyu et al. 2021). In order to better understand the proved lethal effects of this contaminant, *Daphnia magna* should be used in a series of experimental designs to determine cardiovascular and respiratory effects.

Objective

1. Expose *D. magna* to acute and chronic concentrations of 6PPD.
2. Measure heart rate after acute exposure
3. Measure hemoglobin concentration using acute and chronic sample.
4. Measure oxygen consumption

Hypotheses

1. An altered heart rate should be observed due to a stress-related external stimuli.
2. An increased need in oxygen should be in relation to compensation of the metabolic rate.

Specific Aims

1. Toxicity evaluations
2. Cardiovascular approaches
    1. Heart rate
    2. Hemoglobin
3. Oxygen consumption

Analysis of Hypotheses

Daphnia

- Model organism
- Toxicology reports
- Environmental acclimations

6PPD and Quinones

- 6PPD-quinone similar to other quinones as seen in Figure 1 (DTSC 2001)
    - Influence over biological systems comparison

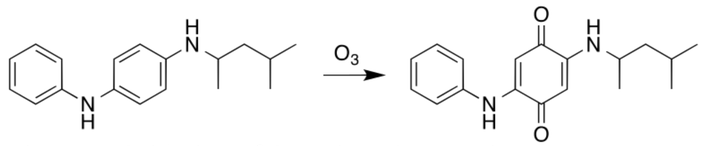

Figure 1: Oxidation of 6PPD into 6PPD-quinone

Heart Rate

- Energy expenditure compensation
    - Other biological systems

Hemoglobin Concentrations

- Oxidation (free radicals)
- Compensation
    - Oxygen consumption
    - Transportation of oxygen

Oxygen Consumption

- Contaminant related stress
- Metabolic rate

Limitations

- Invertebrate vs. vertebrate
    - 2021 study (Zhenyu et al. 2021)

Research Strategy

Aim 1

Toxicity Testing

1. Lab generate 6PPD
2. Calculate acute and chronic concentrations
    1. Acute (ng/L) → 24-hr. exposure
    2. Chronic (ng/L) → 21-day exposure

Aim 2

Heart Rate

1. Acute exposure (24 hrs.) of 6PPD on *D. magna*
2. Measure using microscopy and hand-held tally counter

Hemoglobin

1. Use *D. magna* from toxicity testing
2. Centrifuge for 5 minutes
3. Using spectrophotometry, measure absorbance at 410 nm.

Aim 3

Oxygen Consumption

1. Prepare respirometer based on a previous study seen in Figure 2 (Lampert 1986)
2. Measure pre-existing variables (algae oxygen production/consumption)
3. Use metabolic equation to determine respiratory rate

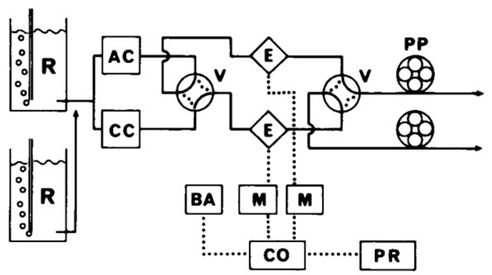

Figure 2: Model of the respirometer used in this assay; R representing the reservoir the water is taken from, AC representing the animal chamber, CC representing the control chamber, V representing four-way valves, E representing oxygen electrodes, PP representing peristaltic pumps, M representing digital/acid base analyzers, BA representing the digital barometer, CO representing the microcomputer, PR representing the printer, and solid lines representing the flow of water.

References

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