UNIVERSITY of WASHINGTON TACOMA

Effects of Perchlorate and Hypoxia on Molecular and Physiological Responses in *Daphnia magna*

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Background

Significance:

Perchlorate is a water-soluble endocrine-disrupting contaminant that is ubiquitous in our environment and known to be toxic to humans, however it is not currently regulated in our drinking water. To understand the effects of perchlorate and hypoxia (low oxygen) on the invertebrate endocrine system, *Daphnia magna* was used as a model organism.

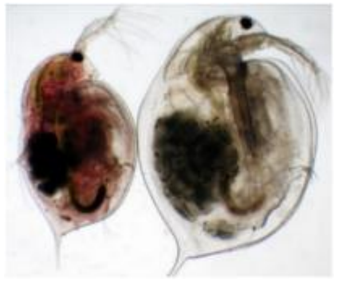


Image credit: Ebert, 2005

Figure 1: Under anoxic conditions *Daphnia Magna* upregulate hemoglobin genes (left). As a comparison, control *Daphnia Magna* under normoxic conditions (right) do not present the upregulation of hemoglobin genes.

Objective:

1. Evaluate transcriptional regulation of hemoglobin genes (*dhb1*, *dhb2*, *dhb3*) in *D.magna* following chronic exposure to combined perchlorate and hypoxia.

2. Evaluate modulation of reproductive strategy in *D. magna* following chronic exposure to combined perchlorate and hypoxia

Hypotheses:

1. Perchlorate and hypoxia co-exposure will cause reproductive modality to be altered due to disruption of juvenile hormone by perchlorate.
2. Hemoglobin genes will be transcriptionally upregulated following co-exposure.

Methods and Materials

Factorial Design:

Combined perchlorate and hypoxia co-exposure.

Time points:

- 2 hour
- 24 hour

Sample size:

- female juvenile
- 3 samples per treatment
- 6 treatment groups

Treatment groups:

Group	Perchlorate (mg/L)	Anoxic or normoxic
1	0	Normoxic
2	0	Anoxic
3	10	Normoxic
4	10	Anoxic
5	100	Normoxic
6	100	Anoxic

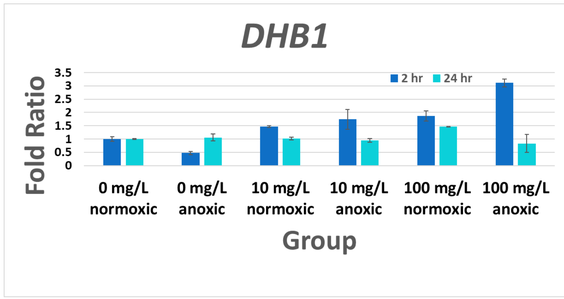
Table 1: Three levels of perchlorate (0 mg/L, 10 mg/L, 100 mg/L) , with or without anoxia were used for this experiment.

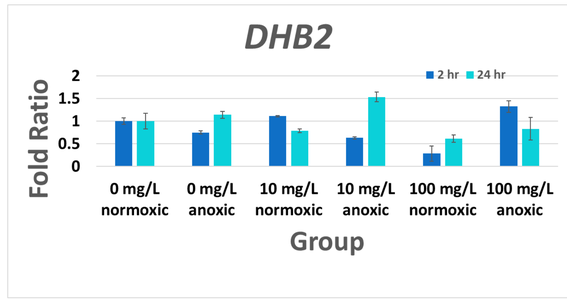
Procedure:

1. RNA extraction
2. cDNA synthesis
3. Polymerase Chain Reaction (PCR)
4. Gel electrophoresis
5. Densitometry analysis using ImageJ
6. Data analysis (fold ratio)

Results and Discussion

Results:





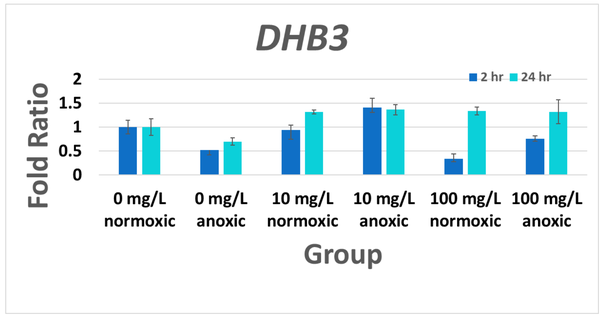


Figure 2: Fold ratios calculated for hemoglobin genes; *DHB1* (a), *DHB2* (b), and *DHB3* (c) by comparing band intensity PCR amplicons in the treated sample compared to the control sample.

Discussion:

- Future direction includes quantitative real-time PCR
- Analysis of reproductive modality following co-exposure
- Morphometric analysis of *D. magna* following co-exposure

References

Ebert D. Ecology, Epidemiology, and Evolution of Parasitism in Daphnia [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2005. Chapter 2, Introduction to Daphnia Biology. Available from: https://www.ncbi.nlm.nih.gov/books/NBK2042/

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