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The Effects of Sodium Perchlorate on *Dsx1* Gene Expression in *Daphnia magna*

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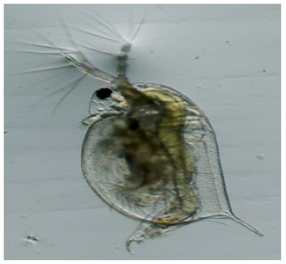
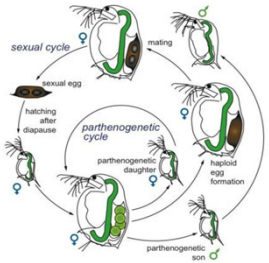


INTRODUCTION

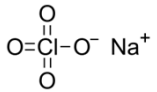
In this study, we use model organism *Daphnia magna* to explore how different concentrations of sodium perchlorate (0, 10, and 100ppm) affect the morphology and expression of a target gene known as the Doublesex gene (*dsx1*). This gene is involved in environmental sex determination and specifically regulates male trait expression in *D. magna*. **The driving interest for this research was understanding how sodium perchlorate exposure effects the growth of the *D. magna* and expression of the *dsx1* gene in *D. magna*.**

Lifecycle of *D. magna*:



Sodium perchlorate is an endocrine disrupting chemical (EDC) that has been commonly used as an oxidizer and found in tap water. This EDC competitively inhibits iodide uptake in the thyroid gland of vertebrates but has been minimally investigated on how it affects the endocrine system of invertebrates.

Sodium Perchlorate Chemical Structure:



The Environmental Protection Agency (EPA) is the entity that governs environmental protection regulations. Further research will help in providing viable data to assist the EPA in determining the maximum contaminant level goal so that they can proceed to enforce a standard of regulation in drinking water.

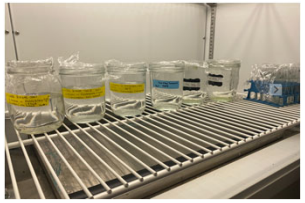
Fenoxycarb Exposure

To obtain male neonates, literature from Tatarazako et al. suggested a concentration of 1000ng/L of fenoxycarb, a chemical that promotes parthenogenesis to gamogenetic reproduction mechanisms in *Daphnia*. Introducing the fenoxycarb during the female *Daphnia's* oocyte development is considered the critical point for efficacy of the chemical (Kato et al., 2018). When introduced to the adult female *Daphnia* jars of a population of ~15, it resulted in 100% male after 4 days. Males were spot checked in each fenoxycarb treated jar using Evos XL core Invitrogen imaging microscope. After 2 days, only 2/5 of the offspring were males, so the whole offspring population were sorted from the jars and were retested 4 days after in which the spot test had 100% success. 9 day post treated fenoxycarb jars also revealed 100% male offspring.

METHODS AND MATERIALS

Housing/Acquisition of *D. magna*:

The *D. magna* used for this study were lab acclimated to a 12-hour light and 12-hour dark photoperiod at 20°C. The *Daphnia* collected were all spot tested to ensure certainty of female sex. The media used for housing the *Daphnia* was an artificial freshwater medium known as Aachener Daphnien Medium or ADaM (Kluttgen et al., 1994). All adult female *Daphnia* were maintained in a population of no greater than 20 animals per 500ml glass jar to avoid overcrowding. Neonate populations did not exceed more than ~100 animals. Adult *Daphnia* jars were fed 3 drops of nannochloropsis sp., and neonate jars were fed 2 drops of nannochloropsis sp. every Monday, Wednesday, and Friday.



Morphometric Assay:

Individual neonate *Daphnia* were collected and put into 10 ml glass tubes with ~7ml of ADaM in them. There were 10 replicates of both female and male neonates at 0 ppm, 10 ppm, and 100ppm. Time points for imaging included at times 0, 2 days, and 1 week after exposure. The morphometric parameters in figure 1 follows a similar protocol to Ranta et al. (1993). The only exception is (d), in which the targeted measurement is the first antennae length. Data was collected through ImageJ, a digital imaging analysis program. Images were processed and saved as TIFF files through the imaging microscope.

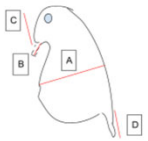


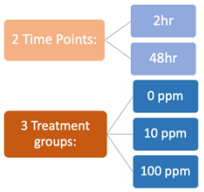
Figure 1: The morphometric analysis of *D. magna* samples are as follows: A) body width, B) first antennae, C) head length, D) caudal spine length

Gene Expression Assay – RNA Extraction + cDNA Synthesis:

Populations of ~70 mixed sex neonate *Daphnia* were exposed to sodium perchlorate at concentrations of 0 ppm, 10 ppm, and 100 ppm and samples were collected at 2 time points of 2 hours and 48 hours. The choice of ~70 *Daphnia* for this experiment was justified through previous experimental work, which suggested ~24 *Daphnia* for each of the 3 replicates of exposures undergoing the RNA purification and cDNA synthesis procedure. There was a total of 18 1.5 ml microcentrifuge tubes that had ~24 neonate *Daphnia* in them, isolated using a glass vacuum Erlenmeyer flask and coffee filter. Following the Trizol-reagent-protocol, homogenization of *Daphnia* tissue was achieved by first using liquid nitrogen to snap freeze the *Daphnia* in the microcentrifuge tube, then pulverizing with a micro pestle and then resuspending in Trizol.

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
Dsx1	CCATTCATCATTACCAAA TCCCTTC	AAGTTTGGTGTAGGGGA GGATGAG
β-actin	CCTGAGCGCA AATACTCCGT	CAGAGAGGCC AAGATGGAGC

Table 1: The forward and reverse primers for the *Dsx1* and *β actin* genes in *D. magna*. The *Dsx1* primers were synthesized according to Kato et al. (2011). The *β-actin* primers were synthesized according to Rider and Leblanc. (2006).



RESULTS

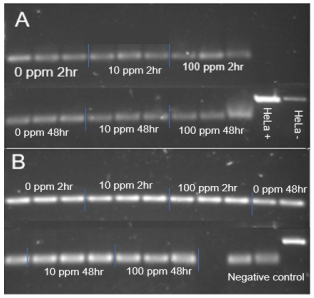


Figure 2: Agarose gel electrophoresis of the PCR of *dsx1*, *ß-actin*, and *HeLa*. **A)** Bands show PCR of *dsx1* on cDNA. All exposure levels show positive PCR results. **B)** The *ß-actin* PCR on cDNA show expression. All bands show positive PCR results.

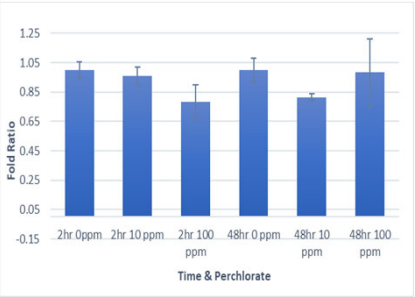


Figure 3: *Dsx1* standardized to expression of house-keeping gene *ß-actin*. The average fold ratios are represented by the bars for each category with standard error represented by the bars.

DISCUSSION

Decrease in body size due to endocrine disruption:

- 15-week perchlorate exposure on maternal *Danio rerio* (zebrafish) resulted in a significant decrease in offspring's lengths of Ceratohyal and Meckel cartilage complexes (Mukhi and Patino 2007).

Interaction of perchlorate and fenoxycarb:

- Studies support that fenoxycarb significantly declines population density (Lu et al. 2020).
- Further research of both chemicals used in tandem.

Lower expression of *dsx1* – effect on female reproduction under stressful conditions:

- Decreased expression of *dsx1* in female *Daphnia pulex* resulted in an increased number of offspring (Lin et al., 2019).
- Lower expression levels of *dsx1* in parthenogenic females (Lin et al. 2019, Wuerz et al. 2019).

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