

Introduction

Tetrahymena thermophila (*T. thermophila*):

- Unicellular
- Eukaryotic ciliate
- Model organism

Bis (2-ethylhexyl) phthalates (DEHP):

- Endocrine disrupting chemical
- Causes reproductive strain

DCL-1 gene:

- Transmits genetic information during conjugation

Hypothesis: DEHP will repress conjugation resulting in a decrease of *DCL-1* expression and a decrease in reproduction rates.

Methods

- **Primer synthesis:** Primers for *DCL-1* were generated using *Tetrahymena* Genome Database and Integrated DNA Technologies Oligoanalyzer.
- **Culturing:** *T. thermophila* cultures were maintained in NEFF media. The control and experimental groups were resuspended in starvation media and DMSO to induce conjugation. The experimental group was exposed to 0.1 μ M of DEHP.
- **Behavioral assay:** Conjugation pairs were counted with a hemocytometer every three hours for a total of 12 hours to assess reproductive behavior.
- **RNA extraction:** RNA was extracted using Qiagen's RNeasy Mini Kit.
- **Reverse transcription:** cDNA was synthesized using RevertAid.
- **qPCR:** Performed twice using PowerUp Sybr Master Mix. *BTU1* gene expression was used as a positive control.

Conjugation Results

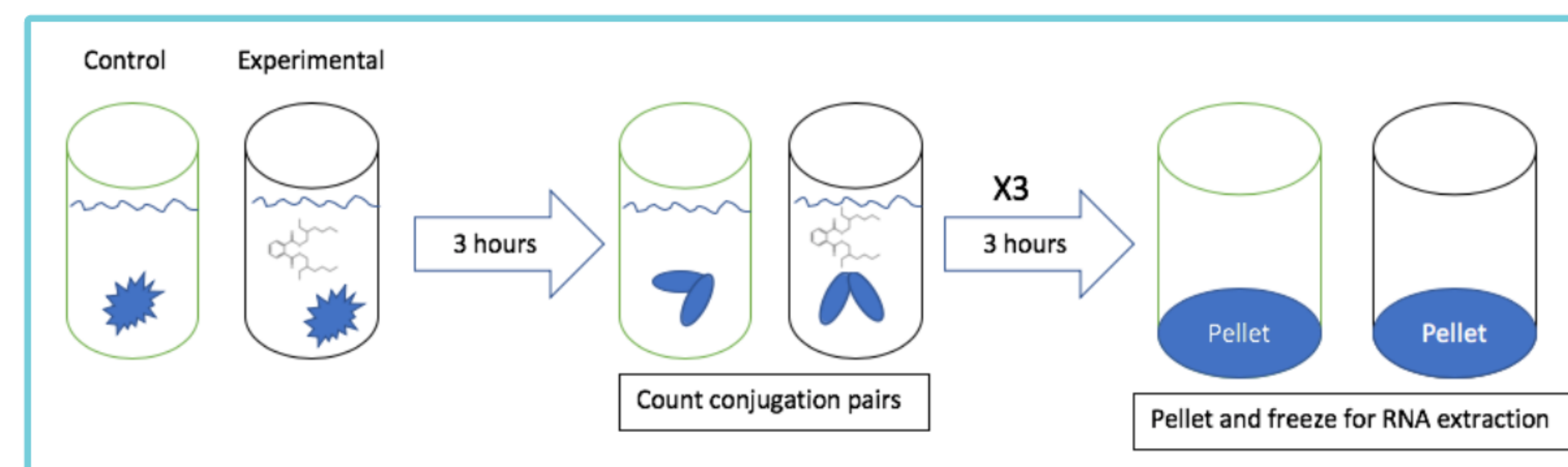


Figure 1. Behavioral assay flow chart.

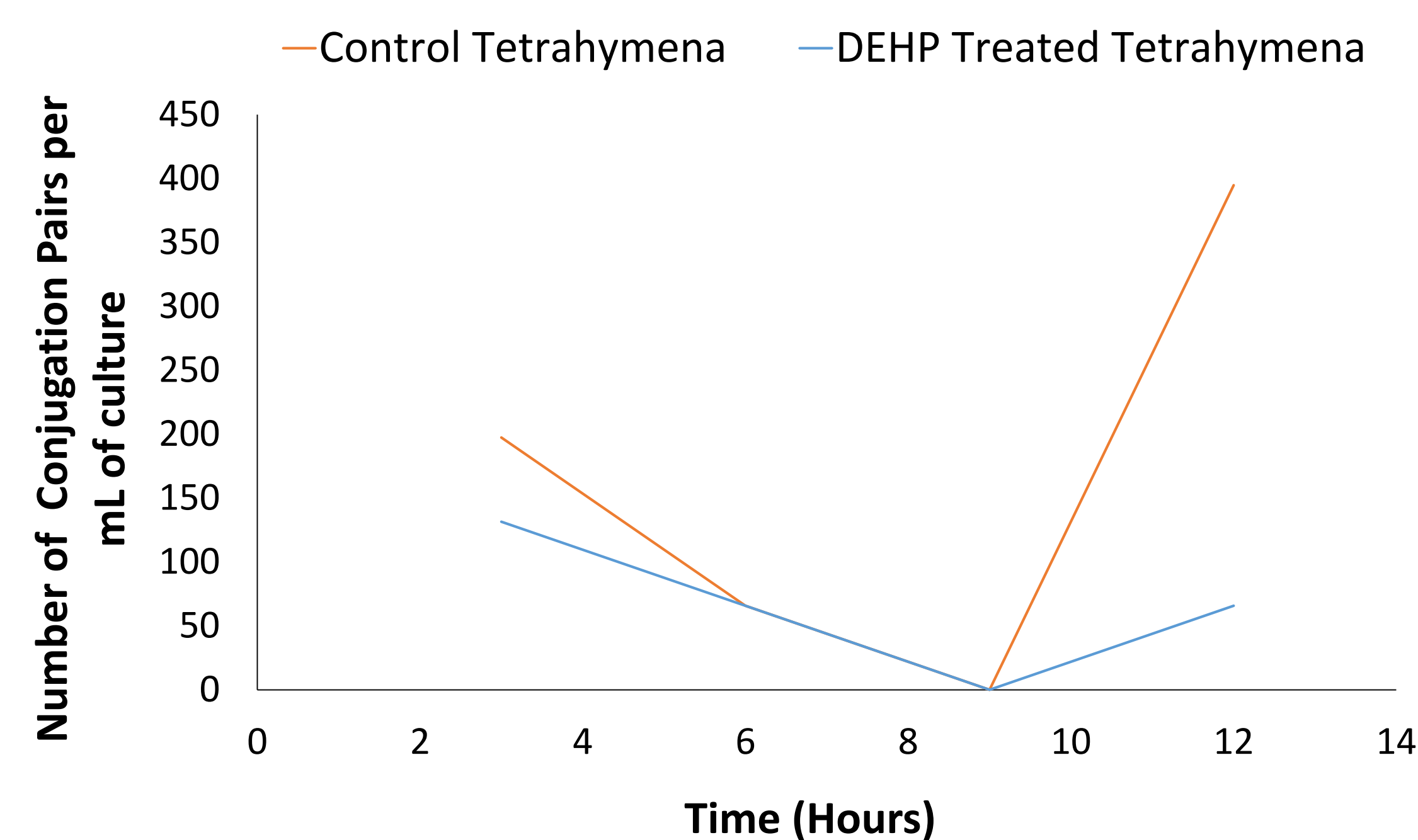


Figure 2. Average number of conjugation pairs over a 12 hour period of DEHP-exposed and control *T. thermophila* (n=2).

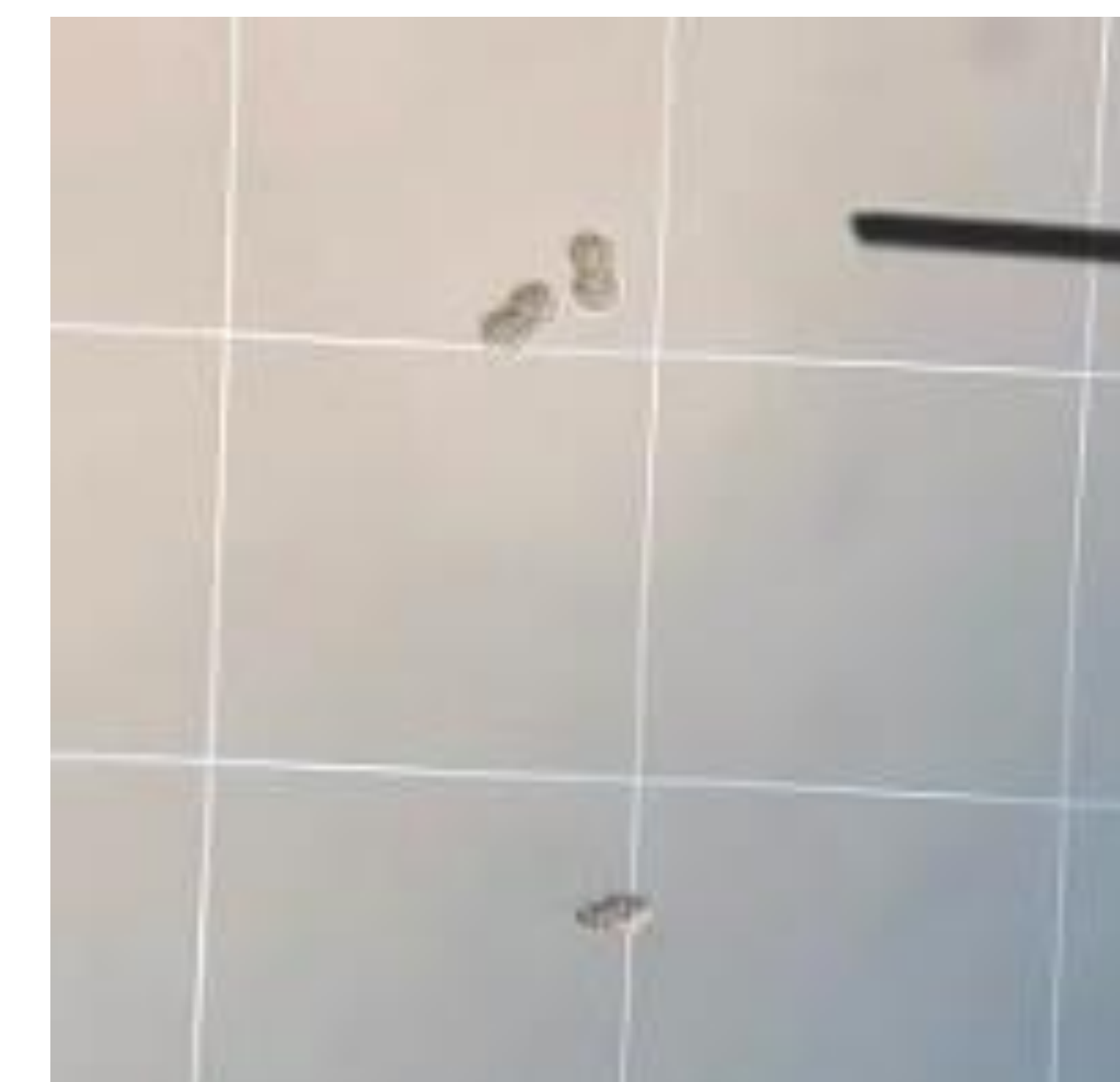


Figure 3. Image of hemocytometer under a microscope demonstrating conjugated *T. thermophila*.

DCL-1 Expression Results

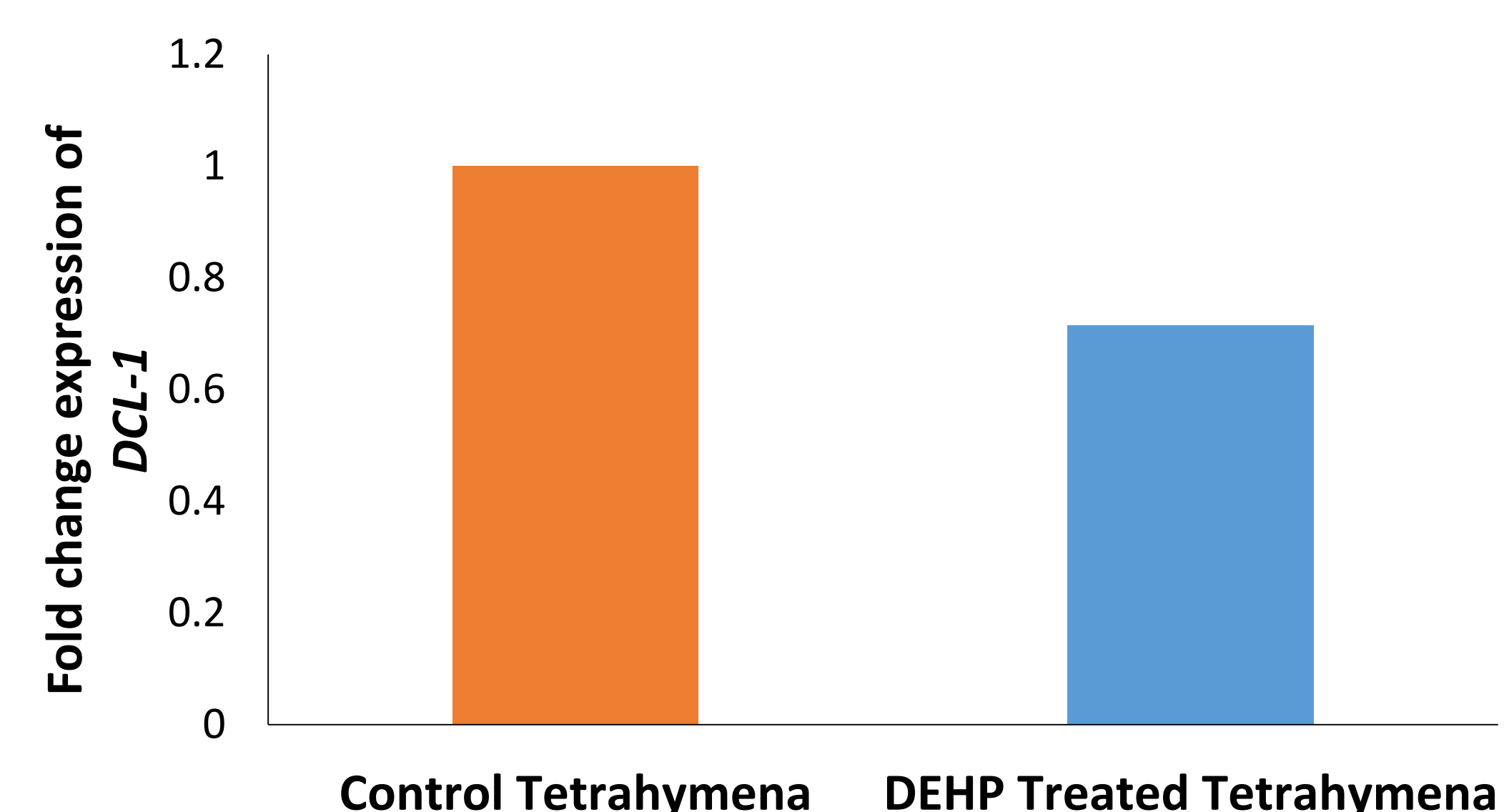


Figure 4. Fold change expression of *DCL-1* between control and DEHP-treated *T. thermophila* (n= 2, p= 0.79).

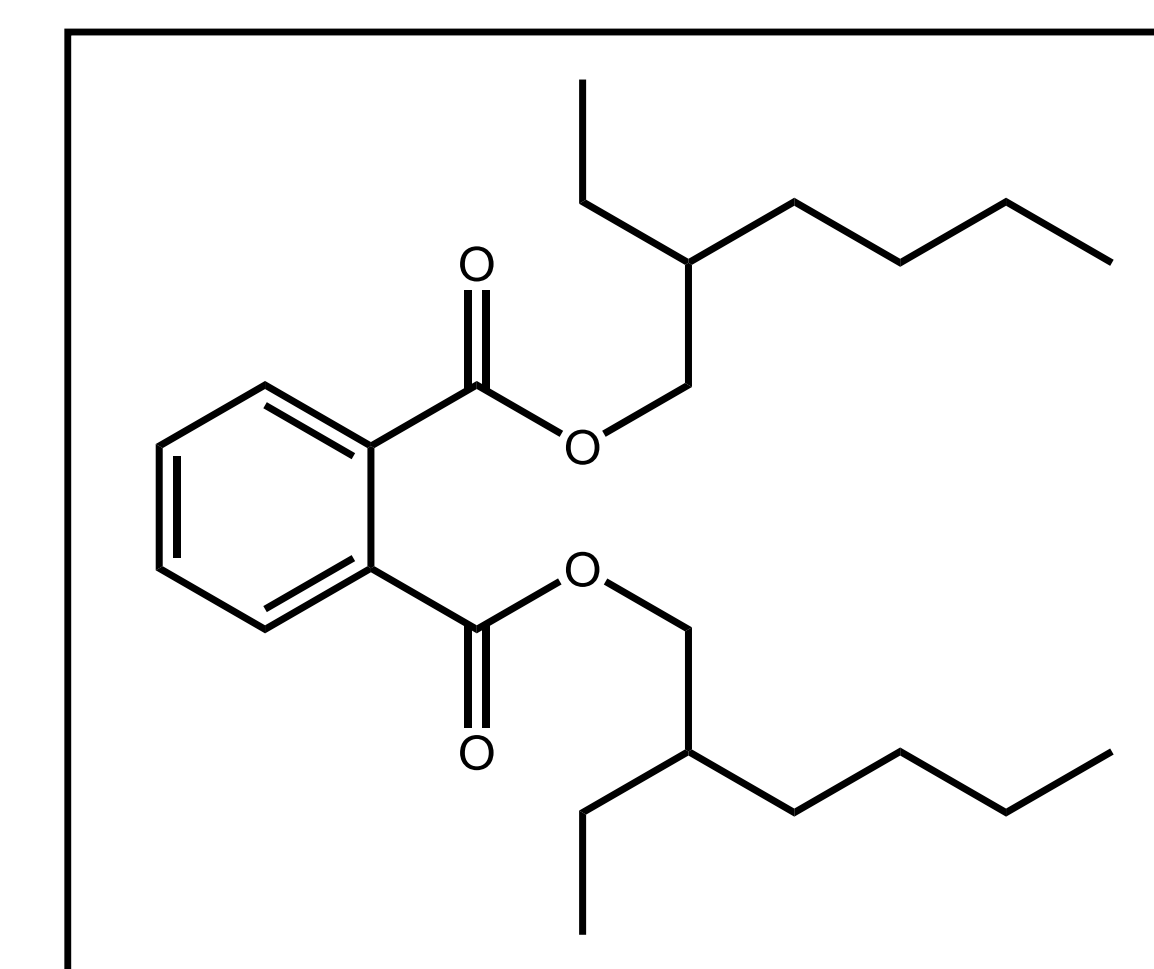


Figure 5. Chemical structure of DEHP.

Conclusion

- DEHP exposure did not significantly alter the number of conjugation pairs, shown in Figure 2.
- Both RT-qPCR rounds demonstrated that *DCL-1* expression did not change significantly after DEHP exposure, as seen in Figure 4.

Our results demonstrate that DEHP does not decrease conjugation or decrease *DCL-1* expression of *T. thermophila*.

Future Directions

- Start with a larger sample of *T. thermophila* cells.
- Alter starvation media recipe in order to promote more conjugation.
- Run RT-qPCR longer to allow *DCL-1* gene to be amplified.

Acknowledgments

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References

1. Niermann, S., Rattan, S., Brehm, E., Flaws, J.A., 2015. Prenatal exposure to di-(2-ethylhexyl) phthalate (DEHP) affects reproductive outcomes in female mice. *Reprod Toxicol* 53, 23-32. <https://doi.org/10.1016/j.reprotox.2015.02.013>
2. Seyoum, A., Pradhan, A., 2018. Effect of phthalates on development, reproduction, fat metabolism and lifespan in *Daphnia magna*. *Sci. Total Environ.* 654, 969-977. <https://doi.org/10.1016/j.scitotenv.2018.11.158>