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Effect of bis (2-ethylhexyl) phthalate on Tetrahymena thermophila DCL-1 Gene Expression and Conjugation

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Effect of bis (2-ethylhexyl) phthalate on *Tetrahymena thermophila DCL-1* Gene Expression and Conjugation

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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 µM 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

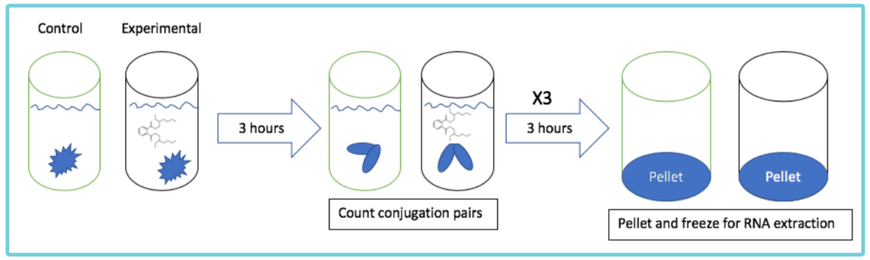

Figure 1. Behavioral assay flow chart.

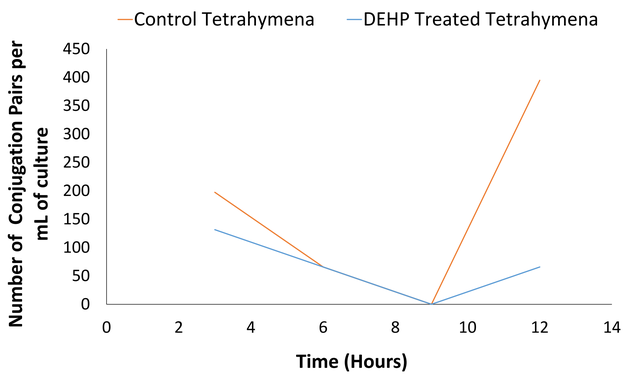

Figure 2. Average number of conjugation pairs over a 12 hour period of DEHP-exposed and control *T. thermophila* (n=2).

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

DCL-1 Expression Results

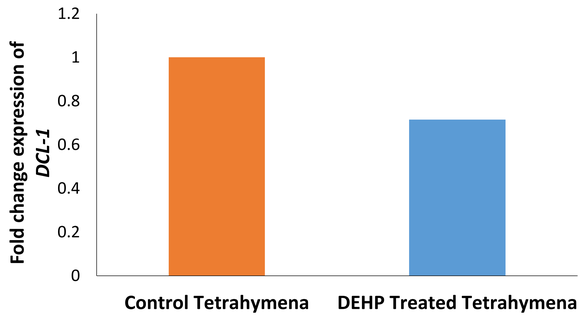

Figure 4. Fold change expression of *DCL-1* between control and DEHP-treated *T. thermophila* (n= 2, p= 0.79).

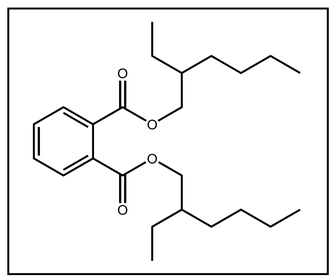

Figure 5. Chemical structure of DEHP.

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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