Bug off! Toxicity of DEET(*N,N-diethyl-m-toluamide*) on *Tetrahymena thermophilia* populations

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Abstract

This study investigates the effects of DEET(N,N-diethyl-m-toluamide), a common insect repellent, on *Tetrahymena*, a model organism used to simulate aquatic microorganisms. Using a micropipette, *Tetrahymena* stock culture was transferred to slides, and a dilution of DEET was added. Observations were recorded using a compound microscope, focusing on behavioral changes and time until death. Initial responses included rapid movement, clumping, and slowed motility. Over time, *Tetrahymena* exhibited cellular damage, shrinking, and eventual cessation of movement, indicating toxicity.

Results showed that DEET exposure led to significant mortality, with varying degrees of cellular breakdown and aggregation patterns. These findings suggest that DEET is at least slightly toxic on aquatic microorganisms, including *Tetrahymena*, which are at the bottom of the food chain. Since these microorganisms serve as a food source for larger organisms, their decline could disrupt the food chain, potentially leading to the downfall of an entire ecosystem.

Background

N,N-diethyl-m-toluamide (DEET) is a widely used insect repellent frequently detected in urban wastewater and runoff. Though it repels insects by interfering with olfactory receptors, its environmental impact —particularly in aquatic systems—is concerning. DEET resists photodegradation, making it persistent in water, and has been shown to cause neurological and biochemical disruption in various freshwater species

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Tetrahymena, a freshwater protozoan with cilia, serves as a valuable model organism in ecotoxicology. These microorganisms help regulate bacterial populations and contribute to nutrient cycling. Their sensitivity to environmental changes makes them ideal bioindicators for assessing chemical toxicity in aquatic ecosystems.

Materials

- Tetrahymena thermophila stock culture
- NEFF growth medium
- 100% DEET Insect repellent
- Distilled Water
- Compound Microscope
- 100uL micropipettes and tips
- Sterile 15mL test tubes
- Stopwatch
- Microscope slides

Methodology

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Culturing Tetrahymena:

1. Add 10 mL of NEFF medium to a sterile test tube using a clean pipette.
2. Inoculate the medium with 3–5 drops of Tetrahymena culture.
3. Incubate at room temperature for 1–2 weeks.

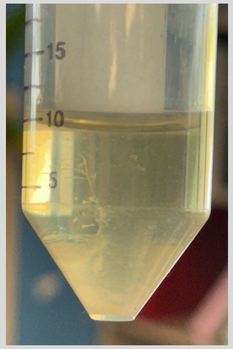
Preparing DEET Dillutions:

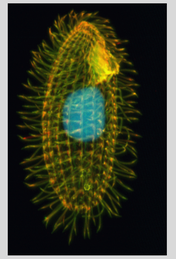
1. Label eleven sterile tubes as D0 through D100 in 10% increments.
2. Prepare 100% DEET (D0) by directly using the insect repellent solution.
3. Prepare dilutions using the following ratios (example for D90: 1 mL DEET + 9 mL distilled water).
4. Repeat to make D80 through D10 accordingly. D100 contains only distilled water (control).
5. Mix each dilution thoroughly before use.

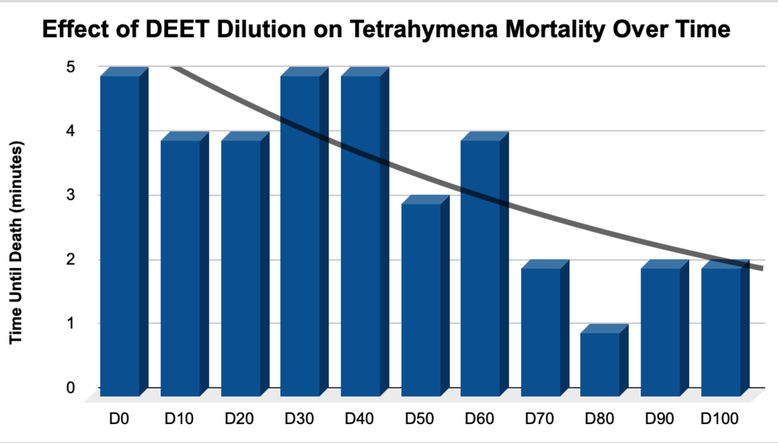
Slide Preparation and Observation:

1. Using a micropipette, transfer 50 µL of Tetrahymena culture onto a microscope slide (no cover slip).
2. Add 1 drop (50 µL) of the DEET dilution onto the same slide.
3. Immediately start a stopwatch and begin observing under a compound microscope.
4. Select 3 random fields of view and observe for one minute per field.
5. Count the number of live (moving) and dead (non-motile) Tetrahymena.
6. Record behavioral changes, mortality rate, and time of death.

DEET	Dilution (Deet : Water)	% Morality
0	0:10	0%
10	1:9	40%
20	2:8	60%
30	3:7	80%
40	4:6	70%
50	5:5	85%
60	4:6	90%
70	7:3	95%
80	8:2	98%
90	9:1	100%
100	10:0	100%







Results

Although initial observations suggested a possible relationship between increasing DEET concentrations and Tetrahymena mortality, the data collected in this experiment is not sufficient to support the original hypothesis. Without multiple trials, it's impossible to determine whether the trends we observed were consistent or simply coincidental.

One obstacle to completing the project as designed was the lack of consistent lab time, which was a result of several uncontrollable disruptions, but impacted our ability to monitor cultures consistently.

We made efforts to begin second and third trials by growing two new Tetrahymena cultures (one from the overcrowded strain, and one from a new colony), but unfortunately, neither culture was successful. Despite following the same procedure that worked the first time, neither culture showed signs of life even after two weeks, although it’s possible we needed to wait just a little bit longer. Without additional trials, we were unable to test for experimental reproducibility, the most fundamental step in confirming our findings.

Resources & Logbook

Conclusion

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