



HOW DO YOU PERFORM A STUDY THAT HAS NEVER BEEN DONE?

APPLICATION NOTE

Acute Oral Fasting for Increased Diet Consumption with *Bombus impatiens*

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INTRODUCTION

My lab mates and I wanted to conduct an acute oral test using dimethoate with *Bombus impatiens*; we were unable to locate published information on an LD50 using dimethoate. We started with an existing protocol that suggested using a dimethoate 40% formulation with *Bombus terrestris*. Since prior testing led us to find that *B. impatiens* and *B. terrestris* react differently to dimethoate and that we were unable to get the 40% formulation,

INCONSISTANT CONSUMPTION WITH REFERENCE PROTOCOL

Rangefinder for first bioassay for dimethoate

Five hours of fasting period before exposure

Concentrations (ug a.i./bee)	Bees (N)	Mortality after exposure (%)			
		24 hrs.	48 hrs.	72 hrs.	96 hrs.
0.25	9	55.5	55.5	66.6	66.6
0.1	9	0	0	0	0
0.025	9	0	0	0	0
0.010	9	0	0	0	3.7
0.0025	9	0	0	0	0
0.0010	9	0	0	0	0
0.00025	9	0	0	0	0
SC	9	0	0	0	0
NC	9	0	0	0	0

Rangefinder for second bioassay for dimethoate

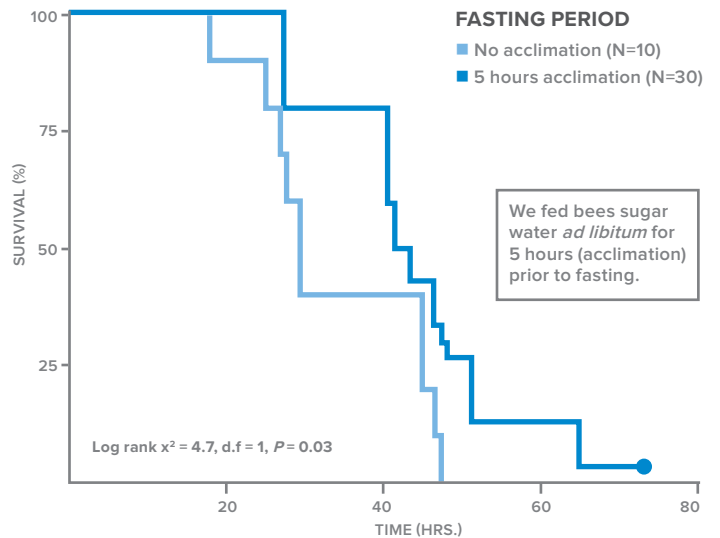
Five hours of fasting period before exposure

Concentrations (ug a.i./bee)	Bees (N)	Mortality after exposure (%)			
		24 hrs.	48 hrs.	72 hrs.	96 hrs.
0.40	9	55	55	55	55
0.35	9	44	44	44	44
0.30	9	22	33	33	33
0.25	9	0	0	0	11
0.20	9	0	0	0	0
0.15	9	0	0	0	0
0.10	9	0	0	0	0
SC	9	0	0	0	0
NC	9	0	0	0	0

■ Problems with varied consumption between individuals.

we decided to use the protocol as a loose guide and began the research with our criteria. The acute oral trials were conducted 4 times with slight tweaks to concentrations and methodology.

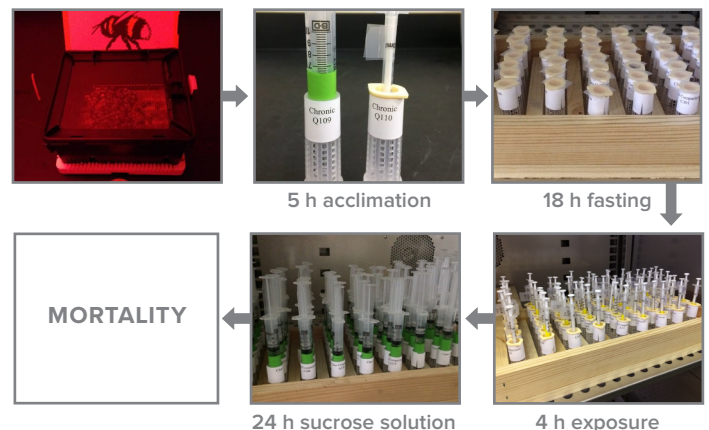
What is the longevity of bumblebees without food?



METHODS

Climate Conditions:

25°C ± 2°C | RH 60% ± 10% | Darkness



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RESULTS

MODIFIED PROTOCOL INCREASED CONSUMPTION

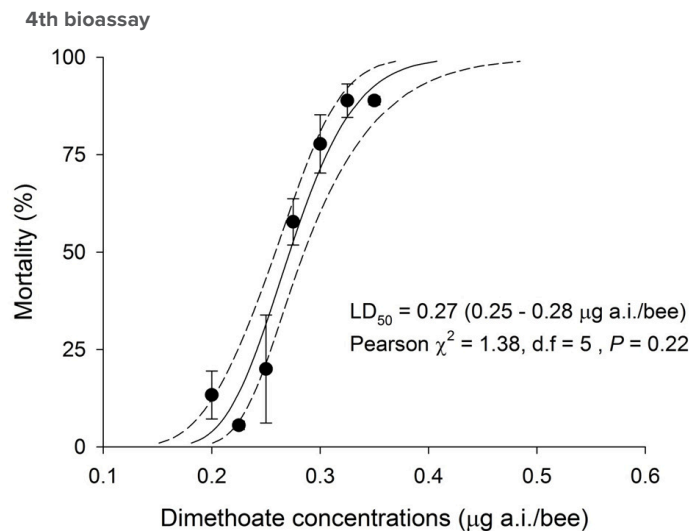
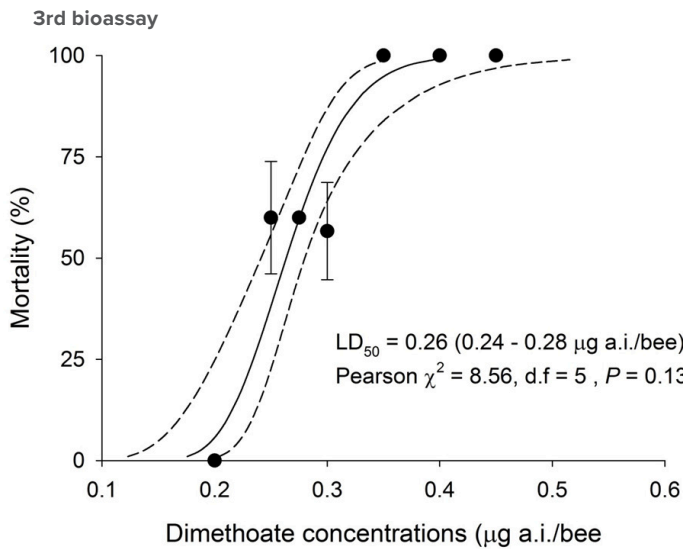
Rangefinder for third bioassay for dimethoate

18-hour fasting period before exposure.

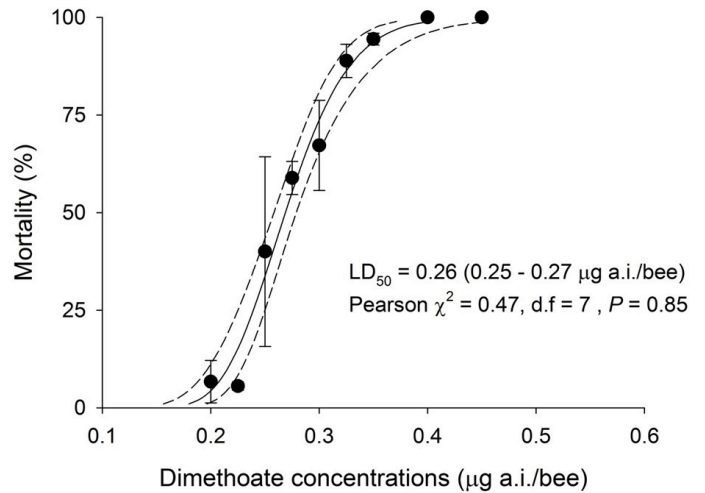
Previous data shows that 100% of bees survived for 24 hours without food, so we increased the fasting period to 18 hours before exposure.

Concentrations (ug a.i./bee)	Bees (N)	Mortality after exposure (%)		
		24 hrs.	48 hrs.	72 hrs.
0.45	14	100	100	100
0.40	15	100	100	100
0.35	16	100	100	100
0.30	16	56	68	68
0.275	15	60	66	66
0.25	11	63	63	63
0.20	11	0	0	0
SC	15	0	0	0
NC	15	0	0	0

■ Considering only bees that consumed higher than 80% of contaminated diet



3rd and 4th bioassay



CONCLUSIONS

Making the changes

At first we followed the protocol pretty closely to see how it would perform. After running the test we noticed that the consumption of the dosed diet was very inconsistent, so we repeated and fine-tuned our concentrations. After a second attempt with the same inconsistent consumption, our mortality was significantly different in the same levels amongst both studies; this was a problem. We initially tried a 5 hour fasting period as opposed to the suggested 2 – 4 hours in the protocol, in which we still were unsuccessful. The next step was to initiate a longevity test to see how long we were able to fast a bumble bee without too much stress or mortality. This test showed us that we could safely push an 18 hour fasting period, so we implemented this 18 hour period on our next trial. By doing this it enabled us to reach a consistently high level of consumption. Therefore, by adding a 5 hour acclimation period with ad libitum 50% sucrose solution and an 18 hour fasting period, we were able to greatly increase consumption and establish a good 24 hour LD50 with dimethoate using *Bombus impatiens*.

REFERENCES

1. Ringtest BB Acute Oral Toxicity, OECD Ringtest Protocol 2015