

Supplementary Material

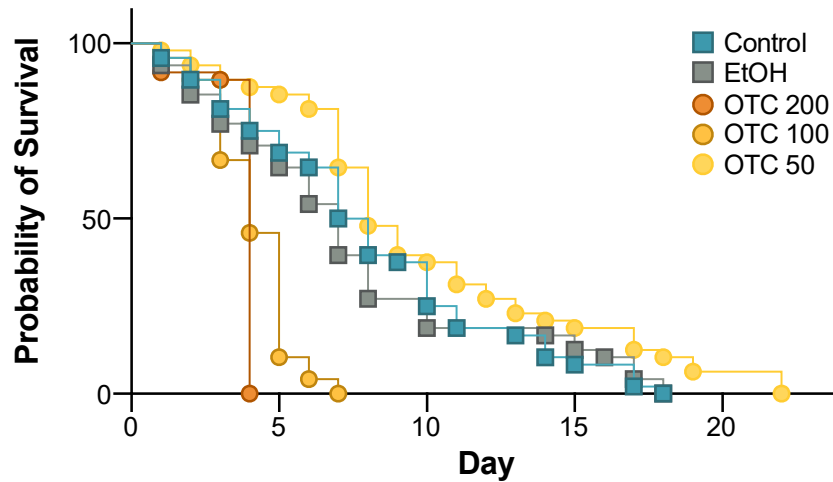


Figure S1. Kaplan-Meier survival curve showing the percent survival of the preliminary *in vitro* rearing toxicity trial to determine a sub-lethal dose of OTC, generated with R. Statistical analysis was scored with a log-rank test implemented in the “survival” package in R.

	Preliminary Diets			Secondary Diets		
	Control 1	EtOH 1	Oxytet 1	Control 2	EtOH 2	Oxytet 2
Royal Jelly	5 g	5 g	5 g	4 g	4 g	4 g
DI Water	5 g	5 g	5 g	4 g	4 g	4 g
Glucose	0	0	0	0.77 g	0.77 g	0.77 g
Fructose	0	0	0	0.77 g	0.77 g	0.77 g
Yeast	0	0	0	0.16 g	0.16 g	0.16 g
Pollen	0	0	0	0.3 g	0.3 g	0.3 g
70% EtOH	0	50μL	0	0	50μL	0
10mg/mL Stock Oxytet	0	0	50μL	0	0	50μL

Table S1. Diet recipes for primary and secondary diets fed to *in vitro* reared drones.

Day #	Amount of Diet fed (μL)	Primary (P) or Secondary (S) Diet
Day 0 (day of grafting)	26	P
Day 1	26	P
Day 2	26	P
Day 3	39	S
Day 4	52	S
Day 5	65	S
Day 6	78	S
Day 7	81	S
TOTAL	= 393μL / larvae	

Table S2. Volume of each diet fed to drone larvae during *in vitro* rearing.

0.05μg/μL OTC concentration * 393μL volume of consumed food = 19.65μg OTC consumed / larva

	Day	Control		EtoH		OTC	
		Alive	Pupated/Emerged	Alive	Pupated/Emerged	Alive	Pupated/Emerged
Larval Stage	0	144		146		144	
	1	144		145		143	
	2	141		141		136	
	3	133		132		127	
	4	132		127		119	
	5	123		118		107	
	6	88		100		88	
Pupation	7	53	7	69	7	53	6
	8	30	29	37	30	32	24
	9	29	29	31	31	28	28
Pupal Stage	10	25		30		22	
	11	25		29		21	
	12	25		29		21	
	13	22		27		18	
	14	22		26		15	
	15	22		26		15	
	16	22		26		15	
	17	22		26		15	
	18	22		26		15	
	19	22		26		15	
	20	22		26		15	
	21	18		22		13	
Emergence	22	17	5	21	7	12	3
	23	17	13	21	17	11	6
	24	14	14	18	18	11	11

Table S3. Number of drones alive each day after grafting until emergence and total number of drones that pupated and emerged.

TOTAL STARTING DRONES PER BATCH			
Batch #	Control	Treatment	Total
1	18	18	36
2	35	35	70
3	40	40	80
Total:	93	93	186
SUCCESSFUL DRONES DISSECTIONS			
Batch #	Control	Treatment	Total
1	10	6	16
2	19	18	37
3	18	10	28
Total:	47	34	81

TOTAL STARTING NEWs PER BATCH			
Batch #	Control	Treatment	Total
1	40	40	80
2	80	80	160
3	80	80	160
Total:	200	200	400
TOTAL END NEWs PER BATCH			
Batch #	Control	Treatment	Total
1	35	39	74
2	69	75	144
3	57	72	129
Total:	161	186	347

Table S4. Total counts of starting and ending drones and NEWs for in-lab OTC 5-day trial with mature drones, accompanied by workers. 81 mature drones split into seminal vesicles, mucous glands and gut samples = 243 samples to determine OTC effect on the local microbiomes.

Sample Processing and Analysis

Quantitative PCR to estimate bacterial abundance

Primers + gBlock	Sequence (5'-3')
EUB338F	ACTCCTACGGGAGGCAGCAG
EUB518R	ACTCCTACGGGAGGCAGCAG
16S gBlocks fragment	GTAACGCTTGACCCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAG CCGGTGCTTATTCGTTAGATACCGTCATAATCTTCTCTAACAAAAGGAGTT TACAATCCTAAACCTTCATCCTCCACGCGCGTTGCTGCTTCAGGCTTT CGCCCATTTAGCAATATTCCTACTGCTGCCTCCCGTAGGAGTCTGGAC CGTGTCTCAGTT

Table S5. Primers and gBlocks fragment used for qPCR.