



Original thinking... applied

Recent developments in bee ecotoxicology

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Talk Outline

- Brief Introduction to Fera
- Guidance on bee ecotoxicology
- Overview of some of the newer bee testing methods to give a feel for how they are conducted
- Questions



Fera Facilities

- Over 30 hectare secure site, with field plots, glasshouses, specialist wildlife unit and experimental apiaries
- 90 Controlled environment facilities
- Cat 3 containment labs
- Cutting edge chemical and molecular analytical tools (Thomson Suite and Molecular Technology Unit)



Recent developments in bee ecotoxicology

- European Food Safety Agency (EFSA) Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)
- The GD outlines a Tiered Risk Assessment Scheme, and covers simple first tier laboratory tests to more complex higher tier semi-field and field studies
- Published 2013 but not yet officially noted
- Commission Regulations (EU) Nos **283/2013** and **284/2013** set out the actual data requirements for both active substances and PPPs respectively.

Testing Scheme Methods

Lifecycle Stage Species	First Tier					Higher Tier	
	Adult			Larval		Brood	Colony
	Acute		Chronic	Acute	Chronic	Semi-field, Field	Field
	Contact	Oral	Oral	Single dose	Repeated Dose		
<i>Apis mellifera</i>	OECD 214	OECD 213	OECD 245*	OECD 237	OECD GD 239	Oomens ,De Reijter OECD GD 75	EPPO 170/ EFSA
<i>Bombus spp</i> ¹	OECD 246*	OECD 247*	Ring Tested 2017-18	N	N	Ring testing/ Developing 2018	EPPO170/ EFSA
Solitary spp ²	Draft OECD GL Being developed	Ring testing/ Developing 2018	N	N	N	Ring testing/ Developing 2018	EPPO170/ EFSA



10 Day chronic adult honey bee test
OECD TG 245

AZHU1001

Test design

- Accepted 9th October 2017. Following Ring Testing and development within the ICPPR WG
- Newly emerged bees used
- Minimum of 5 test item dose rates, 3 replicates per dose, 10 bees per replicate, single toxic reference item (dimethoate) dose rate, ten day testing period
- Feed changed daily – weighed in and out
- Uptake data corrected for evaporation
- Data used to estimate LC_x / LDD_x values and/or NOEC/NOEDD values
- Analytical requirement for dosing solutions and stock



Thoughts on the test

- Pretty straightforward test
- Very low mortality in controls
- Need good source of healthy, well maintained bees is essential.
- Low solubility of test item can be a problem particularly technical.





Bumble bee acute contact and oral toxicity tests
OECD TGs 246 and 247



Test design

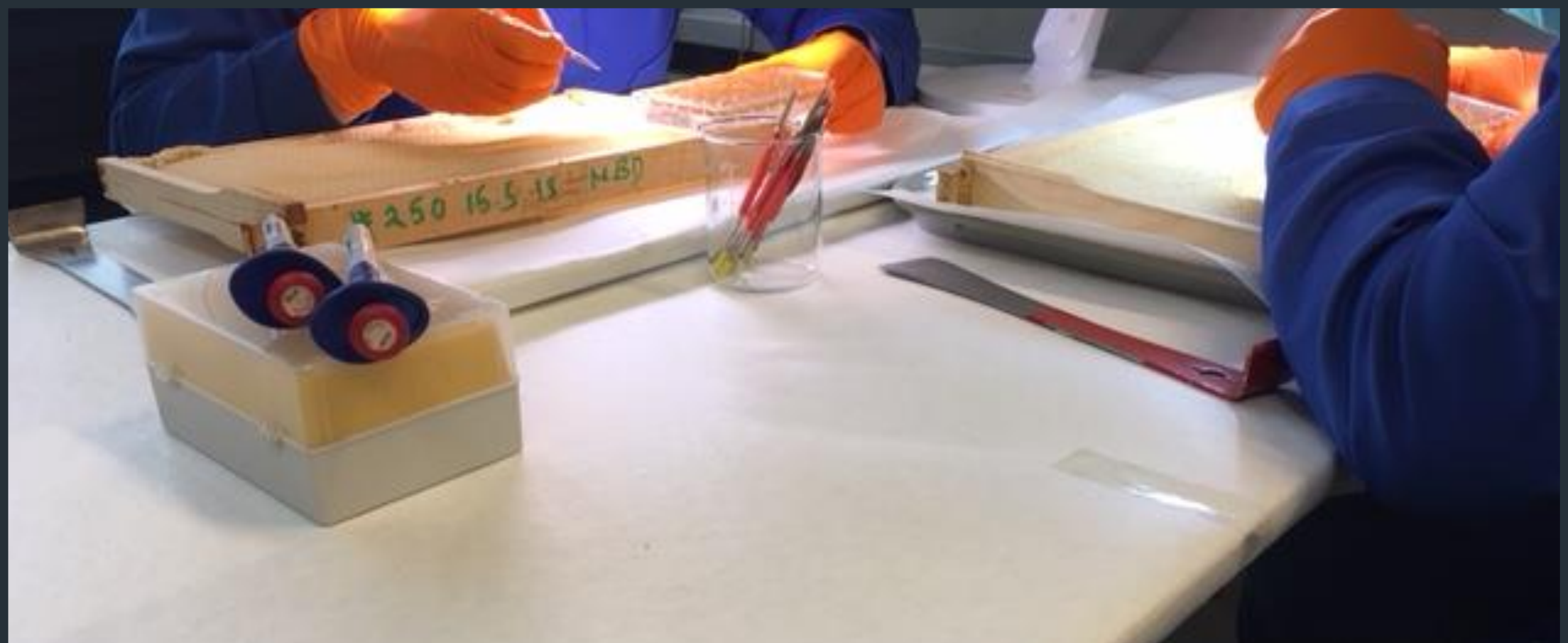
- Based on OECD 213 & 214 Acute honey bee tests
- Ring tested by the ICPPR non-*Apis* WG & Accepted 9th October 2017
- Bees supplied in nests as per pollination units for glasshouses
- Minimum of 5 test item dose rates, 30 individually housed bees per dose rate. Single toxic reference dose rate
- Test period 48-96 hrs
- Data used to estimate LD₅₀ /NOED values
- Analytical requirement for dosing solutions and stock





Thoughts on the test

- Straight-forward and achievable - good control mortality rates.
- Labour intensive due to individual housing
- Sometimes issues with non-feeders (a feeder must eat 80% of the mean dose taken at their dose rate)
- Feeding declines towards the end of the year so although can be supplied all year would not carry out testing past Autumn



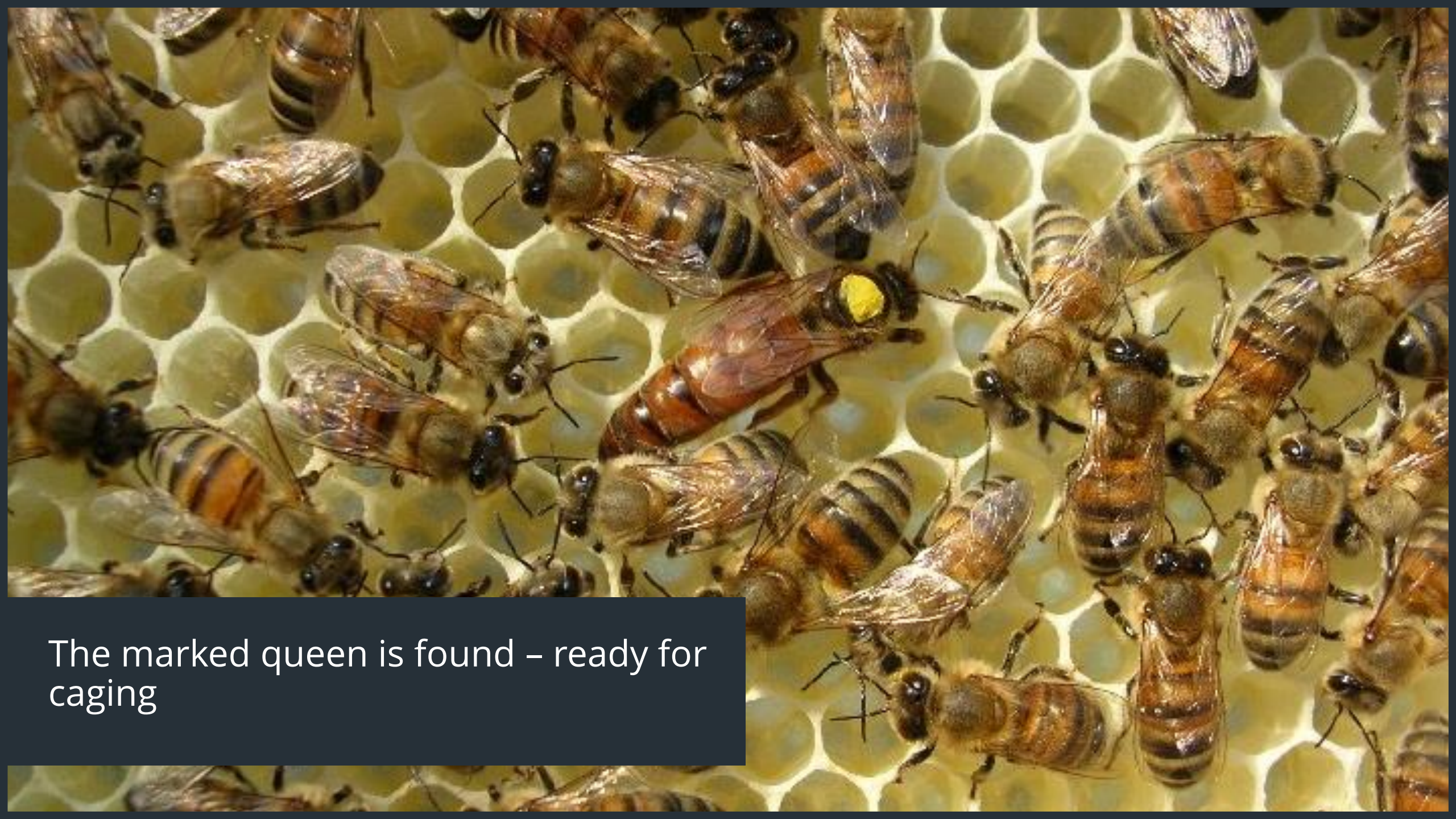
In vitro larval stage honey bee studies

Repeated exposure larval toxicity test - OECD GD 239



Test design

- OECD 239 – Repeated dose guidance document
- Accepted 2016
- Minimum of 5 test item dose rates, 48 larvae per dose rate (16 from each of 3 hives). Single toxic reference dose rate
- Larvae fed dosed artificial diet on D3, D4, D5 & D6
- End points D8 and D22
- Data used to estimate LC_x / LD_x values and/or NOEC/NOED values
- Analytical requirement for stock solutions, dilutions used to dose the larval food & possible also to analyse the dosed feed itself



The marked queen is found – ready for caging



Queen Cage – Housing queen for laying eggs



The frames (3 hives for main test) are brought back to lab larval age should be 12-24 Hrs)



Grafting larvae



Larvae at day 3



Larvae at day 5-6



Larvae at day 8-9



Day 15

Day 22





Thoughts on the test

- 8 day end point fairly straightforward – running for a number of years
- 22-day end point – **Very challenging test system** if inexperienced or not prepared
- There are many points at which the larvae are vulnerable e.g. temperature, desiccation, infection (fungal and bacterial), drowning.
- 2 years of running 22 day studies commercially at Fera – All have been successful and the highest emergence rates seen >90% far exceeding the validity criteria of 70%.

In vitro larval stage honey bee studies

- Example results from 2017 Study:

Treatment (mg/kg larval diet)	Larval Mortality Rate % (D8)	Pupal Mortality rate % (D15)	Adult Emergence Rate % (D22)
62.5	2.1	6.4	91.7
125	2.1	2.1	95.8
250	2.1	4.3	93.8
500	4.2	15.2	81.3
1000	33.3	62.5	25.0
Control (Water) 0	2.1	6.4	91.7
Toxic reference (dimethoate) 10	91.7	100.00	0.00



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Thank you for your time