

OP-121 [Bee Health]

Toxicity tests of mosquito larvicides and adulticides on honey bee larvae (*Apis mellifera*) reared in vitro

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Effective mosquito control is essential to Florida residents, as the state's environment and climate are ideally suited for mosquitos. Unfortunately, some mosquito adulticides and larvicides administered by pesticide applicators could have non-target impacts, including on honey bees (*Apis mellifera*). Research on the effects of mosquito control insecticides on honey bees has primarily focused on the whole colonies in field settings or on adult bees, often neglecting the potential impacts on their developing brood. This study aims to address this critical knowledge gap by evaluating the differential toxicity of mosquito adulticides and larvicides on honey bee larvae reared in vitro and determine the risk posed by these public health chemicals. For that purpose, we exposed honey bee larvae to one of four mosquito adulticide active ingredients (chlorpyrifos, naled, prallethrin, sumithrin), their commercial formulations (Mosquito Mist®, Dibrom®, Duet®) or one larvicide (*Bacillus thuringiensis israelensis* - Bti). Honey bee larvae reared in vitro were exposed to pesticides in their diet, on day three after grafting (acute exposure) or from day two to five after grafting (chronic exposure). Our results showed a high toxicity of Dibrom® (Acute LD50 = 0.803 µg/bee, Chronic LD50 = 0.0027 µg/bee) and Duet® (Acute LD50 = 0.497 µg/bee, Chronic LD50 = 0.034 µg/bee) to honey bee larvae after both exposure ways (LD50 < 2 µg/bee). Mosquito Mist II was found moderately toxic and highly toxic respectively after acute (LD50 = 2.760 µg/bee) and chronic exposure (LD50 = 0.0059 µg/bee). The larvicide Vectobac 12AS was weakly toxic to both types of exposure (LD50 > 11 µg/bee). Based on these endpoints and residues data, we observed a high risk of mosquito adulticides to honey bee larvae after chronic exposure but only a risk of Dibrom® after both exposures. The larvicide may be at risk to honey bee larvae only after acute exposure, but it is still low (RQ = 0.453 for a 0.4 threshold). The resulting data will be used to improve risk assessment for mosquito control products. help mosquito control personnel to make more informed decisions to minimize the non-target impacts of pesticide applications.

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Apimondia 49th – September 24th



Mosquitos and Honey Bees

- Mosquito control is essential to Florida:
 - Economy
 - Citizen health
 - Animal health
- Insecticides necessary
- Honey bees near treated areas
- Florida beekeeper complaints



Objectives & Hypotheses

- Evaluate the toxicities of mosquito control product through toxicological assays
 - Determine the LD₅₀ after an acute exposure
 - Determine the NOAED after a chronic exposure
 - Calculate the RQ of each compound

Objectives & Hypotheses

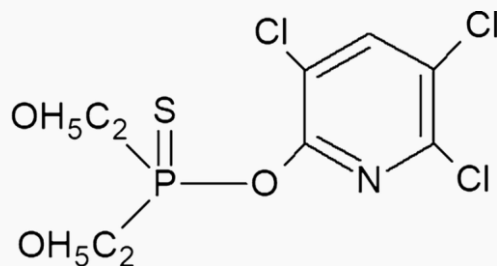
- Evaluate the toxicities of mosquito control product through toxicological assays
 - Determine the LD₅₀ after an acute exposure
 - Determine the NOAED after a chronic exposure
 - Calculate the RQ of each compound
- Hypotheses:
 - Formulated products would be more toxic to honey bee larvae than would active ingredients.
 - The test larvicide would be more toxic to honey bee larvae than would the adulticides.
 - Adulticides would not pose a risk to developing larvae.

Compounds of Interest

Active ingredients

Chlorpyrifos

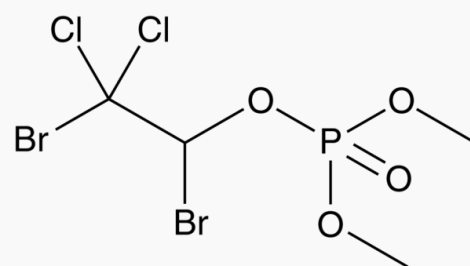
Organophosphate



AChE inhibitor

Naled

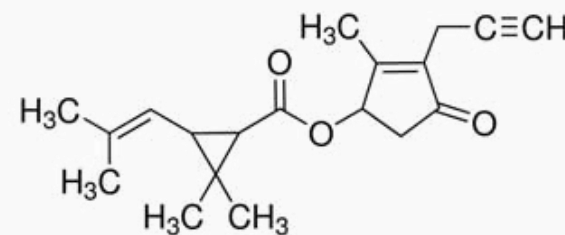
Organophosphate



AChE inhibitor

Prallethrin

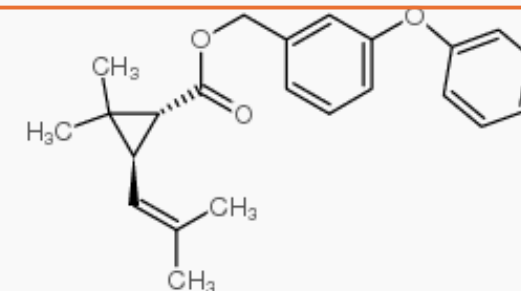
Pyrethroids



Sodium channel modulator

Sumithrin

Pyrethroids



Sodium channel modulator

Formulations

Mosquitomist II®

24.6% of chlorpyrifos

Dibrom®

87.4% of naled

Duet®

5% of sumithrin
1% of prallethrin

Vectobac 12AS®

Biological larvicide
11.61% of *Bacillus thuringiensis israelensis*

Honey bee larvae rearing conditions

- Following Schmehl et al. (2016)

Queen
caging

D-4



Honey bee larvae rearing conditions

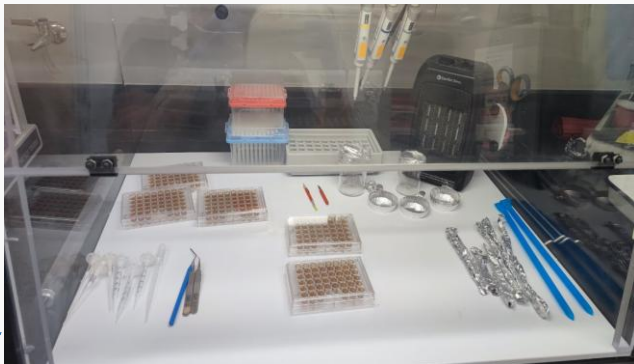
- Following Schmehl et al. (2016)

Queen
caging

Grafting
Diet A

D-4

D0



Honey bee larvae rearing conditions

- Following Schmehl et al. (2016)

Queen
caging

Grafting
Diet A

Diet B

Diet C

Diet C

Diet C

D-4

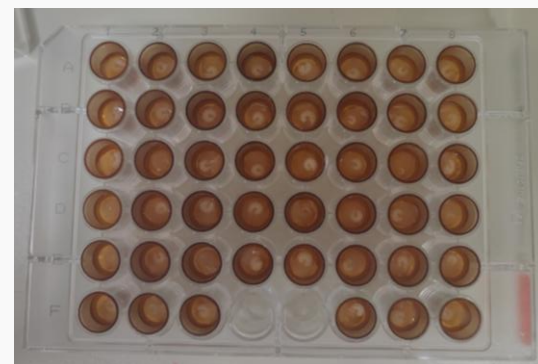
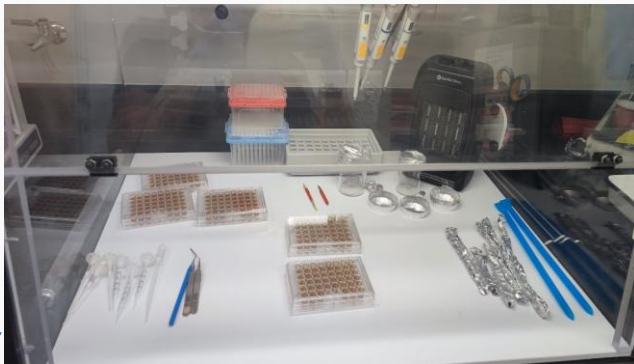
D0

D2

D3

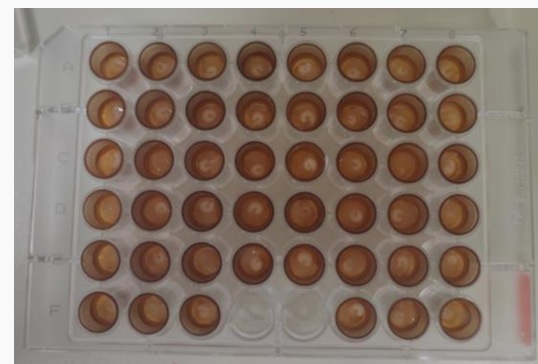
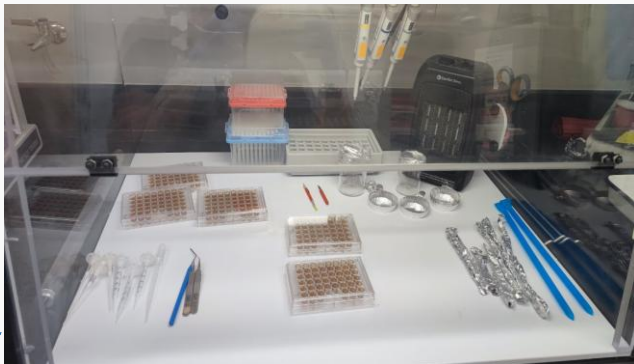
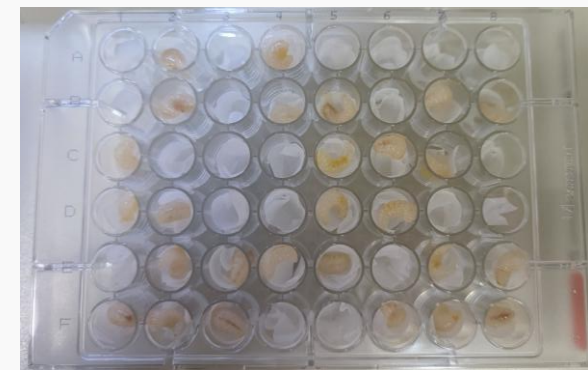
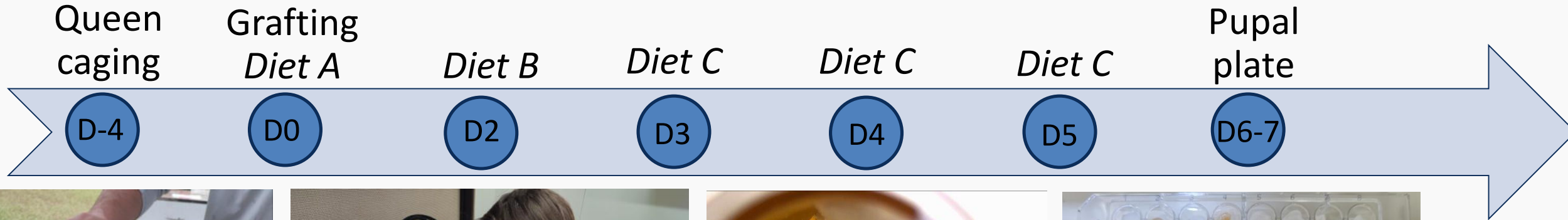
D4

D5



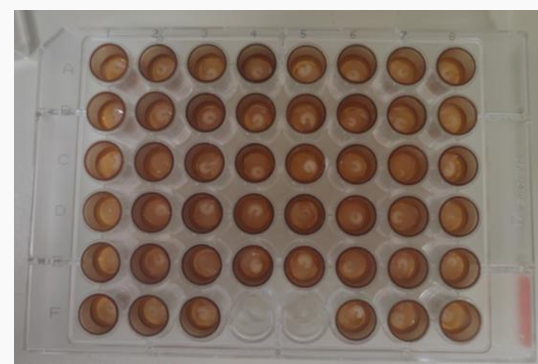
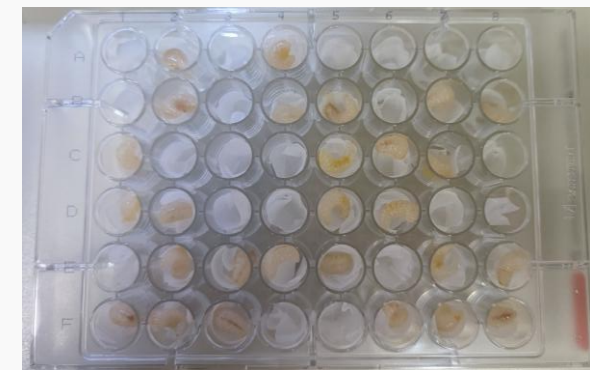
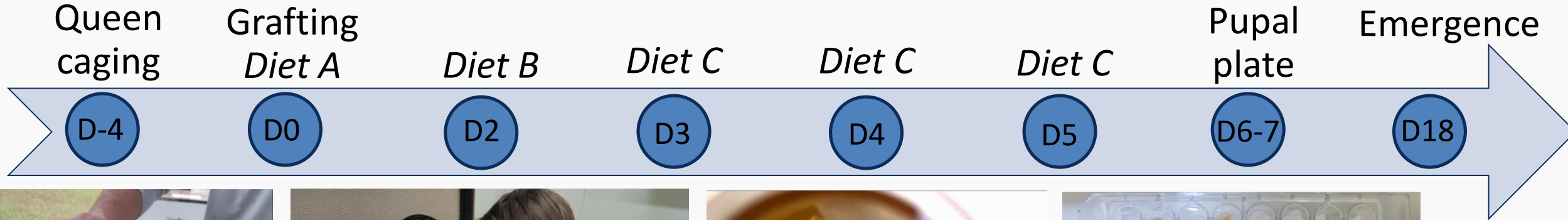
Honey bee larvae rearing conditions

➤ Following Schmehl et al. (2016)

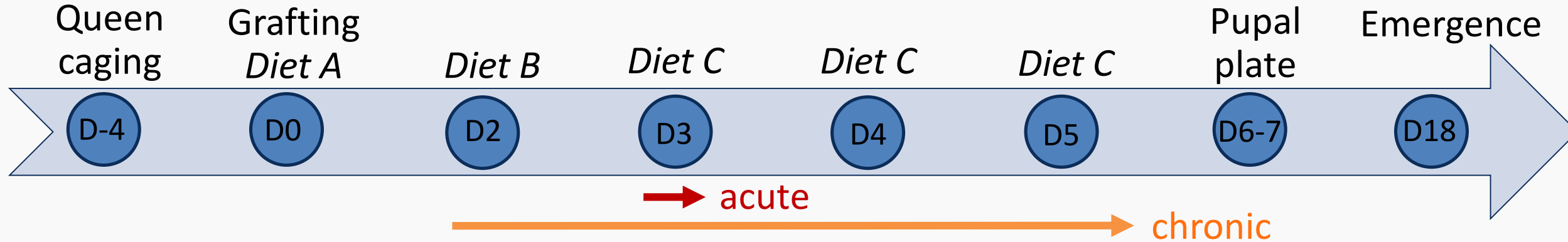


Honey bee larvae rearing conditions

➤ Following Schmehl et al. (2016)

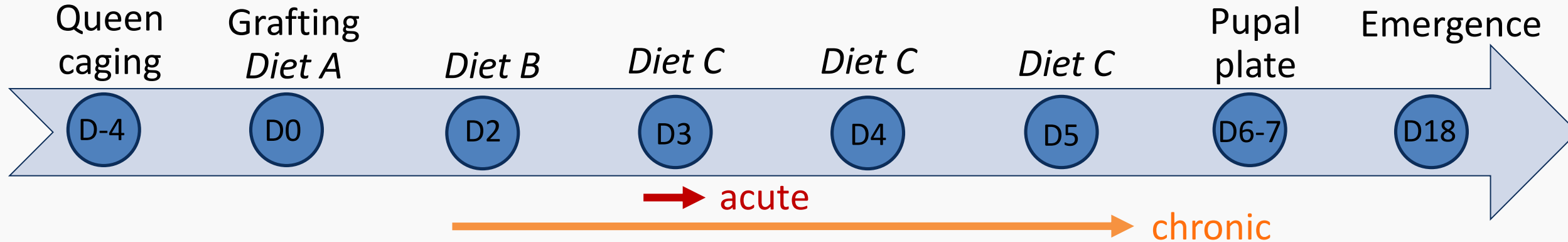


Toxicological assays



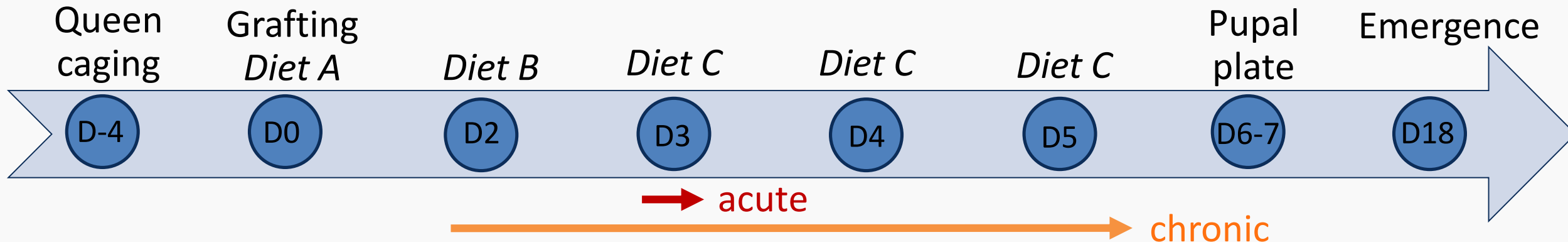
- Acute and chronic exposures
 - Exposed the larvae to pesticides through the diet
 - Larvae – pupae mortality

Toxicological assays



- Acute and chronic exposures
 - Exposed the larvae to pesticides through the diet
 - Larvae – pupae mortality
 - Determined the LD_{50} / NOAED – *probit analysis*
 - Lethal Dose – the dose at which 50% of population dies
 - No-Observed-Adverse-Effect-Dose – the highest dose at which no adverse effects are observed.

Toxicological assays



➤ Acute and chronic exposures

- Exposed the larvae to pesticides through the diet
- Larvae – pupae mortality
- Determined the LD_{50} / NOAED – *probit analysis*
 - Lethal Dose – the dose at which 50% of population dies
 - No-Observed-Adverse-Effect-Dose – the highest dose at which no adverse effects are observed.

- Calculated the RQ: BeeRex model

$$RQ = \frac{\text{Consumption} \times \text{concentration of exposure}}{\text{Endpoint (LD}_{50} \text{ or NOAED)}}$$

Toxicity and Risk



	Active ingredients	N	LD ₅₀ µg a.i./bee (IC95%)
Most toxic	naled	157	0.011 (0.001 – 0.108)
	chlorpyrifos	155	1.617 (0.808 – 3.238)
	prallethrin	184	2.66 (1.561 – 4.533)
Least toxic	sumithrin	164	3.058 (2.089 – 4.475)

Acute
exposure

Toxicity and Risk

	Active ingredients	N	LD ₅₀ µg a.i./bee (IC95%)	Larval NOAED (µg a.i./larva)
Most toxic ↑ ↓ Least toxic	naled	157	0.011 (0.001 – 0.108)	0.0008
	chlorpyrifos	155	1.617 (0.808 – 3.238)	0.02
	prallethrin	184	2.66 (1.561 – 4.533)	0.08
	sumithrin	164	3.058 (2.089 – 4.475)	0.016

Acute
exposure

Chronic
exposure

Toxicity and Risk

LOC = 0.4 LOC = 1

Most toxic



Least toxic

Active ingredients	N	LD ₅₀ µg a.i./bee (IC95%)	Larval NOAED (µg a.i./larva)	RQ (acute)	RQ (chronic)
naled	157	0.011 (0.001 – 0.108)	0.0008	< 0.001	0.059
chlorpyrifos	155	1.617 (0.808 – 3.238)	0.02	< 0.001	0.019
prallethrin	184	2.66 (1.561 – 4.533)	0.08	< 0.001	< 0.001
sumithrin	164	3.058 (2.089 – 4.475)	0.016	< 0.001	< 0.001

Acute
exposure

Chronic
exposure

Risk
Quotient

Toxicity and Risk

Most toxic



Least toxic

Mosquito control products	N	LD ₅₀ µg a.i./bee (IC95%)
Duet®	124	0.497 (0.157 – 0.782)
Dibrom®	160	0.613 (0.376 – 1.253)
MMII®	124	2.853 (2.437 – 3.620)
Vectobac12AS®	163	> 60 *

Acute
exposure

Toxicity and Risk

Most toxic  Least toxic	Mosquito control products	N	LD ₅₀ µg a.i./bee (IC95%)	Larval NOAED (µg a.i./larva)
	Duet®	124	0.497 (0.157 – 0.782)	0.08
	Dibrom®	160	0.613 (0.376 – 1.253)	0.0008
	MMII®	124	2.853 (2.437 – 3.620)	0.0004
	Vectobac12AS®	163	> 60 *	30

Acute
exposure

Chronic
exposure

Toxicity and Risk

LOC = 0.4 LOC = 1

	Mosquito control products	N	LD ₅₀ µg a.i./bee (IC95%)	Larval NOAED (µg a.i./larva)	RQ (acute)	RQ (chronic)
Most toxic ↑ ↓ Least toxic	Duet®	124	0.497 (0.157 – 0.782)	0.08	0.112	0.694
	Dibrom®	160	0.613 (0.376 – 1.253)	0.0008	2.215	16.973
	MMII®	124	2.853 (2.437 – 3.620)	0.0004	0.047	339.1
	Vectobac12AS®	163	> 60 *	30	0.056	0.112

Acute exposure

Chronic exposure

Risk Quotient

➤ Summarize:

- From the most to the least toxic: naled > chlorpyrifos > prallethrin > sumithrin
 - Based on the residues found in pollen and nectar from our field study, the isolated active ingredients are **unlikely to pose a significant risk** to developing larvae: $RQ < LOC$.

➤ Summarize:

- From the most to the least toxic: naled > chlorpyrifos > prallethrin > sumithrin
 - Based on the residues found in pollen and nectar from our field study, the isolated active ingredients are unlikely to pose a significant risk to developing larvae: $RQ < LOC$.
- From the most to the least toxic: Duet® > Dibrom® > MMII® > Vectobac12AS®
 - At **maximum application rates**, adulticides Dibrom® and MMII® pose a **high risk** to developing larvae: $RQ > LOC$.
 - The larvicide (Vectobac12AS®) is relatively non-toxic to developing larvae
→ **no risk**

➤ Hypotheses:

- Formulations would be more toxic to honey bee larvae than would active ingredients.



Duet® > prallethrin & sumithrin



MMII® < chlorpyrifos
Dibrom® < naled

➤ Hypotheses:

- Formulations would be more toxic to honey bee larvae than would active ingredients.



Duet® > prallethrin & sumithrin



MMII® < chlorpyrifos

Dibrom® < naled

- The larvicide would be more toxic to honey bee larvae than would the adulticides.

The larvicide would pose a risk to honey bee larvae.



RQ < 0.4 (acute)

RQ < 1 (chronic)

➤ Hypotheses:

- Formulations would be more toxic to honey bee larvae than would active ingredients.



Duet® > prallethrin & sumithrin



MMII® < chlorpyrifos

Dibrom® < naled

- The larvicide would be more toxic to honey bee larvae than would the adulticides.

The larvicide would pose a risk to honey bee larvae.



RQ < 0.4 (acute)

RQ < 1 (chronic)

- Adulticides would not pose a risk to developing larvae.



Dibrom® (acute & chronic)

MMII® (chronic)

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- Vernaeyah Lane
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- Dena Oliva

