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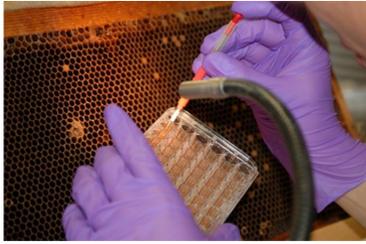


Figure 1: Grafting first instar larvae into a larval plate

Abstract

Several larval honey bee test methods are being developed to assess a growing concern of the potential toxicological effects of pesticides and active GMO proteins on honey bee larvae. The OECD released a draft guideline outlining a 72-hour larval exposure. Longer term studies in the 10 to 21-day range could provide even more valuable data, allowing for the assessment of toxicity throughout more of the development cycle from larva to adult. However, the longer duration exposures present a challenge in meeting acceptable control performance criteria, like development and survival.

Control survival data from larval honey bee tests performed at Smithers Viscient indicate the larval survival criterion of 70% can be regularly achieved over 21 days. Additionally, testing with reference toxicants and several pesticides indicate a clear dose-response can be achieved in tests with durations from 72 hours to 21 days. Consequently, this assay can provide scientifically sound data for the assessment of the toxicity of chemicals to honey bee larvae over their entire metamorphosis. Recommendations on harmonized procedures are provided to allow one study to provide data for larval, pupal and adult survival endpoints.

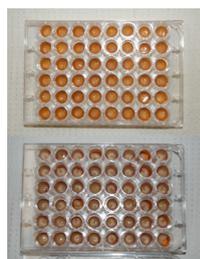


Figure 2: (72H) Larval plates with grafting cell cups containing first instar larvae (day -3 on top) and fourth instar larvae (termination on bottom)



Figure 3: (21-Day) Larval plates with second instar larvae (day 0 on top) and pupation plates with adults (termination on bottom)

Introduction

Protection of honey bee (*Apis mellifera* L.) is paramount in maintaining crop and native plant pollination. Laboratory and field testing is commonly required for registration of pesticides and genetically modified organisms (GMO).

Testing guidelines have been developed with two primary formats; a 72 hour exposure in parallel with the OECD 213 and 214 adult bee acute tests, and a 21 day format to view complete honey bee development.

The objective of this poster is to present an overview of the methods and experiences with the two testing formats over the 2010 - 2013 testing seasons at Smithers Viscient.

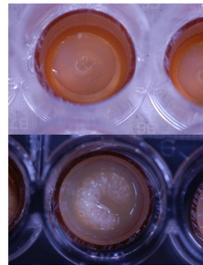


Figure 4: (72H) Test larval plates with grafting cell cups containing first instar larvae (top) and fourth instar larvae (bottom)



Figure 5: (21-Day) Larval plates with second instar larvae (top) and pupation plates with adults (bottom)

Materials and Methods

	OECD	21 Day
Duration	72 Hours	21 Days
Acclimation	3 days	None
Instar (day 0)	3	2
Dosing (days)	Single	Continuous
Diet volume	20-50 μ L	100-200 μ L
Diet removal	None	Vacuum
Diet replacement	Days 1-3	Days 1-6
Number of diets	3	1
Stage at termination	4th instar larva	adult
Endpoints	LD50/NOEC	LD50/NOEC
Units	μ g/larva	μ g/g diet

Discussion and Conclusions

During these assays, the number of surviving control bees during the 72 hour test averaged 94% while the 21 day test averaged 76%. From the survival data, the criteria of 85% and 70% for 72 hour and 21 day tests, respectively, appears achievable.

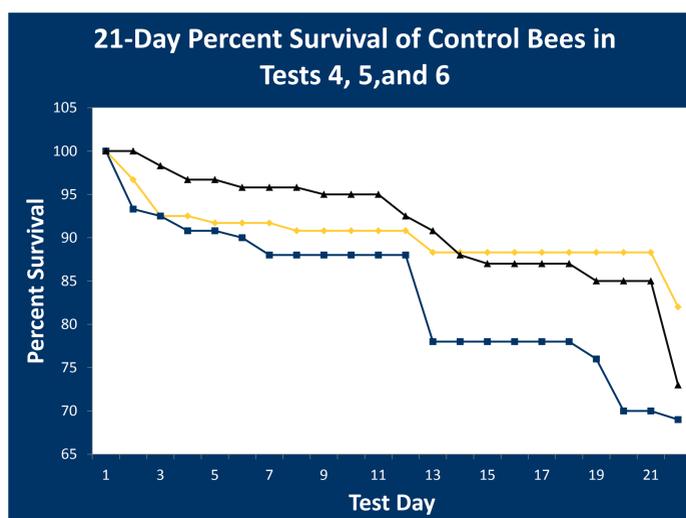
The 21 day test has the potential to assess long-term developmental effects that may not be observed over 72 hours. The two tests, each with their unique methods, have a place in regulatory testing depending upon whether short-term or long-term exposure is expected for a given material.

Recommendations:

- Single diet (OECD Diet A, 21 Day Diet) is adequate for larval bee survival and development (OECD Diets B and C may not be necessary)
- Units expressed as ' μ g/larva'
- Increase the diet volume for the 21 day test to 200 μ L throughout the exposure for consistency

Percent Survival								
Test	OECD 72 Hours		21 Day					
	1	2	1	2	3	4	5	6
N	12	48	24	120	24	120	120	120
Overall Survival	100%	88%	75%	80%	79%	69%	82%	73%

Table 2: Overall control survival of larval bees exposed to control diets.



References

- OECD, 2012 (Draft). OECD Guideline for Testing Chemicals. Honey bee (*Apis mellifera*) larval toxicity test, single exposure.
- Huang, Zachary, 2009. A Standardized Procedure for the In Vitro Rearing of Honey Bee Larvae. CDPR government website (http://cdpr.ca.gov/docs/registration/reevaluation/larval_protocol.pdf), Sacramento, California.
- Vandenburg, J.D. and H. Shimanuki, 1987, Technique for rearing worker honeybees in the laboratory. Journal of Agricultural Research, 26: 90-97.