There's No Space Like Home: Reduction in control mortality by mimicking bee space in the test vessel design for acute Honeybee *Apis mellifera* toxicity testing

Smithers Viscient Authors: Erika Grzebisz & Kirsty-Jo Muddiman

Abstract

Following the OECD Guidelines 213 and 214 Acute Oral and Contact Toxicity Tests, control mortality of two differing test vessel designs were compared for overall percentage mortality and honeybee behaviour. The vessel types consisted of a cylindrical steel cage vs. a clear plastic design. Honeybees from the same supplier hives were used to determine if a change in vessel design would impact upon a number of factors, namely: response to anaesthesia, behaviour, feeding and a reduction in control mortality. In total > 1000 control bees were "exposed" using the steel cage and clear plastic designs for comparison. Perhaps more importantly, a better accuracy for delivery of anaesthesia was devised, dramatically reducing the overall time required for the honeybees to respond to anaesthetic. Additionally, changes in the makeup of the sucrose feeders was investigated and natural honeybee hive behaviour, such as the "wagging dance" and arrangement in symmetrical patterns were observed indicating that the honeybees favoured one test design over the other.

Methods

Experimental Design

Experimental procedures were based on OECD No. 213 & 214 Guideline for Acute Oral and Contact honeybee toxicity testing.

- 48-hour exposure
- Species: *Apis mellifera*
- 10 honeybees per vessel
- 25°C ± 2°C
- 50-70% RH
- 50% (w/v) sucrose solution feed provided
- CO_2 anaesthesia

Clear Dish Design

Design details as follows;

- Holes for feeding and anaesthesia
- Feeder creates trough for uptake of sucrose solution
- Feeder placed in one side of dish to allow for more bee space
- Equally, feeder positioned to allow for more than one bee to feed at once
- Filter paper placed on bottom of vessel to combat natural fouling during test

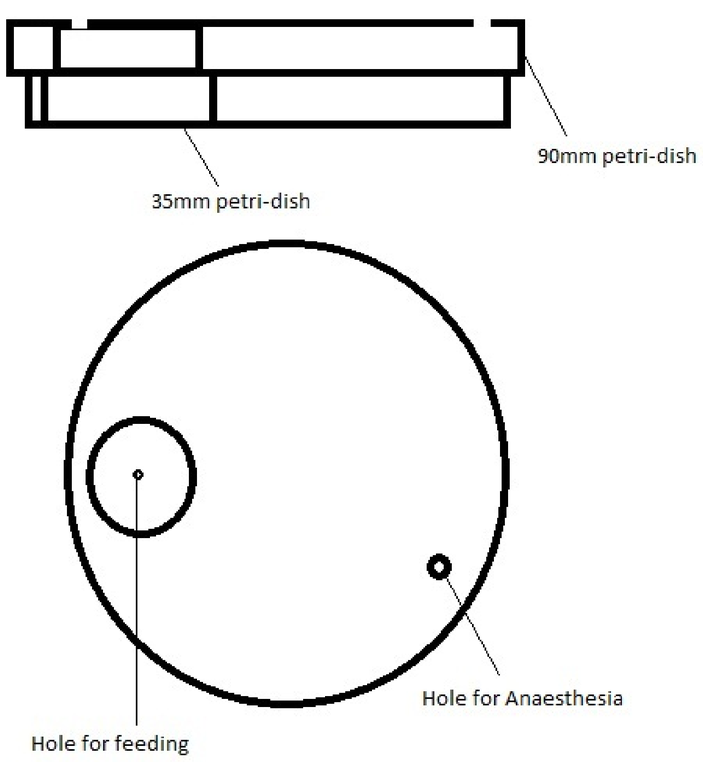

Figure 1: Schematic showing developed dish design

Metal Cage Design

Design details as follows;

- Cylindrical meshed metal cage
- Glass feeder allowing for only a single bee to feed at any given time
- Test utilised more space in the laboratory
- Potential for cross-contamination as cages re-used
- Potential for staff to be stung through the mesh
- Lengthy anaesthesia process

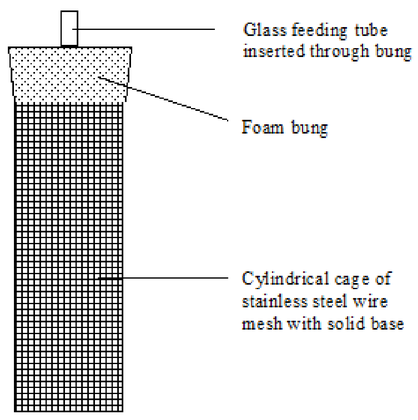

Figure 2: Schematic showing old metal cage design

Figure 3: Depiction of plastic dish design & honeybee contact dosing technique

Results

- Plastic dish design reduced control mortality by 82.16%
- Bee damage through trial flights eradicated
- No risk of cross-contamination as plastic dish is "throw-away"
- Anaesthesia time reduced as the plastic dish design allows for the bees to be closely observed during the administration of the CO_2 - the bees exhibit certain bodily traits when they are fully anaethetised, these traits were never visible when using the metal cage design, but can be closely monitored when using the dish design
- Bees exhibited more "natural" behaviour when in the plastic dish design and generally seemed more content e.g. clustering together, "waggling" etc.

Vessel Type	Number Honeybees Exposed	Number Honeybees Dead	Mean % Control Mortality
Metal Cage	520	22	4.23%
Plastic Dish	530	4	0.75%
Control Mortality Reduction %		82.16%	

Conclusion

- Plastic dish design dramatically reduces control mortality
- Bees do not damage themselves whilst trying to fly
- Plastic dish design allows for the bees to exhibit more "natural" behaviour
- Removes any risk of cross-contamination as "throw-away" design
- Delivery of anaesthesia more accurate, which therefore reduces the time bees are exposed to CO_2
- Increases capacity in the laboratory

References

OECD Guidelines for Testing of Chemicals; Test No. 213 – Honeybees, Acute Oral Toxicity Test and Test No. 214 – Honeybees, Acute Contact Toxicity Test (both September 1998)

Acknowledgements

Claire Burns for laboratory assistance.