A case study of assessing sublethal effects to honeybees for an insecticide considering the EFSA revised bee guidance

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BACKGROUND

Due to calls for greater consideration of sublethal effects to protect bee populations, the EFSA revised Bee Guidance Document (2023) suggests assessing sublethal effects of PPPs as part of the effect-tier assessment. Large scale behavioural changes may interfere with important tasks, such as foraging, which have the potential to translate to colony level effects and hence the Specific Protection Goal (SPG) of colony strength.

We present a 10 day adult honeybee chronic study conducted according to OECD 245 with an insecticide which included 'blind assessments' of sublethal effects and mortality, where the treatment groups were not known to the assessors.

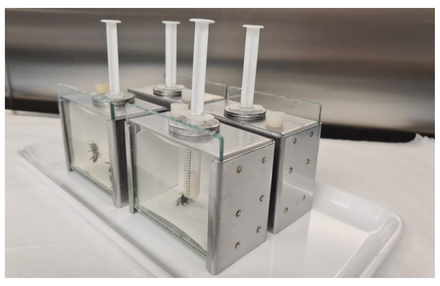
BEHAVIOURAL ASSESSMENTS

In the OECD 245 honeybee adult chronic test guideline, behavioural abnormalities are quantitatively observed (moribund, affected, cramps, apathetic, vomiting).

Some barriers to developing a more comprehensive assessment of sublethal effects include lack of standardisation, lack of proven link to the SPG of colony strength, subjectiveness of visual assessments and potential for unconscious bias.

MATERIAL METHOD SET-UP

- Unmarked bees were kept in stainless steel cages in groups of 10
- Bees were fed with an insecticide in sucrose solution *ad libitum* over 10 consecutive days
- Daily 'blind' assessment of mortality and sublethal effects
- Two labels: visible (front) and concealed (back)
- Treatment groups randomised



CONSIDERATIONS FOR BLIND ASSESSMENTS IN THE LAB

- Identifying test item group numbers must be concealed
- Remove feeders during assessments to avoid visual determination of treatment groups
- Raw data for assessments of mortality and behaviour recorded separately
- Separate observer for sublethal effects only; not involved in other study activities
- Daily randomisation of treatment groups

GENERAL CONSIDERATIONS FOR ASSESSING SUBLETHAL EFFECTS IN THE LAB

- Bees in lab tests are kept in completely artificial environments without normal hive tasks
- Visual assessments are subjective
- Group housed bees can not be individually evaluated (including recovery)
- Behavioural effects can be transient and not dose-dependent

STATISTICAL CONSIDERATIONS

- For the determination of the $NOEC_{behaviour}$ on day 10, the number of affected bees per replicate and assessment time was summed over the test period and compared to the total number of living bees
- A qualitative trend analysis by contrasts (monotonicity of concentration/response, $\alpha = 0.05$) and the Tarone´s test for signs of extra-binomial variance ($\alpha = 0.01$) were carried out. Further to these pre-tests the Cochran Armitage test (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there were statistically significant differences between the data on behaviour of the carrier control and each test item group and to determine the $NOEC_{behaviour}$ of day 10.
- Statistical calculations were made in ToxRat Professional 3.3.0.

Replicate	Treatment group													
	Control		Carrier control		T1		T2		T3		T4		T5	
	Bees alive	Affected bees	Bees alive	Affected bees	Bees alive	Affected bees	Bees alive	Affected bees	Bees alive	Affected bees	Bees alive	Affected bees	Bees alive	Affected bees
1	100	0	100	0	100	63	100	73	100	67	100	41	98	69
2	100	0	99	0	100	73	100	75	93	74	100	80	96	68
3	100	0	100	0	100	82	100	61	100	68	97	82	87	73
4	100	0	100	0	100	75	100	82	100	72	100	69	100	68

Data-set of the study
Bees alive: total number of surviving bees per replicate and assessment time summed up over the exposure period
Affected bees: total number of bees showing effects (affected, cramping, moribund, etc.) per replicate and assessment time summed up over the exposure period

RESULTS

Assessments on mortality and behaviour were carried out as blind assessments. In the control and carrier control group no behavioural abnormalities were observed throughout the test period. During the 10-day test period, behavioural abnormalities (affected bees, moribund bees, cramping bees) were observed in all test item treatment groups, at all assessments and throughout the entire observation period. The proportion of bees showing behavioural abnormalities was not dose-dependent. The affected bees showed reduced coordination, as evidenced by reduced climbing and self-righting ability. Low mortality was observed in the test item treatment groups at the end of the test period indicating bees were neither able to recover from the symptoms, nor that these symptoms led to mortality.

Behavioural effects can be subtle and hard to categorise. Scan the QR codes to watch the behaviour of control and treated bees. What do you see and how would you describe it?

CONCLUSIONS

It is possible to conduct blind assessments for behavioural abnormalities, and we present considerations based on our learnings from this study.

At present, the only way to accurately assess the potential impact of sublethal effects at a colony/population level is higher tier studies such as semi-field or full field studies. Higher tier studies with Apis and Non-Apis bees are being generated with this insecticide in order for us to understand the biological relevance at the colony level of sublethal effects seen in lower tier lab studies.