Assessing sub-lethal effects of pesticides on hypopharyngeal glands: recommendations for a possible ring-test method

Willener Astrid S.T., Volles Claudia and Jeker Lukas
Agroscope, Swiss Bee Research Centre, CH-3003 Bern; www.apis.admin.ch

STATE OF ART

The implementation of the EU Regulation 1107/2009 as well as the Publication and data requirements of the EFSA Bee Guidance Document (EFSA 2013) need further efforts for method development and validation to evaluate the risk of bees and its colony exposed to pesticides for **P**lant **P**rotection **P**roduct authorization in an appropriate and comparable way. Only few data exist on hypopharyngeal gland (HPG), such as its role, its natural development or how to measure its activity[1,2]. The activity of HPG is an adaptive response to the need of brood feeding. Its mechanics is influenced by several factors e.g. diet, age, brood presence, brood stage and seasonal time[1,2,3,4,5].

Testing the effect of pesticides: Several papers have showed an effect of pesticides on HPG size or HPG activity[6,7]. Unfortunately some methodological aspects have not been taken into account. For instance, the conditions for a good development (e.g. pollen or protein access[8]) need to be considered to avoid bias. In addition, the activity of HPG (i.e. nursing) needs to be triggered by e.g. brood presence to assess not only the effect of pesticide on HPG growth, but the inhibition on HPG development due to protein production, too.

METHOD

Protocol:

Freshly hatched bees collected beginning of October (mainly winter bees) from three different colonies were chronically exposed to sub-lethal field realistic concentrations[9] of Clothianidin (**Cl**), Thiamethoxame (**T**), Dimethoate (**D**) or a mix (**M**) of T and Cl). The active ingredients were either dissolved in 50% sucrose solution or pollen patties. Untreated (**C**) and acetone (**A**) controls for both sucrose solution and pollen patties were established. 20 bees were caged during 10 days, fed ad libitum with the treated food (replicate = 2). Food consumption was assessed at least every second day. Bees were killed by chilling on ice, HPG was removed[10], stained[11], mounted on a glass slide and covered. HPG activity was measured via acini size using the software Cell^b from Soft Imaging System of Olympus.

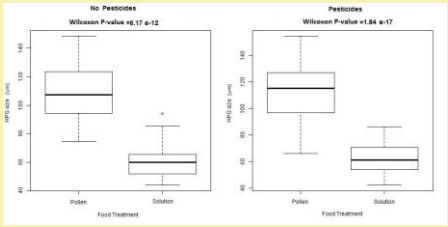
< Nurse bees in a cage fed with pollen (**Left**) and sugar solution (**Right**).

Results and discussion:

Treatment	Concentrations [µg a.i./kg]		Pesticide consumed [µg a.i.*10^{-3} /bee]	
	Pollen[a]	Sucrose solution[b]	Pollen	Sucrose solution
C	0	0	0	0
A	0	0	0	0
Cl	1.99	1.48	0.33 – 0.36	0.44 – 0.46
T	5.24	4.85	1.06 - 1.10	1.36 - 1.37
M	7.23	6.33	1.13 - 1.28	1.74 - 1.85
D	200	NA	35.5 – 39.0	NA

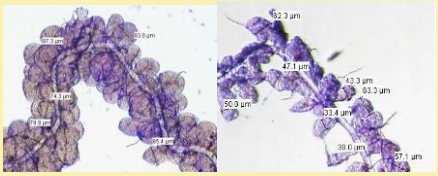
a: Acetone content was 13.5% in all pollen patties, apart from treatment C with 0%
b: Acetone content was 2% in all sucrose solutions, apart from treatment C with 0%

Sugar solution vs pollen patties: HPG from bees fed with sugar solution were statistically significantly smaller when compared to the one fed with pollen. Our results are similar to previous studies using summer bees[8]. This is probably due to the fact that pollen is required for a good development of HPG.



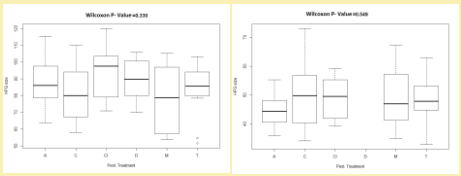
^ Difference in food treatment (pollen vs sucrose solution). Honey bees HPG size fed without (**Left**) and with pesticides (**Right**).

HPG size: In our test, HPG size was below the average found in literature[12] with brood presence. No HPG size data were found in the literature without brood presence. Therefore, our results may be biased to the absence of brood[5], i.e. the development due to activity was not appropriately triggered .



^ HPG of bees fed with pollen (**Left**) and sugar solution (**Right**).

Pesticide effect: No statistically significant effect of the pesticide were found on the HPG size of bees, for both group fed either with sugar solution or pollen patties.



^ HPG size of honey bees fed with and different pesticides in pollen (**Left**) or sucrose solution (**Right**).

CONCLUSION

Our results demonstrate the need of additional research to determine an appropriate method for accurate and comparable results of sub-lethal effects on HPG and its functionality to understand the consequences on colony survival used for risk assessment purposes. Two important points are highlighted and some suggestions for a ring-test are summarized in the table below:

Methodological aspects	Method proposal	Limitations
The conditions for a good HPG development need to be given to avoid bias	• Freshly hatched nursing summer bees ≤ 48h, fed with pesticide-free pollen (potentially protein supplement*) and water • Brood presence	Keeping a constantly new brood may not be possible under laboratory condition, thus the HPG functionality (i.e. HPG development due to protein production) is not tested
The activity of HPG needs to be triggered (e.g. brood presence)	• Measurement of protein quantity in HPG (e.g. relation to HPG size and activity)*. • Head conservation need do be discussed* *more experiments are needed	

REFERENCES [1] Brouwers et al. (1982); [2]Brouwers et al. (1983); [3]Škerl et al. (2015) ,[4]Kuboe et al. (1996), [5]Rahman et al. (2014), [6]Hatjina et al. (2013),[7]Škerl and Gregorc (2010); [8] DeGrandi-Hoffman et al. (2010); [9]Pilling et al., 2013; [10]Carreck et al. (2013); [11]Hartfelder et al. (2013); [12]Maurizio (1954); **ACKNOWLEDGMENT** We would like to thank Melissa Oddie, Selina Bruckner, Peter Neumann and Geoffrey Williams for their scientific input, Kasper Roth as lab technician, Benoit Droz our beekeeper and the UniBE and ZBF team for their help and support.