Oral toxicity test with solitary bees: Data evaluation based on nominal doses or following the feeding behaviour of the test organisms?

Aline Fauser, Thomas Schmidt, Magdaléna Cornement, Bettina Hodapp, Christina Rehberg, Stefan Höger, Stefan Kimmel

Introduction

The request for Bumble bee and Solitary bee species toxicity testing have dramatically increased during the last years due to the regulatory awareness that results on honey bees may not completely cover the risk of exposure to plant protection products. In principle, lower tier oral and contact toxicity tests are designed comparable to the established honey bee tests (OECD 213 & 214, EPPO 170, OCSPP 850.3020), but differ with respect to the biology of the test species (e.g. group testing vs. individual testing, light conditions, mode of food presentation).

Especially oral toxicity tests with the solitary bee species *Osmia bicornis* are tricky since the feeding containers providing them with food are not standardized so far. Additionally individuals vary largely in the consumed amount of test item treated sugar solution (see Tab. 1: Coefficient of Variation (CV) of the final ingested doses within one treatment group, red marked) which resulted to a cloudy dose-response relationship (Fig. 1).
Supported by the non-Apis group of ICPPR (International Commission for Plant Pollinator Relationships) and as part of ongoing development and discussions, we tested a simple feeding system (see Picture 1 & 2) and performed a new assessment scheme using the real ingested doses as grouping parameter for the test organisms.

Methodology

Picture 1: Test system with one solitary bee

How to proceed:

- *Osmia* females (24 to 96 hours old, kept at 4 °C until the number of hatched bees was sufficient for the test)
- Introduction into test vessels without a starving period before (in a lighted area, not in the dark)
- Weighing of feeding vials before application
- Exposition to treated sugar solution for 2-4 hours
- Weighing of feeding vials after application (to exactly measure the amount of sugar solution ingested by the bee)

How to make the treated sugar solution attractive:

- Use of plastic lids ideally Ø 5-10 mm with bee attracting color (e.g. blue, yellow…)
- Feeders should be glued to a piece of paper to avoid overturn during feeding
- Sugar solution should be presented in excess (solitary bees need to "sense" the food)
- 200-250 µL sugar solution per bee (dose calculation based on average food intake
- 20-30 µL, see Tables 1 and 2)

How to assess:

- Normal procedure: test organisms grouped by the nominal dose given
- New procedure: test organisms grouped by ingested doses

Results & Discussion

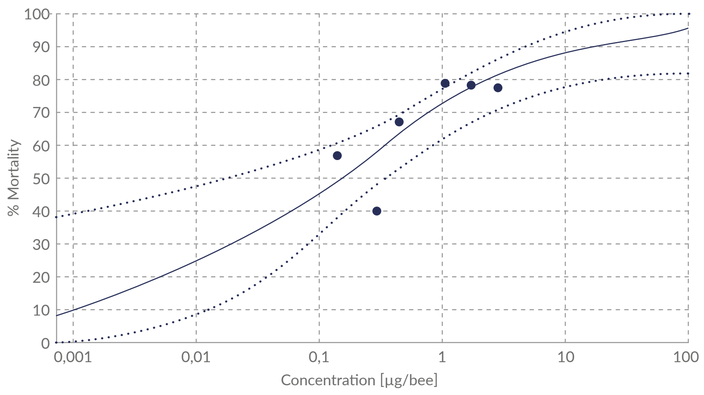

Figure 1: Dose-response relationship of groups of *O. bicornis* exposed to **nominal doses** of 0.125 to 4.0 µg test item per bee.

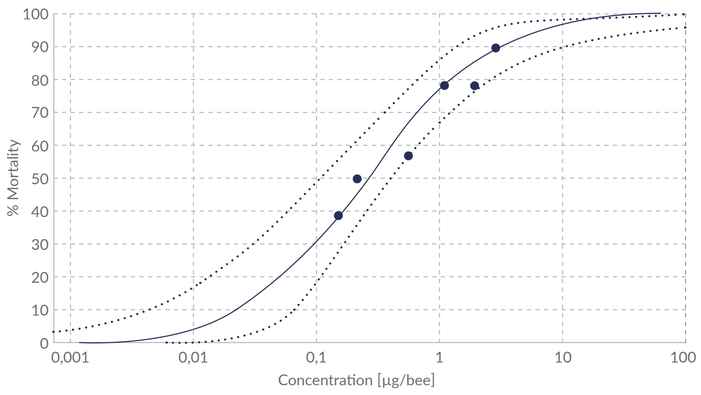

Figure 2: Dose-response relationship of groups of *O. bicornis* exposed to **ingested doses** of 0.171 to 3.4 µg test item per bee.

Treatment group (µg a.i./bee)	N°		Volume (µL)	Weight of bee (mg)	Final dose µg a.i.		No. survived	% survived
					per bee	per g bee		
Control	n=30	mean	23,155	87,963	–	–	28	93
		SD	15,249	9,023	–	–		
		CV	66	10	–	–		
0,125	1	–	40,2	89,6	0,254	2,830	0	0
0,125	2	–	40,3	93,3	0,254	2,724	0	0
0,125	3	–	9,1	79,2	0,057	0,725	0	0
0,125	4	–	48,7	96,6	0,307	3,181	0	0
0,125	5	–	58,9	74,0	0,372	5,022	0	0
0,125	6	–	14,4	76,4	0,091	1,190	1	100
0,125	7	–	25,2	102,3	0,159	1,554	1	100
0,125	8	–	19,7	68,4	0,124	1,819	1	100
0,125	9	–	9,2	81,2	0,058	0,714	1	100
0,125	10	–	44,2	82,4	0,279	3,381	0	0
0,125	11	–	10,8	89,7	0,068	0,759	0	0
0,125	12	–	21,3	95,9	0,135	1,403	1	100
0,125	13	–	16,6	85,8	0,105	1,221	0	0
0,125	14	–	17,5	105,7	0,110	1,041	0	0
0,125	15	–	19,6	76,3	0,123	1,617	0	0
0,125	16	–	32,7	93,9	0,206	2,179	0	0
0,125	17	–	57,9	76,1	0,365	4,800	0	0
0,125	18	–	64,0	103,1	0,404	3,914	1	100
0,125	19	–	16,7	92,2	0,105	1,142	1	100
0,125	20	–	18,6	70,9	0,117	1,657	0	0
0,125	21	–	20,7	112,0	0,131	1,168	1	100
0,125	22	–	58,6	107,2	0,370	3,447	0	0
0,125	23	–	13,7	71,4	0,087	1,214	0	0
0,125	24	–	16,3	89,5	0,103	1,147	1	100
0,125	25	–	48,6	80,5	0,307	3,811	0	0
0,125	26	–	63,6	95,8	0,401	4,185	0	0
0,125	27	–	19,1	89,5	0,121	1,348	1	100
0,125	28	–	10,8	74,5	0,068	0,913	1	100
0,125	29	–	45,9	81,2	0,290	3,568	0	0
0,125	30	–	30,7	93,7	0,194	2,065	1	100
0,125	n=30	mean	30,459	87,610	**0,192**	**2,192**	12	40
		SD	18,141	11,762	0,114	1,299		
		CV	60	13	**60**	**59**		
0,25	n=30	mean	20,722	87,473	**0,261**	**3,052**	17	57
		SD	11,710	12,042	0,147	1,797		
		CV	57	14	**57**	**59**		
0,5	n=30	mean	30,858	88,707	**0,777**	**8,892**	9	30
		SD	16,442	12,545	0,414	4,694		
		CV	53	14	**53**	**53**		
1	n=30	mean	30,987	89,467	**1,561**	**18,021**	6	20
		SD	12,449	14,975	0,627	8,091		
		CV	40	17	**40**	**45**		
2	n=30	mean	29,267	91,787	**2,949**	**32,789**	6	20
		SD	12,969	13,957	1,307	14,961		
		CV	44	15	**44**	**46**		
4	n=30	mean	25,454	88,397	**5,129**	**58,384**	6	20
		SD	10,516	14,023	2,119	23,223		
		CV	41	16	**41**	**40**		

Table 1: Ingested amounts of test item and body weight of groups of *Osmia* (n=30 per group), sorted according to **nominal doses.**

Treatment group (µg a.i./bee)	N°		Volume (µL)	Weight of bee (mg)	Final dose µg a.i.		No. survived	% survived
					per bee	per g bee		
Control	n=30	mean	23,155	87,963	–	–	28	93
		SD	15,249	9,023	–	–		
		CV	66	10	–	–		
0,25	3	–	5,7	103,1	0,072	0,700	0	0
0,125	9	–	9,2	81,2	0,058	0,714	1	100
0,125	3	–	9,1	79,2	0,057	0,725	0	0
0,125	11	–	10,8	89,7	0,068	0,759	0	0
0,125	28	–	10,8	74,5	0,068	0,913	1	100
0,25	24	–	5,9	77,3	0,074	0,961	0	0
0,125	14	–	17,5	105,7	0,110	1,041	0	0
0,125	19	–	16,7	92,2	0,105	1,142	1	100
0,125	24	–	16,3	89,5	0,103	1,147	1	100
0,125	21	–	20,7	112,0	0,131	1,168	1	100
0,125	6	–	14,4	76,4	0,091	1,190	1	100
0,125	23	–	13,7	71,4	0,087	1,214	0	0
0,125	13	–	16,6	85,8	0,105	1,221	0	0
0,125	27	–	19,1	89,5	0,121	1,348	1	100
0,25	12	–	9,8	89,4	0,123	1,378	1	100
0,125	12	–	21,3	95,9	0,135	1,403	1	100
0,25	28	–	13,2	116,0	0,166	1,428	0	0
0,25	5	–	10,1	87,6	0,127	1,454	1	100
0,125	7	–	25,2	102,3	0,159	1,554	1	100
0,125	15	–	19,6	76,3	0,123	1,617	0	0
0,125	20	–	18,6	70,9	0,117	1,657	0	0
0,25	15	–	14,6	110,8	0,184	1,658	1	100
0,25	17	–	12,2	91,3	0,154	1,686	1	100
0,25	6	–	10,1	72,5	0,127	1,757	0	0
0,125	8	–	19,7	68,4	0,124	1,819	1	100
0,25	1	–	15,1	97,6	0,190	1,947	1	100
0,25	7	–	13,3	83,5	0,168	2,009	1	100
0,25	29	–	12,6	79,2	0,159	2,011	0	0
0,125	30	–	30,7	93,7	0,194	2,065	1	100
0,125	16	–	32,7	93,9	0,206	2,197	0	0
0,171	n=30	mean	15,515	88,560	0,124	1,396	17	57
		SD	6,391	12,949	0,042	0,431		
		CV	41	15	**34**	**31**		
0,267	n=30	mean	28,615	88,867	0,291	3,293	14	47
		SD	17,378	12,064	0,068	0,662		
		CV	61	14	**23**	**20**		
0,671	n=30	mean	26,114	89,297	0,679	7,592	12	40
		SD	12,694	13,770	0,237	2,266		
		CV	49	15	**35**	**30**		
1,17	n=30	mean	33,404	91,723	1,532	16,625	6	20
		SD	14,988	14,697	0,408	3,131		
		CV	45	16	**27**	**19**		
2,20	n=30	mean	29,720	88,157	2,681	30,426	5	17
		SD	12,425	13,768	0,688	6,003		
		CV	42	16	**26**	**20**		
3,40	n=30	mean	34,379	86,837	5,562	63,999	2	7
		SD	11,563	12,149	1,763	17,924		
		CV	34	14	**32**	**28**		

Table 2: Ingested amounts of test item and body weight of groups of *Osmia* (n=30 per group), sorted according to **ingested doses.**

Picture 2: Feeding container with one solitary bee

The actual main concern in acute oral tests with solitary bees is the lack of feeding and therefore a reduced amount of test dose ingestion. Making the provided food attractive with colored feeding vials resulted in satisfying feeding rates. Nevertheless the large individual variability of feeding rates should be kept in mind.

This large variability of ingested doses can be easily reduced by sorting the individuals according to their ingested dose from the lowest to the largest value (see Table 2: Coefficient of Variation (CV) of the final ingested doses within one treatment group, green marked) and creating groups of individuals with the 30 lowest ingested doses, then the following larger ingested doses until the last 30 individuals with the largest ingested doses. This procedure reduces the variability of ingested dose within a group by 50 % and increases the significance of the dose-response relationship (Figure 2).

Conclusion

With our feeding method we show that acute oral tests with Solitary bees can be conducted without the addition of bee attractive products (e.g. anise oil) which may have unknown effects on the test substance itself.

Still, further research & development needs to be conducted on this specific test system and setup. We also show that the significance of dose-response relationships can be improved by performing assessments based on solitary bee groups sorted by ingested doses rather than nominal doses. Further research on oral toxicity testing is needed to increase its explanatory power for the risk assessment of plant protection products.

info@ies-ltd.ch | www.ies-ltd.ch