

## References and Footnotes

- <sup>1</sup>The Pollinator Research Task Force (PRTF) was formed in January 2016 and is comprised of ten pesticide registrants (Arysta LifeScience, BASF Corp., Bayer Crop Science LP, Dow AgroSciences LLC DuPont Crop Protection, FMC Corp., Mitsui Chemicals Agro. Inc., Monsanto Co., Syngenta Crop Protection LLC and ValentUSA Corp.) with the focus of mining and generating data to refine and improve pollinator risk assessments in North America and globally where applicable
- <sup>2</sup>OECD Guidance Document on Honey Bee (*Apis mellifera*) Larval Toxicity Test, Repeated Exposure. No. 239. 15 July 2016.
- <sup>3</sup>Daniel R Schmehl, Hudson V. V. Tomé, Ashley N Mortensen, Gustavo Ferreira Martins & James D Ellis (2016): Protocol for the in vitro rearing of honey bee (*Apis mellifera* L.) workers, Journal of Apicultural Research, DOI: 10.1080/00218839.2016.1203530
- <sup>4</sup>Pacific EcoRisk. Results of the honey bee (*Apis mellifera* L.) toxicity following chronic (repeated) exposure international ring test performance. February 2017.
- <sup>5</sup>OECD Guidelines for the Testing of Chemicals: Honeybees, Acute Contact Toxicity Test. No. 214. 12 September 1998.
- <sup>6</sup>OECD Guidelines for the Testing of Chemicals: Honeybees, Acute Oral Toxicity Test. No. 213. 21 September 1998.
- <sup>7</sup>OECD Guidelines for the Testing of Chemicals: Honey bee (*Apis mellifera* L.) Chronic Oral Toxicity Test (10-Day Feeding). 245. 9 October 2017.
- <sup>8</sup>OECD Guidelines for the Testing of Chemicals: Honey Bee (*Apis mellifera*) Larval Toxicity Test, Single Exposure. No. 237. 26 July 2013.
- <sup>9</sup>Aupinel, P., D. Fortini, H. Dufour, J.-N. Tasei, B. Michaud, J.-F. Odoux, and M. Pham-Delegue (2005). Improvement of artificial feeding in a standard *in vitro* method for rearing *Apis mellifera* larvae. Bulletin of Insectology 58, 107-111.
- <sup>10</sup>Aupinel, P., M. Barth, M.P. Chauzat, M. Colli, N. Cougoule, J. Eckert, A. Ehmke, D. Fortini, D. Gladbach, N. Hanewald, M. Janke, S. Kimmel, K. Kleebaum, P. Medrzycki, C. Moreau-Vauzelle, D. Przygoda, U. Riessberger-Gallé, S. Royer, S. Schmitzer, F. Shurr, A. Vincent. Results of the international ring test related to the honey bee (*Apis mellifera*) larval toxicity test, repeated exposure. 17 April 2015.
- <sup>11</sup>Crailsheim, K., R. Brodschneider, P. Aupinel, D. Behrens, E. Genersch, J. Vollmann & U. Riessberger-Gallé (2013) Standard methods for artificial rearing of *Apis mellifera* larvae, Journal of Apicultural Research, 52:1, 1-16, DOI: 10.3896/IBRA.1.52.1.05.

## 2.3 From field to food – Will pesticide contaminated pollen diet lead to a contamination of larval food?

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DOI 10.5073/jka.2018.462.025

### Abstract

The contamination of bee products, by pesticides is an increasing problem of beekeeping in rural areas. Residues of agricultural crop protection chemicals have been found in collected goods of foraging honeybees as well as inside the bee hive, e.g. pollen and bee bread. As pollen is an important ingredient to produce larval food, a contamination with pesticides could entail severe consequences on the colonies well-being. However, the fate of pesticides originating from the pollen during this process is unknown. We designed two experiments to trace possible pesticide residues in royal jelly (RJ) as well as in worker jelly (WJ) back to the protein source. We conducted two field experiments with free flying honeybee colonies where we fed a mixture of commonly found pesticides mixed in high concentrations (34.0-9021.8 µg/kg) into a pollen-honey diet. While feeding, we initiated a queen rearing within the colony to obtain RJ, presumably contaminated with the given pesticides, in the first experiment. In the second experiment, worker larvae were reared during the time the contaminated pollen diet was offered. WJ was harvested on four successive days from larval age three to six. RJ and WJ were subjected to a multi-residue analysis. Seven (out of 13) substances were rediscovered in traces in the RJ. In WJ samples, 6-12 substances (out of 13) were detected in increasing concentrations depending on larval age and pesticide. The increasing number of pollen grains in WJ of older larvae seems to be responsible for the increasing amount of pesticides detected in the WJ samples. However, as there are only few pollen grains in RJ, pollen seems to be a negligible route of contaminating RJ. Considering the facts that (i) the concentrations of pesticides in pollen collected in agricultural areas is usually lower than in our experiments and that (ii) only traces of these residues reach the larval food, we do not expect direct negative effects onto queen or larval development in the field. However, long-term effects, effects on caste differentiation or sub-lethal effects on queen or larval development cannot be excluded. Our experiment gives precise information of the real pesticide contamination of larval food. These results should help to better evaluate the concentrations found in the field and to conduct realistic feeding experiments which may be used for risk assessments or pesticide approval.