4.18 Toxicity assessment of mixtures of neonicotinoids and systemic fungicides or biopesticides in bumblebees (*Bombus terrestris*)

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Abstract

Over the last decades, the decline of bee species has gained much interest as they are pollinators of many crops and wild flowers. This decline of pollinators is multifactorial, and one of the main causes is the use of new-class pesticides such as neonicotinoids in agriculture. Furthermore, in combination with other pesticides, the toxicity can have an antagonistic or synergistic effect. While research has been primarily focused on honeybees, effects are not limited to this specific pollinator and other species such as bumblebees may also be affected.

In this study, we assessed the toxicity of imidacloprid, one of the most widely used neonicotinoids, in combination with a systemic fungicide (difenoconazole) or biopesticide (*Metarhizium anisopliae*) on bumblebees. We exposed standardised microcolonies of *Bombus terrestris* workers, which is a Tier I level test according to EFSA directive, to dilution series of both the individual insecticides (imidacloprid, difenoconazole, *M. anisopliae)* and their binary combinations (imidacloprid + difenoconazole, imidacloprid + *M. anisopliae*). Concentrations were chosen to reflect realistic worse case field exposure concentrations and the parameters which were observed are mortality and reproduction (ejected larvae and number of drones).

Results indicate that the use of biopesticides ($\geq 10^8$ spores of *M. anisopliae*) leads to a chronic toxicity, while no effect was seen when using the system fungicide difenoconazole. Results also confirmed the known toxicity of imidacloprid starting from 0.2 ppm.

Results of binary mixtures indicate a synergistic effect between imidacloprid and *M. anisopliae* for worse case field exposure concentrations (>0.2 ppm imidacloprid and ~10^8 spores of *M. anisopliae*). Although a significant loss in drone production and ejected larvae was observed, for imidacloprid (2 ppm) in combination with difenoconazole (10 ppm), but no synergistic or antagonistic effect was observed

We conclude that imidacloprid is toxic for *B. terrestris* microcolonies (Tier I level) and *M. anisopliae* at realistic worst case exposure estimates (application on foliage). Furthermore, imidacloprid in combination with biopesticide *M. anisopliae* resulted in a synergistic interaction while no interaction was observed for combinations of imidacloprid with the systemic fungicide difenoconazole. Based on these results, further in depth investigation on pesticide mixtures is recommended at higher Tier II or III levels, for example with foraging bumblebees.