Mass Spectrometry-based Detection of Neonicotinoids on Honey Bees

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Introduction

With increasing use of new pesticide formulations worldwide to repel and kill unwanted organisms, negative consequences on non-target species such as honey bees and other pollinators are of increasing concern and not well studied. This study compares the nanotechnology based pesticide (NBP) Safari and its base neonicotinoid, dinotefuran, and their effect on bee death using liquid chromatography tandem mass spectrometry (LC-MS/MS). This research examines whether nanotechnology based pesticides, with particles smaller than pollen, can transfer more readily to the bees than the active ingredient alone and does the NBP have a longer residual toxicity to bees. This transfer can occur in the same manner that pollen is transferred from flowers to bees, or the particles may cling to pollen which is then collected by bees and returned to the hive (Figure 1).

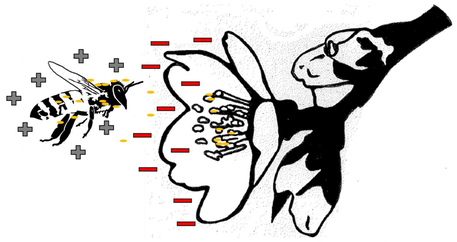

Figure 1. Bees are perfectly designed to attract and collect particles such as pollen. In addition to actively collecting pollen, positively charged bees induce a charge in flowers, which are already negatively charged. This facilitates the transfer of pollen to bees, which are covered with hairs that trap and hold the pollen. Bees may inadvertently collect many other types of particles, some of which have potential adverse effects.

What are Nanotechnology Based Pesticides (NBPs)?

Particulate pesticide formulations are already on the market containing engineered particles in the nano to micro size scale and are commonly applied in agricultural environments. The pesticide active ingredient may be; a) adsorbed onto the particle, b) attached to a particle via ligands, c) encapsulated by or d) entrapped within a polymer matrix (Figure 2). When the pesticides active ingredients are associated with particles, their behavior in the environment changes.

Figure 2. On the left, a schematic representation of different nanodevices for delivery of pesticides, fertilizers or nucleic acids (a) adsorption on nanoparticle; (b) attachment on nanoparticle mediated by different ligands; (c) encapsulation in nanoparticulate polymeric shell; (d) entrapment in polymeric nanoparticle. Ghormande et al, Biotechnology Advances 29 (2011) 792–803. On the right, a scanning electron microscopy image of the nanotechnology based pesticide, Safari.

Safari is an NBP formulated from the neonicotinoid dinotefuran and other proprietary ingredients. The dinotefuran in Safari is associated with irregular particles that vary in size from nanometers to micrometers (Figure 2). Safari has been associated with bumble bee death.

Sample Preparation Method

Leaves were sprayed with dinotefuran or Safari and left outside to be exposed to the elements. Periodically, a set of leaves were picked and honey bees were exposed for 24 hours. To extract dinotefuran or Safari on the exterior of bees in preparation for analysis, 5 bees were placed in 5 mL of distilled water. 1 µg/mL of d3-dinotefuran (Toronto Research Chemicals, Toronto, Canada) was included as an internal standard. The bees were vortexed using a Multi-Tube Holder attached to a Vortex Genie 2 (Scientific Industries, Bohema, NY) for 4 minutes at maximum power. 1 mL of the water was transferred to a 1.5 mL plastic tube, which was vortexed at 5,000 rcf for 5 minutes. 100 µL of the supernatant was transferred to a 300 µL polypropylene autosampler vial (MicroSolv Technology Corp., Eatontown, NJ).

LC-MS/MS Method

- Instrumentation: AB Sciex 3200 QTRAP Mass Spectrometer (Foster City, CA), Shimadzu Prominence Series HPLC (Columbia, MD)
- LC Column: GL Sciences (Tokyo, Japan) Inertsil Phenyl 3 (4.6 mm x 150 mm x 5 µm) with Opti-Solv 2 micron guard column (Optimize Technologies - Portland, OR)
- Internal Standard: d3-dinotefuran
- Mass Spec Conditions: Multiple reaction monitoring (MRM) masses: dinotefuran m/z 203>129, m/z 203>114
  d3-dinotefuran m/z 206>132, m/z 206>117
- Mobile Phase: Solvent A: H_2O with 10 mM ammonium formate and 0.1% formic acid
  Solvent B: Acetonitrile with 0.1% formic acid

Results

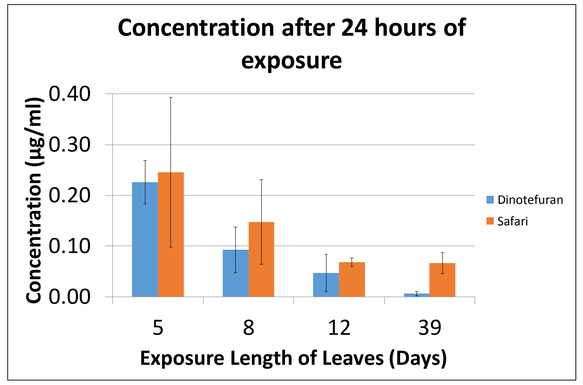

Figure 3. Concentrations of Safari and dinotefuran on honey bees after 24 hours of exposure to treated leaves. Leaves were weathered from 5 to 39 days prior to bee exposure. Results are from three samples of five bees at four time intervals, analyzed by LC-MS/MS.

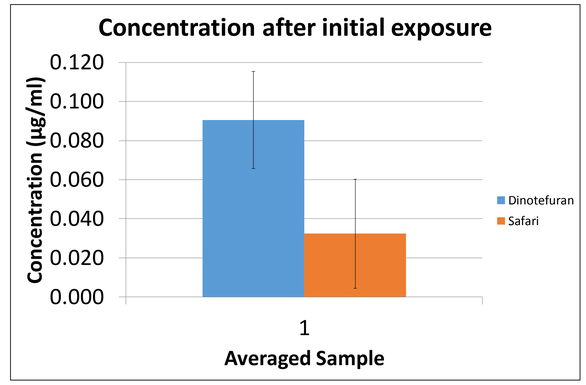

Figure 5. Concentration of Safari and dinotefuran on honey bees after immediate exposure to treated leaves. After one hour of exposure, six samples of five bees each were analyzed with LC-MS/MS.

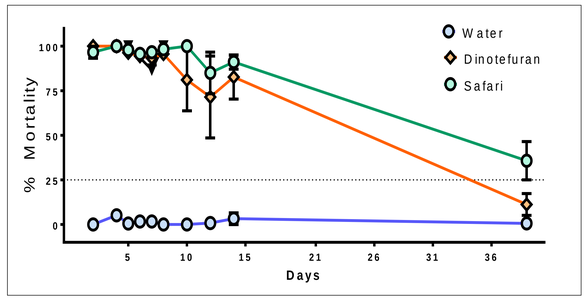

Figure 4. Residual toxicity of Safari compared to dinotefuran. Honey bees were exposed to treated leaves for 24 hours and mortality counted. The results are from ten time points with up to six replicates, consisting of up to 30 bees at each time point.

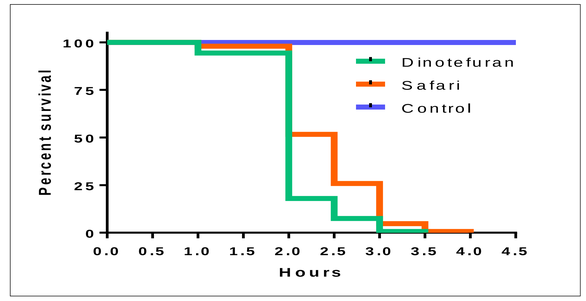

Figure 6. Toxicity of Safari and dinotefuran after leaves were sprayed and bees were immediately introduced. Dead or dying bees were then counted at half hour intervals.

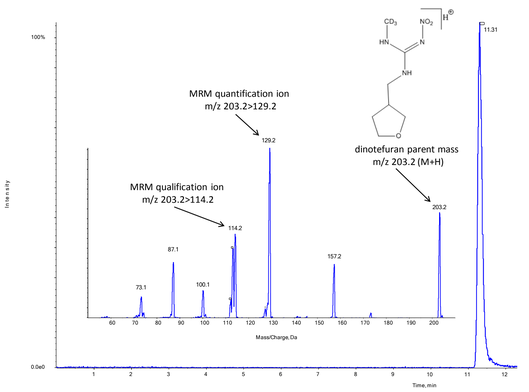

Figure 7. LC-MS/MS spectrum showing retention time and fragmentation pattern of dinotefuran. Limit of quantitation was 0.01 µg/mL.

Conclusion

Utilizing LC-MS/MS, we investigated whether Safari, which is a particulate formulation made from the neonicotinoid dinotefuran and other ingredients, poses more risk to bees than dinotefuran alone. Compared to dinotefuran, it is clear that Safari persists longer in the environment. Safari can then transfer from foliage to bees, and its residues remain lethal to bees for a longer period of time. Interestingly, when bees are exposed to leaves immediately after treatment with Safari or dinotefuran, the bees exposed to dinotefuran die more quickly. We are continuing to investigate how the particulate formulation of Safari plays a role in these differences.

Future Work

- Investigation into additional exposure pathways that may be facilitated by nanotechnology based neonicotinoids.
- Study more pesticides and NBPs that may pose risks to honey bees.

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