Supporting Information

Platform-Independent Suspect Screening Analysis of Pesticides and Transformation Products in Honey Bees across Multiple High-Resolution Mass Spectrometers

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1. Materials and methods

1.1. Liquid chromatography methods

For the LC-QqToF, two instrumental blanks were injected at the beginning of the sequence. Bee extract samples were then analyzed in order of increasing concentration (10 to 100 ng per bee) using MS^1 acquisition only. The reference standard solution was subsequently analyzed in MS^1 mode. MS^2 spectra were then acquired for the 100 ng per bee sample at two collision energies (20 and 30 eV).

For the LC-Q-Orbitrap, one instrumental blank was injected at the beginning of the sequence. Bee extract samples and the reference standard solution were then analyzed in order of increasing concentration using combined MS^1 and MS^2 acquisition.

For the LC-cIMS-MS, the sequence began with one instrument blank, followed by the reference standard solution. A second blank was injected prior to the bee extract samples, which were analyzed from lowest to highest concentration using HDMSe acquisition mode.

No sample randomization or replicate injections were performed, and injections were performed in a single batch for each instrument.

1.2. Application to incurred samples

Honeybees assigned to the 48-hour post-exposure group were kept in plastic experimental cages perforated with one opening (approximately 10 cm^2), covered with a fine mesh to allow air circulation. Throughout the experiment, a syringe containing a 50% *m/v* sucrose solution prepared in distilled water was placed at the top of each cage to provide continuous feeding. Bees were kept in the dark at 25 °C and 60% relative humidity.

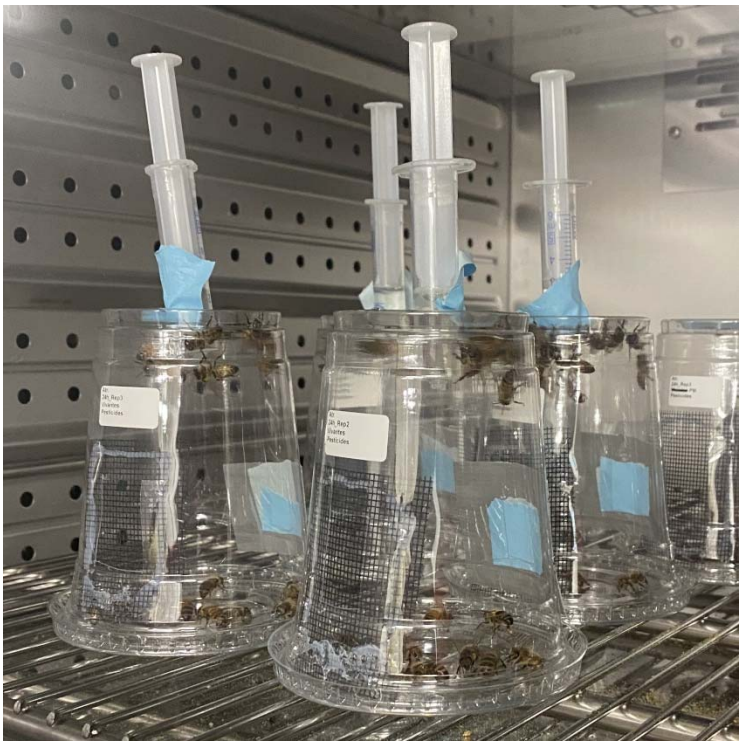

Figure S1. Photograph of the exposure experiment showing experimental cages placed inside the incubator.

Incurred honeybee samples were freeze-dried and manually homogenized using a spatula. Samples were weighted and transferred in a 50 mL polypropylene centrifuge tube. A total of 20 bees (0.6321 g) were extracted for the immediate post-exposure group and 18 bees (0.4165 g) for the 48-hour post-exposure group. Extraction was performed by adding 10 mL of acidified acetonitrile (1% v/v acetic acid) and 7.5 mL of water, followed by manual shaking for 1 min. Afterwards, 4 g of anhydrous $MgSO_4$, 1 g of NaCl, 1 g of $Na_3(citrate)\cdot 2H_2O$ and 0.5 g $Na_2H(citrate)\cdot 1.5H_2O$ were added. Subsequently, the sample was manually shaken for 1 min and then centrifuged at 3,000 rpm ($1409 \times g$) for 5 min. The samples were maintained at -80 °C for 15 minutes then centrifuged again the same way. An aliquot of 1.5 mL of the supernatant was transferred to a 2 mL polypropylene tube containing 70 mg PSA, 100 mg C_{18} and 150 mg anhydrous $MgSO_4$. The mixture was shaken for 1 min and centrifuged at 5,600 rpm ($1409 \times g$) for 5 min.

Aliquots of 67.5 µL (immediate post-exposure) and 675 µL (48-hour post-exposure) of the purified extract were transferred in glass tube and stored at 4 °C overnight. Extracts were then evaporated to dryness under a gentle nitrogen flow and reconstituted in 450 µL of water:methanol (1:1 *v/v*). Finally, the samples were filtered through 0.2 µm PTFE filters into amber glass vials.

2. Results and Discussion

2.1. Reconstitution volume

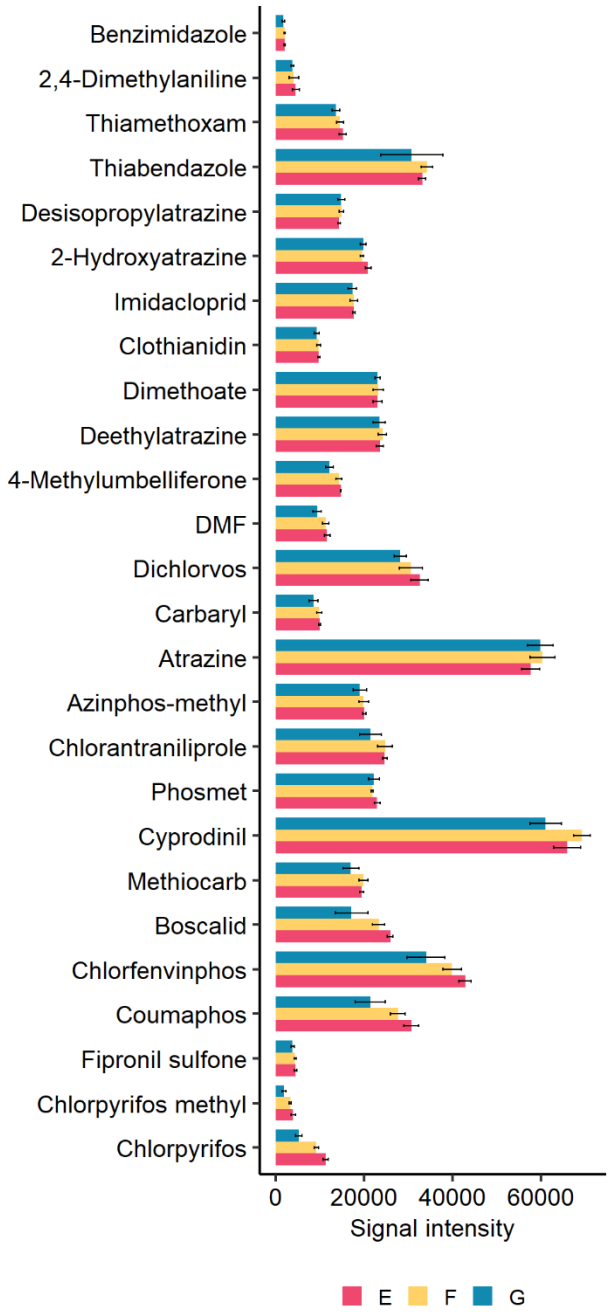

Figure S2. Signal intensities for a subset of 26 representative compounds obtained using different reconstitution volumes. The length of error bars indicates ±1 standard deviation.

2.2. Suspect screening

2.2.1. Exact mass feature detection using MZmine

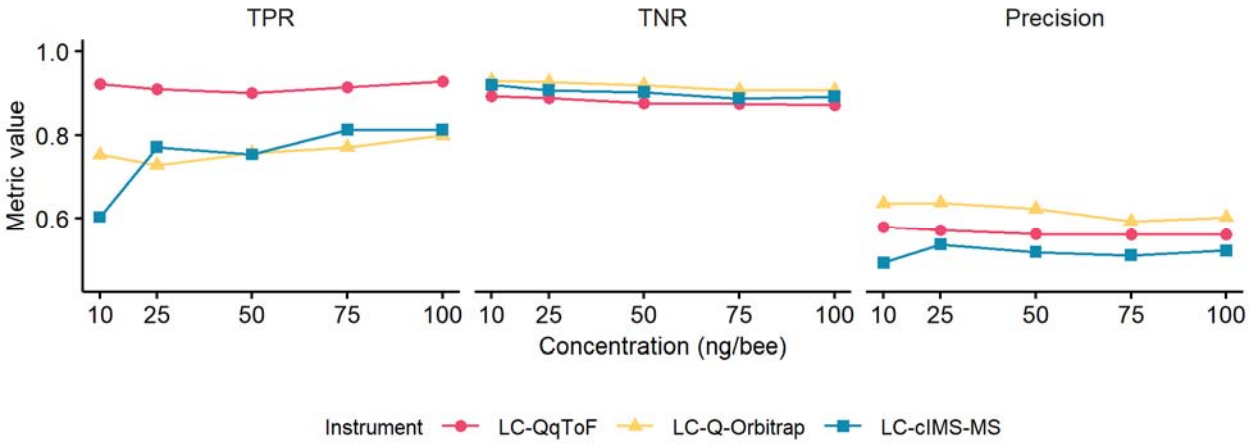

Figure S3. Performance metrics for exact mass-based suspect screening for the three analytical platforms at concentrations ranging from 10 to 100 ng per bee.