Electronic Supplementary Material

Methiocarb metabolites are systemically distributed throughout corn plants grown from coated seeds

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S1. Chemicals and reagents

Pure compounds for the preparation of standard solutions of thiamethoxam, N-desmethyl thiamethoxam, thiamethoxam-d₃, clothianidin, clothianidin-d₃, thiacloprid, thiacloprid-d₄, thiacloprid amide, methiocarb, methiocarb-d₃, methiocarb sulfoxide and methiocarb sulfone were obtained from Sigma-Aldrich (purity >99%, isotopic enrichment >97% for deuterated standards, Pestanal®). Methanol (99.8%, HiPerSolv Chromanorm®, VWR) and acetonitrile (99.9%, LiChrosolv®, VWR) were of HPLC grade and water was purified using a Millipore Milli-Q® equipment. Analytical grade magnesium sulfate anhydrous (99%, AnalAr NORMAPUR®, VWR) and sodium acetate trihydrate (99.0 %, Fluka) were used in the sample preparation step. Primary-secondary amine (PSA) sorbent was obtained from Sigma-Aldrich (Supelco® analytical). 2-chloro-5-(chloromethyl)pyridine, 2-chloroethylamine HCl, triethylamine and KSCN used for thiacloprid imine synthesis, were obtained from Aldrich® chemistry and Fluka® analytical. Thiacecloprid imine, methiocarb phenol, methiocarb sulfoxide phenol and methiocarb sulfone phenol were synthesized to be used as analytical standards.

S2. Synthesis of primary standards

Thiacloprid imine was synthesized using a two-step procedure available in the literature.¹ Briefly, 2-chloro-5-(chloromethyl)pyridine, 2-chloroethylamine HCl and triethylamine were mixed in acetonitrile for 40 h at 25°C. The intermediate 1-[(6-chloro-3-pyridinyl)methyl]-2-chloroethylamine was isolated from the crude reaction product by preparative chromatography and it was mixed with KSCN for 3 h at 95°C in a water/acetonitrile (50:50) solution. Finally, preparative chromatography was used to purify the synthetized thiacloprid imine.

Methiocarb phenol, methiocarb sulfoxide phenol and methiocarb sulfone phenol were synthesized according to a procedure reported in the literature.² Briefly, 5 mg of methiocarb, methiocarb sulfoxide, and methiocarb sulfone pure standards were dissolved in 5 mL of methanol in separated volumetric flasks. 1 mL of NH₃ 1 M was added and the solution was placed in an ultrasonic bath at 50°C for 30 minutes. In the end, 200 µL of a formic acid 5 M were added to neutralize the base. The products obtained were characterized by ¹H-NMR (Bruker 300, only thiacloprid imine) and HRMS (Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer). Their purity was determined by comparing the area of the main peak and total peak areas of the impurities, using a Shimadzu Prominence UFLC-XR chromatograph (SIL 20AC-XR autosampler; CTO-20A column oven; SPD-M20A UV–vis diode-array detector, set at λ = 202 nm) equipped with a Kinetex Biphenyl column (2.6 µm, 100x2.1 mm, Phenomenex).
S3. UHPLC-HRMS method optimization and validation

In order to identify the insecticide degradation products, a suspect screening analysis of guttation drop samples was performed. The acquisition method was a full-scan-data dependent MS² in both ESI+ and ESI- polarities. The analyte identification was based on the accurate mass of the pseudo molecular ion acquired in the full-scan mode, the characteristic isotopic pattern, and the structure was confirmed by the MS² spectra. Once the AI degradation products were identified, a list of target compounds was built for the MS² target analyses with Normalized Collision Energy (NCE) optimized for each analyte (Table S1).

| Analyteɑ | Retention Time (min) | MSX ID | ionization | NCE (eV) | Q1 precursor ion ([m/z])b | Q3 quantification ion ([m/z]) |
|----------|----------------------|--------|------------|----------|--------------------------|-------------------------------|
| thiacloprid imine (a) | 9.80 | 1 | ESI+ | 30 | 228.0357 | 126.0097 |
| methiocarb sulfide hydroxyc | 10.40 | 1 | ESI+ | 30 | 258.0795 | 185.0626 |
| clothianidin urea | 10.49 | 1 | ESI+ | 30 | 206.0149 | 131.9669 |
| desnitro thiamethoxamcd | 10.67 | 1 | ESI+ | 30 | 247.0415 | 131.9691 |
| thiamethoxam nitrosc | 10.85 | 1 | ESI+ | 30 | 276.0316 | 131.9673 |
| methiocarb sulfide phenol (a) | 11.09 | 2 | ESI+ | 40 | 185.0631 | 170.0382 |
| thiacloprid amide hydroxyc | 11.16 | 2 | ESI+ | 40 | 287.0364 | 126.0098 |
| thiamethoxam (a) | 11.34 | 2 | ESI+ | 20 | 292.0266 | 211.0631 |
| methiocarb sulfide (a) | 12.00 | 3 | ESI+ | 15 | 242.0845 | 185.0632 |
| thiacloprid amide (b) | 12.44 | 3 | ESI+ | 15 | 271.0415 | 254.0142 |
| thiamethoxam ureac | 12.51 | 3 | ESI+ | 15 | 265.0520 | 176.9699 |
| clothianidin (b) | 12.65 | 3 | ESI+ | 15 | 250.0160 | 169.0542 |
| methiocarb sulfone phenol (b) | 13.19 | 4 | ESI- | 35 | 199.0434 | 184.0211 |
| thiamethoxam N-desmethy (b) | 13.29 | 4 | ESI+ | 10 | 278.0109 | 131.9660 |
| thiacloprid SOc | 13.35 | 4 | ESI+ | 35 | 237.0538 | 237.0538 |
| thiacloprid hydroxyc | 13.51 | 4 | ESI+ | 35 | 269.0258 | 126.0108 |
| thiacloprid olefin | 13.70 | 4 | ESI+ | 30 | 251.0151 | 126.0106 |
| methiocarb sulfone (c) | 14.12 | 5 | ESI+ | 10 | 258.0795 | 122.0719 |
| thiacloprid (c) | 14.61 | 5 | ESI+ | 35 | 253.0309 | 126.0098 |
| methiocarb phenol (d) | 18.29 | 6 | ESI- | 10 | 167.0536 | 152.0307 |
| methiocarb (d) | 18.75 | 7 | ESI+ | 10 | 226.0896 | 169.0670 |
| thiamethoxam-d3 (a) | 11.34 | 2 | ESI+ | 20 | 295.0454 | 214.0818 |
| clothianidin-d3 (b) | 12.65 | 3 | ESI+ | 15 | 253.0348 | 172.0728 |
| thiacloprid-d4 (c) | 14.61 | 9 | ESI+ | 35 | 257.0560 | 126.0096 |
| methiocarb-d3 (d) | 18.75 | 10 | ESI+ | 10 | 229.1085 | 169.0666 |

ɑ(a), (b), (c) and (d) indicate which internal standard has been used for the quantification.

bthiamethoxam urea was detected as the ammonium adduct, all the other compounds were detected as [M+H]+ in positive or [M-H]- in negative mode.

canalytical standard not available. The quantification is based on the calibration curve of the closest analyte for which the standard was available.

desnitro thiamethoxam was never detected in real samples
Method performance acceptability criteria from EU guidelines were used for assessment. For validation experiments, blank samples obtained from plants treated only with fungicides were used as blank matrices both for leaves and guttations. Grounded leaf samples (four replicates) were spiked at two concentration levels (0.060 and 0.60 µg g⁻¹). The repeatability of the method was determined as the intraday relative standard deviation of these recoveries. Matrix matched calibration solutions were prepared using leaf extracts spiked after extraction. The calibration curve consisted of six points for each tested analyte equivalent to 0.030, 0.060, 0.30, 0.60, 1.5 and 3.0 µg mL⁻¹ together with 0.15 µg mL⁻¹ of IS mixture. The matrix effect was evaluated by comparing the slopes of the matrix-matched calibration curves with those obtained by diluting the analytes in a water/methanol 80:20 solution, as described in other work. Linearity was evaluated in both conditions: an F-test between linear and polynomial regression models applied to the obtained calibration functions was used to assess their linearity. The sensitivity of the method was calculated in terms of method detection and quantification limits (MDL and MQL, respectively), assessed from the matrix-matched calibration curves. For guttation samples, recovery experiments were not performed, but matrix-matched calibration curves were prepared at 0.5, 1, 5, 10, 25, 50 and 100 ng mL⁻¹ together with 2.5 ng mL⁻¹ of IS mixture. MDL and MQL values were calculated using the same method used for leaf samples. The results for method validation are summarized in Table S2. For all the analytes in both matrices, no significant differences were observed between the linear and polynomial regression models (p > 0.072 for leaf samples, p > 0.055 for guttation samples) and so the linear function was used for sample quantification. The matrix effect was considered acceptable for both guttation and leaf samples (Table S2). Considering that the sample preparation applied to guttation samples is a simple dilution, the only potential bias is the matrix effect. Therefore, these results proved that the guttation matrix (xylem sap) does not require clean-up steps prior to injection in order to obtain a proper quantification in UHPLC-ESI-HRMS. Also, the leaf extraction method used fitted the requirements of accuracy and precision for pesticide residue analysis. MDLs for guttation samples were between 0.27 and 8.7 ng mL⁻¹, while MQLs were between 0.81 and 26 ng mL⁻¹. Considering that the typical concentrations of systemic insecticides in guttation drops are in the range of µg mL⁻¹ the first days after sowing and few ng mL⁻¹ in the following month, the MDL and MQL obtained fulfilled the sensitivity required for the quantification of insecticides and their degradation products in guttation drops. MDLs for leaf samples were between 0.81 and 26 ng g⁻¹ and the MQL were between 2.4 and 79 ng g⁻¹. In conclusion, the optimized method allowed us to quantify the target list of analytes both in guttation and leaf samples and it was applied to real samples to assess the presence of insecticides and their degradation products in corn leaves and guttations.
Table S2. Performance of the validated methods for the quantification of insecticides and their degradation products in guttation drop and leaf samples.

| Analyte                    | Guttations                          | Leaves                              |
|----------------------------|-------------------------------------|-------------------------------------|
|                            | $R^2$ | Matrix effect* (%) | MDL (ng/mL) | MQL (ng/mL) | $R^2$ | Matrix effect* (%) | MDL (ng/g) | MQL (ng/g) | Recovery (%) | Mean | RSD | Mean | RSD |
| Methiocarb                 | 0.9997 | 18 | 8.7 | 26 | 0.9975 | 11 | 26 | 79 | 110 | 8.6 | 122 | 5.3 |
| Methiocarb phenol          | 0.9984 | 13 | 1.1 | 3.4 | 0.9907 | 22 | 3.4 | 10 | 95 | 6.8 | 82 | 10 |
| Methiocarb sulfoxide       | 0.9814 | 6.5 | 2.9 | 8.6 | 0.9932 | -21 | 8.6 | 26 | 98 | 8.9 | 98 | 3.2 |
| Methiocarb sulfoxide phenol| 0.9930 | -9.5 | 2.6 | 8.0 | 0.9966 | 14 | 7.9 | 24 | 86 | 3.3 | 101 | 4.5 |
| Methiocarb sulfone         | 0.9901 | 1.6 | 5.1 | 16 | 0.9915 | -10 | 15 | 47 | 71 | 2.1 | 93 | 5.5 |
| Methiocarb sulfone phenol  | 0.9952 | -14 | 1.3 | 4.0 | 0.9931 | -21 | 4.0 | 12 | 126 | 4.2 | 108 | 4.2 |
| Thiacloprid                | 0.9933 | 5.2 | 2.4 | 7.3 | 0.9895 | -7 | 7.2 | 22 | 101 | 3.0 | 109 | 6.0 |
| Thiacloprid amide          | 0.9880 | 17 | 1.9 | 5.7 | 0.9943 | -19 | 5.7 | 17 | 97 | 2.6 | 69 | 16 |
| Thiacloprid imine          | 0.9963 | -4.2 | 0.49 | 1.5 | 0.9932 | -22 | 1.5 | 4.5 | 113 | 10 | 84 | 3.0 |
| Clothianidin               | 0.9998 | 1.9 | 3.4 | 10 | 0.9895 | 13 | 10 | 31 | 79 | 2.4 | 91 | 4.7 |
| Thiamethoxam               | 0.9804 | 4.3 | 1.0 | 3.1 | 0.9977 | 6 | 3.1 | 9.3 | 71 | 3.3 | 113 | 5.5 |
| N-desmethyl thiamethoxan   | 0.9980 | 18 | 1.0 | 3.1 | 0.9494 | -9 | 21 | 64 | 110 | 4.6 | 69 | 4.8 |

* The matrix effect was evaluated by comparing the slopes of the matrix-matched calibration curves with those obtained by diluting the analytes in a water/methanol 80:20 solution.

S4. Thiamethoxam and its metabolites in corn plants grown in pots

Seven guttation samples were collected from plants treated with thiamethoxam. For this insecticide, but also for the other analytes, a significant concentration trend over time was not observed, likely because of the high sample-to-sample variability. The active ingredient (AI) was always detected, and its mean concentration was 5.0±1.6 µg mL$^{-1}$. Also, clothianidin was identified in all the analyzed samples with a mean value of 1.33±0.31 µg mL$^{-1}$. Clothianidin is a well-known thiamethoxam metabolite, but it is also a widely used systemic insecticide. Other metabolites identified were clothianidin urea, N-desmethyl-thiamethoxam, thiamethoxam nitroso and thiamethoxam urea. The insecticide and its degradation products were detected also in corn leaves: the AI was always detected, and its mean concentration was 7.4±3.5 µg g$^{-1}$. Clothianidin was the main metabolite, followed by thiamethoxam urea. All the other thiamethoxam metabolites previously identified in corn guttations were identified also in corn leaves in this study (Table S3).

Corn seedlings, grown from coated seeds, are also a potential exposure route to systemic insecticides for non-target insects, birds and small herbivore mammals that may eat these young plants. Also, unburied coated seeds are dangerous from this point of view in particular for birds. The results obtained from corn leaf analysis revealed that neonicotinoids were present in corn seedling at high concentrations (µg g$^{-1}$ level). This is due to the high amount of AI applied to the seeds with respect to the low weight of the whole plant during the first weeks after sowing. Clothianidin and thiamethoxam urea were the main degradation products identified in plants coated with...
thiamethoxam. Clothianidin is considered moderately toxic for some bird species (e.g. clothianidin LD$_{50}$ for Japanese quail, *Coturnix japonica*, is 423 µg g$^{-1}$).

Table S3. Mean concentrations of thiamethoxam and its metabolites detected in corn guttations and leaves. For calculations, when concentration was below MDL it was considered zero and when it was below MQL the MDL value was assigned.

| Analyte                        | Guttations (µg mL$^{-1}$) | Leaves (µg g$^{-1}$) |
|--------------------------------|---------------------------|----------------------|
|                                | mean | SD | median | 1st quartile | 3rd quartile | mean | SD | median | 1st quartile | 3rd quartile |
| thiamethoxam                   | 5.0  | 1.6| 4.6    | 4.4          | 5.5          | 7.4  | 3.5| 7.3    | 5.5          | 8.0          |
| clothianidin                   | 1.33 | 0.31| 1.32   | 1.26         | 1.39         | 2.0  | 1.0| 1.8    | 1.4          | 2.6          |
| clothianidin urea              | 0.036| 0.018| 0.031 | 0.023        | 0.043        | 0.055| 0.037| 0.042 | 0.030        | 0.070        |
| thiamethoxam urea              | 1.49 | 0.53| 1.80   | 1.08         | 1.90         | 0.49 | 0.37| 0.44   | 0.20         | 0.67         |
| N-desmethyl thiamethoxam       | 0.014| 0.007| 0.017 | 0.008        | 0.019        | 0.059| 0.053| 0.021 | 0.011        | 0.110        |
| thiamethoxam nitroso           | 0.045| 0.022| 0.044 | 0.032        | 0.059        | 0.019| 0.009| 0.019 | 0.013        | 0.023        |
| thiacloprid                    | 1.02 | 0.57| 0.93   | 0.75         | 1.20         | 4.7  | 3.8 | 3.3    | 2.4          | 6.0          |
| thiacloprid amide              | <0.0057| /  | /     | /            | /            | 0.23 | 0.28| 0.11   | 0.066        | 0.26         |
| thiacloprid imine              | 0.0025| 0.0001 | 0.0025 | 0.0025       | 0.0026       | 0.033| 0.029| 0.029 | 0.017        | 0.036        |
| thiacloprid amide hydroxy      | 0.0025| 0.0009| 0.0024 | 0.0021       | 0.0028       | 0.039| 0.033| 0.030 | 0.012        | 0.060        |
| thiacloprid SO                 | 0.0046| 0.0033| 0.0038 | 0.0026       | 0.0058       | 0.0047| 0.0022| 0.0041| 0.0033        | 0.0056       |
| thiacloprid hydroxy            | 0.018 | 0.012| 0.018  | 0.012        | 0.024        | 0.020| 0.017| 0.013 | 0.007        | 0.027        |
| thiacloprid olefin             | 0.117 | 0.079| 0.124  | 0.085        | 0.157        | 0.0084| 0.0077| 0.0062| 0.0045        | 0.0094       |

S5. Thiacloprid and its metabolites in corn plants grown in pots

For thiacloprid, four guttations samples were analyzed. Thiacloprid was always detected and its mean concentration was 1.02±0.57 µg mL$^{-1}$. In addition, five metabolites were identified: thiacloprid amide, thiacloprid hydroxy, thiacloprid olefin, thiacloprid imine, thiacloprid hydroxyl amide and thiacloprid with a sulfur atom substituted by an oxygen atom (thiacloprid SO). In corn leaves, thiacloprid mean concentration was 4.7±3.8 µg g$^{-1}$. In addition, all its metabolites were also detected, the main one being thiacloprid amide but its concentration is much lower if compared to the active ingredient (Table S3).

Few data are available about the toxicity of thiacloprid metabolites and so it is difficult to assess their effects on non-target animals. However, thiacloprid amide is reported to have 15.6 times lower mortality than the parent compound against the pest *Aphis craccivora*. In addition, among the identified metabolites, a modification of the cyano group for thiacloprid often occurred. This may lead to an inversion of selectivity between insects and mammals.

The use of thiacloprid as seed-coating insecticide replacing thiamethoxam, clothianidin and imidacloprid that are currently banned, does not circumvent the environmental problem associated with contamination of guttation drops. In fact, albeit thiacloprid is less toxic for honeybees, the high
content of the active ingredient and its metabolites may constitute a relevant exposure route for wild insects feeding on these contaminated water sources.

S6. References

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