**S1: LC-MS/MS analytical procedures**

*Reagents and solvents*

Analytical standards of carbofuran (purity > 98%) were obtained from Merck KGaA (Darmstadt, Germany). Acetonitrile and methanol (Optima® LC-MS grade) were purchased from Fischer Chemical (Waltham, MA, USA). Internal standard triphenyl phosphate (TPP) and all other chemicals of the best LC-MS available purity grade were purchased from Merck KGaA (Darmstadt, Germany). Agilent Bond Elut QuEChERS EN Method (buffer salt mixture, polypropylene (PP) tubes, and sorbents for dispersive - SPE (dispersive solid phase extraction) including 50 mg primary secondary amine (PSA), 50 mg C18, and 50 mg graphitized carbon black (GCB) were obtained from Agilent Technologies (Santa Clara, CA, USA). The ultra-pure water was obtained from the Millipore Direct-Q3-UV purification system (Merck KGaA, Germany). Stock solution of carbofuran at the concentration of 1000 mgL-1 was prepared by dissolving the analytical standard in methanol and stored at 4°C in a refrigerator. The standard working solution for the matrix-matched calibration was prepared daily before the analysis by diluting the stock solutions with methanol.

*Sample preparation*

The extraction of carbofuran was carried out from lyophilised samples using the modified QuEChERS method. In brief, a representative 1.0-g portion of the liver sample was weighed into a 50-mL polypropylene (PP) tube and mixed thoroughly with 7.5 ml of miliQ water and 200 µL of 1 µg mL-1 TPP (triphenyl phosphate) used as an internal standard. Then, 10 mL of acetonitrile was added and the sample was shaken for 1 min by hand and sonicated for 15 minutes in an ultrasonic bath. Next, 4 g MgSO4 anhydrous, 1 g NaCl, 1 g tri-sodium citrate dehydrate, and 0.5 g disodium hydrogen citrate sesquihydrate were added. The mixture was shaken vigorously by hand for 1 min and then centrifuged for 5 min at 4498 g. A 1-mL aliquot of the supernatant was transferred into a 2-mL centrifuge tube containing 150 mg anhydrous MgSO4, 50 mg PSA, 50 mg C18, and 50 mg GCB and the tube was shaken vigorously for 2 min and centrifuged for 5 min at 12,100 g. The cleaned extract was transferred into an autosampler vial for LC-MS/MS analysis.

*LC-MS/MS analysis*

LC-MS/MS analysis was performed using an Agilent 1290 Infinity LC system coupled to a Q-TOF mass spectrometer model 6550 iFunnel Agilent Technology. The chromatographic separation was carried out on a Zorbax Extend C18 Rapid Resolution analytical column (2.1×100 mm and 1.8 μm particle size, Agilent Technology). Mobile phases A and B were water and acetonitrile respectively, both with 0.1% formic acid. A linear gradient from 5% B to 95% B in 15 min was applied, which was followed by a 95% B hold for 3 min and column conditioning for 4 min at 5%B; the flow rate was 0.5 mL min-1. The injection volume was set to 5 µL and the column temperature was held at 50°C. The QTOF-MS/MS instrument was operated in the Jet Stream positive electrospray ionization mode (ESI+) using the following parameters: capillary voltage 3000 V; nebulizer pressure 40 psi; drying gas 12 L min-1; gas temperature 250°C, and fragmentor voltage 350V. The accurate MS and MS/MS mass spectra were acquired from 100-1000 m/z and 20-1000 m/z, respectively. The product spectrum was collected at two collision energies, 20 and 40. The reference ions of m/z 121.0509 and 922.0098 were used for internal mass correction. LC-QTOF was controlled with Agilent Mass Hunter Data Acquisition (B.09) software and data were processed with Agilent Mass Hunter Qualitative Analysis (B.07) and Agilent Mass Hunter Profinder (B.10) software; both Molecular Feature Extractor and Find by Formula algorithms were used.

*Identification criteria and quantitative analysis*

Confirmation of carbofuran in the liver samples followed by quantitative analysis was carried out according to the SANTE 11813/2017 European Commission guideline for analysis of pesticides. Identification was based on the retention time (±0.2 min), accurate mass with an acceptable ion mass error < 5 ppm, isotopic distribution with a matching score > 95%, and MS/MS spectra with a matching score > 90%. In the first step, the liver sample extract was analyzed by LC-QTOF working in the MS scan. The data were then extracted and screened across an accurate-mass database using the Agilent Mass Hunter Profinder software. The Agilent Pesticide accurate-mass database was used to confirm the presence of carbofuran in the liver sample. The extract was then reanalysed in the second injection in the targeted MS/MS mode at two collision energies, 20 and 40 V. Final confirmation was carried out by comparing the experimental MS/MS spectrum with the MS/MS spectrum obtained by injection of the analytical standard of carbofuran in the LC-Q-TOF system. The quantification of carbofuran was performed for the protonated ion [M+H]+ of m/z 222.1125. The calibration curve was prepared from matrix-matched standards using the internal standard method. The liver samples were spiked with appropriate volumes of the carbofuran working standard to reach the concentrations in the range of 0.05–0.6 mg kg-1. The limit of quantification (LOQ) was determined as the lowest calibration level that was quantified with acceptable accuracy and precision, as described in the guideline SANTE 11813/2017 document (SANTE 2017). All extracts from livers of examined birds were analysed directly after extraction; when it was necessary, the extracts were diluted or concentrated before analysis.

References

SANTE/EU (2017) Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. SANTE/11813/2017. Leg. Depos, 2017, vol. 11813, pp. 1–46.