Is there a risk to honeybees from use of thiamethoxam as a sugar beet seed treatment?

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Abstract
The ban imposed by the European Union on the use of neonicotinoids as sugar beet seed treatments was based on the exposure of bees to residues of neonicotinoids in pollen and nectar of succeeding crops. To address this concern, residues of thiamethoxam (TMX) and clothianidin (CTD) were analyzed in soil collected from fields planted in at least the previous year with thiamethoxam-treated sugar beet seed. This soil monitoring program was conducted at 94 sites across Germany in two separate years. In addition, a succeeding crop study assessed residues in soil, guttation fluid, pollen, and nectar sampled from untreated succeeding crops planted in the season after thiamethoxam seed-treated sugar beet at eight field sites across five countries. The overall mean residues observed in soil monitoring were 8.0 ± 0.5 µg TMX + CTD/kg in the season after the use of treated sugar beet seed. Residue values decreased with increasing time interval between the latest thiamethoxam or clothianidin application before sugar beet drilling and with lower application frequency. Residues were detected in guttation fluid (2.0–37.7 µg TMX/L); however, the risk to pollinators from this route of exposure is likely to be low, based on the reported levels of consumption. Residues of thiamethoxam and clothianidin in pollen and nectar sampled from the succeeding crops were detected at or below the limit of quantification (0.5–1 µg a.i./kg) in 86.7% of pollen and 98.6% of nectar samples and, unlike guttation fluid residues, were not correlated with measured soil residues. Residues in pollen and nectar are lower than reported sublethal adverse effect concentrations in studies with honeybee and bumble bee individuals and colonies fed only thiamethoxam-treated sucrose, and are lower than those reported to result in no effects in honeybees, bumble bees, and solitary bees foraging on seed-treated crops. Integr Environ Assess Manag 2022;18:709–721. © 2021 SYNGENTA Integrated Environmental Assessment and Management published by Wiley Periodicals LLC on behalf of Society of Environmental Toxicology & Chemistry (SETAC).

KEYWORDS: Honeybee, Risk assessment, Seed treatment, Sugar beet, thiamethoxam

INTRODUCTION
Neonicotinoid seed treatments have been an important tool for sugar beet farmers in Europe for the control of arthropods, including aphids spreading virus yellows (a complex of three different viruses that includes beet mild yellowing virus, beet chlorosis virus, and beet yellows virus; Hauer et al., 2017; Hossain et al., 2021). Until 2018, when the EU Commission banned all outdoor use of the neonicotinoid seed treatments containing thiamethoxam, clothianidin, or imidacloprid (European-Commission, 2018a, 2018b, 2018c), almost all conventionally cultivated sugar beet fields used neonicotinoid seed treatments (Hauer et al., 2017). Additional foliar insecticide applications during the growing period, for example, with carbamates or pyrethroids, were conducted only rarely (Hauer et al., 2017; Ladewig et al., 2018). Recent reports from across Europe have revealed that the impact of removing this seed treatment tool from farmers has resulted in up to 50% sugar beet crop loss as a result of virus yellows reported in France (USDA, 2020) and similar problems in other countries (Jha, 2020). Removal of the neonicotinoid seed treatments has also resulted in sugar beet farmers resorting to increased use of other insecticides as foliar sprays (Dewar & Qi, 2021).

The ban imposed by the European Union on the use of neonicotinoids as sugar beet seed treatment was based on concerns about the exposure of bees to residues of neonicotinoids in pollen and nectar from crops planted in fields previously used to grow sugar beet from neonicotinoid-treated seed, that is, succeeding crops...
Sugar beet is a biennial plant that is harvested after its first season of vegetative growth and before flowering. Sugar beet flowers in the second year after vernalization during winter; only in rare cases (<0.05%) do flowering bolters occur in the first year (Hoffmann et al., 2020; Milford, 2006). Exposure via pollen and nectar of the treated crop can thus be excluded with high probability. Guttation fluid exuded from leaves of sugar beet crops has also been demonstrated to be rare (Wirtz et al., 2018). As sugar beet seed is pelleted, dust levels from seed are low and contamination of neighboring flowering crops or weeds from dust generated during drilling is negligible (Forster et al., 2012; Zwercvaegher et al., 2016).

The European Food Safety Authority (EFSA) succeeding crop scenario is based on the potential exposure of bees caused by persistence in soil of pesticide residues from previous-season seed treatments, uptake by subsequent bee-attractive crops planted in the same fields, and movement to pollen and nectar. Based on European soil data (field DT90 data 23.7–307 days; Hilton et al., 2016), thiamethoxam residues would be expected to be almost totally degraded after one year, with negligible residues remaining in soil. Mass recovery in drainage water is less than 2% of seed-applied thiamethoxam (Frame et al., 2021; Wettstein et al., 2016). One of the resulting soil metabolites is clothianidin, which is also an active substance in the class of neonicotinoids, with lower water solubility and mobility than thiamethoxam (Hilton et al., 2016, 2019) and European field DT90 of 387 days (PPDB, 2020). However, although degradation rates of thiamethoxam in the field are comparable between spray application (DT50 18.3 days) and seed treatments (DT50 16.5 days) in side-by-side trials, the metabolism route clearly differs; mean maximum clothianidin concentrations are four-fold lower after seed treatments (3.4%), compared with spray application (17.5%) in field studies (Hilton et al., 2019).

Sugar beet is usually included in a cropping cycle with cereals such as wheat or barley (CIBE, 2018); these are the succeeding crops on 84% of the sugar beet acreage in Germany (Hauer-Jälki et al., 2017) and are not considered bee-attractive (Ctgb, 2015; Requier et al., 2015a, 2015b). Maize, which is wind pollinated but when forage is limited may be visited by honeybees during pollen-shedding (Ctgb, 2015; USDA, 2018), is also grown in sugar beet rotations, but to a much more limited extent for phytosanitary reasons. The EFSA review (EFSA, 2018c) took the default position that bee-attractive succeeding crops are planted after sugar beet. The review’s authors expressed concern regarding the limited data available on subsequent residues in pollen and nectar of bee-attractive crops after use of thiamethoxam as a seed treatment on sugar beet. The lack of flowering crops planted after sugar beet is supported by reported honey residue data. A survey of UK honey after the 2013 ban on uses in flowering crops demonstrated residues of thiamethoxam in 6.5% of honey samples (mean 0.05 µg/kg) reducing to 0% by 2017, and clothianidin in 16.6% (mean 0.12 µg/kg) reducing to 12% by 2017 (mean 0.10 µg/kg; Woodcock et al., 2021).

This study aimed to assess the range of thiamethoxam and clothianidin residues in soil after the use of thiamethoxam-treated seed in sugar beet growing areas of the EU, the implications for uptake into succeeding crops and/or weeds, and the risk posed to honeybees and other bee pollinators. In the first part of the study, in two independent years, the level of residues of thiamethoxam and clothianidin present in soil from 94 fields with succeeding crops after sugar beet in Germany was determined. These data were used to determine the range of residues in soils in which sugar beet had been grown over several years and to assess the representativeness of the fields selected for the succeeding crop study, the second part of the study that analyzed residues in pollen and nectar.

A field study (Thompson et al., 2019) reported thiamethoxam and clothianidin residues in pollen and nectar sampled from succeeding crops and/or weeds after thiamethoxam seed-treated sugar beet: That study was limited in scope to three field sites in Germany and Austria. To better assess the scale of uptake of residues from soil into pollen and nectar for a range of succeeding crops and/or weeds in representative EU sugar beet growing areas, we assessed a further eight field sites across five countries in this study. As the molar mass and acute toxicity of clothianidin and thiamethoxam to honeybees are similar (EFSA, 2018a, 2018c), the residues of each active ingredient were summed to provide a total TMX + CTD residue per sample. These data were compared with previously reported pollen and nectar residue data, and the risk posed to bees is discussed.

**MATERIALS AND METHODS**

**Soil residue monitoring**

In 2017 and 2019, fields were selected in which thiamethoxam seed-treated sugar beet (Cruiser Force, 60 g thiamethoxam per unit of 100 000 seeds) had been grown in 2016 or 2018, respectively, and that were representative of sugar beet cropping areas and succeeding crops in Germany (Table S1). In all, 94 sites were selected, 50 in 2017 and 44 in 2019, 30% in the west, 15% in the east, 31% in the south, and 24% in the north of Germany. With regard to soil texture, 67% of the sites were located on loam soils; the remainder were on sandy soils; or soil textures were not recorded. Winter wheat was the crop grown after sugar beet at 79.8% of the sites, with other cereals making up an additional 7.4%; other crops were potato (two sites), maize (seven sites), fallow (one site), and not recorded (two sites).

The field history and tillage practices, as well as the sugar beet sowing rate, were recorded for each site. The crops planted in 2017 or 2019 after sugar beet (2016 or 2018) were not treated with thiamethoxam or clothianidin. Soil samples were taken in April and July in each year by taking twenty 20 cm soil cores from a subplot (1 ha) within each field in a W-pattern and pooled to form one representative sample (min. 2 kg) for the whole plot. All samples were
placed in cooled conditions (gel packs) until transferred to the freezer (max. −18 °C) and shipped frozen to an analytical laboratory (Eurofins Agroscience Services EcoChem GmbH).

**Succeeding crop study**

Fields with varying soil types at eight trial sites were identified in Germany (two sites), the UK (two sites), Poland (two sites), Austria (one site), and Italy (one site; Table S2). Farm pesticide application records confirmed that neither thiamethoxam nor clothianidin had been applied to these fields in the previous five years at seven sites and at the remaining site (GB-03) in at least the previous three years (further information was not available due to a change in ownership of the farm). A treated (mean 0.59 ± 0.07 ha/plot) and a control plot (mean 0.52 ± 0.05 ha/plot) were established on the field at each trial site.

For the treated seed, thiamethoxam was applied to pelleted sugar beet seed (variety: KWS Vulcania) as Cruiser 600 FS, a flowable concentrate formulation for seed treatment containing nominally 600 g thiamethoxam per liter. The pelleted seed was also treated at a commercial rate with thiram as a protective fungicide. Seed treatment was performed by Syngenta Seeds AB on 1 March 2017. The seeds were treated at a nominal rate of 0.45 mg thiamethoxam per seed (actual rate 0.462 mg thiamethoxam per seed) and were drilled in each treated plot in spring 2017 at a rate of 1.24–1.34 units/ha (equivalent to 57–64 g a.i./ha; Table S2).

Each control plot was drilled with pelleted sugar beet seed treated only with the same rate of thiram as the thiamethoxam-treated seed of the same variety in spring 2017, according to normal commercial practice at the same rate as the treated seed. The control plot was drilled before the treated plot. These control plots were used to provide blank material for analytical methods.

The sugar beet was grown to maturity and harvested according to normal commercial practice (Table S2). In the following spring (i.e., spring 2018), four representative untreated succeeding crops (maize, potato, spring oilseed rape, and *Phacelia tanacetifolia*) were drilled into the plots previously used to grow the sugar beet and cultivated according to normal commercial practice, thus affording four side-by-side control and treated subplots at each trial site. We used spring-sown oilseed rape and *Phacelia* as a worst-case scenario based on timing from harvesting of the sugar beet to drilling. Neither is commonly planted after sugar beet in crop rotations.

**Soil sampling.** Soil samples were collected from all control and treated plots once at 0–3 days, before drilling of the sugar beet seed, and once at 0–1 days, before drilling of the succeeding crops. Additionally, samples of soil were collected from the maize treated subplots once at 0–16 days, after emergence (BBCH 11-16), and once from all treated subplots at 1–8 days, after the start of flowering of each succeeding crop (BBCH 59-67). Soil was sampled using a soil borer, plastic tubes of appropriate size (length 30 cm), and plastic caps to collect soil cores of 5 cm diameter to a depth of 30 cm. For samples taken from the whole plot, 20 soil cores were collected systematically from across the whole plot, following a W-pattern, and pooled to form one representative sample for the whole plot. Samples from the treated subplots comprised 10 soil cores collected from each by following a diagonal line across the subplot and pooled to form one representative sample for each subplot. All samples were placed in cooled conditions (gel packs) until transferred to the freezer (max. −18 °C) and shipped to the analytical laboratory (CEMAS) using a freezer truck.

**Succeeding crop sampling.** Guttation fluid was collected weekly directly from maize plants, from emergence until 42 days after emergence. The fluid was collected by hand from leaves, from at least 12 maize plants across the subplot. A minimum of 1.0 mL of maize guttation fluid was collected per replicate at each sample time point using one-way glass pipettes and transferred into labeled plastic tubes. The collection of the maize guttation fluid was performed in the morning, starting within 3 h of sunrise. If rainfall occurred immediately before sampling, no samples were collected.

For maize and potato, pollen and anthers, respectively, were collected by hand from at least 12 plants, across the subplot at the start, middle, and end of flowering. For maize, pollen was collected by shaking the tassels over a sieve into a plastic bag. Potato anthers were collected by removing the anthers from the blossom with tweezers.

For oilseed rape and *Phacelia*, three insect-proof tunnels (each 108 m²) were placed on each of the treated and control subplots. A honeybee (*Apis mellifera*) colony was placed into each of the tunnels at the start of flowering (BBCH 61–65). At the start, middle, and end of flowering (Table S2) the hives were closed at the beginning of each sampling day and bees were caught in front of the hives or directly from flowers across the entire tunnel and placed in a box containing dry ice. The pollen sacs (corbicular pollen) were collected from the frozen, dead bees and placed in a vessel. For nectar sampling, honey stomachs were collected from 20 frozen, dead bees by dissecting the bees. The honey stomach contents were collected and transferred to a vessel.

All samples were placed in cooled conditions (gel packs) until transferred to the freezer (max. −18 °C) and shipped to the analytical laboratory (CEMAS) using a freezer truck.

**Residue analysis**

Full details of the soil extraction and analysis method are provided in Thompson et al. (2019). In summary, soil was extracted with aqueous ammonium acetate/acetonitrile, centrifuged, and filtered. The extraction solution was concentrated, and the volume was adjusted with water and mixed. An aliquot was then filtered through a PTFE syringe filter, and a 1:1 dilution of the filtrate was prepared with 50 mM ammonium acetate/methanol. Analysis was performed using a high-performance liquid chromatograph (HPLC) with triple quadrupole mass spectrophotometric
(LC-MS/MS) detection. Recovery samples were prepared by fortification of untreated soil samples with a mixture of thiamethoxam and clothianidin (CGA322704), followed by a sample workup as described above.

For the samples from the soil monitoring (analysis conducted by Eurofins Agroscience Services EcoChem GmbH), the limit of detection (LOD) was defined as 30% of the limit of quantification (LOQ), which was 1 µg/kg soil for thiamethoxam and 0.1 µg/kg for clothianidin. The mean recovery for thiamethoxam and clothianidin was 103% and 92% at LOQ and 102% and 95% at 10 times LOQ, respectively. For the samples from the succeeding crop study (analysis conducted by CEMAS), the mean recovery for thiamethoxam at a 1 and 10 µg/kg fortification level was 90%. The mean recovery for clothianidin at 0.1 and 100 µg/kg fortification levels was 94%. In both sets of analyses, a linear detector response was obtained between 0.06 and 100 ng/mL for thiamethoxam and between 0.006 and 10 ng/mL for clothianidin with $r > 0.995$.

Full details of the pollen and anther and nectar extraction and analysis method are provided in Thompson et al. (2019). For guttation fluid, the method followed that for nectar. In summary, pollen and nectar were extracted by vigorous shaking with methanol/0.2% formic acid in ultrapure water. Sample cleanup was performed by solid-phase extraction using Oasis HLB cartridges. Final determination was by HPLC with triple quadrupole mass spectrometric detection (LC-MS/MS). Matrix-matched standards were used for the quantification of thiamethoxam and clothianidin in undiluted extracts of pollen and in a surrogate control nectar matrix (36% sugar solution in ultrapure water).

The matrices used to prepare the matrix-matched standards and determine procedural recoveries contained no residue greater than the LOD (30% LOQ). The LOQ for thiamethoxam was 0.5 µg/kg nectar, 1.0 µg/kg pollen (anther), and 10 ng/L guttation fluid. The LOQ for clothianidin was 1.0 µg/kg nectar, 1.0 µg/kg pollen (anther), and 10 ng/L guttation fluid. Mean recovery in maize, oilseed rape, and *Phacelia* pollen samples fortified at LOQ and 10 times LOQ were 92%, 94%, and 94% respectively, for thiamethoxam, and 100%, 86%, and 92%, respectively, for clothianidin. Mean recovery in potato anther samples fortified at LOQ and 10 times LOQ were 92%, 94%, and 94% respectively, for thiamethoxam, and 100%, 86%, and 92%, respectively, for clothianidin. Mean recovery in potato anther samples fortified at LOQ and 10 times LOQ was 90% for thiamethoxam and 95% for clothianidin. Mean recovery in nectar samples fortified at LOQ and 10 times LOQ was 98% for thiamethoxam and 97% for clothianidin. Mean recovery in guttation samples fortified at LOQ to 5000 times LOQ was 95% for thiamethoxam and 99% for clothianidin.

Where sample size permitted, the sugar content (in degrees Brix [i.e., refractive index]) of nectar samples was measured using a manual refractometer. The refractometer was calibrated with sugar solutions of known concentration before use.

**Statistical analysis of soil monitoring data**

To perform data analyses, where residues were less than the LOD or LOQ, these values were replaced with 50% of LOQ or LOD, respectively. Data were checked for normal distribution and variance homogeneity with SAS Desktop-Version 9.4 (SAS Institute Inc.) according to the recommendations by Kozak and Piepho (2018). Total residue (TMX + CTD) data were normalized by square-root transformation after removing two outliers. Significance of differences was tested by F-statistics based on Restricted Maximum Likelihood estimates of the variance components using SAS proc mixed and post hoc t-tests. Boxplots were created with SigmaPlot 14.4 (Sysstat Software Inc.). For thiamethoxam, no transformation was found to meet the assumptions for F-statistics. Therefore, the Wilcoxon signed rank test for paired comparisons was performed to compare soil residues in April and July using SigmaPlot.

**RESULTS**

**Field monitoring study soil residues**

Field histories were available for 90 of the 94 sites (Table S1). The sugar beet sowing rate ranged from 0.9 to 1.2 (2016) or 1.27 (2018) units of seed per ha (mean and median 1.1 units per ha in both years) which, based on a treatment rate of 60 g thiamethoxam/unit, equates to 54–76 g thiamethoxam/ha (mean and median 66 g thiamethoxam/ha). In the five years before the sugar beet was planted in 2016 or 2018, 20% of the sites had not previously been planted with thiamethoxam or clothianidin-treated seed, 77% had been planted once, and 3% had been planted twice previously. Previous use of thiamethoxam or clothianidin seed treatment in the fields had occurred at least two years earlier, with the majority being used at least three years earlier (92% before the 2016 sowing and 95% before the 2018 sowing; Table S1 and Figure S1). The field histories also revealed that, during the previous five years, thiamethoxam was applied only on sugar beet seed; 48% of the fields had been planted with thiamethoxam or clothianidin-treated seed, 77% had been planted once, and 3% had been planted twice previously. Previous use of thiamethoxam or clothianidin seed treatment in the fields had occurred at least two years earlier, with the majority being used at least three years earlier (92% before the 2016 sowing and 95% before the 2018 sowing; Table S1 and Figure S1). The field histories also revealed that, during the previous five years, thiamethoxam was applied only on sugar beet seed; 48% of the fields had been planted with thiamethoxam or clothianidin-treated seed, 77% had been planted once, and 3% had been planted twice previously. Previous use of thiamethoxam or clothianidin seed treatment in the fields had occurred at least two years earlier, with the majority being used at least three years earlier (92% before the 2016 sowing and 95% before the 2018 sowing; Table S1 and Figure S1). The field histories also revealed that, during the previous five years, thiamethoxam was applied only on sugar beet seed; 48% of the fields had been planted with thiamethoxam or clothianidin-treated seed, 77% had been planted once, and 3% had been planted twice previously. Previous use of thiamethoxam or clothianidin seed treatment in the fields had occurred at least two years earlier, with the majority being used at least three years earlier (92% before the 2016 sowing and 95% before the 2018 sowing; Table S1 and Figure S1). The field histories also revealed that, during the previous five years, thiamethoxam was applied only on sugar beet seed; 48% of the fields had been planted with thiamethoxam or clothianidin-treated seed, 77% had been planted once, and 3% had been planted twice previously. Previous use of thiamethoxam or clothianidin seed treatment in the fields had occurred at least two years earlier, with the majority being used at least three years earlier (92% before the 2016 sowing and 95% before the 2018 sowing; Table S1 and Figure S1).
TABLE 1 Mixed model analysis of variance for factors affecting residue concentration of thiamethoxam + clothianidin in soil taken from fields planted with sugar beet with thiamethoxam seed dressing in the previous year; 84 fields in Germany 2017 and 2019

| Effect | Num | df  | Den df | F value | Prob F |
|--------|-----|-----|--------|---------|--------|
| Year   | 1   | 75.02 | 0.38 | 0.5385 |
| Month  | 1   | 78.79 | 0.24 | 0.6275 |
| Year x Month | 1 | 78.87 | 25.30 | <0.0001 |
| Time since latest application | 2 | 75.35 | 3.50 | 0.0353 |
| Year x time since latest application | 2 | 75.09 | 0.36 | 0.7008 |
| Month x time since latest application | 2 | 78.76 | 0.50 | 0.6098 |
| Tillage | 1 | 74.94 | 4.43 | 0.0386 |
| Year x Tillage | 1 | 75.56 | 4.44 | 0.0384 |
| Month x Tillage | 1 | 79.00 | 0.13 | 0.7217 |
| Tillage x Time since latest application | 2 | 75.24 | 0.47 | 0.6252 |

Abbreviations: df, degrees of freedom; Prob, probability.

both years (Table 2). Residues of thiamethoxam, clothianidin, and TMX + CTD were higher in April 2019 than in April 2017, whereas the July concentrations were close in both years.

The residue values decreased with increasing time interval between the latest thiamethoxam or clothianidin application before the 2016 or 2018 sugar beet drilling and with lower application frequency (Figure S1).

Soil residues at the predominant sandy-loam sites (34 sites) and loam sites (20 sites) were maxima (April or July sampling) of 8.31 ± 0.98 and 9.38 ± 1.25 µg TMX + CTD/kg, respectively, with 6.11 ± 1.59 (clayey-loam; five sites) to 8.54 ± 2.11 (loamy-sand; nine sites) µg TMX + CTD/kg at other sites (Table S1 and Figure S2).

The effect of soil tillage after the 2016 and 2018 sugar beet on TMX + CTD residues was also significant, and a year by tillage interaction was found (Table 1 and Figure 2). Median values of TMX + CTD were very close, independent of soil tillage in 2017 (6.5 and 5.8 µg/kg with plow and mulch tillage, respectively) and with plow tillage in 2019 (3.9 µg/kg), but significantly higher with mulch tillage in 2019 (8.8 µg/kg).

**Succeeding crop soil residues**

Soil samples were collected from treated plots on each field several times, from before drilling of the untreated crops in March/April/May 2018 through to flowering of the last crop on each site in June/July/August (Table S2). Table 3 shows the mean soil residues. Highest soil residues were observed in the treated plots at the two UK sites and the Austrian site. The overall mean residue across the treated plots was 3.14 ± 0.53 µg TMX + CTD/kg soil dry wt (90th centile value 5.07 µg TMX + CTD/kg soil dry wt). Soil from control plots all contained residues below the LOQ for thiamethoxam (1 µg/kg); residues of clothianidin were at or below 1 µg/kg at all sites except at site AT-05, where they were 3.4 and 3.9 µg clothianidin/kg.

**Succeeding crop plant matrices residues**

**Guttation fluid.** Residues increased and then declined in samples of guttation fluid collected from newly emerged maize plants during the 42-day sampling period after emergence (Figure 3). Maximum mean values at the sites occurred 21–35 days after emergence and ranged from 2.01 ± 0.54 µg TMX + CTD/L at the site in Italy (IT-08) to 37.7 ± 1.0 µg TMX + CTD/L at the site in the UK (GB-04). By 42 days after emergence, residues had declined to 0.86 ± 0.24 (German site DE-03) to 17.5 ± 2.7 µg TMX + CTD/L (GB-04). Figure 4 shows that the residues in guttation fluid reflected those in the soils in which the plants were grown (r² = 0.70).

**Pollen and nectar.** Figure 5 shows the distribution of residues in all samples of pollen and nectar. All samples collected from control plots contained residues below the LOQ.

**Maize.** Residues of thiamethoxam and clothianidin in pollen collected from maize were below the LOD (0.3 µg/kg) at five of the eight sites: two sites in Germany (DE-01 and DE-02), and one site each in Austria (AT-07), Italy (IT-08), and the UK (GB-04). Residues of thiamethoxam were all below the LOQ (1 µg/kg) at the remaining three sites. At the three sites where the metabolite clothianidin was detected above the LOQ (1 µg/kg) in pollen samples, two of nine samples each at sites PL-05 and PL-06 contained residues of 1.1–1.2 µg clothianidin/kg and one of seven samples taken at site GB-03 contained 1.1 µg clothianidin/kg.

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Residue concentrations of thiamethoxam (TMX) and clothianidin (CTD) in soil samples (dry matter) from fields in which sugar beet with TMX seed dressing were grown in the previous year

|                | Thiamethoxam (TMX) | Clothianidin (CTD) |
|----------------|---------------------|--------------------|
|                | April | July | p value | April | July | p value |
| 2017 Median    | 2.1   | 1.3  | 0.007   | 3.2   | 5.3  | 0.001   |
| Mean ± SE      | 2.3 ± 0.3 | 1.8 ± 0.3 | 3.5 ± 0.3 | 5.2 ± 0.4 |
| 2019 Median    | 3.4   | 1.4  | 0.001   | 5.2   | 4.3  | 0.037   |
| Mean ± SE      | 4.2 ± 0.6 | 2.2 ± 0.4 | 6.4 ± 0.7 | 5.2 ± 0.5 |

Note: Samples were taken in April and July 2017 (n=49) and 2019 (n=43) from fields across Germany with one to three applications of thiamethoxam or clothianidin in the six years previous. Paired comparison of July vs. April, Wilcoxon signed rank test. Abbreviation: p, significance level.

Oilseed rape. Pollen samples were available from five of the eight sites as a result of crop failure at sites in Germany (DE-01) and the UK (GB-03 and GB-04). At four sites (DE-02, AT-05, PL-06, and IT-08) residues of both thiamethoxam and clothianidin were below the LOQ. At the remaining site in Poland (PL-05), residues of thiamethoxam were detected in all nine samples of pollen (PL-05: mean 2.42 ± 0.14 µg thiamethoxam/kg; maximum 3.1 µg thiamethoxam/kg), but residues of clothianidin were below the LOQ.

Nectar samples were available from the same five sites. At three sites (DE-02, AT-07, and IT-08) residues of both thiamethoxam and clothianidin were below the LOQ (0.5 and 1 µg/kg, respectively). Thiamethoxam residues were at or just above the LOQ in one of nine samples at each of the two sites in Poland (PL-05, 0.6 µg thiamethoxam/kg; PL-06, 0.5 µg thiamethoxam/kg) with residues of clothianidin below the LOQ in all samples. Brix analyses of 48 nectar samples provided a mean sugar content of 33.6% ± 1.1%.

Phacelia. No residues of thiamethoxam and clothianidin were detected above the LOQ in any of the samples of pollen or nectar collected from Phacelia at each of the eight sites. Brix analysis of 69 nectar samples provided a mean sugar content of 32.8% ± 1.2%.

Potato. As it is not possible to collect pollen from potato flowers, anthers were collected as a surrogate. At the two sites in the UK (GB-03, GB-04), the flowers aborted prior to opening and therefore no samples could be collected. At the remaining six sites, residues of thiamethoxam were below the LOQ, but at a single site in Poland (PL-05), residues of clothianidin were detected in all six samples collected (mean 1.95 ± 0.32 µg clothianidin/kg; maximum 3.1 µg clothianidin/kg).

DISCUSSION

Total residues in soil

This soil monitoring dataset is probably the largest directed at neonicotinoid seed treatments with soil sampled from 94 individual fields in Germany. The overall mean thiamethoxam and clothianidin (TMX + CTD) residues observed in soil in the season after use of treated sugar beet seed are in line with those in the previous smaller studies in Germany and Austria (Leisner et al., 2020; Thompson et al., 2019) and with fields previously planted with a range of thiamethoxam or clothianidin seed-treated crops in Ontario, Canada (Schaafsma et al., 2015, 2016).

There were two outliers in the dataset, a single field in each year (2% of the sampled fields) contained residues far in excess of those expected in one of the two samples collected (100.4 µg TMX + CTD/kg in July 2017 vs. 5.35 µg TMX + CTD/kg in April 2017 and 84.0 µg TMX + CTD/kg in April 2019 vs. 12.6 µg TMX + CTD/kg in July 2019). Based on the average sowing rate, the initial concentration in the top 20 cm of soil can be estimated as 21.4 µg thiamethoxam/kg (Botías et al., 2015; Schaafsma et al., 2016). As the soil samples were collected the year after drilling, the most likely explanation of such high residues is contamination during sampling or analysis, although heterogeneous distribution of the seed dressing agents (“hot spots”) such that either residues from pelleted sugar beet seeds may have been captured with the soil samples in the field or retarded releases from the pelleted seeds due to drought may be responsible (Leisner et al., 2020). However, based on the results from the other 98% of samples, it is clear these two outliers are not representative. The apparent increase in TMX + CTD residue between April and July 2017 may also be the result of heterogeneous distribution of residues within the field; residues were lower in April 2017 than April 2019, but residues in July of both years were similar.

Soil residues were similar in the succeeding crop study, where there was no history of use within the previous five years (1.93 ± 0.14 to 5.15 ± 0.24 µg TMX + CTD residues/kg) to those from the soil monitoring where thiamethoxam and clothianidin seed treatment were reported as not being used in the five years before seed-treated sugar beet was grown (5.82 ± 1.2 µg TMX + CTD residues/kg). These residues are also similar to those previously reported for
the center and margins of fields in which thiamethoxam seed-treated sugar beet was grown in the previous year (Jones et al., 2014; Thompson et al., 2019). Use of seed treatments in previous years might have been expected to result in higher residues, but this is not necessarily the case. Higher TMX + CTD residues were observed in the 2019 monitoring study, but not in 2017, suggesting factors other than solely the number of applications affected the soil residues. Two factors that may influence soil residues are rainfall and soil type. Weather data were not available for the individual monitoring sites, and soil type alone did not appear to influence soil residues.

**Persistence of soil residues**

Thiamethoxam residues clearly declined over time after drilling and, as reported by others, tillage (Alletto et al., 2010; de Perre et al., 2015; Li et al., 2018) had an inconsistent effect on residues. In a summary of 18 field dissipation studies performed with thiamethoxam, including multiyear studies, thiamethoxam residues were revealed to be concentrated within 20 cm depth, and cultivation did not result in consistent transport to lower soil layers (Hilton et al., 2016, 2019). In addition, seed-treatment-specific studies where soil cores were taken to 1 m depth demonstrated no detectable thiamethoxam residues below 50 cm (Hilton et al., 2019). These field dissipation studies, together with laboratory data demonstrating the importance of microbial viability of soil for the degradation of thiamethoxam, support the hypothesis that the primary degradation mechanism is microbial with leaching through the soil profile only occurring under extreme conditions (Hilton et al., 2016). In the succeeding crop study, where pre-drilling clothianidin soil residues were established, the estimated half-life of the TMX + CTD residues (based on limited sampling) ranged from 0.29 to 0.57 years, which is in line with the reported 0.27–0.6 years in Ontario, Canada (Schaaafsma et al., 2016). Approximately 6% mol/mol of applied thiamethoxam was represented by clothianidin (based

**TABLE 3** Mean residues of thiamethoxam (TMX) and clothianidin (CTD; µg/kg) detected in soils sampled from control plots and treated plots once before drilling with thiamethoxam-treated sugar beet seed (57.1–64.1 g a.i./ha) in spring 2017, from control plots once before drilling of succeeding crops (spring 2018) and mean ± SE residues of thiamethoxam and clothianidin (µg/kg) detected in soils from treated plots during cultivation of the succeeding crop on the same plots (spring/summer 2018)

| Site Description                      | DE-01 | DE-02 | GB-03 | GB-04 | PL-05 | PL-06 | AT-07 | IT-08 |
|---------------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Control plot: Pre-sugar beet drilling |       |       |       |       |       |       |       |       |
| Thiamethoxam (µg/kg)                  | <LOQ  | <LOQ  | <LOQ  | <LOQ  | <LOQ  | <LOQ  | <LOQ  | <LOQ  |
| Clothianidin (µg/kg)                  | 0.84  | 0.23  | 0.75  | 0.39  | <LOQ  | <LOQ  | 3.4   | 0.18  |
| Treated plot: Pre-sugar beet drilling |       |       |       |       |       |       |       |       |
| Thiamethoxam (µg/kg)                  | <LOQ  | <LOQ  | <LOQ  | <LOQ  | <LOQ  | <LOQ  | <LOQ  | <LOQ  |
| Clothianidin (µg/kg)                  | 0.62  | 0.64  | 0.9   | 0.59  | <LOQ  | <LOQ  | 0.28  | 0.17  |
| Control plot: Pre-succeeding crop drilling |       |       |       |       |       |       |       |       |
| Thiamethoxam (µg/kg)                  | <LOQ  | <LOQ  | <LOQ  | <LOQ  | <LOQ  | <LOQ  | <LOQ  | <LOQ  |
| Clothianidin (µg/kg)                  | 0.85  | 1.0   | 0.95  | 0.67  | <LOQ  | <LOQ  | 3.9   | 0.37  |
| Treated plot: During succeeding crop cultivationb |       |       |       |       |       |       |       |       |
| Thiamethoxam (µg/kg)                  | <LOQ  | <LOQ  | 2.08  | ± 0.57| 3.18  | ± 0.57| 1.21  | ± 0.12| 0.56  | ± 0.06| 1.58  | ± 0.21| <LOQ  |       |
| Clothianidin (µg/kg)                  | 2.80  | ± 0.23| 1.53  | ± 0.11| 2.95  | ± 0.45| 1.00  | ± 0.08| 0.91  | ± 0.07| 1.37  | ± 0.12| 3.58  | ± 0.17| 0.92  | ± 0.12|
| Mean TMX + CTD soil residuea (µg/kg)  | 3.26  | ± 0.26| 2.03  | ± 0.11| 5.03  | ± 0.94| 4.18  | ± 0.60| 2.12  | ± 0.17| 1.93  | ± 0.14| 5.15  | ± 0.24| 1.41  | ± 0.12|

Abbreviation: LOQ, limit of quantification.

a A default of 50% LOQ was used to calculate mean and TMX + CTD residues when a residue less than the LOQ was detected.
b N = 17 except DE-01 N = 12; GB-03 N = 11; GB-04, N = 7.
on limited sampling), which supports the low formation of clothianidin from thiamethoxam when used as a seed treatment (Hilton et al., 2019). The greater persistence of clothianidin in European field studies (PPDB, 2020), when compared with thiamethoxam (geomean DT$_{50}$ 31 days; Hilton et al., 2019), is reflected in the data from this study. Persistence of clothianidin residues does not, however, translate to continual accumulation (Xu et al., 2016).

### Residues in guttation fluid

Sugar beet is recognized as a crop in which guttation rarely occurs, and therefore is not relevant to consider in relation to honeybee exposure (EFSA, 2018a; Schmolke et al., 2018; Wirtz et al., 2018). The thiamethoxam and clothianidin residues in guttation fluid collected from untreated maize plants are orders of magnitude lower than those reported for guttation fluid collected from maize and other seed-treated crops (EFSA 2018c; Girolami et al., 2009; Wirtz et al., 2018). There is a correlation, but not a 1:1 relationship, between residues in soil and guttation water (Figure 4). Residues in soil are extracted by including solvents (in this study 80:20 [v/v] acetonitrile/10 mM ammonium acetate was used), but this may not be representative of residues that are bioavailable. The availability of pesticide residues for uptake by plants is influenced by several factors including soil type, rates of adsorption and desorption to soil particles, as well as water solubility (Pietrzak et al., 2020). The influence of these factors is demonstrated in the case of clothianidin by plant uptake, which represented only 6%–10% of residues present in soil (Xu et al., 2016).

Water consumption by honeybees depends on climatic conditions and the needs of the colony, for example, cooling (Nicolson, 2009; Schmolke et al., 2018), but

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**FIGURE 3** Residues of thiamethoxam and clothianidin in samples of guttation fluid collected over time from sites in (A) Germany, (B) the UK, (C) Poland, and (D) Austria and Italy, after emergence from maize grown from untreated seed in plots previously used to grown sugar beet from thiamethoxam-treated seed.

**FIGURE 4** Comparison of mean total residues of thiamethoxam + clothianidin (µg/L) in samples of guttation fluid collected from maize grown from untreated seed in control fields and in fields where sugar beet had been grown from thiamethoxam-treated seed in the previous season (nominal 60 g a.i./ha) with mean total residues in soil from the same fields (µg/kg).
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maximum water consumption of 11.4 µL/g bee/day, lower than the 10-day no-observed-effect dose in adult honeybees of 2.45 ng thiamethoxam/bee/day (Overmyer et al., 2018). At a colony level, the range of maximum mean concentrations identified in guttation fluid (2.0–37.7 µg/L) is also lower than the no-observed-effect concentration for thiamethoxam fed in sucrose for six weeks (37.5–50 µg a.i./kg sucrose; Overmyer et al., 2018; Thompson et al., 2019); consumption of nectar (sucrose) is at least 20-fold greater than that of water (EFSA, 2013). Therefore, it can be concluded that, although residues in soil are available for uptake into plants and are detected in guttation fluid, this does not pose a significant risk to individual bees or to colonies or populations of bees.

Residues in pollen and nectar

So as to be representative of the range of soil residues present in the soil monitoring study as possible (see above), published pollen and nectar residue data from crops grown after sugar beet on three field sites (Thompson et al., 2019) were combined with those in this study. The total residues (TMX + CTD) in pollen and nectar were lower than the residues in the soil in which the plants were growing and were lower than the residues in guttation fluid (Table 4).

The residues in pollen and nectar from the succeeding crops were lower than those reported for oilseed rape and maize grown from seed treated with thiamethoxam at commercial rates (Figure 6; Botías et al., 2015; David et al., 2016; Filling et al., 2013; Woodcock et al., 2017). Residues of thiamethoxam and clothianidin in pollen and nectar sampled from the succeeding crops were detected at or below the LOQ in 86.6% of pollen and 98.6% of nectar samples and were not correlated with measured soil residues. Correlation of pollen residues with soil residues around the time of flowering might be expected, and has

Collection of guttation fluid is rare (Schmolke et al., 2018). Neither bumblebees nor solitary bees collect water. The exposure of bee pollinators to the residues detected in guttation water from maize, then, based on assumed maximum water consumption of 11.4 µL/bee/day (EFSA, 2013) and the maximum mean residue identified in this study (37.7 µg/L), exposure would be 0.43 ng thiamethoxam/bee/day, lower than the 10-day no-observed-effect dose in adult honeybees of 2.45 ng thiamethoxam/bee/day (Overmyer et al., 2018). At a colony level, the range of maximum mean concentrations identified in guttation fluid (2.0–37.7 µg/L) is also lower than the no-observed-effect concentration for thiamethoxam fed in sucrose for six weeks (37.5–50 µg a.i./kg sucrose; Overmyer et al., 2018; Thompson et al., 2019); consumption of nectar (sucrose) is at least 20-fold greater than that of water (EFSA, 2013). Therefore, it can be concluded that, although residues in soil are available for uptake into plants and are detected in guttation fluid, this does not pose a significant risk to individual bees or to colonies or populations of bees.

![Figure 5](image-url) Summary of distribution of thiamethoxam (TMX) + clothianidin (CTD) residue data in nectar and pollen from succeeding crops and associated soil samples in 2018 (eight sites for Phacelia and maize, six sites for potato, and five sites for oilseed rape). Where residues were below the LOQ, they were set at 50% LOQ for the calculation of TMX + CTD residues.

**TABLE 4** Summary of available data on residues in succeeding crop matrices following thiamethoxam seed-treated crops expressed as mean total thiamethoxam (TMX) + clothianidin (CTD) residues and the mean and median contribution of thiamethoxam to the total residues

| Study | Succeeding crop: All sites | Succeeding crop: Site PL-05 | Succeeding crop study (Thompson et al., 2019) |
|-------|---------------------------|-----------------------------|---------------------------------------------|
| Sample | Mean total residues, µg/kg [N] | % TMX mean (median) [N] | Mean total residues, µg/kg [N] | % TMX mean (median) [N] | Mean total residues, µg/kg [N] | % TMX, mean (median) [N] |
| Soil   | 3.0 ± 0.5 [111] | 37 (33) [111] | 2.1 [1] | 59 (56) [1] | 9.3 ± 0.8 [45] | 24 (23) [45] |
| Maize guttation fluid | 6.2 ± 0.7 [158] | 34 (32) [158] | 5.8 ± 0.7 [21] | 48 (50) [21] | 6.5 ± 1.0 [63] | 23 (21) [63] |
| Oilseed rape pollen | 1.4 ± 0.1 [35] | ND | 2.4 ± 0.1 [9] | 79 (79) [9] | 1.2 ± 0.09 [18] | 28 (26) [5] |
| Phacelia pollen | 1.0 ± 0.2 [63] | ND | 1.0 ± 0.6 [6] | ND | 1.0 ± 0.2 [26] | ND |
| Maize pollen | 1.0 ± 0.2 [63] | ND | 1.6 ± 0.02 [9] | 31 (31) [5] | 1.5 ± 0.1 [27] | 37 (28) [11] |
| Potato anther | 1.2 ± 0.07 [51] | ND | 2.5 ± 0.3 [6] | 22 (24) [6] | 1.1 ± 0.08 [18] | 26 (2) |

Note: Site PL-05 is presented separately from other data from this study as it was the only site where thiamethoxam was detected in pollen in the succeeding crop study. Abbreviations: LOQ, limit of quantification; ND, could not be determined as TMX residue <LOQ.
been observed in seed-treated crops such as oilseed rape, but residues in such studies were higher (mean 3.46 ± 2.98 µg TMX + 13.28 ± 5.73 µg CTD/kg soil, 5.5 ± 1.1 µg TMX + CTD/kg pollen; Botías et al., 2015, 2016) than those reported here. The observed correlation of soil residues with those in guttation fluid in newly emerged maize plants, but not in pollen, is probably caused by both the higher residues observed in guttation fluid and the limited metabolism of pesticide within guttation fluid, as demonstrated by the similar contribution of thiamethoxam to the total residues in soil and guttation fluid, but not maize pollen (Table 4).

The residues observed in the succeeding crops in this study are lower than those reported in pollen collected from arable flowering weeds growing in the margins of fields in which crops were grown from thiamethoxam-treated seed (Botías et al., 2015, 2016; David et al., 2016). More detailed assessment of the residue data within these three publications strongly suggests that, contrary to the authors’ hypotheses, soil residues in the field margin resulting from the seed treatment were not the source of the thiamethoxam residues in weed pollen. Specifically, soil samples collected from the field margin contained thiamethoxam residues 4.8-fold lower than in those collected in the treated crop area, yet thiamethoxam residues in wildflower pollen were up to 26-fold higher than the mean residues in thiamethoxam seed-treated oilseed rape pollen (Botías et al., 2015). In addition, clothianidin residues in field margin soil were 10-fold higher than those of thiamethoxam, and 28% of all weed pollen samples contained 14.4–86.1 µg thiamethoxam/kg, but clothianidin was not detected in any sample where thiamethoxam was above the LOD (Botías et al., 2015). In a similar geographical area (David et al., 2016), seven weed pollen samples were reported as containing residues at or below the LOQ with a single sample of weed pollen containing 21 µg thiamethoxam/kg and no detectable clothianidin residues. In the data reported by Botías et al. (2016), a similar pattern of residues in field margin wildflower foliage occurred; with maximum thiamethoxam residues of 106 µg/kg with no detectable clothianidin residues. In these aforementioned studies, the absence of detectable clothianidin residues in pollen and foliage samples with residues of thiamethoxam of 10–100 µg/kg suggests these residues were not taken up from the soil as the authors suggest, but were from airborne deposition of thiamethoxam, either through spray or dust drift (Krupke et al., 2012). Dust levels vary between crops but, in the context of the current discussion, dust levels during drilling of treated sugar beet seed are very low due to the pelleting process used during treatment (Zwertvaegher et al., 2016).

Is there a risk to bees from residues in pollen and nectar from succeeding crops?

Based on the residue data reported in our analysis, contrary to the concerns expressed by EFSA, no effects would be expected in honeybee colonies foraging on succeeding crops or flowering weeds in field margins. The median and 90th percentile residues in pollen in this study were less than 1 µg TMX + CTD/kg. When other pollen and nectar data from succeeding crops planted after thiamethoxam seed-treated sugar beet (three sites in Germany and Austria; Thompson et al., 2019) are also considered, giving a total of 11 fields, the median pollen residues were less than 1 µg TMX + CTD/kg (90th percentile 1.7 µg TMX + CTD/kg). For nectar, both the median and 90th percentile were less than the 0.75 µg TMX + CTD/kg for this study and the combined dataset. This conclusion is supported by data collected after the ban on the use of thiamethoxam and clothianidin on bee-attractive crops, which demonstrated no detectable (<0.38 µg/kg) residues of thiamethoxam or low (0.75 ± 0.08 µg/kg) residues of clothianidin, respectively, in UK honey, and thus indicated low exposure after use on sugar beet (Woodcock et al., 2021).

Based on the contribution of pollen to the diet of adult bees (Rortais et al., 2005), realistic exposure levels for individuals within colonies would be less than 0.75 µg TMX + CTD/kg diet. These residue levels are two orders of magnitude below those reported to have effects on survival of individual adult honeybee (NOEC: 117 µg thiamethoxam/kg sucrose) or larvae (NOEC: 102 µg thiamethoxam/kg sucrose), or on the development of colonies when fed thiamethoxam-treated sucrose over extended periods (lowest observed effect concentration 100 µg a.i./kg sucrose; Overmyer et al., 2018; Thompson et al., 2019). The residue levels are also orders of magnitude below the concentrations of thiamethoxam in sucrose reported to have effects on homing behavior (NOEC: 14.7 µg a.i./kg sucrose) and immune response (NOEC: 100 µg a.i./kg sucrose) in honeybees (Coulon et al., 2018; Fourrier, 2020).

The residue levels detected in pollen and nectar from succeeding crops are also unlikely to affect foraging non-Apis bees. Based on the contribution of pollen to adult worker bumble bee (Gradish et al., 2019) and queen bumble bee.
bee diet (Pridal & Hofbauer, 1996), realistic exposure levels for individuals would be less than 0.75 µg TMX + CTD/kg diet. Comparing these concentrations with those reported for sublethal effects of thiamethoxam in queen and worker bumble bees after continuous exposure (2.4–10 µg thiamethoxam/kg sucrose; Baron, Jansen, et al., 2017; Baron, Raine, et al., 2017; Dance et al., 2017; Potts et al., 2018; Stanley et al., 2016) reveals they are below those likely to result in adverse effects. Larval non-Apis bees are fed more pollen than honeybees, which would potentially result in exposure close to the 1 µg TMX + CTD/kg diet. Although there are no published laboratory data for effects of thiamethoxam on adult Osmia sp. after oral exposure, larvae exposed to clothianidin in the diet exhibited no effects, including on adult physiological function at 10 µg a.i./kg (Nicholls et al., 2017). Higher residues in pollen and nectar (Figure 6) are detectable in thiamethoxam seed-treated crops; however, under the more realistic conditions of semifield and field studies, no significant effects were detected on colonies or populations of honeybees (Pilling et al., 2013), bumble bees (Thompson, Coulson, Ruddell, Wilkins, Harrington, et al., 2016), or the solitary bees Osmia bicornis (Ruddell et al., 2018), or on the foraging behavior or lifespan of honeybee foragers (Thompson, Coulson, Ruddell, Wilkins, Harkin, 2016) after exposure to seed-treated crops. Effects have been reported on honeybees, bumble bees, and Osmia after use of thiamethoxam as a seed treatment, but these were inconsistent between countries and also associated with the presence of disease and/or other neonicotinoids (Woodcock et al., 2017). The absence of detectable effects in populations of squash bees (Eucerna pruinosa; Willis Chan & Raine, 2021) after the use of thiamethoxam seed treatment in acorn squash (maximum mean soil residues 16.6 µg thiamethoxam/kg and 3.6 µg clothianidin/kg) also suggests the soil residues detected in this study are unlikely to affect similar soil-nesting species.

In summary, residues of thiamethoxam and clothianidin were quantified in soil collected in the season after the use of thiamethoxam as a seed treatment on sugar beet at 94 sites in Germany and eight sites in the UK, Germany, Austria, and Italy. Pollen and nectar collected from a range of crops planted in spring after the sugar beet harvest contained mean and median residues of thiamethoxam and clothianidin in pollen and nectar, which were less than 1 µg TMX + CTD/kg. Although residues in soil were detectable, residues in pollen and nectar of succeeding crops were far below reported no-effect levels for honeybees and a range of non-Apis species. Therefore, contrary to the reported basis for the ban in the EU, residues in soil resulting from use of thiamethoxam as a seed treatment on sugar beet are not likely to result in adverse effects on either honeybee colonies or non-Apis bee populations.

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CONFLICT OF INTEREST

Helen Thompson and Sarah Vaughan are employed by Syngenta, which manufactures and markets thiamethoxam-based seed treatments.

DATA AVAILABILITY STATEMENT

All data are available in the Supporting Information file.

SUPPORTING INFORMATION

The Supporting Information file contains information on sites and residue data generated.

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REFERENCES

Alletto, L., Coquet, Y., Benoit, P., Heddadj, D., & Barriuso, E. (2010). Tillage management effects on pesticide fate in soils. A review. Agronomy for Sustainable Development, 30(2), 367–400.
Baron, G. L., Jansen, V. A. A., Brown, M. J. F., & Raine, N. E. (2017). Pesticide residues in bumblebee colony initiation and increases probability of population extinction. Nature Ecology & Evolution, 1(9), 1308–1316.
Baron, G. L., Raine, N. E., & Brown, M. J. F. (2017). General and species-specific impacts of a neonicotinoid insecticide on the ovary development and feeding of wild bumblebee queens. Proceedings of the Royal Society B: Biological Sciences, 284(1854), 20170123.
Botias, C., David, A., Hill, E. M., & Goulson, D. (2016). Contamination of wild plants near neonicotinoid seed-treated crops, and implications for nontarget insects. Science of the Total Environment, 566–567, 269–278. https://doi.org/10.1016/j.scitotenv.2016.05.065
Botias, C., David, A., Horwood, J., Abdul-Sada, A., Nicholls, E., Hill, E., & Goulson, D. (2015). Neonicotinoid residues in wildflowers, a potential route of chronic exposure for bees. Environmental Science and Technology, 49(21), 12731–12740.
CIBE. (2018). The case for neonicotinoids in pelleted sugar beet seeds. Retrieved May 7, 2021, from www.cibe-europe.eu/img/user05818%20CIBE%20The%20case%20for%20neonicotinoids%20in%20pelleted%20sugar%20beet%20seeds%20April%202018.pdf.
Coulon, M., Schurr, F., Martel, A. C., Cougoule, N., Bégaud, A., Mangoni, P., Dalmon, A., Alaux, C., Le Conte, Y., Thiery, R., Ribière-Chatbert, M., & Dubois, E. (2018). Metabolism of thiamethoxam [a neonicotinoid pesticide] and interaction with the chronic bee paralysis virus in honeybees. Pesticide Biochemistry and Physiology, 144, 10–18.
Ctgb. (2015). Attractiveness of agricultural crops to honeybees for the collection of nectar and/or pollen V2.0. Retrieved May 7, 2021, from https://english.ctgb.nl/documents/decision-documents-board/2015/08/15/attractiveness-of-agricultural-crops-to-honeybees.
Dance, C., Botias, C., & Goulson, D. (2017). The combined effects of a monotonous diet and exposure to thiamethoxam on the performance of bumblebee micro-colonies. Ecotoxicology and Environmental Safety, 139, 194–201.
David, A., Botias, C., Abdul-Sada, A., Nicholls, E., Rotheray, E. L., Hill, E. M., & Goulson, D. (2016). Widespread contamination of wildflower and bee-collected pollen with complex mixtures of neonicotinoids and fungicides commonly applied to crops. Environmental International, 88, 169–178.
de Perre, C., Murphy, T. M., & Lydy, M. J. (2015). Fate and effects of clothianidin in fields using conservation practices. *Environmental Toxicology and Chemistry, 34*(2), 258–265.

Dewar, A. M., & Qi, A. (2021). The virus yellows epidemic in sugar beet in the UK in 2020 and the adverse effect of the EU ban on neonicotinoids on sugar beet production. *Outlook on Pest Management, 32*(4), 53–59.

European Food Safety Authority (EFSA). (2013). Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, Bombus spp. and solitary bees). *EFSA Journal, 11*(7), 3295.

European Food Safety Authority (EFSA). (2018a). Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin considering the uses as seed treatments and granules. *EFSA Journal, 16*(2), 5177.

European Food Safety Authority (EFSA). (2018b). Conclusion on the peer review of the pesticide risk assessment for bees for the active substance imidacloprid considering the uses as seed treatments and granules. *EFSA Journal, 16*(2), 5178.

European Food Safety Authority (EFSA). (2018c). Conclusions on the peer review of the pesticide risk assessment for bees for the active substance thiamethoxam considering the uses as seed treatments and granules. *EFSA Journal, 16*(2), 5179.

European Commission. (2018a). Commission Implementing Regulation (EU) 2018/783 of 29 May 2018 amending Implementing Regulation (EU) No. 540/2011 as regards the conditions of approval of the active substance imidacloprid C/2018/3180. *O J EU 132*, pp. 31–34.

European Commission. (2018b). Commission Implementing Regulation (EU) 2018/784 of 29 May 2018 amending Implementing Regulation (EU) No. 540/2011 as regards the conditions of approval of the active substance clothianidin (Text with EEA relevance.) C/2018/3177. *O J EU 132*, pp. 35–39.

European Commission. (2018c). Commission Implementing Regulation (EU) 2018/785 of 29 May 2018 amending Implementing Regulation (EU) No. 540/2011 as regards the conditions of approval of the active substance thiamethoxam (Text with EEA relevance.) C/2018/3178. *O J EU 132*, pp. 40–44.

Forster, R., Giffard, H., Heimbach, U., Laporte, J-M., Lückmann, J., Nikolakis, A., Pistorius, J., & Vergnet, C. (2012). ICPBR-PLoS One 7(1), e29268.

Fournier, J. (2020). Validation of the Homing flight test in honeybee (*Apis mellifera L.*) after single exposure to sublethal doses of a test chemical. *Results of the international ring test 2018 and 2019. Final report and situationanalyse 2018.*

Frame, S. T., Pearsons, K. A., Elkin, K. R., Saporito, L. S., Presendean, H. E., Karsten, H. D., & Tooker, J. F. (2021). Assessing surface and subsurface transport of neonicotinoid insecticides from no-till crop fields. *Journal of Environmental Quality, 50*(2), 476-484.

Girolami, V., Mazzon, L., Squarzini, A., Mori, N., Marzaro, M., Di Bernardo, A., Greatti, M., Giorio, C., & Tapparo, A. (2009). Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: A novel way of intoxication for bees. *Journal of Economic Entomology, 102*(5), 1808–1815.

Grashid, A. E., van der Steen, J., Scott-Dupree, C. D., Cabrera, A. R., Cutler, G. C., Goulson, D., Klein, O., Lehmann, D. M., Lückmann, J., O’Neill, B., Raine, N. E., Sharma, B., & Thompson, H. (2019). Comparison of pesticide exposure in honey bees (Hymenoptera: Apidae) and bumble bees (Hymenoptera: Apidae): Implications for risk assessments. *Environmental Entomology, 48*(1), 12–21.

Hauer, M., Hansen, A. L., Manderyck, B., Olsson, Å., Raaijmakers, E., Hanse, B., Stockfisch, N., & Märländer, B. (2017). Neonicotinoids in sugar beet cultivation in Central and Northern Europe: Efficacy and environmental impact of neonicotinoid seed treatments and alternative measures. *Crop Protection, 93*, 132–142.

Hauer-Jäkl, M., Nause, N., Trimpler, K., Stockfisch, N., & Märländer, B. (2017). CONVISO® ONE—Ansätze für eine Systemanalyse der Herbizidstrategie. *Sugar Industry, 142*(12), 704–712.

Hilton, M. J., Emburey, S. N., Edwards, P. A., Dougan, C., & Ricketts, D. C. (2019). The route and rate of thiamethoxam soil degradation in laboratory and outdoor incubated tests, and field studies following seed treatments or spray application. *Pest Management Science, 75*(1), 63–78.

Hilton, M. J., Jarvis, T. D., & Ricketts, D. C. (2016). The degradation rate of thiamethoxam in European field studies. *Pest Management Science, 72*(2), 388–397.

Hoffmann, C. M., Koch, H.-J., & Märländer, B. (2020). Sugar beet. In V. O. Sadras & D. Calderini (Eds.), *Crop physiology. Case histories for major crops* (pp. 635–674). Elsevier Inc.

Hossain, R., Menzel, W., Lachmann, C., & Varrelmann, M. (2021). New insights into virus yellows distribution in Europe and effects of beet yellows virus, beet mild yellowing virus, and beet chlorosis virus on sugar beet yield following field inoculation. *Plant Pathology, 70*, 584–593.

Jha, M. (2020). New virus hits Europe, this one threatens sugar crops. *Bloomberg.* Retrieved May 7, 2021, from www.bloomberg.com/news/articles/2021-06-07/onweblisease-threatens-to-destroy-large-chunk-of-europe’s-sugar-crop

Jones, A., Harrington, P., & Turnbull, G. (2014). Neonicotinoid concentrations in arable soils after seed treatment applications in preceding years. *Pest Management Science, 70*(12), 1780–1784.

Kozak, M., & Piepho, H. (2018). What’s normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. *Journal of Agronomy and Crop Science, 204*, 86–98.

Krupke, C. H., Hunt, G. J., Etter, B. D., Andino, G., & Given, K. (2012). Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS One, 7*(1), e92968.

Ladewig, E., Buhre, C., Kentner, C., Stockfisch, N., Varrelmann, M., & Märländer, A.-K. (2018). Pflanzenschutz im zuckerrübenanbau in Deutschland—situationanalyse 2018. *Sugar Industry, 143*(12), 708–722.

Lesiner, J., Guelh, B., Hindersmann, B., & Göckener, B. (2020). Insektilizide in böden unterschiedlicher bewirtschaftung. Nachweis von neonicotinoiden und pyrethroiden. *Bodenschutz, 25*(1), 15–21.

Li, Y., Su, P., Li, Y., Wen, K., Bi, G., & Cox, M. (2018). Adsorption-desorption and degradation of insecticides clothianidin and thiamethoxam in agricultural soils. *Chemosphere, 207*, 708–714.

Milford, G. F. J. (2006). Plant structure and crop physiology. In A. P. Draycott (Ed.), *Sugar Beet (pp. 30–45)*. Blackwell.

Nicholls, E., Fowler, R., Niven, J. E., Gilbert, J. D., & Goulson, D. (2017). Larval exposure to field-realistic concentrations of clothianidin has no effect on development rate, over-winter survival or adult metabolic rate in a solitary bee, *Osmia bicorns*. *PeerJ, 5*, e3417.

Nicolson, S. W. (2009). Water homeostasis in bees, with the emphasis on sociality. *Journal of Experimental Biology, 212*(3), 429–434.

Overmyer, J., Feken, M., Ruddle, N., Boeksh, S., Hill, M., & Thompson, H. (2018). Thiamethoxam honey bee colony feeding study: Linking effects at the level of the individual to those at the colony level. *Environmental Toxicology and Chemistry, 37*(3), 816–828.

Pietrzak, D., Kania, J., Kmiecik, E., Malina, G., & Wętor, K. (2020). Fate of selected neonicotinoid insecticides in soil–water systems: Current state of the art and knowledge gaps. *Chemosphere, 255*, 126981.

Pilling, E., Campbell, P., Coulson, M., Ruddle, N., & Tomer, I. (2013). A four-year field program investigating long-term effects of repeated exposure of honey bee colonies to flowering crops treated with thiamethoxam. *PLoS One, 8*(10), e77193.

Potts, R., Clarke, R. M., Oldfield, S. E., Wood, L. K., Hempel de Ibarra, N., & Cresswell, J. E. (2018). The effect of dietary neonicotinoid pesticides on non-flight thermogenesis in worker bumble bees (Bombus terrestris). *Journal of Insect Physiology, 104*, 33–39.

PPDB. (2020). The Pesticide Properties Database (PPDB). University of Hertfordshire. Retrieved May 7, 2021, from http://sitem.herts.ac.uk/seru/ppdb/ppdb

Pradal, A., & Hofbauer, J. (1996). Laboratory rearing and nutrition of young queens of bumblebee (Bombus terrestris L.) from emergence to diapause. *Scientific Studies, 14*, 125–130.
Requier, F., Odoux, J.-F., Tamic, T., Moreau, N., Henry, M., Decourtye, A., & Bretagnolle, V. (2015a). Floral resources used by honey bees in agricultural landscapes. *Bulletin of the Ecological Society of America, 96*(3), 487–491.

Requier, F., Odoux, J.-F., Tamic, T., Moreau, N., Henry, M., Decourtye, A., & Bretagnolle, V. (2015b). Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds. *Ecological Applications, 36*(1), 71–83.

Ruddle, N., Elston, C., Klein, O., Hamberger, A., & Thompson, H. (2018). Effects of exposure to winter oilseed rape grown from thiamethoxam-treated seed on the red mason bee *Osmia bicornis*. *Environmental Toxicology and Chemistry, 37*(4), 1071–1083.

Schrafsma, A., Limay-Rios, V., Baute, T., Smith, J., & Xue, Y. (2015). Neonicotinoid insecticide residues in surface water and soil associated with commercial maize (corn) fields in southwestern Ontario. *PloS One, 10*(2), e0118139.

Schrafsma, A., Limay-Rios, V., Xue, Y., Smith, J., & Baute, T. (2016). Field-scale examination of neonicotinoid insecticide persistence in soil as a result of seed treatment use in commercial maize (corn) fields in southwestern Ontario. *Environmental Toxicology and Chemistry, 35*(2), 295–302.

Schmolke, A., Kearns, B., & O’Neill, B. (2018). Plant guttation water as a potential route for pesticide exposure in honey bees: a review of recent literature. *Apidologie, 49*(5), 637–890.

Stanley, D. A., Arnold, G., Halm, M. P., & Touffet-Briens, F. (2005). Modes of honeybees exposure to systemic insecticides: Estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie, 36*(1), 81–890.

Rortais, A., Woodcock, B., Ridding, L., Pereira, M. G., Sleep, D., Henrys, P., Peyton, J., Hulmes, S., Hulmes, L., Sárospataki, M., Saure, C., Edwards, M., Genersch, E., Knäbe, S., & Bucheli, T. D. (2016). Leaching of the neonicotinoids thiamethoxam and imidacloprid from sugar beet seed dressings to subsurface tile drains. *Journal of Agricultural and Food Chemistry, 64*(33), 6407–6415.

Wills Chan, D. S., & Raine, N. E. (2021). Population decline in a ground-nesting solitary squash bee (*Eucera pruinosa*) following exposure to a neonicotinoid insecticide treated crop (*Cucurbita pepo*). *Scientific Reports, 11*(1), 4241.

Wirtz, I. P., Hauer-Jákli, M., Schenke, D., Ladewig, E., Märländer, B., Hermbach, U., & Pistorius, J. (2018). Investigations on neonicotinoids in guttation fluid of seed treated sugar beet: Frequency, residue levels and discussion of the potential risk to honey bees. *Crop Protection, 105*, 28–34.

Woodcock, B. A., Bullock, J. M., Shore, R. F., Heard, M. S., Pereira, M. G., Redhead, J., Ridding, L., Dean, H., Sleep, D., Henrys, P., Peyton, J., Hulmes, S., Hulmes, L., Sárospataki, M., Saure, C., Edwards, M., Genensch, E., Knäbe, S., & Wyllie, R. F. (2017). Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. *Science, 356*(6345), 1393–1395.

Woodcock, B. A., Ridding, L., Pereira, M. G., Sleep, D., Newbold, L., Oliver, A., Shore, R. F., Bullock, J. M., Heard, M. S., Gweon, H. S., & Pywell, R. F. (2021). Neonicotinoid use on cereals and sugar beet is linked to continued low exposure risk in honeybees. *Agriculture, Ecosystems & Environment, 308*, 107205.

Xu, T., Dyer, D. G., McConnell, L. L., Bondarenko, S., Allen, R., & Heinemann, O. (2016). Clothianidin in agricultural soils and uptake into corn pollen and canola nectar after multiyear seed treatment applications. *Environmental Toxicology and Chemistry, 35*(2), 311–321.

Zwertvaegher, I. K. A., Foqué, D., Devarrewaere, W., Verboven, P., & Nuyttens, D. (2016). Assessment of the abrasion potential of pesticide-treated seeds using the Heubach test. *International Journal of Pest Management, 62*(4), 348–359.