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Hazard/Risk Assessment

Potential Risk of Residues From Neonicotinoid-Treated Sugar Beet Flowering Weeds to Honey Bees (*Apis mellifera* L.)

Richard Odemer,[a,*] Elsa Friedrich,[b] Ingrid Illies,[c] Stefan Berg,[c] Jens Pistorius,[a] and Gabriela Bischoff[d]

[a]Institute for Bee Protection, Julius Kühn-Institut—Federal Research Centre for Cultivated Plants, Braunschweig, Germany
[b]Apicultural State Institute, University of Hohenheim, Stuttgart, Germany
[c]Institute for Bee Research and Beekeeping, Bavarian State Institute for Viticulture and Horticulture, Veitshöchheim, Germany
[d]Institute for Bee Protection, Julius Kühn-Institut—Federal Research Centre for Cultivated Plants, Berlin, Germany

Abstract: In 2018 the European Union (EU) banned the three neonicotinoid insecticides imidacloprid, clothianidin (CLO), and thiamethoxam (TMX), but they can still be used if an EU Member State issues an emergency approval. Such an approval went into effect in 2021 for TMX-coated sugar beet seeds in Germany. Usually, this crop is harvested before flowering without exposing non-target organisms to the active ingredient or its metabolites. In addition to the approval, strict mitigation measures were imposed by the EU and the German federal states. One of the measures was to monitor the drilling of sugar beet and its impact on the environment. Hence we took residue samples from different bee and plant matrices and at different dates to fully map beet growth in the German states of Lower Saxony, Bavaria, and Baden-Württemberg. A total of four treated and three untreated plots were surveyed, resulting in 189 samples. Residue data were evaluated using the US Environmental Protection Agency BeeREX model to assess acute and chronic risk to honey bees from the samples, because oral toxicity data are widely available for both TMX and CLO. Within treated plots, we found no residues either in pools of nectar and honey crop samples ($n = 24$) or dead bee samples ($n = 21$). Although 13% of beebread and pollen samples and 88% of weed and sugar beet shoot samples were positive, the BeeREX model found no evidence of acute or chronic risk. We also detected neonicotinoid residues in the nesting material of the solitary bee *Osmia bicornis*, probably from contaminated soil of a treated plot. All control plots were free of residues. Currently, there are insufficient data on wild bee species to allow for an individual risk assessment. In terms of the future use of these highly potent insecticides, therefore, it must be ensured that all regulatory requirements are complied with to mitigate any unintentional exposure. *Environ Toxicol Chem* 2023;00:1–11. © 2023 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Treated sugar beet; Neonicotinoid; Thiamethoxam; Clothianidin; Honey bee risk assessment; Pollen nectar residues; Flowering weeds; BeeREX model; Risk quotient

INTRODUCTION

Nearly a decade ago, use of the three neonicotinoid insecticides thiamethoxam (TMX), clothianidin (CLO), and imidacloprid was first restricted in the European Union (EU) by a moratorium (Regulation (EU) No 485/2013). Even after the final ban on field use in 2018 due to the conclusion by the European Food Safety Authority (EFSA, 2018a) that most neonicotinoid pesticide applications pose a risk to wild bees and honey bees, research is ongoing.This is not least because several studies have found that bees are still confronted with these insecticides within their environment (Mitchell et al., 2017; Wintermantel et al., 2020; Woodcock et al., 2021). Moreover, global neonicotinoid use remains high (Bakker et al., 2020), and there is growing evidence that pesticides may play a role in recently reported insect declines (Brühl et al., 2021; Wagner et al., 2021).

The neonicotinoid moratorium that went into effect in 2014 allowed the continued use of treated beet seed because no relevant exposure via nectar, pollen, guttation, or dust drift was assumed to occur when sugar beet is harvested prior to flowering (Epstein et al., 2022). Guttation is a rare event in sugar

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* Address correspondence to richard.odemer@julius-kuehn.de
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beets (Wirtz et al., 2018), and if high- quality seed treatments are provided, exposure to dust drift was also considered negligible (Krahner et al., 2021). With the stricter regulation following EFSA's new conclusion (Regulation (EU) 2018/784), the use of banned neonicotinoids for major crops remained possible due to an emergency approval by a Member State (EFSA, 2021). This need arose from damage by aphids (major pest *Myzus persicae*), which transmit a number of viruses that cause significant damage to plants (Qi et al., 2004). Yellow virus, for example, reduces photosynthesis through leaf damage. These viruses are widespread in Europe, and high losses are expected without preventive measures (Epstein et al., 2022).

Beet growers argue that there are currently no alternative treatments that are as economical and effective as neonicotinoids (Epstein et al., 2022). With Germany being one of the Member States that approved the use of thiamethoxam-treated seeds in 2021, the application came with the following mitigation measures (European Commission [EC], 2021):

- The product may only be used on seeds in professional seed treatment facilities that are included in the governmental facility list from the Julius Kühn-Institute.
- The application rate and sowing density must be reduced, to minimize potentially occurring residues in the soil.
- Only crops that are not attractive to bees must be sown as follow-up crops; flowering weeds in the follow-up crop must be avoided.
- Only crops that are not attractive to bees must be sown as catch crops.
- Nearby beekeepers must be notified prior to sowing.
- The sowing of treated seeds must be accompanied by monitoring studies.

Beyond that, the Federal States involved (7/16) have issued even stricter regulations on bee protection (General Regulation Federal States, 2021). These include, for example, the requirement that only untreated seeds may be used at a distance of at least 45 cm from the edge of the field. In addition, weeds and other plants must not be allowed to flower on the relevant field before and after sowing until the end of 2022. To avoid exposure to seed treatment dust (Krahner et al., 2021), only drift-reduced pneumatic seed drilling machines may be used for sowing.

Because sugar beet is usually harvested before flowering, the most likely route of exposure is adjacent weeds or succeeding crops that take up the active ingredient from the soil and release it to flower visitors. A recent study analyzed CLO + TMX residues in pollen and nectar samples from succeeding crops planted directly at the site where treated sugarbeet had been grown in the previous season (Thompson, Vaughan, et al., 2021). Detections were at or below the limit of quantitation (0.5–1 µg a.i./kg) at eight sites in five countries. By comparison, those levels are lower than concentrations reported as adverse sublethal effects in studies with fed honey bee and bumble bee individuals and colonies (Baron et al., 2017; Coulon et al., 2018; Fourrier, 2020; Stanley et al., 2016).

From field trials with neonicotinoids conducted under realistic exposure conditions with intact honey bee colonies, we know that adverse effects indeed can be absent (Odemer & Rosenkranz, 2020; Odemer et al., 2018; Osterman et al., 2019; Overmyer et al., 2018; Siede et al., 2018). Hence Harwood and Dolezal (2020) suggest that honey bees may be resilient to pesticide stress, and therefore their responses may be context dependent. This leads to the conclusion that effects of insecticides and other pesticides assessed under laboratory conditions may not show their true potential under colony conditions (Tsvetkov & Zayed, 2021). For wild bees, on the other hand, this scenario could be different (Rundlöf et al., 2015).

Despite this controversy, it is still unclear to what extent honey bee colonies and wild pollinators are exposed to neonicotinoid residues from bee and plant matrices in treated sugar beet fields. We therefore monitored residues at four different sites in three states in Germany across a north–south distribution where treated sugar beet was grown, to characterize the realistic exposure of pollinators. Various modeling approaches are currently applied by regulators to compare exposure with toxicity, to allow interpretation of potential hazards (Thompson, 2021). Although the hazard quotient (HQ) is widely used, it has only been validated for foliar sprays and therefore would not be appropriate for seed treatments (Thompson, 2021). Instead, we used the BeeREX model of the US Environmental Protection Agency (USEPA, 2014) to generate acute and chronic risk quotients (RQs) to evaluate whether residues in bee and plant matrices have the potential to pose a risk to honey bees.

MATERIALS AND METHODS

Study sites and test organisms

Three main areas in Germany were considered where emergency approval for TMX-treated sugar beet had been granted in 2021. These areas were within a total of 34 700 ha of treated sugar beet in Lower Saxony (North—JKI), 20 600 ha in Bavaria (Middle—VHH), and 12 000 ha in Baden-Württemberg (South—HOH; EC, 2021; Figure 1).

To sample honey bee matrices, each area included at least one treated (T) plot where four to six full-sized colonies (of *Apis mellifera*) were directly installed and a control (C) plot without neonicotinoid treatment at a sufficient distance (min. 2 km) before drilling. These plots were 6.8 ha in size for JKI-T, 15.3 and 13.6 ha for VHH-T, and 8.8 ha for HOH-T. In addition, a large amount of treated sugar beet was grown in the flight radius of the hives of each treated plot, which was not further measured.

Cocoons from both sexes of the red mason bee *Osmia bicornis* were ordered from a commercial supplier (WAB-Mauerbienenzucht, Konstanz, Germany) and placed together with wooden nest boxes in addition to the bee hives at the two JKI plots, to sample *Osmia* matrices. Each nest box contained 70:30 female:male cocoons. All honey bee colonies in the three main areas and mason bee nesting cavities in the JKI plots were placed close by before or at the time of drilling.

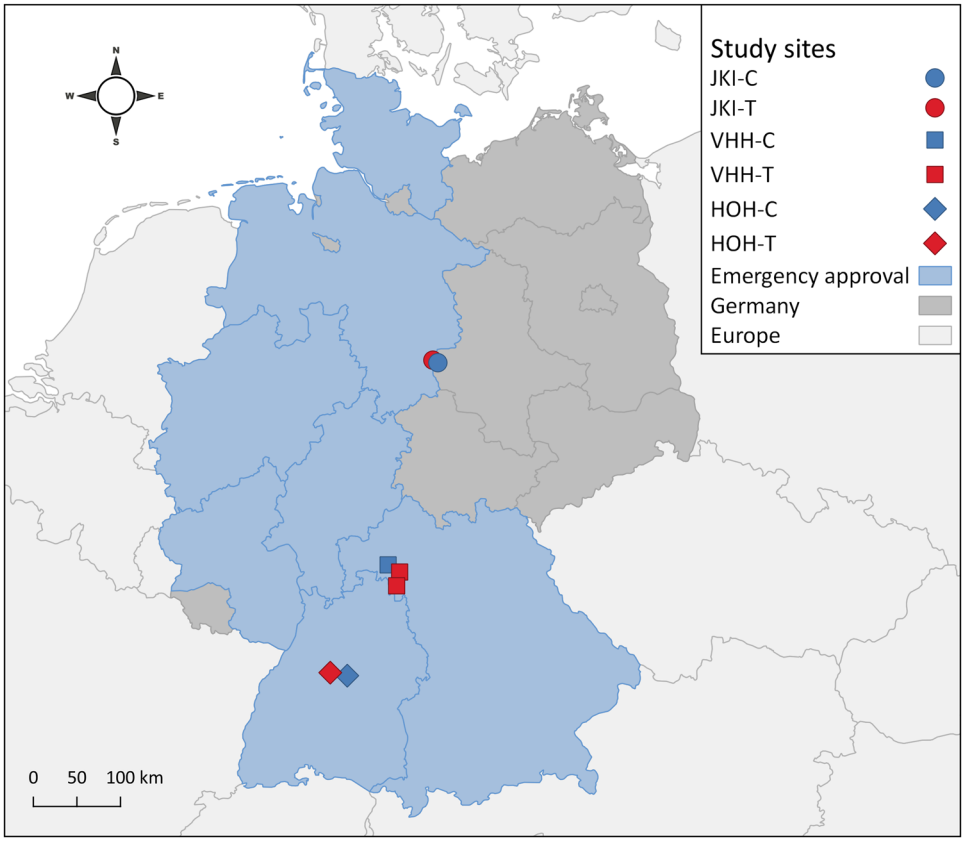

FIGURE 1: Study sites with drilled sugar beet in Germany in the states of Lower Saxony (JKI, circle), Bavaria (VHH, square), and Baden-Württemberg (HOH, diamond). Control (C) and treated (T) plots are colored in dark blue and red, respectively. States where the 2021 emergency approval was applied are highlighted in blue.

Seed treatment

Seeds were dressed with Cruiser 600 FS (approval no. 006034-00; Syngenta Agro GmbH, Frankfurt, Germany), which contained 600 g thiamethoxam/L. The maximum application rate was 82.5 ml/ha, which corresponds to a seed unit of 1.1/ha or 49.5 g TMX/ha (EC, 2021).

Sample collection

JKI. Sample collection at the JKI site covered the period from drilling (March 25) to harvest (September 20–30) for plant and honey bee matrices. We collected nesting cavities of mason bees on November 16 and then took samples from their matrices. Neonicotinoid-treated and untreated seeds and beet leaves at BBCH33 and 49 were sampled as plant matrices (see Meier et al., 2009 and Meier, 2018 for details on the sugar beet BBCH scale [Biologische Bundesanstalt Bundessortenamt and Chemical Industry], used to classify the growth stage of the plant). In addition, sugar beet inflorescences (shoots) and flowering weeds adjacent to or growing directly on the fields were sampled in late July and early August.

Beebread and nectar (which, from capped and uncapped cells, might also be considered as honey) were collected as hive samples at 7–10-day intervals from drilling to harvest. Bee matrices were collected in close proximity to the brood nest to detect potential contamination passing to bee larvae and nurse bees. All samples were immediately stored at −20 °C until further processing and pooled across five colonies/plot.

VHH. Sampling at the VHH site covered the period from drilling (March 27–31) to harvest (September 20–30) for plant and honey bee matrices, similar to the JKI site. Forager bees were sampled 1 day after drilling on two treatment plots and one control plot. The six colonies were equipped with dead bee traps (following Illies et al., 2002), and sampled on 3 days in April and 2 days in May. Shoots of sugar beets and flowering weeds adjacent to or growing directly on the fields were sampled in mid-July. All bee samples were pooled across six colonies/plot and immediately stored at −20 °C until further processing, including plant samples.

HOH. Sampling at the HOH site included collection of forager bees pooled from four colonies in each plot. Corbicular pollen was removed from pollen foragers, wheras the honey crop was seperated from the rest of the body of nectar foragers.

Samples were collected from 1 day after drilling (March 31) to late May (21). Dead bee traps (following Gary, 1967) were installed, and dead bees were sampled once in April and May at the control plot, and twice in March, twice in April, once in May, and once in June at the treatment plot.

In addition, *Phacelia tanacetifolia* was sown on the plot where sugar beet had previously been drilled to adsorb possible TMX + CLO residues from the soil. Just prior to full flowering of the *Phacelia* (BBCH 63–65), two tunnel tents were set up on the treatment plot and one tent at the control plot and each provided with a honey bee colony (hereafter termed semi-field study). The tents were 4 × 10 m and had a total flowering area of 40 m². As just described, pollen and nectar foragers were collected from the tents in late June (26–28) and prepared (honey crop, remaining bodies, corbicular pollen).

Residue analysis

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) was used to quantify and identify TMX, the active ingredient applied with the seed treatment, and its degradation product CLO. Samples analyzed included the matrices described in the three previous sections. The analytical setup included a Nexera X2 HPLC system (Shimadzu Corp., Kyoto, Japan) coupled to a triple-quadrupole mass spectrometer Q TRAP 6500 + (SCIEX, Framingham, USA) equipped with an electrospray ionization source. A detailed description of the methods including sample preparation, processing, quantification, and validation can be found in the Supporting Information.

To jointly account for TMX and CLO residues, we followed the approach of Thompson, Vaughan, et al. (2021). The toxicity and the molar mass of TMX and CLO are very similar (EFSA, 2018b, 2018c; Thompson, Vaughan, et al., 2021; USEPA, 2020), and therefore the residues of each compound were summarized. This provides a total residue of TMX + CLO/sample.

Honey bee risk assessment

To investigate whether measured residue levels in hives and on plots pose a risk to honey bees, we used the Bee Residue Exposure (BeeREX) model to determine a risk quotient (RQ; USEPA, 2012, 2014). The RQ accounts for oral and contact exposure routes from collected pollen or nectar (or both). Different assumptions are made for dietary intake by different life stages of bees (i.e., adult bees, larvae) and different castes (i.e., drones, queens, and workers as foragers, nurses, and others) so that a specific risk can be determined for each assumption (USEPA, 2012, 2014). To obtain the most conservative RQs, the BeeREX model automatically selects the highest values from all castes for acute and chronic risk (i.e., forager bee: 292 mg nectar/day and nurse bee: 9.6 mg pollen/day; USEPA, 2012, 2014). This means that all other castes and life stages were covered by this assumption.

The model utilizes acute (median lethal dose [LD50]) or chronic (no-observed-adverse-effect level [NOAEL]) toxicity data from a compound and relates these to the residue levels found in bee matrices. Their individual consumption/caste and life stage is also taken into account. The level of concern (LOC) to which the acute RQ is compared is set at 0.4 and is based on the historical average dose–response relationship for acute toxicity studies with bees and a 10% mortality rate. A chronic RQ using NOAELs is compared with a LOC of 1 (Thompson, 2021; USEPA, 2012, 2014). For each RQ less than 0.4 (acute) or 1 (chronic), the compound poses little or no risk to bees. If the value is above the LOC, the compound may require higher level testing (e.g., semi-field or field studies; Dai et al., 2018).

Because we summarized the residues of CLO and TMX, we took the most conservative approach and chose the CLO endpoints for further calculations given that they were slightly lower than those of TMX (Table 1).

TABLE 1: Endpoints used for the BeeREX model to calculate acute and chronic risk quotients (RQs)

Endpoint	CLO (µg a.i./bee)	TMX (µg a.i./bee)	Source
Adult contact LD50	0.021[a]	0.024	EFSA (2013a)
Adult oral LD50	0.00368	0.005	Weyman (1998); EFSA (2013a)
Adult oral NOAEL	0.001024	0.00134	Weyman (1998); Henry et al. (2012)
Larval LD50	>0.03	>0.03	USEPA (2020)
Larval NOAEL	0.0032[a]	0.0037	USEPA (2020)

[a]Effects are expressed as "clothianidin equivalent," with thiamethoxam concentrations converted using the molecular weight ratio of clothianidin to thiamethoxam (i.e., ratio = 0.856; USEPA, 2020).
CLO = clothianidin; LD50 = median lethal dose; NOAEL = no-observed-adverse-effect level; TMX = thiamethoxam.

RESULTS

Residue analysis

Overall, 189 pooled samples were analyzed from control and treated plots at the three locations in Germany (Table 2). Samples in the control plots were $n = 76$ and in the treatment $n = 113$. In the open field studies, $n = 18$ samples were positive (for either TMX, or CLO, or both), and in the semi-field study, $n = 9$ samples were positive.

The positive and negative detections of TMX + CLO in all samples except sugar beet seeds, leaves, and *Osmia* matrices are shown in Figure 2. We combined nectar and honey crop samples, beebread and pollen samples, and weed and shoot samples because combinations represent the same matrix and origin. Except for one cross-contaminated sample of sugar beet leaves in the control (<LOQ), residues were found only in samples from the treatment (see also Table 2). The positive leaf sample was not considered in the risk assessment.

In the treated plots, none of the collected nectar + honey samples ($n = 24$) and dead bee samples ($n = 20$) contained TMX or CLO. The samples of beebread + pollen ($n = 23$) contained three positives or 13%, and the samples of weeds + shoots ($n = 17$) contained 15 positives or 88%. All bee matrices were pooled samples from multiple colonies, as described in the *Sample collection* section. Note that the samples from the semi-field trial at HOH were not included in Figure 2 because they do not represent a good agricultural practice scenario. Samples from hives are usually diluted because both nectar and pollen collected are acquired from multiple sources and

TABLE 2: Overview table of all sampled sites and matrices

JKI				HOH				VHH			
Location	Matrix	C (no.)	T (no.)	Location	Matrix	C (no.)	T (no.)	Location	Matrix	C (no.)	T (no.)
Open field	Nectar[a]	17	17	Open field	Bees (r)[a]	9	7	Open field	Dead bees[a]	8	15
	Beebread	16	16		Honey crop[a]	9	7		Weeds	—	4
	Leaves[b]	2	2		Pollen (c)[a]	9	7		Shoots	—	2
	Seeds[b]	1	1		Dead bees[a]	2	6				
	Weeds	—	8								
	Shoots	—	3	Semi-field	Bees (r)	—	5				
					Honey crop	—	5				
Osmia cavities	Mud walls (MW)	1	1		Pollen (c)	—	5				
	Nest entrances[a]	1	1								
	Pollen[a]	1	1								

[a]These matrices had no detectable residues of TMX or CLO, either in the control (C) or in the treated plots (T), and therefore were not considered further in the BeeREX risk assessment.
[b]These matrices had no detectable residues of TMX or CLO in the control (C) plots, and therefore only treated plots (T) were considered further in the BeeREX risk assessment. One leaf sample from the control was positive (less than the limit of quantitation) due to cross-contamination and was not considered in the risk assessment. A total of $n = 189$ samples were analyzed, (C, $n = 76$; T, $n = 113$). In the open field studies, $n = 8$ samples were detected as positive, and in the semi-field study, $n = 9$ samples were detected as positive. All other samples did not contain detectable residues of thiamethoxam (TMX) or clothianidin (CLO). All open field samples were pooled from $n = 5$ (JKI), $n = 4$ (HOH), and $n = 6$ (VHH) full-sized honey bee colonies.
JKI = Lower Saxony; HOH = Baden-Württemberg; VHH = Bavaria.

from different numbers of bees storing them in cells. Therefore samples from the semi-field study were included in the risk assessment to allow comparison of contamination with a single undiluted food source.

The measured residues from the different locations and matrices are shown in Figure 3. The figure indicates that as growth stage progressed from seed to subsequent plant parts (leaves at BBCH 33 and 49), the concentration of TMX + CLO decreased almost 57-fold within 174 days. In Regulation 2017/671, the European Commission set the maximum residue limit (MRL) for honey and other apiculture products at 0.05 mg/kg for both TMX and CLO and at 0.02 mg/kg for sugar beet roots (Regulation (EU) 2017/671). None of the relevant residues measured were above this MRL. Of note, mean residues in weeds and shoots at the VHH sites were approximately 11- to 15-fold higher than at JKI, despite being sampled at a similar time point.

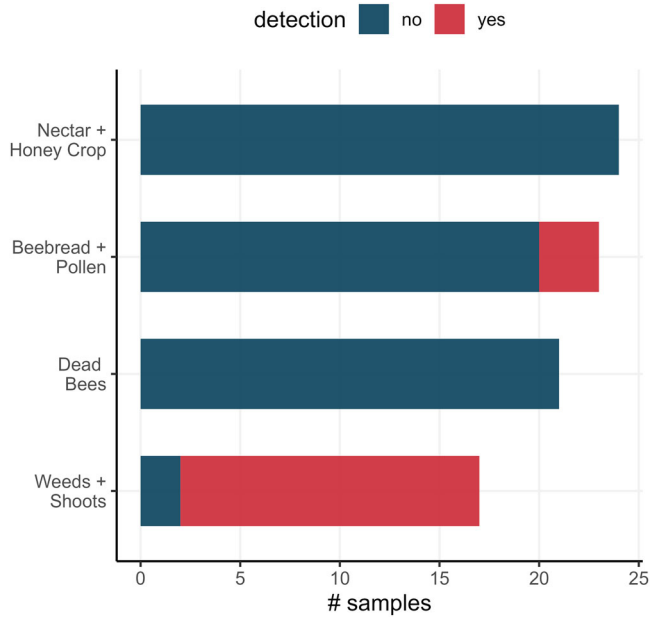

FIGURE 2: Proportion of the positive detected samples in all bee relevant matrices from the treated plots in 2021. Not included are the samples from the semi-field trial at the Baden-Württemberg location. No residues in either pools of nectar and honey crop samples ($n = 24$) or dead bee samples ($n = 20$) were detected. In total, 13% of beebread and pollen samples ($n = 3/23$) and 88% of weed and shoot samples were positive ($n = 15/17$).

Weed species sampled included *Papaver rhoeas*, *Chenopodium album*, *Cirsium arvense*, *P. tanacetifolia*, and *Malva sylvestris*. The plant *P. rhoeas* had the highest single residue value of TMX + CLO for weeds and shoots at 0.016 mg/kg (Figure 3).

The sampled *Osmia* matrices were unconsumed pollen, mud walls between brood cells, and mud walls sealing entrances (after Alkassab et al., 2020). Pooled samples of 34 sealed nest tunnels in the control and 9 nest tunnels in the treatment showed that mud walls between cells in the treatment indeed contained quantifiable residues of TMX + CLO (i.e., 0.001 mg/kg TMX; Figure 3).

Honey bee risk assessment

To assess potential risk to honey bees from the TMX + CLO residues found, BeeREX was used to model both an acute and chronic exposure scenario from dressed seeds and a systemic compound. Because foragers are most likely the first to be exposed in the field (acute exposure), they pass potentially contaminated pollen and/or nectar to hive bees, where the food is then processed to feed larvae and themselves (which may represent chronic exposure; Sponsler & Johnson, 2017).

Because we found residues in plant and mud matrices without evaluating their transfer to bee-relevant food such as pollen or nectar, we conservatively assumed that the bees consumed the plant or mud themselves equivalent to empirical residues in nectar for the BeeREX risk assessment. This must be considered when interpreting the two figures

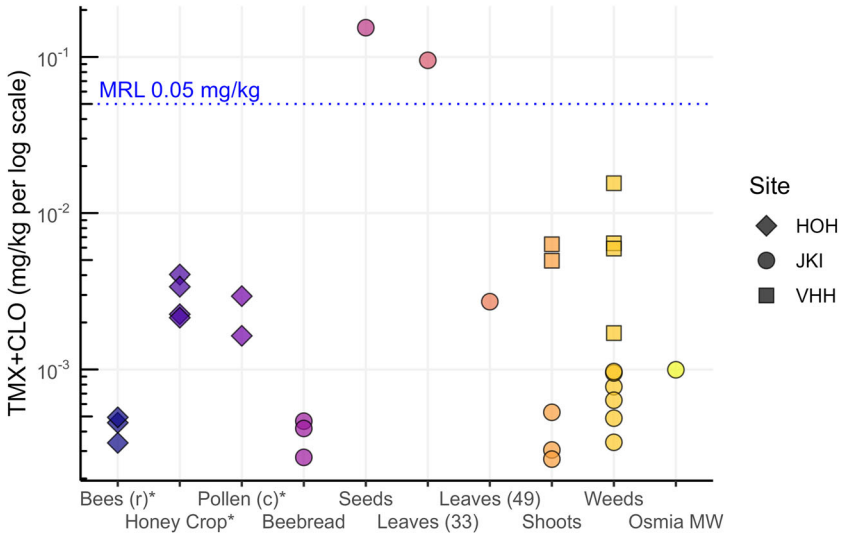

FIGURE 3: Results of measured residues in the different bee and plant matrices. Bees (r) = remaining body parts of prepared bees from which the crop was removed; Pollen (c) = pollen removed from foragers; Leaves at BBCH 33 and 49, and *Osmia* MW = mud walls within *Osmia* nest cavities. The maximum residue level (MRL) of thiamethoxam (TMX) + clothianidin (CLO) for honey and bee products in the European Union is shown as a dotted blue line. The asterisk (*) marks samples from the semi-field study. JKI = Lower Saxony site; VHH = Bavaria site; HOH = Baden-Württemberg site.

(Figures 4 and 5); hence the data points involved are highlighted in a blue square.

Acute risk. The mean acute RQ for honey bees from the residues found in the honey crop, corbicular Pollen (c), and remaining body parts of the prepared Bees (r) from which the crop was removed did not exceed the threshold LOC of 0.4 in the semi-field study. This was also true for beebread, sugar beet leaves (BBCH 49), shoots, weeds, and *Osmia* mud walls from the JKI open field site. Mean RQs of shoots and weeds at the VHH site were slightly higher than the LOC, 0.45 (±0.08) and 0.59 (±0.46), respectively. Seed and early sugar beet leaf stages (BBCH 33) had the highest RQs, at 12.22 and 7.55, respectively (Figure 4).

Chronic risk. Similar to the acute results, the mean chronic RQs for honey bees from the residues in the honey crop, Pollen(c), and Bees (r) did not exceed the threshold LOC of 1.0 in the semi-field study, nor did the mean RQs for beebread, sugar beet leaves (BBCH 49), shoots, weeds, and *Osmia* mud walls from the JKI open field site. Mean RQs of shoots and weeds at the VHH site were higher than the LOC, 1.61 (±0.27) and 2.11 (±1.66), respectively. Seed and early leaf stages of sugar beet (BBCH 33) had the highest RQs, at 43.92 and 27.15, respectively (Figure 5).

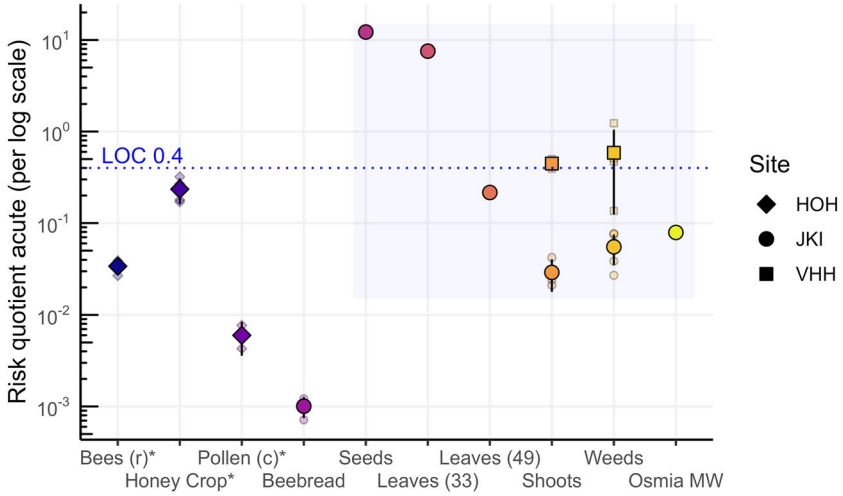

FIGURE 4: The mean acute risk quotients (RQs) for all measured matrices. The BeeREX model assumes acute risk to honey bees at a level of concern (LOC) of 0.4, which is shown as a dotted blue line. The RQs of plant material and *Osmia* mud walls (MW) are highlighted in a blue square because we assumed that bees consumed the whole plants and mud. This is a more conservative assumption from what would have occurred if the residues had transferred to foods such as pollen and nectar and must be considered when interpreting the results. Bees (r)* = remaining body parts of prepared bees from which the crop was removed; Pollen (c)* = pollen removed from foragers; Leaves at BBCH 33 and 49. The asterisk (*) marks samples from the semi-field study.

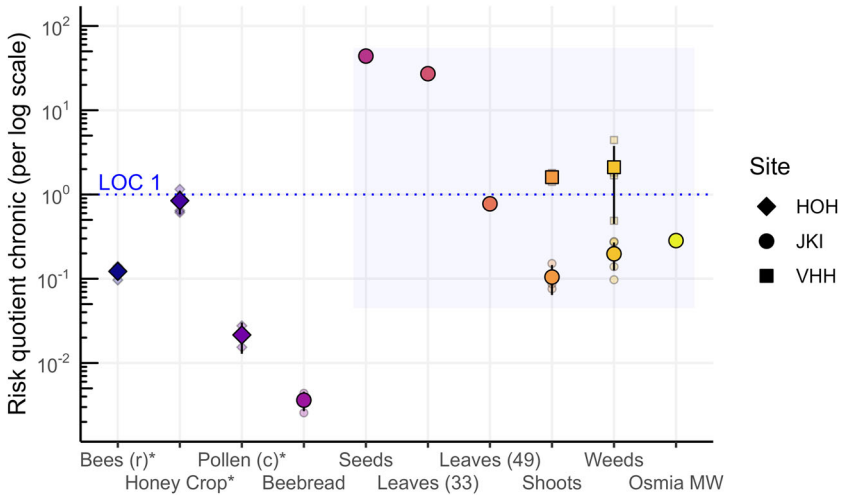

FIGURE 5: The mean chronic risk quotients (RQs) for all measured matrices. The BeeREX model assumes chronic risk to honey bees at a level of concern (LOC) of 1.0, which is shown as a dotted blue line. The RQs of plant material and *Osmia* mud walls (MW) are highlighted in a blue square because we assumed that bees consumed the whole plants and mud. Bees (r)* = remaining body parts of prepared bees from which the crop was removed; Pollen (c)* = pollen removed from foragers; Leaves at BBCH 33 and 49. The asterisk (*) marks samples from the semi-field study.

DISCUSSION

The emergency approval of TMX-treated sugar beet has drawn the attention of beekeepers and nongovernmental organizations. We focused on a cropping system in which treated plants do not result in exposure, but flowering weeds in the field may well be contaminated and therefore cause concern. The extent of exposure to which bees are subjected in the field and the assessment of potential risk from residues are of great importance in identifying routes of exposure and assessing hazards. Monitoring residues in matrices relevant to or even directly from bees, will facilitate our understanding of weather and how they may affect bee colonies.

We analyzed 113 pooled samples collected in and around treated fields from different locations and time points following the whole growing period from drilling to harvest of sugar beet. We did not find any neonicotinoid residues in nectar or dead bee samples during the whole period. Therefore, nectar as a route of exposure was less relevant in our study. However, we measured TMX + CLO in 13% of the pollen samples and in 88% of the weed samples we collected adjacent to or within sugar beet fields.

In a foliar spray scenario, pollen is known to contain not only higher concentrations of neonicotinoids (Dively, 2012), but also a greater number of general pesticide residues than nectar (Démares et al., 2022; Zioga et al., 2020). This is most likely due to the chemical properties and lipophilic nature of most pesticides, which accumulate more rapidly in pollen than in sugar matrices (Mullin et al., 2010). In seed treatments, however, residue concentrations of neonicotinoids are in the same order of magnitude (Thompson, Vaughan, et al., 2021). One reason for this is that in many plants pollen is more likely reached by the spray than covered nectaries at the flower base. Because we did not analyze the palynological spectrum of the samples, we assume that the residues collected via pollen must have come from neighboring weeds that we observed flowering adjacent to or within sugar beet fields. In the semi-field experiment, we confirmed the transfer of the active compounds TMX + CLO from soil to nectar and pollen of *Phacelia* plants. Because this is a known exposure route, farmers were required by regulation to keep weeds and other plants from flowering before and after drilling (until the end of 2022; General Regulation Federal States, 2021). Obviously, not all farmers in our study managed their fields according to this regulation.

The residues we measured in bee-relevant matrices did not exceed the MRL of 0.5 mg/kg TMX + CLO for honey and bee products in the EU (EC, 2017). We also found that average residues in weeds and shoots were 11- to 15-fold higher at the VHH sites than at JKI, even though samples were collected at similar times. One explanation for this could be soil texture and precipitation (site factors), which may have led to faster degradation, demonstrating high variance in exposure levels (Bonmatin et al., 2015; Woodcock et al., 2017). In addition, the plant species sampled may also have had an influence.

Furthermore, our data show that residues in corbicular pollen and nectar from prepared honey stomachs were similar. However, the residues from stored pollen (beebread) were approximately 100-fold lower than those from corbicular pollen. This indicates that dilution is occurring in the hive, because we sampled a pool of different pollen cells, and furthermore, beebread itself may be inhomogeneous. Sponsler and Johnson (2017) argue that knowing the fate of a pesticide and its active ingredients is essential for assessing the potential risk to the bee colony. We agree and suggest that the distribution of active ingredients within different matrices in the hive, including larval food for all three castes (drones, queens, and workers), should be studied in more detail (Farruggia et al., 2022; Wueppenhorst et al., 2022).

An interesting finding was that the residues detected in the *Osmia* mud walls showed transfer of active ingredients into the

nesting material of wild bee species. This confirms the results of a semi-field study by Alkassab et al. (2020), under more realistic conditions, and makes this exposure route potentially relevant for risk assessment. Thompson, Vaughan, et al. (2021) detected 0.008 mg/kg TMX + CLO in German soils 1 year after sugar beet growing. This confirms the exposure level of 0.001 mg/kg in our study, taking into account dilution effects due to the collecting behavior of *Osmia* bees. Particularly with respect to ground-nesting bees, such exposure routes require special attention. Their susceptibility to lethal and sublethal effects of neurotoxic insecticides may be different from that of honey bees (Willis Chan et al., 2019). Schmolke et al. (2021) even showed that solitary bees are potentially more vulnerable to pesticides than honey bees, enabling sublethal effects to have more profound impacts. For non-*Apis* bees, European risk assessment consequently assumes a safety factor that lowers honey bee endpoints by 10-fold (Lewis & Tzilivakis, 2019). This is to account for the difference in sensitivity of the species. However, the consequences of sublethal exposure to pesticides under field conditions for non-*Apis* bees are not well studied and urgently require further investigation (Straub et al., 2022).

The acute RQs used to assess the risk from the residues we found in bees and bee matrices did not exceed the LOC and thus did not indicate a significant risk to honey bees. In particular, when comparing data from the semi-field and field residues, it is evident that exposure from nectar was negligible in the field. Although residues were found in nectar samples from *Phacelia* when planted in tunnels on the sugar beet field, bees did not collect measurable amounts from flowering weeds in and around the sugar beet fields under realistic field conditions. Most likely, these food sources were not predominant and the collected amounts were diluted in the hive by other, more attractive food sources (Sponsler & Johnson, 2017). The RQs of shoots and weeds slightly exceeded the LOC at the VHH site, but only under the assumption that bees would consume the entire plant. Because this is unlikely, even this very conservative scenario would only just trigger a higher test level in the risk assessment. Considering all measured residues, no acute risk to honey bees could be extrapolated at the TMX plots for the sampled matrices.

To also use the BeeREX model for chronic RQs, chronic toxicity data must be obtained. This is not the case for many insecticidal substances, because standardized methods for deriving chronic toxicity endpoints have only recently been introduced (Démares et al., 2022; Organisation for Economic Co-operation and Development [OECD], 2016, 2017). However, neonicotinoids are well studied due to their exceptional toxicity and systemic nature (which attracted public attention), and all relevant endpoints for TMX and CLO were available (Table 1). Although chronic exposure was assumed in this even more conservative risk assessment, the picture was similar to that for the acute risk. We did not detect chronic risk to honey bees at any of our study sites. Similar conclusions were made by Wen et al. (2021), who found CLO residues in nectar and pollen samples collected over 2 years during oilseed rape bloom in China. Previously, Dai et al. (2018) took CLO residues detected in pollen and nectar as a baseline and fed the same amount to in vitro–reared worker larvae accordingly. Acute and chronic RQs determined for CLO in both studies did not exceed LOCs, indicating no risk from these residue levels.

In contrast, Thompson (2021) re-evaluated CLO and TMX residue data from the literature using the BeeREX model. This assumed that the highest levels found in pollen were 75% of those found in nectar. When considering that nectar consumption is higher than pollen, nectar thus represents the more critical matrix (USEPA, 2012, 2014). In fact, CLO and TMX exceeded the acute LOC of 0.4 at 1.8 and 0.93, respectively, when using detected MRLs (Thompson, 2021). But, these levels did not necessarily come from treated seed, and may have been due to other forms of application such as foliar sprays. Under field conditions, however, mixed effects of oilseed rape treated with CLO and TMX on bees have been reported depending on the experimental site (Woodcock et al., 2017). Neonicotinoid exposure caused both adverse and beneficial effects on colony development. This may explain the differences in reported RQs from the literature and confirms a similar effect in our study, in which we found site-specific residue levels in weeds and shoots of sugar beets.

Thompson (2021) highlighted that the RQ approach is a Tier 1 assessment and is intended to determine whether a low level of risk can be concluded from the available exposure data and the toxicity data obtained in laboratory studies. If the RQ thresholds are exceeded, higher tier studies are required. This allows for a more realistic assessment of the risk at the colony level, or corrective action can be specified (Thompson, 2021). Although none of the RQs in our study would trigger a higher tier risk assessment for honey bees, there are currently insufficient data on endpoints for wild bees. This is because standardized tests to assess chemical exposure and effects in a regulatory context are simply better established for honey bees (EFSA, 2013b; USEPA, 2012).

A checklist compiled by Kuhlmann et al. (2014) shows that the European bee fauna includes a total of approximately 1965 species. A growing body of evidence suggests that wild bees (along with other insect pollinators) play an important role in the pollination of crops and native plants, in addition to their overall ecosystem benefit through biodiversity conservation (USEPA, 2012). Although the BeeREX model and other approaches such as the hazard quotient (Stoner & Eitzer, 2013) do not provide a formal risk assessment specifically for wild bee species, considering possible sensitivity and exposure differences compared with honey bees, such inclusion would improve the accuracy of these approaches (EFSA, 2013b; USEPA, 2012). More data on toxicity to bees and effects via different routes of exposure (including sublethal effects in particular) are also urgently needed to update these risk assessment tools accordingly.

CONCLUSIONS

Our study confirms that neonicotinoid residues from sugar beet seed treatments can enter honey bee colonies when

bees forage on flowering weeds near or within treated fields. An assessment of the potential risk to these bees from the presence of such residue levels with the commonly utilized BeeREX model found no evidence of acute or chronic risk. However, we also detected neonicotinoid residues in the mud walls within nests of the solitary bee *O. bicornis* near a treated sugar beet field. Although the emergency approval for TMX-treated sugar beet in Germany was issued with the strict requirement to remove all flowering weeds from the fields, these instructions were not followed by all beet growers. Given the current lack of data on wild bee species and other beneficial pollinator insects to assess their individual risk, care must be taken in future use of such highly potent insecticides. We need to ensure that all regulatory requirements are followed to minimize any unintended exposure that could lead to unacceptable risks for pollinating insects.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5602.

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This article has earned both an Open Data and Open Materials badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at 10.17605/OSF.IO/6U5W7. Learn more about the Open Practices badges from the Center for Open Science: https://osf.io/tvyxz/wiki.

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