Monitoring programme for Thiamethoxam and Clothianidin residues in soil and non-crop vegetation in relation to the application of Cruiser treated seeds in sugar beet fields: Phase II Summary Report

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EXECUTIVE SUMMARY

The objective of this study was to determine if thiamethoxam (TMX) and clothianidin residues were detectable prior to (pre-drilling), during (within growth season), and following (post-harvest), the use of Cruiser treated seeds for the sugar beet crop cultivation season in 2022. Six different sites were selected for the monitoring programme, chosen to be representative of the soil types, geographical locations and climactic conditions used to grow sugar beet in the UK. No claim of GLP compliance is made for the sampling procedures detailed in this summary report.

Both TMX and clothianidin were detected within the growth season and post-harvest soil samples, which would potentially be expected due to the presence of seed casings and ungerminated seeds within the field and could explain the occasional quantified detections above the cited application rate of TMX. The detection of clothianidin in some pre-drilling samples, and comparable levels in the growth season and post-harvest soil samples means that it is not possible to directly attribute these specific detections to the use of the Cruiser seed in this 2022 programme. There is an indication that the persistence of clothianidin residues may be related to soil type, with the more clayey soils showing more continued residue detections.

Residue analysis determined that neither TMX nor clothianidin could be quantifiably detected in any of the pollen or vegetation samples on any sampling occasion. This gives confidence that translocation does not occur into non-target crops and pose a risk to bees and other species within this time frame. Similarly, TMX was not detected in the field margin soil throughout this monitoring programme confirming that the compound does not appear to migrate out of the encapsulated seed or treated fields. Clothianidin was detected within some of the field margins, but only when already present within the baseline, pre-drilling, samples.

Further monitoring of these sites, where possible, is to take place in 2023 to confirm that no continued residue build-up of either TMX or clothianidin can be detected over a longer period, as the current data is inconclusive regarding this possibility at every site monitored in this programme. Additionally, it should be acknowledged that the current analytical methodology employed the standard guideline limit of quantification (LOQ) of 0.01 mg/Kg. Assuming a bulk density of 1.5 g/cm^3, this would equate to 45 g/ha of TMX/clothianidin, which is close to the intended application rate of 51.75 g/ha. A second monitoring programme following the use of Cruiser treated seed, on six sites, in 2023 will employ a lower LOQ (0.001 mg/Kg) for residue analysis to enable more accurate interpretation of the presence of TMX and clothianidin in soil, vegetation and pollen samples.

1 Introduction

In January 2022, HSE approved an Emergency Authorisation for the use of a neonicotinoid seed treatment (Formulated product 'Cruiser', containing the active ingredient thiamethoxam; TMX) on sugar beet grown in the UK under contract to British Sugar. Treated seed is only available for use where the Rothamsted Virus Yellows Risk Forecast model predicts a high risk, with the 19% economic threshold being met. Once treated seed is drilled, several other criteria must be met including a programme of monitoring in soil and vegetation for neonicotinoid residues. Potential issues include the build-up of residues in the soil profile because of the relative persistence of the compounds, migration of residues from the area of use, and translocation to non-target flowering plants that could be a source of food for bees.

On the 1st March 2022, the model forecast an incidence of 68.9% to trigger the use of Cruiser SB seed treatment in 2022. Conditions relating to the maximum use of the Cruiser seed are imposed with the emergency authorisation, including a maximum drilling rate of 115,000 seeds/ha and the maximum dose of the product to be 75 mL/100,000 seeds. Cruiser contains 600 g/L TMX, therefore, the maximum application rate would be 51.75 g/ha TMX. This monitoring programme was devised to provide robust data on thiamethoxam, and the metabolite clothianidin, residues in soils, non-crop vegetation and pollen, to support the continued use of neonicotinoid seed treatments if required by the sugar beet industry until more sustainable solutions become available.

The objective of this study was to determine if TMX and clothianidin residues were detectable prior to (pre-drilling), during, and following (post-harvest), the use of Cruiser treated seeds for the sugar beet crop cultivation season. Six different sites were selected for the monitoring programme that met the following broad requirements:

- Representative of soil type (3 sandy soils, 2 clay soils, and 1 silty soil)
- Differing geographical locations (as much as possible)
- Different expected climatic conditions (e.g., low/high rainfall areas), if possible
- A full pesticide use history (5 years) of the selected sites should ideally be available

The selection of sites, along with obtaining the agreement of the individual growers, was conducted prior to the start of this study (CEA study number 1060845).

Soil sampling was conducted in both the in-field and the field margin areas of the crop field, with non-crop vegetation and pollen samples collected from the field margin area at each site. Samples were stored frozen following collection and all study samples were shipped to the Test Facility for GLP residue analysis of TMX and its primary metabolite clothianidin. No claim of GLP compliance is made for the sampling procedures detailed in this summary report.

2 Materials and Methods

2.1 Target compounds

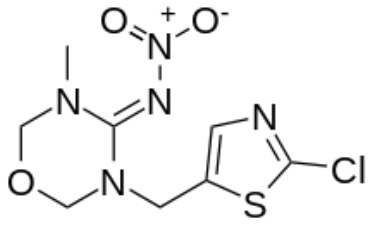
2.1.1 Thiamethoxam

Chemical (IUPAC) Name: Thiamethoxam

CAS Number: 153719-23-4

Chemical formula: $C_8H_{10}ClN_5O_3S$

Structure:



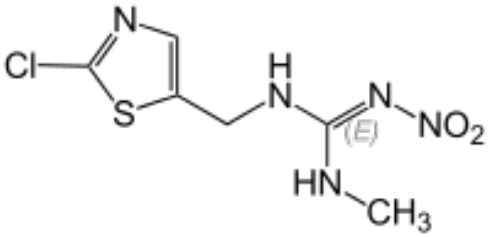
2.1.2 Clothianidin

Chemical (IUPAC) Name: Clothianidin

CAS Number: 210880-92-5

Chemical formula: $C_6H_{18}ClN_5O_2S$

Structure:



2.2 Sampling programme

The in-field soil cores were collected along four transects within the field, spaced to be representative within the planted field area, in a "W" pattern (Figure 1). A trundle wheel was used to mark out this pattern and the in-field core locations recorded on field data sheets. The same pattern was used for each soil sampling occasion, to within 1 m of the core position, to avoid sampling the exact same soil section each time . The field margin soil cores and the vegetation samples were obtained from around the field margins, from each edge and in regular spacings, where possible, as determined by the layout at each individual site.

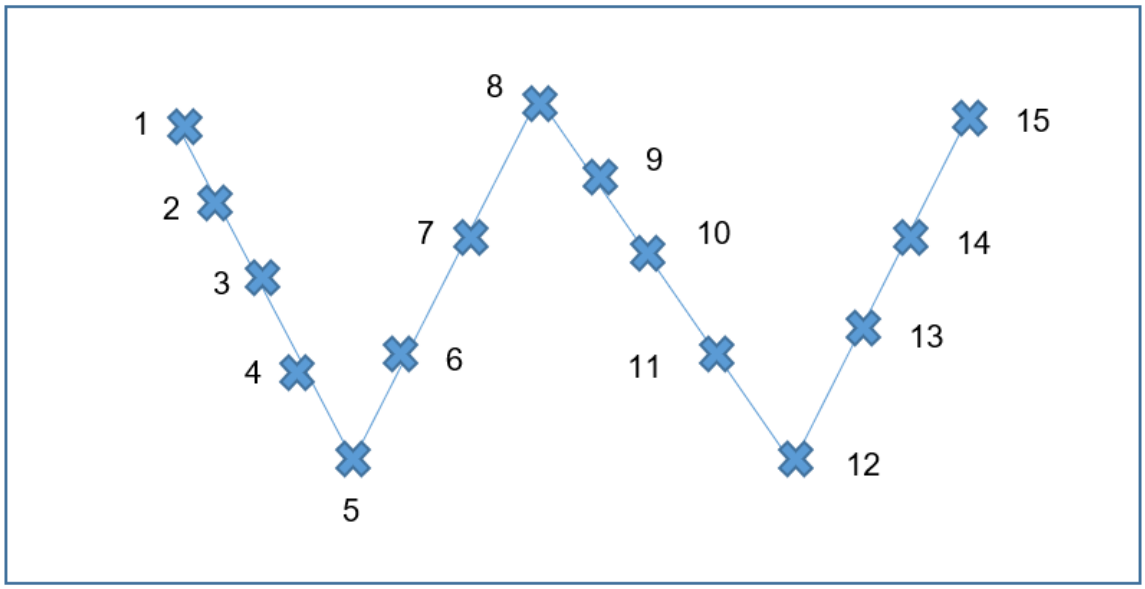


Figure 1. Example schematic of the soil core locations

At each of the six sites, the following sampling regime for soil and field margin vegetation was followed:

2.2.1 Soils

There were three soil sampling occasions: pre-drilling (baseline), within growth season (GS39), and post-harvest (within 1 month). For all six sites 15 in-field cores and 15 field margin cores were obtained on each sampling occasion. For the pre-drilling sampling occasion, 40 cm depth cores (50 mm diameter) were collected and then split into two depths (0-20 and 20-40 cm). All other soil sampling occasions used a 30 cm depth gauge corer sampling approximately a 30 mm diameter soil core (with the exception of the within growth season cores at site 2, where the 40 cm corer was used), due to dry weather conditions preventing the use of the wider 40 cm corer. The 30 cm gauge corer was used in triplicate at each coring position to obtain enough material within the sample replicate.

2.2.2 Field margin vegetation

There were two vegetation sampling occasions: firstly, when most plants were in flower, and secondly, in advance of harvesting (Autumn). For each sampling occasion, 3 individual replicate samples were obtained from each site.

2.2.3 Pollen

There were two pollen sampling occasions, coinciding with the field margin vegetation samplings. For each sampling occasion, approximately 1 Kg of flower heads were obtained from the field margin vegetation at each site.

2.3 Sample Handling and storage

2.3.1 Soil samples

Soil cores were collected as detailed in Section 2.2.1., on each sampling occasion, at all sites. All soil cores were frozen on arrival at the CEA facility. Where 40 cm depth cores were obtained, the frozen cores were split into 0-20 cm and 20-40 cm cores prior to being bulked into composite samples.

The 15 in-field soil cores were bulked to provide 3 composite in-field soil samples and 3 composite field margin samples for analysis. A selective bulking approach was used for the in-field soil cores (Table 1), with randomised bulking for the field margin soil cores.

All bulk samples were assigned a unique sample ID and returned to frozen storage prior to being shipped to the analytical laboratory (frozen) for residue analysis

Table 1. Selective bulking of in-field soil cores

Bulked sample	Sampling Location number				
1	1	2	8	14	15
2	3	6	7	9	13
3	4	5	10	11	12

2.3.2 Vegetation samples

All vegetation samples were frozen on arrival at the CEA facility and remained frozen until being transported (frozen) to the analytical laboratory.

2.3.3 Pollen samples

All flower head samples were frozen on arrival at the CEA facility and remained frozen until being transported (frozen) to the analytical laboratory. The extraction of pollen from the flower heads took place at the analytical test facility.

2.4 Residue analysis

Residue analysis was performed, to GLP, at the Test Facility, Smithers (108 Woodfield Drive, Harrogate, North Yorkshire, HG1 4LS, UK). Sample analysis was carried out under GLP. Smithers are a member of the UK GLP compliance programme. Validated methods were used to analyse the samples for TMX and its metabolite, clothianidin. In addition, soil bulk density analyses for each site, and storage stability studies for each matrix were performed. Full details of all the analytical methods and all the raw data generated will be provided by the analytical Test Facility in a final report.

3 Results

Validated methods[1] for soil, vegetation, and pollen were employed to determine levels of TMX and clothianidin. For both compounds the limit of quantification (LOQ) was 0.01 mg/kg and the limit of detection (LOD) was 0.003 mg/kg. Bulk soil density for each site were also determined at the analytical facility to enable the conversion of residue concentrations from mg/kg to g/ha for the soil samples (Table 2). All analytical runs were QC checked and found to be within the acceptable range for procedural recoveries (raw data provided in the analytical report). Storage stability studies[1] for each matrix confirmed no additional losses occurred between sampling and extraction. Dates of each sampling and subsequent residue analyses are given in Appendix 1.

Table 2. Soil bulk density values and conversion for each site

Site (soil type)	Bulk Density (g/cm^3)	Conversion calculations
1 (sandy)	1.503	***Correction factor*** = (soil core depth [cm]) *100 *bulk density value ***Measured residue in g/ha*** = measured value in mg/kg * correction factor
2 (sandy)	1.576	
3 (sandy)	1.760	
4 (silt)	1.283	
5 (clay)	1.514	
6 (clay)	1.277	

3.1 Soil sample analysis results

The recovery of TMX from soil samples obtained from each site are detailed in Table 3, and the recovery of clothianidin are given in Table 4. All data are presented as the mean of the three bulk samples obtained on each occasion.

The baseline measures of TMX (pre-drilling) were found to be below the LOD, except for Site 4, where residues were detected, however, these values were below the LOQ of the method. Quantifiable levels of TMX were found during the growth season at Sites 2 and 5, and after the harvest in site 6. Detectable levels of TMX, below the LOQ were also determined at Sites 3 and 4 during and after the season and at Site 1 within the growth season. No TMX residues were detected in the field margins at any of the test sites, during or after the sugar beet season.

There were no quantifiable residues of clothianidin detected in the baseline soil cores from Sites 1 to 5 inclusive, although there were some residues detected below the LOQ at some of these sites, both within the field and in the field margin. Site 6 was found to have quantifiable levels of clothianidin within the beet field cores prior to drilling; detectable clothianidin levels were also found in the field margin at this time. Site 6 continued to have quantifiable levels of clothianidin detected in the in-field samples throughout and following the sugar beet season, with comparable residues to the pre-drilling samples.

At all other sites, no quantifiable levels of clothianidin could be detected within the sugar beet fields, or on the field margins, during the growth season or after harvest of the crop. Most of these sites registered detectable, but <LOQ, residues of clothianidin during the growth season within the field. By post-harvest sampling, only <LOQ residues were still detectable at Sites 4 and 5.

It should be acknowledged that the analytical methodology employed the standard guideline LOQ of 0.01 mg/Kg, with an LOD of 0.003 mg/Kg. Assuming a bulk density of 1.5 g/cm^3, this would equate to 45 g/ha (LOQ) and 13.5 g/ha (LOD) of TMX. These levels are below the intended application rate of 51.75 g/ha, however a more sensitive method could provide a more in depth interpretation and understanding of the residue data.

Table 3. Levels of TMX detected in soil cores at each site

Site (soil type)	Sample location	Mean measured Thiamethoxam (mg/Kg [g/ha])		
		1: Pre-drilling	2: Growth Season	3: Post-harvest
1 (sandy)	In-field	0-20 cm: <LOD 20-40 cm: <LOD	>LOD<LOQ *0.005 [22.5]*	<LOD
	Field Margin	0-20 cm: <LOD 20-40 cm: <LOD	<LOD	<LOD
2 (sandy)	In-field	0-20 cm: <LOD 20-40 cm: <LOD	0-20 cm: <LOD 20-40 cm: **0.025 [78.8]**	<LOD
	Field Margin	0-20 cm: <LOD 20-40 cm: <LOD	0-20 cm: <LOD 20-40 cm: <LOD	<LOD
3 (sandy)	In-field	0-20 cm: <LOD 20-40 cm: <LOD	>LOD<LOQ *0.0043 [22.7]*	>LOD<LOQ *0.0086 [45.4]*
	Field Margin	0-20 cm: <LOD 20-40 cm: <LOD	<LOD	<LOD
4 (silt)	In-field	0-20 cm: >LOD<LOQ *0.0048 [12.3]* 20-40 cm: >LOD<LOQ *0.0041 [10.5]*	>LOD<LOQ *0.006 [23.1]*	>LOD<LOQ *0.0078 [30.0]*
	Field Margin	0-20 cm: <LOD 20-40 cm: <LOD	<LOD	<LOD
5 (clay)	In-field	0-20 cm: <LOD 20-40 cm: <LOD	**0.011 [50]**	<LOD
	Field Margin	0-20 cm: <LOD 20-40 cm: <LOD	<LOD	<LOD
6 (clay)	In-field	0-20 cm: <LOD 20-40 cm: <LOD	<LOD	**0.017 [65.1]**
	Field Margin	0-20 cm: <LOD 20-40cm: <LOD	<LOD	<LOD

Unless otherwise stated, soil cores were of 0-30 cm depth. Bold values represent quantified residue levels >LOQ. Italics represent unquantified values (<LOQ)
LOD = 0.003 mg/Kg. LOQ = 0.01 mg/Kg.

Table 4. Levels of Clothianidin detected in soil cores at each site

Site (soil type)	Sample location	Mean measured Clothianidin (mg/Kg [g/ha])		
		1: Pre-drilling	2: Growth Season	3: Post-harvest
1 (sandy)	In-field	0-20 cm: >LOD<LOQ *0.0033 [9.9]* 20-40 cm: <LOD	>LOD<LOQ *0.0051 [23.0]*	<LOD
	Field Margin	0-20 cm: <LOD 20-40 cm: <LOD	<LOD	<LOD
2 (sandy)	In-field	0-20 cm: >LOD<LOQ *0.0034 [10.7]* 20-40 cm: <LOD	0-20 cm: >LOD<LOQ *0.0032 [10.1]* 20-40 cm: >LOD<LOQ *0.0051 [16.1]*	<LOD
	Field Margin	0-20 cm: <LOD 20-40 cm: <LOD	0-20 cm: <LOD 20-40 cm: <LOD	<LOD
3 (sandy)	In-field	0-20 cm: <LOD 20-40 cm: <LOD	<LOD	<LOD
	Field Margin	0-20 cm: <LOD 20-40 cm: <LOD	<LOD	<LOD
4 (silt)	In-field	0-20 cm: >LOD<LOQ *0.0091 [23.4]* 20-40 cm: >LOD<LOQ *0.0091 [23.4]*	**=LOQ** *0.01 [38.5]*	>LOD<LOQ *0.0093 [35.8]*
	Field Margin	0-20 cm: >LOD<LOQ *0.0077 [19.8]* 20-40 cm: >LOD<LOQ *0.0041 [10.5]*	>LOD<LOQ *0.0059 [22.7]*	>LOD<LOQ *0.0045 [17.3]*
5 (clay)	In-field	0-20 cm: >LOD<LOQ *0.0071 [21.5]* 20-40 cm: >LOD<LOQ *0.0041 [12.4]*	>LOD<LOQ *0.0075 [34.1]*	>LOD<LOQ *0.0052 [23.6]*
	Field Margin	0-20 cm: <LOD 20-40 cm: <LOD	<LOD	<LOD
6 (clay)	In-field	0-20 cm: **0.040 [102.2]** 20-40 cm: **0.01 [25.5]** *at LOQ*	**0.026 [99.6]**	**0.026 [99.6]**
	Field Margin	0-20 cm: >LOD<LOQ *0.0077 [19.7]* 20-40 cm: <LOD	>LOD<LOQ *0.0064 [24.5]*	>LOD<LOQ *0.0084 [32.2]*

Unless otherwise stated, soil cores were of 0-30 cm depth. Bold values represent quantified residue levels >LOQ. Italics represent unquantified values (< LOQ)
LOD = 0.003 mg/Kg. LOQ = 0.01 mg/Kg.

3.2 Vegetation sample analysis results

The recovery of TMX and clothianidin from field margin vegetation samples obtained at each site are detailed in Table 5. All samples were determined to be <LOD for both compounds.

Table 5. Levels of TMX and clothianidin detected in field margin vegetation at each site

Site	Mean measured TMX (mg/Kg)		Mean measured Clothianidin (mg/Kg)	
	Full growth	Pre-harvest	Full growth	Pre-harvest
1	<LOD	<LOD	<LOD	<LOD
2	<LOD	<LOD	<LOD	<LOD
3	<LOD	<LOD	<LOD	<LOD
4	<LOD	<LOD	<LOD	<LOD
5	<LOD	<LOD	<LOD	<LOD
6	<LOD	<LOD	<LOD	<LOD

LOD = 0.003 mg/Kg. LOQ = 0.01 mg/Kg.

3.3 Pollen sample analysis

The recovery of TMX and clothianidin from pollen, extracted from field margin flower head samples obtained at each site are detailed in Table 5. All samples were determined to be <LOD for both compounds, except for a detected, but non-quantifiable, residue at Site 4, on the second sampling occasion.

Table 5. Levels of TMX and clothianidin detected in pollen samples from each site

Site	Measured TMX (mg/Kg)		Measured Clothianidin (mg/Kg)	
	Full growth	Pre-harvest	Full growth	Pre-harvest
1	<LOD	<LOD	<LOD	<LOD
2	<LOD	<LOD	<LOD	<LOD
3	<LOD	<LOD	<LOD	<LOD
4	<LOD	>LOD<LOQ *(0.0049)*	<LOD	<LOD
5	<LOD	<LOD	<LOD	<LOD
6	<LOD	<LOD	<LOD	<LOD

LOD = 0.003mg/Kg. LOQ = 0.01 mg/Kg.

4 Discussion

For TMX, only Site 4 had any detectable, although unquantifiable, residues of TMX in the pre-drilling, in-field, soil samples. These low level (<LOQ) residues continued to be detected in the in-field soil samples at this site throughout the monitoring programme. At all other sites, the baseline for TMX was established to be <LOD of the method, therefore any subsequent detections of TMX within the field could be attributed to the use of the Cruiser treated seed. There were three occasions where quantifiable levels of TMX were detected, two of these occurrences (Site 2, 20-40 cm, growth season and Site 6, 0-30 cm, post-harvest) were calculated to be higher than the maximum application rate of 51.75 g/ha. A higher than application rate detection would be possible if the soil cores obtained on these sampling occasions contained a high number of seed casings or ungerminated treated seeds. At Sites 1, 2 and 5, levels of TMX were <LOD in the post-harvest soil samples, indicating low risk of uptake into a succeeding crop, despite the quantifiable, and high, residue detection at Sites 2 and 5 on the previous sampling occasion. Site 6 registered quantifiable levels of TMX at this stage, which could be explained by the presence of seeds or seed casings. Sites 3 and 4, whilst <LOQ, had detectable levels of TMX in the post-harvest samples, with increased residues in comparison to the previous sampling occasion, suggesting a potential residue build up could be occurring. With the soil DT_{50} of TMX in the field reported to be anywhere between 7 and 530+ days[1], continued monitoring of these in-field soils, and potentially the succeeding crops, would be necessary to confirm there is no risk at these sites. No discernible correlation between TMX residue detection and soil type is apparent from this data and the pesticide history provided for each test site showed no TMX based products were used within the last 5 years at any of the locations

No detectable residues were found in the field margin at any site on the pre-drilling sampling occasion, thus, establishing a zero baseline and indicating any subsequent detection of TMX in the field margins could be a direct result of the use of the Cruiser treated seed within the field. As no TMX residues were detected in any of the subsequent soil samplings, at any of the sites, it can be concluded that any migration and translocation of this active ingredient is either very slow or does not occur from the encapsulated treated seed, and contamination of non-target crops is unlikely. This is supported by the lack of any detectable or quantifiable residues of TMX in the field margin vegetation and pollen samples throughout this monitoring programme.

For clothianidin, the residue data is less straightforward than for TMX. In this monitoring programme, clothianidin was monitored as a metabolite of TMX, however, as there were baseline detections of clothianidin in the pre-drilling soil samples, these residue detections are harder to attribute directly to the current use of the Cruiser treated seeds. From the pesticide histories provided, it was determined that Sites 1 and 5 applied products containing clothianidin in 2018 and 2017, respectively, no other test sites used clothianidin based products in the preceding 5 years. The data from this monitoring programme indicated that the silt and clay soil types presented with higher clothianidin residues in comparison to the sandy soil sites. This might be explained by the soil DT_{50} for clothianidin, which has been reported to range from negligible to over 1300 days, with a clay soil type having the longest DT_{50}[2].

For the in-field samples from the sandy soil sites, Site 3 did not have any detectable levels of clothianidin on any of the sampling occasions, indicating that despite the low-level detections of TMX in the growth season and post-harvest soils samples, no detectable breakdown into clothianidin was apparent. At Sites 1 and 2, a small (just above LOD) residue of clothianidin was detected in the baseline

samples, with slightly increased residues detected during the growth season but no detectable residues post-harvest. This was in line with the TMX residue data for these sites and indicates there would be low risk of uptake into any succeeding crop. Site 1 is known to have used a clothianidin based product in 2018, which may explain the detection of this analyte in the pre-drilling samples.

At sites 4 and 5, there were detectable, unquantified, levels of clothianidin found on all 3 sampling occasions. At both sites, the levels detected in the pre-drilling samples were very similar to those found in the growth season and post-harvest samples, however, only Site 5 is known to have previously (2017) applied a clothianidin based product. This suggests that, while clothianidin is persistent within the soil matrix, the use of the Cruiser treated seeds did not increase the residues of clothianidin present in the field. At Site 6, the baseline, pre-drilling, soil samples had quantifiable levels of clothianidin, these were greatest in the pre-drilling soil samples, with a slight reduction seen in the growth season and post-harvest samples, again suggesting these residues are not specifically linked to the Cruiser treated seeds sown in this season. The presence of clothianidin at this site could not be explained using the pesticide history. It may be possible, due to the longevity of this compound in soil, that it remains at these detectable levels following previous use outside of the 5-year history explored, or from spray drift from nearby locations in recent years.

When investigating the field margin soil data, no detectable clothianidin residues were found at any of the three sandy soil sites (Sites 1,2 and 3), or at one of the clay sites (Site 5), on any of the sampling occasions. As with TMX, this suggests that that any migration and translocation of clothianidin is either very slow or does not occur from the encapsulated treated seed, and contamination of non-target crops is unlikely. At Sites 4 and 6, there were detectable, unquantified, levels of clothianidin on all three sampling occasions. At Site 4, the levels were comparable in the pre-drilling samples and in the growth season and post-harvest samples, as with the in-field profile. At Site 6, the residue levels increased slightly through the programme. Although the only detectable clothianidin residues in the field margins were at those sites where the compound was already present, and the lack of any detectable residues of clothianidin in the field margin vegetation and pollen samples throughout this monitoring programme indicates low risk to the field margin crop, continued monitoring of these sites would be necessary to confirm there is no long-term risk at these sites.

5 Conclusions

Both TMX and clothianidin were detected within the growth season and post-harvest soil samples, which would potentially be expected due to the presence of seed casings and ungerminated seeds within the field, and could also explain the quantified detections above the agreed application rate of TMX. The detection of clothianidin in the pre-drilling samples, and comparable levels in the growth season and post-harvest soil samples, means that it is not possible to directly attribute these residues to the use of the Cruiser seed in this 2022 programme.

Residue analysis determined that neither TMX or clothianidin could be quantifiably measured in any of the pollen or vegetation samples on any sampling occasion. This gives confidence that translocation does not occur into the non-target crops and pose a risk to bees, and other sensitive species, within this time frame. Similarly, TMX was not detected in the field margin soil throughout this monitoring programme suggesting that the compound does not migrate out of the encapsulated seed or treated

fields. Clothianidin was detected within the field margins, but only when already present within the baseline, pre-drilling, samples.

Further monitoring of these sites, where possible, is to take place in 2023, to confirm that no continued residue build-up of either TMX or clothianidin can be detected over a longer period as the current data is inconclusive regarding this possibility at every site monitored in this programme. Improving the sensitivity of the analytical method, as is planned for the 2023 monitoring scheme, will provide greater insight into the fate and behaviour of TMX and clothianidin following the application of Cruiser treated seed.

6 References

[1] A. Cashmore. 2023. Method Validation and Sample Analysis of Thiamethoxam and Clothianidin in Soil and Non-Crop Vegetation. Smithers ERS Limited, Study Report No 3203301.

[2] D. Goulson, Review: An overview of the environmental risks posed by neonicotinoid insecticides, J. Appl. Ecol., 2013, 50, 977-987.

7 Appendices

7.1 Appendix 1: Sampling and analysis dates

Table A. Soil sampling and analytical processing dates

Site	Pre-drilling Sampling		Growth season sampling		Post-harvest sampling	
	Sampling Date	Analytical processing date(s)	Sampling Date	Analytical processing date	Sampling Date	Analytical processing date
1	16/03/2022 and 21/03/2022*	16/06/2022 and 27/06/2022	08/08/2022	17/08/2022	22/03/2023	23/03/2023
2	22/03/2022	16/06/2022 and 26/07/2022	22/06/2022	22/08/2022	09/01/2023	24/02/2023
3	06/04/2022	20/06/2022 and 26/07/2022	10/08/2022	17/08/2022	23/02/2023	24/02/2023
4	24/03/2022	20/06/2022 and 27/06/2022	04/08/2022	17/08/2022	12/12/2022	14/12/2022
5	17/03/2022	20/06/2022 and 21/07/2022	02/08/2022	17/08/2022	05/12/2022	14/12/2022
6	25/03/2022	20/06/2022 and 25/06/2022	03/08/2022	17/08/2022	12/12/2022	14/12/2022

*Field margin sampling completed after crop was drilled

Table B. Vegetation sampling and analytical processing dates

Site	Full growth Sampling		Pre-harvest sampling	
	Sampling Date	Analytical extraction date	Sampling Date	Analytical extraction date
1	08/08/2022	24/10/2022	12/09/2022	25/10/2022 and 09/11/2022*
2	22/06/2022	24/10/2022	19/09/2022	25/10/2022
3	10/08/2022	24/10/2022	14/09/2022	26/10/2022
4	04/08/2022	24/10/2022	22/09/2022	26/10/2022
5	02/08/2022	25/10/2022 and 09/11/2022*	15/09/2022	26/10/2022
6	03/08/2022	25/10/2022 and 09/11/2022*	21/09/2022	26/10/2022

*TMX and Clothianidin extracted on different dates

Table C. Pollen (flower heads) sampling and analytical processing dates

Site	Full growth Sampling		Pre-harvest sampling	
	Sampling Date	Analytical extraction date	Sampling Date	Analytical extraction date
1	08/08/2022	27/10/2022	12/09/2022	10/11/2022
2	22/06/2022	27/10/2022	19/09/2022	10/11/2022
3	10/08/2022	27/10/2022	14/09/2022	10/11/2022
4	04/08/2022	27/10/2022	22/09/2022	10/11/2022
5	02/08/2022	27/10/2022	15/09/2022	10/11/2022
6	03/08/2022	27/10/2022	21/09/2022	10/11/2022