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 III 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 SB seed treatment for the sugar beet crop cultivation season in 2023. 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. The Level of Quantification (LOQ) was lowered by a factor of 10, compared to the 2022 cultivation season analysis, to enable a more in depth understanding of the presence of TMX and Clothianidin. No claim of GLP compliance is made for the sampling procedures detailed in this summary report.

Both TMX and clothianidin were detected within the in-field 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. There were detections of clothianidin in the majority of in-field pre-drill samples, and comparable levels in the growth season and post-harvest soil samples meaning that it is not possible to directly attribute these detections to the use of the Cruiser seed in this 2023 programme. There is an indication that the persistence of clothianidin residues may be related to soil type, with the clay type 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 only quantifiable in the field margin soil at one site, in the growth season and post-harvest sampling occasions, throughout this monitoring programme. Clothianidin was detected within four of the six field margins, however, three of these sites (silt and clay soils) also had clothianidin detections in the baseline, pre-drilling, samples.

Whilst the specific sampling fields differed from those monitored during 2022, each site monitored in this current programme was located in the same geographical area, in most cases within the same farm, as those in the 2022 programme. Therefore, some comparison can be made to the data obtained from the 2022 programme. Due to the increase in sensitivity of the analytical methods used in 2023, far more residue detections were found in the 2023 programme soil samples, as would be expected. Unlike in 2022, there were no points during the entire 2023 programme, including both in field and field margin samples, where TMX was determined to be above the intended application rate of 51.75 g/ha. As was found to be the case in this programme, the 2022 data also indicated that the silt and clay soils presented with higher clothianidin residues, but that attributing the clothianidin residues to the use of the Cruiser SB seed was not possible. No quantifiable levels of TMX or Clothianidin could be detected in the vegetation or pollen samples in either monitoring programme.

Further monitoring of the sites that used Cruiser in 2022 was conducted in 2023 (CEA study number 1060953) in an attempt to confirm that no continued residue build-up of either TMX or clothianidin can be detected over a longer period, as the previous data was inconclusive. This data will be presented and discussed in a separate report.



1 Introduction

In January 2023, 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 high risk. 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 2023, the model forecast an incidence of 67.51% to trigger the use of Cruiser SB seed treatment in 2023. 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. This programme follows on from a previous monitoring programme conducted in 2022 (CEA project number 1060849, report number CEA.2457), where each site monitored was located in the same geographical area, in most cases within the same farm, as those in this programme.

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 1060949).

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: $C_8H_{10}CIN_5O_3S$

Structure:

2.1.2 Clothianidin

Chemical (IUPAC) Name: Clothianidin

CAS Number: 210880-92-5

Chemical formula: $C_6H_{18}CIN_5O_2S$

Structure:

$$CI \longrightarrow N \longrightarrow N \longrightarrow N \longrightarrow NO_2$$

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



5

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.

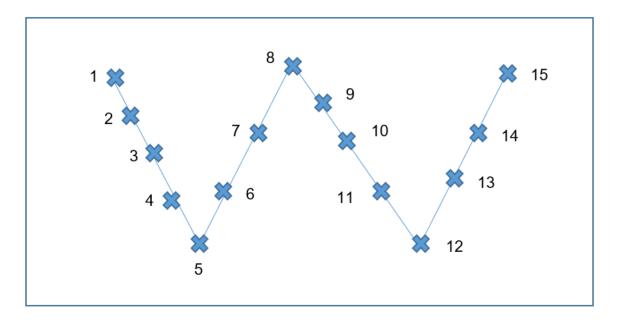


Figure 1. Example schematic of the soil core locations

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 all 3 soil sampling occasions, 40 cm depth cores (50 mm diameter) were collected and then split into two depths (0-20 and 20-40 cm). At Site 5, on the post-harvest occasion, a 30 cm depth gauge corer sampling approximately a 30 mm diameter soil core was used due to frozen, hard ground. At Site 6, in the pre-drilling sampling occasion, the 30 cm corer was used to obtain the field margin samples due to compacted, hard ground. 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 (summer), and secondly, in advance of harvesting (Autumn). For each sampling occasion, 3 individual replicate samples were obtained from within one metre of the field edge at 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 except for one site on the autumn occasion due to cut clearance where around only 600g was obtained.

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. Where it was only possible to obtain 30 cm soil cores, no splitting of the cores was undertaken.

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.

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

Table 1. Selective bulking of in-field soil cores

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 laboratory.



2.4 Residue analysis

Residue analysis was performed, to GLP, at a Test Facility, Smithers ERS Ltd (108 Woodfield Drive, Harrogate, North Yorkshire, HG1 4LS, UK). Validated methods were used to analyse the samples for TMX and its metabolite, clothianidin. 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. The limit of quantification (LOQ) for soil and vegetation analysis was 0.001 mg/kg with the LOQ for pollen set at 0.005 mg/kg. The limit of detection (LOD) was 0.0003 mg/kg for all matrices. Bulk soil density for each site was determined and is presented in Table 2. These values were used for the conversion of residue concentrations from mg/kg to g/ha for the soil samples. All analytical runs were QC checked and found to be within the acceptable range for procedural recoveries (raw data provided in the analytical report). Dates of each sampling and subsequent residue analyses are given in Appendix 1.

Table 2. Conversion formulae for residue analysis from mg/kg to g/ha

Site Number (Soil Type)	Bulk Density (g/cm³)	Conversion calculations
1 (Sand)	1.344	
2 (Sand)	1.289	Correction factor = (soil core depth [cm]) *100
3 (Sand)	1.421	*bulk density value
4 (Silt)	1.185	Measured residue in g/ha = measured value in
5 (Clay)	1.304	mg/kg * correction factor
6 (Clay)	1.058	

3.1 Soil sample analysis results

The recovery of TMX from soil samples obtained from each site are detailed in Table 3, and the recoveries 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 in-field measures of TMX (pre-drilling) were found to be below the LOD for Sites 1, 2 and 5. Residues were detected at Site 3, and in the 20-40 cm samples for Site 6, however, these values were below the LOQ of the method. Quantifiable levels of TMX were found at Site 4 and in the 0-20 cm samples for Site 6. During the growth season, quantifiable levels of TMX were found in the 0-20 cm cores at all sites, except Site 2, where residues were detected but not quantifiable. TMX was



also detected in the 20-40 cm cores for Site 4 only. All sites had quantifiable levels of TMX in the 0-20 cm cores at the post-harvest occasion, Sites 3, 4 and 6 also had detections in the 20-40 cm layer. Only Sites 4 and 6 had levels of TMX above the previous less sensitive LOQ (0.01 mg/Kg), detected in the post-harvest samples.

The field margin only had quantifiable levels of TMX in the growth season and post-harvest phases at Site 4, these detections were below the previous LOQ. No other site had quantifiable levels of TMX during any sampling occasion. In contrast to the previous year, there were no points during the entire programme, including both in field and field margin samples, where TMX was determined to be above the intended application rate of 51.75 g/ha.

For in-field sampling, there were quantifiable residues of clothianidin detected in the baseline soil cores of all sites, except Site 1. All sites had quantifiable levels of clothianidin in the growth season and the post-harvest samples. Clothianidin concentrations remained comparable, or reduced, compared to the baseline values except at Sites 4 and 6.

The edge of field cores showed clothianidin in the baseline cores at the non-sandy sites (4, 5, and 6). Quantifiable levels of clothianidin in the growth season were found at Sites 2, 4, 5, and 6. Clothianidin concentrations in the growth season and post-harvest samples remained comparable to the baseline values, or decreased to below the LOQ. Due to most sites containing clothianidin in the baseline samples, the use of Cruiser SB cannot be directly attributed to these concentrations.

A pesticide history check showed Site 2 to have used a product containing clothianidin in 2018 but no other flagged pesticides were used on the other sites.

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

Site	Sample location	Mean measured Thiamethoxam (mg/Kg [g/ha])			
(soil type)		1: Pre-drilling	2: Growth Season	3: Post-harvest	
	In-field	0-20 cm: <lod< td=""><td>0-20 cm: 0.0022 [5.9]</td><td>0-20 cm: 0.0026 [7.0]</td></lod<>	0-20 cm: 0.0022 [5.9]	0-20 cm: 0.0026 [7.0]	
1 (candy)	III-lielu	20-40 cm: <lod< td=""><td>20-40 cm: <lod< td=""><td>20-40 cm: >LOD<loq< td=""></loq<></td></lod<></td></lod<>	20-40 cm: <lod< td=""><td>20-40 cm: >LOD<loq< td=""></loq<></td></lod<>	20-40 cm: >LOD <loq< td=""></loq<>	
1 (sandy)	Field	0-20 cm: <lod< td=""><td>0-20 cm: <lod< td=""><td>0-20 cm: >LOD<loq< td=""></loq<></td></lod<></td></lod<>	0-20 cm: <lod< td=""><td>0-20 cm: >LOD<loq< td=""></loq<></td></lod<>	0-20 cm: >LOD <loq< td=""></loq<>	
	Margin	20-40 cm: <lod< td=""><td>20-40 cm: <lod< td=""><td>20-40 cm: <lod< td=""></lod<></td></lod<></td></lod<>	20-40 cm: <lod< td=""><td>20-40 cm: <lod< td=""></lod<></td></lod<>	20-40 cm: <lod< td=""></lod<>	
	In-field	0-20 cm: <lod< td=""><td>0-20 cm: >LOD<loq< td=""><td>0-20 cm: 0.0063 [16.2]</td></loq<></td></lod<>	0-20 cm: >LOD <loq< td=""><td>0-20 cm: 0.0063 [16.2]</td></loq<>	0-20 cm: 0.0063 [16.2]	
2 (sandy)		20-40 cm: <lod< td=""><td>20-40 cm: >LOD<loq< td=""><td>20-40 cm: >LOD<loq< td=""></loq<></td></loq<></td></lod<>	20-40 cm: >LOD <loq< td=""><td>20-40 cm: >LOD<loq< td=""></loq<></td></loq<>	20-40 cm: >LOD <loq< td=""></loq<>	
2 (sandy)	Field	0-20 cm: <lod< td=""><td>0-20 cm: <lod< td=""><td>0-20 cm: <lod< td=""></lod<></td></lod<></td></lod<>	0-20 cm: <lod< td=""><td>0-20 cm: <lod< td=""></lod<></td></lod<>	0-20 cm: <lod< td=""></lod<>	
	Margin	20-40 cm: <lod< td=""><td>20-40 cm: <lod< td=""><td>20-40 cm: <lod< td=""></lod<></td></lod<></td></lod<>	20-40 cm: <lod< td=""><td>20-40 cm: <lod< td=""></lod<></td></lod<>	20-40 cm: <lod< td=""></lod<>	
	In-field	0-20 cm: >LOD <loq< td=""><td>0-20 cm: 0.0022 [6.3]</td><td>0-20 cm: 0.0018 [5.1]</td></loq<>	0-20 cm: 0.0022 [6.3]	0-20 cm: 0.0018 [5.1]	
2 (00 00 d) ()		20-40 cm: >LOD <loq< td=""><td>20-40 cm: >LOD<loq< td=""><td>20-40 cm: 0.0021 [6.0]</td></loq<></td></loq<>	20-40 cm: >LOD <loq< td=""><td>20-40 cm: 0.0021 [6.0]</td></loq<>	20-40 cm: 0.0021 [6.0]	
3 (sandy)	Field	0-20 cm: <lod< td=""><td>0-20 cm: <lod< td=""><td>0-20 cm: <lod< td=""></lod<></td></lod<></td></lod<>	0-20 cm: <lod< td=""><td>0-20 cm: <lod< td=""></lod<></td></lod<>	0-20 cm: <lod< td=""></lod<>	
	Margin	20-40 cm: <lod< td=""><td>20-40 cm: <lod< td=""><td>20-40 cm: <lod< td=""></lod<></td></lod<></td></lod<>	20-40 cm: <lod< td=""><td>20-40 cm: <lod< td=""></lod<></td></lod<>	20-40 cm: <lod< td=""></lod<>	
	In field	0-20 cm: 0.0014 [3.3]	0-20 cm: 0.0033 [7.8]	0-20 cm: 0.0100 [23.7]	
4 (silt)	In-field	20-40 cm: 0.0011 [2.6]	20-40 cm: 0.0020 [4.7]	20-40 cm: 0.0024 [5.7]	
		0-20 cm: >LOD <loq< td=""><td>0-20 cm: 0.0015 [3.6]</td><td>0-20 cm: <lod< td=""></lod<></td></loq<>	0-20 cm: 0.0015 [3.6]	0-20 cm: <lod< td=""></lod<>	



	Field Margin	20-40 cm: <lod< th=""><th>20-40 cm: >LOD<loq< th=""><th>20-40 cm: 0.0011 [2.6]</th></loq<></th></lod<>	20-40 cm: >LOD <loq< th=""><th>20-40 cm: 0.0011 [2.6]</th></loq<>	20-40 cm: 0.0011 [2.6]
	In-field	0-20 cm: <lod< td=""><td>0-20 cm: 0.0010 [2.6]</td><td>0-30 cm: 0.0053 [20.7]</td></lod<>	0-20 cm: 0.0010 [2.6]	0-30 cm: 0.0053 [20.7]
5 (clay)		20-40 cm: <lod< td=""><td>20-40 cm: >LOD<loq< td=""><td>0-30 cm. 0.0033 [20.7]</td></loq<></td></lod<>	20-40 cm: >LOD <loq< td=""><td>0-30 cm. 0.0033 [20.7]</td></loq<>	0-30 cm. 0.0033 [20.7]
3 (Clay)	Field	0-20 cm: <lod< td=""><td>0-20 cm: <lod< td=""><td>0-30 cm: <lod< td=""></lod<></td></lod<></td></lod<>	0-20 cm: <lod< td=""><td>0-30 cm: <lod< td=""></lod<></td></lod<>	0-30 cm: <lod< td=""></lod<>
	Margin	20-40 cm: <lod< td=""><td>20-40 cm: <lod< td=""><td>0-30 CIII. CLOD</td></lod<></td></lod<>	20-40 cm: <lod< td=""><td>0-30 CIII. CLOD</td></lod<>	0-30 CIII. CLOD
	In-field	0-20 cm: 0.0029 [6.1]	0-20 cm: 0.0040 [8.5]	0-20 cm: 0.0110 [23.1]
6 (alay)	m-neia	20-40 cm: >LOD <loq< td=""><td>20-40 cm: >LOD<loq< td=""><td>20-40 cm: 0.0016 [3.4]</td></loq<></td></loq<>	20-40 cm: >LOD <loq< td=""><td>20-40 cm: 0.0016 [3.4]</td></loq<>	20-40 cm: 0.0016 [3.4]
6 (clay)	Field	0-30 cm: >LOD <loq< td=""><td>0-20 cm: >LOD<loq< td=""><td>0-20 cm: >LOD<loq< td=""></loq<></td></loq<></td></loq<>	0-20 cm: >LOD <loq< td=""><td>0-20 cm: >LOD<loq< td=""></loq<></td></loq<>	0-20 cm: >LOD <loq< td=""></loq<>
	Margin	0-30 CIII. >LOD <loq< td=""><td>20-40cm: <lod< td=""><td>20-40cm: <lod< td=""></lod<></td></lod<></td></loq<>	20-40cm: <lod< td=""><td>20-40cm: <lod< td=""></lod<></td></lod<>	20-40cm: <lod< td=""></lod<>

Bold values represent quantified residue levels >LOQ. Italicised values include results above previous LOQ (0.01 mg/Kg). LOD = 0.0003 mg/Kg. LOQ = 0.001 mg/Kg.

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

Site	Sample	Mean me	easured Clothianidin (mg/Kg [g/ha])		
(soil type)	location	1: Pre-drilling	2: Growth Season	3: Post-harvest	
	In-field	0-20 cm: >LOD <loq< td=""><td>0-20 cm: 0.0010 [2.7]</td><td>0-20 cm: 0.0016 [4.3]</td></loq<>	0-20 cm: 0.0010 [2.7]	0-20 cm: 0.0016 [4.3]	
1 (candy)	in-neid	20-40 cm: >LOD <loq< td=""><td>20-40 cm: >LOD<loq< td=""><td>20-40 cm: 0.0012 [3.2]</td></loq<></td></loq<>	20-40 cm: >LOD <loq< td=""><td>20-40 cm: 0.0012 [3.2]</td></loq<>	20-40 cm: 0.0012 [3.2]	
1 (sandy)	Field	0-20 cm: >LOD <loq< td=""><td>0-20 cm: >LOD<loq< td=""><td>0-20 cm: >LOD<loq< td=""></loq<></td></loq<></td></loq<>	0-20 cm: >LOD <loq< td=""><td>0-20 cm: >LOD<loq< td=""></loq<></td></loq<>	0-20 cm: >LOD <loq< td=""></loq<>	
	Margin	20-40 cm: >LOD <loq< td=""><td>20-40 cm: >LOD<loq< td=""><td>20-40 cm: >LOD<loq< td=""></loq<></td></loq<></td></loq<>	20-40 cm: >LOD <loq< td=""><td>20-40 cm: >LOD<loq< td=""></loq<></td></loq<>	20-40 cm: >LOD <loq< td=""></loq<>	
	In-field	0-20 cm: 0.0023 [10.3]	0-20 cm: 0.0020 [10.1]	0-20 cm: 0.0025 [10.3]	
2 (sond) ()	in-field	20-40 cm: 0.0020 [11.3]	20-40 cm: 0.0017 [8.2]	20-40 cm: 0.0022 [9.0]	
2 (sandy)	Field	0-20 cm: >LOD <loq< td=""><td>0-20 cm: 0.0026 [6.7]</td><td>0-20 cm: >LOD<loq< td=""></loq<></td></loq<>	0-20 cm: 0.0026 [6.7]	0-20 cm: >LOD <loq< td=""></loq<>	
	Margin	20-40 cm: >LOD <loq< td=""><td>20-40 cm: >LOD<loq< td=""><td>20-40 cm: <lod< td=""></lod<></td></loq<></td></loq<>	20-40 cm: >LOD <loq< td=""><td>20-40 cm: <lod< td=""></lod<></td></loq<>	20-40 cm: <lod< td=""></lod<>	
	In-field	0-20 cm: 0.0023 [6.5]	0-20 cm: 0.0020 [5.7]	0-20 cm: 0.0025 [7.1]	
2 (candy)		20-40 cm: 0.0020 [5.7]	20-40 cm: 0.0017 [4.8]	20-40 cm: 0.0022 [6.3]	
3 (sandy)	Field	0-20 cm: >LOD <loq< td=""><td>0-20 cm: >LOD<loq< td=""><td>0-20 cm: >LOD<loq< td=""></loq<></td></loq<></td></loq<>	0-20 cm: >LOD <loq< td=""><td>0-20 cm: >LOD<loq< td=""></loq<></td></loq<>	0-20 cm: >LOD <loq< td=""></loq<>	
	Margin	20-40 cm: >LOD <loq< td=""><td>20-40 cm: <lod< td=""><td>20-40 cm: <lod< td=""></lod<></td></lod<></td></loq<>	20-40 cm: <lod< td=""><td>20-40 cm: <lod< td=""></lod<></td></lod<>	20-40 cm: <lod< td=""></lod<>	
	In-field	0-20 cm: 0.0066 [15.6]	0-20 cm: 0.0062 [14.7]	0-20 cm: 0.0086 [20.4]	
4 (silt)		20-40 cm: 0.0057 [13.5]	20-40 cm: 0.0041 [9.7]	20-40 cm: 0.0070 [16.6]	
4 (5111)	Field	0-20 cm: 0.0012 [2.8]	0-20 cm: 0.0022 [5.2]	0-20 cm: >LOD <loq< td=""></loq<>	
	Margin	20-40 cm: >LOD <loq< td=""><td>20-40 cm: >LOD<loq< td=""><td>20-40 cm: <lod< td=""></lod<></td></loq<></td></loq<>	20-40 cm: >LOD <loq< td=""><td>20-40 cm: <lod< td=""></lod<></td></loq<>	20-40 cm: <lod< td=""></lod<>	
	i e Calal	0-20 cm: 0.0092 [24.0]	0-20 cm: 0.0062 [16.2]	0.20 am. 0.0000 [27.6]	
F (alay)	In-field	20-40 cm: 0.0044 [11.5]	20-40 cm: 0.0052 [13.6]	0-30 cm: 0.0096 [37.6]	
5 (clay)	Field	0-20 cm: 0.0051 [13.2]	0-20 cm: 0.0063 [16.4]	0.20 cm; 0.0022 [12.0]	
	Margin	20-40 cm: 0.0032 [8.3]	20-40 cm: 0.0042 [11.0]	0-30 cm: 0.0033 [12.9]	
_	In-field	0-20 cm: 0.0440 [93.1]	0-20 cm: 0.0700 [148.1]	0-20 cm: 0.0510 [107.9]	
6 (clay)	iii-lieiu	20-40 cm: 0.0051 [10.8]	20-40 cm: 0.0086 [18.2]	20-40 cm: 0.0081 [17.1]	
6 (clay)	Field	0.20 cm: 0.0152 [47.6]	0-20 cm: 0.0237 [50.1]	0-20 cm: 0.0180 [38.1]	
	Margin	0-30 cm: 0.0152 [47.6]	20-40cm: 0.0055 [11.6]	20-40cm: 0.0094 [19.9]	

Bold values represent quantified residue levels >LOQ. Italicised values include results above previous LOQ (0.01 mg/Kg) or above the calculated application rate (13.3 g/Ha).

LOD = 0.0003 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 <LOQ 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< th=""><th><lod< th=""><th><lod< th=""><th><lod< th=""></lod<></th></lod<></th></lod<></th></lod<>	<lod< th=""><th><lod< th=""><th><lod< th=""></lod<></th></lod<></th></lod<>	<lod< th=""><th><lod< th=""></lod<></th></lod<>	<lod< th=""></lod<>
2	<lod< th=""><th><lod< th=""><th><lod< th=""><th>>LOD<loq< th=""></loq<></th></lod<></th></lod<></th></lod<>	<lod< th=""><th><lod< th=""><th>>LOD<loq< th=""></loq<></th></lod<></th></lod<>	<lod< th=""><th>>LOD<loq< th=""></loq<></th></lod<>	>LOD <loq< th=""></loq<>
3	<lod< th=""><th><lod< th=""><th><lod< th=""><th>>LOD<loq< th=""></loq<></th></lod<></th></lod<></th></lod<>	<lod< th=""><th><lod< th=""><th>>LOD<loq< th=""></loq<></th></lod<></th></lod<>	<lod< th=""><th>>LOD<loq< th=""></loq<></th></lod<>	>LOD <loq< th=""></loq<>
4	>LOD <loq< th=""><th><lod< th=""><th><lod< th=""><th>>LOD<loq< th=""></loq<></th></lod<></th></lod<></th></loq<>	<lod< th=""><th><lod< th=""><th>>LOD<loq< th=""></loq<></th></lod<></th></lod<>	<lod< th=""><th>>LOD<loq< th=""></loq<></th></lod<>	>LOD <loq< th=""></loq<>
5	<lod< th=""><th><lod< th=""><th>>LOD<loq< th=""><th>>LOD<loq< th=""></loq<></th></loq<></th></lod<></th></lod<>	<lod< th=""><th>>LOD<loq< th=""><th>>LOD<loq< th=""></loq<></th></loq<></th></lod<>	>LOD <loq< th=""><th>>LOD<loq< th=""></loq<></th></loq<>	>LOD <loq< th=""></loq<>
6	<lod< th=""><th><lod< th=""><th><lod< th=""><th><lod< th=""></lod<></th></lod<></th></lod<></th></lod<>	<lod< th=""><th><lod< th=""><th><lod< th=""></lod<></th></lod<></th></lod<>	<lod< th=""><th><lod< th=""></lod<></th></lod<>	<lod< th=""></lod<>

LOD = 0.0003 mg/Kg. LOQ = 0.001 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 6. All samples were determined to be <LOD for both compounds, except for a detected, but non-quantifiable, clothianidin residue at Site 6.

Table 6. Levels of TMX and clothianidin detected in pollen samples from each site

Site	Measured TMX (mg/Kg)		Measured Clothianidin (mg/Kg)	
Site	Full growth	Pre-harvest	Full growth	Pre-harvest
1	<lod< th=""><th><lod< th=""><th><lod< th=""><th><lod< th=""></lod<></th></lod<></th></lod<></th></lod<>	<lod< th=""><th><lod< th=""><th><lod< th=""></lod<></th></lod<></th></lod<>	<lod< th=""><th><lod< th=""></lod<></th></lod<>	<lod< th=""></lod<>
2	<lod< th=""><th><lod< th=""><th><lod< th=""><th><lod< th=""></lod<></th></lod<></th></lod<></th></lod<>	<lod< th=""><th><lod< th=""><th><lod< th=""></lod<></th></lod<></th></lod<>	<lod< th=""><th><lod< th=""></lod<></th></lod<>	<lod< th=""></lod<>
3	<lod< th=""><th><lod< th=""><th><lod< th=""><th><lod< th=""></lod<></th></lod<></th></lod<></th></lod<>	<lod< th=""><th><lod< th=""><th><lod< th=""></lod<></th></lod<></th></lod<>	<lod< th=""><th><lod< th=""></lod<></th></lod<>	<lod< th=""></lod<>
4	<lod< th=""><th>>LOD</th><th><lod< th=""><th><lod< th=""></lod<></th></lod<></th></lod<>	>LOD	<lod< th=""><th><lod< th=""></lod<></th></lod<>	<lod< th=""></lod<>
5	<lod< th=""><th><lod< th=""><th><lod< th=""><th><lod< th=""></lod<></th></lod<></th></lod<></th></lod<>	<lod< th=""><th><lod< th=""><th><lod< th=""></lod<></th></lod<></th></lod<>	<lod< th=""><th><lod< th=""></lod<></th></lod<>	<lod< th=""></lod<>
6	<lod< th=""><th><lod< th=""><th>>LOD<loq< th=""><th>>LOD<loq< th=""></loq<></th></loq<></th></lod<></th></lod<>	<lod< th=""><th>>LOD<loq< th=""><th>>LOD<loq< th=""></loq<></th></loq<></th></lod<>	>LOD <loq< th=""><th>>LOD<loq< th=""></loq<></th></loq<>	>LOD <loq< th=""></loq<>

LOD = 0.0003 mg/Kg. LOQ = 0.005 mg/Kg.



4 Discussion

In this programme, the LOQ of the analytical method was reduced to provide more accurate, robust results, compared to the 2022 programme. The previous LOQ was set at the guideline standard 0.01 mg/kg, whereas this season the LOQ was set at 0.001 mg/kg. Therefore, it was expected that an increased number of residue detections would occur within this current programme.

For TMX, only Sites 4 and Site 6 had quantifiable residues in the pre-drilling, in-field, soil samples, meaning the subsequent detections of TMX at these two sites during growth and in the post-harvest samples cannot be directly attributed to the use of the Cruiser SB treated seed. At all other sites, the pre-drilling baseline for TMX was established to be <LOQ of the method, therefore any subsequent quantifiable residues of TMX within these fields could be attributed to the use of the Cruiser SB treated seed.

Quantifiable levels of TMX in the growth season samples, with the exception of Site 2, were detected primarily in the top, 0-20 cm, layer samples. TMX was detected in the post-harvest in field samples at all sites, with an increased number of detections in the 20-40 cm layer on this occasion. This could indicate that TMX may infiltrate vertically through the soil over time. Where quantifiable levels of TMX were detected, no sites were calculated to include TMX levels higher than the maximum application rate of 51.75 g/ha. A higher than application rate detection may be possible if the soil cores obtained on the growth season or post-harvest sampling occasions contained a high number of seed casings or ungerminated treated seeds. There were only two samples (0-20 cm; Sites 4 and 6) which were above the previous, less sensitive, LOQ, both were on the post-harvest sampling occasion. Similarly, TMX was detected at Site 6, post-harvest, during the 2022 programme. All sites had comparable, or increased, residues in comparison to the previous sampling occasion. With the soil DT50 of TMX in the field reported to be anywhere between 7 and 530+ days^[2], continued monitoring of these in-field soils, and potentially the succeeding crops, would be necessary to investigate residues over time 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 quantifiable residues were found in the field margin at any site on the pre-drilling sampling occasion, thus, establishing a true zero baseline and indicating any subsequent detection of TMX in the field margins could be a direct result of the use of the Cruiser SB treated seed within the field. TMX was only quantifiably detected at Site 4 during the growth season and post-harvest sampling occasions, these detection levels were very marginally above the LOQ of the analytical method. No other site had quantifiable TMX residues in the field margin samples, as was the case in the 2022 programme. 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, at any site, throughout this monitoring programme. This conclusion was also drawn from the 2022 monitoring programme.

For clothianidin, the residue detections were more widespread and variable than for TMX. In this monitoring programme, clothianidin was monitored as it is a metabolite of TMX, however, it is also an active ingredient in other pesticidal products. 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 SB treated seeds. In addition, clothianidin was generally detected at both soil depths, rather than primarily in the top layer, including in the detections in the pre-drilling, baseline samples. From the pesticide histories provided, it was determined that Site 2 applied products containing clothianidin in 2018, no other documentation provided indicates that the other test sites used any 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; a trend that was also suggested by the data obtained in the 2022 programme. This might be explained by the soil DT₅₀ for clothianidin, which has been reported to range from negligible to over 1300 days, with a clay soil type having the longest DT₅₀^[2].

For the in-field samples, from the sandy soil sites, Site 1 did not have any quantifiable levels of clothianidin on the pre-drilling sampling occasion. There were very marginal detections of clothianidin (just above the LOQ) in the growth season and post-harvest samples. This follows a similar trend to the TMX detections at this site, and strongly suggests a direct consequence of the Cruiser SB seed use. The very low level detections would not be a major cause for concern in this case.

At Sites 2 and 3, quantifiable residues of clothianidin were detected in the baseline samples, with slightly increased residues detected during the growth season and post-harvest occasions. This was again comparable to the TMX residue data for these sites. Additionally, Site 2 is known to have used a clothianidin based product in 2018, which may explain the increased detection of this analyte in the pre-drilling samples.

At Sites 4, 5, and 6 there were quantifiable levels of clothianidin found, in-field, on all 3 sampling occasions, at both soil depths. The levels of clothianidin varied throughout the growth and post-harvest samplings. Site 4 had a general increase throughout the 3 soil sampling occasions, whilst Site 5 had a reduced level of clothianidin in the growth season which then increased again in the post-harvest season to levels comparable to the pre-drill samples. Site 6 had the greatest levels of clothianidin on all occasions, where levels increased during the growth season and reduced in post-harvest. In this case, all three occasions were above the previous, less sensitive, LOQ (0.01 mg/kg) and above the maximum 51.75 g/ha application rate. The presence of clothianidin at these sites 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. The consistent presence of residues at the 20-40 cm depth also suggests that this may be related to historical clothianidin application, rather than solely as a result of metabolic breakdown of TMX, which was generally found primarily in the upper soil layer samples.

When investigating the field margin soil data, only one quantifiable detection of clothianidin was found at any of the three sandy soil sites (Sites 1, 2 and 3). This was at Site 2, during the growth season, which then reduced to below LOQ in the post-harvest season. Site 2 was known to have used a pesticide containing clothianidin in 2018. This follows a similar trend to the 2022 programme. Quantifiable residues of clothianidin were found in the silt type soil (Site 4) during pre-drilling and growth season but decreased to below LOQ at post-harvest. The clay type soils (Sites 5 and 6) had quantifiable levels of clothianidin on all sampling occasions with a notable increase in the growth stage samples, decreasing again in the post-harvest samples. As with TMX, Site 6 had clothianidin levels above the previous, less sensitive, LOQ but below the intended application rate. This follows the same



trend as the 2022 monitoring where silty soils show some clothianidin detection and clay fields retain clothianidin residues through all sampling occasions.

5 Conclusions

Both TMX and clothianidin were detected within the in-field 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. 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 2023 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 significant 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 quantifiable in the field margin for all sites, except Site 4, suggesting that the compound does not migrate out of the encapsulated seed or treated fields. Clothianidin was detected within the field margins, predominantly in the clay type soils. However, residues were also detected during the pre-drill sampling occasions, hence are unlikely to be a direct effect of the Cruiser SB usage.



6 References

- [1] A. Cashmore. 2024. Method Validation and Sample Analysis of Thiamethoxam and Clothianidin in Soil and Non-Crop Vegetation. Smithers ERS Limited, Study Report No 3203568
- [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

	Pre-drilling Sampling		Growth season sampling		Post-harvest sampling	
Site	Sampling Date	Residue analysis date	Sampling Date	Residue analysis date	Sampling Date	Residue analysis date
1	25/04/2023	08/06/2023	11/07/2023	14/09/2023	21/12/2023	01/02/2024
2	13/04/2023	13/06/2023	41/07/2023	15/09/2023	20/11/2023	02/02/2024
3	06/04/2023	27/06/2023	17/07/2023	19/09/2023	25/01/2024	08/02/2024
4	03/04/2023	04/07/2023	14/07/2023	20/09/2023	01/12/2023	09/02/2024
5	11/04/2023	06/07/2023	20/07/2023	26/09/2023	19/01/2024	20/02/2024
6	12/04/2023	13/07/2023	21/07/2023	27/09/2023	12/12/2023	21/02/2024

Table B. Vegetation sampling and analytical processing dates

6:1	Full growt	h Sampling	Pre-harvest sampling		
Site	Sampling Date	Residue analysis date	Sampling Date	Residue analysis date	
1	11/07/2023	28/09/2023	28/08/2023	14/11/2023	
2	41/07/2023	28/09/2023	05/09/2023	14/11/2023	
3	17/07/2023	28/09/2023	04/10/2023	14/11/2023	
4	14/07/2023	29/09/2023	06/09/2023	15/11/2023	
5	20/07/2023	29/09/2023	13/09/2023	15/11/2023	
6	21/07/2023	29/09/2023	14/09/2023	15/11/2023	



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

	Full growt	h Sampling	Pre-harvest sampling		
Site	Sampling Date	Residue analysis date	Sampling Date	Residue analysis date	
1	11/07/2023	02/10/2023	28/08/2023	13/11/2023	
2	41/07/2023	02/10/2023	05/09/2023	13/11/2023	
3	17/07/2023	02/10/2023	04/10/2023	13/11/2023	
4	14/07/2023	02/10/2023	06/09/2023	13/11/2023	
5	20/07/2023	02/10/2023	13/09/2023	13/11/2023	
6	21/07/2023	02/10/2023	14/09/2023	13/11/2023	

