

# Neonicotinoid concentrations in arable soils after seed treatment applications in preceding years

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## Abstract

**BACKGROUND:** Concentrations of the neonicotinoid insecticides clothianidin, thiamethoxam and imidacloprid were determined in arable soils from a variety of locations in England.

**RESULTS:** In soil samples taken from the central area of fields, concentrations of clothianidin ranged from 0.02 to 13.6  $\mu\text{g kg}^{-1}$ . Thiamethoxam concentrations were between <0.02 and 1.50  $\mu\text{g kg}^{-1}$ , and imidacloprid concentrations between <0.09 and 10.7  $\mu\text{g kg}^{-1}$ . Concentrations of clothianidin and thiamethoxam were lower in soil samples taken from the edges of fields than from the centres of fields, but this difference was less pronounced for imidacloprid.

**CONCLUSION:** This work gives a clear indication of the levels of neonicotinoids in arable soils after typical use of these compounds as seed dressings in the United Kingdom. There was evidence that imidacloprid was more persistent in the soils studied than clothianidin and thiamethoxam. As clothianidin and thiamethoxam have largely superseded imidacloprid in the United Kingdom, neonicotinoid levels were lower than suggested by predictions based on imidacloprid alone.

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**Keywords:** soil; neonicotinoids; clothianidin; thiamethoxam; imidacloprid; honey bee; *Apis mellifera*; bumblebee; pollinator

## 1 INTRODUCTION

There has been considerable interest in recent years in the possible effects of neonicotinoid insecticides on honey bees and other pollinators. Neonicotinoids are water-soluble systemic insecticides that can be applied as seed dressings, thereby avoiding the need for spray applications. Their systemic nature means that they are carried to all parts of the plant, but this can lead to contamination of nectar and pollen,<sup>1</sup> which can, in turn, lead to exposure of pollinators. Blacquiere *et al.*<sup>2</sup> outlined a number of laboratory studies describing effects of neonicotinoids on foraging and learning abilities of bees. Concern has further increased in recent years as a result of studies describing effects of neonicotinoids on foraging abilities of honey bees (*Apis mellifera*) and bumblebees<sup>3–5</sup> and on colony development and queen production in bumblebees.<sup>6</sup> As a result of the growing body of evidence, the European Food Safety Authority (EFSA) reassessed the risk to bees and other pollinators from the three neonicotinoids of greatest concern: clothianidin, thiamethoxam and imidacloprid.<sup>7–9</sup> As well as the reported adverse effect on bees, one of the reasons for concern around neonicotinoids is their reported persistence in soil; for example, clothianidin has a reported half-life of 148–1155 days in aerobic soil.<sup>10</sup> Therefore, soil-dwelling insects may be exposed to neonicotinoids for extended periods, and pollinators may be exposed via contamination of pollen and nectar from subsequent crops. There is limited published information on levels of neonicotinoids in arable soil from fields in which seed treatments have been applied in previous years, and a recent review has suggested that further data are required.<sup>11</sup> Krupke *et al.*<sup>12</sup> determined neonicotinoid levels in soil from fields in which treated

maize seed had been applied the previous year. It was not clear whether the levels detected arose from the treated seed or from deposition of contaminated talc that had been added to ensure a smooth flow of treated seed during planting. Most of the rest of the available data relate only to imidacloprid, the use of which has declined markedly in recent years, while use of clothianidin and thiamethoxam has been increasing.<sup>11</sup> Schmuck *et al.*<sup>13</sup> detected imidacloprid levels of 12.7–17.8  $\mu\text{g kg}^{-1}$  in soil samples taken from two sites at 7–8 months after application of cereals treated with imidacloprid-treated seed. Bonmartin *et al.*<sup>14</sup> found levels of between 1 and >100  $\mu\text{g kg}^{-1}$  of imidacloprid in soils from a range of fields across France in 2005. It has recently been speculated that current levels of clothianidin, thiamethoxam and imidacloprid in soils are likely to be higher than this owing to their widespread use and potential to accumulate.<sup>11</sup>

Neonicotinoids are also mobile in soil,<sup>11</sup> so that pollinators may be exposed via plants growing in field margins if there is significant lateral movement of the pesticide.

In order to provide information enabling both the risk to soil-dwelling arthropods and the risk to pollinators via uptake in subsequent crops and plants in field margins to be assessed,

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some preliminary investigations were conducted to determine the levels of clothianidin, thiamethoxam and imidacloprid in soil samples taken from the central area and from the edge of a number of fields in eastern England to which seeds treated with clothianidin, thiamethoxam and imidacloprid had been applied in the previous 3 years.

## 2 EXPERIMENTAL METHODS

### 2.1 Site selection and soil sampling

This was a preliminary investigation designed to obtain data in a short timescale. The time available did not allow for the design of a systematic sampling regime taking into account such factors as soil type. Instead, farmers across eastern England were contacted, with whom the authors had had previous contact and who the authors believed had used neonicotinoid-treated seed dressing in recent years. As the priority was to estimate the range of concentrations across a wide area, rather than obtain highly accurate measures at each site, and owing to the need to obtain samples quickly, it was decided that replication of sites was more important than replication of sampling within each site. Eighteen sites that were reasonably well spread across the geographic range of interest were eventually selected. Only two soil samples were to be taken from each field, to a depth of 10–15 cm, using a 25 mm diameter corer. One of these was taken from the main body of the field, the second from an area of the field margin not normally in cultivation. Sampling areas were targeted to avoid, as far as possible, vegetation in the sample. Where possible, the 'field edge' sample was taken downslope to increase the likelihood that groundwater from the field had

percolated through the area. Fields were sampled during spring 2013. Each soil core was transferred to a freezer and stored at approximately  $-18^{\circ}\text{C}$  prior to analysis. The farmer was asked to complete a questionnaire detailing application of neonicotinoid seed dressings. Pesticide application records were collected to supplement the questionnaire, when these were readily available.

### 2.2 Chemical analysis

Soil samples were thoroughly mixed, a portion (10 g) of each sample was shaken with methanol (60 mL) for 1 h, the mixture was filtered and the volume of the filtrate was adjusted to 100 mL with methanol. An aliquot (2 mL) of this solution was evaporated to dryness and redissolved in methanol + water (1 + 9 by volume) prior to analysis. This solution was analysed by liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS) using an Agilent 1290 Infinity LC system interfaced to an Agilent 6490 Triple Quad mass spectrometer (Agilent, Sanata Clara, CA). Chromatography was performed on a Waters Acquity UPLC HSS analytical column (Waters, Milford, MA) with a mobile phase consisting of 5 mM of ammonium acetate and methanol programmed from 2% methanol at  $t = 0$  to 98% methanol after 8 min. The solvent flow rate was  $0.5\text{ mL min}^{-1}$ . The MS was operated in positive electrospray mode. For each analyte, two channels were monitored, each consisting of a separate MS/MS transition. One of these channels was used for quantification, with the other used as confirmation. Quantification was by means of external calibration solutions prepared in extracts obtained from control samples, i.e. matrix-matched calibration. Each batch of samples analysed included a control soil sample fortified at  $0.004\text{ mg kg}^{-1}$ , from which recovery values for each analyte could be calculated.

**Table 1.** Soil samples obtained from eastern England during spring 2013

Identifier	Crop	Recent neonicotinoid seed treatments used	
		Seed treatments	Application rate ( $\text{g ha}^{-1}$ )
Lincolnshire 1	Wheat	Clothianidin autumn 2012	80
	OSR	Thiamethoxam autumn 2011	15
Lincolnshire 2	Wheat	Clothianidin autumn 2012	60
	OSR	Clothianidin autumn 2011	19
Lincolnshire 3	Wheat	Clothianidin autumn 2012	60
	OSR	Imidacloprid autumn 2011	10
Nottinghamshire 1	OSR	Clothianidin autumn 2011	24
Lincolnshire 4	Wheat	Clothianidin autumn 2012	$\leq 100$
	Wheat	Clothianidin autumn 2011	79
	OSR	Thiamethoxam autumn 2010	27
Norfolk 1	OSR	Thiamethoxam autumn 2010	$\leq 34$
Cambridgeshire 1	Wheat	Clothianidin autumn 2012	$\leq 100$
Cambridgeshire 2	OSR	Thiamethoxam autumn 2011	$\leq 34$
Nottinghamshire 2	OSR	Thiamethoxam autumn 2011	$\leq 34$
Nottinghamshire 3	OSR	Thiamethoxam autumn 2011	$\leq 34$
Yorkshire 1	OSR	Imidacloprid autumn 2011	$\leq 12$
Cambridgeshire 3	Wheat	Clothianidin autumn 2011	94
	OSR	Clothianidin autumn 2010	$\leq 30$
Cambridgeshire 4	OSR	Clothianidin autumn 2011	$\leq 30$
Cambridgeshire 5	OSR	Clothianidin autumn 2011	$\leq 30$
Norfolk 2	Wheat	Clothianidin autumn 2012	$\leq 100$
	Sugar beet	Thiamethoxam spring 2012	$\leq 78$
Nottinghamshire 4	OSR	Clothianidin autumn 2011	$\leq 30$
Nottinghamshire 5	OSR	Thiamethoxam autumn 2010	$\leq 34$
Yorkshire 2	OSR	Clothianidin autumn 2011	$\leq 30$

### 3 RESULTS

Details of soil samples obtained are given in Table 1. Where the application rate used was known, this has been indicated; in other cases the maximum allowable application rate is listed.

Percentage recovery values from concurrently analysed fortified control samples were 81 and 90% for imidacloprid, 87 and 94% for clothianidin and 72 and 93% for thiamethoxam. Limits of detection were 0.02  $\mu\text{g kg}^{-1}$  for clothianidin and thiamethoxam and 0.09  $\mu\text{g kg}^{-1}$  for imidacloprid. Clothianidin concentrations in soil from the centre of fields in which clothianidin-treated cereal seed had been drilled in Autumn 2012 ranged from 1.8 to 13.7  $\mu\text{g kg}^{-1}$  (Table 2). However, levels of clothianidin of up to 10.3  $\mu\text{g kg}^{-1}$  were detected in soils from the centre of fields in which clothianidin or thiamethoxam (of which clothianidin is a metabolite) had been last applied in Autumn 2011, and 4.5  $\mu\text{g kg}^{-1}$  of clothianidin was detected in soils from the centre of fields in which thiamethoxam had been last applied in Autumn

2010. Clothianidin was detected in one field and thiamethoxam in seven fields to which these chemicals had not been applied in the previous 3 years. Imidacloprid was detected in soil samples from 15 of the 18 fields sampled, at levels of up to 10.7  $\mu\text{g kg}^{-1}$  in the centre of fields, even though this chemical had apparently only been used in two of these fields since 2010.

### 4 DISCUSSION

The results demonstrate that detectable levels of neonicotinoids are present in soil for a considerable time after use in UK conditions (Figure 1). In a number of fields, residues of chemicals that had not apparently been used in the previous 3 years were detected. These residues are unlikely to have arisen from analytical error, as the procedure used, which employed LC-MS/MS detection, was highly specific. Furthermore, the acquisition of data from two MS/MS channels for each chemical allowed for one of these channels to

**Table 2.** Neonicotinoid concentrations (wet weight basis) detected in soil samples<sup>a</sup>

		Imidacloprid ( $\mu\text{g kg}^{-1}$ )	Clothianidin ( $\mu\text{g kg}^{-1}$ )	Thiamethoxam ( $\mu\text{g kg}^{-1}$ )	Total neonicotinoids ( $\mu\text{g kg}^{-1}$ )
Lincolnshire 1	Centre	<b>2.01</b>	13.6	0.76	16.4
	Edge	<0.09	0.30	<0.02	0.3
Lincolnshire 2	Centre	<b>1.40</b>	1.78	<b>0.24</b>	3.42
	Edge	<b>0.63</b>	1.58	<b>0.08</b>	2.29
Lincolnshire 3	Centre	3.95	11.5	<0.02	15.5
	Edge	1.03	1.63	<0.02	2.66
Nottinghamshire 1	Centre	<b>0.55</b>	3.20	<b>0.05</b>	3.8
	Edge	<0.09	0.11	<0.02	0.11
Lincolnshire 4	Centre	<b>1.88</b>	4.71	0.31	6.9
	Edge	<b>1.22</b>	0.81	0.08	2.11
Norfolk 1	Centre	<b>0.33</b>	4.49	<0.02	4.82
	Edge	<b>0.39</b>	4.14	<0.02	4.53
Cambridgeshire 1	Centre	<b>0.74</b>	11.0	<b>0.16</b>	11.9
	Edge	<b>0.15</b>	2.48	<b>0.08</b>	2.71
Cambridgeshire 2	Centre	<b>1.71</b>	10.3	0.64	12.7
	Edge	<b>0.19</b>	1.21	0.11	1.51
Nottinghamshire 2	Centre	<b>0.12</b>	1.12	0.19	1.43
	Edge	<0.09	<0.02	<0.02	
Nottinghamshire 3	Centre	<b>0.89</b>	1.54	1.21	3.64
	Edge	<b>1.88</b>	0.18	0.14	2.2
Yorkshire 1	Centre	1.36	<b>0.02</b>	<0.02	1.38
	Edge	0.38	<0.02	<0.02	0.38
Cambridgeshire 3	Centre	<b>1.66</b>	9.74	<b>0.10</b>	11.5
	Edge	<0.09	0.47	<0.02	0.47
Cambridgeshire 4	Centre	<b>0.39</b>	0.34	<b>0.11</b>	0.84
	Edge	<b>0.64</b>	0.09	<0.02	0.73
Cambridgeshire 5	Centre	<b>10.7</b>	6.69	<b>0.90</b>	18.3
	Edge	<b>6.87</b>	0.25	<b>0.02</b>	7.14
Norfolk 2	Centre	<0.09	4.25	1.50	5.75
	Edge	<0.09	1.57	0.33	1.90
Nottinghamshire 4	Centre	<b>1.50</b>	2.92	<b>0.52</b>	4.94
	Edge	<b>0.38</b>	0.13	<b>0.03</b>	0.54
Nottinghamshire 5	Centre	<0.09	0.32	0.54	0.86
	Edge	<0.09	0.04	<0.02	0.04
Yorkshire 2	Centre	<0.09	0.51	<0.02	0.51
	Edge	<0.09	0.09	<0.02	0.09

<sup>a</sup> Values in bold are those for which residues were detected that were not related to applications in the previous 3 years.

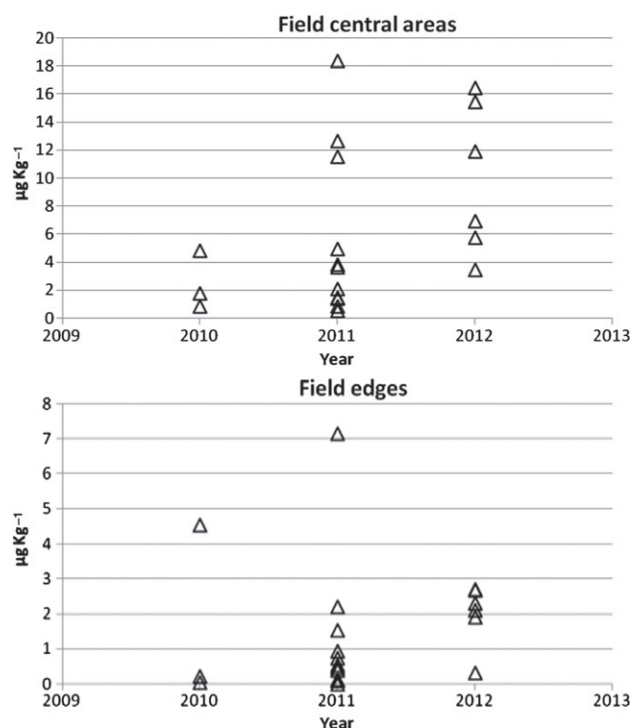
be used for confirmatory purposes. In all cases, residues detected were present in chromatograms from both channels, and there was very good quantitative agreement between channels, giving a very high level of confidence that these residues were actually present. Such residues do not necessarily arise from applications made more than 3 years previously. Some may have resulted from subsequent applications made to surrounding fields, e.g. from dust drift, as it is clear that these chemicals were in widespread use from 2010 to 2012. This may explain the detection of clothianidin in the only field that had not received clothianidin or thiamethoxam in the last 3 years. It is unlikely to be the primary reason why thiamethoxam was detected in seven fields to which it had not been applied for over 3 years, because in all seven fields the levels were higher in the centres than at the edges. Dust drift would be expected to result in concentrations at field edges that were comparable or higher than those at the field centres. It is extremely unlikely that the presence of imidacloprid residues in 13 fields that had not received applications of this chemical in the previous 3 years could have arisen from contamination from surrounding fields, given that imidacloprid use in the United Kingdom has been declining in recent years; amounts used dropped by around 80% between 2005 and 2010,<sup>11</sup> and the application records from the fields included in the present study suggest use has remained relatively low since 2010.

It is possible that, in some cases, applications of neonicotinoids were made but were not recorded in the information supplied on neonicotinoid seed dressing applications. This may be the most obvious explanation of the relatively high levels of imidacloprid detected at 'Cambridgeshire 5'. It may also partly explain the detections of clothianidin and thiamethoxam that were not related to applications in the previous 3 years. The relatively infrequent use of imidacloprid means that unrecorded applications of the chemical are also unlikely to explain the presence of imidacloprid residues in the majority of fields studied. It seems likely that many of the imidacloprid residues and some of the thiamethoxam residues detected arose from applications made more than 3 years ago.

The dissipation rate ( $DT_{50}$ ) of imidacloprid from soil has been reported to vary widely, ranging from 28 to 1250 days,<sup>11</sup> but these estimates include laboratory studies and field studies conducted under very different conditions to those pertaining in the United Kingdom, e.g. in India. Results from a study conducted in the United Kingdom on winter barley seed treated with imidacloprid suggested high persistence, with estimated  $DT_{50}$  values of 1268 and 1233 days,<sup>15</sup> while Bonmartin *et al.*<sup>14</sup> concluded that there was a long persistence of imidacloprid in soils from France. Some estimates of  $DT_{50}$  for imidacloprid can be made for the two fields to which this chemical had been applied in the previous 3 years by comparison with initial concentrations calculated by assuming that the chemical is distributed evenly in the top 10 cm of soil and that the soil bulk density for each site is 1.2. As each soil will have been tilled a number of times since applications of imidacloprid were made, the single samples taken are probably reasonably representative of the whole field. A  $DT_{50}$  of around 500 days would explain the measured concentration in the centre of the 'Lincolnshire 3' field. In 'Yorkshire 1', the application rate is not known, but, if it had been applied at  $10 \text{ g ha}^{-1}$ , then a  $DT_{50}$  of around 210 days would account for the measured concentration in the centre of the field. Imidacloprid was also detected in 13 fields that had not received applications of the chemical in the previous 3 years, with a median concentration (excluding 'Cambridgeshire 5') of  $1.1 \mu\text{g kg}^{-1}$ . A  $DT_{50}$  of around 440 days would result in this

concentration if imidacloprid had been applied at  $10 \text{ g ha}^{-1}$  in Autumn 2009, while a  $DT_{50}$  of around 560 days would give rise to this concentration from an application made, at the same rate, in Autumn 2008. Overall, the results suggest that imidacloprid is highly persistent in the soil in UK conditions, with dissipation rates of the order of a few hundred days.

For clothianidin, the possibility of cross-contamination from subsequent applications to surrounding fields cannot be discounted, and estimates of dissipation rates have therefore not been made for applications prior to Autumn 2012. Clothianidin was applied to six fields in Autumn 2012, but there are some difficulties associated with estimating dissipation times for these fields. The application rate in 'Cambridgeshire 1' is not known but is likely to be  $60\text{--}100 \text{ g ha}^{-1}$ . Four of the fields had received applications of clothianidin or thiamethoxam in the 12 months or so prior to Autumn 2012, so some of the resulting clothianidin residue may have originated from these earlier applications. However, for 'Lincolnshire 1' and 'Lincolnshire 2', the previous applications were at much lower rates than the rates used in Autumn 2012 and are likely to have had little effect on the measured concentration. The  $DT_{50}$  values calculated for the centre of these two fields, plus 'Lincolnshire 3' and 'Cambridgeshire 1' (assuming an application rate of  $60 \text{ g ha}^{-1}$ ), range from 37 to 78 days, with a median of 65 days. The individual  $DT_{50}$  values calculated for each field should be treated with caution because only one sample was taken from the centre and the soil will not have been mixed by tillage since application. In addition, it is possible that the levels present were enhanced by cross-contamination from applications made to spring-sown crops in surrounding fields, which would mean that the  $DT_{50}$  values calculated were overestimates of real values. In spite of these uncertainties, the evidence from this study is that  $DT_{50}$  values for clothianidin in UK soils are lower than reported



**Figure 1.** Total neonicotinoid concentrations ( $\mu\text{g kg}^{-1}$ ) in soil samples from field centres and field edges plotted against last date of drilling with neonicotinoid-treated seed.

values from field studies, which range from 277 to 1386 days.<sup>11</sup> For thiamethoxam, estimates of  $DT_{50}$  values for applications made in autumn 2011 range from 75 to 109 days, with a median of 65 days. However, thiamethoxam was also detected in seven fields to which it had not been applied in the previous 3 years, suggesting that dissipation rates in these fields may have been slower than suggested by the  $DT_{50}$  values calculated. That said, thiamethoxam levels were all below  $2 \mu\text{g kg}^{-1}$ , and it is clear that there is no appreciable build-up of levels of this chemical in UK soils.

The maximum concentrations found in real use scenarios were significantly lower than the maximum concentrations of  $>100 \mu\text{g kg}^{-1}$  of imidacloprid found by Bonmartin *et al.*<sup>14</sup> The reason for the much lower levels found in the present study seems to be that imidacloprid is now used rather sparingly and has been largely superseded by the less persistent clothianidin and thiamethoxam.

It is not clear what concentrations would arise in the pollen and nectar of succeeding crops and wild plants grown in the fields from this study. Schmuck *et al.*<sup>13</sup> found imidacloprid levels of  $<1.5 \mu\text{g kg}^{-1}$  in pollen and nectar from sunflowers grown in soils containing up to  $18 \mu\text{g kg}^{-1}$ , a level similar to the maximum total neonicotinoids concentration detected in the present study. Given their similar persistence and water solubility, it is likely that other neonicotinoids will behave similarly. Of the crops that are attractive to pollinators that are grown from seed dressed with neonicotinoids, only oilseed rape (OSR) is widely grown in the United Kingdom. If transference to pollen and nectar is similar in OSR to sunflowers, then levels in succeeding crops are likely to be  $<1.5 \mu\text{g kg}^{-1}$ , but further information is needed to confirm this.

In every case, the total levels of neonicotinoids that were found in soil from field edges were lower than the levels in the centre of fields. However, examination of results for individual chemicals shows some interesting differences. For clothianidin and thiamethoxam, levels were much lower at the edges of most fields than in the centres, with a small number of exceptions that may be explained by sampling error or by cross-contamination from neighbouring fields. For imidacloprid, this difference was much less marked, and levels at the edges of a number of fields were comparable or even higher than levels in the centres of these fields.

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