The route and rate of thiamethoxam soil degradation in laboratory and outdoor incubated tests, and field studies following seed treatments or spray application

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Abstract

BACKGROUND: The route and rate of degradation of thiamethoxam in the laboratory and field was investigated. The effect of dark incubation versus light/dark cycles, seed treatment versus spray, and watering-in for spray application was explored in side-by-side trials.

RESULTS: Geometric mean DT_50 values were 75.4 days in OECD307 studies, and 18.3 (spray) and 16.5 (seed treatment) days in the field. In laboratory soil core studies DT_50 values were 24.9 to 43.5 days, with the lowest value from the light/dark incubated soil core. Mean clothianid formation was 19.7% applied thiamethoxam [mol/mol] in OECD307 studies and 17.5 (spray) and 3.4% (seed) in field trials.

CONCLUSION: Soil DT_50 values decreased with increasingly realistic tests (laboratory OECD307 to soil cores to soil cores with a light/dark cycle to field trials). The majority of the differences were associated with the soil treatment in OECD307 studies which destroys soil structure and retards the degradation rate; and from the impact on soil pore water movement in light/dark conditions. Degradation rates in the field were comparable between spray application and seed treatments. Maximum clothianid concentrations were four-fold lower for seed treatments than for spray application in field studies.

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Supporting information may be found in the online version of this article.

Keywords: thiamethoxam; clothianidin; laboratory; field; seed; spray

1 INTRODUCTION

Thiamethoxam is a neonicotinoid insecticide used for the control of a wide range of insects. 1 It exhibits low volatility, a high water solubility and a low log K ow. 1,2 The limited number of publicly available K ow values and the two theoretical values calculated by the authors indicate a very high to medium potential for mobility based upon the McCall classification scheme; 2–5 though a compound's actual mobility is also governed by its degradation rate and other dissipation processes. See Table 1.

Thiamethoxam is present in formulations applied worldwide, with products registered in more than 130 countries, including, the United States, Canada, Brazil, Australia, Europe, India and Russia. 6 For use in agriculture, it is commonly formulated in products which are applied as either foliar or bare soil spray applications for the control of target pests in a wide range of crops. It is also commonly used as a seed treatment application prior to sowing of the seed to protect the emerging and growing crop from pests. In recent years the neonicotinoid class of pesticides has been subject to some scrutiny because of their potential risks to bees and other pollinators. 7 Given these concerns, the degree to which thiamethoxam is persistent in soil may be crucial to assessing the uptake of thiamethoxam by crops grown on previously treated fields, and consequently the long-term exposure of non-target insects to thiamethoxam from those rotational crops, and from residues remaining in the soil itself, or transported to surface water bodies from soil via drainflow or run-off. 6,8 Indeed, studies targeted to areas of intensive agriculture and widespread neonicotinoid use in North America have demonstrated the frequent occurrence of thiamethoxam in surface water bodies, typically in the tens of nanograms per litre range, 8–10 though higher concentrations are observed in extreme cases. 8,11 A further study performed in an area...
of Australian agriculture demonstrated a frequency of detection of 27% with a maximum concentration of 0.20 μg/L.12

Little publicly available data exist regarding the route of degradation of thiamethoxam in soil. However, it is known that thi- amethoxam degrades in laboratory aerobic soil studies to form the metabolite CGA322704, commonly known as clothianidin.8,13,14 Clothianidin is also a pesticidically active substance in the neoni- cotinoid class of insecticides and, like thiamethoxam, works as an agonist of the nicotinic acetylcholine receptors (nAChRs) in the central nervous system of insects. Like thiamethoxam, clothianidin has both contact and systemic activity against target pests, and is typically formulated as a seed treatment product.1 Clothianidin exhibits low volatility, a moderate water solubility and a low log Kow of 0.7 at 25 °C.1 The range of Kow values reported in the literature indicate a high to low mobility based upon the McCall classification scheme.3,5,15 See Table 1.

Clothianidin was detected in soil at concentrations up to 11.2 ng/g, run-off water at average concentrations up to 850 ng/L, and groundwater (2 m) at concentrations up to 60 ng/L following applications of clothianidin to maize seeds in Illinois, USA, at rates of up to 0.50 mg clothianidin/seed.16 As for thiamethoxam, other studies targeted to areas of intensive agriculture and widespread neonicotinoid use in North America have demonstrated the frequent occurrence of clothianidin in surface water bodies, typically in the tens of nanograms per litre range, though higher concentrations are observed in extreme cases.8–11 The soil organic carbon content was reported as 2.2–2.5%, however, other details regarding the soil type, soil hydrology, meteorological conditions and other site-specific details were omitted. Goulson17 reported DT50 values of 277 to 1386 days in field dissipation trials performed in the United States. In view of these properties, the formation of clothianidin from thiamethoxam may warrant further investigation, however, the majority of thiamethoxam’s degradation products are known to be non-insecticidally active.6 The structures of thiamethoxam and clothianidin are presented in Table 2.

To our knowledge, the sole publicly available source of information relating to the formation of clothianidin in field studies, or occurrence in the wider environment, following the application of thiamethoxam is the publication of Hilton et al.6 The publication primarily investigated the field dissipation rates of thiamethoxam in European field trials, although concentrations of clothianidin were also monitored. The levels of clothianidin observed were generally too low to kinetically evaluate the rates of formation and decline. Other metabolites were not monitored.

Publicly available data regarding the rate of degradation of thi- amethoxam in soil are sparse. The summary paper of Goulson17 reports laboratory soil degradation DT50 values for thiamethoxam of 34 to 353 days from a number of studies. Additional laboratory soil DT50 values under a range of soil moisture conditions ranged from 46 to 301 days at 25 °C and 15.1 to 21.5 days.19 Goulson17 also reports field DT50 values with a range of 7 to 109 days from a single source. In the study of Hilton et al.6 soil degradation DT50 values of 7.1 to 92.3 days (geomean 31.2 days), following normalisation to 20 °C, were reported from 18 field trials performed throughout Europe. Thiamethoxam soil DT50 values of 1.81 days from field trials performed in Egypt and 12.0–19.1 days in China were also reported.20,21 It should be noted that because the paper of Goulson is a summary paper, it is likely that this data set includes data from some of the trials conducted as part of the Hilton et al.6 study, but also studies from other sources.

The publicly available data set indicates that thiamethoxam degrades more rapidly in the field than in the laboratory. This may be related to unrealistic laboratory incubation conditions, or study design influences, which remove the soil structure and may impair the ability of soils to maintain either specific microbial populations or the overall microbial viability of soil. However, there are no studies available which examine the rate of thiamethoxam degradation in laboratory and field studies and explore and/or demonstrate the reasons for the differences in the degradation rates observed.

Following spray application, any thiamethoxam not intercepted by the target crop is most likely to reach soil, whilst the drilling of treated seeds is likely to result in some dissipation of thi- amethoxam from the treated seed surface to the soil matrix. Thus, following application by either method, soil will be exposed to thiamethoxam; however, the areas of soil exposed will vary depending upon the application method. For spray applications, the initial area of soil exposed will be the soil surface, followed by the upper soil layers following rapid transport in the spray solution away from the immediate soil surface. The initial exposure is therefore largely to upper layers of bulk field soil following spray application. Based upon the earlier Koc values, if thiamethoxam is not degraded in the upper soil layers or dissipated by other mechanisms, thiamethoxam has the potential to be transported in soil pore water, down through the soil column, to expose lower layers of bulk soil (although this is likely to be at low levels).

Following seed treatment applications, some transport of thi- amethoxam would also be anticipated from the seed surface to the surrounding soil. However, although thiamethoxam may again be transported away from the seed, it is the soil imme- diately around the treated seed and roots of the growing plant (rhizosphere) that would be exposed initially and in the highest concentrations.

The narrow area of soil around plant roots is chemically and biologically different for the remaining bulk soil due to secretions from the roots, sloughed off root cells and subsequent coloni- sation by micro-organisms. Therefore, bacterial communities in the rhizosphere form a subset of the total bacteria community present in bulk soils, and hence, a rhizosphere effect can be observed on the microbial community which can also change over time with different plant development stages.22,23 Consequently, thiamethoxam applied as a seed treatment may be subjected to different degradation processes when compared to

| Table 1. Physical/chemical properties of thiamethoxam and clothianidin |
|-------------------------------------------|
| Thiamethoxam | Clothianidin |
|-------------------------------------------|
| Vapour pressure (Pa) | 6.6 × 10^{-9} at 25 °C | 1.3 × 10^{-7} at 25 °C |
| Henry’s Law constant (Pa m^{3}/mol) | 4.7 × 10^{-10} | 2.9 × 10^{-11} at 20 °C |
| Water solubility (g/L) | 4.1 at 25 °C | 0.30–0.34 at 20 °C |
| logK_{ow} | −0.13 | 0.7 |
| K_{oc} (mL/g) | 23.19 | 106.1–631 |

Of these, the narrow area of soil under plant roots is chemically and biologically different for the remaining bulk soil due to secretions from the roots, sloughed off root cells and subsequent colonisation by micro-organisms. Therefore, bacterial communities in the rhizosphere form a subset of the total bacteria community present in bulk soils, and hence a rhizosphere effect can be observed on the microbial community which can also change over time with different plant development stages. Consequently, thiamethoxam applied as a seed treatment may be subjected to different degradation processes when compared to...


| Reference Item | IUPAC name | Molecular mass (g/mol) | Structure |
|----------------|------------|------------------------|-----------|
| Thiamethoxam   | 3-(2-Chloro-thiazol-5-ylmethyl)-5-methyl-[1,3,5]oxadizinan-4-ylidene-N-nitro-amine | 291.7 | ![Structure](image1) |
| CGA322704 (clothianidin) | N-(2-Chloro-thiazol-5-yl-methyl)-N'-methyl-N'-nitroguanidine | 249.7 | ![Structure](image2) |
| CGA355190      | 3-(2-Chloro-thiazol-5-ylmethyl)-5-methyl-[1,3,5]oxadizinan-4-one | 247.7 | ![Structure](image3) |
| NOA459602      | Sodium; 5-(5-methyl-4-nitroimino-[1,3,5]oxadizinan-3-ylmethyl)-thiazole-2-sulfonate | 359.3 | ![Structure](image4) |
| SYN501406      | Sodium; 5-(N'-methyl-N'-nitroguanidinomethyl)-thiazole-2-sulfonate | 317.3 | ![Structure](image5) |
| CGA282149      | nitro-(3-methyl-[1,3,5]oxadizinan-4-yliden)-amine | 160.1 | ![Structure](image6) |
| CGA353042      | 3-Methyl-[1,3,5]oxadizinan-4-ylidenamine | 115.1 | ![Structure](image7) |
spray applied thiamethoxam. If different degradation processes are encountered by spray applied and seed treated thiamethoxam both the route and rate of degradation may be affected. Thus, different degradation rates and metabolites or metabolite concentrations may be observed following spray applications and seed treatments.

A number of factors relating to the degradation rate of thiamethoxam were explored in the study of Hilton et al.\textsuperscript{6}; it was concluded that neither application type, cropped fields versus bare soil, soil pH, soil organic matter content nor repeated annual applications affected the field soil degradation rate. Although The Pesticide Manual for thiamethoxam states that ‘Photolysis applications affected the field soil degradation rate. Although baresoil,soilpH,soilorganicmattercontentnorrepeatedannual

Table 2. continued

| Reference item | IUPAC name | Molecular mass (g/mol) | Structure |
|----------------|------------|-----------------------|-----------|
| NOA404617      | 1-(2-Chloro-thiazol-5-yl)-3-nitro-urea | 236.6 | ![Structure](image) |
| NOA407475      | 3-(2-Chlorothiazol-5-ylmethyl)-5-methyl-[1,3,5]oxadiazinan-4-ylidineamine | 246.7 | ![Structure](image) |

reasons for the differences. We compare the results to determine whether differing processes occur under more realistic field conditions and for different application types (spray applied and seed treatments), and determine whether the maximum formation of the soil metabolite clothianidin and the wider metabolite profile of thiamethoxam, and the rate of degradation of thiamethoxam in soil is affected.

Studies were initially planned on a single soil to determine possible reasons for the differences observed in the route and rate of degradation of thiamethoxam between laboratory and field studies. Side-by-side studies to investigate the degradation of spray applied and seed treated thiamethoxam in the field were also performed. Additional side-by-side method of application studies were also planned for dark laboratory studies; however, these were unsuccessful because seeds did not germinate and/or plants died under the laboratory test conditions. The data presented in this article therefore represent the full and complete available data from the planned experimental studies.

2 EXPERIMENTAL METHODS

Studies simulating a sprayed application were conducted at three tiers of realism. At the first tier, studies were performed in the laboratory in standard regulatory OECD307 studies in five soils, to investigate the route and rate of degradation of thiamethoxam in bulk soil, in the dark at 20 °C under constant moisture conditions.

As an intermediate tier, intact soil cores were collected for a single soil (East Anglia 2) selected from the five soils incubated in accordance with OECD307. The soil cores were incubated under the same standard laboratory conditions used for the OECD307 studies (20 °C in the dark) following a simulated spray application to the surface of the soil core. A comparison of thiamethoxam degradation rates and metabolite profiles was then made to determine whether soil structure, and hence the soil preparation required by the OECD307 guideline, played a role in differences observed between the degradation of thiamethoxam in laboratory and field studies. In addition, to examine the impact of downwards movement, the soil cores from the same soil (East Anglia 2) were incubated following a simulated spray application or rainfall event followed by watering-in (as typically occurs in spray
applications in the field) to distribute the active substance from the soil core surface into the soil core. A final laboratory soil core study performed with the East Anglia 2 soil examined the impact of a light/dark cycle, and consequent downwards movement following a simulated rainfall event and upwards movement through evaporation, on the route and rate of degradation of thiamethoxam following a simulated spray application with watering-in.

The highest tier of realism comprised field dissipation studies, which were performed for spray applications at four sites to allow a comparison of the route and rate of degradation of thiamethoxam in the standard OECD307 laboratory studies to the conditions typically encountered under normal use conditions in the field in Europe. In addition, seed treated field dissipation studies were performed at the same four trial sites, and at the same times, as the spray applied field dissipation trials. This allowed a direct comparison of the route and rate of degradation of thiamethoxam between the two application methods, and hence, whether the different environments immediately experienced by thiamethoxam following seed treatments affected its route and rate of degradation.

An intermediate study was performed for the seed treatment application, in which the degradation of thiamethoxam following a seed treatment application to soil cores from the same four field trial sites used in field dissipation studies was investigated. The soil cores were located in the outdoor area of a glasshouse research facility and allowed a direct comparison between semi-field and field studies to determine whether real differences can be observed; i.e. whether factors such as the collection of a small soil core or the controlled incubation environment affected the observed degradation of thiamethoxam.

Table 3 provides an overview of the studies performed and the test conditions associated with each.

### 2.1 Test compounds and reference standards

Tests 1–4 were conducted with [thiazole-2-14C]-thiamethoxam (Specific activity: 2.18–5.73 MBq/mg; Radiochemical purity: ≥97.0%) and Test 5 with [oxadiazine-4-14C]-thiamethoxam (Specific activity: 5.33 MBq/mg (144.1 μCi/mg); Radiochemical purity: 98.4%). Both test items were obtained from Selcia Ltd (Ongar, UK). The choice of radiolabel was based upon test item availability at the time the studies were performed and did not impact upon the study because the majority of investigated metabolites retained both radiolabels.

Radiochemical test items for use in Tests 1–4 were applied to soil in an aqueous solution. For seed treatment application, the test compound residue was dissolved in Cruiser Blank Formulation (a formulation without the active substance to allow the addition of radiolabelled active substance; in this case, thiamethoxam) and diluted in ultra-pure water. In field dissipation trials a 25% w/w wettable granule (WG) formulation of thiamethoxam was used in broadcast spray application trials (Test 6), while a 30% w/w flowable solution (FS) formulation (Test item codes: 2012-002832, 2012-002951) was used in seed treatment trials (Test 7).

Non-radiolabelled standard reference compounds for thiamethoxam and its potential metabolites, used for the confirmation of their identity, were obtained from Syngenta. Table 2 presents the metabolites for which reference compounds were available. The investigated metabolites represent a comprehensive list of those detected at levels > 5% of applied parent in regulatory soil aerobic degradation studies, anaerobic soil degradation studies and soil photolysis studies.

All other chemicals and reagents were of analytical grade or suitable equivalent.

### 2.2 Soils for laboratory OECD307 and soil core studies and outdoor located soil core studies (Tests 1–5)

OECD307 studies (Test 1) were performed in five soils, the physico-chemical properties for which are presented in Table 4. Soil cores for the laboratory soil core studies (Tests 2–4) were collected from a single site (East Anglia 2), the physico-chemical properties for which are also presented in Table 4. The East Anglia and East Anglia 2 soils were collected from different specific locations and on different days at the same site; hence, the soil characterisations presented, while similar, differ. Soil cores for the outdoor located soil core study (Test 5) were collected from the four sites used in the field dissipation studies, the physico-chemical properties for which are presented in Table 5.

In all cases soils were freshly collected from the upper soil layer (top 30 cm for OECD307 studies and top 7.0–10.0 cm for soil core studies). Soils for Tests 1–4 were collected from sites which had

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**Table 3.** Studies performed to assess the route and rate of degradation of thiamethoxam and associated test conditions (study durations/final sample times are presented in brackets)

| Study Type | Laboratory | Outdoor | Field |
|------------|------------|---------|-------|
| OECD307 | Soil core | Soil core | Field dissipation |
| 1 | Bulk soil (OECD307) – Dark | ✓ (120 DAT) | | |
| 2 | Soil surface/spray applied (soil core) – Dark | ✓ (71 DAT) | | |
| 3 | Soil surface/spray applied – Dark – Watered-in | ✓ (71 DAT) | | |
| 4 | Soil surface/spray applied – Light/dark a – Watered-in | ✓ (71 DAT) | | |
| 5 | Seed treatment – Natural sunlight – Watered-in | ✓ (70 DAT) | | |
| 6 | Soil surface/spray applied – Natural sunlight – Watered-in | ✓ (73–359) | ✓ (62–118) | |
| 7 | Seed treatment – Natural sunlight | | | |

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a Laboratory incubation included a 12 h:12 h light/dark cycle. The light source used was an Atlas Suntest® fitted with a xenon lamp and filters to remove wavelengths < 290 nm. Outdoor located soil cores and field dissipation studies were subjected to natural sunlight.

b DAT = days after treatment. The final sampling was 71 DAT for East Anglia 2 soil, for the four remaining soils the final sample was taken at 120 DAT.

c Field studies were performed for different study durations depending on the specific trial sites. The maximum study duration/latest sample time was 357, 359, 73, 118 DAT for spray applied studies at St Pierre, Meauzac, Markgröningen and Canals, respectively, and 93, 106, 62 and 118 DAT for seed treatment trials at St Pierre, Meauzac, Markgröningen and Canals, respectively.
received no pesticide applications for, at least, the previous 5 years. Neither thiamethoxam nor clothianidin were applied to soils used for Test 5 in the 3 years prior to the trial commencing.

Soil collected for use in the OECD307 guideline studies were stored and pre-incubated in accordance with the requirements of the guideline. Whole soil cores (8.3 cm internal diameter) for use in Tests 2–5 were freshly collected into metal rings using a borer. Following collection, soil cores were placed in a tray containing water (2 cm depth) and stored for at least 8 days, at 20 °C under fluorescent light to promote germination. Prior to application, seedlings were gently removed by hand with minimal disturbance of the soil surface.

2.3 Methods for laboratory OECD307 and soil core studies and outdoor located soil core studies (Tests 1–5)

2.3.1 OECD307 guideline laboratory studies (Test 1)

All studies were performed in accordance with the OECD307 guideline for testing the aerobic transformation in soil of chemicals. With the sole exception of the East Anglia 2 soil, all tests were performed in accordance with the requirements of good laboratory practice (GLP). The test item was applied dropwise in the treatment solution with a pipette to the soil surface (application rate of 107 μg/kg, equivalent to 80 g/ha considering a mean soil bulk density of 1.5 g/cm² and a 3 cm depth of incorporation) into each individual test vessel. Following dosing, soils were thoroughly mixed by stirring. Further methodological details can be found in the Appendix S1.

2.3.2 Laboratory soil column/soil core studies (Tests 2–4)

Three individual experiments were performed with intact soil cores, constant moisture [c. 38% volumetric water content (VWC); approximately equivalent to pF2] and application of [thiazole-2-14C]-thiamethoxam under controlled conditions:

Test 2. 20 °C in the dark; soil surface treatment.
Test 3. 20 °C in the dark; soil surface treatment, followed by a simulated rainfall event.
Test 4. Soil surface treatment, followed by a simulated rainfall event, with incubation with a light/dark cycle (a diurnal cycle with 12 h of light from a xenon-arc lamp followed by 12 h of darkness).

Unprocessed intact soil cores (c. 400 g dry weight equivalent, 8.3 cm diameter, 7.5 cm depth), which were retained within the metal sampling ring, were incubated for up to 71 days. Soil moisture was maintained at 38% VWC by incubation with a hydraulically fed on demand watering system, using capillary action to replace water lost through evaporation. Full details of the experimental design are given in the study of Hand et al. In addition, the soil core surfaces were sprayed daily with water (approximately 0.5 mL) to simulate morning dew.

Test solution was applied to the surface of the soil cores evenly with a pipette at a nominal application rate of 43.3 μg [thiazole-2-14C]-thiamethoxam which corresponded to a surface application rate of 80 g/ha. For Tests 3 and 4 watering-in was performed to simulate a precipitation event after application. Watering-in was carried out 30 min after application: water (c. 53 mL; equivalent to 10 mm) was added gradually to the surface of each core over a period of 3 h.

In Tests 2 and 3, soil cores were incubated in the dark at 20 °C as described. In Test 4, cores were maintained under a 12 h:12 h light/dark cycle. The light source used was an Atlas Suntest® fitted with a xenon lamp and filters to remove wavelengths < 290 nm. The overall light intensity at the start of the incubation period was measured at the sample height over wavelengths of 290 to 800 nm using a Bentham Spectro-radiometer. It was not possible to control temperatures during the light/dark study, instead soil surface temperatures for the light/dark cycle test were monitored.

Further methodological details can be found in the Appendix S1.
2.3.3 Outdoor soil column/soil core studies (Test 5)
A single experiment was performed with unprocessed intact soil cores from four soils and application of [oxadiazine-2-14C]-thiamethoxam as a seed treatment under outdoor conditions. Soil cores were incubated in the outdoor area of a glasshouse facility at Syngenta, Jealott’s Hill, UK. The area had a roof but open sides; therefore, incubated cores were not fully exposed to precipitation, but were subjected to the outdoor local temperatures. The general study design and methodology for outdoor located soil cores was the same as for the laboratory tests described in Section 2.3.2, with the exception that soil cores were reduced to a depth of 5 cm, resulting in a dry soil weight of approximately 300 g per soil core, and soil surfaces were sprayed weekly rather than daily with 1 mL of water. Outdoor located soil cores were acclimatised for 10 days prior to treatment.

The study was specifically designed to ensure that the same amount of test item was applied to each soil core and that each soil core contained the same number of treated seeds. Therefore, [oxadiazine-4-14C]-thiamethoxam was applied as a single drop of application solution to every wheat seed and five seeds were planted per soil core at a depth of approximately 1 cm, to give an application rate of approximately 80 g thiamethoxam/ha. The radioactive recovery for time zero samples of the individual soils gave 103.0–110.1% of theoretical applied radioactivity (see Appendix S1 for full data), demonstrating the target application was reached. A rainfall event was simulated by application of ultrapure water (25 mL per core; equivalent to 5 mm rainfall) over the course of about 2 h immediately after sowing. The cores were then incubated in the outdoor area of a glasshouse facility under natural sunlight. Temperatures were not controlled during the test, instead the temperature and light intensity were monitored.

Further methodological details can be found in the Appendix S1.

2.4 Field study (Tests 6 and 7)

2.4.1 Site selection
Eight soil dissipation studies were performed at four sites in Europe to investigate the route and rate of degradation of thiamethoxam in the field when applied as a spray to the soil surface and as a seed treatment. Four individual test sites located on trial plots at research farms in Germany, Spain, southern France and northern France were selected to provide a broad and representative range of pedo-climatic conditions representative of European agriculture. The sites selected were the same four sites from which soil cores were collected for the outdoor located soil core study (Test 5) in order to allow a direct comparison.

Details of the soil texture and other soil characteristics for each site are presented in Table 5. Details of cropping during the trial, the trial location and application dates for each site are presented in Table 6. The sites were located in typical maize cultivation areas, which are not prone to flooding or erosion, and which have a minimal slope so that overland flow and consequent run-off to areas adjacent to the treated field were negligible. They provide a range of soil characteristics (pH: 5.1–7.9; organic carbon: 0.4–1.2%) and textural classes. Neither thiamethoxam nor clothianidin had been applied to the trial plots in the 3 years prior to application. Trials for spray applied thiamethoxam (with watering-in to remove the influence of surface processes) and for thiamethoxam applied as a seed treatment were performed at all four sites. Applications were made on the same day at each of the trial locations for both application methods.
### Table 6. Thiamethoxam field trial site agronomic characteristics

| Trial name | Location / country | Crop growth stage at application (BBCH) | Application method | Formulation type | Application rates (g/1000 seeds) | Formulation type | Application dates | Application rates (g/ha) |
|------------|-------------------|----------------------------------------|---------------------|-----------------|-----------------------------------|-----------------|-------------------|------------------------|
| France, Silty Clay | St. Pierre, France, North Sandy Loam | 00 | Seed treatment | WG | 0.567 | 62.4 | 10 July 2012 |
| France, Sandy Loam | Meaux, France, South Loam | 00 | Seed treatment | WG | 0.589 | 64.0 | 10 July 2012 |
| Germany, Silt Loam | Markgröningen, Germany | 00 | Seed treatment | WG | 0.557 | 61.0 | 16 July 2012 |
| Spain, Silty Clay | Canals, Spain | 00 | Seed treatment | WG | 0.605 | 67.0 | 16 July 2012 |
| Germany, Silt Loam | Markgröningen, Germany | 00 | Seed treatment | WG | 0.605 | 67.0 | 26 June 2012 |
| Spain, Silty Clay | Canals, Spain | 00 | Seed treatment | WG | 0.605 | 67.0 | 3 July 2012 |

* The application rates of seed treatments in kg/ha were calculated based on the seed application rate (0.567 g thiamethoxam/1000 seeds in the France, Silty Clay trial) and the within-row seed drilling rate (approximately 280 g product/ha equivalent to 70 g thiamethoxam/ha, as described in Table 6.

All trials were conducted in accordance with regulatory guidance in place at the time the trials were performed and the requirements of GLP.26,27 Sites were maintained in accordance with typical agricultural practice.

Spray applications (Test 6) were made in dry conditions at a nominal application rate of 280 g product/ha equivalent to 70 g thiamethoxam/ha, and as described in Table 6.

For seed-treatment trials (Test 7), applications were made to maize seed (Fernandez, Northern Variety; or NK Famoso, Southern Variety) up to 46 days prior to the seed being sown. Maize seed was sown, in moist soil conditions, at a nominal rate of 110 000 seeds/ha which resulted in a nominal application rate of 70 g a.s./ha. Actual application rates at each of the trial sites are presented in Table 6. Within each row, polyvinylchloride (PVC) tubes (80–120 mm diameter) were pushed into the ground approximately 12 cm apart; one seed was pressed 2–3 cm into the ground at the centre of each PVC ring. Full details of crop cultivation, including application of other pesticides, and maintenance is given in the Appendix S1.

For spray applied trials (Test 6), sampling of the treated plots started at the beginning of a sub-plot and moved forward, so that previously sampled areas were not sampled again and to avoid soil being disturbed prior to subsequent samples being taken.

Samples from seed-treatment application (Test 7) plots were taken from inside the PVC rings directly over the seed sowing, one sample per PVC ring, five cores per sub-plot. Maize plants were cut down leaving as little stem and roots as possible but without disturbing the soil surface. The above ground portion of the maize plants were discarded before sampling.

Samples for both spray applied and seed treated trials were collected with a manual corer. Samples collected immediately after application, were collected to a depth of 10 cm. Thereafter samples were collected either to a depth of 30 or 100 cm. Samples were collected from the spray applied and seed-treatment plots up to 714–743 days after treatment (DAT).

Daily weather data (air temperature, air humidity, precipitation, solar radiation, wind speed, soil temperature (at 10 and 20 cm depths, only 10 cm depth reported), soil moisture (at 10 and 20 cm depths, only 10 cm depth reported) were recorded using on-site weather stations. In a few instances missing weather data were taken from a second weather station located up to 2.2 km from the trial area. Weather data are reported in detail in the Appendix S1.

All samples were stored frozen at ≤−18 °C from day of sampling until analysis.

### 2.5 Extraction and analysis

#### 2.5.1 OECD307 and soil cores (Tests 1–5)

Soil cores were prepared for analysis by excavating from the metal ring in 0–1, 1–3, 3–5, 5–7 cm sections. Outdoor located soil cores were extracted without separation into individual soil layers, with the exception of the samples taken for the final time-point. These were separated into four soil layers; however only the top layer (0–2 cm) contained any significant radioactivity and the extracts from the lower layers were combined.

Soil from sacrificed OECD307 samples and the sections of the soil core samples were extracted twice on the day of sampling by shaking in 0.01 M calcium chloride (CaCl₂) for 60 min at room temperature. Extracts were combined. Subsequent soil samples were then extracted once by shaking in acetonitrile/water (80:20 v/v) at room temperature, and then again in acetonitrile/water (80:20 v/v) adjusted to pH 3 with formic acid.
Additional accelerated solvent extraction (ASE) was carried out to investigate the unextracted residues when levels exceeded 10% Applied Radioactivity in the OECD307 samples. The upper section (0–1 cm) soil core samples from 42 DAT onwards, and all seed treatment (Test 5) upper sections, were also extracted via ASE.

All extracts were concentrated by evaporation and re-constituted in acetonitrile/water. The radioactivity in all extracts was radio-assayed with liquid scintillation counting (LSC). In accordance with the OECD307 guideline,24 extracted soils and plant samples were combusted in a sample oxidiser and analysed by LSC. The presence of volatile trap solutions was measured and aliquots radio-assayed with LSC. The mobile phase comprised (A) water with 0.1% formic acid and (B) methanol with 0.5% trifluoroacetic acid in a gradient which varied from 99:1 to 0:100 A:B. The limit of quantification (LOQ) was 0.1%.

The extracts were stored at –20°C and concentrated samples were stored in the refrigerator. The longest elapsed time from sampling to analysis was 6 months.

2.5.2 Field trials (Tests 6 and 7)

All soil cores for individual samples were separated into layers, 0–10, 10–20, 20–30, 30–50, 50–70 and 70–100 cm. In the seed-treatment trials the 0–10 cm soil layers were further separated into 0–5 and 5–10 cm layers. Individual soil cores from the same field and soil layer collected on the same day were then combined to form a single sample, and the composite soil layer samples homogenised prior to sieving through a 0.5 cm sieve. The 0–5 cm soil horizon cores were not bulked or homogenised and no aliquots were taken. Processed samples were stored frozen for up to 1025 days until extraction. Sample extracts were analysed within 35 days, but more typically within 1 week.

Samples were extracted twice by shaking in 10 mM ammonium acetate/acetonitrile (20:80 v/v), filtered, and concentrated by rotary evaporation. Extracts were combined and an aliquot cleaned by filtering through a polytetrafluoroethylene (PTFE) syringe filter. Extracts were analysed by LC–MS/MS using electrospray ionisation techniques. The method was previously validated for all analytes presented in Table 3, all of which demonstrated an LOQ of ≤ 0.001 mg/kg and a limit of detection (LOD) of ≤ 0.0003 mg/kg. This corresponds to ≤ 3% and ≤ 1%, respectively, of the initial thiamethoxam concentration in the upper soil layer of each of the field trials.

2.6 Storage stability

The long-term freezer storage stability of all analytes in soil was demonstrated with freezer-storage stability studies. Freezer storage stability data for thiamethoxam in soil at concentrations of 0.08 and 0.15 mg/kg demonstrated 100% and 104% recovery, respectively, (both n = 4) after 2 years freezer storage. All metabolites were fortified into two soils in duplicate at 0.05 mg/kg and stored in a freezer at –20°C for up to 13 months. The mean recoveries were 92–106% for each metabolite at the end of the 13 months storage period.

2.7 Calculation of degradation rates

The residue data for thiamethoxam were kinetically evaluated using the CAKE version 1.4 tool (version 3.2 for field studies), non-linear regression and a one-compartment, single first order (SFO) model. Where study conditions were not maintained at 20°C and a specific soil moisture content, no correction for temperature and soil moisture was performed for any study, including the outdoor located soil cores or the field dissipation trials. The duplicate samples for each sampling interval from the combined non-harsh extracts were considered in the kinetic evaluations for each soil for OECD307 studies. For the soil core studies the residues in the total soil column were considered. Harsh extracts were considered in addition to non-harsh extracts, where radioactive residues exceeded 5% of applied thiamethoxam. The SFO model assumes that the entire chemical is contained within a single compartment and degrades at the same rate. The following equation describes SFO kinetics:

\[ M = M_0 \ e^{-kt} \]

where \( M \) represents the total amount of chemical present at time \( t \); \( M_0 \) the total amount of chemical present at time \( t = 0 \); \( k \) is a rate constant.

The suitability of the fit of the models was evaluated both visually, based on a graphical plot of the degradation and in a plot of the residuals, and statistically, with the chi-squared error and t-test statistics.

2.5.3 Field trials (Tests 6 and 7)

For field dissipation studies the total residues were calculated, as described, for each of the triplicate samples (one for each sub-plot) as the total across all analysed soil layers. Samples below the LOQ for each analysed soil layer were accounted for according to FOCUS guidance.28,29

In two of the field trial sites SFO kinetics did not provide a good description of the degradation of thiamethoxam and bi-phasic kinetics were explored. Double first order in parallel (DFOP) kinetics, which considers two competing first order decline processes, provided the best fit. DFOP kinetics are described by the following equation:

\[ M = M_0 \left[ g \ e^{-kt_1} + (1 - g) e^{-kt_2} \right] \]

where \( M \) represents the total amount of chemical present at time \( t \); \( M_0 \) the total amount of chemical present at time \( t = 0 \); \( k_1 \) the rate constant in compartment 1; \( k_2 \) the rate constant in compartment 2; \( g \) is the fraction of \( M_0 \) applied to compartment 1.

Kinetic evaluations were not reported for clothianidin because reliable kinetic fits would not be possible in the majority of soils due to the need for more of a decline phase. In the majority of laboratory studies and some field studies, clothianidin was observed at its maximum concentration at the final time-point in the study. Where this was not the case, the decline phase consisted of one to two time-points with the sole exception of the France sandy loam outdoor soil core study.

3 RESULTS

3.1 Incubation conditions and weather data

Laboratory studies (OECD307 and soil core) for Tests 1 and 2 were incubated at 20 ± 2°C and with a soil moisture content maintained at pH2 (OECD307) or a mean moisture content of 38 to 40% VWC (soil cores), where 38% VWC was estimated to be approximately pH2. Although the mean moisture content was close to the target of 38% VWC, the actual moisture content of the soil cores varied, due to evaporation and constant replenishment.
from the reservoir by capillary action. Actual soil core moisture contents were 31.9% to 44.3% VWC over the course of the trials.

In Test 4 the light/dark cycle employed using the filtered xenon lamp resulted in increased temperatures during the 12 h light phase of 25 to 34 °C. In the 12 h dark phase temperatures were maintained at 20 ± 2 °C. A higher degree of variation was also observed in the soil core moisture content for Test 4 (31.3–52.0% VWC), although the mean moisture content was close to the target at 39.5% VWC. The mean measured light intensity at the soil surface was 61.2 W/m² (300–400 nm). Actual temperature and soil moisture contents are presented in the accompanying Appendix S1.

In outdoor soil core studies (Test 5) and field dissipation studies (Tests 6 and 7) soil moisture and temperature was monitored rather than controlled. Recorded results are presented in the accompanying Appendix S1. In the outdoor soil cores of Test 5, light intensities and soil core temperatures demonstrated diurnal variation, with typical peak daytime light intensities of 40 to 60 W/m² (250–400 nm) and air temperatures of approximately 8 to 38 °C (mean approximately 20 °C). Soil core temperatures varied from approximately 5 to 36 °C with mean values around 15–20 °C depending on the soil type. Soil moisture levels displayed regular small short-term variability for all four soils studied, however, general trends were also observed. For the Germany, Sandy Loam soil, the soil core moisture was typically 30–40% VWC and for the France, Silty Clay Loam soil core 40–50% VWC; both soils displayed a gradual increase in soil moisture over the course of the study. For the France, Sandy Loam soil cores a gradual increase in soil moisture from around 25–33% VWC was observed up to 21 August whereupon a sharp increase in soil moisture to 40% VWC was observed and subsequent variation from 40 to 45% VWC for the remainder of the study. Moisture in the Spain, Silty Clay core demonstrated a gradual decrease in soil moisture from around 50 to 35% VWC.

Full details of daily weather and soil temperature and moisture data from field dissipation studies (Tests 6 and 7) are presented in the Appendix S1. Average air temperatures during the field phase of all of the trials were similar to the long-term average for the individual sites. Precipitation was more erratic with total monthly precipitation frequently > 100 mm and air temperatures of approximately 8 to 38 °C (mean approximately 20 °C). Soil core temperatures varied from approximately 5 to 36 °C with mean values around 15–20 °C depending on the soil type. Soil moisture levels displayed regular small short-term variability for all four soils studied, however, general trends were also observed. For the Germany, Sandy Loam soil, the soil core moisture was typically 30–40% VWC and for the France, Silty Clay Loam soil core 40–50% VWC; both soils displayed a gradual increase in soil moisture over the course of the study. For the France, Sandy Loam soil cores a gradual increase in soil moisture from around 25–33% VWC was observed up to 21 August whereupon a sharp increase in soil moisture to 40% VWC was observed and subsequent variation from 40 to 45% VWC for the remainder of the study. Moisture in the Spain, Silty Clay core demonstrated a gradual decrease in soil moisture from around 50 to 35% VWC.

Full details of daily weather and soil temperature and moisture data from field dissipation studies (Tests 6 and 7) are presented in the Appendix S1. Average air temperatures during the field phase of all of the trials were similar to the long-term average for the individual sites. Precipitation was more erratic with total monthly precipitation frequently > 20% higher or lower than the long-term monthly average. Where rainfall was significantly lower than the long-term monthly average, the trial plot was additionally irrigated. Soil temperatures at 10 cm depth in field trials also demonstrated diurnal variations and though only daily mean soil moisture values are presented, day-to-day variation is observed.

### 3.2 Thiamethoxam DT50/DT90

Raw residue data and values used for the kinetic fitting for all trials are presented in the Appendix S1. Calculated end-points from the kinetic evaluations are shown in Table 7. All analyses from samples collected from treated plots in field trials prior to the first application displayed concentrations < LOQ, with the sole exception of the Germany, Silt Loam seed treatment application which displayed a background clothianidin concentration of 1% of the maximum clothianidin concentration, or 0.05% of the applied thiamethoxam residue. Therefore, all kinetic evaluations were concluded to be unaffected by background concentrations, or contamination from an external source.

All SFO kinetic fits were considered acceptable on the basis of the chi-squared error value (< 12.0% for laboratory studies and < 17.5% for semi-field and field studies) and visual fits, with the exception of the two spray applied field dissipation trials performed at France, Sandy Loam and France, Silty Clay Loam. For these two trials alone biphasic DFOP kinetics were employed, which gave a good fit to the residue data, again on the basis of the chi-squared error value (< 10.0%) and the visual fits.

### 3.3 Maximum metabolite formation

Only one metabolite was ever observed with mean maximum concentrations > 10% of applied thiamethoxam [mol/mol]. The maximum concentrations from all studies, of clothianidin are presented in Table 8. Additionally, the metabolites CGA355190, CGA353042 and NOA459602 were observed with mean maximum concentrations > 3% [mol/mol]. Full results for these metabolites are also presented in the Appendix S1.

### 4 DISCUSSION

#### 4.1 Degradation rate of thiamethoxam

The majority of SFO kinetic fits were considered good on the basis of the statistical and visual assessments. However, the two spray applied field dissipation trials (Test 7) performed at France, Sandy Loam and France, Silty Clay Loam did not demonstrate a good fit to the thiamethoxam residue data using SFO kinetics and consequently bi-phasic DFOP kinetics were employed which showed a good fit to the data.

Full mass balances were obtained in Test 1 OECD307 studies; volatile CO₂ concentrations were 8.1–34.1% applied thiamethoxam [mol/mol] and unextracted radioactivity was 3.8–14.5% applied thiamethoxam [mol/mol]. However, it was not possible to obtain full mass balances in Tests 2–5 soil core studies because, due to the study design, it was not possible to trap volatile compounds. This does not impact upon the calculated degradation rates for thiamethoxam because thiamethoxam is not volatile, and the only noted volatile compound formed from the degradation of thiamethoxam is CO₂. In the same way, full mass balances could not be obtained from field dissipation studies (Tests 6 and 7). A further possible additional dissipation mechanism from such studies is leaching through the soil profile. However, degradation rates were calculated based upon all thiamethoxam residues observed in the analysed soil layers and for all samples it can be concluded that the vast majority of extractable thiamethoxam residues remained in the 1 m deep soil profile. In the field studies the coring was carried out to 1 m and no detectable residues of thiamethoxam were observed below 30 cm in spray applied studies or below 50 cm in seed applied studies. Within the study design the LOD was set to detect thiamethoxam and clothianidin at levels < 1% and < 0.1% levels of the applied dose, respectively. It can therefore be concluded that leaching is not a significant loss mechanism of thiamethoxam in these field dissipation studies. Because thiamethoxam has a high water solubility and the calculated K₁ values indicate a very high to medium mobility, it might be assumed that thiamethoxam would be transported to greater depths in the soil column in soil pore water. This is further supported by the regular occurrence of thiamethoxam in surface water bodies in areas of intensive agriculture. However, the rapid degradation of thiamethoxam in both the spray applied and seed treated field dissipation studies reported here (see Table 7) means that thiamethoxam was typically degraded before leaching to deeper soil layers could occur. This is consistent with previous field dissipation studies which have also demonstrated that the majority of thiamethoxam is retained in the upper soil layers and that leaching of thiamethoxam through the soil profile...
Table 7. DT50 values (days) for thiamethoxam in laboratory, outdoor and field studies with chi-squared and r² presented in brackets.

| Laboratory | Outdoor | Field |
|------------|---------|-------|
| Soil core – Spray – Watered-in | Soil core – Spray – Light/dark – Watered-in | Soil core – Seed – Natural sunlight – Watered-in |
| OECD 307 Test 1 | 23.5 (4.0/0.987) | 10.2 (7.8/0.989) |
| East Anglia Test 1 | 146.3 (3.9/0.984) | 113.6 (8.1/0.978) |
| East Anglia Test 2 | 95.3 (4.6/0.992) | 4.2 (7.5/0.977) |
| Germany, Silt – – – – | 40.1 (6.0/0.938) | 24.9 (9.5/0.882) |
| France, Sandy Loam – – – – | 102.7 (8.0/0.951) | 29.9 (7.8/0.951) |
| Spain, Silty Clay – – – – | 25.8 (7.3/0.954) | – |
| n | 2 | 2 | 2 |
| Minimum | 4 | 1 | 4 |
| Maximum | 4 | 4 | 4 |
| Arithmetic mean | 19.2 | 12.4 | 29.9 |
| Geometric mean | 17.0 | 10.3 | 22.8 |
| Standard deviation | 2.0 | 2.0 | 3.0 |
| Relative standard deviation (% of geometric mean) | 11.8 | 19.2 | 13.6 |

Note: Number of soils: – study not performed. To parallel (DFOP) kinetics since single first order (SFO) kinetics did not provide an acceptable fit. The DT50 value reported is calculated from the overall DT90/3.32 which allows back calculation from the reported DT90 to a DT50 assuming SFO kinetics, and a comparison to be made to the other SFO DT50 values. The full reported DT50 and DT90 values were: France, Sandy Clay Loam g = 0.736; DT50 (overall) 0.578; DT90 (overall) 55.7 d; k1 DT50 0.046 d⁻¹; k2 DT50 22.1 d⁻¹.

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Table 8. Maximum observed mean clothianidin concentrations (% of applied thiamethoxam [mol/mol])

|                | Laboratory |                      | Outdoor |                      | Field |                |
|----------------|------------|-----------------------|---------|-----------------------|-------|-----------------|
|                | OECD307    | Soil core – Spray     | Soil core – Spray – Watered-in | Soil core – Spray – Light/dark – Watered-in | Soil core – Seed – Natural sunlight – Watered-in | Spray – Watered-in | Seed treatment |
| Test number    | Test 1     | Test 2                | Test 3  | Test 4                | Test 5 | Test 6          | Test 7          |
| Gartenacker    | 32.7 (119 DAT)a | –                    | –      | –                     | –     | –              | –              |
| 18 Acres       | 10.3 (119 DAT)a | –                    | –      | –                     | –     | –              | –              |
| East Anglia    | 26.7 (93 DAT) | –                    | –      | –                     | –     | –              | –              |
| Sarpy          | 18.0 (60 and 118 DAT)a | –            | –      | –                     | –     | –              | –              |
| East Anglia 2  | 10.8 (71 DAT)a | 9.6 (71 DAT)a       | 10.6 (71 DAT)a | 9.9 (21 DAT) | –     | 17.3 (73 DAT)b | 2.1 (28 DAT) |
| Germany, Silt Loam | –                | –                    | –      | –                     | –     | 4.4 (21 DAT)   | –              |
| France, Silty Clay Loam | –                | –                    | –      | –                     | –     | 4.5 (43 DAT)   | –              |
| France, Sandy Loam | –                | –                    | –      | –                     | –     | 2.1 (7 DAT)    | –              |
| Spain, Silty Clay | –                | –                    | –      | –                     | –     | 5.2 (70 DAT)b  | –              |
| n              | 5          | 1                     | 1      | 1                     | 1     | 4              | 4              |
| Maximum        | 32.7       | 9.6                   | 10.6   | 9.9                   | 9.9   | 5.2            | 19.0           | 4.5             |
| Minimum        | 10.3       | 9.6                   | 10.6   | 9.9                   | 9.9   | 2.1            | 16.3           | 2.1             |
| Arithmetic mean| 19.7       | 9.6                   | 10.6   | 9.9                   | 9.9   | 4.1            | 17.5           | 3.4             |
| Standard deviation| 9.8       | –                     | –      | –                     | –     | 1.3            | 1.1            | 1.2             |
| Relative standard deviation (% of geometric mean) | 49.9 | – | – | – | 33.3 | 64 | 36.2 |

a Study termination.
b Initial thiamethoxam concentration determined from analysis of soil in application trays.
Note: n, number of soils.
will only occur under extreme conditions comprising soils with a very low organic carbon content and extreme rainfall.20,30–32 Consequently, we conclude that field DT$_{50}$ values reported here represent the degradation of thiamethoxam rather than loss through other dissipation mechanisms.

The thiamethoxam DT$_{50}$ values derived from the OECD307 studies conducted on typical European soils lie within the typical range of thiamethoxam DT$_{50}$ values reported for laboratory studies in the literature which were derived from a range of temperature and soil moisture conditions.17–19 Full details of soils, soil treatment and incubation are not reported in the available literature studies. However, the single soil from New Delhi, India, reported in the study of Gupta et al.18 includes a range of soil moisture conditions from completely dry to flooded and soil treatment which includes soil drying and sieving through a 2 mm sieve, as required by the OECD307 guideline. A range of thiamethoxam DT$_{50}$ values are reported for the different soil moisture conditions (46–301 days); when thiamethoxam DT$_{50}$ values for a soil moisture of pF2 alone are considered the range is significantly reduced to 91.2–94.1 days, which is consistent with the values reported here.

For field studies examining the degradation of thiamethoxam, the majority of the values in the literature originate from the study of Hilton et al.6 which was performed at 18 typical European trial sites, which encompassed a range of pedoclimatic conditions and examined both seed treatments and spray applications. A direct comparison is difficult because in that study degradation rates were normalised to a temperature of 20°C, however all DT$_{50}$ values reported here are lower than the geometric mean value reported in that study (31.2 days), though the values are not untypical of the range of thiamethoxam DT$_{50}$ values reported in that study (7.1 to 92.3 days).

The values reported here for the OECD307 and field studies are considered to be typical of the values found in the literature. Therefore, the effects observed on soils investigated in this study are likely to be observed in other typical agricultural soils.

The results presented in Table 7 demonstrate that in general the degradation rates of thiamethoxam increase, and the calculated DT$_{50}$ values decrease, with increasing reality of the test. A comparison of the thiamethoxam DT$_{50}$ values observed in standard regulatory dark laboratory OECD307 studies (Test 1) to those from spray applied field dissipation studies (Test 6) demonstrated much faster degradation rates in the field (geometric mean DT$_{50}$ values were 18.3 days in spray applied field studies and 75.4 days in OECD307 studies). This confirmed the apparent effect noted from information reported in previous literature.6,17–21

The geometric mean DT$_{50}$ value of 75.4 days for the OECD307 studies (Test 1) compares to 40.1 days for East Anglia 2 laboratory soil cores incubated in the dark under the same conditions following simulated spray applications (Test 2). However, in order to deduce the impacts of particular variables it is appropriate to examine studies in which the soil type remains the same. Therefore, a comparison of thiamethoxam DT$_{50}$ values in soil core tests performed with the East Anglia 2 soil alone was made. The thiamethoxam DT$_{50}$ value in the East Anglia 2 soil for the Test 1 OECD307 study was 85.7 days, which compared to the DT$_{50}$ value of 40.1 days for the spray applied East Anglia 2 soil core incubated under the same conditions. For the comparison of laboratory incubated soils in OECD307 studies to those in soil cores, the effect of temperature and soil moisture conditions can be excluded since the conditions were similar in this regard for all dark laboratory studies. The only differences relate to the treatment of soil for the OECD307 studies, by air-drying and sieving, which removes soil structure. Information in the literature suggests that total soil microbial biomass is not significantly affected by the soil treatments employed in the OECD307 test.33–35 However, specific effects on fungal hyphae have been noted, which can result in a reduction in fungal networks thereby reducing fungal dependent microbial communities.34,35 Therefore, the removal of soil structure, may, in this case, also remove, or reduce, the specific microbial communities which are additionally responsible for the degradation of thiamethoxam. The increasing reality of the tests when moving from the sieved soil in the OECD307 studies to the soil cores therefore appears to retain some of the soil function responsible for thiamethoxam degradation, which is lost with the soil treatment employed for OECD307 studies.

Watering-in immediately after a simulated spray application made a negligible difference to the degradation rate of thiamethoxam in the East Anglia 2 soil cores, with a soil core DT$_{50}$ value for thiamethoxam of 43.5 days reported for Test 3 compared to 40.1 days for Test 2. However, the introduction of the light/dark regime following simulated spray application and watering-in increased the degradation rate of thiamethoxam, with the DT$_{50}$ value from the soil core in Test 4 being 24.9 days. It is possible that the increased degradation rate under these conditions arises due to the increased temperature due to the irradiation methodology employed. However, the temperature recorded was at the soil surface; deeper soil layers were not subjected to the same direct light exposure and the initial watering-in would be expected to transport the applied thiamethoxam from the immediate soil surface and the direct light exposure. While the deeper soil layers are anticipated to exhibit a temperature increase compared to the non-light exposed tests, the soil temperature in those deeper layers is also anticipated to be lower than the temperature at the soil surface. Therefore, while the effect of an increased soil temperature cannot be completely excluded on the degradation rate increase, other factors may also be contributing to the reduction in the DT$_{50}$ value of thiamethoxam observed. In Test 4, though mean moisture contents of the soil cores under the light/dark regime were comparable to those of the other laboratory soil core studies, a larger degree of variation in the soil moisture was observed (see Section 3.1).

The variation in moisture contents of the laboratory soil cores in Test 4 is as a result of light and/or heat exposure experienced at the soil core surface in these tests which also creates an upward movement of water from deeper soil layers through the soil profile by evaporation and subsequent capillary action. Overnight cooling resulted in downward movement of water. Since thiamethoxam has a high water solubility, this constant short-range movement of water therefore allows the transport of any solubilised thiamethoxam through the soil profile to other areas, where degradation rates may be greater and/or additional degradation or dissipation processes or mechanisms may occur.

The transport of thiamethoxam through the soil profile is supported by the observed levels of unextracted residues (see Appendix S1) in the OECD307 and soil core studies, which generally increased as the studies moved closer to reality. The effect is particularly evident when data for the East Anglia 2 soil cores alone are examined. Maximum unextracted residues following ASE for Tests 2–4 for the soil cores were 6.91%, 9.84% and 14.73% applied thiamethoxam, respectively; demonstrating that the watered-in (Test 3) and watered-in light/dark (Test 4) laboratory tests showed the highest soil binding. It therefore seems apparent that the increased short-range movement of water through the soil profile results either in the transport of solubilised thiamethoxam to a
greater number of binding sites in soil in the immediate vicinity, and counter-intuitively results in greater binding of thiamethoxam to soil; or in the increased transport of thiamethoxam to areas of soil where microbial, or other degradation mechanisms, may occur, possibly at a faster rate, resulting in metabolites that are more readily adsorbed to soil.

The highest unextracted residues in outdoor located soil cores were 23.3 – 32.8%. While these studies were performed on different soils and with seed treated as opposed to simulated spray applications, four soils were studied each of which displayed increased amounts of unextracted residues when compared to the indoor located soil cores in Tests 2 – 4. These results, therefore appear to support the conclusions from comparison of the East Anglia 2 soil cores. It was not possible to detect unextracted residues in field trials since radiolabelled test material could not be applied in field studies.

Thiamethoxam DT₅₀ values were also much lower in outdoor located soil cores than those reported in OECD307 studies and the indoor located soil cores. A direct comparison of the degradation rates from outdoor located soil cores to those from laboratory OECD307 and indoor located soil core studies is complicated because of the different treatment methods employed and the different soils used. However, the fact that thiamethoxam degradation rates are comparable between seed treated and spray applied field dissipation studies (see following paragraph) suggests that a comparison is valid.

Although measured mean air temperatures in the outdoor located soil core studies were slightly lower, or the same as, those in the standard OECD307 studies, peak temperatures were again significantly higher than those in the standard laboratory studies, as were the measured soil moisture contents. Both of these factors may again influence the enhanced degradation rate as described earlier for Test 4 as conditions move from laboratory OECD307 studies and closer to the reality experienced in the field.

Field trials performed in Hilton et al.° demonstrated a lower geometric mean DT₅₀ value for seed-treatment trials versus spray applied trials (25.2 versus 32.5 days), though the effect was not statistically significant. In the field trials reported here overall geometric mean values were 16.5 and 18.3 days for the seed treatment and spray application, respectively, with two of the four field trial locations demonstrating a more rapid thiamethoxam degradation for seed treatments and two locations demonstrating a more rapid thiamethoxam degradation for spray applied trials.

The geometric mean DT₅₀ value for the four seed-treated outdoor located soil cores performed with the same soils as the field dissipations studies (Test 5) was lower than in the seed-treated field dissipation studies, being 11.8 days. However, the standard deviations suggest that the effect is not significant and that the degradation rates in the outdoor located soil cores and the field dissipation studies are comparable. Indeed, for the France Sandy Loam the thiamethoxam DT₅₀ in Test 5 was notably faster than in the seed treated field studies, while for the Germany Silt Loam the rate of degradation was also quicker in Test 5 than Test 7. In the Spain Silty Clay the converse was true with thiamethoxam degradation being much faster in the field than in Test 5. For the France Silty Clay Loam the difference in thiamethoxam degradation rates was negligible between Tests 5 and 7. Thus, it is likely that the effect of the soil core when compared to bulk soil in the field is minor in seed treated studies and other factors, probably relating to the specifics of the soil temperature or moisture content experienced in the studies, are responsible for any differences in degradation rates observed in specific soils.

Overall, the studies suggest that the two main influencing factors for the differences between the degradation rates of thiamethoxam in standard laboratory OECD307 studies and field dissipation studies relate to the destruction of soil structure in the regulatory studies and the constant movement of water due to diurnal temperature differences and consequent short-range transport of thiamethoxam (though the increases in peak temperature at the soil surface cannot be completely excluded). These two factors result in a significant overestimation of the DT₅₀ value of thiamethoxam in the field by the OECD307 studies typically used to support regulation.

4.2 Route of degradation of thiamethoxam

With the exception of clothianidin, the maximum observed formations for the metabolites were consistently too low to allow any conclusions to be made regarding the route of degradation in soil of thiamethoxam. Therefore, only the formation of clothianidin is discussed further here.

The maximum observed formation of clothianidin in laboratory studies was frequently observed at study termination and may therefore have increased further had studies been performed for a longer duration. Hence results for the metabolite formation from these studies should be interpreted with care. However, comparison of the maximum clothianidin formations in studies performed with the East Anglia 2 soil did not demonstrate a significant effect on the maximum formation of clothianidin relating to the study type (OECD307 versus soil core). In addition, mean maximum clothianidin formation in the East Anglia 2 soil core studies did not appear to be affected by the increasing reality of the tests. Therefore, it appears that, unlike the rate of thiamethoxam degradation, neither the soil treatment employed for the OECD307 study nor the movement of water created by the light/dark cycle impact the route of degradation of thiamethoxam. This effect is apparently confirmed when the mean maximum formations of 19.7% (n = 5) and 17.5% (n = 4) applied thiamethoxam [mol/mol] for Tests 1 (OECD307 studies) and 6 (spray applied field dissipation studies), respectively, are compared.

Comparison of mean clothianidin formations following application of thiamethoxam as a seed treatment in the outdoor located soil core and field dissipation studies – Tests 5 (n = 4) and 7 (n = 4) – demonstrated lower formations of clothianidin (mean formations: 4.1% and 3.4% applied thiamethoxam [mol/mol] respectively) when compared to the spray applied field dissipation study (Test 6). Seed-treated dissipation studies displayed maximum clothianidin concentrations ranging from 2.1 to 4.5% [mol/mol] thiamethoxam, while spray applied thiamethoxam studies displayed maximum clothianidin concentrations of 16.3 to 19.0% [mol/mol] thiamethoxam. Side-by-side comparisons at individual field trial sites demonstrated at least a four-fold reduction in the maximum observed clothianidin concentrations. This is despite the fact that degradation rates of thiamethoxam were comparable between the seed treated and spray applied field studies.

As discussed in Section 4.1, leaching is a potential dissipation mechanism from field dissipation studies. However, for all samples in these studies, all extractable thiamethoxam and the vast majority of extractable metabolite residues remained in the 1 m deep soil core (see Appendix S1). In some instances small amounts of clothianidin were observed in the lowest soil layer analysed in the soil core and therefore, small amounts of clothianidin may have
leached to deeper soil layers than those analysed. The concentration of clothianidin residues in the lowest analysed soil layers never exceeded 1% of applied thiamethoxam [mol/mol] (and were typically < 0.1% applied thiamethoxam [mol/mol]) in seed applied studies or 3% of applied thiamethoxam [mol/mol] in spray applied studies. Leached amounts would be anticipated to be even lower, and therefore, it is unlikely that the maximum observed concentrations of clothianidin would be affected significantly were lower soil layers to be analysed.

In this case, the difference in the maximum observed formations of clothianidin in the thiamethoxam seed treated field studies when compared to spray applied thiamethoxam field studies suggests that different or additional competing degradation mechanisms occur which are associated with the rhizosphere of treated seed (which are widely reported to be highly active microbial zones).\cite{12,22,23} This is despite thiamethoxam degradation rates being comparable in seed treatment and spray applied field studies. Alternatively, the zones in which clothianidin is formed following seed treatments may have a higher degree of microbial activity towards clothianidin resulting in its more rapid degradation and therefore its lower maximum observed formation.

Based upon these results, we conclude that routes of thiamethoxam degradation in bulk field soil and the rhizosphere differ, which results in much lower clothianidin formation for applications to treated seed compared to spray applications in the field.

5 CONCLUSION

Spray applied thiamethoxam degraded more rapidly in the field than in standard regulatory OECD307 laboratory tests. Stepwise evaluation of the impact of study design on the degradation rates of thiamethoxam was investigated. Comparison of OECD307 standard regulatory studies with soil core studies under the same conditions clearly demonstrated decreased DT$_{50}$ values of thiamethoxam (85.7 to 40.1 days). We conclude that the majority of the difference between the degradation rates is likely to be associated with the artificial soil treatment employed in OECD307 studies which destroys bulk soil structure and is likely to impact microbial population viability and numbers. This results in the retardation of the degradation rate of thiamethoxam and provides unrealistically high DT$_{50}$ values. Watering-in of thiamethoxam in soil cores produced a negligible impact on the observed thiamethoxam DT$_{50}$ values (40.1 and 43.5 days for the non-watered-in and watered-in tests, respectively).

As the soil core studies became more realistic to the field situation, with the introduction of a light/dark regime, a further clear decrease in the thiamethoxam DT$_{50}$ values was observed; 40.1 to 24.9 days for dark soil cores and light/dark soil cores, respectively. The introduction of this intermittent source of heat and light creates constant movement of water in the soil column, which in turn enhances the short-range transport of thiamethoxam to areas of soil where microbial, or other degradation mechanisms, may occur and thereby increases degradation. However, though mean soil temperatures are comparable between the studies, an effect from the increased temperature observed at the soil surface during the irradiation periods cannot be completely excluded.

The additional complexity of comparing seed treatments to spray applied thiamethoxam in side-by-side field trials at four sites demonstrated similar DT$_{50}$ values for thiamethoxam (geometric mean DT$_{50}$ values of 18.3 and 16.5 days for spray applied and seed treated thiamethoxam, respectively). Therefore, we conclude that there is little impact of the application method on the degradation rate of thiamethoxam in the field. Soil cores were collected from the same four field trial sites and thiamethoxam was incubated as a seed treatment in outdoor located studies in a glasshouse facility. These additional tests showed comparable data-sets to the field data (geometric mean DT$_{50}$ value of 11.8 days was calculated for the outdoor located studies) demonstrating no additional key factors relating to the soil core structure and the structure of bulk soil in the field influencing the degradation rate of thiamethoxam between soil cores and field studies.

We conclude that the majority of the difference between the degradation rates of thiamethoxam in soil in laboratory OECD307 studies and in field dissipation studies are a result of the soil treatment employed in OECD307 studies, as well as from the constant movement of solubilised thiamethoxam in soil pore water due to diurnal light/dark conditions. The application type and watering-in of thiamethoxam demonstrated negligible effects on degradation rates.

With the exception of clothianidin, the maximum observed formations for the metabolites were consistently too low to allow any conclusions to be made regarding the route of degradation in soil of thiamethoxam.

When comparing spray application in laboratory OECD307 standard regulatory studies with dark soil cores and soil cores incubated under a light/dark cycle, as well as with spray applied field data, the maximum observed levels of clothianidin remained consistent. Even though the DT$_{50}$ values of the parent significantly decreased with the increasing realism of the tests, this had little or no apparent effect on the maximum observed formation of clothianidin. However, the side-by-side field dissipation spray and seed treatment studies demonstrated consistent lower formation of clothianidin in the seed treated studies. Despite the application rates of thiamethoxam being the same for both application methods, the maximum levels of clothianidin seen in the seed treated studies were at least four-times lower than in the spray applied trials. The outdoor soil core studies also showed that for seed treated cores the same low levels of clothianidin formation occurred and that the maximum observed clothianidin formations were consistent with the field data.

Thus, we conclude that the presence of the differing rhizospheric soil composition experienced by seed treated thiamethoxam affects either the route of degradation of thiamethoxam (resulting in clothianidin being formed in lower concentrations) or the rate of degradation of metabolites (i.e. lower clothianidin DT$_{50}$ values resulting in lower maximum observed clothianidin concentrations) when compared to bulk soil, though degradation rates of thiamethoxam remain comparable.

It is clear from the data presented here that the OECD307 guidance can be a highly conservative study design for the evaluation of the aerobic rate of degradation of chemicals in agricultural soils. The processing of soil (sieving, moisture maintenance and storage) in itself may have a significant impact on the abundance and activity of the microbial communities in soils, which can lead to an underestimation of the rate of degradation for some chemicals. Other processes, including the presence of light (both as a direct process of chemical breakdown and as a promoter of microbial biomass), upward movement and rhizosphere enriched microbial environments all also play an important role in the degradation of chemicals in soil. While the OECD307 guideline remains an appropriate guideline for first-tier regulatory assessments, this study has demonstrated that, for some compounds, higher-tier environmental risk assessments informed by more realistic laboratory
or outdoor/semi-field degradation studies may be warranted as an alternative, or intermediate, approach to full field dissipation studies. Before such studies play a regular role in the regulatory assessment of chemicals, and thereby enable a more realistic and relevant evaluation of chemical degradation in soil, further work is needed to develop standardised higher-tier study protocols or guidelines (possibly similar to those presented here). Any standardised higher-tier studies should allow for the consideration of the possible effects of soil processing, diurnal light variation and the application method of the chemical being studied.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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