Document Title

Summary of the fate and behaviour in the environment for spiroxamine

Data Requirement(s)

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Section 7: Fate and behaviour in the environment

According to the Guidance Document SANCO/10181/2013 for applicants on preparing dossiers for the approval of a chemical active substance

Date

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ERM
On behalf of Bayer AG
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Version history

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[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4, 'How to revise an Assessment Report'

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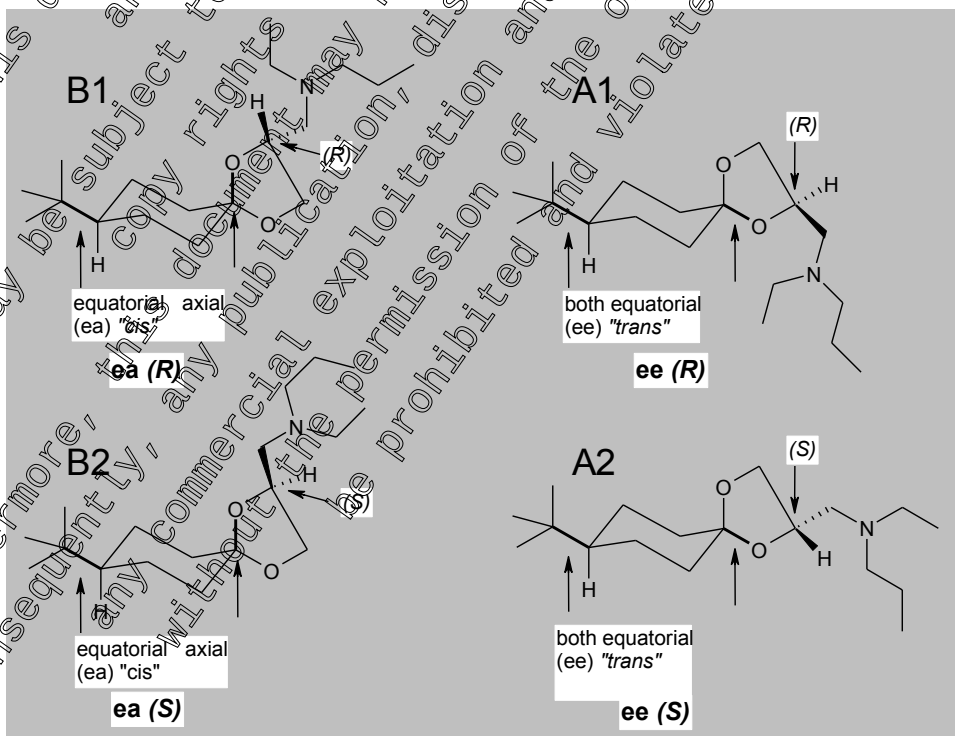
CA 7 FATE AND BEHAVIOUR IN THE ENVIRONMENT

Spiroxamine was included in Annex I to Council Directive 91/414/EEC in 1999 (Directive 1999/73/EC, Entry into Force on 1 September 1999). This Supplementary Dossier contains data which were not submitted at the time of the Annex I inclusion and first renewalof spiroxamine under Council Directive 91/414/EEC and which were therefore not evaluated during the EU review and first renewal. However, all studies submitted for the first approval and subsequent first renewal of spiroxamine have also been summarised according to current guidance and included in the dossier. Where studies meet relevant validity criteria, new robust study summaries have been provided in the appropriate dossier section. However, where studies do not meet relevant validity criteria and are not considered acceptable, less detailed summaries have been provided alongside discussions of study deficiencies. All relied upon study reports are submitted in Document K for this second renewal of approval dossier or in Document K for the previous Annex I inclusion and first renewal submissions.

All data which were already submitted by Bayer AG (former Bayer CropScience) for the Annex I inclusion and first renewal under Council Directive 91/414/EEC are contained in the draft Re-Assessment Report (RAR) 2010 and its revised RAR 2017, and are included in the Baseline Dossier provided by Bayer AG.

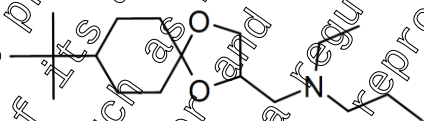
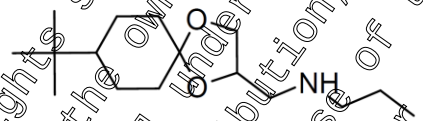
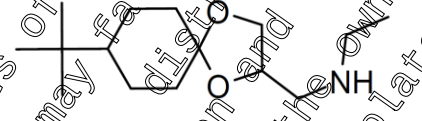
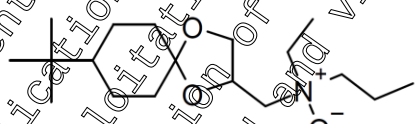
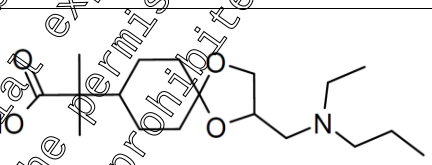
Relevant information for classification as detailed in the "Combined Draft (Renewal) Assessment Report prepared according to Regulation (EC) N° 1107/2009 and Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008 – Volume 1, Level 2" is provided in Document N1, Sections 8.2 and 8.3, and highlighted in light grey

Spiroxamine consists of four isomers (two diastereomers each with its corresponding two enantiomers which are in a 1:1 ratio) as shown in the schematic below. The isomer nomenclature presented in some historical documentation may differ with respect to the A/B and corresponding trans/cis notation as a result of a discrepancy in referencing, which is discussed in detail in position paper M-761468-01-1 (see CA 1.7/01). It is recommended that the stereo assignments depicted here, together with the A and B notation should be used exclusively going forward to ensure continuity of information throughout the dossier.



This document reviews the fate and behaviour in the environment, including additional data and the appropriate risk assessments, for spiroxamine.

The structures of the active substance spiroxamine and of all associated metabolites are presented in the N3 document. A full copy of the N3 document is provided in Appendix 1 for reference only. Studies to investigate the fate and behaviour of spiroxamine have been performed with [cyclohexyl-1-^{14}C]-spiroxamine, [1,3-dioxolane-4-^{14}C]-spiroxamine radiolabelled or non-radiolabelled (with or without formulation) test substance. The structure of spiroxamine and the radiolabel positions is summarised below.

Name used in this document	Structure	Equivalent names used in some study reports
[cyclohexyl-1-^{14}C]-spiroxamine		[cyclohexyl-1-^{14}C]- KWG 4168
[1,3-dioxolane-4-^{14}C]-spiroxamine		[1,3-dioxolane-4-^{14}C]- KWG 4168
Non radiolabelled spiroxamine		KWG 4168
M01 (spiroxamine-desethyl)		KWG 4168 – desethyl; FHW 0104H; KWG 4557
M02 (spiroxamine-despropyl)		KWG 4168 – despropyl; KWG 4669; WAK 6174
M03 (spiroxamine-N-oxide)		KWG 4168 - N-oxide; WAK 6301; WAK 6301/1
M06 (spiroxamine-acid)		KWG 4168 – acid; WAK 5708; WAK 5708/P

* Denotes position of [^{14}C]-radiolabel

Details of the literature search undertaken can be found in M-CA Section 9. If a relevant, scientifically peer-reviewed, open literature reference has been identified for spiroxamine or its major metabolites, it has been discussed within the relevant data point.

CA 7.1 Fate and behaviour in soil

Previously, information on the aerobic route of degradation of spiroxamine was obtained from studies performed with spiroxamine radiolabelled in either the cyclohexyl or dioxolane position (KCA 7.1.1.1/01 (M-006135-01-1); KCA 7.1.1.1/02 (M-006141-01-1); KCA 7.1.1.1/04 (M-006148-01-1) & KCA 7.1.1.1/05 (M-303803-01-1)). An additional study (KCA 7.1.1.1/03 (M-006096-01-1)) is also included for completeness but is not relied on for endpoint setting. Although conducted some years ago, these legacy studies are considered fully adequate for defining the rate and route of degradation of spiroxamine in soil.

The route of degradation of spiroxamine was consistent in all studies and driven via de-alkylation of the amine moiety and/or oxidation reactions of the alkyl chains resulting in identification of the soil metabolites M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl) and M03 (spiroxamine-N-oxide, please see Figure 7.1-1). Ultimately, spiroxamine was mineralized with up to 50% applied radioactivity (AR) being observed as CO_2 and bound residue of approximately 20% AR. M01 (spiroxamine-desethyl) was observed in all soils with a maximum occurrence of 8.8% in the Mondheim 3 soil (KCA 7.1.1.1/02 (M-006141-01-1)). M02 (spiroxamine-despropyl) was found at lower overall percentages than M01 (spiroxamine-desethyl), but was still observed in all studies with a maximum formation of 5.8% AR with the highest formation typically occurring close to study end. M03 (spiroxamine-N-oxide) was found only >5% AR in the wolf ranch soil (KCA 7.1.1.1/04 (M-006148-01-1)) where the maximum observed %AR was 7.9%. Other minor metabolites were found in the studies, of which no single component accounted for >3.5% AR, which included M06 (spiroxamine-acid), M11 (spiroxamine-desethyl-acid), M12 (spiroxamine-despropyl acid) and M15 (spiroxamine-ketone). Overall, the fate and behaviour of spiroxamine was well described in the historical studies submitted in the previous renewal.

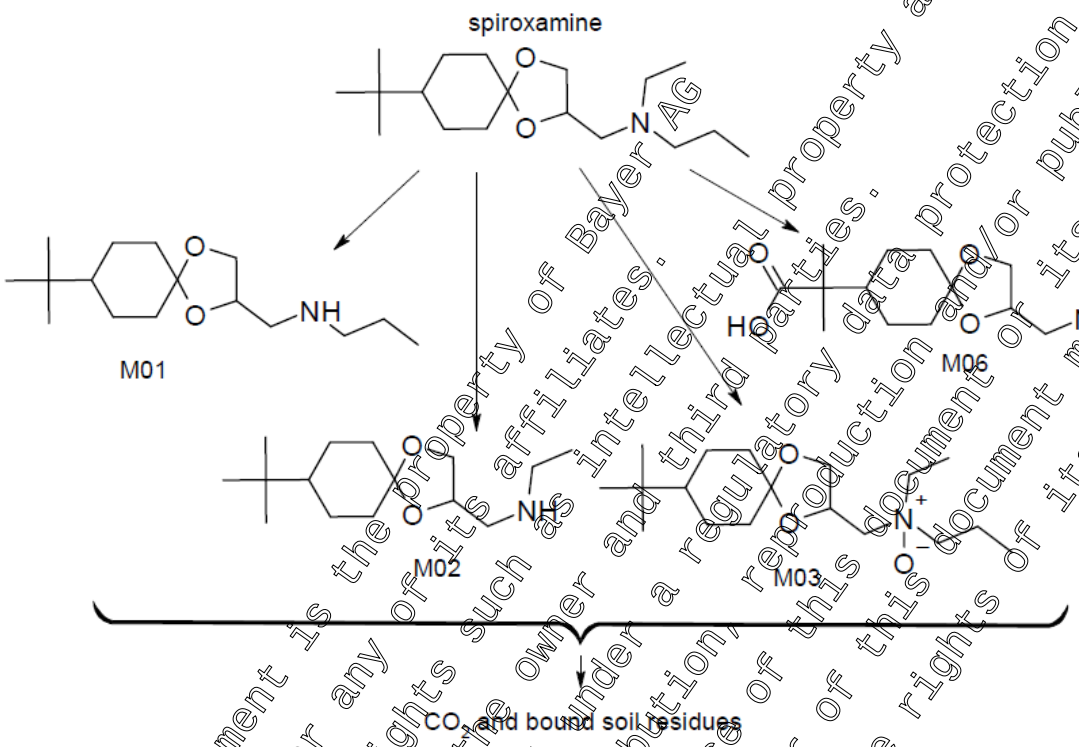
In order to clearly define the potential for stereochemical interconversion or stereochemical specific degradation, a new aerobic route study was conducted in four soils (KCA 7.1.1.1/06 (M-762349-01-1)) taking into consideration the stereochemistry guidance (EFSA, 2019). In this new study, quantification of spiroxamine and metabolites occurred using both chiral and achiral methods, with only the achiral results available at this time for consideration. The chiral analysis has been technically challenging the data are unavailable at the point of submission but will be submitted as part of the top-up submission or soon as completed.

However, considering the achiral analysis (i.e. analysis for total spiroxamine), we see a consistent picture of spiroxamine degradation with the metabolism profile previously reported in the legacy studies. The degradation of [^{14}C-cyclohexyl]-spiroxamine was investigated in 4 EU soils (Longwoods, Refesol-03G; Refesol-02A and Speyer 6S). Spiroxamine degraded ultimately to CO_2 and bound residue with the formation of the relevant metabolites, M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl) and M03 (spiroxamine-N-oxide) identified as major soil metabolites. M01 (spiroxamine-desethyl) was seen at a maximum formation of 12% AR in the Longwoods soil, and at levels >5% observed in all other soils. Likewise, M02 (spiroxamine-despropyl) was also observed in all soils >5% with a maximum observed value of 9.2%AR in the Longwoods soil whilst M03 (spiroxamine-N-oxide) was consistently observed in all four soils but was only found >5% AR in the Longwoods soil with a maximum observation of 7.2% AR. The only notable new observation versus the previous evaluation was that of M06 (spiroxamine-acid), previously M06 was observed only at a maximum of 3.5% (in KCA 7.1.1.1/01 (M-006135-01-1)); the most recent data show M06 at a maximum of 5.3%AR at the final time point in the Refesol-02A soil thus triggering further evaluation and risk assessment. A number of additional minor metabolites were observed, but these were not characterised due to their low levels in all soils.

In conclusion, from the studies submitted to support the route of degradation of spiroxamine, studies conducted on parent radiolabelled in the cyclohexyl or dioxolane positions fully define the behaviour of the active substance. Spiroxamine is degraded by de-alkylation of the amine moiety with further oxidation reactions of side chains, forming the relevant metabolites M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl), M03 (spiroxamine-N-oxide) and M06 (spiroxamine-acid) which are thus considered in the definition of residue for soil. The observation of M06 (spiroxamine-acid) at the final timepoint of the aerobic degradation of Refesol-02A >5% (KCA 7.1.1.1/06 (M-762349-01-1)) is a new

observation as this metabolite had only previously been observed at a maximum %AR of 3.5% (KCA 7.1.1.1/01 (M-006135-01-1)). No other metabolites were observed at concentrations approaching 5% and it can be considered that the route of degradation of spiroxamine has been fully characterised.

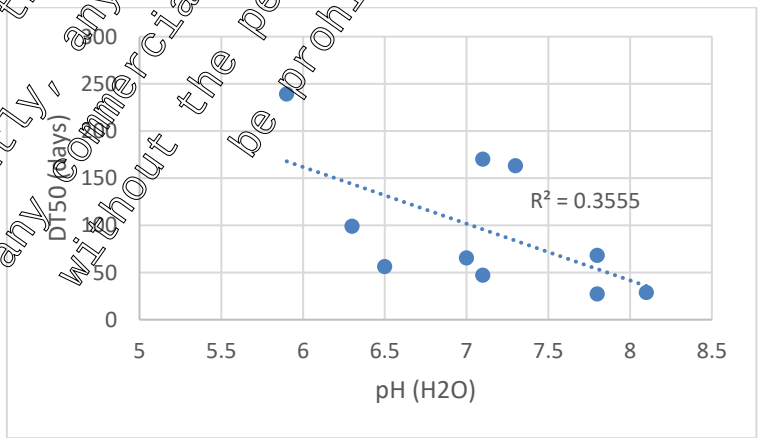
Figure 7.1-1: Aerobic soil degradation pathway for Spiroxamine



The spiroxamine data from all previous and new studies were considered of sufficient quality to evaluate degradation kinetics and the kinetic evaluation was conducted in accordance to FOCUS (2014) and normalised using a Q_{10} of 2.58 (EFSA 2007). The spiroxamine persistence DT_{50} values ranged from 7.2 to 142 days and DT_{90} values ranged from 80.9 to 696 days (KCA 7.1.2.1.1/09 (M-763139-01-1)). The persistence followed biphasic kinetics and the worst case DT_{50}/DT_{90} was subsequently used to assess spiroxamine accumulation. The geometric mean of the modelling DT_{50} values was calculated to be 75.4 days, with the acceptable model fits demonstrating biphasic behaviour. The outcome of the chiral analysis of spiroxamine degradation is ongoing at time of submission and will be provided, along with a definition of any Uncertainty Factor (UF) in Doc N5, at a later date.

An assessment of pH dependency of spiroxamine degradation in the laboratory is presented below:

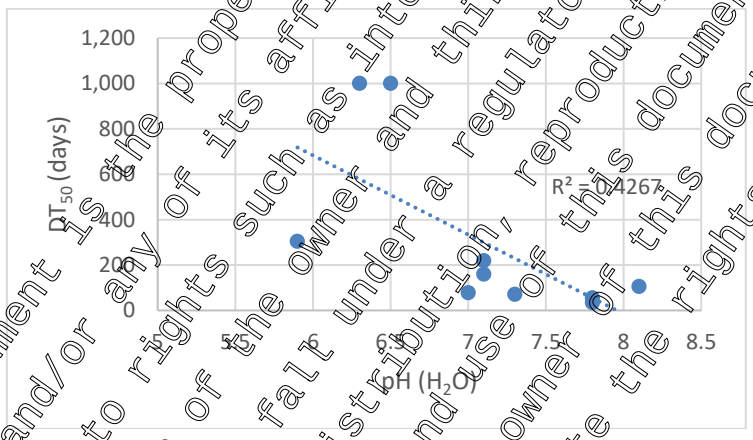
Figure 7.1-2: Evaluation of pH dependency for spiroxamine



As shown, a potential weak relationship between soil DT_{50} and pH was observed ($R^2 = 0.356$), but evaluation using the German decision input tree did not conclude that the data demonstrated a statistically significant pH dependence and recommended the use of the geomean DT_{50} value in modelling estimates. As such, no pH dependency has been included in the risk assessment.

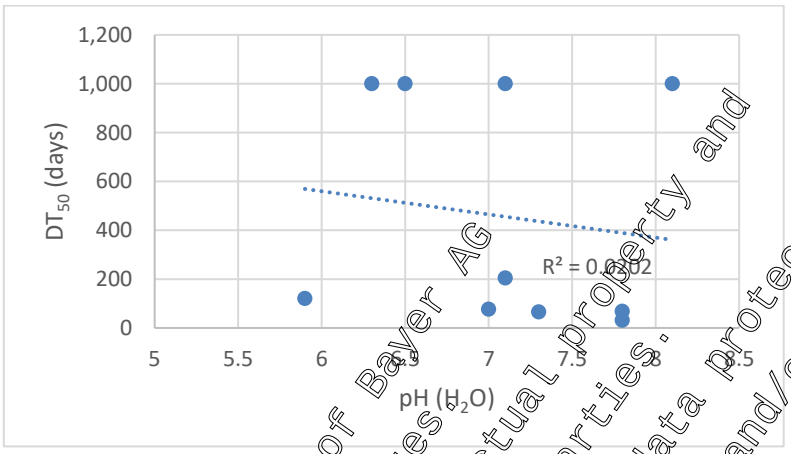
The DT_{50} values for M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl), M03 (spiroxamine-N-oxide) and M06 (spiroxamine-acid) have also been determined from these parent studies where the metabolite was observed at levels sufficient for a kinetic evaluation (KCA 7.1.2.1.1/09 (M-763139-01-1)). M01 (spiroxamine-desethyl) persistence DT_{50} values ranged from 28.9 to 555 days and DT_{90} value ranged from 95.8 to >1,000 days. The observation of $DT_{50persistence}$ endpoints for M01 of >60 days triggers a requirement to investigate the degradation in the field. The geometric mean of the modelling DT_{50} values was found to be 160 days, with the arithmetic mean of the formation fractions from parent determined as 0.183. Evaluation of pH dependency was impacted by having only 5 acidic soils of which 2 evaluations resulted in default endpoints of 1000 days. As such pH dependency was not established and modelling endpoints, established from the geomean of the aerobic lab studies, will be used for PEC calculations.

Figure 7.1-3: Evaluation of pH dependency for M01



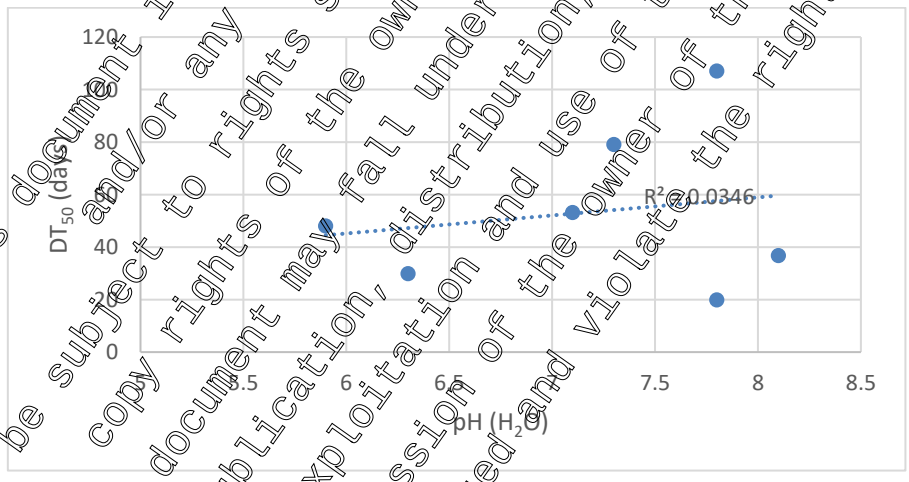
The M02 persistence DT_{50} values ranged from 26.6 to 1,000 days (FOCUS default) and DT_{90} values ranged from 88.2 to 3,320 days (FOCUS default). A number of soils did not have acceptable fits for M02 which resulted in the assignment of the FOCUS default of 1,000 days as the persistence endpoint as a conservative approach (KCA 7.1.2.1.1/09 (M-763139-01-1)). Once again, the observation of $DT_{50persistence}$ endpoints for M02 >60 days triggers a requirement to investigate degradation in the field. The geometric mean of the modelling DT_{50} values was determined to be 219.1 days with the arithmetic mean of the formation fractions from parent calculated as 0.139. No evidence of pH dependence was observed and, as such, modelling endpoints established from the geomean of the aerobic lab studies will be used for all PEC calculations.

Figure 7.1-4: Evaluation of pH dependency for M02



The M03 persistence DT_{50} values ranged from 16.7 to 107 days and DT_{90} values ranged from 55.4 to 358 days. All study data included a clear decline phase allowing for an accurate determination of persistence DT_{50}, with only two soils yielding $DT_{50persistence} > 60$ days. As such, the requirement for a field study on M03 is triggered, although the risk assessment has been finalised considering only lab data. The geometric mean of the modelling DT_{50} values was 46.4 days and the arithmetic mean of the formation fractions from parent was 0.149. No evidence of pH dependence was observed and modelling endpoints established from the geomean of the aerobic lab studies will be used for all PEC calculations.

Figure 7.1-5: Evaluation of pH dependency for M03



Acceptable fitting could only be performed for persistence endpoints in one soil for M06 resulting in the assignment of a number of default values. The persistence DT_{50} values, therefore, ranged 49.6 days to 1,000 days whilst DT_{90} values was 165 days to 3,320 days. The geometric mean of modelling DT_{50} values was calculated at 479.6days. The formation fraction from parent was 0.0947. A specific rate study to define the appropriate DT_{50} for M06 is currently ongoing but for this assessment, the conservative M06 endpoints identified in KCA 7.1.2.1.1/09 (M-763139-01-1) are used to provide an initial investigation into M06. This will be refined upon completion of the M06 rate study and assessment of the data by FOCUS kinetics (2014). The current data package did not adequately define whether a field study was required for M06 as a reliable fit could only be established for a single soil. No evaluation of pH dependency could be conducted.

In conclusion, the large number of studies performed on spiroxamine allows for a clear definition of the degradative behaviour of spiroxamine for the purposes of conducting a risk assessment. Spiroxamine consistently follows biphasic kinetics resulting in a number of studies giving DT_{50} persistence values

>60 days and as such the requirement to assess spiroxamine degradation in the field is triggered. Modelling endpoints for spiroxamine, M01, M02, M03 and M06 were successfully established for the purposes of conducting a risk assessment, with no pH dependency observed and the endpoints based on the geomean of all data points. Field studies are triggered for M01, M02, M03 and potentially for M06. In order to provide further information on the degradation of M06, a laboratory study on the rate of degradation of M06 (spiroxamine-acid) is currently in progress to allow a further refinement of the degradative behaviour for inclusion in the risk assessment:

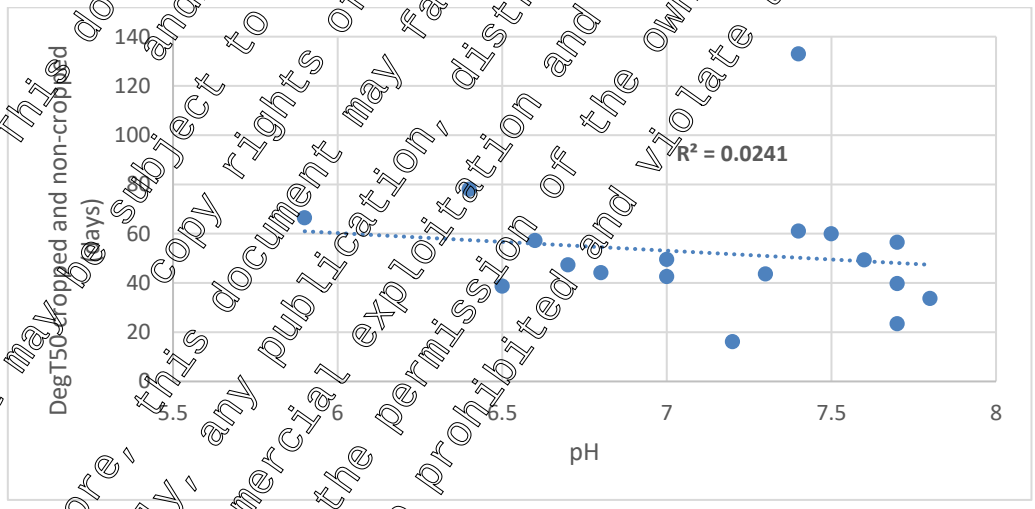
- Rate of degradation of M06 (spiroxamine-acid) in 3 soils.

Nevertheless, the large number of studies on spiroxamine fully define the route of spiroxamine degradation under aerobic conditions in soil and this pathway is presented in Figure 7.1-1.

In order to address potential spiroxamine persistence, a number of field dissipation studies, which have previously been reviewed and accepted, have been further considered for an assessment of persistence and derivation of modelling endpoints of spiroxamine, M01 and M02. No new field studies have been performed. In total, 18 valid field dissipation trials were conducted across 5 studies (KCA 7.1.2.2.1/01 (M-006116-01-1); KCA 7.1.2.2.1/02 (M-006126-01-1); KCA 7.1.2.2.1/03 (M-006127-01-1); KCA 7.1.2.2.1/04 (M-006128-01-1) & KCA 7.1.2.2.1/05 (M-006129-01-1)) conducted in representative agricultural fields across the EU. These dissipation trials followed the "legacy" design and as such were either applied to bare soil (without exclusion of surface processes) or applied to cropped plots. For an assessment of persistence, a kinetic analysis to determine DT_{50} and DT_{90} values for comparison with relevant study triggers and persistence criteria was performed using non-normalised data, in accordance with the flowcharts for persistence/trigger endpoints provided by FOCUS (2014). The spiroxamine persistence/trigger DT_{50} values ranged from 0.5 to 59.6 days and DT_{90} values ranged from 43.5 to 433 days. The modelled field persistence DT_{50} values were all <60 days and as such spiroxamine was classified as non-persistent.

An evaluation of pH dependency of spiroxamine degradation in the field is presented below:

Figure 7.1-6: Evaluation of potential pH dependency of spiroxamine field degradation



As outlined in the previous evaluation, no pH dependency was noted for the field degradation of spiroxamine, and as such a geomean $DegT_{50,matrix}$ was applied in the risk assessment.

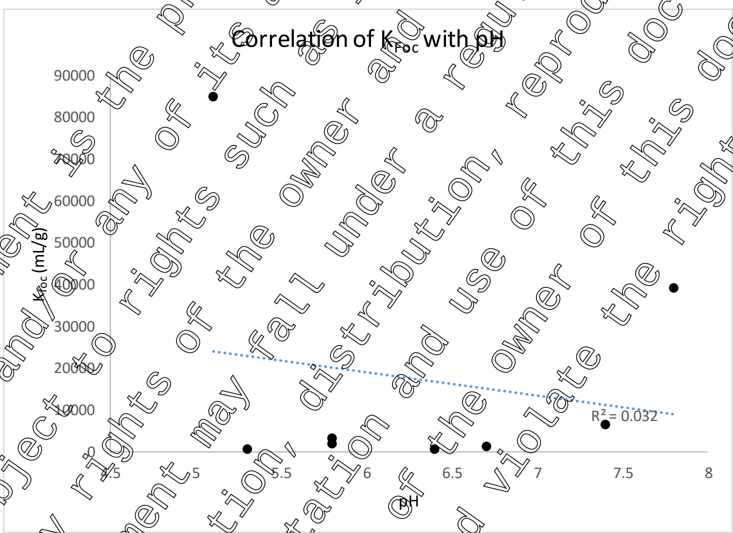
M01 persistence/trigger DT_{50} values ranged from 17.8 to 223 days and DT_{90} values ranged from 59 to 742 days whilst M02 persistence/trigger DT_{50} values ranged from 21 to 161 days and DT_{90} values ranged from 69.6 to 533 days. Overall, both metabolites were considered persistent in soil.

To determine $DegT_{50,\ matrix}$ values for spiroxamine, a kinetic assessment was performed following the flowcharts for calculating modelling endpoints provided by EFSA (2014), and using data that have been

normalised to reference conditions (20°C and pF 2 soil moisture content) and subject to time step normalisation procedures. These endpoints were used for the selection of an appropriate modelling value for use with regulatory Predicted Environmental Concentration (PEC) models. Modelling $DegT_{50.matrix}$ values (at 20°C and pF 2) ranged from 23.4 to 78 days, with a geometric mean of 43.8 days. Overall, the rate of degradation of spiroxamine in the field appeared faster than degradation in the lab studies, and this was confirmed through an evaluation using the EFSA $DegT_{50.matrix}$ endpoint selector resulting in the selection of the $DegT_{50.matrix}$ endpoint as the appropriate modelling endpoint for groundwater and surface water for spiroxamine.

The mobility in soil of spiroxamine and its degradation products relevant for assessment was studied by batch equilibrium tests on a variety of different soils. The adsorption and desorption of spiroxamine has been investigated in two studies (KCA 7.1.3.1.1/01 and KCA 7.1.3.1.1/02) which were evaluated during the previous EU review. Following a review of the study reliability using the EFSA 106 checklist v2.0, it can be concluded that the adsorption endpoints from these two batch equilibrium studies are reliable and appropriate for use in risk assessment. The mobility of spiroxamine was investigated in a total of ten soils of which two were excluded from derivation of the geomean as a consequence of the organic carbon content being below the guideline requirements. Spiroxamine K_{foc} values ranged from 85,008 to 659 ml/g with no evidence of a relationship with soil pH.

Figure 7.1-7: pH dependent sorption of Spiroxamine to soil



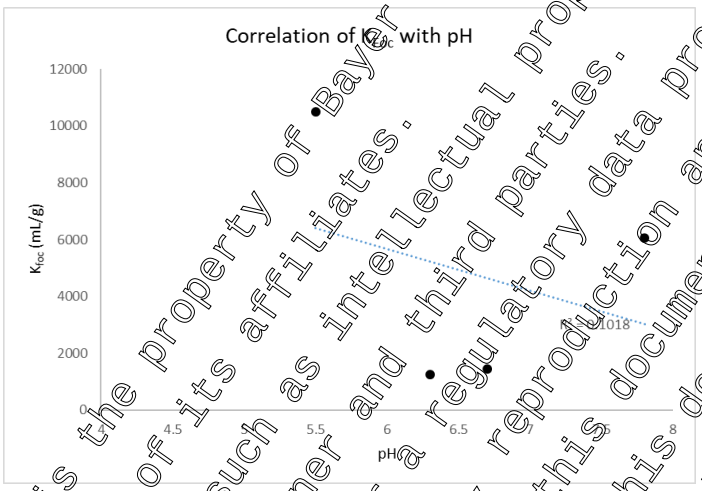
There was no significant correlation between soil sorption parameter K_{foc} with soil pH for parent spiroxamine (R^2 =0.032), therefore no pH dependence was concluded. The study data indicated that the two clay soils gave very high sorption estimates indicating that the binding of spiroxamine to clays was significant, potentially mediated via binding to charged sites on the clay. Sorption to non-clay soils yielded K_{foc} values ranging from 659 to 6417 mL/g and these interactions were considered to be mediated via soil organic matter. Considering the importance of the two sorption mechanisms, an overall geomean of the data has been used for modelling purposes to reflect both aspects of spiroxamine sorption behaviour. Overall, spiroxamine exhibits low mobility in soil according to the McCall mobility classification. No information on the individual sportive behaviour of the spiroxamine isomers was determined.

The adsorption and desorption of metabolites of spiroxamine have been investigated in three studies (KCA 7.1.3.1.2/01 to KCA 7.1.3.1.1/03) which were evaluated during the previous EU review. Sorption of M01, M02 and M03 was measured in four soil types and the outcome of the studies reviewed for reliability using the EFSA 106 checklist v2.0. It was concluded that despite some minor deviations the outcome of the adsorption/desorption studies were reliable and suitable for use in risk assessment. Some differences between the calculated Kfoc values and those reported were noted, but this was attributed to

the use of averages in the original calculations. Since differences were minor, for consistency the reported values from the report were used in the risk assessment.

Adsorption of M01 was investigated in 4 different soils with K_{foc} ranging from 1237 to 10511 L/kg (KCA 7.1.3.1.2/01 (M-006084-01-1)). Adsorption was shown to be generally correlated with organic carbon content, and M01 (spiroxamine-desethyl) exhibits medium to immobile mobility in soil according to the McCall mobility classification. As part of the risk assessment, potential relationships between pH and sorption were investigated:

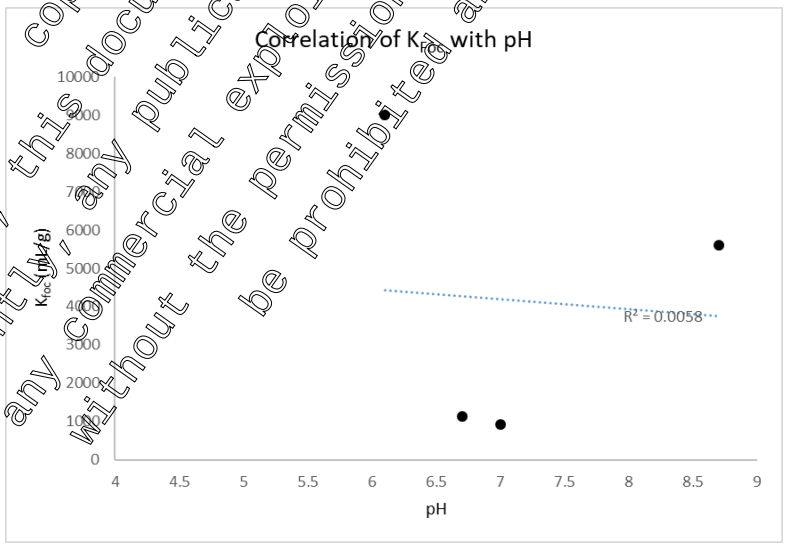
Figure 7.1-8: Evaluation of pH dependent sorption for M01 (spiroxamine-desethyl)



No significant correlation was observed between soil sorption parameter K_{foc} with soil pH for M01 (spiroxamine-desethyl) (R^2=0.102); therefore no pH dependence was concluded. The geomean of the four soils was considered appropriate for use in the risk assessment as previously concluded (EFSA Journal (2010);8(10):1719)).

Adsorption of M02 was investigated in 4 different soils with K_{foc} ranging from 916.7 to 8993.6 L/Kg (KCA 7.1.3.1.2/02 (M-006086-01-1)). Adsorption was shown to be generally correlated with organic carbon content, and M02 (spiroxamine-despropyl) exhibits medium to immobile mobility in soil according to the McCall mobility classification. As part of the risk assessment, potential pH dependency was investigated:

Figure 7.1-9: Evaluation of pH dependent sorption of M02 (spiroxamine-despropyl)

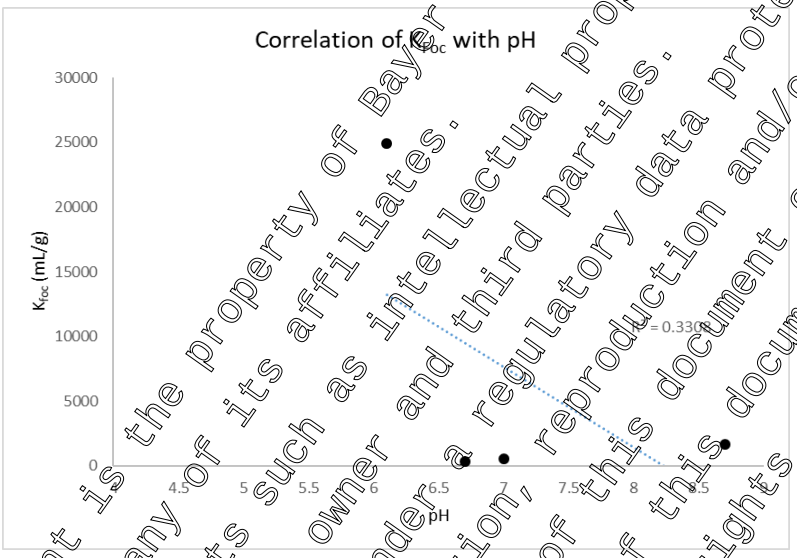


No significant correlation between soil sorption parameter K_{foc} with soil pH for M02 (spiroxamine-

despropyl) was observed (R^2=0.005), therefore no pH dependence was concluded. The geomean of the four soils was thus considered appropriate for use in the risk assessment as previously concluded (EFSA Journal (2010);8(10):1719)).

Adsorption of M03 was also determined in 4 different soils, with the K_{foc} ranging from 350.5 to 24892.5 L/Kg (KCA 7.1.3.1.2/03 (M-006089-01-1)). Adsorption was shown to be generally correlated with organic carbon content and M03 (spiroxamine-N-oxide) exhibits medium to immobile mobility in soil according to the McCall mobility classification.

Figure 7.1-10: Evaluation of pH dependent sorption of M03 (spiroxamine-N-oxide)



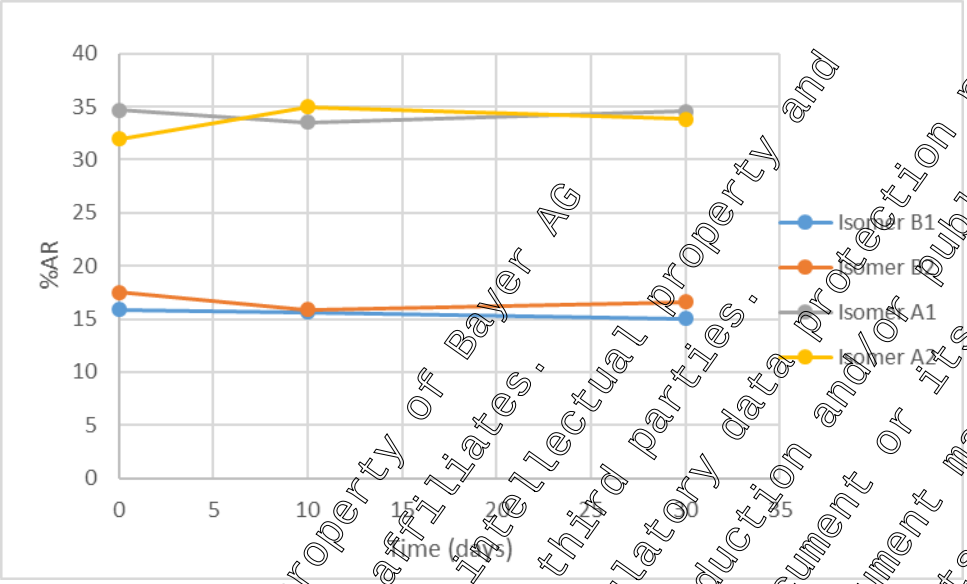
No significant correlation between soil sorption parameter K_{foc} with soil pH for M03 (spiroxamine -N-oxide) was observed (R^2=0.331), therefore no pH dependence was concluded. The geomean of the four soils was thus considered appropriate for use in the risk assessment as previous concluded (EFSA Journal (2010);8(10):1719)).

One further study is being conducted to provide soil sorption properties for metabolite M06 (spiroxamine-acid) and will be provided as soon as available. As such, in order to provide a basis for the risk assessment, an estimated K_{foc} value of 3.2 L/Kg for M06 for use in the preliminary risk assessment was obtained from KocWIN. This will be updated upon completion of the M06 OECD106 study.

The high sorption displayed by spiroxamine and its metabolites is reflected in the outcome of column leaching studies investigating the leaching behaviour of aged residue of spiroxamine in soil. These studies demonstrated that in soil column studies, aged residues of spiroxamine did not significantly leach to the column percolate with only 0.2 %AR being found in the leachate. The major residue in this leachate was found to be M03 (N-oxide) representing only 0.03% of the applied radioactivity in column leachates. Overall, leaching behaviour of spiroxamine (including individual isomers) or its major soil metabolites is not envisaged.

In addition to consideration of aerobic degradation, the rate and route of degradation of spiroxamine, including potential changes in the stereoisomer composition, was investigated in a single anaerobic soil. Following a period of incubation of 30 days under aerobic conditions, the degradation of spiroxamine proceeded via the aerobic pathway in Figure 7.1-1, with formation of M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl), M03 (spiroxamine-N-oxide) and M06 (spiroxamine-acid) to >3% AR prior to flooding the vessels. During the anaerobic (flooded) phase, almost no further degradation of spiroxamine or the metabolites occurred, although levels of M06 (spiroxamine-acid) increased slightly from 4.7 to 9.9% AR. Analysis of spiroxamine using chiral methods also demonstrated that, under anaerobic conditions, the relative proportions of each isomer did not change significantly during either the aerobic or anaerobic phases (see Figure 7.1-11 and 12).

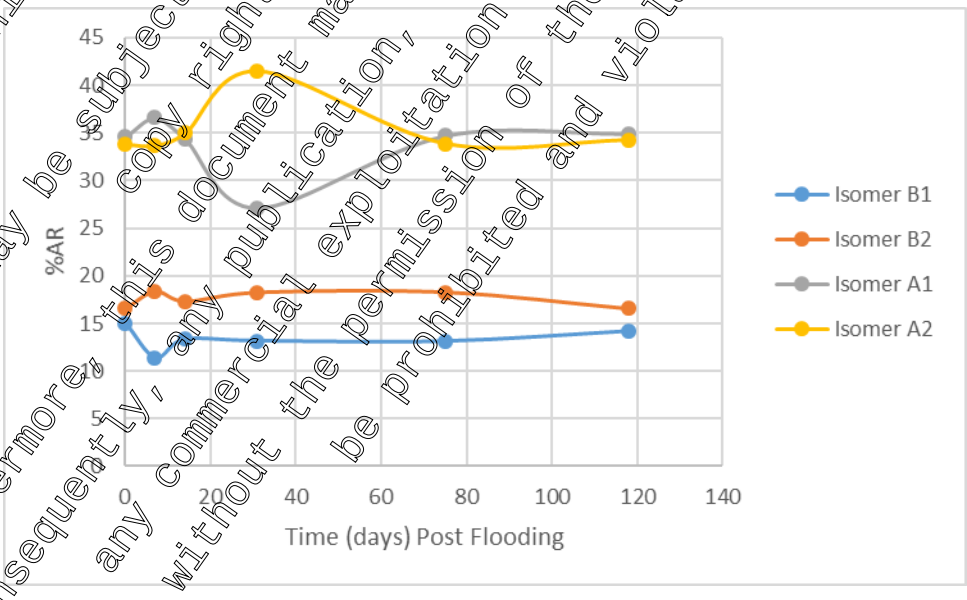
Figure 7.1-11: Evaluation of relative composition of spiroxamine isomer following 30 days aerobic incubation phase (KCA 7.1.1.2/02 (M-762348-01-1))



From the chiral analysis of the aerobic phase, no evidence of a change in stereoisomer ratio was observed indicating that for the 30 days aerobic incubation, isomer specific degradation or interconversion was not observed. As outlined, the study data from KCA 7.1.1.1/06 (M-762349-01-1) is being analysed using chiral techniques to provide further information on potential changes in spiroxamine isomer ratios and to define whether an Isomer Uncertainty Factor needs to be applied.

Considering the anaerobic (flooded) phase, no significant changes in stereoisomer composition were noted between the start and end of the flooded phase. As such, no isomer UF has been considered on the basis of this analysis.

Figure 7.1-12: Evaluation of relative composition of spiroxamine isomer following 120 days anaerobic incubation phase (KCA 7.1.1.2/02 (M-762348-01-1))



It was noted that a single time point (31 Days Post Flooding) demonstrated a significant change in %se for isomers A1 and A2, but this was not considered sufficient justification of an UF (EFSA, 2019). However, it is not clear that the change at 31 DPF is reliable, and that the performance of the method performance is essential in order to identify, reliably, the relatively small changes needed to trigger a

significant %**se**. As such, the analytical methodology is being revised for some studies and this has delayed the submission of this data. The data will be submitted at top-up or as soon as it is available.

Finally, a soil photolysis study is presented, which confirms that soil photodegradation is not a significant process for spiroxamine with an estimated photolytic DT_{50} of approximately 119 days (KCA 7.1.1.3/01 (M-006150-01-1)). During the conduct of the study, M01 (spiroxamine-desethyl) and M02 (spiroxamine-despropyl) were identified as relevant metabolites as they are being observed >5% at two consecutive time points with the maximum formation being 9.1 and 6.1% AR respectively. In addition, M03 (spiroxamine-N-oxide) was observed at 6.2% AR but not >5% at two consecutive time points whilst M15 (spiroxamine-ketone) was observed at 2.7% AR. Other minor metabolites, including M05, were observed but not > 1.7% AR. Considering the DT_{50} for photolysis, it is likely that a combination of this slow rate with movement from the soil surface renders the process of photolysis as insignificant, with aerobic degradation the major soil degradation mechanism.

CA 7.1.1 Route of degradation in soil

Use of plant protection products containing the active substance spiroxamine will potentially result in contact with soil, therefore the route of degradation in soil of the active substance under aerobic, anaerobic and sunlight conditions is considered under Points CA 7.1.1.1 (aerobic), CA 7.1.1.2 (anaerobic) and CA 7.1.1.3 (soil photolysis) in laboratory studies according to the data requirements laid down in Commission Regulation (EU) No 283/2013.

In accordance with the data requirements defined by EC Regulation 283/2013, for soil degradation studies included under Point CA 7.1.1, metabolites are considered major if they exceed 10% AR at a single timepoint or exceed 5% AR on two consecutive sampling intervals or exceed 5% AR and are rising at the end of the study. If metabolites do meet any of these criteria, they are considered minor and are not included as part of the definition of residue for that compartment.

Overview:

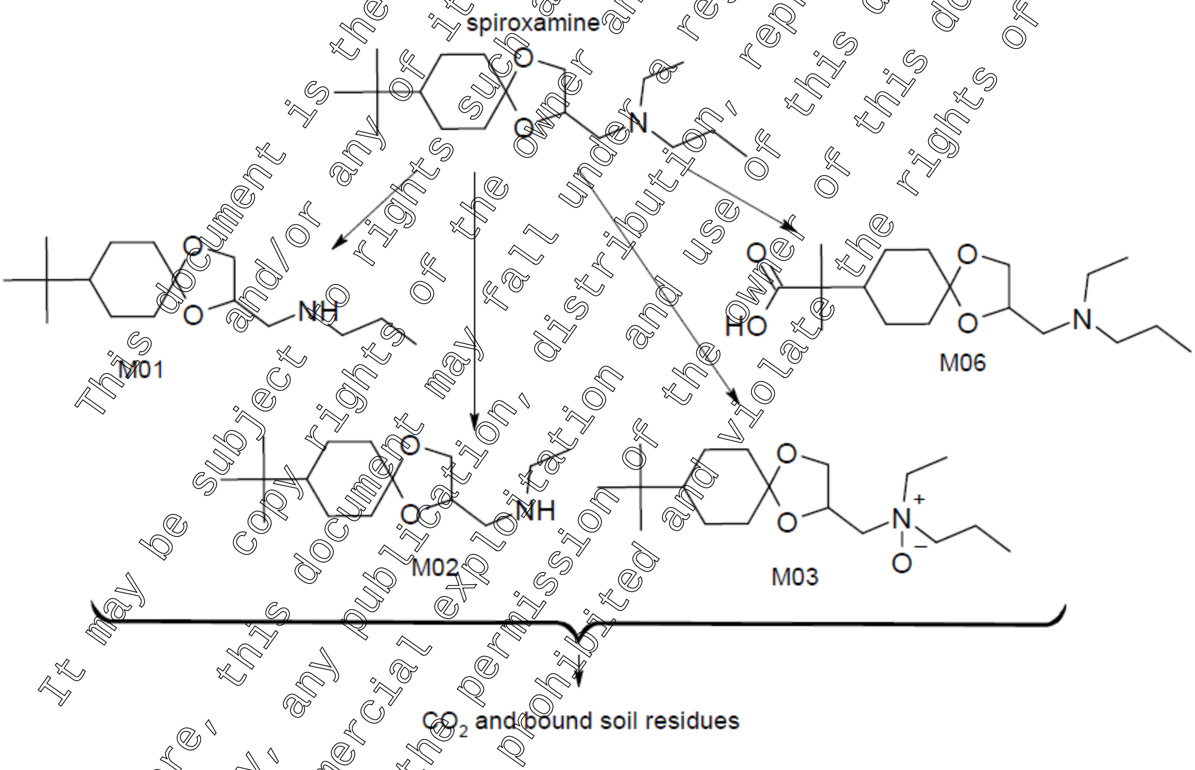
The route of spiroxamine degradation in soil has been assessed for aerobic degradation in six studies of which 5 were previously evaluated in the last EU review. A single new study has been conducted to address the new data requirements (EFSA, 2019). In aerobic soil, spiroxamine degraded ultimately to CO_2 and bound residue with the formation of a number of metabolites; M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl), M03 (spiroxamine-N-oxide) and M06 (spiroxamine-acid) present as major metabolites. The major soil metabolite was identified M01 (spiroxamine-desethyl) and was seen at a maximum formation of 12% AR in the Longwoods soil, and levels >5% AR in all other soils. Likewise, M02 (spiroxamine-despropyl) was also observed in all soils >5% with a maximum observed value of 9.2%AR in the Longwoods soil. M03 (spiroxamine-N-oxide) was consistently observed in all four soils but was only found >5% AR (seen at two consecutive timepoints) in the Longwoods soil with a maximum observation of 7.2% AR. However, M06 (spiroxamine-acid), previously observed but always at low levels, was observed at >5%AR at the final timepoint in the Refesol-02A soil, triggering further evaluation and risk assessment. No other metabolite was identified at >5%AR.

The active substance spiroxamine contains two stereogenic centres and therefore the new aerobic soil degradation study was established to include an investigation in accordance with the requirements of EFSA 2019. The active substance spiroxamine and the associated environmental metabolites each have 4 possible isomers (except for metabolite M03 (spiroxamine-N-oxide) which has three stereogenic centres and hence eight possible isomers). To simplify the analytical challenge associated with separation of so many components, the approach adopted in the new study was to quantify separate isomers only for parent spiroxamine and to address any possible change in isomeric composition of metabolites by incorporating a worst-case UF (Uncertainty Factor) in the environmental risk assessment. The outcome of the chiral analysis of spiroxamine degradation is ongoing at time of submission and will be provided, along with a definition of the UF in Doc N5, in a top up submission. The achiral analysis in this study has been completed and the interim study report (KCA 7.1.1.1/06 (M-762349-01-1)) presents the outcome of this analysis and the achiral study data has been considered in this evaluation.

In addition to consideration of aerobic degradation, the rate and route of degradation of spiroxamine, and potential changes in the stereoisomer composition, was investigated in a single anaerobic soil (KCA 7.1.1.2/02 (M-762348-01-1)). Following a period of incubation of 30 days under aerobic conditions, the metabolic profile observed was similar to that previously reported in the aerobic studies (with formation of M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl), M03 (spiroxamine-N-oxide) and M06 (spiroxamine-acid) to >3% AR). During the anaerobic phase, almost no further degradation of spiroxamine or the metabolites occurred, although levels of M06 (spiroxamine-acid) increased from 4.7 to 9.9% AR. Analysis of spiroxamine using chiral methods also demonstrated that under anaerobic conditions the relative proportions of each isomer did not change significantly.

Further, a soil photolysis study is presented to confirm that soil photodegradation is not considered significant for spiroxamine with estimated photolytic DT_{50}s of approximately 119 days (KCA 7.1.1.3/01 (M-006150-01-1)). During the conduct of the study, M01 (spiroxamine-desethyl) and M02 (spiroxamine-despropyl) were identified as relevant metabolites being observed at >5% on two consecutive timepoints with the maximum formation being 9.1 and 6.1% AR respectively. In addition, M03 (spiroxamine-N-oxide) was observed at 6.2% AR but not >5% at two consecutive timepoints. M15 (spiroxamine-ketone) was observed at 2.7% AR and other minor metabolites, including M05, were also observed but not > 1.7% AR. On the basis of this study, soil photolysis was not considered a significant degradation pathway for spiroxamine.

Figure 7.1.1-1: Proposed biotransformation pathway in soil



CA 7.1.1.1 Aerobic degradation

In view of the proposed use pattern for spiroxamine, as a post-emergence spray applied fungicide for application to cereals crops and vines, potential exposure to aerobic conditions in soil is envisaged. The degradation of spiroxamine in aerobic soil has been investigated in five studies (KCA 7.1.1.1/01 to KCA 7.1.1.1/05) all of which were evaluated during the previous EU review. In addition, one new study has

been conducted, mainly to address the new requirements of EFSA, 2019[1].

Substance	Report reference	Document no.	Test material used	Comment
Spiroxamine	KCA 7.1.1.1/01; KCA 7.1.2.1.1/01	M-006135-01-1	[cyclohexyl-1-^{14}C]-spiroxamine	Submitted for first approval of spiroxamine, 1999. Reviewed under UP. Considered valid and acceptable.
Spiroxamine	KCA 7.1.1.1/02; KCA 7.1.2.1.1/02	M-006141-01-1	[cyclohexyl-1-^{14}C]-spiroxamine	
Spiroxamine	KCA 7.1.1.1/03; KCA 7.1.2.1.1/03	M-006096-01-1	n.a.	
Spiroxamine	KCA 7.1.1.1/04; KCA 7.1.2.1.1/04	M-006148-01-1	[cyclohexyl-1-^{14}C]-spiroxamine	Submitted for first renewal of spiroxamine, 2010. Reviewed under UP. Considered valid and acceptable.
Spiroxamine	KCA 7.1.1.1/05; KCA 7.1.2.1.1/05	M-303803-01-1	[1,3-dioxolane-4-^{14}C]-spiroxamine	
Spiroxamine	KCA 7.1.1.1/06; KCA 7.1.2.1.1/08	M-762349-01-1	[cyclohexyl-1-^{14}C]-spiroxamine	New data not yet reviewed under UP.

n.a. not applicable

The aerobic degradation of spiroxamine in soil has been investigated in a total of six studies at 20°C. Some supplemental studies are additionally provided under Point KCA 7.1.2.1.1 but these studies do not indicate the formation of any further major metabolites. Previously evaluated studies have been conducted using both [cyclohexyl-1-^{14}C]-spiroxamine and [1,3-dioxolane-4-^{14}C]-spiroxamine and show a consistent degradation pathway throughout the studies and very little to no separation of the two possible radiolabelling positions. Therefore, the new aerobic soil degradation study was conducted using [cyclohexyl-1-^{14}C]-spiroxamine only.

A number of minor deviations are reported for the investigation of bound residue in the studies evaluated previously, but considering the extensive extraction schemes employed in KCA 7.1.1.1/05 (M-303803-01-1) and KCA 7.1.1.1/06 (M-762349-01-1) these minor deviations are considered insignificant for the understanding of the behaviour of spiroxamine in soil. The metabolic profile of spiroxamine degradation in KCA 7.1.1.1/06 (M-762349-01-1) validates the observations of the previous studies, with the confirmation of the major metabolites as M01 (desthexyl-spiroxamine), M02 (despropyl-spiroxamine) and M03 (spiroxamine-N-oxide) confirmed, and the additional recognition of M06 (spiroxamine-acid) as a major metabolite as a consequence of a single soil having M06 >5% at the final timepoint. Overall, it can be considered that the studies provided outline the rate and route of spiroxamine degradation in aerobic soils.

The active substance spiroxamine contains two stereogenic centres and the new aerobic soil degradation study was set up to include an investigation in accordance with the requirements of EFSA 2019[1]. The active substance spiroxamine and the associated environmental metabolites each have 4 possible isomers (except for metabolite M03 (spiroxamine-N-oxide) which was three stereogenic centres and hence eight possible isomers). To simplify the analytical challenge associated with separation of so many components, the approach adopted in the new study was to quantify separate isomers only for parent spiroxamine and to address any possible change in isomeric composition of metabolites in environmental residues by incorporating a worst-case UF (Uncertainty Factor) in the relevant environmental risk assessments. The outcome of the chiral analysis of spiroxamine degradation is ongoing at time of submission and will be provided, along with a definition of the UF in Doc N5, as part of a top up submissiont at a later date. The achiral analysis has been completed and as such the interim study report (KCA 7.1.1.1/06

[1] EFSA (2019). Guidance of EFSA on risk assessments for active substances of plant protection products that have stereoisomers as components or impurities and for transformation products of active substances that may have stereoisomers. EFSA Journal 2019;17(8):5804, 33 pp.

([M-762349-01-1]) presents the outcome of this analysis and the data has been considered in this evaluation.

New studies, not previously evaluated

Data Point:	KCA 7.1.1.1/06
Report Author:	[redacted]; [redacted]
Report Year:	2020
Report Title:	[14C]-spiroxamine: Route and rate of degradation in four soils under aerobic conditions at 20°C - Interim report
Report No:	VC/19/055
Document No:	M-762349-01-1
Guideline(s) followed in study:	(EU) No. 283/2013(EC) no. 1107/2009 OECD 307 (2002)
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The route and rate of degradation of spiroxamine was investigated in four European soils (Longwoods, sandy loam; Refesol-02-A, silt loam; Refesol-03-G, clay loam; Speyer 6S, clay) under laboratory aerobic conditions. Soil samples (100 g dw^{-1}) were treated with [cyclohexyl-1-^{14}C]-spiroxamine (radiochemical purity 99.6%) at a nominal application rate of 2.0 mg/kg soil dry weight (reported as equivalent to 750 g/ha assuming a mixing depth of 2.5 cm and soil density of 1.5 g/cm^3, otherwise equivalent to 1500 g/ha assuming a mixing depth of 5 cm) and incubated (20°C, pF 2) for 120 days in the dark.

Duplicate treated samples of each soil were removed for analysis after 0, 3, 7, 14, 30, 44, 59, 80, 97 and 120 days of incubation. Soil samples were extracted four times at room temperature with acetonitrile/water/35% ammonia solution 80/20/1 v/v/v, by addition of solvent, vigorous mechanical shaking and centrifugation. Soil extracts were analysed, without concentration, primarily by reverse phase HPLC. Confirmatory analysis was conducted by normal-phase TLC on selected samples. Degradation products were also confirmed using LC-MS on selected samples.

The material balances ranged from 99.2 to 105.1% of applied radioactivity (% AR) indicating a complete mass balance. The amounts of unextractable radioactivity increased to a maximum 16.9% AR by DAT 120. Mineralisation to carbon dioxide was a major pathway, steadily increasing throughout the study to a maximum of 16.3 to 69.9% AR by DAT 120. No significant levels of organic volatiles were observed.

Following application of [cyclohexyl-1-^{14}C]-spiroxamine, the amount of parent in the soil extracts of the Longwoods soil decreased from 101.2% AR at DAT 0 to 5.8% AR by the end of the study at DAT 120. The amount of parent in the soil extracts of the Refesol-02-A, Refesol-03-G and Speyer 6S soils decreased from 102.2, 95.4 and 99.1% AR at DAT 0 to 50.6, 55.1 and 56.0% AR respectively by the end of the study at DAT 120. DT_{50} values for the degradation of spiroxamine were calculated in the report, however a re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (FOCUS 2014), was performed in the report presented under point KCA 7.1.2.1.1/09 ([M-763139-01-1]).

Degradation of [cyclohexyl-1-^{14}C]-spiroxamine was accompanied by the formation of 4 major metabolites: M01 (spiroxamine-desethyl, maximum 12.0% AR), M02 (spiroxamine-despropyl, maximum 9.2% AR), M03 (spiroxamine-N-oxide, maximum 7.2% AR) and M06 (spiroxamine-acid, maximum 5.3% AR). Some other metabolites were detected typically <1% AR but all <5% AR.

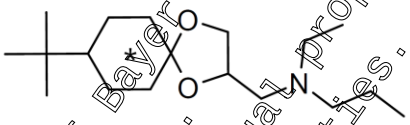
**1 Additional work is currently being conducted on the chiral analysis of the samples for parent spiroxamine. This work will be submitted in the final report for the study (currently report included is an interim report) and will be supplied as part of a top up submission (estimate August 2021).

I. Materials and Methods

A. Materials

1. Test Items

[cyclohexyl-1-^{14}C]-spiroxamine



* Denotes position of [^{14}C]-radiolabel

Specific Activity: 4.26 MBq/mg

Radiochemical Purity: 99.6% (HPLC)

2. Test System (soil)

The study was performed using four test soils as characterised in Table CA 7.1.1.1-1.

Table CA 7.1.1.1-1: Physico-chemical properties of test soil

Parameter	Soil			
Soil Designation:	Longwoods	Refesol-02-A	Refesol-03-G	Speyer 6S
Geographic Location				
City	Longwoods quarry, Lincs. [B]	Northrhine, Westphalia [B]	Northrhine, Westphalia [B]	Sieb-eldingen, Rheinland-Pfalz [B]
Country	UK	Germany	Germany	Germany
Textural Classification (USDA)	Sandy loam	Silt loam	Clay loam	Clay
Sand [50 - 2000 µm] (%)	50	16	30	18
Silt [2 – 50 µm] (%)	38	66	40	28
Clay [< 2 µm] (%)	12	18	30	54
pH				
in H_2O (1:1)	7.8	7.1	5.9	7.3
in 0.01M $CaCl_2$ (1:1)	7.5	6.7	5.6	7.1
Organic Matter (%)*	2.4	1.7	7.6	2.8
Organic Carbon (%)	1.4	1.0	4.4	1.6
Cation Exchange Capacity (meq/100 g)	11.3	11.4	12.7	19.8
Water Holding Capacity (g H_2O per 100g dry soil)				
pF 2.0 (0.1 bar, w/w %)	20.0	35.9	41.1	30.7
pF 2.5 (0.33 bar, w/w %)	11.0	19.9	30.0	25.7

Parameter	Soil			
Soil Designation:	Longwoods	Refesol-02-A	Refesol-03-G	Speyer 6S
Soil Microbial Biomass (mg org C/100 g soil) [A]				
Initial, DAT 0	31.8 (2.3)	17.3 (1.7)	48.2 (1.1)	32.1 (2.0)
Final, 100 DAT 120	29.2 (2.1)	12.4 (1.2)	35.2 (0.8)	23.3 (1.5)

* Calculated by multiplying organic carbon content by 1.724 (not reported)
A Biomass as a percentage of organic carbon content provide in parentheses
B Location known free of pesticide use for ≥3 years and used within 3 months from sampling.

The test soils were handled in accordance with ISO 18400-102 and 105 prior to use.

B. Study Design

1. Experimental Conditions

Tests were performed in flow through systems consisting of glass flasks each containing 100 g soil and attached to an ethylene glycol trap to collect organic volatiles followed by two potassium hydroxide traps (2M) to collect carbon dioxide.

Soil samples (100 g) (prepared within 3 months of sampling) were pre-acclimatised to the incubation conditions (dark, 20°C and pF 2 moisture content) for 5-6 days before application of the test substance. The study was conducted at a concentration of 2.0 mg/kg dry weight of soil (reported as equivalent to 750 g/ha assuming a mixing depth of 2.5 cm and soil density of 1.5 g/cm^3, otherwise equivalent to 1500 g/ha assuming a mixing depth of 5 cm). Application of [cyclohexyl-1-^{14}C]-spiroxamine in a solvent (acetonitrile, <170 µL) was made to the soil surface. Soil samples were lightly agitated after application to aid distribution throughout the soil and to allow solvent evaporation. Soil moisture was maintained by addition of water (every *ca.* 3 weeks).

Additional samples for each soil were treated with an equivalent amount of blank solvent only to monitor microbial activity at the beginning and end of the incubation period.

2. Sampling

Duplicate samples for each soil were removed for analysis after 0, 3, 7, 14, 30, 44, 59, 80, 97 and 120 days of incubation. Untreated samples were analysed for biomass at the beginning and end of the experiment.

3. Analytical Procedures

Soil samples were extracted four times at room temperature with acetonitrile/water/35% ammonia solution 80/20/1 v/v/v, by addition of solvent, vigorous mechanical shaking and centrifugation. Radioactivity in extracts was determined by liquid scintillation counting (LSC). Soil extracts were analysed (without concentration) by HPLC with radio-detection. Degradation products were identified by comparison of the retention times of reference standards. Confirmatory analysis using an alternative technique was conducted by TLC with co-chromatography against reference items on selected samples. Degradation products were also confirmed using LC-MS on selected samples.

Volatile radioactivity in volatile traps was quantified by LSC. Carbon dioxide in the potassium hydroxide traps was confirmed by barium carbonate precipitation.

Following homogenisation, non-extractable residues (NER) in extracted soils were determined by combustion.

Additionally, soil extracts were separately analysed using a chiral HPLC method. This work is on-going and will be submitted in the final report for the study (currently interim) as soon as possible

4. Determination of degradation kinetics

The degradation kinetics of data within this report are subject of a separate modelling study. DT_{50} and DT_{90} values for the degradation of spiroxamine and its metabolites have been re-calculated from the

reported data following the recommendations of the FOCUS work group (FOCUS 2014[2]). Full details are provided in the report under point KCA 7.1.2.1.1/09 (M-763139-01-1).

II. Results and Discussion

A. Data

The distribution and characterisation of radioactivity for the test soils incubated at 20°C following application of [cyclohexyl-1-^{14}C]-spiroxamine are summarised in Table CA 7.1.1.1-2 to Table CA 7.1.1.1-5.

Table CA 7.1.1.1-2: Degradation of [cyclohexyl-1-^{14}C]-spiroxamine at 20°C in Longwoods soil under aerobic conditions [% AR]

Compound	Replicate	Incubation time (DAT)									
		0	3	7	14	30	44	59	80	97	120
Spiroxamine	A	101.8	80.6	65.8	51.6	32.5	22.5	16.7	13.0	6.7	5.5
	B	100.6	82.7	67.6	53.5	28.9	21.5	16.8	10.8	7.5	6.0
	Mean	101.2	81.6	66.7	52.6	30.7	22.0	16.8	11.9	7.1	5.8
M01 (spiroxamine desethyl)	A	1.6	6.1	8.1	11.2	11.2	12.1	9.5	6.4	5.0	3.5
	B	1.1	4.8	8.7	10.6	10.2	11.8	9.7	7.2	4.1	3.4
	Mean	1.3	5.4	8.4	10.9	10.7	12.0	9.6	6.8	4.6	3.4
M02 (spiroxamine despropyl)	A	0.8	4.2	6.6	8.1	10.3	8.2	6.6	5.0	3.6	2.2
	B	0.6	4.3	6.7	8.3	8.2	8.3	7.0	5.0	3.1	2.3
	Mean	0.7	4.3	6.6	8.2	9.2	8.2	6.9	5.0	3.3	2.2
M03 (spiroxamine N-oxide)	A	n.d.	6.0	7.1	7.2	5.6	5.8	4.9	2.8	2.8	1.5
	B	1.2	5.1	6.7	7.2	7.0	5.9	4.1	3.1	1.8	1.5
	Mean	0.6	5.5	6.9	7.2	6.3	5.9	4.5	2.9	2.3	1.5
M06 (spiroxamine acid)	A	n.d.	1.4	1.9	3.2	2.2	2.4	4.2	1.0	1.7	2.0
	B	n.d.	1.3	1.8	3.2	3.8	2.4	3.6	2.1	2.8	1.9
	Mean	n.d.	1.4	1.8	3.2	3.0	2.4	3.9	1.5	2.3	2.0
Total other metabolites	Mean	n.d.	1.7	3.6	1.9	5.4	1.5	0.9	n.d.	n.d.	n.d.
Total extractable radioactivity	Mean	101.8	99.9	94.1	84.0	65.3	51.9	42.6	28.1	19.6	14.9
Non-extractable radioactivity	Mean	0.4	2.7	4.9	7.1	10.2	13.7	14.0	16.1	16.2	16.6
Carbon dioxide	Mean	n.a.	n.d.	4.1	6.6	26.3	37.4	47.1	57.6	66.3	69.9
Other volatiles	Mean	n.a.	0.1	0.3	0.1	0.4	0.1	0.2	0.4	0.3	0.2
Total AR	Mean	104.2	102.6	103.4	97.8	102.3	103.2	103.8	102.2	102.3	101.6

n.a.: not analysed, n.d.: not detected, DAT: days after treatment

[2] FOCUS (2014). Generic guidance for estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration. Version 1.1 (18-Dec-2014).

Table CA 7.1.1.1-3: Degradation of [cyclohexyl-1-^{14}C]-spiroxamine at 20°C in Refesol-02-A soil under aerobic conditions [% AR]

Compound	Replicate	Incubation time (DAT)									
		0	3	7	14	30	44	59	80	97	120
Spiroxamine	A	103.0	96.5	88.8	85.1	75.2	66.6	66.7	65.0	54.6	50.1
	B	102.1	97.4	89.3	80.1	76.5	67.0	64.1	61.6	54.6	50.6
	Mean	102.5	97.0	89.0	82.6	75.9	66.8	65.4	63.3	54.6	50.6
M01 (spiroxamine desethyl)	A	0.7	1.9	3.5	4.2	6.9	7.2	6.9	7.2	10.3	9.5
	B	0.0	1.9	3.0	4.6	6.7	8.8	7.3	7.1	9.8	8.7
	Mean	0.3	1.9	3.3	4.4	6.8	8.0	7.1	7.2	10.1	9.1
M02 (spiroxamine despropyl)	A	0.0	1.7	2.9	3.0	5.5	4.7	5.8	4.4	6.7	7.2
	B	0.7	1.5	3.1	3.9	3.8	5.5	5.9	6.3	6.4	7.0
	Mean	0.3	1.6	3.0	3.5	4.7	5.1	5.9	5.4	6.6	7.1
M03 (spiroxamine N-oxide)	A	n.d.	1.3	3.3	3.2	2.8	4.4	4.3	3.8	3.8	4.2
	B	n.d.	1.2	3.1	3.3	2.7	3.1	3.4	3.4	4.0	2.3
	Mean	n.d.	1.2	3.2	3.3	2.8	3.8	3.8	3.4	3.9	3.3
M06 (spiroxamine acid)	A	n.d.	n.d.	1.1	1.0	3.1	4.4	2.4	2.4	4.2	5.7
	B	n.d.	n.d.	0.4	3.0	2.6	2.3	3.7	2.8	4.2	4.9
	Mean	n.d.	n.d.	0.7	2.0	2.9	3.4	3.0	2.6	4.2	5.3
Total other metabolites	Mean	n.d.	n.d.	0.3	n.d.	n.d.	0.6	n.d.	n.d.	0.2	n.d.
Total extractable radioactivity	Mean	102.2	101.7	99.5	95.9	92.9	87.6	85.2	81.8	79.5	75.3
Non-extractable radioactivity	Mean	0.5	2.4	3.2	4.4	4.4	6.4	5.5	5.9	7.8	7.2
Carbon dioxide	Mean	n.a.	0.2	1.6	4.0	7.1	9.6	12.6	15.2	17.8	20.5
Other volatiles	Mean	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	0.1	0.1
Total AR	Mean	103.7	104.2	104.4	104.3	104.5	103.6	103.3	103.0	105.1	103.1

n.a.: not analysed, n.d.: not detected, DAT: days after treatment

Table CA 7.1.1.1-4: Degradation of [cyclohexyl-1-^{14}C]-spiroxamine at 20°C in Refesol-03-G soil under aerobic conditions [% AR]

Compound	Replicate	Incubation time (DAT)									
		0	3	7	14	30	44	59	80	97	120
Spiroxamine	A	95.6	86.3	80.4	77.1	71.5	69.4	66.7	66.4	56.8	54.9
	B	95.2	85.7	76.6	75.8	71.3	67.0	66.3	64.8	58.7	55.4
	Mean	95.4	86.0	78.5	76.4	71.4	68.2	66.5	65.6	57.7	55.1
M01 (desethyl)	A	3.2	5.0	6.2	6.1	8.1	7.3	8.9	7.7	10.4	9.7
	B	3.3	5.3	6.7	5.9	7.9	9.2	8.7	8.2	10.2	10.7
	Mean	3.2	5.2	6.5	6.0	8.0	8.2	8.8	7.9	10.3	10.2
M02 (despropyl)	A	2.9	5.3	5.6	4.7	6.0	6.6	6.6	5.4	7.9	7.0
	B	2.3	4.8	5.4	5.5	5.4	6.5	5.6	5.5	7.1	6.1
	Mean	2.6	5.1	5.5	5.1	5.7	6.5	6.1	5.4	7.5	6.5
M03 (N-oxide)	A	1.6	4.0	4.1	3.9	5.2	3.6	3.8	2.5	4.1	3.3
	B	2.0	4.5	4.0	4.6	3.7	3.7	3.5	2.6	3.4	3.3
	Mean	1.8	4.3	4.1	4.3	4.4	3.6	3.7	2.6	3.7	3.3
M06 (acid)	A	n.d.	n.d.	0.6	3.5	1.6	1.6	1.3	2.3	2.5	3.2
	B	n.d.	n.d.	0.5	3.7	2.1	2.1	2.1	2.9	2.0	2.5
	Mean	n.d.	n.d.	0.6	3.6	1.8	1.8	1.7	2.6	2.2	2.8
Total other metabolites	Mean	n.d.	n.d.	0.1	n.d.	0.9	n.d.	n.d.	n.d.	n.d.	0.4
Total extractable radioactivity	Mean	103.1	n.d.	95.2	95.4	92.2	88.4	86.8	84.1	81.5	78.4

Compound	Replicate	Incubation time (DAT)									
		0	3	7	14	30	44	59	80	97	120
Non-extractable radioactivity	Mean	1.2	3.5	4.5	5.6	5.6	7.2	7.0	7.6	9.0	8.8
Carbon dioxide	Mean	n.a.	n.d.	1.6	3.1	5.8	7.5	9.5	11.2	13.7	16.3
Other volatiles	Mean	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total AR	Mean	104.3	104.1	101.3	104.1	103.6	103.2	103.3	103.3	103.5	103.5

n.a.: not analysed, n.d.: not detected, DAT: days after treatment

Table CA 7.1.1.1-5: Degradation of [cyclohexyl-1,^{14}C]-spiroxamine at 20°C in Speyer 6S soil under aerobic conditions [% AR]

Compound	Replicate	Incubation time (DAT)									
		0	3	7	14	30	44	59	80	97	120
Spiroxamine	A	98.6	94.2	91.1	88.9	80.2	75.9	73.1	70.2	59.4	55.6
	B	99.5	93.6	91.7	87.3	85.0	74.2	72.0	69.6	60.3	56.5
	Mean	99.1	93.9	91.4	88.1	82.6	75.1	72.6	69.9	59.8	56.0
M01 (desethyl)	A	1.7	3.1	3.2	3.7	4.1	5.5	5.5	4.7	7.2	5.7
	B	1.9	3.1	3.3	4.2	3.6	5.3	5.5	5.1	5.8	6.0
	Mean	1.8	3.1	3.3	3.7	3.8	5.4	5.5	4.9	6.5	5.9
M02 (despropyl)	A	1.4	2.4	2.6	2.4	3.5	3.7	4.4	3.5	5.0	4.1
	B	1.2	2.3	3.0	2.7	2.5	3.2	3.7	3.5	5.7	3.6
	Mean	1.3	2.3	2.8	2.5	3.0	3.5	4.1	3.5	5.3	3.9
M03 (N-oxide)	A	1.4	2.1	2.4	1.7	3.4	2.6	3.0	2.9	4.3	4.3
	B	1.0	2.1	2.3	2.6	n.d.	3.8	3.0	2.3	3.3	3.7
	Mean	1.2	2.1	2.3	2.2	1.7	3.2	3.0	2.6	3.8	4.0
M06 (acid)	A	n.d.	n.d.	0.3	1.5	3.6	3.3	2.4	3.1	3.4	4.1
	B	n.d.	n.d.	0.4	1.4	3.0	4.3	3.5	2.8	3.5	4.3
	Mean	n.d.	n.d.	0.3	1.5	3.3	3.8	2.9	2.9	3.5	4.2
Total other metabolites	Mean	n.d.	n.d.	0.7	0.6	n.d.	n.d.	0.3	n.d.	n.d.	0.1
Total extractable radioactivity	Mean	103.4	101.4	100.9	98.6	94.5	91.8	88.4	83.8	78.9	74.1
Non-extractable radioactivity	Mean	0.9	2.1	2.6	3.5	3.6	5.2	4.6	5.8	7.8	7.4
Carbon dioxide	Mean	n.a.	n.d.	0.6	1.6	5.0	7.5	10.0	14.2	17.3	17.6
Other volatiles	Mean	n.a.	n.d.	n.d.	n.d.	n.d.	0.1	n.d.	n.d.	0.1	0.1
Total AR	Mean	104.3	104.1	104.1	103.6	103.1	103.7	103.1	103.8	104.1	99.2

n.a.: not analysed, n.d.: not detected, DAT: days after treatment

B. Material Balance

Mass balances ranged from 101.6 to 104.2, 103.0 to 105.1, 101.3 to 104.3 and 99.2 to 104.3% AR for the Longwoods, Refesol-02-A, Refesol-03-G and Speyer 6S soils, respectively.

C. Extractable and Non-Extractable Residues

Extractable radioactivity declined most significantly in the Longwoods soil. For this soil extractable radioactivity decreased from 101.8% AR at DAT 0 to 14.9% AR by DAT 120. Non-extractable residues (NER) for the Longwoods soil increased from 0.4% AR at DAT 0 to 16.9% AR by DAT 120. Further investigation of NER showed the majority of the applied radioactivity associated with the humin fraction.

Extractable radioactivity for the three remaining soils declined more steadily and comprised 103.2, 103.1 and 103.4% AR at DAT 0 and declined to 75.3, 78.4 and 74.1% AR by DAT 120 for the Refesol-02-A, Refesol-03-G and Speyer 6S soils, respectively. Non-extractable residues (NER) for these soils increased from 0.5, 1.2 and 0.9% AR at DAT 0 to maximums of 7.8, 9.0 and 7.8% AR at

DAT 97 and then declined to 7.2, 8.8 and 7.4% AR by DAT 120, respectively.

D. Volatile Radioactivity

Levels of ^{14}C-carbon dioxide formed in the Longwoods soil increased during incubation to 69.9% AR by DAT 120. Levels of ^{14}C-carbon dioxide formed for the three remaining soils ranged from 16.3 to 20.3% AR by DAT 120. Other volatile radioactivity was ≤0.4% AR for all soils at all time points.

E. Degradation of Parent Compound

Following application of [cyclohexyl-1-^{14}C]-spiroxamine, the amount of parent in the soil extracts of the Longwoods soil decreased from 101.2% AR at DAT 0 to 5.8% AR by the end of the study at DAT 120. The amount of parent in the soil extracts of the Refesol 02-A, Refesol 03-G and Speyer 6S soils decreased from 102.2, 95.4 and 99.1% AR at DAT 0 to 50.6, 55.1 and 56.0% AR by the end of the study at DAT 120.

Degradation of [cyclohexyl-1-^{14}C]-spiroxamine was accompanied by the formation of the degradation products M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl), M03 (spiroxamine-N-oxide) and M06 (spiroxamine-acid). M01 was detected at a maximum of 12.0% AR at DAT 44 in the Longwoods soil and maximums ranging from 6.5 to 10.3% AR at DAT 97 in the other soils. M02 was detected at a maximum of 9.2% AR at DAT 30 in the Longwoods soil and maximums ranging from 5.3 to 7.5% AR around DAT 97-120 in the other soils. M03 was detected at a maximum of 7.2% AR at DAT 14 in the Longwoods soil and maximums ranging from 3.9 to 4.4% AR over the course of incubation in the other soils. M06 was detected at a maximum of 3.9% AR at DAT 59 in the Longwoods soil and maximums ranging from 3.6 to 5.3% AR between at DAT 14-120 in the other soils. Some other metabolites were detected typically <1% AR but all <5% AR.

F. Degradation Kinetics

Degradation rates determined in the report are superseded by the report presented under point KCA 7.1.2.1.1/09 (M-763139-01-1).

G. Isomers of Parent Compound

Additional work is being conducted on the chiral analysis of the samples for parent spiroxamine. This work will provide the quantified amounts of each individual isomer of spiroxamine in all samples. This work will be submitted in the final report of this study (currently this is interim) as soon as possible.

III. Conclusions

Spiroxamine degraded in soil under aerobic conditions (20°C, pF 2) with 5.8% of applied radioactivity remaining as parent compound in the Longwoods soil at DAT 120 and with 50.6 to 56.0% of applied radioactivity remaining as parent compound in the other three soils. The amounts of unextractable radioactivity increased to 16.9% AR by DAT 120 in one soil but remained <10% AR in the other three soils. Mineralisation to carbon dioxide was a major pathway, steadily increasing to 69.9% AR in one soil and comprised 16.3 to 20.3% AR in the other three soils. No significant levels of organic volatiles were observed. The metabolic pathways involved de-alkylation of the amine moiety to form metabolites: M01 (spiroxamine-desethyl, maximum 12.0% AR) and M02 (spiroxamine-despropyl, maximum 9.2% AR); oxidation of the amine moiety to form metabolite M03 (spiroxamine-N-oxide, maximum 7.2% AR); oxidation of a methyl group to form M06 (spiroxamine-acid, maximum 5.3% AR).

A re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) is performed in the report presented under point KCA 7.1.2.1.1/09 (M-763139-01-1).

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to study guideline(s) OECD 307 (required guideline). The study is considered valid to assess the aerobic degradation of [cyclohexyl-1-^{14}C]-spiroxamine in soil.

Existing studies, previously evaluated

Data Point:	KCA 7.1.1.1/01
Report Author:	[redacted]
Report Year:	1994
Report Title:	Aerobic degradation of KWG 4168 in BBA soil 2.2
Report No:	PF4027
Document No:	M-006135-01-1
Guideline(s) followed in study:	BBA: Part IV, 4-1; BBA: Part IV, 4-1
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The route and rate of degradation of spiroxamine was investigated in one European soil (BBA 2.2, loamy sand) under laboratory aerobic conditions. Soil samples (100 g dw) were treated with [cyclohexyl-1-^{14}C]-spiroxamine (radiochemical purity >99%) at an application rate of 0.766 mg/kg soil dry weight (equivalent to 575 g/ha assuming 5 cm depth and 1.5 g/cm^3 soil density) and incubated (20°C, 40% MWHC) for 100 days in the dark.

Single treated samples were removed for analysis after 0, 1, 3*, 7, 14*, 30*, 59 and 100* days of incubation (except for intervals marked * when duplicate samples were taken). Soil samples were extracted three times at room temperature under agitation using acetonitrile for 30 minutes, and once using water. Soil extracts were analysed, without concentration, primarily by normal phase TLC. Confirmatory analysis was conducted by reverse-phase TLC.

The material balances ranged from 99.1 to 101.5% of applied radioactivity (% AR). The amounts of unextractable radioactivity increased to 18.5% AR by DAT 100. No significant levels of organic volatiles were observed. Mineralisation to carbon dioxide was a major pathway, steadily increasing throughout the study, and accounted for 21.9% AR by DAT 100.

After 100 days aerobic degradation, spiroxamine degraded to 40.1% AR. A DT_{50} value for the degradation of spiroxamine was calculated in the report, however, a re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014), was performed in the report presented under point KCA 7.1.2.1.1/09 (M-763139-01-1).

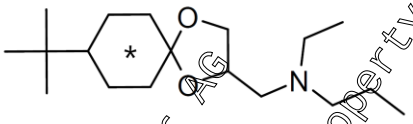
Two major metabolites were observed formed by de-alkylation of the amine moiety: M01 (spiroxamine-desethyl, maximum 8.1% of AR); M02 (spiroxamine-despropyl, maximum 5.6% AR). Additional minor metabolites observed were M03 (spiroxamine-N-oxide, maximum 3.3% of AR); M06 (spiroxamine-acid, maximum 3.5% AR). No other metabolites were observed >1.8% AR.

I. Materials and Methods

A. Materials

1. Test Items

[cyclohexyl-1-^{14}C]-spiroxamine



* Denotes position of [^{14}C]-radiolabel

Specific Activity: 2.59 MBq/mg

Radiochemical Purity: >99% (TLC at time of treatment)

2. Test Soil

The study was performed using one test soil as characterised in Table CA 7.1.1.1-6. No soil history is available.

Table CA 7.1.1.1-6: Physico-chemical properties of test soil

Parameter		Soil
Soil Designation		BBA 2.2
Geographic Location City Country		Speyer, Rhineland-Palatinate Germany
Textural Classification (USDA)		Loamy sand
Sand [50 - 2000 µm]	(%)	83.0
Silt [2 – 50 µm]	(%)	13.0
Clay [< 2 µm]	(%)	4.0
pH in H_2O (1:1) in 0.01M $CaCl_2$ (1:1)		 6.3 5.5
Organic Matter (%)		2.15
Organic Carbon (%)*		1.25
Cation Exchange Capacity (meq/100 g)		10.0
Maximum Water Holding Capacity (g H_2O per 100g dry soil)		17.67
Soil Microbial Biomass (mg organic C/kg soil) Initial, untreated (0 DAT) Final, untreated (100 DAT) Final, treated (100 DAT)		 256 211 201

* Calculated by multiplying organic carbon content by 1.724 (not reported)

B. Study Design

1. Experimental Conditions

Tests were performed in sealed static systems consisting of glass flasks each containing 100 g soil with solid traps of oil-coated (2% paraffin oil in hexane) quartz glass wool for sorption of volatile organic compounds and soda lime for absorption of evolved carbon dioxide.

The study was conducted at a concentration of approximately 0.766 mg/kg dry weight of soil. The test

concentration was based on a field rate of 750 g a.s./ha assuming 10 cm soil depth and 1.0 g/cm^3 soil density (note - this is equivalent to a field rate of 575 g a.s./ha, assuming a mixing depth of 5 cm and a soil density of 1.5 g/cm^3). Application of the re-purified [cyclohexyl-1-^{14}C]-spiroxamine in solvent (acetonitrile) was made to a subsample of soil, which was then added to the bulk soil and mixed thoroughly after solvent evaporation, for 1.5 hours before use in the experiment. After mixing, samples of 100 g dry weight equivalent of soil was weighed into incubation vessels. Soil moisture was adjusted at 40% MWHC, at the time of application (i.e. no pre-acclimatisation), and maintained during incubation with periodic additions of water (30 days). The soil samples were incubated at a temperature of 20°C under aerobic conditions.

Two untreated samples were incubated alongside test systems at under the same conditions to monitor microbial activity at the beginning and end of the study.

2. Sampling

Prior to opening the sealed static incubation vessels either for sampling or maintenance of moisture, possibly volatile components were flushed into the trap attachment by introducing water saturated air (10 mins). Single treated samples were removed for analysis after 0, 1, 3*, 7, 14*, 30*, 59 and 100* days of incubation (except for intervals marked * when duplicate samples were taken).

Untreated microbial biomass samples were analysed at the beginning and end of the experiment. Additionally, the biomass in treated soil was analysed at the end of the experiment.

3. Analytical Procedures

Soil samples were extracted three times at room temperature under agitation using acetonitrile and once using water (30 mins each). Liquid extracts were separated by centrifugation, filtered and radio-assayed by Liquid Scintillation Counting (LSC) directly. Extracted soil debris and filter papers were air-dried and analysed by combustion LSC.

Aliquots of combined soil extracts were analysed by TLC against reference standards using two methods:

- Normal phase: Silica gel plates and an acetonitrile:water:25% ammonia (80:18:2, v/v/v) solvent system
- Reverse phase: RP-18 plates with two solvent systems (used sequentially in 1 dimension):
  - i) n-hexane:dichloromethane:2-propyl alcohol:25% ammonia (30:70:10:2, v/v/v/v) and a saturated tank system (developed until solvent front at 14 cm)
  - ii) chloroform:ethyl alcohol (50:50 v/v) and an unsaturated tank system (developed until solvent front at 7 cm)

The TLC plates were evaluated using a linear analyser to determine the quantities of radiolabelled test substance and its degradation products.

Volatile radioactivity in volatile traps was determined by LSC (organic volatiles were extracted using ethyl acetate and aliquots quantified by LSC; $^{14}CO_2$ was liberated from soda lime through addition of 18% (w/w) hydrochloric acid and absorbed by a suitable cocktail and quantified by LSC).

4. Determination of degradation kinetics

The degradation kinetics of data within this report are subject of a separate modelling study. DT_{50} and DT_{90} values for the degradation of spiroxamine and its metabolites have been re-calculated from the reported data following the recommendations of the FOCUS work group (FOCUS 2014[2]). Full details are provided in in the report under point KCA 7.1.2.1.1/09 (M-763139-01-1).

II. Results and Discussion

A. Data

The distribution and characterisation of radioactivity for the test soil incubated at 20°C following application of [^{14}C]-spiroxamine are summarized in Table CA 7.1.1.1-7. Mean values are used for replicate

samples.

Table CA 7.1.1.1-7: Degradation of [^{14}C]-spiroxamine in BBA 2.2 soil at 20°C under aerobic conditions [% AR]

Compound	Replicate	Incubation time (DAT)							
		0	1	3	7	14	30	59	100
Spiroxamine	A	89.4	88.5	85.1	78.9	74.2	58.3	48.8	40.5
	B	-	-	83.8	-	72.3	61.7	-	40.1
	Mean	89.4	88.5	84.5	78.9	73.3	60.0	48.8	40.3
M01 (spiroxamine-desethyl)	A	n.d.	0.7	1.4	2.3	4.1	4.6	5.2	5.4
	B	-	-	1.7	-	3.7	5.3	-	5.6
	Mean	n.d.	0.7	1.6	2.3	3.9	5.0	5.2	5.5
M02 (spiroxamine-despropyl)	A	n.d.	n.d.	0.6	1.1	2.5	3.5	5.2	5.4
	B	-	-	0.7	-	2.6	-	-	5.6
	Mean	n.d.	n.d.	0.7	1.1	2.6	3.5	5.2	5.5
M03 (spiroxamine-N-oxide)	A	n.d.	0.7	1.1	1.5	1.9	4.0	1.9	1.6
	B	-	-	1.5	-	1.9	2.0	-	1.8
	Mean	n.d.	0.7	1.3	1.5	1.9	3.3	1.9	1.7
M06 (spiroxamine-acid)	A	n.d.	n.d.	n.d.	0.8	1.6	2.3	3.5	3.1
	B	-	-	n.d.	-	1.3	2.9	-	3.1
	Mean	n.d.	n.d.	n.d.	0.8	1.6	2.6	3.5	3.1
Unknown	Mean	1.0	n.d.	n.d.	n.d.	n.d.	1.8	0.3	0.4
Total extractable radioactivity	Mean	90.4	89.9	88.0	84.6	83.2	76.1	66.9	59.1
Non-extractable residue [A]	Mean	9.6	10.5	12.0	12.6	13.0	14.8	17.0	18.5
^{14}C-Carbon dioxide including other volatiles [B]	Mean	n.a.	0.7	1.6	2.8	4.9	9.0	15.2	21.9
Total radioactivity	Mean	100.0	101.1	101.5	100.0	101.1	99.9	99.1	99.5

n.a.: not analysed, n.d.: not detected, DAT: days after treatment, LOD: 0.1% AR
All values expressed as percentage of applied radioactivity (% AR)
A Value includes recovery from paper filters used in centrifugation of extracts
B Other volatile radioactivity was < 0.1%AR

B. Material Balance

Recoveries of initial radioactivity ranged from 99.1 to 101.5%. Values presented are % of initial recovery at time = 0 rather than % applied radioactivity. This is not considered to have had any impact on the study conclusions.

C. Extractable and Non-Extractable Residues

Total extractable radioactivity decreased from 90.4% AR at 0 DAT to 59.1% AR by DAT 100. The total of non-extractable residues (NER) increased from 9.6% AR at DAT 0 to 18.5% AR by the end of the study (DAT 100).

D. Volatile Radioactivity

Levels of ^{14}C-carbon dioxide formed increased to 21.9% AR by DAT 100. Other volatile radioactivity was < 0.1% AR at all timepoints.

E. Degradation of Parent Compound

Following application of [^{14}C]-spiroxamine in BBA 2.2 soil, the amount of parent in the total soil extracts decreased from a maximum of 89.4% at 0 DAT to 40.1% AR by 100 DAT. In addition to parent material, two major metabolites were observed. M01 (spiroxamine-desethyl) increased over the course of the incubation period, and accounted for a maximum of 8.1% of applied radioactivity at 100 DAT. M02 (spiroxamine-despropyl) was detected at a maximum of 5.6% of applied radioactivity at 100 DAT. Other metabolites observed were: M03 (spiroxamine-N-oxide) was detected at a maximum of 3.3% of applied radioactivity at DAT 30 and declined to 1.7% AR by DAT 100; M06 (spiroxamine-acid) was detected at a maximum of 3.5% of applied radioactivity at DAT 59 and declined to 3.1% AR by DAT 100. No other metabolites were observed >1.8% AR.

F. Degradation kinetics

Degradation rates determined in the report are superseded by the report presented under point KCA 7.1.2.1.1/09 (M-763139-01-1).

III. Conclusions

Spiroxamine degraded in BBA 2.2 soils under aerobic conditions at 20°C with 40.1% of the applied radioactivity remaining as parent compound after 100 days. The amounts of unextractable radioactivity increased to 18.5% AR by DAT 100. No significant levels of organic volatiles were observed throughout the study. Mineralisation to carbon dioxide was a major pathway, steadily increasing throughout the study, and accounted for 21.9% AR by DAT 100. The primary metabolic pathway involved de-alkylation of the amine moiety to form two major metabolites M01 (spiroxamine-desethyl, maximum 8.1% AR) and M02 (spiroxamine-despropyl, maximum 5.6% AR). Additional minor metabolites observed were M03 (spiroxamine-N-oxide, maximum 3.3% of AR) and M06 (spiroxamine-acid, maximum 3.5% AR).

A re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) is performed in the report presented under point KCA 7.1.2.1.1/09 (M-763139-01-1).

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to study guideline(s) BBA: Part IV, 4-1 (similar to current required guideline, with only minor differences). The study is considered valid to assess the aerobic degradation of [cyclohexyl-1-^{14}C]-spiroxamine in soil.

Data Point:	KCA 7.1.1.1/02
Report Author:	[redacted]
Report Year:	1995
Report Title:	Aerobic degradation and metabolism of KWG 4168 in soil
Report No:	PF4034
Document No:	M-006141-01-1
Guideline(s) followed in study:	BBA: Part IV, 4-1
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017) The degradation rates of spiroxamine ([redacted] (1997) and [redacted] (2008)) were included in the following survey (M-036125-01-1)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The route and rate of degradation of spiroxamine was investigated in two European and one US soil (Laacherhof/silt loam, Monheim 3/sandy loam and Howe/sandy loam, respectively) under laboratory aerobic conditions. Soil samples (100 g dry weight) were treated with [cyclohexyl-1-^{14}C]-spiroxamine (radiochemical purity >99%) at an application rate of 0.5 mg/kg soil (equivalent to 375 g a.s./ha assuming 5 cm soil depth and 1.5 g/cm^3 soil density) and incubated at 20°C and 40% Maximum Water Holding Capacity, MWHC (except US soil incubated at a moisture of 75% of 1/3rd bar moisture, equivalent to 48% MWHC) for 100 days in the dark.

Duplicate (Laacherhof) or singlicate (Monheim 3 and Howe) samples were removed for analysis after 0, 1, 3, 7, 14, 30, 60, and 100 days of incubation. Soil samples were extracted three times at room temperature under agitation using acetonitrile for 30 minutes, and once under reflux for 6 hours, also using acetonitrile. Liquid extracts were radio-assayed by Liquid Scintillation Counting (LSC) directly. Extracted soil debris was air-dried and analysed by combustion LSC. Liquid extracts were analysed and quantified by normal phase TLC and quantitatively confirmed by reverse-phase TLC. Levels of parent spiroxamine were also confirmed by reverse-phase HPLC (selected samples). Metabolite identity was also confirmed by isolation and GC-MS (selected samples).

The material balances ranged from 94.8 to 98.0% AR in Laacherhof soil, 95.0 to 97.5% AR in Monheim 3 soil and 93.6 to 97.5% AR in Howe soil. Levels of NER increased to maximum levels of 17.0 to 23.9% AR by DAT 30-100 and subsequently declined in some soils. Mineralization to carbon dioxide was a major pathway, demonstrated by the high amount of radioactivity recovered in the volatile traps for all soils (maximum of 44.6% AR in Laacherhof, 30.5% AR in Monheim 3 and 35.2% AR for Howe by the end of the study). No significant levels of organic volatiles were observed.

After 100 days incubation at 20°C, [^{14}C]-spiroxamine degraded to 14.3% of the radioactivity applied in Laacherhof soil, to 27.4% in Monheim 3 soil, and to 24.9% in Howe soil. A re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) was performed in the report presented under point KCA 7.1.2.1.1/09 (M-763139-01-1).

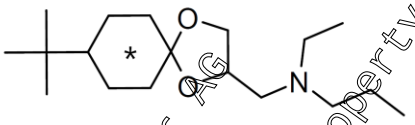
The primary metabolic pathway involved de-alkylation of the amine moiety to form two major metabolites, M01 (spiroxamine-desethyl, maximum 8.8% of AR in Monheim 3 soil) and M02 (spiroxamine-despropyl, maximum 5.8% AR in Monheim 3 soil). Additional minor metabolites observed were M03 (spiroxamine-N-oxide, maximum 2.5% AR in Laacherhof soil only) M06 (spiroxamine-acid, maximum 1.6% of AR in Laacherhof soil) and M07 (spiroxamine-hydroxy acid, maximum 0.2% AR in Laacherhof soil).

I. Materials and Methods

A. Materials

1. Test Items

[cyclohexyl-1-^{14}C]-spiroxamine



* Denotes position of [^{14}C]-radiolabel

Specific Activity: 2.59 MBq/mg

Radiochemical Purity: >99% (HPLC)

2. Test Soil

The study was performed using three test soils as characterized in Table CA 7.1.1.1-8. No soil history is available.

Table CA 7.1.1.1-8: Physico-chemical properties of test soil

Parameter		Soil		
Soil Designation		Laacherhof	Monheim 3	Howe
Geographic Location				
City		Monheim	Monheim	Howe
Country		Germany	Germany	Indiana, USA
Textural Classification (USDA)		Silt loam	Sandy loam	Sandy loam
Sand [50 - 2000 µm]	(%)	36.9	58.2	65.5
Silt [2 – 50 µm]	(%)	51.1	31.0	26.3
Clay [< 2 µm]	(%)	12.0	10.8	8.2
pH				
in H_2O (1:1)		8.1	6.5	7.1
in $CaCl_2$ (1:1)		7.3	6.3	6.8
Organic Matter (%) *		1.55	3.41	1.87
Organic Carbon (%)		0.9	1.98	1.09
Cation Exchange Capacity (meq/100 g)		10.0	10.0	10.0
Water Holding Capacity (g H_2O per 100g dry soil)				
40% of maximum		13.1	16.6	11.3
75% of 1/3 bar		-	-	13.7
Soil Microbial Biomass (mg C/kg soil)				
Initial, untreated (0 DAT)		307	257	285
Final, untreated (100 DAT)		275	202	250
Initial, treated (0 DAT)		402	246	347
Final, treated (100 DAT)		271	167	247

* Calculated by multiplying organic carbon content by 1.724 (not reported)

B. Study Design

1. Experimental Conditions

Tests were performed in sealed static systems consisting of glass flasks each containing 100 g soil (dry weight) with solid traps of oil-coated (2% paraffin oil in hexane) quartz glass wool for sorption of organic volatiles and soda lime for adsorption of evolved carbon dioxide.

The study was conducted at a concentration of approximately 0.5 mg/kg dry weight of soil. The test concentration was based on a field rate of 750 g a.s./ha assuming a 10 cm soil depth and 1.5 g/cm^3 soil density (note – this is equivalent to 375 g a.s./ha assuming 5 cm soil depth and 1.5 g/cm^3 soil density). Application of [^{14}C]-spiroxamine, dissolved in acetonitrile/triethylamine (100/0.5 v/v), was made to a subsample of each soil, which was then added to the bulk soil and mixed thoroughly, after solvent evaporation, for 2 hours before use in the experiment. After mixing, 100 g dry weight equivalent of soil was weighed into incubation vessels. Soil moisture was adjusted to 40% MWHC at the time of application i.e. no pre-acclimatisation (75% of 1/3 bar for Howe soil, equivalent to 48% MWHC) and maintained at this level during incubation with periodic additions of water (30 days). The soil samples were incubated at a temperature of 20 ± 2°C under aerobic conditions.

Additional samples in Laacherhof soil treated with 10 and 100 fold increased application rates were prepared with 500 g soil and incubated under the same conditions as test vessels only for use in identification of parent spiroxamine and metabolites.

Two treated (applied at the same rate) and two untreated soil samples were incubated alongside test systems under the same conditions to monitor microbial activity at the beginning and end of the study.

2. Sampling

Prior to opening the sealed static incubation vessels, either for sampling or maintenance of moisture, possibly volatile components were flushed into the trap attachment by introducing water saturated air (10 mins). Test samples (duplicates for Laacherhof soil; single samples for Monheim 3 and Howe soils) were removed for analysis after 0, 1, 3, 7, 14, 30, 60 and 100 days of incubation.

Treated and untreated microbial biomass samples were analysed at the beginning and end of the experiment for each soil.

3. Analytical Procedures

Soil samples were extracted three times at room temperature under agitation with acetonitrile for 30 minutes. Liquid extracts were radio-assayed by Liquid Scintillation Counting (LSC) directly. Extracted soil debris was air-dried and analysed by combustion LSC. Soil NER was further refluxed for 6 hours with acetonitrile and quantified by LSC separately.

The combined room temperature soil extracts were analysed (without any prior work-up or concentration) by TLC against reference standards using the main primary (normal phase) method below:

- Silica gel plates and an acetonitrile:water:25% ammonia (either i) 320:18:2, v/v/v or ii) 80:18:2, v/v/v) solvent system

Quantitative confirmatory analysis was conducted using the following reverse phase TLC method:

- RP-18 plates with two solvent systems (used sequentially in 1 dimension):
  - n-hexane:dichloromethane:2-propyl alcohol:25% ammonia (30:70:10:2, v/v/v) and a saturated tank system (developed until solvent front at 14 cm)
  - chloroform:ethyl alcohol (50:50 v/v) and an unsaturated tank system (developed until solvent front at 6 cm)

The NER reflux extracts were analysed separately, however, the methodology used is unclear.

The TLC plates were evaluated using a linear analyser to determine the quantities of radiolabelled test

substance and its degradation products. Confirmatory analysis of soil extracts to determine parent spiroxamine levels was also conducted by reverse-phase HPLC against reference standards with a LiChrosphere 100 RP-18 column and mobile phase of 20% water (with 0.09% o-phosphoric acid and 0.3% triethylamine) and 80% acetonitrile (isocratic).

Additional soil samples, treated at x10 and x100 application rates, were analysed as follows:

- for isolation of metabolites M01 and M02: soil samples treated at the x10 rate were extracted with acetonitrile after DAT 69 and 71, respectively. Soil extracts were concentrated under vacuum and analysed using the normal phase TLC primary analytical method using solvent system i). The radioactive substance corresponding to the reference standards were extracted from the silica gel with acetonitrile.
- for isolation of metabolites M03 and M06: soil samples treated at the x100 rate were extracted with acetonitrile after DAT 16 and 10, respectively. Soil extracts were concentrated (solvent evaporated at ambient temperature) and analysed using the normal phase TLC primary analytical method using solvent system ii). The radioactive substance corresponding to the reference standards were extracted from the silica gel with acetonitrile (plus methanol) and ethanol, respectively.

The isolates above were then analysed by GC-MS against authentic reference standards to confirm metabolite identity and structure.

Volatile radioactivity in volatile traps was extracted using ethyl acetate and aliquots quantified by LSC. $^{14}CO_2$ was liberated from soda lime through addition of 18% (w/w) hydrochloric acid and absorbed by a suitable cocktail and quantified by LSC. Identity of $^{14}CO_2$ was further confirmed by conversion to [^{14}C]-benzoic acid by means of the Grignard reaction.

4. Determination of degradation kinetics

The degradation kinetics of data within this report are subject of a separate modelling study. DT_{50} and DT_{90} values for the degradation of spiroxamine and its metabolites have been re-calculated from the reported data following the recommendations of the FOCUS work group (FOCUS 2014[2]). Full details are provided in in the report under point KCA 7.1.2.1.1/09 (M-763139-01-1).

II. Results and Discussion

The distribution and characterisation of radioactivity for the test soils incubated at 20°C following application of [^{14}C]-spiroxamine are summarized in Table CA 7.1.1.1-9 to Table CA 7.1.1.1-11.

Table CA 7.1.1.1-9: Degradation of [^{14}C]-spiroxamine in Laacherhof soil at 20°C under aerobic conditions [% AR]

Compound	Replicate	Incubation time (DAT)							
		0	1	3	7	14	30	60	100
Spiroxamine	A	75.3	71.3	67.8	61.1	47.8	41.0	28.1	15.0
	B	76.3	72.2	66.5	60.2	52.2	41.5	29.4	14.3
	Mean	75.8	71.8	67.2	60.7	50.0	41.3	28.8	14.7
M01 (spiroxamine-desethyl)	A	0.5	1.4	2.9	4.4	5.2	6.0	7.3	7.2
	B	0.7	1.4	2.9	4.3	5.7	6.1	6.6	7.0
	Mean	0.6	1.4	2.9	4.4	6.5	6.1	7.0	7.1
M02 (spiroxamine-despropyl)	A	0.4	1.0	1.9	2.7	3.6	3.9	4.9	5.6
	B	0.7	1.0	1.8	2.8	3.6	3.8	4.3	5.7
	Mean	0.6	1.0	1.9	2.8	3.6	3.9	4.6	5.7
M03 (spiroxamine-N-oxide)	A	0.7	0.8	1.4	1.7	2.4	2.1	1.8	1.8
	B	0.8	0.9	1.3	1.7	2.5	1.8	1.7	1.9
	Mean	0.8	0.9	1.4	1.7	2.5	2.0	1.8	1.9
M06 (spiroxamine-acid)	A	<0.1	0.2	1.1	1.6	1.2	1.0	0.6	0.7
	B	<0.1	0.2	1.0	1.6	1.6	0.5	0.3	0.7
	Mean	n.d.	0.2	1.1	1.6	1.4	0.8	0.5	0.7
M11 (spiroxamine-desethyl acid)	A	<0.1	<0.1	<0.1	0.1	<0.1	0.1	0.1	<0.1
	B	<0.1	<0.1	<0.1	0.1	<0.1	0.1	0.1	<0.1
	Mean	n.d.	n.d.	n.d.	0.1	n.d.	0.1	0.1	n.d.
Ambient extract	-	77.7	75.2	74.3	71.2	63.9	54.0	42.6	30.0
Total extractable radioactivity	-	77.7	75.2	74.3	71.2	63.9	54.0	42.6	30.0
Non-extractable radioactivity (including reflux and filter papers) [A]	-	17.1	20.6	19.4	20.6	32.1	26.3	22.9	20.2
Reflux extract	-	2.9	8.7	6	6.7	6.7	7.7	4.9	1.9
^{14}C-Carbon dioxide including other volatiles [A]	-	n.a.	1.0	3.0	5.7	9.4	17.6	31.2	44.6
Total radioactivity	-	94.8	96.8	96.7	97.4	95.4	97.9	96.7	94.7

n.a.: not analysed, n.d.: not detected above LOD, DAT: days after treatment, LOD: 0.1% AR
A Max filter paper value of 1.8%AR
B Other volatile radioactivity was <0.1%AR

Table CA 7.1.1.1-10: Degradation of [^{14}C]-spiroxamine in Monheim 3 soil at 20°C under aerobic conditions [% AR]

Compound	Incubation time (DAT)							
	0	1	3	7	14	30	60	100
Spiroxamine	72.3	73.5	71.5	65.0	57.7	43.6	34.3	27.4
M01 (spiroxamine-desethyl)	0.6	0.9	1.7	3.9	5.8	6.9	8.8	7.7
M02 (spiroxamine-despropyl)	0.5	0.7	1.1	2.3	3.4	4.6	5.8	5.4
Ambient extract	73.9	75.9	75.7	73.5	69.2	57.9	53.5	43.5
Total extractable radioactivity	73.9	75.9	75.7	73.5	69.2	57.9	53.5	43.5

Compound	Incubation time (DAT)							
	0	1	3	7	14	30	60	100
Reflux extract	7.0	5.9	6.8	5.7	7.2	6.6	3.5	3.9
Non-extractable radioactivity (including reflux and filter papers) [A]	21.7	20.8	19.1	20.4	21.5	24.7	21.4	21.0
^{14}C-Carbon dioxide including other volatiles [B]	n.a.	0.8	1.9	3.6	6.5	12.5	21.2	26.6
Total radioactivity	95.6	97.5	96.7	97.5	97.3	95.1	96.1	91.1

n.a.: not analysed, n.d.: not detected above LOD, DAT: days after treatment, LOD: 0.1% AR
A Max filter paper value of 2.2%AR
B Other volatile radioactivity was <0.1%AR

Table CA 7.1.1.1-11: Degradation of [^{14}C]-spiroxamine in Howe soil at 20°C under aerobic conditions [% AR]

Compound	Incubation time (DAT)							
	0	1	3	7	14	30	60	100
Spiroxamine	75.1	71.5	68.9	64.5	57.9	45.4	34.1	24.9
M01 (spiroxamine-desethyl)	0.6	0.5	1.2	2.1	2.6	3.5	3.9	3.4
M02 (spiroxamine-despropyl)	0.1	0.4	0.7	0.8	1.5	2.5	3.9	2.6
Ambient extract	76.1	73.5	72.3	69.8	65.6	53.6	45.6	34.8
Total extractable radioactivity	76.1	73.5	72.3	69.8	65.6	53.6	45.6	34.8
Reflux extract	3.9	3.4	6.5	1.0	6.2	6.3	2.8	1.4
Non-extractable radioactivity (including reflux and filter papers) [A]	21.1	22.8	22.4	24.2	26.5	31.7	25.5	23.6
^{14}C-Carbon dioxide including other volatiles [B]	n.a.	0.6	1.5	2.9	5.4	12.2	22.9	35.2
Total radioactivity	97.2	96.9	96.2	96.9	97.5	97.5	94.0	93.6

n.a.: not analysed, n.d.: not detected above LOD, DAT: days after treatment, LOD: 0.1% AR
A Max filter paper value of 1.7%AR
B Other volatile radioactivity was 0.1%AR

B. Material Balance

Material balances ranged from 94.8 to 97.9% AR in Laacherhof soil, 95.0 to 97.5% AR in Monheim 3 soil and 93.6 to 97.5% AR in Howe soil.

C. Extractable and Non-Extractable Residues

For samples of Laacherhof soil total extractable radioactivity decreased from 77.7% AR at DAT 0 to 30.0% AR by DAT 100. The total of non-extractable residues (NER) increased from 17.1% AR at DAT 0 to 20.2% AR by the end of the study (DAT 100).

For samples of Monheim 3 soil total extractable radioactivity decreased from 73.9% AR at 0 DAT to 43.5% AR after 100 days incubation. NER was 21.7% AR on DAT 0 and showed a slight overall decrease to 21.0% AR by DAT 100.

For samples of Howe soil total extractable radioactivity decreased from 76.1% AR at DAT 0 to 34.8% AR after 100 days of incubation. NER was 21.1% AR at 0 DAT and increased to 23.6% AR by DAT 100.

D. Volatile Radioactivity

Levels of ^{14}C-carbon dioxide increased to 44.6, 30.5 and 35.2% AR for the Laacherhof, Monheim 3 and Howe soils, respectively. Other volatile radioactivity was < 0.1% AR at all timepoints for all soils.

E. Degradation of Parent Compound

Following application of [^{14}C]-spiroxamine in Laacherhof soil, the amount of parent in the total soil extracts decreased from a maximum of 75.8% AR at DAT 0 to 14.7% AR by DAT 100. In addition to parent material, M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl), M03 (spiroxamine-N-oxide), M06 (spiroxamine-acid) and M11 (spiroxamine-desethyl acid) were detected. Levels of M01 increased over the course of the incubation period, and accounted for a maximum of 7.1% of applied radioactivity at DAT 100. M02 was detected at a maximum of 5.7% of applied radioactivity at DAT 100. M03 was detected at a maximum of 2.5% of applied radioactivity at DAT 14. M06 was detected at a maximum of 1.6% at DAT 7. Metabolite M11 was detected at a maximum of 0.2% at DAT 30.

Following application of [^{14}C]-spiroxamine in Monheim 3 soil, the amount of parent in the total soil extracts decreased from 72.3% at DAT 0 to 27.4% AR by DAT 100. In addition to parent material, M01 (spiroxamine-desethyl) and M02 (spiroxamine-despropyl) were detected. Levels of M01 increased over the course of the incubation period and accounted for a maximum of 8.8% of applied radioactivity at DAT 60. M02 increased to a maximum of 5.8% of applied radioactivity at DAT 60.

Following application of [^{14}C]-spiroxamine in Howe soil, the amount of parent in the total soil extracts decreased from 75.1% at DAT 0 to 24.9% AR by DAT 100. In addition to parent material, M01 (spiroxamine-desethyl) and M02 (spiroxamine-despropyl) were detected. Levels of M01 increased over the course of the incubation period and accounted for a maximum of 3.9% of applied radioactivity at DAT 60. M02 was detected at a maximum of 3.9% of applied radioactivity at DAT 60.

Analysis of the reflux extracts (containing a maximum 7.7% AR in Laacherhof soil) seems to have been conducted separately and were reported to comprise mostly M15 (spiroxamine-ketone) without providing full details. The report postulated that M15 was formed from parent spiroxamine during the reflux process.

F. Degradation kinetics

Degradation rates determined in the report are superseded by the report presented under point KCA 7.1.2.1.1/09 (M-763139-01-1).

III. Conclusions

Spiroxamine degraded in Laacherhof, Monheim 3 and Howe soils under aerobic conditions at 20°C, with 14.7%, 27.4% and 24.9% of the applied radioactivity remaining as parent compound in these respective soils after 100 days. The amounts of unextractable radioactivity increased to an overall maximum of 31.7% AR by DAT 30 in Howe soil and subsequently declined to 23.6% AR by DAT 100. No significant levels of organic volatiles were observed in the study. Mineralisation to carbon dioxide was a significant pathway, steadily increasing throughout the study in all soils, accounting for an overall maximum of 44.6% AR in Laacherhof soil after 100 days. The primary metabolic pathway involved dealkylation of the amine moiety to form two major metabolites, M01 (spiroxamine-desethyl, maximum 8.8% of AR in Monheim 3 soil) and M02 (spiroxamine-despropyl, maximum 5.8% AR in Monheim 3 soil). Additional minor metabolites observed were M03 (spiroxamine-N-oxide, maximum 2.5% AR in Laacherhof soil only) M06 (spiroxamine-acid, maximum 1.6% of AR in Laacherhof soil) and M11 (spiroxamine-desethyl acid, maximum 0.2% AR in Laacherhof soil).

A re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) is performed in the report presented under point KCA 7.1.2.1.1/09 (M-763139-01-1).

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to study guideline(s) BBA: Part IV, 4-1 (similar to required guideline, minor differences). The study is considered valid to assess the aerobic degradation of [cyclohexyl-1-^{14}C] spiroxamine in soil.

Data Point:	KCA 7.1.1.1/03
Report Author:	[redacted]
Report Year:	1994
Report Title:	[Cyclohexyl-1-14C] KWG 4168 residues in following crops
Report No:	PF4043
Document No:	M-006096-01-1
Guideline(s) followed in study:	US EPA §165-1 Confined accumulation studies on rotational crops, 1983
Deviations from current test guideline:	Yes. OECD 502 guideline (January 2007) requires three plant-back intervals for succeeding crops. The longest interval of 270 to 365 days to represent crops sown the following year was not conducted in this study.
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2013)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A full summary of this study is provided under Point KCA 6.6.1/01. The study is also referenced at this location as potentially containing relevant information (i.e. information of the degradation of the active substance in soil). The study was also previously provided under this location and, therefore is provided for completeness. However, the study does not fulfil modern requirements and was not previously used of setting of endpoints and is therefore not addressed further.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to address a different data requirement, but potentially contains information relevant to Point KCA 7.1.1.1. The study is provided for completeness only and considered supplemental information only.

Data Point:	KCA 7.1.1.1/04
Report Author:	[redacted]
Report Year:	1997
Report Title:	Degradation and metabolism of KWG 4168 in soil aerobic soil metabolism
Report No:	PF4274
Document No:	M-006148-01-1
Guideline(s) followed in study:	US EPA Pesticide Assessment Guidelines, Subdivision N § 162-1: Aerobic soil metabolism studies (1982)
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The route and rate of degradation of spiroxamine was investigated in one US soil (Wolf Ranch, a loam) under laboratory aerobic conditions. Soil samples (100 g dw[3]) were treated with [cyclohexyl-1-^{14}C]-spiroxamine (radiochemical purity >99%) at an application rate of 0.89 mg/kg soil (equivalent to a single application of 668 g a.s./ha assuming 5 cm soil depth and 1.5 g/cm^3 soil density) and incubated at 20ºC and 75% of 1/3rd bar moisture (equivalent to 48% MWHC) for 360 days in the dark.

Duplicate samples were removed for analysis after 0, 1, 3, 7, 14, 30, 58, 90, 120, 181, 269 and 360 days of incubation. Soil samples were extracted three times at room temperature under agitation using acetonitrile for 30 minutes, and once using a SoxTec extraction system for 1 hour, using boiling methanol. Liquid extracts were radio-assayed by Liquid Scintillation Counting (LSC) directly. Extracted soil debris was air-dried and analysed by combustion LSC. Liquid extracts were analysed and quantified by normal phase TLC and quantitatively confirmed by reverse-phase TLC. Levels of parent spiroxamine were also confirmed by GC/MS and metabolite identity was confirmed by isolation and HPLC-MS/MS.

The material balances ranged from 95.1 to 100.5% AR. Levels of NER increased to maximum levels of 19.7% AR by DAT 360. Mineralization to carbon dioxide was a major pathway, demonstrated by the high amount of radioactivity recovered in the volatile traps for all soils (maximum of 53.5% AR by the end of the study). No significant levels of organic volatiles were observed.

After 360 days incubation at 20°C, [^{14}C]-spiroxamine degraded to 12.0% of the radioactivity applied. A re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) was performed in the report presented under point KCA 7.1.2.1.1/09 (M-763139-01-1).

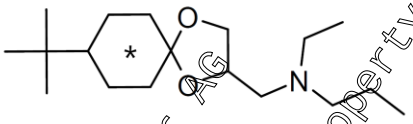
Two major soil metabolites were observed via de-alkylation of the amine moiety to form metabolite M01 (spiroxamine-desethyl, maximum 6.1% AR) and oxidation of the tertiary amine to form metabolite M03 (spiroxamine-N-oxide, maximum 7.9% AR). Other minor metabolites observed were M02 (spiroxamine-despropyl, maximum 4.2% AR) and M06 (spiroxamine-acid), M11 (spiroxamine-desethyl acid), M12 (spiroxamine-despropyl acid) and M15 (spiroxamine-ketone) all observed at levels <1% AR.

I. Materials and Methods

A. Materials

1. Test Items

[cyclohexyl-1-^{14}C]-spiroxamine



* Denotes position of [^{14}C]-radiolabel

Specific Activity:	3.63 MBq/mg
Radiochemical Purity:	>99% (HPLC)

2. Test Soil

The study was performed using one test soil as characterized in Table CA 7.1.1.1-12. No pesticide of the same chemical class was used for two years before sampling for this study.

Table CA 7.1.1.1-12: Physico-chemical properties of test soil

Parameter	Soil
Soil Designation	Wolf Ranch
Geographic Location	
City	Fresno, California
Country	USA
Textural Classification (USDA)	Loam
Sand [50 - 2000 µm] (%)	29.7
Silt [2 – 50 µm] (%)	45.1
Clay [< 2 µm] (%)	25.2
pH	
in H_2O (1:1)	7.8
in $CaCl_2$ (1:1)	8.7
Organic Matter (%) *	1.67
Organic Carbon (%)	0.97
Cation Exchange Capacity (meq/100 g)	19.0
Water Holding Capacity (g H_2O per 100g dry soil)	
40% of maximum	18.8
Soil Microbial Biomass (mg C/kg soil)	
Initial, untreated (0 DAT)	320
Mid point, treated (90 DAT)	290
Mid point, untreated (90 DAT)	272
Final, treated (360 DAT)	110
Final, untreated (360 DAT)	135

* Calculated by multiplying organic carbon content by 1.724 (not reported)

B. Study Design

1. Experimental Conditions

Tests were performed in sealed static systems consisting of glass flasks each containing 100 g soil (dry weight) with solid traps of oil-coated (2% paraffin oil in hexane) quartz glass wool for sorption of organic volatiles and soda lime for adsorption of evolved carbon dioxide.

The study was conducted at a concentration of approximately 0.89 mg/kg dry weight of soil. The test concentration was based on a single application of 2 kg a.s./ha (field rate of four applications of 500 g a.s./ha per year) assuming a 15 cm soil depth and 1.5 g/cm^3 soil density (note – this is equivalent to a single application of 668 g a.s./ha assuming 5 cm soil depth and 1.5 g/cm^3 soil density). Application of [^{14}C]-spiroxamine, dissolved in acetonitrile, was made to a subsample of each soil, which was then added to the bulk soil and mixed thoroughly, after solvent evaporation, for 2.5 hours before use in the experiment. After mixing, 100 g dry weight equivalent of soil was weighed into incubation vessels. Soil moisture was adjusted to 75% of 1/3 bar at the time of application (i.e. no pre-acclimatisation) and maintained at this level during incubation with periodic additions of water (30 days). The soil samples were incubated at a temperature of 20 ± 1°C under aerobic conditions.

Two treated (applied at the same rate) and three untreated soil samples were incubated alongside test systems under the same conditions to monitor microbial activity at the beginning, during and at the end of the study.

2. Sampling

Prior to opening the sealed static incubation vessels, either for sampling or maintenance of moisture, possibly volatile components were flushed into the trap attachment by introducing water saturated air (10 mins). Duplicate test samples were removed for analysis after 0, 1, 3, 7, 14, 30, 58, 90, 120, 181, 269 and 360 days of incubation.

Treated and untreated microbial biomass samples were analysed at 0, 90 and 360 days of incubation.

3. Analytical Procedures

Soil samples were extracted three times at ambient temperature under agitation with acetonitrile for 30 minutes and combined. Following this extraction series the remaining radioactivity in the soil was quantified by combustion. Subsequently, a second extraction was performed of the soil residue with boiling methanol for 60 minutes, and a methanol rinse for a further 60 minutes, both in a SoxTec system. Soil extracts were radio-assayed by Liquid Scintillation Counting (LSC). The soil residue remaining after the second soil extraction was re-quantified in the 1, 30, 120 and 270 days "A" replicates samples only; for the remaining samples it was calculated by difference (due to good agreement with measured values). The soil extracts from the first and second extraction procedures were analysed separately.

The combined ambient temperature soil extracts were analysed (without any prior work-up or concentration) using the main primary TLC normal phase method below against reference standards:

- Silica gel plates and an acetonitrile:water:25% ammonia (80:18:2, v/v/v) solvent system.

A quantitative confirmatory analysis was conducted using the following reverse phase TLC method:

- RP-18 plates with two solvent systems (used sequentially in 1 dimension):
  - n-hexane:dichloromethane:2-propyl alcohol:25% ammonia (30:70:10:2, v/v/v/v) and a saturated tank system (developed until solvent front at 14 cm)
  - chloroform:ethyl alcohol (50:50 v/v) and an unsaturated tank system (developed until solvent front at 6 cm)

The TLC plates were evaluated using a linear analyser to determine the quantities of radiolabelled test substance and its degradation products. Additional confirmatory analysis of soil extracts to confirm spiroxamine and metabolites by MS was also conducted on single replicates of samples from 90 DAT (spiroxamine and M03 (spiroxamine-N-oxide)) and 120 DAT (M01 (spiroxamine-desethyl) and M02

(spiroxamine-despropyl)). These samples extracts were concentrated and chromatographed using the normal phase TLC method above, excised from the TLC plate, extracted and analysed by GC/MS (spiroxamine) or HPLC/MS/MS (metabolites) to confirm identity.

Volatile radioactivity in volatile traps and polyurethane plugs was extracted using ethyl acetate and aliquots quantified by LSC. $^{14}CO_2$ was liberated from soda lime through addition of 18% (w/w) hydrochloric acid and absorbed by a suitable cocktail and quantified by LSC. Identity of $^{14}CO_2$ was further confirmed by conversion to [^{14}C]-benzoic acid by means of the Grignard reaction.

4. Determination of degradation kinetics

The degradation kinetics of data within this report are subject of a separate modelling study. DT_{50} and DT_{90} values for the degradation of spiroxamine and its metabolites have been re-calculated from the reported data following the recommendations of the FOCUS work group (FOCUS 2014[2]). Full details are provided in in the report under point KCA 7.1.2.1.1/09 (M-763139-01-1).

II. Results and Discussion

A. Data

The distribution and characterisation of radioactivity for the test soils incubated at 20°C following application of [^{14}C]-spiroxamine is summarised in Table CA 7.1.1.1-13.

Table CA 7.1.1.1-13: Degradation of [^{14}C]-spiroxamine in Wolf Ranch soil at 20°C under aerobic conditions [% AR]

Compound	Rep.	Incubation time (DAT)											
		0	**1**	**3**	**7**	**14**	**30**	**58**	**90**	**120**	**181**	**269**	**360**
Spiroxamine	A	88.0	83.2	79.0	79.3	75.3	66.3	50.2	40.8	33.0	21.5	14.7	11.9
	B	85.3	79.8	81.3	79.4	79.2	70.5	55.2	40.7	34.3	21.7	12.7	12.1
	Mean	**86.7**	**81.5**	**80.2**	**79.4**	**77.3**	**68.4**	**52.7**	**40.8**	**33.7**	**21.6**	**13.7**	**12.0**
M01 (spiroxamine-desethyl)	A	n.d.	0.2	0.7	1.0	2.5	3.7	4.8	6.2	5.3	3.7	3.3	3.8
	B	0.2	0.1	0.7	0.9	2.8	4.1	4.9	5.9	5.1	4.2	2.8	3.7
	Mean	**0.1**	**0.2**	**0.7**	**1.0**	**2.7**	**3.9**	**4.9**	**6.1**	**5.2**	**4.0**	**3.1**	**3.8**
M02 (spiroxamine-despropyl)	A	n.d.	0.1	0.5	0.4	1.8	2.3	3.0	4.3	3.9	2.4	2.6	2.7
	B	0.1	0.1	0.2	0.4	1.8	2.3	3.4	4.0	3.9	2.9	2.2	2.8
	Mean	**0.1**	**0.1**	**0.4**	**0.4**	**1.8**	**2.3**	**3.2**	**4.2**	**3.9**	**2.7**	**2.4**	**2.8**
M03 (spiroxamine-N-oxide)	A	n.d.	0.1	0.5	1.4	2.9	5.0	6.2	8.0	7.3	7.4	5.6	6.6
	B	n.d.	n.d.	0.5	0.9	3.2	5.3	6.4	7.5	7.5	8.3	5.4	6.6
	Mean	**-**	**0.1**	**0.5**	**1.2**	**3.1**	**5.2**	**6.3**	**7.8**	**7.4**	**7.9**	**5.5**	**6.6**
M06 (spiroxamine-acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	0.3	0.3	0.1	0.5	0.3
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	0.2	0.4	0.2	0.3	n.d.
	Mean	**-**	**-**	**-**	**-**	**-**	**-**	**0.1**	**0.3**	**0.4**	**0.2**	**0.4**	**0.2**
M11 (spiroxamine-desethyl acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	0.1	0.1	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	0.3	0.1	0.1
	Mean	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.2**	**0.2**	**0.1**	**0.1**
M12 (spiroxamine-despropyl acid)	A	n.d.	0.1	0.1	n.d.	n.d.	n.d.	0.1	0.1	n.d.	n.d.	0.3	0.1
	B	n.d.	0.1	n.d.	n.d.	n.d.	n.d.	0.1	0.3	0.2	0.2	0.2	0.2
	Mean	**-**	**0.1**	**0.1**	**-**	**-**	**-**	**0.1**	**0.2**	**0.1**	**0.1**	**0.3**	**0.2**
M15 (spiroxamine-ketone)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5	n.d.	0.6	1.3	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2	n.d.	n.d.	1.3	n.d.
	Mean	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.4**	**-**	**0.3**	**1.3**	**-**
Unknown 1	A	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	0.2	0.4	0.4	1.9	0.6	0.6

Compound	Rep.	Incubation time (DAT)											
		0	**1**	**3**	**7**	**14**	**30**	**58**	**90**	**120**	**181**	**269**	**360**
	B	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	0.2	0.5	0.4	0.3	0.6	0.6
	Mean	**-**	**-**	**-**	**-**	**-**	**0.1**	**0.2**	**0.5**	**0.4**	**1.1**	**0.6**	**0.6**
Unknown 2	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	0.1
	Mean	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**		
Ambient extract (including filter papers) [A]	A	66.6	66.0	65.4	67.0	66.5	62.0	48.4	42.3	36.5	26.2	19.5	19.4
	B	64.4	63.8	67.2	65.6	66.5	62.1	52.4	42.3	37.5	26.6	17.2	19.7
	Mean	**65.5**	**64.9**	**66.3**	**66.3**	**66.5**	**62.1**	**50.4**	**42.3**	**37.0**	**26.4**	**18.4**	**19.6**
Hot extract	A	21.5	17.7	15.5	15.1	16.0	15.4	16.1	18.3	14.1	11.8	9.6	6.7
	B	21.2	16.4	15.6	16.0	20.6	20.2	17.8	16.9	14.5	11.5	8.7	6.6
	Mean	**21.4**	**17.1**	**15.6**	**15.6**	**18.3**	**17.8**	**17.0**	**17.6**	**14.3**	**11.7**	**9.2**	**6.7**
Total extractable radioactivity	A	88.1	83.7	80.9	82.1	82.5	77.4	64.5	60.6	50.6	38.0	29.1	26.1
	B	85.6	80.2	82.8	81.6	87.1	82.3	70.2	59.2	52.0	38.1	25.9	26.3
	Mean	**86.9**	**82.0**	**81.9**	**81.9**	**84.8**	**79.9**	**67.4**	**59.9**	**51.3**	**38.1**	**27.5**	**26.2**
Non-extractable radioactivity (including filter papers) [A]	A	10.7	15.7[B]	16.0	15.2	14.1	15.9[B]	16.2	16.9	18.1[B]	19.3	21.5[B]	19.6
	B	10.2	14.7	17.6	14.4	9.6	12.9	15.1	18.2	16.6	19.3	20.4	19.8
	Mean	**10.5**	**15.2**	**16.8**	**14.8**	**11.9**	**14.4**	**15.7**	**17.6**	**17.4**	**19.3**	**21.0**	**19.7**
^{14}C-Carbon dioxide including other volatiles [C]	A	n.a.	0.1	0.3	0.9	1.9	5.0	12.2	19.6	28.5	41.3	52.4	53.8
	B	n.a.	0.1	0.3	0.9	1.9	4.9	12.0	18.5	29.0	40.2	51.6	53.2
	Mean	**n.a.**	**0.1**	**0.3**	**0.9**	**1.9**	**5.0**	**12.1**	**19.1**	**28.8**	**40.8**	**52.0**	**53.5**
Total radioactivity	A	98.8	97.7	97.2	98.2	98.5	98.7	92.9	97.1	95.7	98.6	103.1	99.5
	B	95.8	95.0	100.7	96.9	98.6	100.1	97.3	95.9	97.6	97.6	97.9	99.3
	Mean	**97.3**	**96.4**	**99.0**	**97.6**	**98.6**	**99.4**	**95.1**	**96.5**	**96.7**	**98.1**	**100.5**	**99.4**

Rep.: Replicate, n.a.: not analysed, n.d.: not detected above LOD, DAT: days after treatment, LOD: 0.1% AR
A Max filter paper value of 1.8%AR
B Other volatile radioactivity was max 0.2% AR for replicate A at 58 DAT. All other timepoints were <0.1% AR.

B. Material Balance

Material balances ranged from 95.1 to 100.5% AR in Wolf Ranch soil.

C. Extractable and Non-Extractable Residues

For samples of Wolf Ranch soil total extractable radioactivity decreased from 86.9% AR at DAT 0 to 26.2% AR by DAT 360. The total of non-extractable residues (NER) increased from 10.5% AR at DAT 0 to 19.7% AR by the end of the study (DAT 360).

D. Volatile Radioactivity

Levels of ^{14}C-carbon dioxide increased to 53.5% AR at the end of the study. Other volatile radioactivity was < 0.1% AR at all timepoints, except for a single replicate at DAT 58 of 0.2% AR. This is included in the ^{14}C-carbon dioxide values.

E. Degradation of Parent Compound

Following application of [^{14}C]-spiroxamine in Wolf Ranch soil, the amount of parent in the total soil extracts decreased from a maximum of 86.7% AR at DAT 0 to 12.0% AR by DAT 360. In addition to parent material, M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl), M03 (spiroxamine-N-oxide), M06 (spiroxamine-acid), M11 (spiroxamine-desethyl acid), M12 (spiroxamine-despropyl acid) and M15 (spiroxamine-ketone) were detected. Levels of M01 (spiroxamine-desethyl) increased over the course of the incubation period, and accounted for a maximum of 6.1% AR at DAT 90. M02 (spiroxamine-despropyl) was detected at a maximum of 4.2% AR at DAT 90. M03 (spiroxamine-N-oxide) was

detected at a maximum of 7.9% of AR at DAT 181. M06 (spiroxamine-acid) was detected at a maximum of 0.4% AR at DAT 120 (and DAT 269). M11 (spiroxamine-desethyl acid) was detected at a maximum of 0.2% AR at DAT 120 (and DAT 181). M12 (spiroxamine-despropyl acid) was detected at a maximum of 0.3% AR at DAT 269. M15 (spiroxamine-ketone) was detected at a maximum of 1.3% AR at DAT 269.

F. Degradation kinetics

Degradation rates determined in the report are superseded by the report presented under point KCA 7.1.2.1.1/09 (M-763139-01-1).

III. Conclusions

Spiroxamine degraded in Wolf Ranch soil under aerobic conditions at 20°C, with 12.0% of the applied radioactivity remaining as parent compound after 360 days. The amounts of unextractable radioactivity increased to an overall maximum of 21.0% AR by DAT 269 and subsequently declined to 19.7% AR by DAT 360. No significant levels of organic volatiles were observed. Mineralisation to carbon dioxide was a significant pathway, steadily increasing throughout the study and accounting for an overall maximum of 53.5% AR after 360 days. The primary metabolic pathway involved de-alkylation of the amine moiety to form two major metabolites, M01 (spiroxamine-desethyl, maximum 6.8% of AR at DAT 90) and M02 (spiroxamine-despropyl, maximum 4.2% AR). An additional major metabolite observed was M03 (spiroxamine-N-oxide, maximum 7.9% AR), formed by oxidation of the tertiary amine. Other metabolites formed include M06 (spiroxamine-acid, maximum 0.4% AR), M11 (spiroxamine-desethyl acid, maximum 0.2% AR), M12 (spiroxamine-despropyl acid, maximum 0.3% AR) and M15 (spiroxamine-ketone, maximum 1.3% AR).

A re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) is performed in the report presented under point KCA 7.1.2.1.1/09 (M-763139-01-1).

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to study guideline(s) US EPA Pesticide Assessment Guidelines, Subdivision N § 162-1: Aerobic soil metabolism studies (1982) (similar to required guideline, minor differences). The study is considered valid to assess the aerobic degradation of [cyclohexyl-1-^{14}C]-spiroxamine in soil.

Data Point:	KCA 7.1.1.1/05
Report Author:	[redacted]
Report Year:	2008
Report Title:	[1,3-Dioxolane-4-14C]spiroxamine: Metabolic screening for degradation pathways under aerobic conditions in soil
Report No:	MEF-08/214
Document No:	M-303803-01-1
Guideline(s) followed in study:	EU 95/36/EC amending 91/414/EEC; OECD 307; US EPA, Subdivision N, Section 162-1
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The route and rate of degradation of spiroxamine was investigated in one European soil (Hoefchen am Hohenseh, silt loam) under laboratory aerobic conditions. Soil samples (100 g dry weight) were treated with [1,3-dioxolane-4-^{14}C]-spiroxamine (radiochemical purity 100% at the time of application) at an application rate of 2.09 mg/kg dry weight of soil (equivalent to nominal 1500 g/ha assuming 5 cm soil depth and 1.5 g/cm^3 soil density) and incubated (20°C, 55% maximum water holding capacity) for 120 days in the dark.

Duplicate samples were removed for analysis after 0, 1, 3, 7, 16, 31, 58, 88 and 120 days of incubation. Soil samples were extracted three times at room temperature under agitation using acetonitrile/water for 30 minutes, and twice under reflux for 30 minutes, also using acetonitrile/water. Liquid extracts were analysed by reverse-phase HPLC, confirmed by normal phase TLC, run against reference standards to confirm metabolite identity.

The material balances ranged from 98.0 to 103.4% of applied radioactivity (% AR). Non-extractable radioactivity increased to 33.1% AR by DAT 120. No significant levels of organic volatiles were observed. Mineralisation to carbon dioxide was a major pathway, steadily increasing and accounting for 40.5% AR by DAT 120.

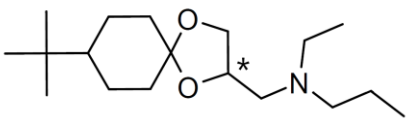
After 120 days incubation at 20°C, [^{14}C]-spiroxamine degraded to 8.2% AR. A DT_{50} for spiroxamine was given in the report, however, a re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) was performed in the report presented under point KCA 7.1.2.1.1/09 (M-763139-01-1).

Two major metabolites were observed formed by de-alkylation of the amine moiety: M01 (spiroxamine-desethyl, maximum 7.9% of AR); M02 (spiroxamine-despropyl, maximum 9.2% AR at DAT 31). No other metabolites were observed >2.9% AR.

I. Materials and Methods

A. Materials

1. Test Items



* Denotes position of [^{14}C]-radiolabel

[1,3-dioxolane-4-^{14}C]-spiroxamine

Specific Activity: 4.09 MBq/mg

Radiochemical Purity: 100% (as measured at time of application to the soils)

2. Test Soil

The study was performed using one test soil as characterized in Table CA 7.1.1.1-14, which had not been treated with any pesticides within the previous five years.

Table CA 7.1.1.1-14: Physico-chemical properties of test soil

Parameter		Soil
Soil Designation		**Hoefchen am Hohensch**
Geographic Location		
City		Burscheid, North Rhine-Westfalia
Country		Germany
Textural Classification (USDA)		Silt loam
Sand [50 - 2000 µm]	(%)	11.0
Silt [2 – 50 µm]	(%)	70.9
Clay [< 2 µm]	(%)	18.1
pH		
in H_2O (1:1)		7.0
in 0.01M $CaCl_2$ (1:1)		6.6
Organic Matter (%)*		4.10
Organic Carbon (%)		2.38
Cation Exchange Capacity (meq/100g)		16.3
Water Holding Capacity (g H_2O per 100g dry soil)		
Maximum		55.1
0.33 bar		25.0
Soil Microbial Biomass (mg C/kg soil)		
Initial, untreated (0 DAT)		1217
Final, untreated (122 DAT)		682
Final, solvent only (122 DAT)		602

* Calculated by multiplying organic carbon content by 1.724 (not reported)

B. Study Design

1. Experimental Conditions

The study was performed in static systems consisting of flasks each containing 100 g dry weight of soil attached to solid traps containing a polyurethane plug for adsorption of organic volatiles and soda lime for adsorption of carbon dioxide. Soil moisture was adjusted to 55% MWHC. Soil samples were pre-acclimatised to the study conditions (dark, 20°C) for 5 days prior to application of the test substance.

The study was conducted at a nominal soil concentration of 2.0 mg/kg dry weight of soil (actual study concentration was 2.09 mg/kg dw). The nominal test concentration was based on a field rate of 750 g a.s./ha assuming 2.5 cm soil depth and 1.5 g/cm^3 soil density (note - this is equivalent to a field rate of 1500 g a.s./ha, assuming a mixing depth of 5 cm and a soil density of 1.5 g/cm^3). [^{14}C]-spiroxamine dissolved in methanol and diluted with an equal volume of analytical grade water was applied dropwise to soil in each vessel, which were then shaken for 30 minutes to allow evaporation of solvent and mixing. Soil moisture was maintained monthly. Samples were incubated over 120 days in the dark at 20.1 ± 0.2°C and aerobic conditions.

Two untreated samples and one sample treated with solvent only were incubated alongside test systems under the same conditions to monitor microbial biomass at the start and end of the study.

2. Sampling

Duplicate vessels were removed for analysis after 0, 1, 3, 7, 16, 31, 58, 88 and 120 days of incubation.

Untreated microbial biomass samples were analysed at the beginning and end of the experiment. The solvent treated biomass sample was analysed at the end of the experiment.

3. Analytical Procedures

Soil samples were extracted three times at room temperature under agitation using acetonitrile/water (80:20, v/v) for 30 minutes, and twice under reflux for 30 minutes, also using acetonitrile/water (80:20, v/v). Liquid extracts from ambient and reflux were centrifuged and combined separately, concentrated and analysed by reverse phase HPLC using a Purospher RP18e column and a gradient system using mobile phases 0.5% triethylamine in water / 0.5% triethylamine in acetonitrile (0 and 1 DAT only) or 0.05% triethylamine in water / 0.05% triethylamine in acetonitrile (all other timepoints). Samples were run against reference standards for identification purposes. The effluent was passed through a UV detector (210 + 230 + 254 nm) to detect reference standards, and a radioactivity detector to determine the quantities of radiolabelled test substance and its degradation products.

Confirmatory analysis was performed using TLC with silica gel plates and an automated multiple development method using mobile phases of methanol + 1% NH3 (25%) and dichloromethane, again run against reference standards for identification. TLC plates were evaluated using a linear analyser to determine the quantities of radiolabelled test substance and its degradation products.

Volatile radioactivity in volatile traps was extracted using ethyl acetate and aliquots quantified by LSC. $^{14}CO_2$ was liberated from soda lime through addition of 18% (w/w) hydrochloric acid. Identity of $^{14}CO_2$ was determined by conversion to [^{14}C]-barium carbonate.

4. Determination of degradation kinetics

The degradation kinetics of data within this report are subject of a separate modelling study. DT_{50} and DT_{90} values for the degradation of spiroxamine and its metabolites have been re-calculated from the reported data following the recommendations of the FOCUS work group (FOCUS 2014[2]). Full details are provided in in the report under point KCA 7.1.2.1.1/09 (M-763139-01-1).

II. Results and Discussion

A. Data

The distribution and characterisation of radioactivity for the test soils incubated at 20°C following application of [^{14}C]-spiroxamine are summarized in Table CA 7.1.1.1-15.

Table CA 7.1.1.1-15: Mass balance of [^{14}C]-spiroxamine in Hoefchen am Hohenseh soil at 20°C under aerobic conditions [% AR]

Compound	Replicate	Incubation time (DAT)								
		0	1	3	7	16	31	58	88	120
Spiroxamine	A	89.6	81.5	64.7	55.3	42.0	27.2	22.7	12.9	7.7
	B	89.1	82.0	62.6	54.8	40.9	28.6	21.5	10.8	8.6
	Mean	89.3	81.8	63.7	55.1	41.5	27.9	22.1	11.9	8.2
HPLC-P1	A	n.d.	n.d.	1.3	1.9	3.8	4.6	2.1	3.5	3.7
	B	n.d.	n.d.	1.4	2.0	3.4	4.0	2.4	3.3	3.6
	Mean	n.d.	n.d.	1.3	1.9	3.6	4.3	2.2	3.4	3.6
HPLC-P2	A	0.6	0.8	2.2	2.3	2.6	1.4	0.2	n.d.	n.d.
	B	0.6	0.9	2.1	2.6	3.1	1.3	0.3	n.d.	0.2
	Mean	0.6	0.8	2.1	2.5	2.8	1.4	0.3	n.d.	0.1
HPLC-P3	A	0.3	1.1	1.5	1.3	1.1	1.2	0.5	0.4	0.2
	B	0.2	1.0	1.4	1.8	1.2	1.1	0.3	0.2	0.2
	Mean	0.3	1.0	1.4	1.6	1.2	1.1	0.4	0.3	0.2
HPLC-P4	A	0.4	1.1	1.7	1.3	1.5	1.3	0.6	0.4	n.d.
	B	0.4	1.2	2.3	1.4	1.7	1.4	0.4	0.4	n.d.
	Mean	0.4	1.1	2.0	1.4	1.6	1.3	0.5	0.4	n.d.
HPLC-P5	A	0.4	1.3	1.7	1.6	1.7	1.8	0.5	0.4	0.4
	B	0.6	1.1	1.9	2.6	1.2	1.8	0.5	0.3	0.5
	Mean	0.5	1.2	1.8	2.1	1.5	1.8	0.5	0.3	0.5
HPLC-P6	A	0.3	1.2	1.8	1.5	1.4	1.5	0.6	0.5	n.d.
	B	0.7	1.0	1.9	1.7	1.5	1.4	0.6	0.3	0.3
	Mean	0.5	1.1	1.8	1.6	1.4	1.5	0.6	0.4	0.1
M01 (spiroxamine-desethyl)	A	0.3	1.5	2.7	4.4	6.2	7.3	8.0	6.0	3.9
	B	0.5	1.7	2.9	4.8	6.0	7.2	7.7	4.7	4.9
	Mean	0.4	1.6	2.8	4.6	6.1	7.2	7.9	5.3	4.4
M02 (spiroxamine-despropyl) combined isomers A + B	A	0.6	n.d.	3.0	4.5	6.1	9.4	7.3	6.9	4.1
	B	0.7	0.7	2.8	4.8	6.9	9.0	7.8	4.9	5.3
	Mean	0.6	0.7	2.9	4.7	6.6	9.2	7.5	5.9	4.8
M02 (spiroxamine-despropyl) isomer A (cis)	Mean	0.3	0.4	1.4	2.5	3.6	5.2	4.1	3.3	2.9
M02 (spiroxamine-despropyl) isomer B (trans)	Mean	0.3	0.4	1.5	2.2	3.0	4.0	3.4	2.5	1.9
Ambient extract	Mean	74.6	71.1	55.9	54.4	50.8	45.5	36.9	25.3	19.9
Reflux extract	Mean	17.9	18.4	24.1	21.3	16.7	12.5	7.1	5.3	4.6

Compound	Replicate	Incubation time (DAT)								
		0	1	3	7	16	31	58	88	120
Total extractable radioactivity [A]	Mean	92.5	89.5	80.0	75.7	67.6	58.0	44.0	30.6	24.5
Non-extractable radioactivity	Mean	10.9	11.7	19.0	23.1	24.4	26.1	30.3	34.1	33.1
^{14}C-Carbon dioxide including other volatiles [B]	Mean	n.a.	0.2	0.8	2.0	6.9	13.8	24.6	34.5	40.5
Total radioactivity	Mean	103.3	101.3	99.7	100.9	98.9	97.9	98.8	99.1	98.1

n.a.: not analysed, n.d.: not detected above LOD, DAT: days after treatment, LOD: 0.2% AR
A Sum of ambient and reflux extracts
B Other volatile radioactivity was <0.1%AR

Table CA 7.1.1.1-16: Characterisation of [^{14}C]-spiroxamine in soil after 120 days at 20°C under aerobic conditions

Total bound residues (%AR)	Humin (% of bound residues)	Humic acid (% of bound residues)	Fulvic acid (% of bound residues)
33.9	46.5	29.5	24.4

B. Material Balance

Material balances ranged from 98.0 to 103.4% AR.

C. Extractable and Non-Extractable Residues

Total extractable radioactivity decreased from 92.6% AR at DAT 0 to 24.5% AR by DAT 120. The total of non-extractable residues (NER) increased from 10.9% AR at DAT 0 to a maximum of 34.1% AR by DAT 88 and then declined to 33.1% AR by the end of the study (DAT 120). Further characterisation of NER in a selected soil sample is presented in Table CA 7.1.1.1-16, the majority of applied radioactivity was associated with the humin fraction.

D. Volatile Radioactivity

Levels of ^{14}C-carbon dioxide formed ranged from 0.2% AR (DAT 1) to 40.5% AR (DAT 120) during incubation. Other volatile radioactivity was < 0.1% AR at all timepoints.

E. Degradation of Parent Compound

Following application of [^{14}C]-spiroxamine the amount of parent in the total soil extracts decreased from a maximum of 89.4% AR at DAT 0 to 8.2% AR by DAT 120. In addition to parent material, two major metabolites were observed. M01 (spiroxamine-desethyl) accounted for a maximum of 7.9% of applied radioactivity at DAT 58 and M02 (spiroxamine-despropyl) accounted for a maximum of 9.2% AR at DAT 31. No other metabolites were observed >2.9% AR.

F. Degradation kinetics

Degradation rates determined in the report are superseded by the report presented under point KCA 7.1.2.1.1/09 (M-763139-01-1).

III. Conclusions

Spiroxamine degraded in Hoefchen am Hohenseh soil under aerobic conditions at 20°C, with 8.2% AR remaining as parent compound after 120 days. The amount of unextractable radioactivity increased to a maximum of 34.1% AR by DAT 88 and then declined to 33.1% AR by the end of the study (DAT 120). Mineralisation to carbon dioxide was a major pathway, steadily increasing throughout the study, accounting for 40.5% AR at DAT 120. The primary metabolic pathway involved de-alkylation of the amine moiety to form M01 (spiroxamine-desethyl, maximum 7.9% AR, DAT 58), M02 (spiroxamine-despropyl, maximum 9.3% AR at DAT 31). No other metabolites were observed >2.9% AR.

A re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) is performed in the report presented under point KCA 7.1.2.1.1/09 (M-763139-01-1).

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to study guideline(s) OECD 307 (required guideline). The study is considered valid to assess the aerobic degradation of [1,3-dioxolane-4-^{14}C]-spiroxamine in soil.

CA 7.1.1.2 Anaerobic degradation

In view of the proposed use pattern for spiroxamine as a post-emergence spray applied fungicide for application to cereals crops and vines, exposure to anaerobic conditions in soil is not envisaged. However, the degradation of spiroxamine in anaerobic soil has been investigated in one study (KCA 7.1.1.2/01) which was evaluated during the previous EU review. As the existing study was not fully conducted according to the required study guideline, one new study has been conducted to fulfil this requirement. The new study was also conducted to address the new requirements of EFSA, 2019[1].

Substance	Report reference	Document no.	Test material used	Comment
Spiroxamine	KCA 7.1.1.2/01; KCA 7.1.2.1.3/01	M-006010-02-1	[cyclohexyl-1-^{14}C]-spiroxamine	Submitted for first approval of spiroxamine, 1999. Reviewed under UP. Considered valid and acceptable.
Spiroxamine	KCA 7.1.1.2/02; KCA 7.1.2.1.3/02	M-762348-01-1	[cyclohexyl-1-^{14}C]-spiroxamine	New data not yet reviewed under UP.

The anaerobic degradation of spiroxamine in soil has been investigated in two studies at 20°C. The new anaerobic soil degradation study was conducted with [cyclohexyl-1-^{14}C]-spiroxamine only, however, as essentially no degradation was observed under actual anaerobic conditions this is considered sufficient to fully investigate the degradation pathway.

New studies, not previously evaluated

Data Point:	KCA 7.1.1.2/02
Report Author:	[redacted]; [redacted]
Report Year:	2021
Report Title:	[14C]-spiroxamine: Route and rate of degradation in soil under anaerobic conditions at 20°C
Report No:	VC/19/053
Document No:	M-762348-01-1
Guideline(s) followed in study:	Commission Regulation (EU) No. 283/2013in accordance with Regulation (EC) No. 1107/2009 OECD 307
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The route and rate of degradation of spiroxamine was investigated in one European soil (Refesol-02-A, silt loam) under laboratory anaerobic conditions. Soil samples (100 g dw[3]) were treated with [cyclohexyl-1-^{14}C]-spiroxamine (radiochemical purity 99.6%) at a nominal application rate of 2.0 mg/kg soil dry weight (reported as equivalent to 750 g/ha assuming a mixing depth of 2.5 cm and soil density of 1.5 g/cm^3, otherwise equivalent to 1500 g/ha assuming a mixing depth of 5 cm) and incubated (20°C, pF 2) for 30 days under aerobic conditions in the dark before being flooded to a 2 cm depth of water.

Duplicate samples for each soil were removed for analysis after 0, 10 and 30 days after treatment (DAT) and for 7, 14, 31, 75 and 118 days post flooding (DPF). Overlying water (post flooding only) was decanted from the underlying soil and combined with acetonitrile. Soil samples were extracted four times at room temperature with acetonitrile/water/35% ammonia solution 80/20/1 v/v/v, by addition of solvent, vigorous mechanical shaking and centrifugation. The overlying water and soil extracts were combined and analysed, without concentration, primarily by reverse-phase HPLC. Confirmatory analysis was conducted by normal-phase TLC on selected samples. Degradation products were also confirmed using LC-MS on selected samples.

The material balances ranged from 100.6 to 109.4% AR indicating a complete mass balance. Under the initial aerobic conditions, the amount of unextractable radioactivity increased to 6.5% AR and mineralisation to carbon dioxide increased to 7.1-15.8% AR by DAT 30. Post flooding, unextractable radioactivity from the soil increased to 10.5% AR and mineralisation to carbon dioxide increased to 15.6% AR by DPF 118. No significant levels of organic volatiles were observed.

Following application of [cyclohexyl-1-^{14}C]-spiroxamine, the amount of parent in the soil extracts decreased from 99.9% AR at DAT 0 to 65.7% AR by DAT 30 (time of flooding). During the flooded phase, the amount of parent in the combined overlying water and soil extracts did not change from a total of 62.3% AR at DPF 7 to 62.6% AR at DPF 118. There was no appreciable decline during the flooded phase in the level of spiroxamine. No further kinetic evaluation was conducted, spiroxamine is stable under anaerobic conditions.

As such, under flooded conditions degradation of [cyclohexyl-1-^{14}C]-spiroxamine was minimal and there was no appreciable increase in the level observed of any metabolites.

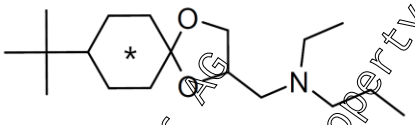
[3] dry-weight

I. Materials and Methods

A. Materials

1. Test Items

[cyclohexyl-1-^{14}C]-spiroxamine



* Denotes position of [^{14}C]-radiolabel

Specific Activity: 4.26 MBq/mg (34.4 mCi/mmol, 1273 MBq/mmol)

Radiochemical Purity: >99.4% (HPLC)

2. Test System (soil)

The study was performed using one test soil as characterised in Table CA 7.1.1.1-1. The test soil was from the same batch as that used for study KCA 7.1.1.1/06 (M-762349-01-1).

Table CA 7.1.1.2-1: Physico-chemical properties of test soil

Parameter	Soil
Soil Designation:	**Retesol-02-A**
Geographic Location	
City	Northrhine, Westphalia [B]
Country	Germany
Textural Classification (USDA)	Silt loam
Sand [50 - 2000 µm] (%)	16
Silt [2 – 50 µm] (%)	66
Clay [< 2 µm] (%)	18
pH	
in H_2O (1:1)	7.1
in 0.01M $CaCl_2$ (1:1)	6.7
Organic Matter (%)*	1.7
Organic Carbon (%)	1.0
Cation Exchange Capacity (meq/100 g)	11.4
Water Holding Capacity (g H_2O per 100 g dry soil)	
pF 2.0 (0.1 bar, w/w %)	35.9
pF 2.5 (0.33 bar, w/w %)	19.9
Soil Microbial Biomass (mg org C/100 g soil) [A]	
Initial, DAT 0	15.2 (1.5)
Final, 100 DAT 120	0.49 (0.5)

* Calculated by multiplying organic carbon content by 1.724 (not reported)
A Biomass as a percentage of organic carbon content provide in parentheses
B Location known free of pesticide use for previous 3 years and used within 3 months from sampling.

The test soils were handled in accordance with ISO 18400-102 and 105 prior to use.

B. Study Design

1. Experimental Conditions

Tests were performed in flow through systems consisting of glass flasks each containing 100 g soil (dry weight) and attached to an ethylene glycol trap to collect organic volatiles followed by two potassium hydroxide traps (2M) to collect carbon dioxide.

Soil samples (100 g) were pre-acclimatised to the initial aerobic incubation conditions (dark, 20°C and pF 2 moisture content) for 13-15 days before application of the test substance (within 3 months of sampling). The study was conducted at a concentration of 2.0 mg/kg dry weight of soil (reported as equivalent to 750 g/ha assuming a mixing depth of 2.5 cm and soil density of 1.5 g/cm^3, otherwise equivalent to 1500 g/ha assuming a mixing depth of 5 cm). Application of [cyclohexyl-1-^{14}C]-spiroxamine in a solvent (acetonitrile, 201 µL) was made to the soil surface. Soil samples were lightly agitated after application to aid distribution throughout the soil and to allow solvent evaporation.

After 30 days (at the time the study was being conducted, information was available from the first few sampling intervals of the associated study KCA 7.1.1.1/06 (M-762349-01-1) which indicated that the degradation rate of the active substance in Refesol-02-A soil under aerobic conditions was >30 days), the soil samples were flooded to a 2 cm depth of water.

Additional samples for each soil were treated with an equivalent amount of blank solvent only to monitor microbial activity at the beginning and end of the incubation period.

2. Sampling

Duplicate samples for each soil were removed for analysis after 0, 10 and 30 days after treatment (DAT) and for 7, 14, 31, 75 and 128 days post flooding (DPF). Untreated samples were analysed for biomass at the beginning and end of the experiment.

3. Analytical Procedures

Overlying water (post flooding only) was decanted from the underlying soil and combined with acetonitrile. Soil samples were extracted four times at room temperature with acetonitrile/water/35% ammonia solution 80/20/1 v/v/v by addition of solvent, vigorous mechanical shaking and centrifugation. Radioactivity in extracts was determined by liquid scintillation counting (LSC). The overlying water and soil extracts were combined and analysed (without concentration) by HPLC with radio-detection. Degradation products were identified by comparison of the retention times of reference standards. Confirmatory analysis using an alternative technique was conducted by TLC with co-chromatography against reference items on selected samples. Degradation products were also confirmed using LC-MS on selected samples.

Volatile radioactivity in volatile traps was quantified by LSC. Any radioactivity in the polyurethane foam bung was extracted using acetonitrile and quantified by LSC. Carbon dioxide in the potassium hydroxide traps was confirmed by barium carbonate precipitation.

Following homogenisation, non-extractable residues (NER) in extracted soils were determined by combustion.

4. Determination of degradation kinetics

No significant degradation of parent spiroxamine was observed under anaerobic conditions and therefore no degradation rate could be calculated (assumed stable under anaerobic conditions).

II. Results and Discussion

A. Data

The distribution and characterisation of radioactivity for the test soils incubated at 20°C following application of [cyclohexyl-1-^{14}C]-spiroxamine are summarized in Table CA 7.1.1.2-2.

Table CA 7.1.1.2-2: Degradation of [cyclohexyl-1-^{14}C]-spiroxamine at 20°C in Refesol-02-A soil under anaerobic conditions [% AR]

Compound	Replicate	Incubation time (DAT)							
		DAT			DPF				
		0	10	30	7	14	31	75	118
Spiroxamine	A	100.1	74.3	64.4	61.5	65.2	63.1	65.0	61.9
	B	99.7	76.5	67.0	62.9	65.7	60.9	55.3	63.3
	Mean	99.9	75.4	65.7	62.2	65.5	62.0	60.2	62.6
M01 (desethyl)	A	0.5	4.2	7.1	7.1	6.7	8.2	7.3	7.7
	B	0.2	4.4	6.9	7.1	7.1	7.1	6.6	6.1
	Mean	0.3	4.3	7.0	7.1	6.9	7.6	6.9	6.9
M02 (despropyl)	A	0.4	3.1	5.1	5.4	4.9	6.4	4.6	4.8
	B	0.5	2.8	5.4	5.1	5.8	5.2	4.3	4.6
	Mean	0.4	3.0	5.3	5.3	5.4	5.8	4.4	4.7
M03 (N-oxide)	A	n.d.	3.5	3.3	3.3	3.0	1.6	n.d.	n.d.
	B	0.2	3.1	3.2	3.2	1.0	2.6	n.d.	n.d.
	Mean	0.1	3.3	3.3	3.2	2.0	1.8	n.d.	n.d.
M06 (acid)	A	n.d.	6.1	5.8	5.6	4.8	4.3	6.2	7.7
	B	n.d.	6.0	5.2	5.4	4.6	6.4	13.6	7.8
	Mean	n.d.	6.1	5.5	5.5	4.7	5.4	9.9	7.7
M11 (desethyl acid)	A	n.d.	0.3	n.d.	1.3	n.d.	1.0	n.d.	n.d.
	B	n.d.	n.d.	0.4	1.2	0.9	0.8	2.1	n.d.
	Mean	n.d.	0.2	0.2	1.3	0.5	1.0	1.1	n.d.
Minor unknowns (total)	A	n.d.	1.3	n.d.	0.9	n.d.	0.6	n.d.	0.9
	B	n.d.	1.5	n.d.	n.d.	n.d.	n.d.	1.3	1.1
	Mean	n.d.	1.4	n.d.	0.5	n.d.	0.3	0.6	1.0
Overlying Water	Mean	-	-	-	8.2	6.3	5.9	5.5	6.1
Soil extracts	Mean	100.7	93.6	86.9	76.8	78.6	77.9	77.6	76.7
(sub-total)	Mean	100.7	93.6	86.9	85.0	84.9	83.8	83.1	82.9
PU bung*	Mean	n.a.	n.d.	n.d.	n.d.	0.2	0.1	0.4	0.4
Volatile traps**	Mean	n.a.	2.9	8.7***	15.5	14.3	15.5	14.6	15.6
Non extracted soil residue	Mean	0.5	4.1	6.5	5.8	6.8	9.3	10.0	10.5
Total AR	Mean	101.2	100.6	102.2	106.3	106.2	108.7	108.1	109.4

n.a.: not analysed, n.d.: not detected, DAT: days after treatment, DPF: days post flooding
* shown to comprise parent spiroxamine. ** Almost entirely carbon dioxide. *** Content range 7.1-15.8% AR for other traps sampled at the same time

B. Material Balance

Mass balances ranged from 100.6 to 109.4% AR.

C. Extractable and Non-Extractable Residues

Extractable radioactivity from the soil declined from 100.7 at DAT 0 to 86.9% AR by DAT 30. Extractable radioactivity from the soil further declined to 76.7% AR by DPF 118. Radioactivity in the overlying water increased to 8.2% AR at DPF 7 and subsequently declined to 6.1% AR after DPF 118. Non-extractable residues (NER) increased from 0.5% AR at DAT 0 to 6.5% AR by DAT 30 and further increased to 10.55 AR by DPF 118. Further investigation of NER at the end of the stud (i.e. DPF 118) showed the majority of the applied radioactivity associated with the humin fraction.

D. Volatile Radioactivity

Significant levels of volatile degradates were evolved during the aerobic phase, reaching levels of 7.1 to 15.8% AR by 30 DAT. During the anaerobic phase, the amount of volatility evolved from the 30 DAT sampling interval was at most 1.1% AR.

E. Degradation of Parent Compound

Following application of [cyclohexyl-1-^{14}C]-spiroxamine, the amount of parent in the soil extracts decreased from 99.9% AR at DAT 0 to 65.7% AR by DAT 30 (time of flooding). During the flooded phase, the amount of parent in the combined overlying water and soil extracts changed from a total of 62.2% AR at DPF 7 to 62.6% AR at DPF 118.

Under flooded conditions degradation of [cyclohexyl-1-^{14}C]-spiroxamine was minimal and there was no appreciable increase in the level observed of any metabolites.

F. Degradation Kinetics

There was no appreciable decline during the flooded phase in the level of spiroxamine. No further kinetic evaluation was conducted, spiroxamine is reasonably stable under anaerobic conditions.

G. Isomers of Parent Compound

The isomeric composition of parent spiroxamine throughout the study is presented in Table CA 7.1.1.2-3 (content as actual % AR) and Table CA 7.1.1.2-4 (content as normalised ratio).

Table CA 7.1.1.2-3: Isomeric content of [cyclohexyl-1-^{14}C]-spiroxamine at 20°C in Refesol-02-A soil under anaerobic conditions [% AR]

Compound	Incubation time (DAT)							
	DAT			DPF				
	0 [A]	10 [A]	30 [A]	7 [A]	14 [A]	31 [B]	75 [C]	118 [C]
Spiroxamine B1	15.9	11.6	9.7	7.0	8.7	8.0	7.9	8.9
Spiroxamine B2	17.5	11.8	10.7	11.3	11.3	11.1	11.0	10.4
Spiroxamine A1	34.7	24.9	22.3	22.5	22.4	16.5	20.9	21.9
Spiroxamine A2	32.0	26.0	21.8	20.7	22.8	25.2	20.4	21.5
Spiroxamine (total)	100.1	74.3	64.4	61.5	65.2	60.9	60.2	62.6

DAT: days after treatment, DPF: days post flooding
A Replicate A only; B - Replicate B only; C – Mean of replicates A and B

Table CA 7.1.1.2-4: Isomeric composition of [cyclohexyl-1-^{14}C]-spiroxamine at 20°C in Refesol-02-A soil under anaerobic conditions [percentage content]

Compound	Incubation time (DAT)							
	DAT			DPF				
	0 [A]	10 [A]	30 [A]	7 [A]	14 [A]	31 [B]	75 [C]	118 [C]
Spiroxamine B1	15.9	15.6	15.0	11.4	13.3	13.1	13.2	14.3
Spiroxamine B2	17.4	15.9	16.5	18.3	17.3	18.3	18.4	16.7
Spiroxamine A1	34.6	33.5	34.6	36.6	34.4	27.1	34.7	34.7
Spiroxamine A2	32.0	35.0	33.9	33.7	35.0	41.4	33.8	34.4
Spiroxamine (total) % AR	100.1	74.3	64.4	61.5	65.2	60.9	60.2	62.6

DAT: days after treatment, DPF: days post flooding
A Replicate A only; B - Replicate B only; C – Mean of replicates A and B

There was no significant change in the isomeric composition of the overall spiroxamine present in the samples over the duration of the study i.e. 16/17/35/32 at DAT 0 to 14/17/35/34 at DPF 118.

III. Conclusions

Spiroxamine did not degraded significantly in soil under anaerobic conditions (20°C).

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to study guideline(s) OECD 307 (required guideline). The study is considered valid to assess the anaerobic degradation of [cyclohexyl-1-^{14}C]-spiroxamine in soil.

Existing studies, previously evaluated

Data Point:	KCA 7.1.1.2/01
Report Author:	[redacted]
Report Year:	1998
Report Title:	Anaerobic aquatic metabolism of the active ingredient KWG 4168
Report No:	PF4288
Document No:	M-006010-02-1
Guideline(s) followed in study:	US EPA Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, § 162-3, Anaerobic Aquatic Metabolism Studies
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The details of this study are fully summarised under point KCA 7.2.2.3.06.

CA 7.1.1.3 Soil photolysis

In view of the proposed use pattern for spiroxamine, as a post-emergence spray applied fungicide for application to cereals crops and vines, exposure to sunlight on soil surfaces is envisaged. However, the active substance spiroxamine has a low UV absorbance (molar decadic absorption > 10 L per mol per cm) therefore studies investigating soil photolysis are not required.

Nevertheless, an existing study investigating the degradation of spiroxamine under sunlight which was evaluated during the previous EU review is available (KCA 7.1.1.3/01) and has therefore been included.

Substance	Report reference	Document no.	Comment
Spiroxamine	KCA 7.1.1.3/01	M-006150-01-1	Submitted for first approval of spiroxamine, 1999. Reviewed under UP. Considered valid and acceptable.

The soil photolysis of spiroxamine has been investigated in one study at 25°C. In addition, a further study has been conducted to determine the quantum yield.

Existing studies, previously evaluated

Data Point:	KCA 7.1.1.3/01
Report Author:	[redacted]
Report Year:	1995
Report Title:	Photolysis of KWG 4168 on soil surfaces (according to EPA guidelines)
Report No:	PF4076
Document No:	M-006150-01-1
Guideline(s) followed in study:	EPA, Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate § 163-1, Photodegradation studies on soil
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The photolytic degradation of spiroxamine on soil surfaces was investigated in air-dried loam soil, prepared as a thin-layer within glass incubation vessels with a thickness of about 2 mm. The test item, [cyclohexyl-1-^{14}C]-spiroxamine, dissolved in acetonitrile/water (1+99 v/v), was applied evenly to the soil surface at an application rate of 12.43 mg/kg (the target application rate of 13.3 was reported as equivalent to an annual application rate of 2 kg a.s./ha based on a soil mixing depth of 1 cm and soil density of 1.5 g/cm^3). Treated soil samples were exposed to artificial irradiation from a Xenon lamp (with < 280 nm cut-off filter) with continuous irradiation for a period of 17 days at 25 ± 1ºC. Two dark control samples wrapped in aluminium foil were treated at the same application rate and incubated under the same conditions.

Material balances were 100.5 to 106.1% AR for irradiated samples and 101.0% AR for dark controls.

Levels of spiroxamine reduced over the study period in irradiated samples from 100.6 to 60.8% AR. The only significant degradation products observed were M01 (spiroxamine-desethyl, max 9.1% AR) and M02 (spiroxamine-despropyl, max 6.1% AR) i.e. metabolites already observed in studies investigating degradation under aerobic conditions. Other degradation products observed in minor amounts were M03 (spiroxamine-N-oxide, maximum 6.2% AR), M15 (spiroxamine-ketone, maximum 2.7% AR, M05 (spiroxamine-hydroxy, maximum 0.4% AR and other unknown metabolites detected at maximum individual occurrence of 2.0% AR.

In the dark controls, M01 (spiroxamine-desethyl) is formed at 2.4% AR, M02 (spiroxamine-despropyl) is formed at 1.6% AR, M03 (spiroxamine-N-oxide) is formed at 2.1% AR, M05 (spiroxamine-hydroxy) is formed at 0.1% AR. All other unknowns are present at <0.3% AR.

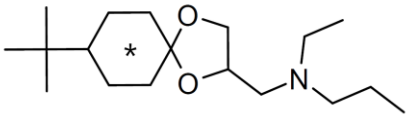
The reported (non FOCUS) degradation rate of spiroxamine under sunlight was equivalent to 119 solar days (Phoenix location). Photolysis of the active substance on soil is not a significant degradation pathway.

I. Materials and Methods

A. Materials

1. Test Items

[Cyclohexyl-1-^{14}C]-spiroxamine



* Denotes position of [^{14}C]-radiolabel

Specific Activity: 3.63 MBq/mg

Radiochemical Purity: 99.4% (checked by TLC at time of application)

2. Test System (soil)

The study was performed using one test soil as characterized in Table CA 7.1.1.3-1.

Table CA 7.1.1.3-1: Physico-chemical properties of test soil

Parameter		Soil
Soil Designation		Wolf Ranch
Geographic Location		
City		Fresno County
Country		California, USA
Textural Classification (USDA)		Loam
Sand [50 - 2000 µm]	(%)	29.7
Silt [2 – 50 µm]	(%)	45.1
Clay [< 2 µm]	(%)	25.2
pH		
in H_2O (1:1)		8.7
in $CaCl_2$ (1:1)		7.8
Organic Carbon (%)		0.97
Cation Exchange Capacity (meq/100 g)		19
Water Holding Capacity (g H_2O per 100g dry soil)		
75% of 1/3 bar		15.2
Microbial biomass (mg microbial C/kg soil)		
At 0 DAT		237

B. Study Design

1. Experimental Conditions

Soil was air dried to a soil moisture of approximately 10% and sieved to 2mm. Prior to use, soil was acclimatised at 20-22°C for 48 hours. Portions of fresh soil (3 g dry weight) were added to borosilicate glass incubation vessels, fitted with quartz glass covers, to give a soil depth of *ca*. 2mm (surface area 10.2 cm^2). The soil samples were incubated inside static glass units with a trap for volatile products containing a polyurethane plug and soda lime (to trap $^{14}CO_2$) connected to each unit.

The test item [cyclohexyl-1-^{14}C]-spiroxamine (37.29 µg), dissolved in acetonitrile/water (240 µL, 1+99 v/v), was applied evenly to the soil surface (equivalent to an actual application rate of 12.43 µg/g soil dry weight). Units were irradiated continuously with light from an Heraeus Suntest xenon lamp, with two dark control samples tightly wrapped in aluminium foil to block light. The target application rate was described as 13.3 mg/kg based on an application rate of 2 kg/ha, 1cm depth and density of 1.5 g/cm^3 (this is equivalent to 2.67 mg/kg using default assumptions of 5 cm depth and density of 1.5 g/cm^3). The temperature of both irradiated and non-irradiated samples was maintained at a temperature of 25 ± 1 ºC

throughout the incubation period. The photolytic degradation was studied with continuous illumination under artificial sunlight for a period of up to 17 days. The artificial sunlight was provided by a xenon arc lamp with filters to cut off any radiation below 280nm.

2. Sampling

Duplicate vessels were taken from both irradiated and non-irradiated systems after 0, 3, 7, 11 and 17 days (equivalent to 80 days sunlight Phoenix location). The duplicate dark controls were also taken for analysis at 17 days.

3. Analytical Procedures

Prior to opening, headspace in the vessels were purged into volatile traps under vacuum. The polyurethane plugs were extracted with ethanol and analysed by LSC. $^{14}CO_2$ was liberated from soda lime by addition of 18% HCl and captured in scintillation cocktail for LSC analysis.

Soil samples were extracted at ambient temperature four times with acetonitrile (5 mL) under agitation for 30 mins. SoxTec extraction was subsequently performed using methanol (30 mL) at 170°C. Radioactivity extracted from soil was quantified by liquid scintillation counting (LSC). Unextracted radioactivity was determined by combustion and LSC.

Soil extracts were analysed without concentration using the primary TLC method:

- Normal phase (saturated system) with acetonitrile/water/25% ammonia (80/18/2, v/v/v) mobile phase.

Confirmatory analysis (parent and metabolites) was conducted using the additional TLC methods:

- Normal phase (unsaturated system) with dichloromethane/ethyl-acetate (90/20, v/v) mobile phase.
- Reverse phase first run (saturated system) with n-hexane/dichloromethane/2-propanol/ammonia (30/70/10/2, v/v/v/v) and second run (unsaturated system) with chloroform/ethanol (50/50, v/v) mobile phase.
- Normal phase (saturated system) with acetonitrile/water/25% ammonia (95/5/0.6, v/v/v)

Degradation products were identified by comparison of the retention times of reference standards. The detection limit for a single peak in the extracts was ≤0.3% of applied radioactivity.

4. Determination of degradation kinetics

The degradation rate of spiroxamine under irradiated conditions was estimated using Timme G. *et al* (1986)[4] and converted to equivalent days sunlight under Phoenix location conditions.

The radiation intensity was measured via radiometer and actinometer (uranyl oxalate, 0.01M uranyl nitrate and 0.05M oxalic acid) at the beginning and end of the test. These intensity measurements were used to determine that 5.1 hours irradiation in the Suntest unit equalled 1 solar day at Phoenix, Arizona, USA.

II. Results and Discussion

A. Data

The distribution and characterisation of radioactivity under irradiated conditions and in dark controls following application of [cyclohexyl-1-^{14}C]-spiroxamine is summarized in Table CA 7.1.1.3-2.

[4] Timme G., Frehse H. & Laska V. (1986). Zur statistischen Interpretation und graphischen Darstellung des Abbauverhaltens von Pflanzenschutzmittel-Ruckstanden, Part II. Pflanzenschutz-Nachrichten Bayer 39, 188-204, 1986.

Table CA 7.1.1.3-2 Degradation of [cyclohexyl-1-^{14}C]-spiroxamine at 25°C in Wolf Ranch soil irradiated under aerobic conditions [% AR]

Compound	Rep.	Incubation time (DAT)				
		0	3	7	11	17
Spiroxamine	A	-	-	-	-	-
	B	-	-	-	-	-
	Mean	**100.6**	**88.8**	**71.9**	**62.6**	**60.8**
M01 (spiroxamine-de-sethyl)	A	-	-	-	-	-
	B	-	-	-	-	-
	Mean	**0.6**	**2.7**	**7.9**	**8.6**	**9.1**
M02 (spiroxamine-despropyl)	A	-	-	-	-	-
	B	-	-	-	-	-
	Mean	**0.5**	**2.0**	**5.6**	**5.9**	**6.1**
M03 (spiroxamine-N-ox-ide)	A	-	-	-	-	-
	B	-	-	-	-	-
	Mean	**0.5**	**1.5**	**4.3**	**6.2**	**4.7**
M15 (spiroxamine-ke-tone)	A	-	-	-	-	-
	B	-	-	-	-	-
	Mean	**1.8**	**2.7**	**2.4**	**1.4**	**1.4**
M05 (spiroxamine-hy-droxy)	A	n.d.	-	-	-	-
	B	n.d.	-	-	-	-
	Mean	**-**	**0.3**	**0.4**	**0.1**	**0.1**
UK1	A	n.d.	-	-	-	-
	B	n.d.	-	-	-	-
	Mean	**-**	**0.3**	**0.9**	**0.2**	**0.7**
UK2	A	n.d.	-	-	-	-
	B	n.d.	-	-	-	-
	Mean	**-**	**0.4**	**0.8**	**0.7**	**0.9**
UK3	A	n.d.			-	
	B	n.d.	-	-	-	
	Mean	**-**	**0.3**	**1.7**	**1.3**	**1.2**
Diffuse radioactivity & minor unknowns	A	n.d.	-	-	-	-
	B	n.d.	-	-	-	-
	Mean	**-**	**0.1**	**0.9**	**1.5**	**2.0**
Ambient extract	A	81.96	66.67	65.60	58.81	60.74
	B	88.36	68.93	66.47	58.35	61.79
	Mean	**85.16**	**67.8**	**66.04**	**58.58**	**61.27**
Soxtec	A	19.57	30.73	31.28	27.30	27.73
	B	18.14	31.62	30.08	32.45	23.05
	Mean	**18.86**	**31.18**	**30.68**	**29.88**	**25.39**
Total Extractables[A]	A	101.53	97.40	96.88	86.11	88.47
	B	106.50	100.55	96.55	90.80	84.84
	Mean	**104.02**	**98.98**	**96.71**	**88.46**	**86.65**
Non-extractables	A	3.51	2.99	5.23	12.45	12.11
	B	0.74	3.04	4.89	8.04	13.93
	Mean	**2.12**	**3.01**	**5.06**	**10.24**	**13.02**
$^{14}CO_2$	A	0.0	0.50	1.53	1.85	1.88
	B	0.0	0.93	1.42	1.71	1.95
	Mean	**0.0**	**0.72**	**1.47**	**1.78**	**1.92**

Compound	Rep.	Incubation time (DAT)				
		0	3	7	11	17
Other volatiles	A	0.0	0.08	0.06	0.06	0.04
	B	0.0	0.06	0.07	0.07	0.04
	Mean	**0.0**	**0.07**	**0.07**	**0.07**	**0.04**
Total radioactivity	A	105.04	100.95	103.66	100.46	102.50
	B	107.24	104.57	102.91	100.61	100.75
	Mean	**106.14**	**102.76**	**103.29**	**100.54**	**101.63**

n.d.: not detected, DAT: days after treatment,
* Complete resolution was not achieved between metabolites M01 and M05 during LC analysis. An estimation of the separate amounts was made during the study but this must be viewed as approximate
A Sum of ambient and SoxTec extractions

B. Material Balance

Material balances ranged from 100.5 to 106.1% AR for irradiated samples and 101.03% AR for samples incubated in the dark.

C. Extractable and Non-Extractable Residues

The majority of the applied radioactivity was extractable throughout the study. For irradiated samples treated with [cyclohexyl-1-^{14}C]-spiroxamine, total extractable radioactivity ranged from 104.02% AR at DAT 0 to 86.65% AR at DAT 17. The total of non-extractable residues (NER) increased throughout the study, from 2.12% AR at DAT 0 to 13.02% AR by the end of the study (DAT 17).

For samples incubated in the dark with [cyclohexyl-1-^{14}C]-spiroxamine, total extractable radioactivity was 90.14% AR at DAT 17. The total of non-extractable residues (NER) was 7.88% AR at DAT 17.

D. Volatile Radioactivity

Radiolabelled carbon dioxide accounted for 0.72 to 1.92% of the applied radioactivity in the irradiated samples. In samples incubated in the dark, $^{14}CO_2$ accounted for 2.99% AR. Only trace amounts of radioactivity were recovered from the polyurethane plug (maximum of 0.07% of applied).

E. Degradation of Parent Compound

Levels of spiroxamine reduced over the study period in irradiated samples from 100.6 to 60.8% AR. The only significant degradation products observed were M01 (spiroxamine-desethyl, max 9.1% AR) and M02 (spiroxamine-despropyl, max 6.1% AR) i.e. metabolites already observed in studies investigating degradation under aerobic conditions. Other degradation products observed in minor amounts were M03 (spiroxamine-N-oxide, maximum 6.2% AR), M15 (spiroxamine-ketone, maximum 2.7% AR, M05 (spiroxamine-hydroxy, maximum 0.4% AR and other unknown metabolites detected at maximum individual occurrence of 2.0% AR.

In the dark controls, M01 (spiroxamine-desethyl) is formed at 2.4% AR, M02 (spiroxamine-despropyl) is formed at 1.6% AR, M03 (spiroxamine-N-oxide) is formed at 2.1% AR, M05 (spiroxamine-hydroxy) is formed at 0.1% AR. All other unknowns are present at <0.3% AR.

F. Degradation Kinetics

An experimental DT$_{50}$ of 25.4 days irradiated exposure was calculated. Correcting the half-life for solar days gives an estimation of the environmental half-life under solar conditions at Phoenix of 119 days. The experimental data has not been re-evaluated according to the FOCUS guidance document on degradation kinetics (FOCUS, 2014) as the minimal degradation observed was sufficient to confirm that photolysis of the active substance on soil is not a significant degradation pathway.

III. Conclusions

The degradation rate of [cyclohexyl-1-^{14}C]-spiroxamine was only slightly enhanced in the presence of sunlight. The only significant degradation products observed were M01 (spiroxamine-desethyl, max 9.1% AR) and M02 (spiroxamine-despropyl, max 6.1% AR) i.e. metabolites already observed in studies investigating degradation under aerobic conditions. Other degradation products observed in minor

amounts were M03 (spiroxamine-N-oxide, maximum 6.2% AR), M15 (spiroxamine-ketone, maximum 2.7% AR, M05 (spiroxamine-hydroxy, maximum 0.4% AR and other unknown metabolites detected at maximum individual occurrence of 2.0% AR.

The reported (non FOCUS) degradation rate of spiroxamine under sunlight was equivalent to 109 solar days (Phoenix location). Photolysis of the active substance on soil is not a significant degradation pathway.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to study guideline(s) EPA 163-1 (similarto required guideline). The study is considered valid to assess the Photodegradation of [cyclohexyl-1-^{14}C]-spiroxamine on soil.

CA 7.1.2 Rate of degradation in soil

Use of plant protection products containing the active substance spiroxamine will potentially result in contact with soil, therefore the rate of degradation in soil of the active substance and all significant metabolites (as defined under Point CA 7.4.1) is considered under Point CA 7.1.2. according to the data requirements laid down in Commission Regulation (EU) No 283/2013. Laboratory studies investigating the rate of degradation of the active substance and any significant metabolites under aerobic conditions are considered under Points CA 7.1.2.1.1 and CA 7.1.2.1.2, respectively. Similarly, laboratory studies investigating the rate of degradation of the active substance and any significant metabolites under anaerobic conditions are considered under Points CA 7.1.2.1.3 and CA 7.1.2.1.4, respectively. Additionally, soil dissipation studies investigating the rate of degradation of the active substance and any significant metabolites under field conditions are considered under Points CA 7.1.2.2.

Overview:

New and existing laboratory route and rate studies considered reliable were evaluated for degradation rates normalized to reference conditions following FOCUS kinetics (2014). DT_{50} and DT_{90} values were calculated both for an evaluation of persistence and for comparison with relevant study triggers. Modelling DT_{50} values and formation fractions (f.f.) were calculated for use in deriving endpoints for predicting environmental concentrations. The data were used to attempt calculation of kinetic endpoints for spiroxamine and its metabolites M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl), M03 (spiroxamine-N-oxide) and M06 (spiroxamine-acid).

Input data were generated according to the data handling recommendations of FOCUS (2014a). The kinetic modelling of the laboratory data was conducted using the CAKE v3.4 software package. The FOCUS (2014a) flowcharts for calculating persistence and modelling endpoints have been followed. The spiroxamine calculated DT_{50} values ranged from 7.2 to 142 days and DT_{90} values ranged from 81.2 to 696 days. The geometric mean of the kinetic analysis DT_{50} value was 75.4 days. M01 (spiroxamine-desethyl) persistence DT_{50} values ranged from 28.9 to 555 days and DT_{90} value range from 95.8 to >1,000 days. The geometric mean DT_{50} value was 168.6 days. The arithmetic mean of the formation fractionfrom parent was 0.183. The M02 (spiroxamine-despropyl) persistence DT_{50} values ranged from 26.6 to the FOCUS default value of 1,000 days and DT_{90} values ranged from 88.2 to 3,320 days (again the FOCUS default). The geometric mean DT_{50} value was 219.1 days. The arithmetic mean of the formation fractions from parent was 0.138. The M03 (spiroxamine-N-oxide) persistence DT_{50} values ranged from 16.7 to 107 days and DT_{90} values ranged from 55.4 to 358 days. The geometric mean DT_{50} value was 46.4 days. The arithmetic mean of the formation fractions from parent was 0.149. Acceptable fitting could only be performed for persistence endpoints in one soil for M06 (spiroxamine-acid) resulting in a number of presented default values. The persistence DT_{50} value was 49.6 days to 1,000 days whilst DT_{90} values was 165 days to 3,320 days. The geometric mean of modelling DT_{50} values was, therefore, calculated as 479.6 days but this is highly influenced by the default values and the real DT_{50}

is likely much shorter (based on the one reliable kinetic fit). The formation fraction from parent was 0.0947.

The dissipation behaviour of spiroxamine was investigated in Europe in eighteen field sites across five studies. The study design for each study did not exclude surface loss processes such as photolysis or volatilisation; therefore, they are considered to follow the "legacy" study design according to EFSA (2014) guidance. The data from these studies are considered appropriate to derive persistence/trigger and modelling endpoints and were analysed using the CAKE version 3.4 (2020) software package to derive suitable kinetics. Thiskinetic analysis to determine DT_{50} and DT_{90} values for comparison with relevant study triggers and persistence criteria was performed using non-normalised data, in accordance with the flowcharts for persistence/trigger endpoints provided by FOCUS (2014). Likewise, the flowcharts for calculating modelling endpoints provided by EFSA (2014), and using data that have been normalised to reference conditions (20°C and pF 2 soil moisture content) and subject to timestep normalisation procedures, were followed to derive $DegT_{50,matrix}$ values; these endpoints were used for the selection of appropriate modelling values for use with regulatory Predicted Environmental Concentration (PEC) models.

The spiroxamine persistence/trigger DT_{50} values ranged from 0.5 to 59.6 days and DT_{90} values ranged from 43.5 to 433 days. The spiroxamine modelling DT_{50} values (at 20°C and pF 2) ranged from 16.1 to 133 days, with a geometric mean of 42.9 days. If not including cropped trials, modelling DT_{50} values (at 20°C and pF 2) ranged from 23.4 to 78 days, with a geometric mean of 43.8 days.

M01 (spiroxamine-desethyl) persistence trigger DT_{50} values ranged from 17.8 to 223 days and DT90 values ranged from 59 to 742 days. Modelling DT_{50} values ranged from 23.4 to the default 1,000 days, with a geometric mean of 66.2 days. Formation fractions were not determined as formation may have been affected by photolytic factors according to EFSA (2014). If not including cropped trials, modelling DT_{50} values (at 20°C and pF 2) ranged from 23.4 to 1,000 days, with a geometric mean of 89.8 days.

M02 (spiroxamine-despropyl) persistence trigger DT_{50} values ranged from 21 to 161 days and DT_{90} values ranged from 69.6 to 533 days. Modelling DT_{50} values ranged from 27.7 to 196 days, with a geometric mean of 69.1 days. Formation fractions were not determined as formation may have been affected by photolytic factors according to EFSA (2014). If not including cropped trials, modelling DT_{50} values (at 20°C and pF 2) ranged from 44.3 to 196 days, with a geometric mean of 93.8 days.

An assessment of the statistical difference of the kinetic evaluation of the lab and field studies was performed using the EFSA endpoint XL. This assessment determined that the field studies were statistically different to the lab dataset and as such modelling endpoints are taken from the field studies in isolation (KCA 7.1.2.2.1/12 (M-763140-01-1)). For tier 1, modelling endpoints have been taken from the lab endpoints (KCA 7.1.2.1.1/09 (M-763139-01-1)).

Table CA 7.1.2-1: Overall summary of best-fit kinetic parameters (persistence endpoints) for aerobic degradation of spiroxamine in soil (laboratory studies)

Soil	pH (H2O)	Temp (°C) / % MWHC	DT_{50} / DT_{90} (days)	Normalised DT_{50} / DT_{90} (days)	χ^2 error (%)	Kinetic model
BBA 2.2/Speyer 2.2 (KCA 7.1.1.1/01 (M-006135-01-1))	6.3	20 / 40[2]	66.6 / 296[1]	66.6/296[1]	3.2	DFOP
Laacherhof (KCA 7.1.1.1/02 (M-006141-01-1))	8.1	20 / 40	22.5 / 128[1]	14.2 / 80.9	3.3	DFOP
Monheim 3 (KCA 7.1.1.1/02 (M-006141-01-1))	6.5	20 / 40	33.2 / 176[1]	30.2 / 160.2	5.0	DFOP
Howe (KCA 7.1.1.1/02 (M-006141-01-1))	7.1	20 / (75% 0.33 bar)	29.9 / 168[1]	23.6 / 132.7[1]	3.8	DFOP
Wolf Ranch (KCA 7.1.1.1/04 (M-006148-01-1))	7.8	20 / (75% of 0.33 bar)	75 / 299[1]	53.0 / 211.1[1]	3.6	DFOP
Hoefchen am Hohenseh (KCA 7.1.1.1/05 (M-303803-01-1))	7.0	20 / 35	7.2 / 93.6	7.2 / 93.6	4.4	DFOP
Longwoods (KCA 7.1.1.1/06 (M-762349-01-1)0)	7.8	20 / pF 2.0	13.1 / 81.2	13.1 / 81.2	3.2	DFOP
Refesol 02-A (KCA 7.1.1.1/06 (M-762349-01-1))	7.1	20 / pF 2.0	116 / 510[1]	116 / 510[1]	1.9	DFOP
Refesol 03-G (KCA 7.1.1.1/06 (M-762349-01-1))	5.9	20 / pF 2.0	142[1] / 696[1]	1421 / 696[1]	1.5	DFOP
Speyer 6S (KCA 7.1.1.1/06 (M-762349-01-1))	7.3	20 / pF 2.0	137[1] / 514[1]	1371 / 514[1]	1.7	DFOP
Worst case			142[1] / 696[1]	142[1] / 696[1]		

[1] Interpret with care – extrapolated beyond experimental period

[2] Moisture value above pF 2.0

Table CA 7.1.2-2: Overall summary of best-fit kinetic parameters (persistence endpoints) for aerobic degradation of spiroxamine in soil (field studies)

Study	Soil (USDA)	Location	pH (CaCl2)	DissT50 (days)	DissT90 (days)	χ2 error (%)	Kinetics
KCA 7.1.2.2.1/01 (M-006116-01-1)	Silt loam	Höfchen	6.5	13.8	145	11.8	DFOP
	Loam	Laacher Hof	6.8	32.6	196	6.3	DFOP
	Sandy loam	Elm Farm/ Thurston	7.5	0.8	197	7.4	DFOP
	Loamy sand	Pakenham	7.3	0.5	132	8.2	DFOP
KCA 7.1.2.2.1/02 (M-006126-01-1)	Silt loam	Höfchen	6.4	56.6	393	8.1	FOMC
	Sandy loam	Laacher Hof	6.6	20.1	127	9.5	DFOP
	Sandy loam	Maasen	5.9	8.4	271	10.6	DFOP
	Silt loam	Swisttal-Hohn	6.7	7.9	84.7	7.9	FOMC
	Clay loam	Albig	7.8	6.0	74.5	6.3	DFOP
KCA 7.1.2.2.1/03 (M-006127-01-1)	Sandy loam	Elm Farm/ Thurston	7.4	1.46	132	5.2	DFOP
	Sandy loam	Pakenham	7.0	9.5	199	6.3	DFOP
	Sandy loam	Elm Farm/ Thurston	7.4	9.1	433	6.7	DFOP
	Sandy loam	Pakenham	7.0	1.2	247	9.2	DFOP
	Silt loam	Touf-freville	7.2	6.1	58.4	3.8	DFOP
KCA 7.1.2.2.1/04 (M-006128-01-1)	Loam	Laudun	7.7	2.1	43.5	9.1	DFOP
	Silty clay loam	Filetto	7.6	59.6	295	16.9	DFOP
KCA 7.1.2.2.1/05 (M-006129-01-1)	Loam	Laudun	7.7	2.1	93.3	3.9	DFOP
	Sandy loam	Nogarole Rocca	7.9	3.5	43.8	1.9	DFOP
			WorstCase	9.1	433		
			pH dependence	No			

CA 7.1.2.1 Laboratory studies

CA 7.1.2.1.1 Aerobic degradation of the active substance

The rate of aerobic degradation in soil of the active substance has been investigated, as part of the studies listed under Point CA 7.1.1.1, in five studies (KCA 7.1.2.1.1/01 to KCA 7.1.2.1.1/05) which were evaluated during the previous EU review. In addition:

- one new study (KCA 7.1.2.1.1/08 (M-762349-01-1)) has been conducted mainly to address the new requirements of EFSA, 2019[1]
- two further studies were included during the last evaluation and therefore they have been included for completeness (KCA 7.1.2.1.1/06 (M-036125-01-1) provides a comparison of laboratory and field degradation studies each individually summarised more fully elsewhere and KCA 7.1.2.1.1/07 (M-347082-01-1) provides a kinetic evaluation of degradation rates from laboratory soil studies which has since been superseded)
- one new study (KCA 7.1.2.1.1/09 (M-763139-01-1)) has been conducted to provide an up-to-

date kinetic assessment of degradation rates observed in all laboratory studies to modern requirements (FOCUS 2014[2])

Substance	Report reference	Document no.	Comment
Spiroxamine	KCA 7.1.1.1/01; KCA 7.1.2.1.1/01	M-006135-01-1	Submitted for first approval of spiroxamine, 1999. Reviewed under UP. Considered valid and acceptable.
Spiroxamine	KCA 7.1.1.1/02; KCA 7.1.2.1.1/02	M-006141-01-1	
Spiroxamine	KCA 7.1.1.1/03; KCA 7.1.2.1.1/03	M-006141-01-1	
Spiroxamine	KCA 7.1.1.1/04; KCA 7.1.2.1.1/04	M-006148-01-1	Submitted for first renewal of spiroxamine, 2010. Reviewed under UP. Considered valid and acceptable.
Spiroxamine	KCA 7.1.1.1/05; KCA 7.1.2.1.1/05	M-303803-01-1	
Spiroxamine	KCA 7.1.2.1.1/06; KCA 7.1.2.2.1/06	M-036125-01-1	
Spiroxamine	KCA 7.1.2.1.1/07	M-347082-01-1	
Spiroxamine	KCA 7.1.1.1/06; KCA 7.1.2.1.1/08	M-762349-01-1	New data not yet reviewed under UP.
Spiroxamine	KCA 7.1.2.1.1/09	M-763139-01-1	

The rate of degradation of spiroxamine in soil under aerobic conditions in laboratory studies has been investigated in ten soils. A new kinetic evaluation has been performed (see KCA 7.1.2.1.1/09 (M-763139-01-1) below) and the resulting best fit kinetic parameters (persistence endpoints) are summarised in Table CA 7.1.2-1.

New studies, not previously evaluated

Data Point:	KCA 7.1.2.1.1/08
Report Author:	[redacted]
Report Year:	2020
Report Title:	[14C]-spiroxamine: Route and rate of degradation in four soils under aerobic conditions at 20°C - Interim report
Report No:	VC/19/055
Document No:	M-762349-01-1
Guideline(s) followed in study:	(EU) No. 283/2013(EC) No. 1107/2009 OECD 307 (2002)
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The details of this study are fully summarised under point KCA 7.1.1.1/06.

Existing studies, previously evaluated

Data Point:	KCA 7.1.2.1.1/01
Report Author:	[redacted]
Report Year:	1994
Report Title:	Aerobic degradation of KWG 4168 in BBA soil 2.2
Report No:	PF4027
Document No:	M-006135-01-1
Guideline(s) followed in study:	BBA: Part IV, 4-1
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The details of this study are fully summarised under point KCA 7.1.1.1/01.

Data Point:	KCA 7.1.2.1.1/02
Report Author:	[redacted]
Report Year:	1995
Report Title:	Aerobic degradation and metabolism of KWG 4168 in soil
Report No:	PF4034
Document No:	M-006141-01-1
Guideline(s) followed in study:	BBA: Part IV, 4-1
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017) The degradation rates of spiroxamine ([redacted] (1997) and [redacted] (2008)) were included in the following survey (M-036125-01-1).
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The details of this study are fully summarised under point KCA 7.1.1.1/02.

Data Point:	KCA 7.1.2.1.1/03
Report Author:	[redacted]
Report Year:	1994
Report Title:	[Cyclohexyl-1-14C] KWG 4168 residues in following crops
Report No:	PF4043
Document No:	M-006096-01-1
Guideline(s) followed in study:	US EPA §165-1 Confined accumulation studies on rotational crops, 1983
Deviations from current test guideline:	Yes. OECD 502 guideline (January 2007) requires three plant-back intervals for succeeding crops. The longest interval of 270 to 365 days to represent crops sown the following year was not conducted in this study.
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The details of this study are fully summarised under point KCA 7.1.1.1/03.

Data Point:	KCA 7.1.2.1.1/04
Report Author:	[redacted]
Report Year:	1997
Report Title:	Degradation and metabolism of KWG 4168 in soil aerobic soil metabolism
Report No:	PF4274
Document No:	M-006148-01-1
Guideline(s) followed in study:	US EPA Pesticide Assessment Guidelines, Subdivision N, 162-1: Aerobic soil metabolism studies (1982)
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The details of this study are fully summarised under point KCA 7.1.1.1/04.

Data Point:	KCA 7.1.2.1.1/05
Report Author:	[redacted]
Report Year:	2008
Report Title:	[1,3-Dioxolane-4-14C]spiroxamine: Metabolic screening for degradation pathways under aerobic conditions in soil
Report No:	MEF-08/214
Document No:	M-303803-01-1
Guideline(s) followed in study:	EU 95/36/EC amending 91/414/EEC; OECD 307; US EPA, Subdivision N, Section 162-1
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The details of this study are fully summarised under point KCA 7.1.1.1/05.

Data Point:	KCA 7.1.2.1.1/06
Report Author:	[redacted]
Report Year:	2000
Report Title:	Dissipation of spiroxamine in soils - survey of results from studies conducted under field and laboratory conditions
Report No:	MR-251/00
Document No:	M-036125-01-1
Guideline(s) followed in study:	BBA: Part IV, 4-1
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

This study was previous considered during the evaluation of spiroxamine (RAR (2010), RAR (2017) and is therefore included again for completeness. The study compares laboratory versus field degradation rates derived in other studies namely the laboratory degradation rates observed in studies KCA 7.1.1.1/01 (M-006135-01-1); KCA 7.1.1.1/02 (M-006141-01-1); KCA 7.1.1.1/04 (M-006148-01-1); KCA 7.1.1.1/05 (M-303803-01-1) and KCA 7.1.1.1/06 (M-762349-01-1) versus the rates observed in field studies KCA 7.1.2.2.1/01 (M-006116-01-1); KCA 7.1.2.2.1/02 (M-006126-01-1); KCA 7.1.2.2.1/03 (M-006127-01-1); KCA 7.1.2.2.1/04 (M-006128-01-1); and KCA 7.1.2.2.1/05 (M-006129-01-1). Evaluation of the degradation rates observed in laboratory and field studies is superseded by the new kinetic evaluations performed in studies KCA 7.1.2.1.1/09 (M-763139-01-1) and KCA 7.1.2.2.1/12 (M-763140-01-1), respectively.

Assessment and conclusion by applicant:

The study and its data are considered as supplementary data with no use in risk assessment.

Data Point:	KCA 7.1.2.1.1/07
Report Author:	[redacted]
Report Year:	2009
Report Title:	Kinetic evaluation of laboratory soil degradation studies with KWG4168-N-oxide to determine input parameters for model calculations
Report No:	MEF-09/309
Document No:	M-347082-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

This study was previous considered during the evaluation of spiroxamine (RAR (2010), RAR (2017)

and is therefore included again for completeness. The study performs a kinetic evaluation according to FOCUS 2006[5] of the degradation of spiroxamine metabolite M03, where observed, reported in studies KCA 7.1.1.1/01 (M-006135-01-1); KCA 7.1.1.1/02 (M-006141-01-1); and KCA 7.1.1.1/04 (M-006148-01-1). The kinetic evaluation reported in this study is superseded by the new kinetic evaluation performed in study KCA 7.1.2.1.1/09 (M-763139-01-1) on all the same laboratory soil degradation studies.

Assessment and conclusion by applicant:

The study and its data are considered as supplementary data with no use in risk assessment.

Data Point:	KCA 7.1.2.1.1/09
Report Author:	[redacted]
Report Year:	2021
Report Title:	Spiroxamine: Kinetic assessment of laboratory aerobic soil studies
Report No:	0471836-KIN2
Document No:	M-763139-01-1
Guideline(s) followed in study:	FOCUS 2006/FOCUS 2014
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The degradation of spiroxamine and the formation of its metabolites has been investigated in the laboratory in five [cyclohexyl-1-^{14}C] or [1,3-dioxolane-^{14}C]-spiroxamine applied studies with eight EU soils and two US soils incubated under aerobic conditions (see studies under CA 7.1.1.1/01 (M-006135-01-1), CA 7.1.1.1/02 (M-006141-01-1), CA 7.1.1.1/04 (M-006148-01-1), CA 7.1.1.1/05 (M-303803-01-1) and CA 7.1.1.1/06 (M-762349-01-1)).

The kinetic endpoints from these studies according to the guidance of FOCUS (2014[2]) were re-calculated. DT_{50} and DT_{90} values were calculated for evaluation of persistence and for comparison with relevant study triggers. Modelling DT_{50} values and formation fractions (f.f.) were calculated for use in deriving endpoints for predicting environmental concentrations. The data were used to attempt calculation of kinetic endpoints for spiroxamine and its metabolites M01, M02, M03 and M06. Input data were generated according to the data handling recommendations of FOCUS (2014). The kinetic modelling of the laboratory data was conducted using the CAKE v3.4 software package. The FOCUS flowcharts for calculating persistence and modelling endpoints have been followed.

The derived persistence and modelling endpoints for spiroxamine, M01, M02, M03 and M06 are summarised in Table CA 7.1.2.1.1-1 and Table CA 7.1.2.1.1-2, respectively.

I. Materials and Methods

The soil degradation data presented in studies under CA 7.1.1.1/01 (M-006135-01-1), CA 7.1.1.1/02 (M-006141-01-1), CA 7.1.1.1/04 (M-006148-01-1), CA 7.1.1.1/05 (M-303803-01-1) and CA 7.1.1.1/06

[5] FOCUS (2006) "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp.

([M-762349-01-1](#)) has been re-evaluated according FOCUS guidelines (2006, 2014).

Input data were generated according to the data handling recommendations of FOCUS (2014a). Where true replicates were available for each sampling time, all values for each soil were used individually in the optimisation. The amounts of parent at time zero were set to the initial mass balance value (i.e., the total radioactivity recovered in the 0 day soil as a percentage of the radioactivity applied) corrected for radiochemical purity. The initial amounts of metabolites in parent dosed studies were set to 0%. If necessary, the handling of values below the LOD and LOQ was performed according to the procedure recommended by FOCUS (2014a) as follows:

- All values between LOD and LOQ were set to the actual measured value. If the actual measured concentration was not reported, 0.5 × (LOQ + LOD) was used.
- All samples < LOD just after detectable amounts were set to 0.5 × LOD.
- All samples after the first non-detect (< LOD) were omitted unless positive detections above LOQ were made later in the experiment. In that case, samples were included up to the first non-detect (< LOD) which is not followed by later positive samples above LOQ. For metabolites the same procedure was applied to samples before the first detectable amount.

All values reported as not detected (n.d.), 0%, or not reported (n.r.) were considered as < LOD. For time zero, the mass balance is used as the parent residue value corrected for radiochemical purity. Full details of the reported residues (% AR) and the values considered in the fittings are detailed in the study report (pages 20-26).

The kinetic modelling of the laboratory data was conducted using the CAKE (version 3.4) software package. The data were evaluated with the single first order (SFO) and first order multi-compartment (FOMC) models, and if necessary, with the double first order in parallel (DFOP) and hockey-stick (HS) models. Data were directly fitted, unweighted, with the complete usable data set and unconstrained initial concentration (M_0). To give the best chance of finding the global minimum (i.e., the true best-fit values) the model default initial parameters were examined and amended if necessary to provide appropriate starting values, as recommended by FOCUS.

Kinetic assessments were performed using the degradation schemes presented in Figure 7.1.2.1.1-1. As CAKE v3.4 can only simulate 3 primary metabolites, M06 was modelled in a separate run. In the initial fitting, all possible flows to sink compartments were included. Based on the results of the initial estimation, flows were kept or removed for simplification of the degradation scheme, as recommended by FOCUS (2014).

Figure 7.1.2.1.1-1: Route of degradation in aerobic soil

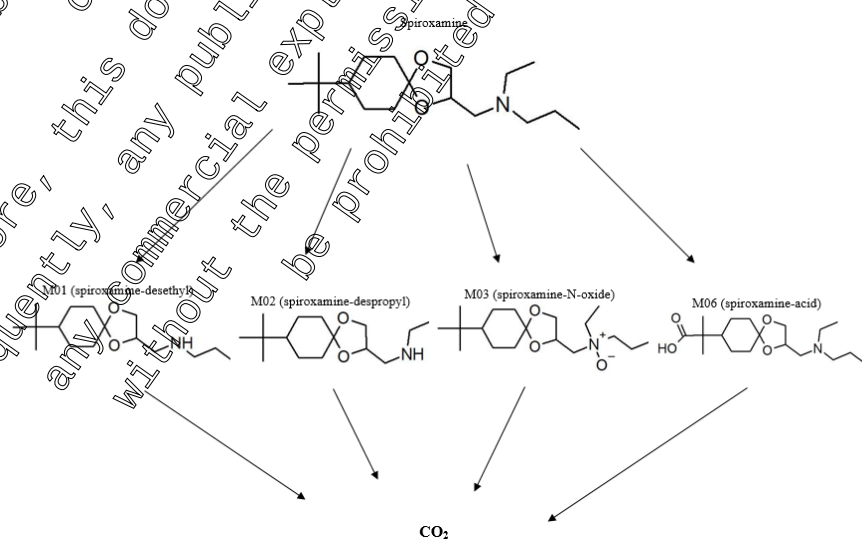
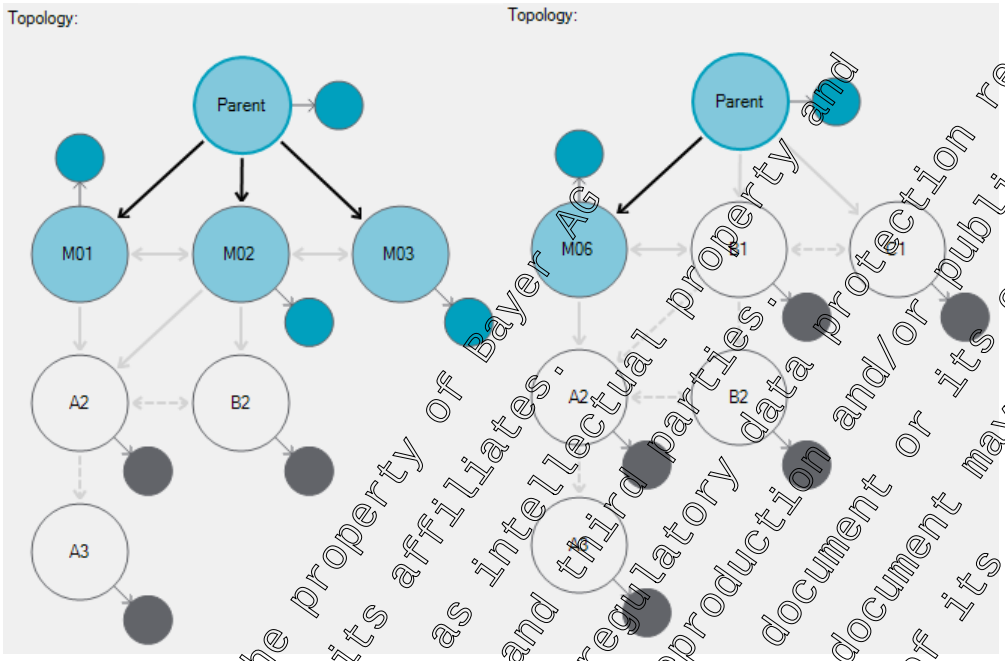


Figure 7.1.2.1.1-2: Degradation scheme used in kinetic analysis of all soils treated with cyclohexyl- or dioxolane- labelled spiroxamine



The acceptability of kinetic fits was judged both visually and according to the χ^2 error and the t-test functions as recommended by FOCUS. The visual assessment is recommended as the main tool for assessing goodness of fit. However, it is also recommended that a χ^2 error of less than 15% and a t-test probability of greater than 95% ($p < 0.05$) for estimated degradation rate constants indicate an acceptable fit. The χ^2 error was not considered as an absolute cut-off criterion as FOCUS guidance indicates that there will be cases where the error is higher than 15% but the fit still represents a reasonable description of the degradation behaviour. In such situations, examination of plots of residuals for systematic error is considered important.

The t-test assesses whether degradation rate constants differs significantly from zero (i.e., no degradation). Alternatively, confidence intervals can be examined. In this assessment, the t-test was chosen for assessing confidence in rate constants. When fitting the FOMC model, FOCUS guidance indicates that the t-test is not appropriate as a measure of confidence for the gamma-distribution parameters α and β. Therefore, if a FOMC fit indicated slow degradation, confidence intervals for β were examined to determine if they were high compared to the parameter estimate, which would indicate that the parameter estimate was not reliable.

When calculating modelling endpoints for a metabolite, it was considered important to derive a formation fraction wherever possible. In the FOCUS flowsheets, if the SFO fit for a metabolite is not considered acceptable, a case-by-case decision is required. The first option given is to assess the decline of the metabolite after its maximum ('top-down' method). However, this method does not allow formation fraction assessment. The second option given is to fix the formation fraction to a worst-case value (usually 1) and use this in combination with a worst-case DT_{50} (usually 1000 days). However, this method almost always results in a clear overestimation of observed metabolite residues. The final option given is to use alternative – but conservative – estimates that describe the observed patterns. In this assessment, alternative – but conservative – estimates were chosen, implemented by fixing the formation fraction to the fitted value and using this in combination with a conservative DT_{50}, or vice versa. The parameter to amend and its value were chosen based on expert judgement and iterative amendments to give a conservative description of the observed metabolite residue pattern.

The FOCUS (2014a) flowcharts for calculating persistence and modelling endpoints were followed.

If necessary, the kinetic endpoints derived for each soil were normalised to FOCUS reference conditions

(soil temperature of 20°C and soil moisture content equal to pF 2) following the procedures recommended by FOCUS.

II. Results and Discussion

Each soil has been considered following the steps in the flowcharts and the considerations are discussed in detail in Appendix 3: (of this document). Parent endpoints were derived from kinetic fits of parent only data (i.e., not including metabolite data) in order to avoid changes in metabolite evaluations influencing parent endpoints. Metabolite endpoints, including formations fractions, were derived from final kinetic fits (i.e., including all data). The full outputs from all kinetic fits used, including the initial parameters and flows, are presented in full in the study report (Appendix 2 of the study report).

The resulting persistence or best-fit endpoints are presented in Table CA 7.1.2.1.1-1.

The resulting modelling endpoints are presented in Table CA 7.1.2.1.1-2.

It is noted that the rate of degradation of spiroxamine varies between soils, with a range of DT_{50} values between 7.2 to 142 days for persistence, and 27.1 to 239 days for modelling. The route of degradation was consistent to some extent, with M01, M02 and M03 present in most tested soils. M06 is a new metabolite only identified at levels exceeding 5% AR in a new suite of studies performed to address new data requirements. Examination of the data indicates no obvious correlation with any soil properties that could potentially influence route or rate of degradation (e.g., pH).

Table CA 7.1.2.1.1-1: Summary of persistence endpoints for spiroxamine and metabolites M01, M02, M03 and M06

Soil properties			Spiroxamine				M01				M02			
Soil name	pH (H_2O)	Temp (°C) / % MWHC	DT_{50} / DT_{90} (days)	Normalised DT_{50} / DT_{90} (days)	χ^2 error (%)	Kinetic model	DT_{50} / DT_{90} (days)	Normalised DT_{50} / DT_{90} (days)	χ^2 error (%)	Kinetic model	DT_{50} / DT_{90} (days)	Normalised DT_{50} / DT_{90} (days)	χ^2 error (%)	Kinetic model
CA 7.1.1.1/01 (M-006135-01-1) 1994														
BBA 2.2/ Speyer 2.2	6.3	20 / 40[2]	66.6 / 296[1]	66.6/ 296[1]	3.2	DFOP	555[1] / 1,840[1]	555[1] / 1,840[1]	5.5	DFOP/ SFO	1,000 / 3,320[3]	1,000 / 3,320[3]	NA	DFOP/ SFO
CA 7.1.1.1/02 (M-006141-01-1) 1995														
Laacherhof	8.1	20 / 40	22.5 / 128[1]	14.2 / 80.9	3.3	DFOP	167[1] / 554[1]	105.5[1] / 350.1	6.2	DFOP/ SFO	1,000 / 3,320[3]	1,000 / 3,320[3]	NA	DFOP/ SFO
Monheim 3	6.5	20 / 40	33.2 / 176[1]	30.2 / 160.2	3.0	DFOP	366[1] / 1,220[1]	543.3[1] / 1801.8[1]	6.41	DFOP/ SFO	1,000 / 3,320[3]	1,000 / 3,320[3]	NA	DFOP/ SFO
Howe	7.1	20/ 75% 1/3 bar	29.9 / 168[1]	23.6 / 132.7[1]	3.8	DFOP	202[1] / 672[1]	159.6[1] / 530.9[1]	17.7	DFOP/ SFO	1,000 / 3,320[3]	1,000 / 3,320[3]	NA	DFOP/ SFO
CA 7.1.1.1/04 (M-006148-01-1) 1997														
Wolf Ranch	7.8	20/ 75% 1/3 bar	75 / 299[1]	53.0 / 211.1[1]	3.6	DFOP	78.2 / 260[1]	55.2 / 183.6[1]	18.1	DFOP/ SFO	95.4 / 317[1]	67.4 / 223.8[1]	19.0	DFOP/ SFO
CA 7.1.1.1/05 (M-303803-01-1) 2008														
Hoefchen am Hohenseh	7.0	20 / 55[2]	7.2 / 93.6	7.2 / 93.6	4.4	DFOP	78.7 / 261[1]	78.7 / 261[1]	10.9	DFOP/ SFO	78.8 / 262[1]	78.8 / 262[1]	14.8	DFOP/ SFO
CA 7.1.1.1/06 (M-762349-01-1) 2020														
Longwoods	7.8	20 / pF 2.0	13.1 / 81.2	13.1 / 81.2	3.2	DFOP	28.9 / 95.8	28.9 / 95.8	5.9	DFOP/ SFO	26.6 / 88.2	26.6 / 88.2	3.3	DFOP/ SFO
Refesol 02-A	7.1	20 / pF 2.0	116 / 510[1]	116 / 510[1]	4.9	DFOP	219[1] / 727[1]	219[1] / 727[1]	9.3	DFOP/ SFO	204[1] / 678[1]	204[1] / 678[1]	5.6	DFOP/ SFO
Refesol 03-G	5.9	20 / pF 2.0	142[1] / 696[1]	142[1] / 696[1]	1.5	DFOP	350[1] / 1,160[1]	350[1] / 1,160[1]	5.8	DFOP/ SFO	128 / 426[1]	128 / 426[1]	7.6	DFOP/ SFO
Speyer 6S	7.3	20 / pF 2.0	137[1] / 514[1]	137[1] / 514[1]	1.7	DFOP	70.7 / 235[1]	70.7 / 235[1]	7.4	DFOP/ SFO	65.2 / 217[1]	65.2 / 217[1]	11.4	DFOP/ SFO
Worst-case :			142[1] / 696[1]	142[1] / 696[1]			555[1] /1,840[1]	555[1] /1,840[1]			1,000/ 3,320	1,000/ 3,320		

Table CA 7.1.2.1.1-1: Summary of persistence endpoints for spiroxamine and metabolites M01, M02, M03 and M06 (continued)

Soil properties			M03				M06			
Soil name	**pH (H_2O)**	**Temp (°C) / % MWHC**	**DT_{50} / DT_{90} (days)**	**Normalised DT_{50} / DT_{90} (days)**	**χ^2 error (%)**	**Kinetic model**	**DT_{50} / DT_{90} (days)**	**Normalised DT_{50} / DT_{90} (days)**	**χ^2 error (%)**	**Kinetic model**
CA 7.1.1.1/01 (M-006135-01-1) 1994										
BBA 2.2/ Speyer 2.2	6.3	20 / 40[2]	29.8 / 98.8	29.8 / 98.8	18.0	DFOP / SFO	Not observed			
CA 7.1.1.1/02 (M-006141-01-1) 1995										
Laacherhof	8.1	20 / 40	58.1 / 193[1]	36.7 / 122[1]	10.7	DFOP / SFO	Not observed			
Monheim 3	6.5	20 / 40	Not observed				Not observed			
Howe	7.1	20 / (75% 1/3 bar)	Not observed				Not observed			
CA 7.1.1.1/04 (M-006148-01-1) 1997										
Wolf Ranch	7.8	20 / (75% 1/3 bar)	152 / 507[1]	107 / 358[1]	15.1	DFOP / SFO	Not observed			
CA 7.1.1.1/05 (M-303803-01-1) 2008										
Hoefchen am Hohenseh	7.0	20 / 55[2]	Not observed				Not observed			
CA 7.1.1.1/06 (M-762349-01-1) 2020										
Longwoods	7.8	20 / pF 2.0	16.7 / 55.4	16.7 / 55.4	8.1	DFOP / SFO	49.6 / 165[1]	49.6 / 165[1]	18.5	DFOP / SFO
Refesol 02-A	7.1	20 / pF 2.0	53.1 / 176[1]	53.1 / 176[1]	11.2	DFOP / SFO	1,000 / 3,320[3]	1,000/3,320[1,3]	16.1	DFOP / SFO
Refesol 03-G	5.9	20 / pF 2.0	49.9 / 166[1]	49.9 / 166[1]	9.3	DFOP / SFO	1,000 / 3,320[3]	1,000/3,320[1,3]	32.1	DFOP / SFO
Speyer 6S	7.3	20 / pF 2.0	79 / 262[1]	79 / 262[1]	14.3	DFOP / SFO	191[1] / 635[1]	191[1] / 635[1]	23.5	DFOP / SFO
		Worst-case :	152 / 507[1]	107 / 358[1]			1,000 / 3,320[1]	1,000/ 3,320[1]		

- component not observed in corresponding soil
1 - Interpret with care – extrapolated beyond experimental period
2 - Moisture value above pF 2.0
3 - FOCUS default

Table CA 7.1.2.1.1-2: Summary of modelling endpoints for spiroxamine and metabolites M01, M02, M03 and M06

Soil properties			Spiroxamine				M01				
Soil name	**pH (H_2O)**	**Temp (°C) / % MWHC**	**$DT_{50\ mod}$ (days)**	**$DT_{50\ mod}$ 20°C / pF 2 (days)**	**χ^2 error (%)**	**Kinetic model**	**$DT_{50\ mod}$ (days)**	**$DT_{50\ mod}$ 20°C / pF 2 (days)**	**f.f. from parent**	**χ^2 error (%)**	**Kinetic model**
CA 7.1.1.1/01 (M-006135-01-1) 1994											
BBA 2.2/ Speyer 2.2	6.3	20 / 40[2]	98.9	98.9	3.2	DFOP	1,000[3]	1,000[3]	0.162	NA	SFO
CA 7.1.1.1/02 (M-006141-01-1) 1995											
Laacherhof	8.1	20 / 40	45.4	28.7	3.3	DFOP	167[2]	105.5	0.137	6.2	SFO
Monheim 3	6.5	20 / 40	61.7	56.1	5.0	DFOP	1,000[3]	1,000[3]	0.150	NA	SFO
Howe	7.1	20 / (75% 1/3 bar)	59.5	47.0	3.8	DFOP	202[1]	159.6	0.090	NA	SFO
CA 7.1.1.1/04 (M-006148-01-1) 1997											
Wolf Ranch	7.8	20 / (75% 1/3 bar)	96.3	68.0	3.6	DFOP	78.2	55.2	0.182	18.1	SFO
CA 7.1.1.1/05 (M-303803-01-1) 2008											
Hoefchen am Hohen-seh	7.0	20 / 55[2]	65.4	65.4	7.0	FOMC	78.0	78.0	0.134	7.54	SFO
CA 7.1.1.1/06 (M-762349-01-1) 2020											
Longwoods	7.8	20 / pF 2.0	27.1	27.1	3.7	FOMC	34	34	0.246	7.55	SFO
Refesol 02-A	7.1	20 / pF 2.0	170	170[1]	1.9	DFOP	219[2]	219[2]	0.225	9.3	SFO
Refesol 03-G	5.9	20 / pF 2.0	239	239[1]	1.5	DFOP	304[2]	304[2]	0.258	5.8	SFO
Speyer 6S	7.3	20 / pF 2.0	163	163[1]	1.7	DFOP	70.7	70.7	0.242	7.4	SFO
		Geometric mean :		75.4			Geometric mean :	168.6	-		
		pH dependence :		No			Arithmetic mean :	-	0.183		
							pH dependence :	No			

1 Interpret with care – extrapolated beyond experimental period
2 Moisture value above pF 2.0
3 Conservative value (default)

Table CA 7.1.2.1.1-2: Summary of modelling endpoints for spiroxamine and metabolites M01, M02, M03 and M06 (continued)

Soil properties			M02					M03				
Soil name	**pH (H_2O)**	**Temp (°C) / % MWHC**	**$DT_{50\ mod}$ (days)**	**$DT_{50\ mod}$ 20°C / pF 2 (days)**	**f.f.$_{from\ parent}$**	**χ^2 error (%)**	**Kinetic model**	**$DT_{50\ mod}$ (days)**	**$DT_{50\ mod}$ 20°C / pF 2 (days)**	**f.f.$_{from\ parent}$**	**χ^2 error (%)**	**Kinetic model**
CA 7.1.1.1/01 (M-006135-01-1) 1994												
BBA 2.2/ Speyer 2.2	6.3	20 / 40[2]	1,000[3]	1,000[3]	0.104	9.39	SFO	29.8	29.8	0.125	18.0	SFO
CA 7.1.1.1/02 (M-006141-01-1) 1995												
Laacherhof	8.1	20 / 40	1,000[3]	1,000[3]	0.08	3.26	SFO	58.1	36.7	0.06	10.7	SFO
Monheim 3	6.5	20 / 40	1,000[3]	1,000[3]	0.1	NA	SFO	Not observed				
Howe	7.1	20 / (75% 1/3 bar)	1,000[3]	1,000[3]	0.07	NA	SFO	Not observed				
CA 7.1.1.1/04 (M-006148-01-1) 1997												
Wolf Ranch	7.8	20 / (75% 1/3 bar)	95.4	67.4	0.113	19.0	SFO	152	107	0.171	15.1	SFO
CA 7.1.1.1/05 (M-303803-01-1) 2008												
Hoefchen am Hohen-seh	7.0	20 / 55[2]	75.9	75.9	0.147	8.21	SFO	Not observed				
CA 7.1.1.1/06 (M-762349-01-1) 2020												
Longwoods	7.8	20 / pF 2.0	312	312	0.196	4.85	SFO	19.8	19.8	0.199	14.9	SFO
Refesol 02-A	7.1	20 / pF 2.0	204[1]	204[1]	0.166	5.6	SFO	53.1	53.1	0.160	11.2	SFO
Refesol 03-G	5.9	20 / pF 2.0	120	120	0.224	7.6	SFO	48.0	48.0	0.192	9.3	SFO
Speyer 6S	7.3	20 / pF 2.0	65.2	65.2	0.183	11.4	SFO	79.0	79.0	0.137	14.3	SFO
		Geometric mean :		219.4				Geometric mean :	46.4	-		
		Arithmetic mean :		-	0.138			Arithmetic mean :	-	0.149		
		pH dependence :		No				pH dependence :	No	-		

1 Interpret with care – extrapolated beyond experimental period
2 Moisture value above pF 2.0
3 Conservative value (default)

Table CA 7.1.2.1.1-2: Summary of modelling endpoints for spiroxamine and metabolites M01, M02, M03 and M06 (continued)

Soil properties			M06				
Soil name	**pH (H_2O)**	**Temp (°C) / % MWHC**	**$DT_{50\ mod}$ (days)**	**$DT_{50\ mod}$ 20°C / pF 2 (days)**	**f.f.$_{from\ parent}$**	**χ^2 error (%)**	**Kinetic model**
CA 7.1.1.1/06 (M-762349-01-1) 2020							
Longwoods	7.8	20 / pF 2.0	52.9	52.9	0.059	19.1	SFO
Refesol 02-A	7.1	20 / pF 2.0	1,000[3]	1,000[3]	0.096[3]	NA	SFO
Refesol 03-G	5.9	20 / pF 2.0	1,000[3]	1,000[3]	0.060[3]	NA	SFO
Speyer 6S	7.3	20 / pF 2.0	1,000[3]	1,000[3]	0.170	NA	SFO
		Geometric mean :		479.6			
		Arithmetic mean :		750	0.095		
		pH dependence :		No			

1 Interpret with care – extrapolated beyond experimental period
2 Moisture value above pF 2.0
3 Conservative value (default)

III. Conclusions

Persistence and modelling endpoints representing the degradation rates of spiroxamine and its metabolites M01, M02, M03 and M06 in a laboratory aerobic soil studies have been calculated in accordance with the guidance of FOCUS (2014). For M01, M02 and M06 alternative – but conservative – estimates were chosen in cases where acceptable fits were not possible. This was implemented by fixing the formation fraction to the fitted value and using this in combination with a conservative DT_{50} or vice versa. The parameter to amend and its value were chosen based on expert judgement and iterative amendments to give a conservative description of the observed metabolite residue pattern.

The spiroxamine persistence DT_{50} values ranged from 7.2 to 142 days and DT_{90} values ranged from 80.9 to 696 days. The geometric mean of the modelling DT_{50} values was 75.4 days.

M01 persistence DT_{50} values ranged from 28.9 to 555 days and DT_{90} value range from 95.8 to >1,000 days. The geometric mean of the modelling DT_{50} values was 168.6 days. The arithmetic mean of the formation fractions from parent was 0.183.

The M02 persistence DT_{50} values ranged from 26.6 to 1,000 days (FOCUS default) and DT_{90} values ranged from 88.2 to 3,320 days (FOCUS default). The geometric mean of the modelling DT_{50} values was 219.1 days. The arithmetic mean of the formation fractions from parent was 0.138.

The M03 persistence DT_{50} values ranged from 16.7 to 107 days and DT_{90} values ranged from 55.4 to 358 days. The geometric mean of the modelling DT_{50} values was 46.4 days. The arithmetic mean of the formation fractions from parent was 0.149.

Acceptable fitting could only be performed for persistence endpoints in one soil for M06 resulting in a number of presented default values. The persistence DT_{50} value was 49.6 days to 1,000 days whilst DT_{90} values was 165 days to 3,320 days. The geometric mean of modelling DT_{50} values was 479.6days. The formation fraction from parent was 0.095.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to guideline(s) FOCUS 2006, 2014 (required guideline). The study is considered valid to assess best fit and modelling DT_{50} values for spiroxamine and associated metabolites in laboratory soil studies.

CA 7.1.2.1.2 Aerobic degradation of metabolites, breakdown and reaction products

The rate of aerobic degradation in soil of metabolites of spiroxamine has been investigated, as part of the studies listed under Point CA 7.1.1.1, in five studies conducted with the active substance (KCA 7.1.2.1.1/01 to KCA 7.1.2.1.1/05) which were evaluated during the previous EU review. These studies have been sufficient to address the requirements for metabolites, however, for metabolite M06 (spiroxamine-acid) which is now included in the definition of the residue for risk assessment in soil (see CA 7.4.1) and where it was not always possible to derive acceptable degradation parameters for use in modelling, a new study is being conducted and will be provided as soon as possible.

Data Point:	KCA 7.1.2.1.2/01
Report Author:	TBA
Report Year:	TBA
Report Title:	TBA
Report No:	TBA
Document No:	TBA
Guideline(s) followed in study:	OECD Guideline for Testing of Chemicals, No. 307; Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
Remarks previous evaluation:	Not applicable (New study, not previously submitted)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

**2 A study investigating the aerobic degradation in soil of metabolite applied M06 is currently on-going and will be supplied as part of a top up submission (estimate September 2021).

CA 7.1.2.1.3 Anaerobic degradation of the active substance

The rate of anaerobic degradation in soil was investigated as part of the studies listed under Point CA 7.1.1.2, in two studies (KCA 7.1.1.2/01 and KCA 7.1.1.2/02) which were evaluated during the previous EU review. These studies sufficiently address the data requirement.

New studies, not previously evaluated

Data Point:	KCA 7.1.2.1.3/02
Report Author:	[redacted]; [redacted]
Report Year:	2021
Report Title:	[14C]spiroxamine: Route and rate of degradation in soil under anaerobic conditions at 20°C
Report No:	VC 19/053
Document No:	M-762348-01-1
Guideline(s) followed in study:	Commission Regulation (EU) No. 283/2013in accordance with Regulation (EC) No. 1107/2009 OECD 307
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The details of this study are fully summarised under point KCA 7.1.1.2/02.

Existing studies, previously evaluated

Data Point:	KCA 7.1.2.1.3/01
Report Author:	[redacted]
Report Year:	1998
Report Title:	Anaerobic aquatic metabolism of the active ingredient KWG 4168
Report No:	PF4288
Document No:	M-006010-02-1
Guideline(s) followed in study:	US EPA Pesticide Assessment Guidelines, Subdivision N, Chemistry Environmental Fate, § 162-3: Anaerobic Aquatic Metabolism Studies
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The details of this study are fully summarised under point KCA 7.2.2.3/06.

CA 7.1.2.1.4 Anaerobic degradation of metabolites, breakdown and reaction products

The degradation rate of any metabolites of spiroxamine under anaerobic conditions is adequately investigated in the studies conducted with the active substance under Point CA 7.1.2.1.2. Therefore, no further studies are provided.

CA 7.1.2.2 Field studies

CA 7.1.2.2.1 Soil dissipation studies

Based on the information presented under Points CA 7.1.1.1 and CA 7.1.2.1 and the summary of DT_{50} values presented in Table CA 7.1.2-1, either the laboratory soil DT_{50} of the active substance and metabolites M01 and M02 exceeds 60 days or the DT_{90} exceeds 200 days. Consequently, soil dissipation studies have been conducted with analysis to these components. Additionally, either the laboratory soil DT_{50} of metabolite M03 exceeds 60 days or the DT_{90} exceeds 200 days, however, this had only occured in one soil from an older study. However, the observation of M03 DT_{50} >60 days is confirmed in the Speyer 6S soil from KCA 7.1.1.1/06 (M-762349-01-1) confirmining the requirement for a field study. For M06, only a single reliable kinetic fit could be established which indicated a DT_{50} < 60 days, but potential requirements for a field dissipation study can only be confirmed after the completion of the M06 rate study which is currently being conducted but is unavailable at the time of submission. This is considered a data gap, rather than an issue that prevents finalisation of the risk assessment, as worst-case PEC values based on laboratory data can still be adequately derived.

The field dissipation of spiroxamine has been investigated at a total of eighteen (n=18) locations in five 'legacy' European trials (KCA 7.1.2.2.1/01 to KCA 7.1.2.2.1/05) which were evaluated during the previous EU review. In addition:

- three further studies were included during the last evaluation to address stability of stored samples from the field dissipation trials
- one further study was included during the last evaluation and therefore has been included for completeness (KCA 7.1.2.2.1/06 (M-036125-01-1) provides a comparison of laboratory and field degradation studies summarised more fully elsewhere)
- two further studies were included during the last evaluation to address the kinetic evaluation of

the degradation observed in the field dissipation trails and have therefore been included for completeness. These evaluations have been superseded by a new study conducted to modern requirements (KCA 7.1.2.2.1/12 (M-763140-01-1))

- one new study (KCA 7.1.2.2.1/12 (M-763140-01-1)) has been conducted to provide an up-to-date kinetic assessment of degradation rates observed in field dissipation trials to modern requirements (FOCUS 2014[2] and EFSA 2014[6])

Substance	Report reference	Document no.	Comment
Spiroxamine	KCA 7.1.2.2.1/01; KCA 7.1.4.3/01	M-006116-01-1	Submitted for first approval of spiroxamine, 1999. Reviewed under UP. Considered valid and acceptable.
Spiroxamine	KCA 7.1.2.2.1/02; CA 7.1.4.3/02	M-006126-01-1	
Spiroxamine	KCA 7.1.2.2.1/03; CA 7.1.4.3/03	M-006127-01-1	
Spiroxamine	KCA 7.1.2.2.1/04	M-006128-01-1	Submitted for first renewal of spiroxamine, 2010. Reviewed under UP. Considered valid and acceptable.
Spiroxamine	KCA 7.1.2.2.1/05	M-006129-01-1	
Spiroxamine	KCA 7.1.2.1.1/06; KCA 7.1.2.2.1/06	M-036125-01-1	
Storage stability			
Spiroxamine	KCA 7.1.2.2.1/07	M-006082-01-1	Submitted for first approval of spiroxamine, 1999. Reviewed under UP. Considered valid and acceptable.
Spiroxamine	KCA 7.1.2.2.1/08	M-006079-01-1	Submitted for first renewal of spiroxamine, 2010. Reviewed under UP. Considered valid and acceptable.
Spiroxamine	KCA 7.1.2.2.1/09	M-006074-01-1	
Kinetic evaluation			
Spiroxamine	KCA 7.1.2.2.1/10	M-293744-01-1	Submitted for first renewal of spiroxamine, 2010. Reviewed under UP. Considered valid and acceptable.
Spiroxamine	KCA 7.1.2.2.1/11	M-302004-01-1	
Spiroxamine	KCA 7.1.2.2.1/12	M-763140-01-1	New data not yet reviewed under UP

6 EFSA (2014) EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662, 37 pp., doi:10.2903/j.efsa.2014.3662.

Existing studies, previously evaluated

Data Point:	KCA 7.1.2.2.1/01
Report Author:	[redacted]
Report Year:	1995
Report Title:	Dissipation of KWG 4168 in soils under field conditions (Germany and Great Britain)
Report No:	RA-2078/93
Document No:	M-006116-01-1
Guideline(s) followed in study:	BBA Guideline IV-4.1 (1986)
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Soil dissipation of spiroxamine was studied after application as an EC formulation containing 494 g/L to soil under field conditions (some with and some without vegetation) for up to 258 days at four sites located in Germany and Great Britain (Höfchen, silt loam; Laacherhof, loam; Elm Farm, sandy loam and Pakenham, loamy sand).

Spiroxamine was applied at a single application or 1.5 L/ha (0.75 kg a.s./ha) in spring 1993.

Duplicate samples were collected immediately after the treatment and at intervals up to 258 days after the treatment (DAT). Samples were taken at the Höfchen site, at 0, 7, 14, 27, 60, 90, 120, 151, 180 and 239 DAT, at Laacherhof at 0, 7, 14, 28, 56, 90, 122, 152, 180 and 231 DAT, at Elm Farm at 0, 7, 14, 29, 56, 90, 120, 151, 180 and 258 DAT and at Pakenham at 0, 7, 14, 28, 55, 91, 120, 148, 177 and 239 DAT.

Twenty soil core samples were taken from the treated as well as ten soil core samples from the control plot directly post application. Core samples were taken to a depth of 30 cm and segmented into 10 cm soil layers prior to homogenisation and analysis. Soil samples were analysed for spiroxamine and the metabolites M01 (spiroxamine-desethyl) and M02 (spiroxamine-despropyl). Soil samples were extracted by Soxtec extraction with a solution of methanol/water/ammonia (25%) (8:2:0.1. v/v/v). Parent and metabolites were quantitatively determined by liquid chromatography with MS-MS detection. A limit of quantification (LOQ) of 5 µg/kg and a limit of detection (LOD) of 2 µg/kg was reported for spiroxamine and the metabolites.

Starting from an application rate of 0.75 kg a.i./ha and a soil density of 1.5 kg/L the theoretical total concentration in a 10 cm layer is 500 µg/kg soil. The concentration of spiroxamine in the 0-10 cm layer found immediately after application were 327, 262, 223 and 238 µg/kg for Höfchen, Laacherhof, Elm Farm and Pakenham trial sites, respectively. This equates that at day zero 65.4%, 52.3%, 44.6% and 47.6% of the applied amount was recovered.

During the whole test duration, nearly all residues remained in the 0-10 cm layers of the soil. The maximum concentration of spiroxamine was observed for the Höfchen (30122/1) trial site at 327 µg/kg at 0 DAT. Only on the Pakenham site was spiroxamine found in the 10-20 cm layer, the maximum value was 25.3 µg/kg at Day 0. No mobility into soil layers below 20 cm was observed. The metabolites M01 and M02 were detected at all sites. The metabolite M01 reached a maximum of 38.1 µg/kg at DAT 28 in the Pakenham trial site. The metabolite M02 reached a maximum of 43.7 µg/kg at DAT 28 in the Pakenham trial site in the 0-10 cm layer. In both the 10-20 cm and 20-30 cm layer for the metabolites M01 and

M02, no residues were found at levels greater than LOQ (5 µg/kg) at any time point.

The DT_{50} and DT_{90} of spiroxamine determined in the study was reported for each site. However, a re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) and the EFSA guidance on deriving $DegT_{50}$ values from laboratory and field dissipation studies (EFSA 2014), was performed in the report presented under point KCA 7.1.2.2.1/12 (M-763140-01-1).

I. Materials and Methods

A. Materials

1. Test Items

Spiroxamine (product code 30-0122915, Suspension concentrate formulation (494 g/L spiroxamine)

Certificate of analysis: Analysed and certified at March 17, 1999 by Dr. GroB, Bayer AG, PF-E/ET, D-51368 Leverkusen

Batch no.: FL 04023/0089 A, FAR 166-00

Purity: Not Stated

2. Trial locations & Soils

The study was performed at two different trial sites in Germany (Höfchen and Laacherhof) and Great Britain (Elm Farm and Pakenham). Site soil is characterised in Table CA 7.1.2.2.1-1. The field soil dissipation trial consisted of one treated and one untreated plot on each site. No pesticide history was given for previous years before the trial for either site.

Table CA 7.1.2.2.1-1: Location, site description and climatic data of test sites

Trial designation	301221/1	30124/8	30262/7	30263/5
Soil Designation	Höfchen	Laacherhof	Elm Farm	Pakenham
Vegetation	Bare soil	With vegetation [A]	With vegetation [A]	With vegetation [A]
Geographic Location	Deutsche Versuchsgüter 2 D-51399 Burscheid Höfchen Flur4011	Deutsche Versuchsgüter 3 D-40789 Monheim Laacherhof Flur715	Elm Farm Development Station GB Bury St. Edmunds Thurston, Suffolk Field 6, Block 4	Elm Farm Development Station GB Bury St. Edmunds Thurston, Suffolk Field 6, Block 4
Country	Germany	Germany	Great Britain	Great Britain
Textural Classification (USDA)	Silt loam	Loam	Sandy loam	Loamy sand
Sand [50 - 2000 µm] (%)	17.3	51.3	65.2	83.3
Silt [2 - 50 µm] (%)	69.9	34.0	19.1	10.9
Clay [< 2 µm] (%)	12.8	14.7	15.7	5.8
pH in $CaCl_2$	6.5	6.8	7.5	7.3
Organic matter (%)	1.57	1.86	1.96	1.51
Organic carbon (%)	0.97	1.08	1.14	0.88

Trial designation	301221/1	30124/8	30262/7	30263/5
Cation Exchange Capacity (meq/100 g)	15.0	10.0	13.0	8.0
Soil Moisture capacity (g/100g soil)	44.6	33.4	34.1	34.8
Size of plot (m^2)	225	75	325	325
Spray equipment	Agrotop Spaying-Boom	Agrotop Spaying-Boom	Knapsack Sprayer Fredo 2	Knapsack Sprayer Fredo 2
Nozzle type	Teejet XR 11004 VS	Lumarck 03-110 F	Teejet 8003 VS	Teejet 8003 VS

A The report does not provide details on crop type and extent of coverage for the covered sites in the study. However for study KCA 7.1.2.2.1/04 (M-006128-01-1), which uses some of the same sites, the cover crop was spring barley.

B. Study Design

1. Experimental Conditions

A single application of a nominal 0.75 kg a.s./ha as an emulsifiable concentrate (EC) was performed to bare soil on 22nd April 1993 (Höfchen) and to vegetation on 30th April 1993 (Laacherhof), 9th July 1993 (Elm Farm) and 9th July 1993 (Pakenham).

During the trials, soil cultivation and maintenance was performed according to the usual local agricultural practice. No irrigation was carried out on any of the sites. Weather data was collected at a location near to the site during the study period; this data includes the mean air temperature, rainfall and sunshine hours.

Soil dissipation of spiroxamine was studied for 258 days.

2. Sampling

Trials were sampled immediately after treatment and at intervals up to 258 days after the treatment. Samples were taken at the Höfchen site, at 0, 7, 14, 27, 60, 90, 120, 151, 180 and 239 days after treatment (DAT), at Laacherhof at 0, 7, 14, 28, 56, 90, 122, 152, 180 and 231 DAT, at Elm Farm at 0, 7, 14, 29, 56, 90, 120, 151, 180 and 258 DAT and at Pakenham at 0, 7, 14, 28, 55, 91, 120, 148, 177 and 239 DAT. Control samples (10 cores) were taken on the first and on the last sampling day from the control plots.

At each sampling point, 20 cores were collected using a pushing sampling system (Wacker Hammer) down to a depth of 30 cm (diameter 50 mm) per sampling interval. Locations of sampling were statistically distributed over the plot to get representative samples. Samples were transported in a cooler box containing dry ice before being stored at -18°C until analysis at the laboratory.

The frozen soil cores were cut into 10 cm segments. Ten (control sample) to twenty (treated samples) of such segment of one later were milled and carefully homogenised.

3. Analytical Procedures

Soil samples were analysed for spiroxamine and the metabolites M01 (spiroxamine-desethyl) and M02 (spiroxamine-despropyl). Replicate sub-samples were analysed by Method 00374 (RA-607/94) (M-019207-02-1), involving liquid chromatography.

In summary, the method involved Soxtec extraction with refluxing with a solution of methanol/water/ammonia (25%) (8:2:0.1, v/v/v). After solvent evaporation to the aqueous remainder the internal standard were added. A limit of quantification (LOQ) of 5 µg/kg was reported, and a limit of detection (LOD) of 2 µg/kg for spiroxamine and the metabolites.

Procedural recovery samples were analysed. Untreated soil samples were fortified with known amounts of spiroxamine, M01 and M02 and carrying these samples through the procedure alongside the treated samples.

4. Determination of degradation kinetics

The DT_{50} and DT_{90} of spiroxamine determined in the study were reported for each site. However, a re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (20142) and the EFSA guidance on deriving $DegT_{50}$ values from laboratory and field dissipation studies (EFSA 20145), was performed in the report presented under point KCA 7.1.2.2.1/12 (M-763140-01-1).

II. Results and Discussion

A. Analytical Methodology

Full details and acceptable validation data to support this method (Method 00374 (RA-607/94)) are presented in Document M-CA 4, Section 4.1.2 (M-019207-02-1). The method complies with the EU regulatory requirements outlined within SANCO/3029/99 rev. 4 and is suitable for the determination of spiroxamine and its metabolites in soil samples by HPLC/MS/MS.

Analytical performance was determined by analysing known amounts of spiroxamine and internal standards. The mean recovery of spiroxamine, M01 and M02 was 87.1 ± 13%, 89.8 ± 5.8% and 91.1 ± 6.7% of the nominal treatment rate respectively, over a fortification range of 5 to 500 µg/kg.

B. Data

Total residues detected in each treated plot are presented in the report. The results for spiroxamine and its metabolites are presented below as soil residue concentrations (on a µg/kg dry weight basis) for each of the treated plots in Table CA 7.1.2.2.1-2 to Table CA 7.1.2.2.1-3.

Table CA 7.1.2.2.1-2: Residues of spiroxamine in 0-30 cm horizons of soil at Höfchen expressed as µg/kg

Depth [cm]	Replicate	DAT									
		0	**7**	**14**	**27**	**60**	**90**	**120**	**151**	**180**	**239**
0-10	A	337	200	180	143	73.2	36.2	38.3	38.6	41.0	33.4
	B	307	248	169	154	71.6	40.7	35.6	45.6	46.9	31.7
	C	341	194	-	-	-	-	-	-	-	-
	D	325	228	-	-	-	-	-	-	-	-
	Mean	**327**	**210**	**175**	**148**	**72.4**	**38.4**	**36.9**	**42.1**	**44.0**	**32.5**
10-20	A	<LOQ	<LOQ	n.d.	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.
	B	<LOQ	<LOQ	n.d.	n.d.	<LOQ	<LOQ	<LOQ	n.d.	n.d.	<LOQ
	Mean	**<LOQ**	**<LOQ**	**n.d.**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**n.d.**	**n.d.**	**<LOQ**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-3: Residues of M01 (spiroxamine-desethyl) in 0-30 cm horizons of soil at Höfchen expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**27**	**60**	**90**	**120**	**151**	**180**	**239**
0-10	A	14.4	30.9	21.7	19.8	8.5	6.2	5.9	7.4	7.9	5.1
	B	18.2	33.5	22.2	21.4	10.7	6.6	<LOQ	7.5	7.9	6.0
	C	10.5	27.2	-	-	-	-	-	-	-	-
	D	10.5	33.0	-	-	-	-	-	-	-	-
	Mean	**13.4**	**31.1**	**21.9**	**20.6**	**9.6**	**6.4**	**<LOQ**	**7.5**	**7.9**	**5.5**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-4: Residues of M02 (spiroxamine-despropyl) in 0-30 cm horizons of soil at Höfchen expressed as µg/kg (mean)

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**27**	**60**	**90**	**120**	**151**	**180**	**239**
0-10	A	14.1	31.7	24.8	22.8	9.7	7.4	6.8	6.8	7.2	5.2
	B	17.0	34.9	24.4	23.6	10.5	7.4	5.9	6.6	7.2	5.7
	C	9.7	28.5	-	-	-	-	-	-	-	-
	D	9.7	36.0	-	-	-	-	-	-	-	-
	Mean	**12.6**	**32.8**	**24.6**	**23.2**	**10.1**	**7.4**	**6.4**	**6.7**	**7.2**	**5.4**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-5: Residues of spiroxamine in 0-30 cm horizons of soil at Laacherhof expressed as µg/kg

Depth [cm]	Replicate	DAT									
		0	7	14	28	56	90	122	152	180	231
0-10	A	241	210	181	156	100	48.6	42.5	39.9	39.0	24.2
	B	284	200	161	169	114	51.0	43.6	37.4	35.5	26.3
	C	254	207	-	-	-	-	-	-	-	-
	D	268	247	-	-	-	-	-	-	-	-
	Mean	**262**	**216**	**171**	**162**	**107**	**49.8**	**43.0**	**38.6**	**37.3**	**25.2**
10-20	A	n.d.	n.d.	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	<LOQ	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-6: Residues of M01 (spiroxamine-desethyl) in 0-30 cm horizons of soil at Laacherhof expressed as µg/kg

Depth [cm]	Replicate	DAT									
		0	7	14	28	56	90	122	152	180	231
0-10	A	7.5	19.2	27.6	33.0	29.3	15.8	16.8	14.3	14.6	9.1
	B	6.3	18.8	27.1	33.0	32.6	17.5	15.7	13.2	14.1	10.3
	C	9.0	20.2	-	-	-	-	-	-	-	-
	D	6.1	20.3	-	-	-	-	-	-	-	-
	Mean	**7.2**	**19.6**	**27.3**	**33.0**	**31.0**	**16.7**	**16.2**	**13.7**	**14.4**	**9.7**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-7: Residues of M02 (spiroxamine-despropyl) in 0-30 cm horizons of soil at Laacherhof expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**28**	**56**	**90**	**122**	**152**	**180**	**231**
0-10	A	7.0	18.1	26.2	29.7	25.8	13.9	14.7	12.9	13.0	8.0
	B	6.4	17.2	25.0	29.6	28.6	14.6	14.1	11.7	12.2	9.4
	C	8.5	18.6	-	-	-	-	-	-	-	-
	D	6.2	18.8	-	-	-	-	-	-	-	-
	Mean	**7.0**	**18.2**	**25.6**	**29.7**	**27.2**	**14.2**	**14.4**	**12.3**	**12.6**	**8.7**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-8: Residues of spiroxamine in 0-30 cm horizons of soil at Elm Farm expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**29**	**56**	**90**	**120**	**151**	**180**	**258**
0-10	A	247	86.0	90.1	85.8	54.5	43.8	39.8	27.5	27.2	16.4
	B	241	93.2	122	84.0	56.4	47.4	41.8	27.0	25.0	15.3
	C	181	81.9	-	-	-	-	-	-	-	-
	D	223	109	-	-	-	-	-	-	-	-
	Mean	**223**	**92.5**	**106**	**84.9**	**55.5**	**45.6**	**40.8**	**27.2**	**26.1**	**15.8**
10-20	A	7.6	<LOQ	<LOQ	5.3	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.
	B	5.4	5.5	<LOQ	<LOQ	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.
	Mean	**6.5**	**<LOQ**	**<LOQ**	**<LOQ**	**n.d.**	**<LOQ**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	5.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-9: Residues of M01 (spiroxamine-desethyl) in 0-30 cm horizons of soil at Elm Farm expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**29**	**56**	**90**	**120**	**151**	**180**	**258**
0-10	A	5.5	35.3	34.1	35.9	30.6	18.4	16.9	10.1	9.3	5.9
	B	<LOQ	34.8	40.5	39.7	28.8	19.3	16.0	10.1	8.8	5.9
	C	<LOQ	37.5	-	-	-	-	-	-	-	-
	D	<LOQ	35.8	-	-	-	-	-	-	-	-
	Mean	**<LOQ**	**35.8**	**37.3**	**37.8**	**29.7**	**18.9**	**16.5**	**10.1**	**9.0**	**5.9**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-10: Residues of M02 (spiroxamine-despropyl) in 0-30 cm horizons of soil at Elm Farm (20151/7) expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**29**	**56**	**90**	**120**	**151**	**180**	**258**
0-10	A	5.9	36.6	36.8	38.0	32.8	21.5	19.0	11.8	13.5	8.7
	B	5.5	37.3	42.4	40.4	32.1	21.7	19.1	11.7	12.5	8.3
	C	5.6	38.8	-	-	-	-	-	-	-	-
	D	<LOQ	37.2	-	-	-	-	-	-	-	-
	Mean	**<LOQ**	**37.5**	**39.6**	**39.2**	**32.4**	**21.6**	**19.0**	**11.8**	**13.0**	**8.5**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-11: Residues of spiroxamine in 0-30 cm horizons of soil at Pakenham expressed as µg/kg

Depth [cm]	Replicate	DAT									
		0	7	14	28	55	91	120	148	177	239
0-10	A	236	76.2	89.1	50.7	65.2	47.3	28.4	16.7	16.7	12.9
	B	234	87.5	89.3	80.2	67.9	54.4	27.5	14.2	14.2	11.9
	C	248	81.4	-	-	-	-	-	-	-	-
	D	233	68.2	-	-	-	-	-	-	-	-
	Mean	**238**	**78.3**	**89.2**	**65.4**	**66.5**	**50.9**	**28.0**	**15.5**	**15.4**	**12.4**
10-20	A	25.4	7.5	10.3	<LOQ	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.
	B	25.2	8.1	10.5	8.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**25.3**	**7.8**	**10.4**	**5.3**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	<LOQ	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	<LOQ	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-12: Residues of M01 (spiroxamine-desethyl) in 0-30 cm horizons of soil at Pakenham expressed as µg/kg

Depth [cm]	Replicate	DAT									
		0	7	14	28	55	91	120	148	177	239
0-10	A	8.9	35.1	32.1	41.5	39.7	32.0	15.5	8.4	8.9	7.1
	B	13.2	38.9	38.1	34.7	35.7	31.4	13.9	8.5	8.9	6.9
	C	10.4	38.4	-	-	-	-	-	-	-	-
	D	10.0	47.5	-	-	-	-	-	-	-	-
	Mean	**10.6**	**40.0**	**35.1**	**38.1**	**37.7**	**31.7**	**14.7**	**8.4**	**8.9**	**7.0**
10-20	A	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**<LOQ**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-13: Residues of M02 (spiroxamine-despropyl) in 0-30 cm horizons of soil at Pakenham expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**28**	**55**	**91**	**120**	**148**	**177**	**239**
0-10	A	9.0	40.0	36.6	45.5	46.1	39.1	20.8	10.4	11.7	9.5
	B	13.5	43.0	43.1	41.9	41.7	39.8	20.3	11.2	11.2	8.9
	C	10.9	43.1	-	-	-	-	-	-	-	-
	D	10.1	51.1	-	-	-	-	-	-	-	-
	Mean	**10.9**	**44.3**	**39.8**	**43.7**	**43.9**	**39.4**	**20.5**	**10.8**	**11.4**	**9.2**
10-20	A	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

C. Residues

No residue of spiroxamine, M01 and M02 above the LOQ of the analytical method could be found in the control samples.

Starting from an application rate of 0.75 kg a.s./ha and a soil density of 1.5 kg/l the theoretical total concentration in a 10 cm layer is 500 µg/kg soil. The concentration of spiroxamine in the 0-10 cm layer found immediately after application were 327, 262, 223 and 238 µg/kg for Höfchen, Laacherhof, Elm Farm and Pakenham trial sites, respectively. This equates that at day zero 65.4%, 52.3%, 44.6% and 47.6% of the applied amount was recovered.

For Höfchen (30122/1) trial site the mean residue concentration in the 0-10 cm soil segment decreased from 327 µg/kg at 0 DAT to 32.5 µg/kg at 239 DAT. In the 10-20 cm and 20-30 cm layer spiroxamine was not found at levels great than LOQ at any time point. For the metabolite, M01 in the 0-10 cm soil segment concentrations reached a maximum of 31.1 µg/kg at 7 DAT and decreased to 5.5 µg/kg at 239 DAT. For the metabolite, M02 in the 0-10 cm soil segment concentrations reached a maximum of 32.8 µg/kg at 7 DAT and decreased to 5.4 µg/kg at 239 DAT. In both the 10-20 cm and 20-30 cm layer for the metabolites M01 and M02, no residues were found at levels greater than LOQ (5 µg/kg) at any time point.

For Laacherhof (30124/8) trial site the mean residue concentration in the 0-10 cm soil segment decreased from 262 µg/kg at 0 DAT to 22.2 µg/kg at 231 DAT. In the 10-20 cm and 20-30 cm layer spiroxamine was not found at levels great than LOQ at any time point. For the metabolite, M01 in the 0-10 cm soil segment concentrations reached a maximum of 33.0 µg/kg at 28 DAT and decreased to 9.7 µg/kg at 231 DAT. For the metabolite, M02 in the 0-10 cm soil segment concentrations reached a maximum of 29.7 µg/kg at 28 DAT and decreased to 8.7 µg/kg at 231 DAT. In both the 10-20 cm and 20-30 cm layer for the metabolites M01 and M02, no residues were found at levels greater than LOQ (5 µg/kg) at any time point.

For Elm Farm (20151/7) trial site the mean residue concentration in the 0-10 cm soil segment decreased from 223 µg/kg at 0 DAT to 15.8 µg/kg at 258 DAT. In the 10-20 cm layer spiroxamine was found on one occasion, with the maximum value of 6.5 µg/kg found at DAT 0. In the 20-30 cm layer spiroxamine was not found at levels great than LOQ at any time point. For the metabolite, M01 in the 0-10 cm soil segment concentrations reached a maximum of 37.8 µg/kg at 29 DAT and decreased to 5.9 µg/kg at 258 DAT. For the metabolite, M02 in the 0-10 cm soil segment concentrations reached a maximum of 39.6 µg/kg at 14 DAT and decreased to 8.5 µg/kg at 231 DAT. In both the 10-20 cm and 20-30 cm layer for

the metabolites M01 and M02, no residues were found at levels greater than LOQ (5 µg/kg) at any time point.

For Pakenham (30263/5) trial site the mean residue concentration in the 0-10 cm soil segment decreased from 238 µg/kg at 0 DAT to 12.4 µg/kg at 239 DAT. In the 10-20 cm layer spiroxamine was found in small amounts, with the maximum value of 25.3 µg/kg found at DAT 0 and decreasing to 5.3 µg/kg at DAT 28. For the metabolite, M01 in the 0-10 cm soil segment concentrations reached a maximum of 38.1 µg/kg at 28 DAT and decreased to 7.0 µg/kg at 239 DAT. For the metabolite, M02 in the 0-10 cm soil segment concentrations reached a maximum of 43.7 µg/kg at 28 DAT and decreased to 9.2 µg/kg at 239 DAT. In both the 10-20 cm and 20-30 cm layer for the metabolites M01 and M02, no residues were found at levels greater than LOQ (5 µg/kg) at any time point.

F. Degradation kinetics

Degradation rates determined in the report are superseded by the report presented under point KCA 7.1.2.2.1/12 (M-763140-01-1).

III. Conclusion

Following a single application of spiroxamine at a nominal application rate of 0.75 kg a.i/ha to under field conditions without vegetation (Höfchen) and with vegetation (Laacherhof Film Farm and Pakenham) in spring 1993. The decline of spiroxamine and the formation and decline of its metabolites M01 and M02 was followed for up to 258 days after application at four sites in Germany and the UK.

During the whole test duration, nearly all residues remained in the 0-10 cm layers of the soil. The maximum concentration of spiroxamine was observed for the Höfchen (30122/1) trial site at 327 µg/kg at 0 DAT. Only in the Pakenham site was spiroxamine found in the 10-20 cm layer, the maximum value was 25.3 µg/kg at Day 0. No mobility into soil layers below 20 cm was observed. The metabolites M01 and M02 were detected at all sites. The metabolite M01 reached a maximum of 38.1 µg/kg at DAT 28 in the Pakenham trial site. The metabolite M02 reached a maximum of 43.7 µg/kg at DAT 28 in the Pakenham trial site in the 0-10 cm layer. In both the 10-20 cm and 20-30 cm layer for the metabolites M01 and M02, no residues were found at levels greater than LOQ (5 µg/kg) at any time point.

A re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) is performed in KCA 7.1.2.2.1/12 (M-763140-01-1).

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study is considered valid to assess the dissipation of spiroxamine under field conditions in soil. The study meets the requirements to assess field persistence of spiroxamine and its metabolites, and to derive parent soil $DegT_{50matrix}$ values for legacy field studies as defined by EFSA (2014). It is not suitable for assessing metabolite soil $DegT_{50matrix}$ values as the design did not minimise soil surface processes immediately after application as required by EFSA (2014).

Data Point:	KCA 7.1.2.2.1/02
Report Author:	[redacted]
Report Year:	1995
Report Title:	Dissipation of KWG 4168 in soils under field conditions
Report No:	RA-2002/94
Document No:	M-006126-01-1
Guideline(s) followed in study:	BBA Guideline IV-4.1 (1986)
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Soil dissipation of spiroxamine was studied after application as an EC formulation containing 491.4 g/L to bare soil plots under field conditions for up to 270 days at five trial sites in Germany (Höfchen, silt loam; Laacherhof, sandy loam; Maasen, sandy loam; Swisttal-Hohn, silt loam and Albig, clay loam/silty clay loam).

In spring 1994 a single application of a nominal 0.75 kg a.s./ha as an emulsifiable concentrate formulation was performed to bare soil.

Twenty soil core samples were taken from the treated as well as ten soil core samples from the control plot directly post application. Core samples were taken to a depth of 30 cm and segmented into 10 cm soil layers prior to homogenisation and analysed. Samples were taken at the Höfchen site, at 0, 7, 14, 29, 56, 89, 118, 155, 190 and 270 days after treatment (DAT), at Laacherhof at 0, 7, 14, 28, 59, 87, 120, 152, 181 and 240 DAT, at Maasen at 0, 7, 14, 30, 60, 90, 120, 150, 180 and 240 DAT, at Swisttal-Hohn at 0, 7, 14, 30, 58, 94, 120, 150, 181 and 252 DAT and at Albig at 0, 7, 14, 30, 60, 90, 120, 150, 180 and 240 DAT.

Soil samples were analysed for spiroxamine and the metabolites M01 (spiroxamine-desethyl) and M02 (spiroxamine-despropyl). Soil samples were extracted by Soxtec extraction with a basic solution of methanol/water/ammonia (25%) (8:2:0.1 v/v/v). Parent and metabolites were quantitatively determined by liquid chromatography with MS-MS detection. A limit of quantification (LOQ) of 5 µg/kg and a limit of detection (LOD) of 2 µg/kg was reported for spiroxamine and the metabolites.

Starting from an application rate of 0.75 kg a.s./ha and a soil density of 1.5 kg/L the theoretical total concentration in a 10 cm layer is 500 µg/kg soil. The concentration of spiroxamine in the 0-10 cm layer found immediately after application were 365, 395, 284, 325 and 387 µg/kg for Höfchen, Laacherhof, Maasen, Swisttal-Hohn and Albig trial sites, respectively. This equates that at day zero 73.0%, 79.0%, 56.8%, 65.0% and 77.4% of the applied amount was recovered from each site, respectively.

During the whole test duration, nearly all residues remained in the 0-10 cm layers of the soil. The maximum concentration of spiroxamine was observed for the Albig trial site at 386 µg/kg at 0 DAT. Only in the Höfchen was spiroxamine found in the 10-20 cm layer, the maximum value of 9.4 µg/kg was reported at 89 DAT. No mobility into soil layers below 20 cm was observed. The metabolites M01 and M02 were detected at all sites. The metabolite M01 reached a maximum of 45.8 µg/kg at 14 DAT in Swisttal-Hohn in the 0-10 cm layer. The metabolite M02 reached a maximum of 43.6 µg/kg at 7 DAT in Swisttal-Hohn in the 0-10 cm layer.

The DT_{50} and DT_{90} of spiroxamine determined in the study was reported for each site. However, a re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) and the EFSA guidance on deriving DegT50 values from laboratory and field dissipation

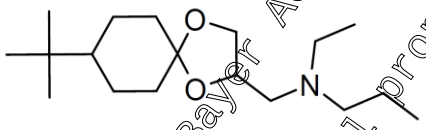
studies (EFSA 2014), was performed in the report presented under point KCA 7.1.2.2.1/12 (M-763140-01-1).

I. Materials and Methods

A. Materials

1. Test Items

Spiroxamine (product code 0122915, Suspension concentrate formulation (491.4 g/L spiroxamine)



Certificate of analysis: Analysed and certified at December 16, 1993 by Dr. GroB, Bayer AG, PF-E/FT, D-51368 Leverkusen

Batch no.: FL 04023.0089 A, FAR 210

Purity: Not Stated

2. Trial locations & Soils

The study was performed in Germany at five different sites. Site soil is characterised in Table CA 7.1.2.2.1-14. The field soil dissipation trial consisted of one treated and one untreated plot on each site. No pesticide history was given for previous years before the trial for either site.

Table CA 7.1.2.2.1-14: Location, site description and climatic data of test sites

Trial designation	40006/8	40007/6	40008/4	40009/2	40010/06
Soil Designation	Hörchen	Laachenhof	Maasen	Swisttal-Hohn	Albig
Vegetation	Bare soil	Bare soil	Bare soil	Bare soil	Bare soil
Geographic Location Country	Deutsche Versuchsgüter 2 D-51399 Burscheid Hörchen Flur 4018 Germany	Deutsche Versuchsgüter 3 D-40789 Monheim Laachenhof Flur 715 Germany	Deutschland Nordost D-27249 Maasen An der Scheune Flur 79/7 Germany	Deutsche Versuchsgüter 1 D-53913 Swisttal-Hohn Flur 790, J. Brünker Germany	Deutschland Südweat, D-55234 Albig, Flur 37 Nr. 25 Im Odernheimer Weg Germany
Textural Classification (USDA)	Silt loam	Sandy loam	Sandy loam	Silt loam	Clay loam/ silty clay loam
Sand [50 - 2000 µm] (%)	6.5	70.8	66.4	27.6	20.0
Silt [2 – 50 µm] (%)	76.6	20.1	30.4	60.4	51.1
Clay [< 2 µm] (%)	16.9	9.1	3.2	12.0	28.9
pH (in 0.01 M $CaCl_2$ solution)	6.4	6.6	5.9	6.7	7.8
Organic matter (%)	1.50	2.08	2.18	1.72	2.41
Organic carbon (%)	0.87	1.21	1.27	1.00	1.40

Trial designation	40006/8	40007/6	40008/4	40009/2	40010/06
Cation Exchange Capacity (meq/100 g)	1.50	8.0	8.0	9.0	17
Soil Moisture capacity (g/100g soil)	39.2	40.6	32.1	36.7	44.9
Size of plot (m^2)	227	255	200	255	200

B. Study Design

1. Experimental Conditions

A single application of a nominal 0.75 kg a.s./ha as an emulsifiable concentrate (EC) was performed to bare soil on 21st April 1994 (Höfchen), 26h April 1994 (Laacherhof), 10th May 1994 (Maasen), 10th May 1994 (Swisttal-Hohn) and 21st May 1994 (Albig).

During the studies on bare soil mechanical weed control was performed. No plant protection products or irrigation was carried out and any of the trail sites. Weather data was collected at a location near to the site during the study period; this data includes the mean air temperature, rainfall and sunshine hours.

Soil dissipation of spiroxamine was studied for a maximum of 270 days.

2. Sampling

Duplicate samples were collected immediately after the treatment and at intervals up to 270 days after the treatment. Core samples were taken to a depth of 30 cm (diameter 50 mm) and segmented into 10 cm soil layers prior to homogenisation and analysed. Samples were taken at the Höfchen site, at 0, 7, 14, 29, 56, 89, 118, 155, 190 and 270 days after treatment (DAT), at Laacherhof at 0, 7, 14, 28, 59, 87, 120, 152, 181 and 240 DAT, at Maasen at 0, 7, 14, 30, 60, 90, 120, 150, 180 and 240 DAT, at Swisttal-Hohn at 0, 7, 14, 30, 58, 91, 120, 150, 181 and 252 DAT and at Albig at 0, 7, 14, 30, 60, 90, 120, 150, 180 and 240 DAT. The control plots were sampled at the beginning and end of the study.

At each sampling point 20 cores were taken using a pushing sampling system (Wacker Hammer) down to a depth of 30 cm (diameter 50 mm) per sampling interval. Locations of sampling were statistically distributed over the plot to get representative samples. Samples were transported in a cooler box containing dry ice, before being stored at -18°C until analysis at the laboratory.

The frozen soil cores were cut into 10 cm segments. Ten (control sample) to twenty (treated samples) of such segment of one later were milled and carefully homogenised.

3. Analytical Procedures

Soil samples were analysed for spiroxamine and the metabolites M01 (spiroxamine-desethyl) and M02 (spiroxamine-despropyl). Replicate sub-samples were analysed by Method 00374 (RA-607/94) (M-019267-02-1), using HPLC-MS/MS.

In summary, the method involved Soxtec extraction with refluxing methanol/water/ammonia (25%) (8:2:0.1, v/v/v). After solvent evaporation to the aqueous remainder the internal standard were added. The LOQ (5 µg/kg) of the method was 2 µg/kg for parent and metabolites.

Procedural recovery samples were analysed. Untreated soil samples were fortified with known amounts of spiroxamine, M01 and M02 and carrying these samples through the procedure alongside the treated samples.

4. Determination of degradation kinetics

The DT_{50} and DT_{90} of spiroxamine determined in the study was reported for each site. However, a re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) and the EFSA guidance on deriving $DegT_{50}$ values from laboratory and field dissipation

studies (EFSA 2014), was performed in the report presented under point KCA 7.1.2.2.1/12 (M-763140-01-1).

II. Results and Discussion

A. Analytical Methodology

Full details and acceptable validation data to support this method (Method 00374 (RA-607/94)) are presented in Document M-CA 4, Section 4.1.2 (M-019207-02-1). The method complies with the EU regulatory requirements outlined within SANCO/3029/99-rev. 4 and is suitable for the determination of spiroxamine and its metabolites in soil samples by HPLC/MS/MS.

Analytical performance was determined by analysing known amounts of spiroxamine and internal standards. The mean recovery of spiroxamine, M01 and M02 was 87.1 ± 13%, 89.8 ± 5.8% and 91.1 ± 6.7% respectively, over a fortification range of 5 to 500 µg/kg.

B. Data

Total residues detected in each treated plot are presented in the report. The results for spiroxamine and its metabolites are presented below as soil residue concentrations (on a µg/kg dry weight basis) for each of the treated plots in Table CA 7.1.2.2.1-15 to Table CA 7.1.2.2.1-29.

Table CA 7.1.2.2.1-15: Residues of spiroxamine in 0-30 cm horizons of soil at Höfchen expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**29**	**56**	**89**	**118**	**155**	**190**	**270**
0-10	A	303	277	327	250	179	125	84.4	81.9	58.2	59.2
	B	364	345	302	249	197	128	90.5	75.5	66.0	60.8
	C	452	294	-	-	-	-	-	-	-	-
	D	343	314	-	-	-	-	-	-	-	-
	Mean	**365**	**308**	**315**	**249**	**188**	**127**	**87.5**	**78.7**	**62.1**	**60.0**
10-20	A	n.d.	n.d.	<LOQ	5.7	7.9	10.8	6.7	<LOQ	<LOQ	n.d.
	B	<LOQ	n.d.	n.d.	6.4	7.0	8.0	7.2	<LOQ	<LOQ	n.d.
	Mean	**<LOQ**	**n.d.**	**<LOQ**	**6.0**	**7.0**	**9.4**	**7.0**	**<LOQ**	**<LOQ**	**n.d.**
20-30	A	n.d.	n.d.	<LOQ	<LOQ	<LOQ	<LOQ/6.2	<LOQ	6.2	<LOQ	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	7.1/5.1	n.d.	<LOQ	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-16: Residues of M01 (spiroxamine-desethyl) in 0-30 cm horizons of soil at Höfchen expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**29**	**56**	**89**	**118**	**155**	**190**	**270**
0-10	A	12.4	14.7	19.2	22.5	19.6	19.2	16.9	13.2	13.1	10.8
	B	13.3	15.9	18.9	23.5	20.4	19.9	16.9	13.5	12.4	12.1
	C	12.0	15.9	-	-	-	-	-	-	-	-
	D	9.6	18.6	-	-	-	-	-	-	-	-
	Mean	**11.8**	**16.3**	**19.0**	**23.0**	**20.0**	**19.6**	**16.5**	**13.4**	**12.7**	**11.4**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-17: Residues of M02 (spiroxamine-despropyl) in 0-30 cm horizons of soil at Höfchen expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**29**	**56**	**89**	**118**	**155**	**190**	**270**
0-10	A	11.0	13.5	17.4	22.4	19.7	18.6	15.7	10.2	12.0	10.3
	B	12.5	14.5	18.6	24.7	20.7	19.7	15.7	10.6	11.0	10.7
	C	11.0	14.2	-	-	-	-	-	-	-	-
	D	8.8	17.0	-	-	-	-	-	-	-	-
	Mean	**10.8**	**14.8**	**18.0**	**23.5**	**20.2**	**19.2**	**15.6**	**10.4**	**11.5**	**10.5**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	5.4	n.d.	<LOQ	8.9/n.d.	<LOQ	<LOQ	<LOQ	n.d.
	Mean	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-18: Residues of spiroxamine in 0-30 cm horizons of soil at Laacherhof expressed as µg/kg

Depth [cm]	Replicate	DAT									
		0	7	14	28	59	87	120	153	181	240
0-10	A	385	223	233	180	87.3	70.7	38.7	31.7	30.6	28.3
	B	408	260	247	179	98.0	68.5	39.1	33.4	29.6	28.2
	C	422	240	-	-	-	-	-	-	-	-
	D	366	263	-	-	-	-	-	-	-	-
	Mean	**395**	**247**	**240**	**180**	**92.6**	**69.6**	**38.9**	**32.5**	**30.1**	**28.3**
10-20	A	n.d.	n.d.	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	<LOQ	<LOQ	n.d.	<LOQ	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**<LOQ**	**<LOQ**	**<LOQ**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	<LOQ	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**<LOQ**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-19: Residues of M01 (spiroxamine-desethyl) in 0-30 cm horizons of soil at Laacherhof expressed as µg/kg

Depth [cm]	Replicate	DAT									
		0	7	14	28	59	87	120	153	181	240
0-10	A	10.3	16.7	23.8	30.7	20.6	25.3	17.7	16.2	16.2	17.3
	B	8.9	19.2	26.3	26.8	24.6	25.8	18.6	17.5	15.6	16.7
	C	11.0	20.5	-	-	-	-	-	-	-	-
	D	7.3	20.3	-	-	-	-	-	-	-	-
	Mean	**9.4**	**19.2**	**25.1**	**28.7**	**22.6**	**25.6**	**18.1**	**16.9**	**15.9**	**17.0**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-20: Residues of M02 (spiroxamine-despropyl) in 0-30 cm horizons of soil at Laacherhof expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**28**	**59**	**87**	**120**	**153**	**181**	**240**
0-10	A	10.7	15.4	22.1	30.5	18.9	21.6	15.4	13.6	13.4	14.1
	B	8.8	17.0	24.9	26.9	20.6	22.5	15.1	15.6	13.5	14.1
	C	10.5	18.2	-	-	-	-	-	-	-	-
	D	7.1	18.7	-	-	-	-	-	-	-	-
	Mean	**9.3**	**17.3**	**23.5**	**28.7**	**19.8**	**22.1**	**15.4**	**14.6**	**13.5**	**14.1**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-21: Residues of spiroxamine in 0-30 cm horizons of soil at Maasen expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**30**	**60**	**90**	**120**	**150**	**180**	**240**
0-10	A	286	140	136	155	86.1	67.1	69.2	48.2	66.5	74.1
	B	271	171	142	133	87.1	75.9	63.6	44.0	62.7	54.5
	C	276	163	-	-	-	-	-	-	-	-
	D	305	150	-	-	-	-	-	-	-	-
	Mean	**284**	**156**	**139**	**144**	**86.6**	**71.5**	**66.4**	**46.1**	**64.6**	**64.3**
10-20	A	n.d.	n.d.	n.d.	<LOQ	<LOQ	6.4	<LOQ	<LOQ	<LOQ	n.d.
	B	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	<LOQ	n.d.	<LOQ
	Mean	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**
20-30	A	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	6.9	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-22: Residues of M01 (spiroxamine-desethyl) in 0-30 cm horizons of soil at Maasen expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**30**	**60**	**90**	**120**	**150**	**180**	**240**
0-10	A	5.4	13.4	16.4	19.8	15.0	18.6	19.2	14.1	20.2	20.8
	B	<LOQ	16.7	18.5	18.8	15.9	17.8	20.1	13.8	18.8	16.3
	C	9.2	15.8	-	-	-	-	-	-	-	-
	D	12.1	15.6	-	-	-	-	-	-	-	-
	Mean	**7.3**	**15.4**	**17.4**	**19.3**	**15.5**	**18.2**	**19.7**	**14.0**	**19.5**	**18.5**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-23: Residues of M02 (spiroxamine-despropyl) in 0-30 cm horizons of soil at Maasen expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**30**	**60**	**90**	**120**	**150**	**180**	**240**
0-10	A	7.5	12.1	15.6	19.9	12.6	13.5	16.0	11.0	14.4	19.3
	B	5.3	15.0	16.7	18.2	13.7	12.9	14.5	10.1	15.1	13.2
	C	9.3	14.6	-	-	-	-	-	-	-	-
	D	11.1	13.8	-	-	-	-	-	-	-	-
	Mean	**7.8**	**13.9**	**16.1**	**19.0**	**13.0**	**13.2**	**15.2**	**10.6**	**14.7**	**16.3**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-24: Residues of spiroxamine in 0-30 cm horizons of soil at Swisttal-Hohn expressed as µg/kg

Depth [cm]	Replicate	DAT									
		0	7	14	30	58	91	120	150	181	252
0-10	A	338	181	142	68.6	50.2	35.4	26.5	11.9	11.4	8.9
	B	328	184	142	72.0	50.3	28.6	28.6	11.8	11.1	8.8
	C	289	169	-	-	-	-	-	-	-	-
	D	345	182	-	-	-	-	-	-	-	-
	Mean	**325**	**179**	**142**	**70.3**	**50.3**	**32.0**	**27.6**	**11.9**	**11.2**	**8.9**
10-20	A	n.d.	<LOQ	<LOQ	<LOQ	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	<LOQ	<LOQ	n.d.	<LOQ	<LOQ	<LOQ	n.d.
	Mean	**n.d.**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**n.d.**	**<LOQ**	**<LOQ**	**<LOQ**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-25: Residues of M01 (spiroxamine-desethyl) in 0-30 cm horizons of soil at Swisttal-Hohn expressed as µg/kg

Depth [cm]	Replicate	DAT									
		0	7	14	30	58	91	120	150	181	252
0-10	A	8.0	41.9	41.4	38.3	23.5	22.0	19.5	7.9	8.4	5.3
	B	7.3	47.7	50.2	32.2	24.3	19.3	19.2	8.0	8.1	6.2
	C	7.1	44.9	-	-	-	-	-	-	-	-
	D	6.9	45.8	-	-	-	-	-	-	-	-
	Mean	**7.3**	**45.1**	**45.8**	**35.2**	**23.9**	**20.7**	**19.3**	**7.9**	**8.3**	**5.8**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-26: Residues of M02 (spiroxamine-despropyl) in 0-30 cm horizons of soil at Swisttal-Hohn expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**30**	**58**	**91**	**120**	**150**	**181**	**252**
0-10	A	8.0	41.4	38.7	38.3	26.7	22.8	20.2	8.9	9.7	6.6
	B	7.6	45.8	47.5	31.2	26.9	20.7	19.7	9.4	9.9	7.7
	C	7.2	43.4	-	-	-	-	-	-	-	-
	D	7.5	43.7	-	-	-	-	-	-	-	-
	Mean	**7.6**	**43.6**	**43.1**	**34.8**	**26.8**	**21.7**	**20.1**	**9.1**	**9.8**	**7.1**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	<LOQ	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**<LOQ**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-27: Residues of spiroxamine in 0-30 cm horizons of soil at Albig expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**30**	**60**	**90**	**120**	**150**	**180**	**240**
0-10	A	383	209	135	103	42.6	40.0	29.6	15.8	7.6	<LOQ
	B	405	200	128	102	41.1	41.6	23.5	14.8	8.7	7.7
	C	392	207	-	-	-	-	-	-	-	-
	D	366	187	-	-	-	-	-	-	-	-
	Mean	**386**	**201**	**131**	**102**	**41.9**	**40.8**	**26.5**	**15.3**	**8.2**	**5.1**
10-20	A	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**<LOQ**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-28: Residues of M01 (spiroxamine-desethyl) in 0-30 cm horizons of soil at Albig expressed as µg/kg

Depth [cm]	Replicate	DAT									
		0	7	14	30	60	90	120	150	180	240
0-10	A	21.1	40.3	30.6	55.1	31.4	22.0	20.2	15.8	7.1	<LOQ
	B	22.5	40.1	40.2	58.6	25.5	28.3	23.2	15.7	9.0	6.4
	C	19.6	26.0	-	-	-	-	-	-	-	-
	D	31.0	37.6	-	-	-	-	-	-	-	-
	Mean	**23.5**	**36.0**	**35.4**	**56.9**	**28.4**	**25.2**	**21.9**	**15.7**	**8.0**	**<LOQ**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-29: Residues of M02 (spiroxamine-despropyl) in 0-30 cm horizons of soil at Albig expressed as µg/kg

Depth [cm]	Replicate	DAT									
		0	7	14	30	60	90	120	150	180	240
0-10	A	23.5	41.4	35.4	50.8	30.2	25.9	26.4	18.9	9.8	<LOQ
	B	21.6	46.8	38.9	53.1	29.8	30.0	19.8	17.6	9.1	8.3
	C	15.4	29.1	-	-	-	-	-	-	-	-<LOQ
	D	31.8	38.9	-	-	-	-	-	-	-	-
	Mean	**22.6**	**39.0**	**37.2**	**52.0**	**29.8**	**28.0**	**23.1**	**18.3**	**9.5**	**5.4**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	<LOQ
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	<LOQ	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**<LOQ**	**<LOQ**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ
	B	n.d.	LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ
	Mean	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

C. Residues

No residue concentration of spiroxamine, M01 and M02 above the LOQ (5 µg/kg) of the analytical method could be found in the control samples.

Starting from an application rate of 0.75 kg a.s./ha and a soil density of 1.5 kg/L the theoretical total concentration in a 10 cm layer is 500 µg/kg soil. The concentration of spiroxamine in the 0-10 cm layer found immediately after application were 365, 395, 284, 325 and 387 µg/kg for Höfchen, Laacherhof, Maasen, Swisttal-Hohn and Albig trial sites, respectively. This equates that at day zero 73.0%, 79.0%, 56.8%, 65.0% and 77.4% of the applied amount was recovered from each site, respectively.

For the Höfchen trial site the mean residue concentration in the 0-10 cm soil segment decreased from 365 µg/kg at 0 DAT to 60.0 µg/kg at 270 DAT. In the 10-20 cm layer spiroxamine was found on four occasion at values greater than the LOD, with the maximum value of 9.4 µg/kg found at DAT 89. In the

20-30 cm layer spiroxamine was not found at levels great than the LOQ (5 µg/kg) at any time point. For the metabolite, M01 in the 0-10 cm soil segment concentrations reached a maximum of 23.0 µg/kg at 29 DAT and decreased to 11.4 µg/kg at 270 DAT. For the metabolite, M02 in the 0-10 cm soil segment concentrations reached a maximum of 23.5 µg/kg at 29 DAT and decreased to 10.5 µg/kg at 270 DAT. In both the 10-20 cm and 20-30 cm layer for the metabolites M01 and M02, no residues were found at levels greater than the LOQ (5 µg/kg) at any time point.

For Laacherhof trial site the mean residue concentration in the 0-10 cm soil segment decreased from 247 µg/kg at 0 DAT to 28.3 µg/kg at 240 DAT. In the 10-20 cm and 20-30 cm layer spiroxamine was not found at levels great than the LOQ (5 µg/kg) at any time point. For the metabolite, M01 in the 0-10 cm soil segment concentrations reached a maximum of 28.7 µg/kg at 28 DAT and decreased to 17.0 µg/kg at 240 DAT. For the metabolite, M02 in the 0-10 cm soil segment concentrations reached a maximum of 28.7 µg/kg at 28 DAT and decreased to 14.1 µg/kg at 240 DAT. In both the 10-20 cm and 20-30 cm layer for the metabolites M01 and M02, no residues were found at levels greater than the LOQ (5 µg/kg) at any time point.

For Maasen trial site the mean residue concentration in the 0-10 cm soil segment decreased from 284 µg/kg at 0 DAT to 64.3 µg/kg at 240 DAT. In the 10-20 cm and 20-30 cm layer spiroxamine was not found at levels great than the LOQ (5 µg/kg) at any time point. For the metabolite, M01 in the 0-10 cm soil segment concentrations reached a maximum of 19.7 µg/kg at 120 DAT and decreased to 18.5 µg/kg at 240 DAT. For the metabolite, M02 in the 0-10 cm soil segment concentrations reached a maximum of 19.0 µg/kg at 30 DAT and decreased to 16.3 µg/kg at 240 DAT. In both the 10-20 cm and 20-30 cm layer for the metabolites M01 and M02, no residues were found at levels greater than the LOQ (5 µg/kg) at any time point.

For Swisttal-Hohn trial site the mean residue concentration in the 0-10 cm soil segment decreased from 325 µg/kg at 0 DAT to 8.9 µg/kg at 252 DAT. In the 10-20 cm and 20-30 cm layer spiroxamine was not found at levels great than the LOQ (5 µg/kg) at any time point. For the metabolite, M01 in the 0-10 cm soil segment concentrations reached a maximum of 45.8 µg/kg at 14 DAT and decreased to 5.8 µg/kg at 252 DAT. For the metabolite, M02 in the 0-10 cm soil segment concentrations reached a maximum of 43.6 µg/kg at 7 DAT and decreased to 7.1 µg/kg at 252 DAT. In both the 10-20 cm and 20-30 cm layer for the metabolites M01 and M02, no residues were found at levels greater than the LOQ (5 µg/kg) at any time point.

For Albig trial site the mean residue concentration in the 0-10 cm soil segment decreased from 386 µg/kg at 0 DAT to 5.1 µg/kg at 240 DAT. In the 10-20 cm and 20-30 cm layer spiroxamine was not found at levels great than the LOQ (5 µg/kg) at any time point. For the metabolite, M01 in the 0-10 cm soil segment concentrations reached a maximum of 56.9 µg/kg at 30 DAT and decreased to <5 µg/kg at 240 DAT. For the metabolite, M02 in the 0-10 cm soil segment concentrations reached a maximum of 39.0 µg/kg at 7 DAT and decreased to 5.4 µg/kg at 240 DAT. In both the 10-20 cm and 20-30 cm layer for the metabolites M01 and M02, no residues were found at levels greater than the LOQ (5 µg/kg) at any time point.

F. Degradation kinetics

Degradation rates determined in the report are superseded by the report presented under point KCA 7.1.2.2.1/12 (M-763140-01-1).

III. Conclusion

Following a single application of spiroxamine at a nominal application rate of 0.75 kg a.s./ha to bare soil in spring 1994. The decline of spiroxamine and the formation and decline of its metabolites M01 and M02 was followed for up to 270 days after application at 5 sites in Germany.

During the whole test duration, nearly all residues remained in the 0-10 cm layers of the soil. The maximum concentration of spiroxamine was observed for the Albig trial site at 386 µg/kg at 0 DAT. Only in the Höfchen was spiroxamine found in the 10-20 cm layer, the maximum value of 9.4 µg/kg was reported at 89 DAT. No mobility into soil layers below 20 cm was observed. The metabolites M01 and

M02 were detected at all sites. The metabolite M01 reached a maximum of 45.8 µg/kg at 14 DAT in Swisttal-Hohn in the 0-10 cm layer. The metabolite M02 reached a maximum of 43.6 µg/kg at 7 DAT in Swisttal-Hohn in the 0-10 cm layer.

A re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) is performed in KCA 7.1.2.2.1/12 (M-763140-01-1).

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study is considered valid to assess the dissipation of spiroxamine under field conditions in soil. The study meets the requirements to assess field persistence of spiroxamine and its metabolites, and to derive parent soil $DegT_{50matrix}$ values for legacy field studies as defined by EFSA (2014). It is not suitable for assessing metabolite soil $DegT_{50matrix}$ values as the design did not minimise soil surface processes immediately after application as required by EFSA (2014).

Data Point:	KCA 7.1.2.2.1/03
Report Author:	[redacted]
Report Year:	1995
Report Title:	Dissipation of KWG 4168 in soils under field conditions (Great Britain and France)
Report No:	RA-2132/94
Document No:	M-006127-01-1
Guideline(s) followed in study:	BBA Guideline IV-4.1 (1986)
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RARA (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Soil dissipation of spiroxamine was studied after application as an EC formulation containing 491.4 g/L to spring cereal crop under field conditions for up to 244 days at five trial sites in Great Britain (Elm Farm; sandy loam and Old Hall Farm; sandy loam) and France (Touffreville, silt loam). Spiroxamine was applied as an emulsifiable concentrate formulation at a single application at two different applications rates of either 3.0 L/ha (1.5 kg a.s./ha; sites 40097/1 and 40099/8) or 1.5 L/ha (0.75 kg a.s./ha; sites 40100/5, 40101/3 and 40193/5) in spring 1994.

Twenty soil core samples were taken from the treated as well as ten soil core samples from the control plot directly post application. Core samples were taken to a depth of 30 cm (except trial 40193/5 which from 119-234 DAT was sampled to 50 cm depth) and segmented into 10 cm soil layers prior to homogenisation and analysed. Samples were taken at the Elm Farm (trial 40097/1 and 40100/5) site, at 0, 7, 14, 28, 56, 90, 121, 149, 182 and 244 days after treatment (DAT), for the Old Hall Farm (trial 40097/1 and 40101/3) site, at 0, 7, 14, 28, 55, 90, 120, 150, 181 and 240 DAT and for the Touffreville (trial 40193/5) at 0, 7, 14, 28, 56, 88, 119, 146, 182 and 234 DAT.

Soil samples were analysed for spiroxamine and the metabolites M01 (spiroxamine-desethyl) and M02 (spiroxamine-despropyl). Soil samples were extracted by Soxtec extraction with a basic solution of methanol/water/ammonia (25%) (8:2:0.1 v/v/v). Parent and metabolites were quantitatively determined

by liquid chromatography with MS-MS detection. A limit of quantification (LOQ) of 5 µg/kg was reported, and a limit of detection (LOD) of 2 µg/kg for spiroxamine and the metabolites.

Starting from an application rate of 1.5 (0.75) kg spiroxamine/ha and a soil density of 1.5 kg/L the theoretical total concentration in a 10 cm layer is 1000 (500) µg/kg soil. The concentration of spiroxamine in the 0-10 cm layer found immediately after application were 479, 684, 246, 327 and 309 µg/kg for Elm Farm (40097/1), Old Hall Farm (40099/8), Elm Farm (40100/5), Old Hall Farm (40101/3) and Touffreville (40193/5) trial sites, respectively. This equates that at day zero 47.9%, 68.4%, 49.2%, 65.4% and 61.8% of the applied amount was recovered from each site, respectively.

During the test duration, nearly all residues remained in the 0-10 cm layers of the soil. However, spiroxamine was also found in the 10-20 cm layer in four of the trial sites investigated, the maximum value of 32.2 µg/kg found at DAT 0 at the Old Hall Farm trial site (40099/8, 1.5 kg a.s./ha application). In the 20-30 cm layer spiroxamine was found on one occasion at values greater than the LOQ, with the maximum value of 8.6 µg/kg found at 0 DAT at the Old Hall Farm trial site (40101/3, 0.75 kg a.s./ha application). No mobility into soil layers below 30 cm was observed. The metabolites M01 and M02 were detected at all sites. The metabolite M01 reached a maximum of 99.9 µg/kg at 14 DAT in the Old Hall Farm trial site (40099/8, 1.5 kg a.s./ha application) in the 0-10 cm layer. The metabolite M02 reached a maximum of 121 µg/kg at 7 DAT in the Elm Farm trial site (40097/1, 1.5 kg a.s./ha application) in the 0-10 cm layer.

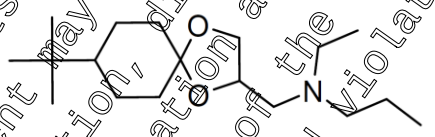
The DT_{50} and DT_{90} of spiroxamine determined in the study was reported for each sitesoil. However, a re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) and the EFSA guidance on deriving DegT50 values from laboratory and field dissipation studies (EFSA 2014), was performed in the report presented under point KCA 7.1.2.2.1/12 (M-763140-01-1).

I. Materials and Methods

A. Materials

1. Test Items

Spiroxamine (product code 0122915, Suspension concentrate formulation (491.4 g/L spiroxamine)



Certificate of analysis:	Analysed and certified at December 16, 1993 by Dr. GroB, Bayer AG, PF-E/FT, D-51368 Leverkusen
Batch no:	FL 04023/0089 A, FAR 210
Purity:	Not stated

2. Trial locations & Soils

The study was performed in Great Britain and France at five different sites. Site soil is characterised in Table CA 7.1.2.2.1-30. The field soil dissipation trial consisted of one treated and one untreated plot on each site. No pesticide history was given for previous years before the trial for either site.

Table CA 7.1.2.2.1-30: Location, site description and climatic data of test sites

Trial designation	40097/1	40099/8	40100/5	40101/3	40193/5
Soil Designation	Elm Farm	Old Hall Farm	Elm Farm	Old Hall Farm	Touffreville
Geographic Location	Elm Farm, Development Station GB Bury St. Edmunds Thurston, Suffolk Field 7, Block 4	Old Hall Farm, Pakenham GB Bury St. Edmunds, Suffolk	Elm Farm, Development Station GB Bury St. Edmunds Thurston, Suffolk Field 7, Block 4	Old Hall Farm, Pakenham GB Bury St. Edmunds, Suffolk	La Ferme du Plessis, F-27440, Touffreville, La Pointe Aux, Normands
Country	Great Britain	Great Britain	Great Britain	Great Britain	France
Vegetation	With vegetation [A]	With vegetation [A]	With vegetation [A]	With vegetation [A]	With vegetation [A]
Textural Classification (USDA)	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Silt loam
Sand [50 - 2000 µm] (%)	56.8	74.0	56.8	74.0	12.9
Silt [2 – 50 µm] (%)	24.9	15.6	24.9	15.6	70.8
Clay [< 2 µm] (%)	18.3	10.4	18.3	10.4	16.3
pH (in 0.01 M $CaCl_2$ solution)	7.4	7.0	7.4	7.0	7.2
Organic matter (%)	1.86	3.23	1.86	3.23	2.22
Organic carbon (%)	1.08	1.88	1.08	1.88	1.29
Cation Exchange Capacity (meq/100 g)	14	13	14	13	12
Soil Moisture capacity (g/100g soil)	37.4	45.8	37.4	45.8	42.4
Size of plot (m^2)	325	325	325	325	360

A Treatment to the soil plots was made approximately 1 month after sowing of spring barley (or spring wheat for Touffreville 40193/5). The report documents ca 5-10% ground coverage at the time of application and that sampling was conducted between seed rows

The trails in Great Britain were conducted under spring barley, in France the test was done under spring wheat. Details are shown in Table CA 7.1.2.2.1-31 below.

Table CA 7.1.2.2.1-31: Details of crop used at each trail location

Trial No.	Crop	Variety	Date of Sowing	Application rate of spiroxamine [kg/ha]	Seed Rate [kg/ha]	Growth Stage at application [BBCH]	Crop Covering of the Soil [%]	Date of Harvest
40097/1 (Elm Farm)	Spring Barley	Alexis	30th March 1994	1.5	180	13	5	30th October 1994
40099/8 (Old Hall Farm)	Spring Barley	Alexis	21st March 1994	1.5	180	21-22	6	3rd October 1994
40100/5 (Elm Farm)	Spring Barley	Alexis	30th March 1994	0.75	180	13	5	30th October 1994
40101/3 (Old Hall Farm)	Spring Barley	Alexis	21st March 1994	0.75	180	21-22	6	3rd October 1994
40193/5 (Touffreville)	Spring Wheat	Ysathis	28th March 1994	0.7	180	13	5-10	20th October 1994

B. Study Design

1. Experimental Conditions

A single application of a nominal 0.75 kg spiroxamine/ha as a as an emulsifiable concentrate (EC) was performed to bare soil on 4th May 1994 (40097/1, Elm Farm) and 26th April 1994 (40099/8, Old Hall Farm). A single application of a nominal 1.5 kg spiroxamine/ha as a as an emulsifiable concentrate (EC) as performed to bare soil on 4th May 1994 (40100/5, Elm Farm), 26th April 1994 (40101/3, Old Hall Farm) and 29th April 1994 (40193/5, Touffreville). Soil dissipation of spiroxamine was studied for a maximum of 244 days.

Weather data was collected at a location near to the site during the study period; this data includes the mean air temperature, rainfall and sunshine hours. The soil cultivation and the agronomic and maintenance activates on the test plots were conducted according to the usual local agricultural practise. During the studies on covered soils plant protection products were used on the treated and on the control plot for maintenance.

2. Sampling

Twenty soil core samples were taken from the treated and control plots directly post application and at different intervals up to 244 days after the treatment. Core samples were taken to a depth of 30 cm (except trial 40193/5 which from 119-234 DAT was sampled to 50 cm depth, diameter 50 mm) and segmented into 10 cm soil layers prior to homogenisation and analysed. Samples were taken at the Elm Farm (trial 40097/1 and 40100/5) site, at 0, 7, 14, 28, 56, 90, 121, 149, 182 and 244 days after treatment (DAT), for the Old Hall Farm (trial 40097/1 and 40101/3) site, at 0, 7, 14, 28, 55, 90, 120, 150, 181 and 240 DAT and for the Touffreville (trial 40193/5) at 0, 7, 14, 28, 56, 88, 119, 146, 182 and 234 DAT. The control plots were sampled at the beginning and end of the study.

At each sampling point 20 cores were taken using a pushing sampling system (Wacker Hammer) down to a depth of 30 cm (diameter 50 mm) per sampling interval. Locations of sampling were statistically distributed over the plot to get representative samples. Samples were transported in a cooler box containing dry ice, before being stored at -18°C until analysis at the laboratory.

The frozen soil cores were cut into 10 cm segments. Ten (control sample) to twenty (treated samples) of such segment of one later were milled and carefully homogenised.

3. Analytical Procedures

Soil samples were analysed for spiroxamine and the metabolites M01 (spiroxamine-desethyl) and M02 (spiroxamine-despropyl). Replicate sub-samples were analysed by Method 00374 (RA-607/94) (M-019207-02-1, involving liquid chromatography.

In summary, the method involved Soxtec extraction with refluxing methanol/water/ammonia (25%) (8:2:0.1, v/v/v). After solvent evaporation to the aqueous remainder the internal standard were added. LOQ (5 µg/kg)of the method was 2 µg/kg for parent and metabolites.

Procedural recovery samples were analysed. Untreated soil samples were fortified with known amounts of spiroxamine, M01 and M02 and carrying these samples through the procedure alongside the treated samples.

4. Determination of degradation kinetics

The DT_{50} and DT_{90} of spiroxamine determined in the study was reported for each site. However, a re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (20142) and the EFSA guidance on deriving $Deg T_{50}$ values from laboratory and field dissipation studies (EFSA 20145), was performed in the report presented under point KCA 7.1.2.2.1/12 (M-763140-01-1).

II. Results and Discussion

A. Analytical Methodology

Full details and acceptable validation data to support this method (Method 00374 (RA-607/94)) are presented in Document M-CA 4, Section 4.1.2 (M-019207-02-1). The method complies with the EU regulatory requirements outlined within SANCO/3029/99 rev. 4 and is suitable for the determination of spiroxamine and its metabolites in soil samples by HPLC/MS/MS.

Analytical performance was determined by analysing known amounts of spiroxamine and internal standards. The mean recovery of spiroxamine, M01 and M02 was 87.1 ± 13%, 89.8 ± 5.8% and 91.1 ± 6.7% respectively, over a fortification range of 5 to 500 µg/kg.

B. Data

The concentration of the total residues of spiroxamine and its metabolites are presented in the report. The concentrations of the single compounds are summarised in Table CA 7.1.2.2.1-32 to Table CA 7.1.2.2.1-46.

Table CA 7.1.2.2.1-32: Residues of spiroxamine in 0-30 cm horizons of soil at Trial 400971/1 (Elm Farm) expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**28**	**56**	**90**	**121**	**149**	**182**	**244**
0-10	A	516	125	122	83.8	93.0	77.1	52.9	43.0	38.5	30.8
	B	474	127	119	87.3	89.2	72.1	47.9	45.2	45.1	28.9
	C	504	121	-	-	-	-	-	-	-	-
	D	421	142	-	-	-	-	-	-	-	-
	Mean	**479**	**129**	**121**	**85.6**	**91.1**	**74.6**	**50.4**	**44.1**	**41.8**	**29.9**
10-20	A	7.6	n.d.	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	5.8	n.d.
	B	7.0	n.d.	n.d.	8.3	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
	Mean	**7.3**	**n.d.**	**n.d.**	**5.4**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-33: Residues of M01 (spiroxamine-desethyl) in 0-30 cm horizons of soil at Trial 400971/1 (Elm Farm) expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**28**	**56**	**90**	**121**	**149**	**182**	**244**
0-10	A	39.8	90.2	87.1	72.3	86.2	52.7	39.3	27.9	24.4	18.7
	B	27.6	95.8	74.9	74.2	75.5	55.4	38.2	29.2	27.1	18.5
	C	28.6	90.6	-	-	-	-	-	-	-	-
	D	30.6	93.8	-	-	-	-	-	-	-	-
	Mean	**31.6**	**92.6**	**81.0**	**73.3**	**80.9**	**54.1**	**38.7**	**28.5**	**25.8**	**18.6**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-34: Residues of M02 (spiroxamine-despropyl) in 0-30 cm horizons of soil at Trial 400971/1 (Elm Farm) expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**28**	**56**	**90**	**121**	**149**	**182**	**244**
0-10	A	36.5	119	89.5	82.4	89.4	67.6	46.8	34.8	30.7	25.7
	B	25.9	123	80.3	84.1	78.6	70.6	48.2	35.7	33.1	24.7
	C	26.7	124	-	-	-	-	-	-	-	-
	D	27.9	120	-	-	-	-	-	-	-	-
	Mean	**29.3**	**121**	**84.9**	**83.2**	**84.0**	**69.1**	**47.5**	**35.3**	**31.9**	**25.2**
10-20	A	n.d.	n.d.	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	<LOQ	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-35: Residues of spiroxamine in 0-30 cm horizons of soil at Trial 40099/8 (Old Hall Farm) expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**28**	**55**	**90**	**120**	**150**	**181**	**240**
0-10	A	693	398	288	211	223	196	123	79.7	88.8	52.4
	B	634	457	278	211	212	197	118	77.3	81.5	59.4
	C	714	426	-	-	-	-	-	-	-	-
	D	694	457	-	-	-	-	-	-	-	-
	Mean	**684**	**434**	**283**	**211**	**217**	**197**	**120**	**78.5**	**85.1**	**55.9**
10-20	A	33.4	13.6	<LOQ	19.3	<LOQ	n.d.	n.d.	n.d.	n.d.	<LOQ
	B	31.1	13.7	<LOQ	16.2	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**32.2**	**13.5**	**<LOQ**	**17.7**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**
20-30	A	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-36: Residues of M01 (spiroxamine-desethyl) in 0-30 cm horizons of soil at Trial 40099/8 (Old Hall Farm) expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**28**	**55**	**90**	**120**	**150**	**181**	**240**
0-10	A	17.5	76.9	103	88.8	89.0	85.8	57.4	46.4	46.2	31.8
	B	15.1	81.2	96.9	90.7	90.0	78.1	56.7	44.6	41.6	34.9
	C	36.9	78.3	-	-	-	-	-	-	-	-
	D	15.6	84.8	-	-	-	-	-	-	-	-
	Mean	**21.3**	**80.3**	**99.9**	**89.8**	**82.0**	**57.1**	**45.5**	**43.9**	**43.9**	**33.3**
10-20	A	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-37: Residues of M02 (spiroxamine-despropyl) in 0-30 cm horizons of soil at Trial 40099/8 (Old Hall Farm) expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**28**	**55**	**90**	**120**	**150**	**181**	**240**
0-10	A	16.2	81.4	108	98.5	96.1	90.2	63.8	51.7	48.1	33.4
	B	14.9	85.6	105	101	95.5	84.1	62.8	49.7	43.5	39.6
	C	33.2	81.4	-	-	-	-	-	-	-	-
	D	15.7	88.5	-	-	-	-	-	-	-	-
	Mean	**20.0**	**84.2**	**106**	**99.7**	**95.8**	**87.2**	**63.3**	**50.7**	**45.8**	**36.5**
10-20	A	n.d.	<LOQ	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	<LOQ	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**<LOQ**	**n.d.**	**<LOQ**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-38: Residues of spiroxamine in 0-30 cm horizons of soil at Trial 40100/5 (Elm Farm) expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**28**	**56**	**90**	**121**	**149**	**182**	**244**
0-10	A	226	149	130	87.7	81.2	82.8	64.8	52.2	54.6	51.9
	B	253	143	126	84.0	81.5	74.5	64.1	57.5	57.4	58.2
	C	267	155	-	-	-	-	-	-	-	-
	D	240	144	-	-	-	-	-	-	-	-
	Mean	**246**	**148**	**128**	**85.8**	**81.4**	**78.7**	**64.4**	**54.9**	**56.0**	**55.1**
10-20	A	7.6	<LOQ	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	B	8.9	n.d.	n.d.	<LOQ	<LOQ	<LOQ	n.d.	n.d.	<LOQ	n.d.
	Mean	**8.3**	**<LOQ**	**n.d.**	**<LOQ**	**<LOQ**	**<LOQ**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	<LOQ
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**<LOQ**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-39: Residues of M01 (spiroxamine-desethyl) in 0-30 cm horizons of soil at Trial 40100/5 (Elm Farm) expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**28**	**56**	**90**	**120**	**151**	**180**	**258**
0-10	A	24.6	43.3	38.0	37.8	26.6	23.4	29.9	20.5	17.3	18.0
	B	15.8	46.6	37.8	35.0	29.3	25.8	29.3	18.6	15.9	20.4
	C	15.6	45.3	-	-	-	-	-	-	-	-
	D	13.8	41.2	-	-	-	-	-	-	-	-
	Mean	**17.5**	**44.1**	**37.9**	**36.4**	**27.9**	**24.6**	**29.6**	**19.5**	**16.6**	**19.2**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-40: Residues of M02 (spiroxamine-despropyl) in 0-30 cm horizons of soil at Trial 40100/5 (Elm Farm) expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**28**	**56**	**90**	**121**	**149**	**182**	**244**
0-10	A	23.3	49.8	41.9	41.4	29.1	26.3	29.8	22.2	20.4	19.3
	B	15.2	55.8	41.6	39.4	31.5	27.4	29.7	21.9	18.4	21.0
	C	15.4	53.2	-	-	-	-	-	-	-	-
	D	13.2	41.7	-	-	-	-	-	-	-	-
	Mean	**16.8**	**50.1**	**41.7**	**40.4**	**30.3**	**26.8**	**29.8**	**22.1**	**19.4**	**20.2**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-41: Residues of spiroxamine in 0-30 cm horizons of soil at Trial 40101/3 (Old Hall Farm) expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**28**	**55**	**90**	**120**	**150**	**181**	**240**
0-10	A	350	229	146	131	113	106	57.7	50.2	43.2	45.4
	B	312	223	146	137	89.1	133	74.1	53.1	44.0	47.7
	C	346	214	-	-	-	-	-	-	-	-
	D	300	223	-	-	-	-	-	-	-	-
	Mean	**327**	**222**	**146**	**134**	**101**	**120**	**65.9**	**51.7**	**43.6**	**46.6**
10-20	A	9.9	<LOQ	<LOQ	n.d.	5.3	5.1	n.d.	n.d.	n.d.	n.d.
	B	8.3	11.2	<LOQ	n.d.	5.1	<LOQ	n.d.	n.d.	n.d.	n.d.
	Mean	**9.1**	**6.9**	**<LOQ**	**n.d.**	**5.2**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	12.0	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ
	B	5.2	LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**8.6**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-42: Residues of M01 (spiroxamine-desethyl) in 0-30 cm horizons of soil at Trial 40101/3 (Old Hall Farm) expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**28**	**55**	**90**	**120**	**150**	**181**	**240**
0-10	A	7.6	30.1	31.4	34.2	26.2	27.9	26.4	20.0	17.8	19.2
	B	6.5	32.9	30.2	33.3	28.4	36.0	27.8	19.1	17.4	19.3
	C	7.8	31.4	-	-	-	-	-	-	-	-
	D	6.1	31.2	-	-	-	-	-	-	-	-
	Mean	**7.0**	**31.4**	**30.8**	**33.8**	**27.3**	**32.0**	**27.0**	**19.55**	**17.6**	**19.3**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-43: Residues of M02 (spiroxamine-despropyl) in 0-30 cm horizons of soil at Trial 40101/3 (Old Hall Farm) expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**28**	**55**	**90**	**120**	**150**	**181**	**240**
0-10	A	7.7	29.3	34.9	39.0	32.5	31.2	30.8	19.9	18.8	21.4
	B	6.5	31.3	33.3	38.9	33.1	38.2	30.8	19.1	19.1	20.6
	C	7.7	30.0	-	-	-	-	-	-	-	-
	D	6.2	30.6	-	-	-	-	-	-	-	-
	Mean	**7.0**	**30.3**	**34.1**	**38.9**	**32.8**	**34.7**	**30.8**	**19.5**	**19.0**	**21.0**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	<LOQ	5.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**<LOQ**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-44: Residues of spiroxamine in 0-30 cm horizons of soil at Trial 40193/5 (Touffreville) expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**28**	**56**	**88**	**119**	**146**	**182**	**234**
0-10	A	342	168	98.8	52.7	40.4	21.5	<LOQ	7.2	<LOQ	<LOQ
	B	285	156	112	62.8	30.9	22.2	8.2	7.1	5.1	<LOQ
	C	309	153	94.6	68.1	38.2	22.2	10.7	-	-	-
	D	300	148	101	53.9	31.5	21.1	8.8	-	-	-
	Mean	**309**	**156**	**102**	**59.4**	**35.3**	**21.7**	**7.5**	**7.2**	**<LOQ**	**<LOQ**
10-20	A	9.5	n.d.	5.1	n.d.	n.d.	<LOQ	n.d.	n.d.	<LOQ	6.3
	B	5.8	<LOQ	8.0	<LOQ	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.
	Mean	**7.7**	**<LOQ**	**6.5**	**<LOQ**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**<LOQ**	**<LOQ**
20-30	A	<LOQ	n.d.	<LOQ	<LOQ	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.
	B	5.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**<LOQ**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
30-40	A	-	-	-	-	-	-	n.d.	n.d.	n.d.	n.d.
	B	-	-	-	-	-	-	n.d.	n.d.	n.d.	n.d.
	Mean	**-**	**-**	**-**	**-**	**-**	**-**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
40-50	A	-	-	-	-	-	-	n.d.	n.d.	n.d.	n.d.
	B	-	-	-	-	-	-	n.d.	n.d.	n.d.	n.d.
	Mean	**-**	**-**	**-**	**-**	**-**	**-**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-45: Residues of M01 (spiroxamine-desethyl) in 0-30 cm horizons of soil at Trial 40193/5 (Touffreville) expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**28**	**56**	**88**	**119**	**146**	**182**	**234**
0-10	A	18.2	25.9	17.8	20.0	11.3	8.9	n.d.	<LOQ	n.d.	<LOQ
	B	13.5	21.5	22.1	13.7	10.3	8.2	<LOQ	<LOQ	n.d.	<LOQ
	C	18.0	27.4	18.2	13.9	12.1	8.5	<LOQ	-	-	-
	D	16.1	25.7	16.6	14.8	10.3	7.9	<LOQ	-	-	-
	Mean	**16.4**	**25.1**	**18.7**	**15.6**	**11.0**	**8.4**	**<LOQ**	**<LOQ**	**n.d.**	**<LOQ**
10-20	A	5.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ
	B	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**<LOQ**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**
20-30	A	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
30-40	A	-	-	-	-	-	-	n.d.	n.d.	n.d.	n.d.
	B	-	-	-	-	-	-	n.d.	n.d.	n.d.	n.d.
	Mean	**-**	**-**	**-**	**-**	**-**	**-**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
40-50	A	-	-	-	-	-	-	n.d.	n.d.	n.d.	n.d.
	B	-	-	-	-	-	-	n.d.	n.d.	n.d.	n.d.
	Mean	**-**	**-**	**-**	**-**	**-**	**-**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-46: Residues of M02 (spiroxamine-despropyl) in 0-30 cm horizons of soil at Trial 40193/5 (Touffreville) expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**28**	**56**	**88**	**119**	**146**	**182**	**234**
0-10	A	19.4	28.6	20.3	17.2	12.5	9.8	<LOQ	<LOQ	<LOQ	<LOQ
	B	14.2	23.3	23.5	13.5	12.1	10.3	<LOQ	5.6	<LOQ	<LOQ
	C	20.5	29.8	19.2	14.2	12.8	10.1	<LOQ	-	-	-
	D	17.2	28.4	17.9	14.8	11.5	9.4	<LOQ	-	-	-
	Mean	**17.8**	**27.5**	**20.2**	**14.9**	**12.2**	**9.9**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**
10-20	A	7.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ
	B	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ
	Mean	**<LOQ**	**<LOQ**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**
20-30	A	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	<LOQ	<LOQ
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**<LOQ**
30-40	A	-	-	-	-	-	-	n.d.	n.d.	n.d.	n.d.
	B	-	-	-	-	-	-	n.d.	n.d.	n.d.	n.d.
	Mean	**-**	**-**	**-**	**-**	**-**	**-**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
40-50	A	-	-	-	-	-	-	n.d.	n.d.	n.d.	n.d.
	B	-	-	-	-	-	-	n.d.	n.d.	n.d.	n.d.
	Mean	**-**	**-**	**-**	**-**	**-**	**-**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

C. Residues

No residue concentration of spiroxamine, M01 and M02 above LOQ (5 µg/kg) of the analytical method could be found in the control samples.

Starting from an application rate of 1.5 (0.75) kg spiroxamine/ha and a soil density of 1.5 kg/L the theoretical total concentration in a 10 cm layer is 1000 (500) µg/kg soil. The concentration of spiroxamine in the 0-10 cm layer found immediately after application were 479, 684, 246, 327 and 309 µg/kg for Elm Farm (40097/1), Old Hall Farm (40099/8), Elm Farm (40100/5), Old Hall Farm (40101/3) and Touffreville (40193/5) trial sites, respectively. This equates that at day zero 47.9%, 68.4%, 49.2%, 65.4% and 61.8% of the applied amount was recovered from each site, respectively.

For the Elm Farm (trial site 40097/1) the mean residue concentration in the 0-10 cm soil segment decreased from 479 µg/kg at 0 DAT to 29.9 µg/kg at 244 DAT. In the 10-20 cm layer spiroxamine was found on two occasion at values greater than the LOD, with the maximum value of 7.3 µg/kg found at 0 DAT. In the 20-30 cm layer spiroxamine was not found at levels great than LOQ (5 µg/kg) at any time point. For the metabolite, M01 in the 0-10 cm soil segment concentrations reached a maximum of 92.6 µg/kg at 7 DAT and decreased to 18.6 µg/kg at 244 DAT. For the metabolite, M02 in the 0-10 cm soil segment concentrations reached a maximum of 121 µg/kg at 7 DAT and decreased to 25.2 µg/kg at 244 DAT. In both the 10-20 cm and 20-30 cm layer for the metabolites M01 and M02, no residues were found at levels greater than LOQ (5 µg/kg) at any time point.

For the Old Hall Farm (trial site 40099/8) the mean residue concentration in the 0-10 cm soil segment decreased from 684 µg/kg at 0 DAT to 55.9 µg/kg at 240 DAT. In the 10-20 cm layer spiroxamine was found on three occasion at values greater than the LOD, with the maximum value of 32.2 µg/kg found at 0 DAT. In the 20-30 cm layer spiroxamine was not found at levels great than LOQ (5 µg/kg) at any time point. For the metabolite, M01 in the 0-10 cm soil segment concentrations reached a maximum of 99.9 µg/kg at 14 DAT and decreased to 33.3 µg/kg at 240 DAT. For the metabolite, M02 in the 0-10 cm

soil segment concentrations reached a maximum of 106 µg/kg at 14 DAT and decreased to 36.5 µg/kg at 240 DAT. In both the 10-20 cm and 20-30 cm layer for the metabolites M01 and M02, no residues were found at levels greater than LOQ (5 µg/kg) at any time point.

For the Elm Farm (trial site 40100/5) the mean residue concentration in the 0-10 cm soil segment decreased from 246 µg/kg at 0 DAT to 55.1 µg/kg at 244 DAT. In the 10-20 cm and 20-30 cm layer spiroxamine was not found at levels great than LOQ (5 µg/kg) at any time point. In the 10-20 cm layer spiroxamine was found on one occasion at values greater than the LOD, with the maximum value of 8.3 µg/kg found at 0 DAT. In the 20-30 cm layer spiroxamine was not found at levels great than LOQ (5 µg/kg) at any time point. For the metabolite, M01 in the 0-10 cm soil segment concentrations reached a maximum of 44.1 µg/kg at 7 DAT and decreased to 19.2 µg/kg at 244 DAT. For the metabolite, M02 in the 0-10 cm soil segment concentrations reached a maximum of 50.1 µg/kg at 7 DAT and decreased to 20.2 µg/kg at 244 DAT. In both the 10-20 cm and 20-30 cm layer for the metabolites M01 and M02, no residues were found at levels greater than LOQ (5 µg/kg)at any time point.

For the Old Hall Farm (trial site 40101/3) site the mean residue concentration in the 0-10 cm soil segment decreased from 327 µg/kg at 0 DAT to 46.6 µg/kg at 240 DAT. In the 10-20 cm layer spiroxamine was found on three occasions at values greater than the LOD, with the maximum value of 9.1 µg/kg found at 0 DAT. In the 20-30 cm layer spiroxamine was found on one occasion at values greater than the LOD, with the maximum value of 8.6 µg/kg found at 0 DAT. For the metabolite, M01 in the 0-10 cm soil segment concentrations reached a maximum of 33.8 µg/kg at 28 DAT and decreased to 19.3 µg/kg at 240 DAT. For the metabolite, M02 in the 0-10 cm soil segment concentrations reached a maximum of 38.9 µg/kg at 28 DAT and decreased to 21.0 µg/kg at 240 DAT. In both the 10-20 cm and 20-30 cm layer for the metabolites M01 and M02, no residues were found at levels greater than LOQ (5 µg/kg) at any time point.

For the Touffreville (trial site 40193/5) the mean residue concentration in the 0-10 cm soil segment decreased from 309 µg/kg at 0 DAT to <5 µg/kg at 234 DAT. In the 10-20 cm layer spiroxamine was found on two occasions at values greater than the LOD, with the maximum value of 7.7 µg/kg found at 0 DAT. In the 20-30, 30-40, 40-50 cm layer spiroxamine was not found at levels great than LOQ (5 µg/kg) at any time point. For the metabolite, M01 in the 0-10 cm soil segment concentrations reached a maximum of 25.1µg/kg at 7 DAT and decreased to <LOQ at 234 DAT. For the metabolite, M02 in the 0-10 cm soil segment concentrations reached a maximum of 27.5 µg/kg at 7 DAT and decreased to <LOQ at 234 DAT. In both the 10-20, 20-30, 30-40 and 40-50 cm layer for the metabolites M01 and M02, no residues were found at levels greater than LOQ (5 µg/kg) at any time point.

F. Degradation kinetics

Degradation rates determined in the report are superseded by the report presented under point KCA 7.1.2.2.1/12 (M-763140-01-1).

III. Conclusion

Spiroxamine was applied at a single application at two different applications rates of either 3.0 L/ha (1.5 kg a.s./ha) or 1.5 L/ha (0.75 kg a.s./ha) in spring 1994 to spring cereal crops. The decline of spiroxamine and the formation and decline of its metabolites M01 and M02 was followed for up to 244 days after application at 5 sites in Great Britain and France.

During the test duration, nearly all residues remained in the 0-10 cm layers of the soil. However, spiroxamine was also found in the 10-20 cm layer in four of the trial sites investigated, the maximum value of 32.2 µg/kg found at DAT 0 at the Old Hall Farm trial site (40099/8, 1.5 kg a.s./ha application). In the 20-30 cm layer spiroxamine was found on one occasion at values greater than the LOQ, with the maximum value of 8.6 µg/kg found at 0 DAT at the Old Hall Farm trial site (40101/3, 0.75 kg a.s./ha application). No mobility into soil layers below 30 cm was observed. The metabolites M01 and M02 were detected at all sites. The metabolite M01 reached a maximum of 99.9 µg/kg at 14 DAT in the Old Hall Farm trial site (40099/8, 1.5 kg a.s./ha application) in the 0-10 cm layer. The metabolite M02 reached a maximum of 121 µg/kg at 7 DAT in the Elm Farm trial site (400971/1, 1.5 kg a.s./ha application) in the 0-10 cm layer.

A re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) is performed in KCA 7.1.2.2.1/12 (M-763140-01-1).

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study is considered valid to assess the dissipation of spiroxamine under field conditions in soil. The study meets the requirements to assess field persistence of spiroxamine and its metabolites, and to derive parent soil $DegT_{50matrix}$ values for legacy field studies as defined by EFSA (2014). It is not suitable for assessing metabolite soil $DegT_{50matrix}$ values as the design did not minimise soil surface processes immediately after application as required by EFSA (2014).

Data Point:	KCA 7.1.2.2.1/04
Report Author:	[redacted]
Report Year:	1996
Report Title:	Dissipation of KWG 4168 in soils under field conditions (France and Italy)
Report No:	RA-2048/94
Document No:	M-006128-01-1
Guideline(s) followed in study:	BBA Guideline IV-4.1 (1986)
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Soil dissipation of spiroxamine was studies after application as an EC formulation containing 491.4 g/L to bare soil plots under field conditions for up to 240 days at two trial sites in Laudun, France and Filetto, Italy.

A single application of a nominal 400 g spiroxamine/ha as an emulsifiable concentrate formulation was performed to bare soil on 21st June 1994 (Laudun, France) or 22nd August 1994 (Filetto, Italy).

The initial dissipation of spiroxamine was rapid for both sites, showing a slower dissipation phase after 7 DAT. Residues of spiroxamine were detected mainly in the 0-10 cm soil horizon throughout the trial. Residues of spiroxamine were found above the LOQ (5 µg/kg) in the 10-20 cm horizon once at Laudun at 0 DAT, and once in the 20-30 cm horizon at Filetto at 7 DAT.

M01 (spiroxamine-desethyl) was only detected above LOQ once at Laudun (6.86 µg/kg at 7 DAT in the 0-10 cm horizon only). At Filetto, M01 (spiroxamine-desethyl) was detected above LOQ at 14, 31 and 60 DAT in the 0-10cm horizon only. Peak levels of 8.39 µg/kg were found at 31 DAT before declining below LOQ at 91 DAT. No residues of spiroxamine were found above the LOQ (5 µg/kg) in 10-20 or 20-30 cm horizons on either site.

M02 (spiroxamine-despropyl) was detected above LOQ at Laudun at 0 and 7 DAT before dropping below LOQ at 16 DAT (peak of 7.55 µg/kg at 7 DAT in the 0-10 cm horizon only). At Filetto, M02 (spiroxamine-despropyl) was detected above LOQ at 14, 31 and 60 DAT in the 0-10cm horizon only. Peak levels of 8.72 µg/kg were found at 31 DAT before declining below LOQ at 91 DAT. No residues of spiroxamine were found above the LOQ (5 µg/kg) in 10-20 or 20-30 cm horizons on either site.

The DT_{50} and DT_{90} of spiroxamine determined in the study was reported for each soil. However, a re-

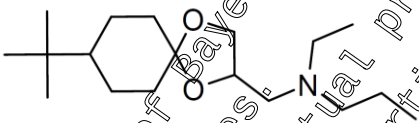
evaluation of the degradation kinetics in accordance with the FOCUS guidance document on degradation kinetics (2014) and the EFSA guidance on deriving DegT50 values from laboratory and field dissipation studies (EFSA 2014), was performed in the report presented under point KCA 7.1.2.2.1/12 ([M-763140-01-1](#)).

I. Materials and Methods

A. Materials

1. Test Items

Spiroxamine (KWG 4168 500 EC), emulsifiable concentrate (491.4g/L spiroxamine)



Product no.:	0122915
Batch no.:	FL-04023/0089A
Certificate of analysis:	FAR 210 (16 December 1993)

2. Trial locations & soils

The study was performed at two different trial sites in Laudun-l'ardoise, France (loam) and Filetto, Italy (silty clay loam). Site soil is characterized in Table CA 7.1.2.2.1-47. The field soil dissipation trial consisted of one treated and one untreated plot on each site. Plot measurements in the report given as 228 m^2 and 480m^2 in Laudun and Filetto, respectively (it was not specified if this referred to the entire area used for the trial or the treated area only). No pesticide history was given for previous years before the trial for either site.

Table CA 7.1.2.2.1-47: Location, site description and climatic data of test sites

Trial designation	40198/6	40424/1
Soil Designation	Laudun	Filetto
Vegetation	Vines	Vines
Geographic Location Country	Laudun-l'ardoise France	Filetto, Ravenna Italy
Textural Classification (USDA) Sand [50 - 2000 µm] (%) Silt [2 – 50 µm] (%) Clay [< 2µm] (%)	Loam 44.5 41.5 14.0	Silty clay loam 9.5 51.2 39.3
pH in $CaCl_2$	7.7	7.6
Organic Matter (%)	1.34	2.22
Organic carbon (%)	0.78	1.29
Cation Exchange Capacity (meq/100 g)	10	17
Soil Moisture capacity (g/100g soil)	37.0	46.8

B. Study Design

1. Experimental Conditions

A single application of a nominal 400 g spiroxamine/ha as an emulsifiable concentrate (EC) formulation was performed to bare soil on 21st June 1994 at Laudun and 22nd August 1994 at Filetto. Application confirmation was performed by calculation using the DAT 0 samples.

During the trials, soil cultivation and maintenance was performed according to the usual local agricultural practice. At Laudun, applications of glyphosate, copper, sulphur and azoxystrobin were used for maintenance of the vines. Application of mancozeb only was made for maintenance of vines at Filetto. No irrigation was carried out on either site. Products used for maintenance were listed, with none containing active substances in the same chemical family as spiroxamine.

Average temperatures, rainfall and sunlight are given for sampling dates themselves and time periods between sampling points at each site from weather stations an unknown distance from the trial sites. Total rainfall for Laudun is 513 mm for 11th June – 31st December 1994 and 83 mm for 1st January 20th February 1995. Total rainfall for Filetto from 22nd August – 19th December 1994 is 237 mm and 138 mm between 21st December 1994 – 19th April 1995. Temperatures and rainfall for both sites represent broadly typical conditions.

Soil dissipation of spiroxamine was studies for 240 days.

2. Sampling

At 0, 7, 16, 28, 58, 91, 119, 149, 175 and 240 days after treatment (DAT) at Laudun and 0, 7, 14, 31, 60, 91, 119, 150, 182 and 240 days at Filetto, 20 cores were taken at locations around the plot (statistically distributed to ensure representative samples), down to a depth of 30 cm (diameter of approximately 50 mm) using a 'Wacker Hammer'. Soil cores were split into 10 cm sections, homogenised and frozen (-18°C) before storage and analysis. Control plots were sampled at the beginning and the end of the study.

3. Analytical Procedures

Soil samples were extracted at using a Soxtec extractor with boiling methanol/water/ammonia (25%), 80/20/1 (v/v/v). Extracts were re-dissolved in methanol and quantified using HPLC/MS/MS. Specific details of the method are given in MCA Section 4 (Method no. 00374 (RA-607/94) (M-019207-02-1)). The limit of quantification (LOQ) is given as 5 µg/kg and limit of detection (LOD) of 2 µg/kg for spiroxamine and metabolites.

Method validation was performed separately with a mixture of standard soils (soil 2.1/soil 2.2/soil 2.3, 1/1/1, v/v/v), fortified with spiroxamine, M01 (spiroxamine-desethyl) or M02 (spiroxamine-despropyl). The mean recoveries were 98.8% (relative standard deviation, RSD, 3.96%) for spiroxamine, 99.7% (RSD 2.72%) for M01 (spiroxamine-desethyl) and 101% (RSD 2.71%) for M02 (spiroxamine-despropyl).

In addition, concurrent recoveries were run with soil from control plots from each site fortified with spiroxamine, M01 (spiroxamine-desethyl) or M02 (spiroxamine-despropyl). Mean spiroxamine recoveries were 88.7 and 83.8% (RSD 18.9 and 18.4%) for Laudun and Filetto, respectively. Mean M01 (spiroxamine-desethyl) recoveries were 91.3 and 86.5% (RSD 8.4 and 9.0%) for Laudun and Filetto, respectively. Mean M02 (spiroxamine-despropyl) recoveries were 95.7 and 93.1% (RSD 9.3 and 9.6%) for Laudun and Filetto, respectively.

4. Determination of degradation kinetics

The DT_{50} and DT_{90} of spiroxamine determined in the study was reported for each site. However, a re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) and the EFSA guidance on deriving DegT50 values from laboratory and field dissipation studies (EFSA 2014), was performed in the report presented under point KCA 7.1.2.2.1/12 (M-763140-01-1).

II. Results and Discussion

A. Analytical Methodology

Full details and acceptable validation data to support this method (Method 00374 (RA-607/94)) are presented in Document M-CA 4, Section 4.1.2 (M-019207-02-1). The method complies with the EU regulatory requirements outlined within SANCO/3029/99 rev. 4 and is suitable for the determination of

spiroxamine and its metabolites in soil samples by HPLC/MS/MS.

B. Data

The results for spiroxamine and its metabolites are presented below as soil residue concentrations (on a µg/kg dry weight basis) for each of the treated plots in Table CA 7.1.2.2.1-55 to Table CA 7.1.2.2.1-60.

Table CA 7.1.2.2.1-48: Mean residues of spiroxamine in 0-30 cm horizons of soil at Laudun expressed as µg/kg

Depth [cm]	Replicate	DAT									
		0	**7**	**16**	**28**	**58**	**91**	**119**	**149**	**175**	**240**
0-10	A	65.6	45.0	14.0	29.7	13.3	11.1	8.69	7.28	<LOQ	5.55
	B	63.4	33.0	15.8	26.6	15.7	12.1	9.77	7.20	<LOQ	<LOQ
	C	59.0	31.4	-	-	-	-	-	-	-	-
	D	55.0	23.5	-	-	-	-	-	-	-	-
	Mean	**60.8**	**33.2**	**14.9**	**28.2**	**14.5**	**11.6**	**9.23**	**7.24**	**<LOQ**	**<LOQ**
10-20	A	6.96	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	n.d.	n.d.	n.d.
	B	6.36	n.d.	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**6.66**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**
20-30	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	13.4	n.d.	n.d.
	B	<LOQ	n.d.	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	C	5.45	-	-	-	-	-	-	<LOQ	-	-
	D	5.31	-	-	-	-	-	-	n.d.	-	-
	Mean	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-49: Mean residues of M01 (spiroxamine-desethyl) in 0-30 cm horizons of soil at Laudun expressed as µg/kg

Depth [cm]	Replicate	DAT									
		0	**7**	**16**	**28**	**58**	**91**	**119**	**149**	**175**	**240**
0-10	A	6.02	7.77	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	n.d.
	B	6.03	6.40	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n..d
	C	5.35	7.06	-	-	-	-	-	-	-	-
	D	<LOQ	6.20	-	-	-	-	-	-	-	-
	Mean	**<LOQ**	**6.86**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**n.d.**	**n.d.**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-50: Mean residues of M02 (spiroxamine-despropyl) in 0-30 cm horizons of soil at Laudun expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**16**	**28**	**58**	**91**	**119**	**149**	**175**	**240**
0-10	A	6.51	8.60	5.03	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.
	B	6.50	7.15	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	n.d.
	C	5.88	7.33	-	-	-	-	-	-	-	-
	D	<LOQ	7.13	-	-	-	-	-	-	-	-
	Mean	**5.35**	**7.55**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-51: Mean residues of spiroxamine in 0-30 cm horizons of soil at Filetto expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	**7**	**14**	**21**	**60**	**91**	**119**	**150**	**182**	**240**
0-10	A	92.9	63.4	72.3	71.9	40.0	26.0	22.5	16.2	21.0	29.5
	B	107.2	66.5	62.6	67.1	38.8	28.9	25.0	16.5	21.1	28.5
	C	92.0	68.1	-	-	-	-	-	-	-	-
	D	99.2	77.1	-	-	-	-	-	-	-	-
	Mean	**97.8**	**68.8**	**67.5**	**69.5**	**39.4**	**27.5**	**23.8**	**16.4**	**21.1**	**29.0**
10-20	A	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	<LOQ
	B	<LOQ	<LOQ	n.d.	<LOQ	n.d.	n.d.	<LOQ	<LOQ	n.d.	n.d.
	Mean	**<LOQ**	**<LOQ**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**<LOQ**	**<LOQ**	**n.d.**	**<LOQ**
20-30	A	n.d.	6.15	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	C	-	5.16	-	-	-	-	-	-	-	-
	D	-	9.88	-	-	-	-	-	-	-	-
	Mean	**n.d.**	**6.00**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-52: Mean residues of M01 (spiroxamine-desethyl) in 0-30 cm horizons of soil at Filetto expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	7	14	31	60	91	119	150	182	240
0-10	A	<LOQ	<LOQ	7.09	8.35	5.01	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	B	<LOQ	5.15	7.56	8.43	5.78	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	C	<LOQ	<LOQ	-	-	-	-	-	-	-	-
	D	<LOQ	7.13	-	-	-	-	-	-	-	-
	Mean	**<LOQ**	**<LOQ**	**7.33**	**8.39**	**5.40**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-53: Mean residues of M02 (spiroxamine-despropyl) in 0-30 cm horizons of soil at Filetto expressed as µg/kg

Depth [cm]	Repli-cate	DAT									
		0	7	14	31	60	91	119	150	182	240
0-10	A	<LOQ	<LOQ	7.30	8.87	5.36	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	B	<LOQ	<LOQ	7.47	8.56	5.99	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	C	<LOQ	<LOQ	-	-	-	-	-	-	-	-
	D	<LOQ	6.47	-	-	-	-	-	-	-	-
	Mean	**<LOQ**	**<LOQ**	**7.39**	**8.72**	**5.68**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**
10-20	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

C. Residues

Analysis of control samples showed no residues above the limit of quantification (LOQ) of 5 µg/kg at either site.

The average initial concentration of spiroxamine in soil samples taken immediately after application was 60.8 and 97.8 µg/kg in Laudun and Filetto respectively. Within the report, an application rate of 400 g/ha, assuming a soil density of 1.5 g/cm3 and depth of 10 cm, is given as 267 mg/kg soil. Therefore, according to the report, the measured soil concentrations at 0 DAT were 12 and 20% of nominally applied spiroxamine for Laudun and Filetto sites, respectively.

The initial dissipation of spiroxamine was rapid for both sites, showing a slower dissipation phase after 7 DAT. Residues of spiroxamine were detected mainly in the 0-10 cm soil horizon throughout the trial. Residues of spiroxamine were found above the LOQ (5 µg/kg) in the 10-20 cm horizon once at Laudun

at 0 DAT, and once in the 20-30 cm horizon at Filetto at 7 DAT.

M01 (spiroxamine-desethyl) was only detected above LOQ once at Laudun (6.86 µg/kg at 7 DAT in the 0-10 cm horizon only). At Filetto, M01 (spiroxamine-desethyl) was detected above LOQ at 14, 31 and 60 DAT in the 0-10cm horizon only. Peak levels of 8.39 µg/kg were found at 31 DAT before declining below LOQ at 91 DAT. No residues of spiroxamine were found above the LOQ (5 µg/kg) in 10-20 or 20-30 cm horizons on either site.

M02 (spiroxamine-despropyl) was detected above LOQ at Laudun at 0 and 7 DAT before dropping below LOQ at 16 DAT (peak of 7.55 µg/kg at 7 DAT in the 0-10 cm horizon only). At Filetto, M02 (spiroxamine-despropyl) was detected above LOQ at 14, 31 and 60 DAT in the 0-10cm horizon only. Peak levels of 8.72 µg/kg were found at 31 DAT before declining below LOQ at 91 DAT. No residues of spiroxamine were found above the LOQ (5 µg/kg) in 10-20 or 20-30 cm horizons on either site.

D. Kinetic Analysis

Degradation rates determined in the report are superseded by the report presented under point KCA 7.1.2.2.1/12 (M-763140-01-1).

III. Conclusions

Following a single application of spiroxamine at a rate of nominal rate of 400 g/ha to bare soil applied on 21st June 1994 (Laudun) and 22nd August 1994 (Filetto), decline of spiroxamine, and the formation and decline of M01 (spiroxamine-desethyl) and M02 (spiroxamine-despropyl) was followed for up to 360 days after application at 2 trial sites in Laudun, France and Filetto, Italy. A re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) is performed in KCA 7.1.2.2.1/12 (M-763140-01-1).

M01 (spiroxamine-desethyl) was detected at a peak level of 8.39 µg/kg in the 0-10 cm horizon at Filetto.

M02 (spiroxamine-despropyl) was detected at a peak level of 8.72 µg/kg in the 0-10 cm horizon at Filetto.

No residues of spiroxamine or its metabolites were found above the LOQ (5 µg/kg) in 10-20 or 20-30 cm horizons on either site.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283-2013.

The study is considered valid to assess the dissipation of spiroxamine under field conditions in soil. The study meets the requirements to assess field persistence of spiroxamine and its metabolites, and to derive parent soil $DegT_{50matrix}$ values for legacy field studies as defined by EFSA (2014). It is not suitable for assessing metabolite soil $DegT_{50matrix}$ values as the design did not minimise soil surface processes immediately after application as required by EFSA (2014).

Data Point:	KCA 7.1.2.2.1/05
Report Author:	[redacted]
Report Year:	1996
Report Title:	Dissipation of KWG 4168 in soils under field conditions (France and Italy)
Report No:	RA-2127/95
Document No:	M-006129-01-1
Guideline(s) followed in study:	BBA Guideline IV-4.1 (1986)
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Soil dissipation of spiroxamine was studied after application as an EC formulation containing 498.5 g/L to bare soil plots under field conditions for up to 360 days at two trial sites in Laudun, France and Nogarole Rocca, Italy.

A single application of a nominal 400 g spiroxamine/ha as an emulsifiable concentrate formulation was performed to bare soil in spring/summer 1995.

The initial dissipation of spiroxamine was rapid for both sites, with Laudun showing a slower dissipation phase after 14 DAT, whilst Nogarole Rocca showed continued rapid dissipation. Residues of spiroxamine were detected in the 0-10 cm soil horizon throughout the trial. No residues of spiroxamine were found above the LOQ (5 µg/kg) in 10-20 or 20-30 cm horizons on either site.

M01 (spiroxamine-desethyl) was formed initially rapidly as spiroxamine dissipated, and then declined throughout the study. Peak levels of 27.9 µg/kg were found at 7 DAT at Laudun and 15.7 µg/kg at the same time point at Nogarole Rocca, before declining below LOQ at 180 and 91 DAT, respectively. Residues of M01 (spiroxamine-desethyl) were detected in the 0-10 cm soil horizon throughout the trial. No residues of spiroxamine were found above the LOQ (5 µg/kg) in 10-20 or 20-30 cm horizons on either site.

M02 (spiroxamine-despropyl) was formed initially rapidly as spiroxamine dissipated, and then declined throughout the study. Peak levels of 29.0 µg/kg were found at 7 DAT at Laudun and 15.8 µg/kg at the same time point at Nogarole Rocca, before declining below LOQ at 180 and 91 DAT, respectively. Residues of M02 (spiroxamine-despropyl) were detected in the 0-10 cm soil horizon throughout the trial. No residues of spiroxamine were found above the LOQ (5 µg/kg) in 10-20 or 20-30 cm horizons on either site.

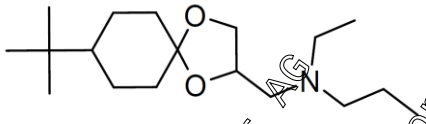
The DT_{50} and DT_{90} of spiroxamine determined in the study was reported for each site. However, a re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) and the EFSA guidance on deriving DegT50 values from laboratory and field dissipation studies (EFSA 2014), was performed in the report presented under point KCA 7.1.2.2.1/12 (M-763140-01-1).

I. Materials and Methods

A. Materials

1. Test Items

Spiroxamine (KWG 4168 500 EC), emulsifiable concentrate (498.5g/L spiroxamine)



Product no.:	0168478
Batch no.:	FL 0402300435
Certificate of Analysis:	FAR 335 (19-Apr-1995)

2. Trial locations & soils

The study was performed at two different trial sites in Laudun-l'ardoise, France (loam) and Nogarole Rocca, Filetto, Italy (sandy loam). Site soil is characterized in Table CA 7.1.2.2.1-54. The field soil dissipation trial consisted of one treated and one untreated plot on each site. Plot measurements in the report given as 300 m^2 and 768m^2 in Laudun and Nogarole Rocca, respectively (it was not specified if this referred to the entire area used for the trial or the treated area only). No pesticide history was given for previous years before the trial for either site.

Table CA 7.1.2.2.1-54: Location, site description and climatic data of test sites

Trial designation	50135/2	50136/0
Soil Designation	Laudun	Nogarole Rocca
Vegetation	Bare soil	Bare soil
Geographic Location	Laudun-l'ardoise	Nogarole Rocca, Filetto
Country	France	Italy
Textural Classification (USDA)	Loam	Sandy loam
Sand [50 - 2000 µm] (%)	41.6	74.3
Silt [2 – 50 µm] (%)	39.2	19.9
Clay [< 2 µm] (%)	19.2	5.8
pH in $CaCl_2$	7.7	7.7
Organic Matter (%)	2.00	0.65
Organic carbon (%)	1.16	0.38
Cation Exchange Capacity (meq/100 g)	15.0	10.0
Soil Moisture capacity (g/100g soil)	40.5	38.4

B. Study Design

1. Experimental Conditions

A single application of a nominal 400 g spiroxamine/ha as an emulsifiable concentrate (EC) formulation was performed to bare soil on 5th October 1995 at Laudun and 7th March 1995 at Nogarole Rocca. Application confirmation was performed by calculation using the DAT 0 samples.

During the trials, soil cultivation and maintenance was performed according to the usual local agricultural practice. At Laudun, 3 applications of glyphosate were used to keep the site free of weeds. No plant protection products were used during the trial at Nogarole Rocca. No irrigation was carried out on either site.

Monthly average temperatures, rainfall and sunlight are given for time periods close to sampling points at each site from weather stations an unknown distance from the trial sites. In addition, temperature, rainfall and sunlight data were taken on sampling dates. Total rainfall for Laudun is 659 mm for 21st April – 31st December 1995 and 2984 mm for 1st January – 31st May 1996. It is noted in the report that the rainfall in January, February and March 1996 was extraordinarily heavy in the south of France. Total rainfall for Nogarole Rocca from 7th March – 28th December 1995 is 185.4 mm and 338.8 mm between 29th December 1995 – 27th June 1996. Temperatures for both sites and rainfall for Nogarole Rocca represent broadly typical conditions.

Soil dissipation of spiroxamine was studied for 360 days.

2. Sampling

At 0, 7, 14, 30, 62, 91, 120, 180, 308 and 358 days after treatment (DAT) at Laudun and 0, 7, 14, 30, 60, 91, 120, 178, 268 and 360 days at Nogarole Rocca, 20 cores were taken at locations around the plot (statistically distributed to ensure representative samples), down to a depth of 30 cm (diameter of 50 mm) using a 'Wacker Hammer'. At 0 DAT a replicate was also taken with an 'RA Piercer' (down to 10 cm). Soil cores were split into 10 cm sections, homogenised and frozen (-18°C) before storage and analysis. The control plots were sampled at the beginning and end of the study.

3. Analytical Procedures

Soil samples were extracted at using a Soxtec extractor with boiling methanol/water/ammonia (25%), 80/20/10 (v/v/v). Extracts were re-dissolved in methanol and quantified using HPLC/MS/MS. Specific details of the method are given in MCA Section 4 (Method no. 00374 (RA-607/94), M-019207-02-1). The limit of quantification (LOQ) is given as 5 µg/kg and limit of detection (LOD) of 2 µg/kg for spiroxamine and metabolites.

Method validation was performed separately with a mixture of standard soils (soil 2.1/soil 2.2/soil 2.3, 1/1/1, v/v/v), fortified with spiroxamine, M01 (spiroxamine-desethyl) or M02 (spiroxamine-despropyl). The mean recoveries were 84.3% (relative standard deviation, RSD, 11.8%) for spiroxamine, 89.4% (RSD 5.7%) for M01 (spiroxamine-desethyl) and 91.1% (RSD 6.3%) for M02 (spiroxamine-despropyl).

In addition, concurrent recoveries were run with soil from control plots from each site fortified with spiroxamine, M01 (spiroxamine-desethyl) or M02 (spiroxamine-despropyl). Mean spiroxamine recoveries were 87.3 and 81.3% (RSD 23.5 and 11.3%) for Laudun and Nogarole Rocca, respectively. Mean M01 (spiroxamine-desethyl) recoveries were 86.1 and 88.7% (RSD 18.4 and 3.9%) for Laudun and Nogarole Rocca, respectively. Mean M02 (spiroxamine-despropyl) recoveries were 90.2 and 96.1% (RSD 14.5 and 5.6%) for Laudun and Nogarole Rocca, respectively.

4. Determination of degradation kinetics

The DT_{50} and DT_{90} of spiroxamine determined in the study was reported for each site. However, a re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014[2]) and the EFSA guidance on deriving DegT50 values from laboratory and field dissipation studies (EFSA, 2014[6]) was performed in the report presented under point KCA 7.1.2.2.1/12 (M-763140-01-1).

II. Results and Discussion

A. Analytical Methodology

Full details and acceptable validation data to support this method are presented in Document M-CA 4, Section 4.1.2 (Method no. 00374 (RA-607/94), M-019207-02-1). The method complies with the EU regulatory requirements outlined within SANCO/3029/99 rev. 4 and is suitable for the determination of spiroxamine and its metabolites in soil samples by HPLC/MS/MS.

B. Data

The results for spiroxamine and its metabolites are presented below as soil residue concentrations (on a µg/kg dry weight basis) for each of the treated plots in Table CA 7.1.2.2.1-55 to Table CA 7.1.2.2.1-60

Table CA 7.1.2.2.1-55: Residues of spiroxamine in 0-30 cm horizons of soil at Laudun expressed as µg/kg

Depth [cm]	Replicate	DAT										
		0 [A]		7	14	30	62	91	120	180	308	358
0-10	A	295	206	53.3	46.0	49.9	35.9	19.5	19.8	10.2	<LOQ	<LOQ
	B	207	233	71.0	67.9	53.0	22.0	19.3	23.9	9.30	<LOQ	<LOQ
	C	296	233	56.7	-	-	-	-	-	-	-	-
	D	287	217	73.3	-	-	-	-	-	-	-	-
	Mean	**271**	**222**	**63.6**	**57.0**	**51.4**	**28.9**	**19.4**	**21.9**	**9.76**	**<LOQ**	**<LOQ**
10-20	A	n.d.		n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	6.16	n.d.	n.d.
	B	<LOQ		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	C	-		-	-	-	-	-	-	n.d.	-	-
	D	-		-	-	-	-	-	-	n.d.	-	-
	Mean	**<LOQ**		**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**	**n.d.**
20-30	A	n.d.		n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	C	-		-	<LOQ	-	-	-	-	-	-	-
	D	-		-	n.d.	-	-	-	-	-	-	-
	Mean	**n.d.**		**n.d.**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

A two samples for 0 DAT only (one using 'Wacker Hammer' and one using 'RA Piercer' pushing sampling system)
DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-56: Residues of M01 (spiroxamine-desethyl) in 0-30 cm horizons of soil at Laudun expressed as µg/kg

Depth [cm]	Replicate	DAT										
		0 [A]		7	14	30	62	91	120	180	308	358
0-10	A	5.80	<LOQ	33.5	17.1	16.2	12.2	6.37	8.45	<LOQ	<LOQ	<LOQ
	B	<LOQ	<LOQ	31.9	20.9	10.5	9.09	6.42	7.61	<LOQ	<LOQ	<LOQ
	C	5.93	<LOQ	27.7	-	-	-	-	-	-	-	-
	D	5.77	<LOQ	18.4	-	-	-	-	-	-	-	-
	Mean	**5.8**	**<LOQ**	**27.9**	**19.0**	**13.8**	**10.7**	**6.40**	**8.03**	**<LOQ**	**<LOQ**	**<LOQ**
10-20	A	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	C	-		-	-	-	-	-	-	n.d.	-	-
	D	-		-	-	-	-	-	-	n.d.	-	-
	Mean	**n.d.**		**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	C	-		-	n.d.	-	-	-	-	-	-	-
	D	-		-	n.d.	-	-	-	-	-	-	-
	Mean	**n.d.**		**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

A two samples for 0 DAT only (one using 'Wacker Hammer' and one using 'RA Piercer' pushing sampling system)
DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-57: Residues of M02 (spiroxamine-despropyl) in 0-30 cm horizons of soil at Laudun expressed as µg/kg

Depth [cm]	Replicate	DAT										
		0 [A]		7	14	30	62	91	120	180	308	358
0-10	A	5.90	<LOQ	34.4	18.7	16.0	12.1	7.13	8.38	<LOQ	<LOQ	<LOQ
	B	<LOQ	<LOQ	32.9	21.1	14.1	10.1	7.58	7.83	<LOQ	<LOQ	<LOQ
	C	5.93	<LOQ	28.0	-	-	-	-	-	-	-	-
	D	<LOQ	<LOQ	20.5	-	-	-	-	-	-	-	-
	Mean	**<LOQ**	**<LOQ**	**29.0**	**19.9**	**15.1**	**11.1**	**7.36**	**8.11**	**<LOQ**	**<LOQ**	**<LOQ**
10-20	A	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	C	-		-	-	-	-	-	n.d.	-	-	-
	D	-		-	-	-	-	-	n.d.	-	-	-
	Mean	**n.d.**		**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
20-30	A	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	C	-		-	n.d.	-	-	-	-	-	-	-
	D	-		-	n.d.	-	-	-	-	-	-	-
	Mean	**n.d.**		**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

A two samples for 0 DAT only (one using 'Wacker Hammer' and one using 'RA Piercer' pushing sampling system)
DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-58: Residues of spiroxamine in 0-30 cm horizons of soil at Nogarole Rocca expressed as µg/kg

Depth [cm]	Replicate	DAT										
		0 [A]		7	14	30	60	91	120	178	268	360
0-10	A	162	162	73.7	52.5	31.6	9.80	<LOQ	n.d.	<LOQ	<LOQ	<LOQ
	B	189	172	61.1	34.1	30.3	9.04	<LOQ	n.d.	<LOQ	<LOQ	<LOQ
	C	211	182	63.8	-	-	-	-	-	-	-	-
	D	174	167	64.7	-	-	-	-	-	-	-	-
	Mean	**184**	**171**	**65.8**	**43.3**	**31.0**	**9.42**	**<LOQ**	**n.d.**	**<LOQ**	**<LOQ**	**<LOQ**
10-20	A	<LOQ		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	<LOQ		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**<LOQ**		**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**
20-30	A	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**		**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

A two samples for 0 DAT only (one using 'Wacker Hammer' and one using 'RA Piercer' pushing sampling system)
DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-59: Residues of M01 (spiroxamine-desethyl) in 0-30 cm horizons of soil at Nogarole Rocca expressed as µg/kg

Depth [cm]	Replicate	DAT										
		0[A]		7	14	30	60	91	120	178	268	360
0-10	A	<LOQ	<LOQ	11.7	14.7	14.7	6.39	n.d.	n.d.	n.d.	n.d.	n.d.
	B	<LOQ	<LOQ	17.8	15.0	11.4	5.90	<LOQ	n.d.	n.d.	n.d.	n.d.
	C	<LOQ	<LOQ	14.1	-	-	-	-	-	-	-	-
	D	<LOQ	<LOQ	19.0	-	-	-	-	-	-	-	-
	Mean	**LOQ**	**LOQ**	**15.7**	**14.8**	**13.1**	**6.15**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
10-20	A	<LOQ		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.33	n.d.
	B	<LOQ		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**<LOQ**		**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**
20-30	A	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**		**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

A two samples for 0 DAT only (one using 'Wacker Hammer' and one using 'RA Piercer' pushing sampling system)
DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

Table CA 7.1.2.2.1-60: Residues of M02 (spiroxamine-despropyl) in 0-30 cm horizons of soil at Nogarole Rocca expressed as µg/kg

Depth [cm]	Replicate	DAT										
		0[A]		7	14	30	60	91	120	178	268	360
0-10	A	<LOQ	<LOQ	12.0	14.6	13.4	6.39	<LOQ	n.d.	n.d.	n.d.	n.d.
	B	<LOQ	<LOQ	18.4	14.6	11.2	6.11	<LOQ	n.d.	n.d.	n.d.	n.d.
	C	<LOQ	<LOQ	13.8	-	-	-	-	-	-	-	-
	D	<LOQ	<LOQ	18.9	-	-	-	-	-	-	-	-
	Mean	**LOQ**	**LOQ**	**15.8**	**14.6**	**12.3**	**6.25**	**<LOQ**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
10-20	A	<LOQ		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.35	n.d.
	B	<LOQ		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**<LOQ**		**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**<LOQ**	**n.d.**
20-30	A	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**		**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

A two samples for 0 DAT only (one using 'Wacker Hammer' and one using 'RA Piercer' pushing sampling system)
DAT: days after treatment, LOQ (limit of quantitation) = 5 µg/kg, LOD (limit of detection) = 2 µg/kg, n.d. = not detected above LOD

C. Residues

Analysis of control samples from the Nogarole Rocca site, showed no residues above the limit of quantification (LOQ) of 5 µg/kg. However, control samples from the Laudun site, showed a single analytical replicate value of 6.81 µg/kg of spiroxamine at 0 DAT at 0-10 cm depth. All other samples taken from the control plot at Laudun showed no detectible residues of spiroxamine or its metabolites.

The average initial concentration of spiroxamine in soil samples taken immediately after application was 247 and 178 µg/kg in Laudun and Nogarole Rocca respectively. Within the report, an application rate of 400 g/ha, assuming a soil density of 1.5 g/cm^3 and depth of 10 cm, is given as 267 µg/kg soil. Therefore, according to the report, the measured soil concentrations at 0 DAT were 93 and 67% of nominally applied spiroxamine for Laudun and Nogarole Rocca sites, respectively.

The initial dissipation of spiroxamine was rapid for both sites, with Laudun showing a slower dissipation phase after 14 DAT, whilst Nogarole Rocca showed continued rapid dissipation. Residues of spiroxamine were detected mainly in the 0-10 cm soil horizon throughout the trial. No residues of spiroxamine were found above the LOQ (5 µg/kg) in 10-20 or 20-30 cm horizons on either site.

M01 (spiroxamine-desethyl) was formed initially rapidly as spiroxamine dissipated, and then declined throughout the study. Peak levels of 27.9 µg/kg were found at 7 DAT at Laudun and 15.7 µg/kg at the same time point at Nogarole Rocca, before declining below LOQ at 180 and 91 DAT respectively. Residues of M01 (spiroxamine-desethyl) were detected mainly in the 0-10 cm soil horizon throughout the trial. No residues of spiroxamine were found above the LOQ (5 µg/kg) in 10-20 or 20-30 cm horizons on either site.

M02 (spiroxamine-despropyl) was formed initially rapidly as spiroxamine dissipated, and then declined throughout the study. Peak levels of 29.0 µg/kg were found at 7 DAT at Laudun and 15.8 µg/kg at the same time point at Nogarole Rocca, before declining below LOQ at 180 and 91 DAT, respectively. Residues of M02 (spiroxamine-despropyl) were detected mainly in the 0-10 cm soil horizon throughout the trial. No residues of spiroxamine were found above the LOQ (5 µg/kg) in 10-20 or 20-30 cm horizons on either site.

D. Kinetic Analysis

Degradation rates determined in the report are superseded by the report presented under point KCA 7.1.2.2.1/12 (M-763140-01-1).

III. Conclusions

Following a single application of spiroxamine at a rate of nominal rate of 400 g/ha to bare soil in spring/ summer, 1995, the decline of spiroxamine and the formation and decline of M01 (spiroxamine-desethyl) and M02 (spiroxamine-despropyl) was followed for up to 360 days after application at 2 trial sites in Laudun, France and Nogarole Rocca, Italy. A re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) is presented in KCA 7.1.2.2.1/12 (M-763140-01-1).

M01 (spiroxamine-desethyl) was detected at a peak level of 27.9 µg/kg in the 0-10 cm horizon.

M02 (spiroxamine-despropyl) was detected at a peak level of 29.0 µg/kg in the 0-10 cm horizon.

No residues of spiroxamine or its metabolites were found above the LOQ (5 µg/kg) in 10-20 or 20-30 cm horizons on either site.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study is considered valid to assess the dissipation of spiroxamine under field conditions in soil. The study meets the requirements to assess field persistence of spiroxamine and its metabolites, and to derive parent soil $DegT_{50matrix}$ values for legacy field studies as defined by EFSA (2014). It is not suitable for assessing metabolite soil $DegT_{50matrix}$ values as the design did not minimise soil surface processes immediately after application as required by EFSA (2014).

Data Point:	KCA 7.1.2.2.1/06
Report Author:	[redacted]; [redacted]
Report Year:	2000
Report Title:	Dissipation of spiroxamine in soils - survey of results from studies conducted under field and laboratory conditions
Report No:	MR-251/00
Document No:	M-036125-01-1
Guideline(s) followed in study:	BBA: Part IV, 4-1
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The details of this study are fully summarised under point KCA 7.1.2.1.1/06.

Storage stability

Data Point:	KCA 7.1.2.2.1/07
Report Author:	[redacted]
Report Year:	1996
Report Title:	Storage stability of KWG 4168 and the metabolites KWG 4557 (desethyl-KWG 4168) and KWG 4689 (despropyl-KWG 4168) in soil
Report No:	MR-535/96
Document No:	M-006982-01-1
Guideline(s) followed in study:	None quoted
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCA 7.1.2.2.1/08
Report Author:	[redacted]
Report Year:	1997
Report Title:	Storage stability of KWG 4168 and the metabolites desethyl-KWG 4168 (KWG 4557) and despropyl-KWG 4168 (KWG 4669) in soil
Report No:	MR-3/97
Document No:	M-006079-01-1
Guideline(s) followed in study:	None quoted
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCA 7.1.2.2.1/09
Report Author:	[redacted]
Report Year:	1998
Report Title:	Storage stability of KWG 4168 and the metabolites desethyl-KWG 4168 (KWG 4557), despropyl-KWG 4168 (KWG 4669) and KWG 4168 N-oxide (WAK 6301) in soil
Report No:	MR-38/98
Document No:	M-006074-01-1
Guideline(s) followed in study:	None quoted
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The storage stability of spiroxamine and metabolites M01, M02 and later also M03 in soil was investigated under frozen conditions over 3 studies.

Untreated soil samples were fortified with spiroxamine and metabolites M01, M02 and M03 and stored frozen for a period up to 2 years.

Storage stability of residues of spiroxamine and metabolites M01, M02 and M03 in soil under frozen conditions was shown to be acceptable for storage periods of up to 2 years.

I. Materials and Methods

A. Materials

1. Test Items

Substance	Study	Details
Spiroxamine:	KCA 7.1.2.2.1/07 (M-006082-01-1), 535/96 KCA 7.1.2.2.1/08 (M-006079-01-1), MR 3/97 KCA 7.1.2.2.1/09 (M-006074-01-1), MR 38/98	CoA 920522ELB01 (4-Mar-94); purity 99.0% (isomer A 53%, isomer B 46%)
M01 (spiroxamine-desethyl	KCA 7.1.2.2.1/07 (M-006082-01-1), 535/96 KCA 7.1.2.2.1/08 (M-006079-01-1), MR 3/97	CoA 921103ELB02 (24-Nov-94); purity 98.0% (isomer A 56%, isomer B 42%)
	KCA 7.1.2.2.1/09 (M-006074-01-1), MR 38/98	CoA 921103ELB02 (24-Nov-94); purity 98.0% (isomer A 56%, isomer B 42%) CoA 921103ELB02 (5-Jan-98); purity 98.0% (isomer A 56%, isomer B 42%)
M02 (spiroxamine-despropyl)	KCA 7.1.2.2.1/07 (M-006082-01-1), 535/96 KCA 7.1.2.2.1/08 (M-006079-01-1), MR 3/97	CoA 921103ELB03 (24-Nov-94); purity 98.0% (isomer A 55%, isomer B 43%)
	KCA 7.1.2.2.1/09 (M-006074-01-1), MR 38/98	CoA 921103ELB03 (24-Nov-94); purity 98.0% (isomer A 55%, isomer B 43%) CoA 921103ELB03 (12-Dec-97); purity 98.0% (isomer A 55%, isomer B 43%)
M03 (spiroxamine-N-oxide)	KCA 7.1.2.2.1/09 (M-006074-01-1), MR 38/98	CoA 950209ELB01 (2-May-95); purity 93.0% CoA M00190 (6-Feb-97)

2. Test System (soil)

The storage stability study was performed using a mixture of the BBA 2.1, 2.2 and 2.3 soils below (1:1:1 by weight).

Table CA 7.1.2.2.1-61: Physico-chemical properties of test soil

Parameter		Soil			
Soil Designation:		**BBA 2.1 (sp 149)**	**BBA 2.1 (sp 1121)**	**BBA 2.2**	**BBA 2.3**
Batch		Sp 149	Sp 1121	Sp 2110a	Sp 3121
Used for study(s):		KCA 7.1.2.2.1/07 (M-006082-01-1), 535/96	KCA 7.1.2.2.1/08 (M-006079-01-1), MR 3/97 KCA 7.1.2.2.1/09 (M-006074-01-1), MR 38/98	KCA 7.1.2.2.1/07 (M-006082-01-1), 535/96 KCA 7.1.2.2.1/08 (M-006079-01-1), MR 3/97 KCA 7.1.2.2.1/09 (M-006074-01-1), MR 38/98	
Textural Classification (USDA)		Loamy sand	Sand	Loamy sand	Sandy loam
Sand [50 - 2000 µm]	%	85.9	89.4	86.2	66.8
Silt [2 – 50 µm]	%	8.8	10.5	7.7	22.4
Clay [< 2 µm]	%	5.3	0.1	6.1	10.8
pH in 0.01M $CaCl_2$		5.4	5.3	6.2	6.6
Organic Matter (%)		0.98	0.98	4.44	2.48
Organic Carbon (%)		0.57	0.57	2.58	1.44

Other soils were also used for method validation purposes.

B. Study Design

1. Experimental Conditions

Samples (25 g, except 30 g for study KCA 7.1.2.2.1/09 (M-006074-01-1), MR 38/98) of soil (a mixture of BBA 2.1, 2.2 and 2.3) were fortified with the test substances and stored frozen (-18 to -25°C), see Table CA 7.1.2.2.1-62. In studies KCA 7.1.2.2.1/07 (M-006082-01-1), 535/96 and KCA 7.1.2.2.1/08 (M-006079-01-1), MR 3/97 each sample was treated with spiroxamine and metabolites M01 and M02. However, for study KCA 7.1.2.2.1/09 (M-006074-01-1), MR 38/98 one sample was treated with spiroxamine and a further samples was treated with metabolites M01, M02 and M03.

The storage stability of spiroxamine and metabolites M01, M02 and M03 were investigated for at least 639 days.

2. Sampling

The sampling intervals taken for the various studies are detailed in Table CA 7.1.2.2.1-68. At each sampling occasion whole treated samples were taken for analysis along with untreated controls.

3. Analytical Procedures

Details of analytical procedures for the three studies are presented in Table CA 7.1.2.2.1-63. An overview of the concurrent recoveries conducted during the course of the analysis of the storage stability samples is provided in Table CA 7.1.2.2.1-68.

II. Results and Discussion

A. Analytical Methodology

Full details and acceptable validation data to support the methods used in these studies are presented in Document M-CA 4, Section 4.1.2 (Method 00352 (M-090916-01-1); 00374 (RA-607/94) (M-019207-02-1); 00433 (M-015066-01-2)). The methods comply with the EU regulatory requirements outlined within SANCO/3029/99 rev. 4 and is suitable for the determination of spiroxamine and its metabolites in soil samples by GC/MS and HPLC-MS/MS.

Details of the recovery data generated during method validation are provided in Table CA 7.1.2.2.1-63

to Table CA 7.1.2.2.1-67.

B. Data

The storage stability of spiroxamine and metabolites M01, M02 and M03 in soil under frozen conditions is presented in Table CA 7.1.2.2.1-68.

Table CA 7.1.2.2.1-62: Experimental overview of soils used and treatment levels for the various studies

Study	Analytical method used	Experimental phase					
		Method validation		Storage stability			
		Soil(s) used	Treatment level (µg/kg) recoveries	Soil(s) used	Duration of frozen storage (days) and no. of sampling intervals	Treatment level (µg/kg) of storage stability samples	Treatment level (µg/kg) of concurrent recoveries
KCA 7.1.2.2.1/07 (M-006082-01-1), 535/96	00352	BBA 2.1, BBA 2.3, R 30262.7	Spx: 10 - 500 M01 and M02: 20 - 300	Mix 2.1, 2.2, 2.3	0 – 121 days (28-Jun-94*)	Spx: 400 M01: 400 M02: 400	Spx: 401.6, 418.8 M01: 387.2, 390.4 M02: 400.8, 427.6
	00374	BBA 2.1, BBA 2.2, BBA 2.3, Höfchen, Mix 2.1, 2.2, 2.3	Spx: 3.34 - 534 M01: 3.60 - 576 M02: 4.40 - 844		150 – 730 days		Spx: 116.6 – 534.4 M01: 86.1 – 575.4 M02: 88.1 – 843.2
KCA 7.1.2.2.1/08 (M-006079-01-1), MR 3/97	00374	BBA 2.1, BBA 2.2, BBA 2.3, Höfchen, Mix 2.1, 2.2, 2.3	Spx: 3.34 - 534 M01: 3.60 - 576 M02: 4.40 - 844	Mix 2.1, 2.2, 2.3	0 – 726 days (n=14) (22-Dec-94*)	Spx: 134 M01: 144 M02: 211	Spx: 86.0 - 234 M01: 86.1 - 172 M02: 85.4 - 211
KCA 7.1.2.2.1/09 (M-006074-01-1), MR 38/98	00433	Fresno, Watsonville, Mix 2.1, 2.2, 2.3	Spx: 5.585 - 262.5 M01: 5.850 – 272.7 M02: 5.706 – 287.7 M03: 4.682 – 246.0	Mix 2.1, 2.2, 2.3	0 – 639 days (n=10) (24-Apr-96*)	Spx: 165.7 M01: 144.2 M02: 153.8 M03: 158.9	Spx: 114.9 – 165.7 M01: 119.4 – 144.2 M02: 124.6 – 153.8 M03: 107.6 – 158.9

* Treatment date

Table CA 7.1.2.2.1-63: Experimental overview of analytical methods used for the various studies

Study	Analytical method used	Method report	Method details	Validation details	LOQ
KCA 7.1.2.2.1/07 (M-006082-01-1), 535/96 used for sampling intervals 0 to 121 days	00352	Method 00352 for gas chromatographic determination of KWG 4168 and the metabolites KWG 4557 and KWG 4669 in soil. RA-294/94 (1-Jun-94)	Soil samples were extracted with boiling methanol. The solvent was evaporated and the residue was re-dissolved in 2-propanol. Quantitative determination was done by gas chromatography with mass selective detector (MSD) in the single-ion-monitoring-mode.	See Table CA 7.1.2.2.1-64 for soils used and fortification levels of recovery samples performed for method validation	Spx: 10 µg/kg M01 and M02: 20 µg/kg
KCA 7.1.2.2.1/07 (M-006082-01-1), 535/96 used for sampling intervals 150 to 730 days KCA 7.1.2.2.1/08 (M-006079-01-1), MR 3/97	00374	Method 00374 for Liquid Chromatographic Determination of KWG 4168 and the Metabolites KWG 4557 and KWG 4669 in Soil. MR-607/94 (6-Dec-94)	Soil samples were extracted in a Soxtec hot extraction equipment with boiling methanol / water / ammonia (25%) (800 + 200 + 10 parts by volume). After solvent evaporation to the aqueous remainder the internal standard was added. The active substance and the metabolites were quantitatively determined by liquid chromatography with MS-MS detection.	See Table CA 7.1.2.2.1-65 for soils used and fortification levels of recovery samples performed for method validation See Table CA 7.1.2.2.1-66 for soils used and fortification levels of recovery samples performed for method validation	Spx, M01 and M02: 5 µg/kg
KCA 7.1.2.2.1/09 (M-006074-01-1), MR 38/98	00433	Method 00433 (MR-248/96) for Liquid Chromatographic Determination of KWG 4168 and the Metabolites Desethyl-KWG 4168 (KWG 4557), Despropyl-KWG 4168 (KWG 4669) and KWG 4168 N-Oxide (WAK6301) in Soil. MR-248/96 (15-Apr-97)	Soil samples were extracted with a mixture of methanol / water / ammonia (25%) (800 + 200 + 10 parts by volume) during 60 minutes on a mechanical shaker and filtered. From the filtrate an aliquot was concentrated in a Turbo Vap to the aqueous remainder and internal standard was added. After centrifugation the quantitative determination of the active substance and the metabolites was done by high performance liquid chromatography using MS/MS detection.	See Table CA 7.1.2.2.1-67 for soils used and fortification levels of recovery samples performed for method validation	Spx, M01 and M02: 5 µg/kg

Table CA 7.1.2.2.1-64: Method validation (GC/MS method 00352) of spiroxamine and soil metabolites M01 and M02 in frozen soil for study KCA 7.1.2.2.1/07 (M-006082-01-1) [%]

Spiroxamine					M01					M02				
Soil	Fortification level (µg/kg)	Recovery values (%)	Mean recovery (%)	RSD	Soil	Fortification level (µg/kg)	Recovery values (%)	Mean recovery (%)	RSD	Soil	Fortification level (µg/kg)	Recovery values (%)	Mean recovery (%)	RSD
2.1	10	84.9, 129.1, 73.3	95.8	31	2.1	20	91.7, 77.2, 81.9	83.6	9	2.1	20	73.9, 63.5, 78.6	72.0	11
	20	102.4, 85.5, 94.6	94.2	9		100	83.0, 85.2, 74.5	80.9			100	87.4, 85.4, 77.5	83.4	6
	100	97.7, 96.8, 84.5	93.0	8		500	71.9, 77.5, 77.1	75.5	4		500	67.2, 72.9, 72.5	70.9	5
	500	79.6, 87.9, 85.7	84.4	5										
2.3	10	100.2, 110.7	105.5	7	2.3	20	96.4, 87.8, 96.0	93.4	5	2.3	20	77.5, 74.3, 74.7	75.5	2
	20	103.3, 90.1, 104.6	99.3	8		100	85.1, 67.0, 55.6	69.2	22		100	77.5, 74.3, 74.7	61.5	18
	100	105.1, 74.6, 71.4	83.7	22		500	88.6, 92.3, 82.4	87.8	6		500	67.2, 72.9, 72.5	73.3	5
	500	101.8, 106.2, 93.5	100.5	6										
R 30262/7	10	115.3, 108.9, 93.5	105.9	11	R 30262/7	20	111.1, 104.3, 95.5	93.4	8	R 30262/7	20	93.9, 92.1, 85.5	90.5	5
	20	129.1, 120.6, 109.2	119.6	8		100	69.6, 66.4, 69.1	68.4	2		100	62.5, 60.4, 62.5	61.8	2
	100	103.8, 88.1, 101.6	97.8	9		500	84.3, 62.9, 67.7	71.6	16		500	70.6, 56.7, 62.4	63.2	11
	500	94.3, 88.2, 89.9	90.8	3										
		Overall mean:	97.3	14.5			Overall mean:	81.6	16.1			Overall mean:	72.5	14.7

Table CA 7.1.2.2.1-65: Method validation (LC-MS/MS method 00374) of spiroxamine and soil metabolites M01 and M02 in frozen soil for study KCA 7.1.2.2.1/07 (M-006082-01-1) [%]

Spiroxamine					M01					M02				
Fortification level (µg/kg)	Soil	Recovery values (%)	Mean recovery (%)	RSD	Fortification level (µg/kg)	Soil	Recovery values (%)	Mean recovery (%)	RSD	Fortification level (µg/kg)	Soil	Recovery values (%)	Mean recovery (%)	RSD
3.34	2.1	98.4, 93.5, 103	103	11.2	3.60	2.1	90.2, 93.4, 92.3	94.7	5.38	5.27	2.1	86.5, 94.3, 89.7	94.1	10.0
	2.2	132, 115, 99.7				2.2	90.5, 96.4, 86.7				2.2	89.4, 84.5, 82.6		
	2.3	101, 94.5, 91.0				2.3	94.7, 95.3, 105				2.3	89.6, 98.7, 111		
	Höfchen	149[A], 130, 102				Höfchen	135[A], 95.7, 101				Höfchen	133[A], 101, 108		
5.68	Mix 2.1, 2.2, 2.3	84.1, 84.9, 91.2 103, 108, 111	97.0	12.2	4.304	Mix 2.1, 2.2, 2.3	104, 101, 103 98.1, 96.1, 95.6	100	3.92	4.404	Mix 2.1, 2.2, 2.3	105, 107, 101 102, 103, 100	103	2.49
5.84	Mix 2.1, 2.2, 2.3	102, 106, 101 99.5, 97.6, 100	101	2.83	4.977	Mix 2.1, 2.2, 2.3	83.9, 85.0, 96.8 87.5, 91.9, 104	91.5	8.47	5.272	Mix 2.1, 2.2, 2.3	87.4, 89.1, 94.7 92.0, 93.1, 116	95.4	11.0
33.4	2.1	88.0, 86.2, 82.8	84.0	4.40	36.0	2.1	95.6, 87.3, 90.7	88.7	4.61	52.7	2.1	96.9, 93.3, 96.9	92.7	3.93
	2.2	81.1, 76.9, 82.2				2.2	85.5, 82.1, 82.1				2.2	88.5, 85.0, 89.9		
	2.3	87.9, 89.7, 85.7				2.3	91.9, 90.6, 92.8				2.3	95.4, 94.5, 95.8		
	Höfchen	83.1, 79.9, 83.3				Höfchen	87.2, 88.8, 89.2				Höfchen	90.5, 92.6, 93.2		
113.6	Mix 2.1, 2.2, 2.3	80.4,88.1, 82.6 77.9, 74.4, 83.5	81.2	5.86	86.08	Mix 2.1, 2.2, 2.3	101, 102, 102 98.8, 97.3, 99.7	100	1.75	88.08	Mix 2.1, 2.2, 2.3	101, 101, 101 99.7, 99.9, 102	101	0.92
116.8	Mix 2.1, 2.2, 2.3	101, 100, 101 95.4, 99.0, 100	99.3	2.11	98.88	Mix 2.1, 2.2, 2.3	87.0, 92.2, 87.3 83.5, 85.3, 86.0	86.9	3.38	105.4	Mix 2.1, 2.2, 2.3	85.7, 88.5, 90.1 87.0, 89.41, 88.1	88.1	1.82
134	2.1	87.6, 80.8, 90.3	84.4	4.35	144	2.1	93.2, 85.5, 92.3	89.9	4.20	211	2.1	92., 87.9, 92.5	91.3	3.37
	2.2	87.6, 86.9, 80.3				2.2	88.9, 87.4, 81.8				2.2	93.6, 90.0, 83.7		
	2.3	87.1, 82.2, 83.8				2.3	94.7, 91.6, 90.8				2.3	93.3, 94.8, 91.0		
	Höfchen	78.5, 81.7, 83.6				Höfchen	88.1, 90.1, 94.1				Höfchen	91.1, 90.4, 93.9		
454.4	Mix 2.1, 2.2, 2.3	90.5, 92.5, 79.3 85.0, 84.3, 96.6	88.1	7.12	344.3	Mix 2.1, 2.2, 2.3	99.4, 96.0, 98.4	99.2	1.99	352.3	Mix 2.1, 2.2, 2.3	99.5, 95.9, 96.3 103, 104, 99.8	99.8	3.34

Spiroxamine					M01					M02				
Fortification level (µg/kg)	Soil	Recovery values (%)	Mean recovery (%)	RSD	Fortification level (µg/kg)	Soil	Recovery values (%)	Mean recovery (%)	RSD	Fortification level (µg/kg)	Soil	Recovery values (%)	Mean recovery (%)	RSD
467.2	Mix 2.1, 2.2, 2.3	98.1, 93.9, 92.0 101, 101, 94.6	96.8	3.96	395.2	Mix 2.1, 2.2, 2.3	94.6, 93.2, 88.6 88.6, 88.5, 93.8	91.2	3.22	421.6	Mix 2.1, 2.2, 2.3	95.5, 94.4, 91.5 91.2, 91.7, 97.2	93.6	2.66
534	2.1	80.9, 84.5, 86.1	78.5	7.62	576	2.1	90.7, 89.7, 92.1	86.6	5.70	844	2.1	87.1, 87.4, 90.6	86.7	4.83
	2.2	83.1, 77.0, 71.2				2.2	87.0, 81.9, 76.9				2.2	86.0, 84.7, 80.0		
	2.3	84.4, 83.7, 75.6				2.3	92.1, 91.3, 88.1				2.3	92.3, 93.6, 86.6		
	Höfchen	73.1, 72.9, 69.2				Höfchen	84.4, 83.8, 81.3				Höfchen	81.7, 82.0, 88.0		
		Overall mean:	90.1	12.1			Overall mean:	92.0	6.82			Overall mean:	93.6	7.40

A Excluded from mean (identified as an outlier)

Table CA 7.1.2.2.1-66: Method validation of spiroxamine and soil metabolites M01 and M02 in frozen soil for study KCA 7.1.2.2.1/08 (M-006079-01-1) [%]

Spiroxamine					M01					M02				
Fortification level (µg/kg)	Soil	Recovery values (%)	Mean recovery (%)	RSD	Fortification level (µg/kg)	Soil	Recovery values (%)	Mean recovery (%)	RSD	Fortification level (µg/kg)	Soil	Recovery values (%)	Mean recovery (%)	RSD
3.34	2.1	98.4, 93.6, 103	103	11.2	3.60	2.1	90.2, 93.4, 92.3	94.7	5.4	4.40	Mix 2.1, 2.2, 2.3	105, 107, 101 102, 103, 100	103	2.5
	2.2	132, 115, 99.7				2.2	90.5, 96.4, 86.7							
	2.3	101, 94.5, 91.0				2.3	94.7, 95.3, 105			5.27	2.1	86.5, 94.3, 89.7	94.1	10.0
	Höfchen	149, 130, 102				Höfchen	135, 95.7, 101				2.2	89.4, 84.5, 82.6		
5.68	Mix 2.1, 2.2, 2.3	84.1, 84.9, 91.2 103, 108, 111	97.0	12.2	4.30	Mix 2.1, 2.2, 2.3	104, 104, 103 98.1, 96.1, 95.6	100	3.9		2.3	89.6, 98.7, 111		
5.84	Mix 2.1, 2.2, 2.3	102, 106, 101 99.5, 97.6, 100	101	2.8	4.98	Mix 2.1, 2.2, 2.3	83.9, 85.0, 96.8 87.5, 91.9, 104	91.5	8.5		Höfchen	133, 101, 108		

Spiroxamine					M01					M02				
Fortification level (µg/kg)	Soil	Recovery values (%)	Mean recovery (%)	RSD	Fortification level (µg/kg)	Soil	Recovery values (%)	Mean recovery (%)	RSD	Fortification level (µg/kg)	Soil	Recovery values (%)	Mean recovery (%)	RSD
33.4	2.1	88.0, 86.2, 82.8	84.0	4.4	6.71	Mix 2.1, 2.2, 2.3	86.5, 97.8, 89.2	90.2	6.6	5.27	Mix 2.1, 2.2, 2.3	87.4, 89.1, 94.7 92.0, 93.1, 116	95.4	11.0
	2.2	81.1, 76.9, 82.2				Höfchen	94.6, 91.9, 104			6.41	Mix 2.1, 2.2, 2.3	97.7, 92.9, 83.0	91.6	5.4
	2.3	87.9, 89.7, 85.7			36.0	2.1	95.6, 87.3, 90.7	88.7	4.6		Höfchen	91.6, 94.2, 90.0		
	Höfchen	83.1, 79.9, 83.3				2.2	85.5, 82.1, 82.1			52.7	2.1	96.9, 93.3, 96.9	92.7	3.9
86.0	Mix 2.1, 2.2, 2.3	85.5, 82.0, 86.9	79.0	8.4		2.3	91.9, 90.6, 92.8				2.2	88.5, 85.0, 89.9		
	Höfchen	71.4, 73.8, 74.6				Höfchen	87.2, 88.8, 89.2				2.3	95.4, 94.6, 95.8		
114	Mix 2.1, 2.2, 2.3	80.4, 88.1, 82.8 77.9, 74.4, 83.5	81.2	5.9	86.1	Mix 2.1, 2.2, 2.3	101, 102, 102 98.8, 97.3, 99.7	100	1.8		Höfchen	90.5, 92.6, 93.2		
117	Mix 2.1, 2.2, 2.3	101, 100, 101 95.4, 99.0, 100	99.3	2.1										
134	2.1	87.6, 80.8, 90.3	84.4	4.4	89.5	Mix 2.1, 2.2, 2.3	86.4, 90.4, 91.7	85.9	3.4	85.4	Mix 2.1, 2.2, 2.3	87.4, 86.9, 87.3	85.7	2.1
	2.2	87.6, 86.9, 80.3				Höfchen	77.7, 85.5, 83.5				Höfchen	82.9, 84.6, 85.0		
	2.3	87.1, 82.2, 85.8			98.9	Mix 2.1, 2.2, 2.3	87.0, 92.2, 87.2	86.9	4.2	88.1	Mix 2.1, 2.2, 2.3	101, 101, 101 99.7, 99.9, 102	101	0.9
	Höfchen	78.5, 81.5, 83.6								105	Mix 2.1, 2.2, 2.3	85.7, 88.5, 90.1 87.0, 89.4, 88.1	88.1	1.8

Spiroxamine					M01					M02				
Fortification level (µg/kg)	Soil	Recovery values (%)	Mean recovery (%)	RSD	Fortification level (µg/kg)	Soil	Recovery values (%)	Mean recovery (%)	RSD	Fortification level (µg/kg)	Soil	Recovery values (%)	Mean recovery (%)	RSD
384	Mix 2.1, 2.2, 2.3	78.8, 81.3, 80.2 68.7, 72.7, 71.1	75.5	7.0	144	2.1	93.2, 83.5, 92.1	89.8	4.2	211	2.1	92.9, 87.9, 92.5	91.3	3.4
454	Mix 2.1, 2.2, 2.3	90.5, 92.5, 79.3 85.0, 84.6, 96.6	88.1	7.1		2.2	88.9, 87.4, 81.8				2.2	93.6, 90.0, 83.7		
467	Mix 2.1, 2.2, 2.3	98.1, 93.9, 92.0 101, 101, 94.6	96.8	4.0		2.3	94.7, 91.6, 90.8				2.3	93.3, 94.8, 91.0		
534	2.1	80.9, 84.5, 86.1	78.5	7.6		Höfchen	88.1, 90.1, 94.1				Höfchen	91.1, 90.4, 93.9		
	2.2	83.1, 77.0, 71.2			344	Mix 2.1, 2.2, 2.3	99.4, 96.0, 98.4 100, 102, 99.2	99.3	2.0	342	Mix 2.1, 2.2, 2.3	94.5, 93.2, 93.6	92.3	2.5
	2.3	73.1, 72.9, 69.2			358	Mix 2.1, 2.2, 2.3	92.2, 87.9, 96.7	90.7	3.8		Höfchen	88.9, 93.6, 89.9		
	Höfchen	73.1, 72.9, 69.2				Höfchen	89.5, 87.2, 90.9			352	Mix 2.1, 2.2, 2.3	99.5, 95.9, 91.5 103, 104, 99.8	99.8	3.3
-	-	-	-	-	395	Mix 2.1, 2.2, 2.3	94.6, 93.2, 88.6 88.6, 88.5, 93.8	91.2	3.2	422	Mix 2.1, 2.2, 2.3	95.5, 94.4, 91.5, 91.2, 91.7, 97.2	93.6	2.7
-	-	-	-	-	576	2.1	90.7, 89.7, 92.1	86.6	5.7	844	2.1	87.1, 87.4, 90.6	86.7	4.8
-	-	-	-	-		2.2	87.0, 81.9, 76.9				2.2	86.0, 84.7, 80.0		
-	-	-	-	-		2.3	92.1, 91.3, 88.1				2.3	92.3, 93.6, 86.6		
-	-	-	-	-		Höfchen	84.4, 83.8, 81.3				Höfchen	81.7, 82.0, 88.0		
		Overall mean:	88.1	12.6			Overall mean:	91.5	6.8			Overall mean:	92.9	7.2

Table CA 7.1.2.2.1-67: Method validation of spiroxamine and soil metabolites M01, M02 and M03 in frozen soil for study KCA 7.1.2.2.1/09 (M-006074-01-1) [%]

Soil	Spiroxamine				M01			
	Fortification level (µg/kg)	Recovery values (%)	Mean recovery (%)	RSD	Fortification level (µg/kg)	Recovery values (%)	Mean recovery (%)	RSD
Fresno	5.585	85.9, 90.0, 96.7	85.2	9.1	5.850	100.2, 93.7, 91.8	90.0	7.4
Watsonville		74.9, 79.2, 84.8				84.2, 82.0, 88.5		
Mix 2.1, 2.2, 2.3	4.923	99.2, 88.6, 89.1	92.3	6.5	5.115	92.3, 102, 91.1	95.1	6.3
Fresno	55.85	79.3, 97.1, 80.8	82.5	8.8	58.50	85.3, 86.2, 81.3	83.3	2.4
Watsonville		81.0, 78.8, 77.9				82.7, 81.4, 83.2		
Mix 2.1, 2.2, 2.3	105.0	87.5, 84.2, 78.2	83.3	5.7	109.1	84.2, 90.7, 94.1	89.7	5.6
Mix 2.1, 2.2, 2.3	262.5	91.8, 90.9, 91.2	91.3	0.5	272.7	86.2, 93.7, 92.6	90.8	4.5
		Overall mean:	86.1	8.1		Overall mean:	88.9	6.7

Soil	M02				M03			
	Fortification level (µg/kg)	Recovery values (%)	Mean recovery (%)	RSD	Fortification level (µg/kg)	Recovery values (%)	Mean recovery (%)	RSD
Fresno	5.700	81.8, 97.1, 96.8	91.9	8.8	4.682	98.6, 95.5, 99.0	96.7	2.6
Watsonville		90.7, 85.8, 93.5				97.4, 92.3, 97.0		
Mix 2.1, 2.2, 2.3	5.395	105, 91.2, 104	100	7.7	4.613	106, 104, 87.1	99.0	10
Fresno	57.00	83.1, 95.7, 81.4	84.4	6.7	46.82	92.1, 91.8, 93.1	92.4	1.5
Watsonville		80.7, 82.4, 83.1				90.3, 92.8, 94.4		
Mix 2.1, 2.2, 2.3	115.1	90.2, 89.1, 89.6	88.3	2.7	98.41	93.9, 89.1, 90.7	91.2	2.7
Mix 2.1, 2.2, 2.3	287.7	93.1, 98.0, 91.1	94.1	3.8	246.0	97.4, 98.0, 97.7	97.7	0.3
		Overall mean:	90.5	8.0		Overall mean:	95.2	4.9

Table CA 7.1.2.2.1-68: Storage stability of spiroxamine and soil metabolites M01, M02 and M03 in frozen soil [%]

Duration (days)	Spiroxamine		M01		M02		M03	
	Stability (%)	RSD (%)	Stability (%)	RSD (%)	Stability (%)	RSD (%)	Stability (%)	RSD (%)
KCA 7.1.2.2.1/07 (M-006082-01-1)								
0	118.4	7.9	101.6	6.7	94.9	5.1	-	-
30	124.5	1.3	98.8	2.1	92.6	2.4	-	-
59	78.3[B]	13.3	55.8[B]	19.9	47.1[B]	20.7	-	-
101	128.2	5.7	117.8	12.0	89.1	9.0	-	-
121	97.0	4.8	87.6	2.4	88.0	4.7	-	-
150	88.6	2.9	89.8	1.8	89.8	2.4	-	-
175	84.7	3.6	86.3	2.4	88.5	2.4	-	-
240	87.8	2.7	88.0	3.9	96.0	3.6	-	-
300	72.2	2.5	84.1	3.1	84.6	3.1	-	-
359	95.6	2.8	96.2	1.2	99.0	2.1	-	-
419	90.6	3.0	88.7	1.8	95.2	2.3	-	-
479	76.5	3.3	78.0	2.8	82.1	2.4	-	-
538	81.7	2.2	83.5	1.1	84.2	2.3	-	-
618	89.7	7.0	89.4	3.5	90.7	2.1	-	-
658	90.1	4.2	89.3	2.5	90.2	3.3	-	-
730	84.7	2.0	87.4	1.6	88.7	1.6	-	-
mean	93.3	18.0	89.2	14.2	87.9	12.4	-	-
Concurrent recovery (%)[A]	116[C] 85.0[D] (96.0)	17.1[C] 9.0[D] (19.1)	99.9[C] 88.6[D] (92.2)	20.9[C] 7.7[D] (14.4)	86.2[C] 91.9[D] (90.1)	24.1[C] 6.4[D] (13.4)		
KCA 7.1.2.2.1/08 (M-006079-01-1)								
0	83.1	6.6	81.8	2.6	89.2	4.1	-	-
34	78.6	3.2	80.6	1.5	81.7	3.4	-	-
90	82.5	4.4	80.4	7.1	85.1	3.3	-	-
120	73.2	14.2	80.6	15.9	82.1	15.4	-	-
181	100	0.8	101	1.9	98.8	1.4	-	-
243	90.0	1.7	93.7	0.4	90.6	0.7	-	-
300	76.8	2.3	83.3	1.9	83.4	0.8	-	-
361	85.4	1.8	86.4	2.0	86.1	1.8	-	-
441	91.6	3.4	91.7	4.3	91.6	3.9	-	-
481	87.6	1.4	93.6	0.7	92.1	1.3	-	-
540	76.0	1.9	78.7	8.4	80.5	3.2	-	-
601	88.0	3.8	88.8	4.5	90.6	4.3	-	-
662	86.6	1.9	87.8	4.5	88.7	3.6	-	-
726	89.7	4.5	94.8	7.4	97.3	9.2	-	-
mean	84.9	9.3	87.4	9.1	88.4	7.8	-	-
Concurrent recovery (%)[A]	84.1	7.6	87.1	8.6	90.0	7.4	-	-

Duration (days)	Spiroxamine		M01		M02		M03	
	Stability (%)	RSD (%)	Stability (%)	RSD (%)	Stability (%)	RSD (%)	Stability (%)	RSD (%)
KCA 7.1.2.2.1/09 (M-006074-01-1)								
0	85.5	4.1	92.7	2.4	96.0	1.6	97.5	1.9
30	77.8	6.0	87.6	2.0	88.2	0.8	96.9	2.8
61	78.0	2.1	83.5	2.6	86.4	3.5	91.4	1.8
120	80.2	3.6	88.1	2.0	90.5	1.9	95.2	3.5
177	72.9	5.5	86.2	1.9	86.3	3.7	90.2	1.3
268	73.9	4.0	84.4	3.1	86.7	0.8	92.7	3.6
355	78.1	3.2	87.8	2.1	90.5	1.2	92.9	2.6
427	77.1	2.5	85.0	3.2	85.7	3.7	89.2	5.6
555	76.4	2.6	85.2	1.8	88.0	1.2	92.9	6.2
639	79.7	3.8	85.6	2.3	85.4	0.8	90.2	1.2
mean	78.0	5.5	86.6	3.6	88.4	4.0	92.9	4.3
Concurrent recovery (%)[A]	81.0	9.1	88.2	4.1	88.5	4.5	89.1	6.7

- not applicable (metabolite not included)

A Recovery samples spiked and analysed at the same time as the storage stability samples
B The mean result presented excluded some outliers
C Overall mean and RSD value for concurrent recoveries using the GC/MS method at sampling interval 0-121 days
D Overall mean and RSD value for concurrent recoveries using the LC-MS/MS method at sampling interval 150-730 days

III. Conclusions

Storage stability of residues of spiroxamine and metabolites M01, M02 and M03 in soil under frozen conditions was shown to be acceptable for storage periods of up to 2 years.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study is considered valid to assess the storage stability of spiroxamine in soil under frozen conditions.

Kinetic evaluation

Data Point:	KCA 7.1.2.2.1/10
Report Author:	[redacted]; [redacted]
Report Year:	2007
Report Title:	Kinetic modelling analysis of spiroxamine and its metabolites KWG 4557 and KWG 4669 from field soil residue studies conducted in Europe
Report No:	VC/07/007A
Document No:	M-293744-01-1
Guideline(s) followed in study:	EU Council Directive 91/414/EEC, as amended by Commission Directive 95/36/EC of July 1995, Section 5, Point 7.1.1
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

This study was previous considered during the evaluation of spiroxamine (RAR (2010), RAR (2017)) and is therefore included again for completeness. The study performs a kinetic evaluation according to FOCUS 2006[5] of soil dissipation studies reported in studies KCA 7.1.2.2.1/01 (M-006116-01-1); KCA 7.1.2.2.1/02 (M-006126-01-1); KCA 7.1.2.2.1/03 (M-006127-01-1); KCA 7.1.2.2.1/04 (M-006128-01-1); and KCA 7.1.2.2.1/05 (M-006129-01-1). The kinetic evaluation reported in this study is superseded by the new kinetic evaluation performed in study KCA 7.1.2.2.1/12 (M-763140-01-1) on all the same soil dissipation studies.

Assessment and conclusion by applicant:

The study and its data are considered as supplementary data with no use in risk assessment.

Data Point:	KCA 7.1.2.2.1/11
Report Author:	[redacted]; [redacted]
Report Year:	2008
Report Title:	Kinetic modelling analysis of spiroxamine and its metabolites KWG 4557 and KWG 4669 from field soil residue studies conducted in Europe normalised to 20°C and pF2
Report No:	VC/08/019
Document No:	M-302004-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

This study was previous considered during the evaluation of spiroxamine (RAR (2010), RAR (2017)) and is therefore included again for completeness. The study performs a kinetic evaluation according to FOCUS 2006[5] of soil dissipation studies reported in studies KCA 7.1.2.2.1/01 (M-006116-01-1); KCA

7.1.2.2.1/02 (M-006126-01-1); KCA 7.1.2.2.1/03 (M-006127-01-1); KCA 7.1.2.2.1/04 (M-006128-01-1); and KCA 7.2.2.3/05 (M-006129-01-1). The kinetic evaluation reported in this study is superseded by the new kinetic evaluation performed in study KCA 7.1.2.2.1/12 (M-763140-01-1) on all the same soil dissipation studies.

Assessment and conclusion by applicant:

The study and its data are considered as supplementary data with no use in risk assessment.

Data Point:	KCA 7.1.2.2.1/12
Report Author:	[redacted]
Report Year:	2021
Report Title:	Spiroxamine: Kinetic assessment of field soil dissipation studies
Report No:	0471836-KIN3
Document No:	M-763140-01-1
Guideline(s) followed in study:	FOCUS 2006; FOCUS 2014
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The dissipation behaviour of spiroxamine was investigated in the field in five studies at eighteen European sites (see studies KCA 7.1.2.2.1/01 (M-006116-01-1), KCA 7.1.2.2.1/02 (M-006126-01-1), KCA 7.1.2.2.1/03 (M-006127-01-1), KCA 7.1.2.2.1/04 (M-006128-01-1) and KCA 7.1.2.2.1/05 (M-006129-01-1).

The study design for each study did not exclude loss processes such as photolysis or volatilisation; therefore, they are considered to follow the "legacy" study design according to EFSA (2014) guidance. The data from these studies were considered appropriate to derive persistence/trigger and modelling endpoints and were analysed using the CAKE version 3.4 (2020) software package. A kinetic analysis to determine DT_{50} and DT_{90} values for comparison with relevant study triggers and persistence criteria was performed using non-normalised data, in accordance with the flowcharts for persistence/trigger endpoints provided by FOCUS (2014). To determine $Deg1_{50,\ matrix}$ values, a kinetic assessment was performed following the flowcharts for calculating modelling endpoints provided by EFSA (2014), and using data that have been normalised to reference conditions (20°C and pF 2 soil moisture content) and subject to timestep normalisation procedures. These endpoints were used for the selection of an appropriate modelling value for use with regulatory Predicted Environmental Concentration (PEC) models.

The derived persistence and modelling endpoints for spiroxamine, M01 and M02 are summarised in Table CA 7.1.2.2.1-69 and Table CA 7.1.2.2.1-70, respectively.

I. Materials and Methods

The dissipation behaviour of spiroxamine was investigated in the field in five studies at eighteen European sites (see studies KCA 7.1.2.2.1/01 (M-006116-01-1), KCA 7.1.2.2.1/02 (M-006126-01-1), KCA 7.1.2.2.1/03 (M-006127-01-1), KCA 7.1.2.2.1/04 (M-006128-01-1) and KCA 7.1.2.2.1/05 (M-006129-01-1). It is noted that some studies are performed on sites at the same location at the same time. These have been fitted separately, and the individual endpoints used for persistence/trigger purposes. For modelling, the geomean of DT_{50mod} values from the individual fits for these sites have been used for final endpoints.

The study design for each study did not exclude loss processes such as photolysis or volatilisation;

therefore, they are considered to follow the “legacy” study design according to EFSA (2014) guidance.

Daily rainfall, air temperature, soil temperature and soil moisture values were not provided in the study reports.

A. Normalisation to reference conditions

Kinetic analysis to generate persistence/trigger endpoints was initially performed using the data as reported (i.e. not normalised to reference conditions).

Kinetic analysis to generate modelling endpoints was performed with data that have been normalised to reference conditions (20°C and moisture at pF 2), and subject to a timestep normalisation procedure increasing or reducing the length of each day in the study, depending on the soil temperature and moisture, according to FOCUS guidance (FOCUS, 2014). These procedures were conducted in KCA 7.1.2.2.1/11 (M-302004-01-1). Daily rainfall and air temperature data were used from weather stations <20km from test sites (summarised in the study report). In order to estimate soil temperature and soil moisture values, the PEARL v2.2.2 model was used. PEARL meteorological files were generated from the available daily weather data (minimum and maximum air temperature, rainfall and PET) and input files were set up with van Geneuchten parameters estimated with the HYPRES database, using soil characterisation data obtained for the study. There was no crop chosen for the PEARL simulations as the applications was made to bare soil. The PEARL model was run using the site-specific soil properties and meteorological data and the estimated daily soil temperature and volumetric moisture content stored in an output file. PEARL input files are available in the study report (Appendix 3). An example of the timestep normalisation is also provided in the study report (Appendix 5).

B. Data handling

Input data were generated according to the data handling recommendations made in the FOCUS guidance for degradation kinetics (FOCUS, 2014).

True individual replicates were not taken in any of the studies. The reported measured concentrations (mg/kg dry soil) were converted to g/ha and handled in accordance with the FOCUS guidance on values below the limit of detection (LOD) or limit of quantification (LOQ) according to individual depths and time. Spiroxamine residues in individual horizons at each sampling time were summed before conversion to g/ha, with metabolite values corrected for molecular weight differences before conversion.

The handling of values below the LOD and LOQ was performed according to the procedure recommended by (FOCUS, 2014) as follows:

- All values between LOD and LOQ were set to the actual measured value. If the actual measured concentration was not reported, 0.5 × (LOQ + LOD) was used.
- All samples <LOD were set to 0.5 × LOD.
- All samples after the first non-detect (<LOD) were omitted unless positive detections above LOQ were made later in the experiment. In that case, samples were included up to the first non-detect (<LOD) which is NOT followed by later positive samples above LOQ.

C. Input data

Sampling interval values in actual days were used for persistence/trigger endpoint kinetics assessments and normalised days were used for modelling endpoint kinetics assessments.

The studies were conducted according to the “legacy” design, without measures to eliminate surface loss processes. In the modelling endpoint kinetics assessment for these studies, in accordance with EFSA (2014) guidance, the datapoints before 10 mm cumulative rainfall were excluded from the SFO kinetic fit since the study design did not exclude surface processes. The normalised times at which >10 mm cumulative rainfall was measured were determined (see Table 3-6 in the study report).

Sampling interval values in actual days were used for persistence/trigger kinetics assessments. Sampling intervals normalised to soil moisture at pF 2 and a temperature of 20°C were used for the modelling

kinetics assessments. The data used for conversion to g/ha and subsequently input into the kinetic models for each field trial site in the soil dissipation studies are presented in the study report (Table 3-7 to Table 3-24, pages 23-40).

D. Kinetic modelling

The kinetic modelling of the field data was conducted using CAKE version 3.4 (2020).

In the first instance, the data were directly fitted, un-weighted, with the complete data set and unconstrained initial concentration (M_0). The acceptability of kinetic fits was judged both visually and according to the χ^2 error and the t-test functions as recommended by FOCUS (2014). It is recommended that a χ^2 error of 15% or less indicates an acceptable fit, although for data that may include intrinsically variable data (e.g. field data) higher values can be tolerated if the visual fit is acceptable or good.

The FOCUS Kinetics guidelines state that the confidence that can be assigned to a parameter must be assessed and a t-test probability of greater than 95% ($p<0.05$) for estimated degradation rate constants indicate robust estimates. When fitting the first-order multi-compartment model (FOMC), the t-test is not appropriate as a measure of confidence (FOCUS, 2014).

The FOCUS (2014) flowcharts for calculating persistence/trigger and modelling endpoints and the EFSA (2014) flowcharts for calculating modelling endpoints were followed, as appropriate. Each dataset has been considered following the steps in the flowcharts and the considerations are discussed in detail below. The full outputs from the persistence/trigger and modelling kinetic fits (including the initial settings) are shown in the study report (Appendices 6 and 7, respectively). For persistence endpoints, kinetic fits for both spiroxamine and metabolites M01 and M02 were performed following FOCUS (2014), whilst for modelling endpoints fits for spiroxamine were considered according to EFSA (2014). For modelling endpoints of M01 and M02, fitting of the decline curve is performed to derive DT_{50} values only.

It is noted that some studies are performed on sites at the same location at the same time. These have been fit separately, and the separate endpoints used for persistence/trigger purposes. For modelling, the geomean of DT_{50mod} values from the separate fits for these sites have been used for final endpoints.

Kinetic assessments were performed using the degradation schemes presented in Figure 7.1.2.2.1-1 and Figure 7.1.2.2.1-2.

Figure 7.1.2.2.1-1: Route of degradation in aerobic soil

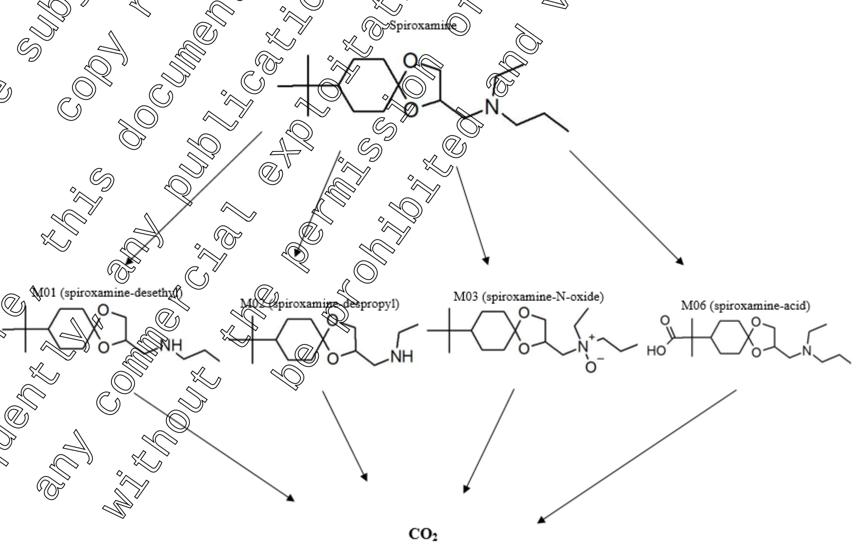
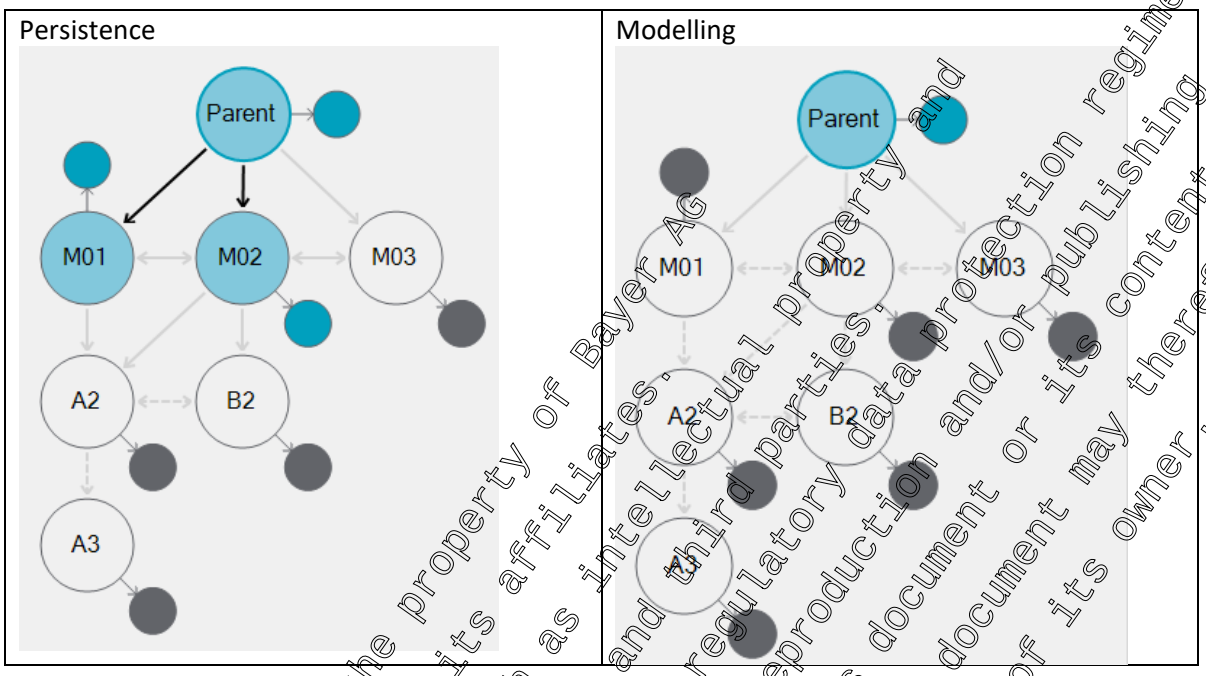


Figure 7.1.2.2.1-2: Degradation scheme used in kinetic analysis of all soils treated with spiroxamine



The acceptability of kinetic fits was judged both visually and according to the χ^2 error and the t-test functions as recommended by FOCUS. The visual assessment is recommended as the main tool for assessing goodness of fit. However, it is also recommended that a χ^2 error of less than 15% and a t-test probability of greater than 95% ($p < 0.05$) for estimated degradation rate constants indicate an acceptable fit. The χ^2 error was not considered as an absolute cut-off criterion as FOCUS guidance indicates that there will be cases where the error is higher than 15%, but the fit still represents a reasonable description of the degradation behaviour. In such situations, examination of plots of residuals for systematic error is considered important.

The t-test assesses whether degradation rate constants differ significantly from zero (i.e., no degradation). Alternatively, confidence intervals can be examined. In this assessment, the t-test was chosen for assessing confidence in rate constants. When fitting the FOMC model, FOCUS guidance indicates that the t-test is not appropriate as a measure of confidence for the gamma-distribution parameters α and β. Therefore, if a FOMC fit indicated slow degradation, confidence intervals for β were examined to determine if they were high compared to the parameter estimate, which would indicate that the parameter estimate was not reliable.

When calculating modelling endpoints for a metabolite, it was considered important to derive a formation fraction wherever possible. In the FOCUS flowsheets, if the SFO fit for a metabolite is not considered acceptable, a case-by-case decision is required. The first option given is to assess the decline of the metabolite after its maximum ('top-down' method). However, this method does not allow formation fraction assessment. The second option given is to fix the formation fraction to a worst-case value (usually 1) and use this in combination with a worst-case DT_{50} (usually 1000 days). However, this method almost always results in a clear overestimation of observed metabolite residues. The final option given is to use alternative – but conservative – estimates that describe the observed patterns. In this assessment, alternative – but conservative – estimates were chosen, implemented by fixing the formation fraction to the fitted value and using this in combination with a conservative DT_{50}, or vice versa. The parameter to amend and its value were chosen based on expert judgement and iterative amendments to give a conservative description of the observed metabolite residue pattern.

The FOCUS (2014) flowcharts for calculating persistence and modelling endpoints were followed.

If necessary, the kinetic endpoints derived for each soil were normalised to FOCUS reference conditions

(soil temperature of 20°C and soil moisture content equal to pF 2) following the procedures recommended by FOCUS.

II. Results and Discussion

A. Persistence/trigger endpoints

The kinetic evaluation was conducted using CAKE version 3.4 (2020) following the FOCUS (2014) decision flowchart for persistence/trigger endpoints. A summary of the fits achieved and decisions taken is provided (Appendix 4.1:) for each soil in Appendix 4.1.1 to Appendix 4.1.18.

The resulting persistence or best-fit endpoints are presented in Table CA 7.1.2.2.1-69.

The spiroxamine persistence/trigger DT_{50} values ranged from 0.5 to 59.6 days and DT_{90} values ranged from 43.5 to 433 days.

M01 persistence/trigger DT_{50} values ranged from 17.8 to 223 days and DT_{90} values ranged from 59 to 742 days.

M02 persistence/trigger DT_{50} values ranged from 21 to 161 days and DT_{90} values ranged from 69.6 to 533 days.

B. Modelling endpoints

The kinetic evaluation was conducted using CAKE version 3.4 (2020) following the EFSA (2014) decision flowcharts for calculating $DegT_{50,matrix}$ values. A summary of the fits achieved and decisions taken is provided (Appendix 4.2:) for each soil in Appendix 4.2.1 to Appendix 4.2.18. For modelling endpoints of M01 and M02, fitting of the decline curve is performed to derive DT_{50} values only.

The resulting modelling endpoints are presented in Table CA 7.1.2.2.1-70.

The spiroxamine modelling DT_{50} values (at 20°C and pF 2) ranged from 16.1 to 133 days, with a geometric mean of 42.9 days. If not including cropped trials, modelling DT_{50} values (at 20°C and pF 2) ranged from 23.4 to 78 days, with a geometric mean of 43.8 days.

M01 modelling DT_{50} values ranged from 23.4 to 1,000 days (default), with a geometric mean of 66.2 days. Formation fraction was not determined as formation may have been affected by photolytic factors according to EFSA (2014). If not including cropped trials, modelling DT_{50} values (at 20°C and pF 2) ranged from 23.4 to 1,000 days, with a geometric mean of 89.8 days.

M02 modelling DT_{50} values ranged from 27.7 to 196 days, with a geometric mean of 69.1 days. Formation fraction was not determined as formation may have been affected by photolytic factors according to EFSA (2014). If not including cropped trials, modelling DT_{50} values (at 20°C and pF 2) ranged from 44.3 to 196 days, with a geometric mean of 93.8 days.

Table CA 7.1.2.2.1-69: Summary of persistence endpoints for spiroxamine and metabolites M01 and M02

Soil properties			Spiroxamine				M01				M02			
Soil type	**Soil name**	**pH ($CaCl_2$)**	**DissT50 (days)**	**DissT90 (days)**	**χ^2 error (%)**	**Kinet-ics**	**DissT50 (days)**	**DissT90 (days)**	**χ^2 error (%)**	**Kinet-ics**	**DissT50 (days)**	**DissT90 (days)**	**χ^2 error (%)**	**Kinet-ics**
CA 7.1.2.2.1/01 (M-006116-01-1) 1995														
Silt loam	Höfchen	6.5	13.8	145	11.8	DFOP	18.1	60.2	16.6	SFO	21.0	69.6	16.5	SFO
Loam	Laacher Hof	6.8	32.6	196	6.3	DFOP	50.3	167	11.9	SFO	48.3	160	10.8	SFO
Sandy loam	Elm Farm/ Thurston	7.5	0.8	197	7.4	DFOP	51.4	171	12.0	SFO	57.7	192	10.7	SFO
Loamy sand	Pakenham	7.3	0.5	132	8.2	DFOP	57.4	191	13.5	SFO	63.8	212	13.7	SFO
CA 7.1.2.2.1/02 (M-006126-01-1) 1995														
Silt loam	Höfchen	6.4	56.6	393	8.1	FOMC	130	432	6.9	SFO	135	447	7.2	SFO
Sandy loam	Laacher Hof	6.6	20.1	127	9.5	DFOP	125	416	7.4	SFO	122	406	5.7	SFO
Sandy loam	Maasen	5.9	8.4	271	10.6	DFOP	223	742	8.5	SFO	161	533	12.4	SFO
Silt loam	Swisttal-Hohn	6.7	7.9	84.7	7.9	FOMC	40	133	10.0	SFO	49.2	164	8.8	SFO
Clay loam	Albig	7.8	6.0	74.5	6.3	DFOP	52.3	174	21.7	SFO	63.3	210	18.7	SFO
CA 7.1.2.2.1/03 (M-006127-01-1) 1995														
Sandy loam	Elm Farm/ Thurston	7.4	1.46	132	5.2	DFOP	74.8	248	7.95	SFO	75.9	252	9.7	SFO
Sandy loam	Pakenham	7.0	9.5	199	6.3	DFOP	72.6	241	5.2	SFO	73.2	243	4.0	SFO
Sandy loam	Elm Farm/ Thurston	7.4	9.1	433	6.7	DFOP	74.8	248	7.95	SFO	75.9	252	14.8	SFO
Sandy loam	Pakenham	7.0	11.2	247	9.20	DFOP	96.8	322	10.2	SFO	103	342	10.8	SFO
Silt loam	Touffreville	7.2	6.1	58.4	3.8	DFOP	22.6	75.2	16.4	SFO	21.3	70.7	14.6	SFO
CA 7.1.2.2.1/04 (M-006128-01-1) 1996														
Loam	Laudun	7.7	2.1	43.5	9.1	DFOP	Too few data points				Too few data points			
Silty clay loam	Filetto	7.6	59.6	295	16.9	DFOP	17.8	59.0	12.5	SFO	18.1	60.1	11.4	SFO
CA 7.1.2.2.1/05 (M-006129-01-1) 1996														
Loam	Laudun	7.7	2.1	93.3	3.9	DFOP	30.9	103	17.7	SFO	28.9	96.1	19.1	SFO
Sandy loam	Nogarole Rocca	7.7	3.5	43.8	1.9	DFOP	28.8	95.6	3.5	SFO	28.5	94.8	3.3	SFO
	Worst-case :		59.6	433			223	742			161	533		
	pH dependence :		No				No				No			

Table CA 7.1.2.2.1-70: Summary of modelling endpoints for spiroxamine and metabolites M01 and M02

Soil properties				Spiroxamine				M01				M02			
Soil type	Soil name	Crop (Y/N	pH ($CaCl_2$)	Individ. trial modelling $DegT_{50}$ (days)	Final modelling $DegT_{50}$ (days)	χ^2 error (%)	Kinetics	Individ. trial modelling $DegT_{50}$ (days)	Final modelling $DegT_{50}$ (days)	χ^2 error (%)	Kinetics	Individ. trial modelling $DegT_{50}$ (days)	Final modelling $DegT_{50}$ (days)	χ^2 error (%)	Kinetics
CA 7.1.2.2.1/01 (M-006116-01-1) 1995															
Silt loam	Höfchen	N	6.5	38.7	54.9	11.1	DFOP[3]	52.3	72.1	5.2	SFO	52.3	76.5	9.6	SFO
Loam	Laacher Hof	Y	6.8	44.1	50.2	10.1	SFO	59.2	99.4	10.5	SFO	66.3	96.5	9.7	SFO
Sandy loam	Elm Farm/ Thurston	Y	7.5	59.8	78.6	8.4	SFO	48.9	65.3	3.0	SFO	56.7	69.7	4.1	SFO
Loamy sand	Pakenham	Y	7.3	43.6	45.1	10.4	SFO	59.5	71.9	15.1	SFO	66.6	72	15.8	SFO
CA 7.1.2.2.1/02 (M-006126-01-1) 1995															
Silt loam	Höfchen	N	6.4	78.0	78.0	8.2	SFO	99.3	-[2]	5.0	SFO	112	-[2]	6.3	SFO
Sandy loam	Laacher Hof	N	6.6	57.2	-[2]	4.3	SFO	167	-[2]	5.6	SFO	140	-[2]	4.8	SFO
Sandy loam	Maasen	N	5.9	66.4	66.4	11.0	SFO	No fit	1,000 default	NA	NA	196	196	12.2	SFO
Silt loam	Swisttal-Hohn	N	6.7	47.3	47.3	10.6	SFO	53.8	53.8	11.2	SFO	64.4	64.4	10.1	SFO
Clay loam	Albig	N	7.8	33.7	33.7	8.2	DFOP	45.9	45.9	22.2	SFO	70.4	70.4	16.5	SFO
CA 7.1.2.2.1/03 (M-006127-01-1) 1995															
Sandy loam	Elm Farm/ Thurston	Y	7.4	61.0	-[2]	8.6	SFO	59.3	-[2]	8.3	SFO	61	-[2]	10.5	SFO
Sandy loam	Pakenham	Y	7.0	42.6	-[2]	7.3	SFO	101.0	-[2]	4.6	SFO	57.7	-[2]	3.7	SFO
Sandy loam	Elm Farm/ Thurston	Y	7.4	133	-[2]	7.0	DFOP	96.2	-[2]	10.5	SFO	98	-[2]	10.0	SFO
Sandy loam	Pakenham	Y	7.0	49.5	-[2]	12.0	SFO	96.2	-[2]	9.6	SFO	97.3	-[2]	11.0	SFO
Silt loam	Touffreville	Y	7.2	16.1	16.1	4.8	HS	28.9	28.9	11.6	SFO	28.1	28.1	9.8	SFO

Soil properties				Spiroxamine				M01				M02			
Soil type	Soil name	Crop (Y/N	pH ($CaCl_2$)	Individ. trial model-ling $DegT_{50}$ (days)	Final model-ling $DegT_{50}$ (days)	χ^2 er-ror (%)	Kinet-ics	Individ. trial model-ling $DegT_{50}$ (days)	Final model-ling $DegT_{50}$ (days)	χ^2 er-ror (%)	Kinet-ics	Individ. trial model-ling $DegT_{50}$ (days)	Final model-ling $DegT_{50}$ (days)	χ^2 er-ror (%)	Kinet-ics
CA 7.1.2.2.1/04 (M-006128-01-1) 1996															
Loam	Laudun	Y	7.7	56.5	47.4	12.2	SFO	Too few observations				Too few observations			
Silty clay loam	Filetto	Y	7.6	49.3	49.3	17.8	SFO	17.8	59.0	12.5	SFO	27.7	27.7	5.1	SFO
CA 7.1.2.2.1/05 (M-006129-01-1) 1996															
Loam	Laudun	N	7.7	39.7	-[2]	12.7	SFO	30.9	103	17.7	SFO	151	151	6.3	FOMC
Sandy loam	Nogarole Rocca	N	7.7	23.4	23.4	5.6	SFO	28.8	95.6	3.5	SFO	44.3	44.3	5.3	SFO
		Geometric mean*			42.9/ 43.8				66.2/ 89.8				69.1/ 93.8		
		pH dependence :			No				No				No		

NA = not applicable
[1] Sites at the same location are treated as replicates due to trials being the same locations and application times.
[2] Geomean of replicate sites given with first replicate site
[3] expert judgment
* Geomean for all plots and non-cropped only presented respectively

III. Conclusions

Persistence/trigger and modelling endpoints (DT_{50} and DT_{90}) representing the degradation rate of spiroxamine in field soil dissipation studies were calculated in accordance with FOCUS (2014) and EFSA (2014) guidance.

The spiroxamine persistence/trigger DT_{50} values ranged from 0.5 to 59.6 days and DT_{90} values ranged from 43.5 to 433 days. The spiroxamine modelling DT_{50} values (at 20°C and pF 2) ranged from 16.1 to 133 days, with a geometric mean of 42.9 days. If not including cropped trials, modelling DT_{50} values (at 20°C and pF 2) ranged from 23.4 to 78 days, with a geometric mean of 43.8 days.

M01 persistence/trigger DT_{50} values ranged from 17.8 to 223 days and DT_{90} values ranged from 59 to 742 days. Modelling DT_{50} values ranged from 23.4 to 1,000 days (default), with a geometric mean of 66.2 days. Formation fraction was not determined as formation may have been affected by photolytic factors according to EFSA (2014). If not including cropped trials, modelling DT_{50} values (at 20°C and pF 2) ranged from 23.4 to 1,000 days, with a geometric mean of 89.8 days.

M02 persistence/trigger DT_{50} values ranged from 21 to 161 days and DT_{90} values ranged from 69.6 to 533 days. Modelling DT_{50} values ranged from 27.7 to 196 days, with a geometric mean of 69.1 days. Formation fraction was not determined as formation may have been affected by photolytic factors according to EFSA (2014). If not including cropped trials, modelling DT_{50} values (at 20°C and pF 2) ranged from 44.3 to 196 days, with a geometric mean of 93.8 days.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to guideline(s) FOCUS 2006, 2014 (required guideline). The study is considered valid to assess best fit and modelling DT_{50} values for spiroxamine and associated metabolites in field soil studies.

CA 7.1.2.2.2 Soil accumulation studies

Soil dissipation studies conducted under Point CA 7.1.2.2.1 show that $DisT90_{field}$ for spiroxamine and metabolites M01 and M02 can exceed one year. Additionally, in the absence of further information $DisT90_{field}$ for spiroxamine metabolite M03 is also assumed to exceed one year. The potential for accumulation of spiroxamine and metabolites M01, M02, M03 and M06 in soil is addressed by consideration of worst-case soil accumulation PECs, see CP 9.1.3.

Soil accumulation studies are therefore not considered necessary.

CA 7.1.3 Adsorption and desorption in soil

CA 7.1.3.1 Adsorption and desorption

Use of plant protection products containing the active substance spiroxamine will result in contact with soil, therefore the soil sorption characteristics of the active substance and major metabolites (as defined under Point CA 7.4.1) are investigated in laboratory studies according to the data requirements laid down in Commission Regulation (EU) No 283/2013 under Points CA 7.1.3.1.1 and CA 7.1.3.1.2, respectively.

Overview:

The mobility in soil of spiroxamine and its degradation products relevant for assessment was studied by batch equilibrium tests on a variety of different soils. A summary of the resulting modelling soil sorption parameters are presented below:

Table CA 7.1.3.1-1: Overview of soil sorption modelling parameters for spiroxamine and associated metabolites

Compound	K_{foc} [A] [mL/g]	1/n [B] [-]	Sorption dependant on soil pH (Y/N)
spiroxamine	4111 (geomean, n=8)	0.892 (average, n=8)	N
M01	3271 (geomean, n=4)	0.848 (average, n=4)	N
M02	2695 (geomean, n=4)	0.878 (average, n=4)	N
M03	1677 (geomean, n=4)	0.900 (average, n=4)	N
M06	Study on-going	Study on-going	Study on-going

A Geometric mean
B Arithmetric mean

The adsorption and desorption of metabolites of spiroxamine have been investigated in three studies (KCA 7.1.3.1.2/01 to KCA 7.1.3.1.1/03) which were evaluated during the previous EU review. Sorption of M01, M02 and M03 was established in four soil types and the outcome of the studies reviewed for reliability using the EFSA 106 checklist v2.0. It was concluded that despite some minor deviations the outcome of the adsorption/desorption studies were reliable and suitable for use in risk assessment. Some differences between the calculated K_{foc} values and those reported were noted, but this was attributed to the use of averages in the original calculations. Since differences were minor, for consistency the reported values from the report were used in the risk assessment.

Adsorption of M01 was investigated in 4 different soils with Kfoc ranging from 1237 to 10511 L/kg ([redacted], 1996). Adsorption was shown to be generally correlated with organic carbon content, and M01 (spiroxamine-desethyl) exhibits medium to immobile mobility in soil according to the McCall mobility classification. No significant correlation was observed between soil sorption parameter K_{foc} with soil pH for M01 (spiroxamine-desethyl) (R^2=0.102), therefore no pH dependence was concluded. The geomean of the four soils was considered appropriate for use in the risk assessment as previously concluded (EFSA Journal (2010);8(10):1719)).

Adsorption of M02 was investigated in 4 different soils with Kfoc ranging from 916.7 to 8993.6 L/Kg (Fent, 1996b). Adsorption was shown to be generally correlated with organic carbon content, and M02 (spiroxamine-despropyl) exhibits medium to immobile mobility in soil according to the McCall mobility classification. No significant correlation between soil sorption parameter K_{foc} with soil pH for M02 (spiroxamine-despropyl) was observed (R^2=0.005), therefore no pH dependence was concluded. The geomean of the four soils was thus considered appropriate for use in the risk assessment as previously concluded (EFSA Journal (2010);8(10):1719)).

Adsorption of M03 was also determined in 4 different soils, with the K_{foc} ranging from 350.5 to 24892.5 L/Kg (Fent, 1997). Adsorption was shown to be generally correlated with organic carbon content and M03 (spiroxamine-N-oxide) exhibits medium to immobile mobility in soil according to the McCall mobility classification. No significant correlation between soil sorption parameter K_{foc} with soil pH for M03 (spiroxamine-N-oxide) was observed (R^2=0.331), therefore no pH dependence was concluded. The geomean of the four soils was thus considered appropriate for use in the risk assessment as previous concluded (EFSA Journal (2010);8(10):1719)).

One further study is being conducted to provide soil sorption properties for metabolite M06 (spiroxamine-acid) and will be provided as soon as available. As such, in order to provide a basis for the risk assessment, an estimated K_{foc} value of 3.2 L/Kg for M06 for use in the preliminary risk assessment was obtained from KocWIN. This will be updated upon completion of the M06 OECD106 study.

The high sorption displayed by spiroxamine and its metabolites is reflected in the outcome of column leaching studies investigating the leaching behaviour of aged residue of spiroxamine in soil. These studies demonstrated that in soil column studies, aged residues of spiroxamine did not significantly leachate to the column percolate with only 0.2 %AR being found in the leachate. The major residue in the leachate

was found to be M03 (N-oxide) representing only 0.03% of the applied radioactivity in column leachates. Overall, leaching behaviour of spiroxamine (including individual isomers) or its major soil metabolites is not envisaged.

CA 7.1.3.1.1 Adsorption and desorption of the active substance

The adsorption and desorption of spiroxamine has been investigated in two studies (KCA 7.1.3.1.1/01 and KCA 7.1.3.1.1/02) which were evaluated during the previous EU review.

The applicant considers the adsorption endpoints from batch equilibrium studies are appropriate for use in risk assessment.

Substance	Report reference	Document no.	Comment
Spiroxamine	KCA 7.1.3.1.1/01	M-006189-01-2	Submitted for first approval of spiroxamine, 1999. Reviewed under UP. Considered valid and acceptable.
Spiroxamine	KCA 7.1.3.1.1/02	M-006186-02-1	Submitted for first renewal of spiroxamine, 2010. Reviewed under UP. Considered valid and acceptable.

The resulting soil sorption parameters for the active substance spiroxamine are summarised below:

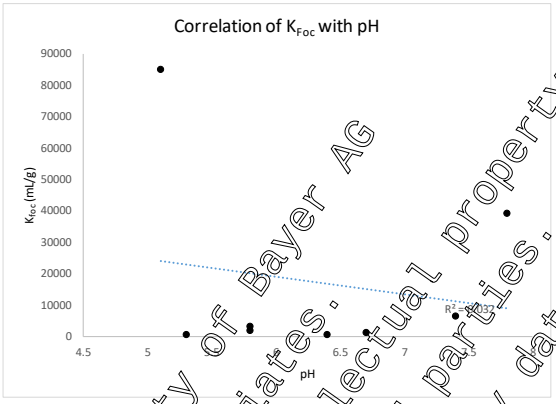
Table CA 7.1.3.1.1-1: Overall summary of Freundlich soil adsorption parameters for spiroxamine

Study	Soil name	Soil properties			Soil sorption parameters		
		Texture	pH[A]	OC (%)	K_f (L/kg)	K_{foc} (L/kg)	1/n
KCA 7.1.3.1.1/01 M-006189-01-2	Laacherhof AXXa (0-30cm)	Loamy sand	6.4	1.8	12.78	710	0.785
	Standard Soil 2.1	Sand	5.3	0	4.61	659	0.768
	Hoefchen "im Tal"	Silt loam	5.8	2.4	44.98	1874	0.831
	Clay soil LUFA Speyer	Silty clay	7.4	0.6	41.07	6417	0.885
	Laacherhof AXXa (30-60 cm)	Loamy sand	6.3	0.3	7.25	2415*[B]	0.833*[B]
KCA 7.1.3.1.1/02 M-006186-02-1	Vero Beach	Sand	6.7	0.2	8.55	4276*[C]	1.06*[C]
	Grape Vineyard	Sandy loam	5.8	0.45	14.47	3216	1.05
	Howe	Sandy loam	6.7	1.12	15.09	1347	1.02
	Wolf Ranch	Loam	7.8	0.97	381.65	39346	1.02
	Stanley	Silty clay	5.1	1.05	892.59	85008	1.01
						4111 (geo-mean, n=8)	0.892 (mean, n=8)

* Value excluded from calculations (i.e. geometric mean and arithmetic mean)
A pH (0.01M $CaCl_2$) unless otherwise stated
B Excluded for modelling endpoints due to being sub-soil
C Excluded for modelling endpoints as oc<0.3%

The correlation for the active substance spiroxamine of soil sorption parameter K_{foc} with soil pH is presented below:

Figure 7.1.3.1.1-1: Correlation of K_{foc} with soil pH for spiroxamine



There was no strong correlation between soil sorption parameter K_{foc} with soil pH for parent spiroxamine (R^2=0.032), therefore no pH dependence was concluded (note the dissociation constant, pKa, value for parent spiroxamine is 6.9, see CA 2.8).

Existing studies, previously evaluated

Data Point:	KCA 7.1.3.1.1/01
Report Author:	[redacted]
Report Year:	1995
Report Title:	Adsorption/Desorption of KWG 4168 on soils
Report No:	PF4038
Document No:	M-006189-01-2
Guideline(s) followed in study:	EPA Ref.: 163-1, Leaching and Adsorption/Desorption
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption and desorption of [cyclohexyl-1-^{14}C]-spiroxamine on four European soils and one European subsoil was studied using the batch equilibrium method with a tiered approach.

Parameter	Soil				
Soil Designation	**Laacherhof AXXa (0-30cm)**	**Standard Soil 2.1**	**Hoefchen "im Tal"**	**Clay soil LUFA Speyer**	**Laacherhof AXXa (30-60 cm)**
Textural Classification (USDA)	Loamy sand	Sand	Silt loam	Silty clay	Loamy sand
pH ($CaCl_2$)	6.4	5.3	5.8	7.4	6.3
Organic carbon (%)	1.8	0.7	2.4	0.64	0.3

Preliminary tests were performed to determine the appropriate soil:solution ratios and equilibrium time and to evaluate stability of the test substance in $CaCl_2$ solution (without soil, potential for sorption of the test substance to test vessels and any effects of the inclusion of a biocide in solution.

In the preliminary tests, soil:solution ratios of 1:10 and equilibrium time 24 hours was determined for all soils and the subsoil. After shaking the test substance in $CaCl_2$ without soil, recoveries of 94.3 – 97.1% AR were reported, indicating sorption to test vessels was not occurring. Stability of spiroxamine was also monitored in $CaCl_2$ solution without soil and results show >99% AR present as unchanged spiroxamine after 9 days.

Freundlich isotherm tests were performed with all soils using the indirect and parallel methods. A soil:solution ratio of 1:10, and 24 hours equilibrium time for adsorption and desorption were used for all soils. For the adsorption step, appropriate solutions of the test substance were applied to the samples and the samples were shaken for 24 hours at 20 ± 1°C in the dark. Following the adsorption step, the samples were removed from the shaker, centrifuged, and the adsorption supernatants removed. Chromatographic analysis of supernatants of adsorption from Standard soil 2.1, Hoefchen "im Tal", Clay soil LUFA Speyer at 5.0 mg/L show >90% AR identified as spiroxamine. Chromatographic analysis of supernatants of adsorption in the subsoil Laacherhof AXXa 30-60 cm at 5.0 and 0.5 mg/L also showed >90% AR identified as spiroxamine.

For the desorption step, a 20 ml volume of fresh 0.01 M $CaCl_2$ solution was added and the samples were shaken for a further 24 hours at 20 ± 1°C in the dark. Following the desorption step, the samples were removed from the shaker, centrifuged, and the desorption supernatants removed. The desorption supernatants were analysed by LSC. The soil samples were combusted in a sample oxidiser and analysed by LSC to determine the radioactivity present. Chromatographic analysis of supernatants of desorption in the subsoil Laacherhof AXXa 30-60 cm at 5.0 and 0.5 mg/L also showed >90% AR identified as spiroxamine.

Mass balances ranged from 81.8-98.8% AR. The amount of radioactivity adsorbed on the soil at adsorption equilibrium ranged from 25.0-93.6% AR. After the adsorption phase, 50.67 – 82.44% AR was adsorbed to Laacherhof AXXa 0-30cm soil, 24.95 – 60.70% AR adsorbed to Standard soil 2.1, 81.86 – 93.61% AR to Hoefchen "im Tal", 81.61 – 95.90% AR.

Freundlich adsorption coefficients (Kf) ranged from 4.61-44.98 L/kg. When normalised to organic carbon, Freundlich adsorption coefficients (Kfoc) ranged from 658.8-6417.1 L/kg, indicating that spiroxamine exhibits low mobility in soil according to the McCall mobility classification (McCall et al 1981[7]). The 1/n values ranged from 0.768-0.885, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range. The OECD 106 Checklist (v2) was used to evaluate the study; however, all final parameters are taken from the study report. The evaluation confirmed acceptability for all soils according to the quality criteria and therefore acceptable for regulatory use.

[7] McCall P.J., Laskowski D.A., Swann R.L. and Dishburger H.J. (1981). Measurement of sorption coefficients of organic chemicals and their use, in environmental fate analysis, in Test Protocols for Environmental Fate and Movement of Toxicants. Proceeding of AOAC Symposium, AOAC.

Soil	Laacherhof A XX a, 0-30 cm (Loamy sand)	Standard soil 2.1 (Sand)	Hofchen "im Tal" (Silt loam)	Clay soil LUFA Speyer (Silty clay)	Laacherhof A XX a, 30-60 cm (Loamy sand)
OC (%)	1.8	0.7	2.4	0.64	0.3
pH ($CaCl_2$)	6.4	5.3	5.8	7.4	6.3
K_f (L/kg)	12.78	4.61	44.98	41.07	7.25
K_{foc} (L/kg)	709.9	658.8	1874.0	6417.1	2415.3
1/n	0.785	0.768	0.831	0.885	0.833
R^2	1.000	0.999	1.000	1.000	1.000

I. Materials and Methods

A. Materials

1. Test Items

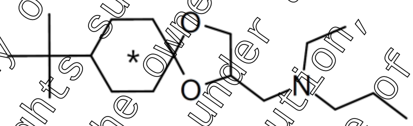
Test substance: [cyclohexyl-1-^{14}C]-spiroxamine

Lot/Batch No.: KML2039
7551/12

Specific activity: 2.59 MBq/mg

Radiochemical purity: >99%

Structure:



*Denotes position of [^{14}C]-radiolabel

2. Test System (soil)

Four European soils and one sub-soil with contrasting organic carbon, pH and clay content were used with details as shown below:

Table CA 7.1.3.1.1-2: Physico-chemical properties of test soils

Parameter	Soil				
Soil Designation	Laacherhof AXXa (0-30cm)	Standard Soil 2.1	Hoefchen "im Tal"	Clay soil LUFA Speyer	Laacherhof AXXa (30-60 cm)
Geographic Location					
City	Monheim	Jockgrim	Burscheid	Südpfalz	Monheim
Country	Germany	Germany	Germany	Germany	Germany
Textural Classification (USDA)	Loamy sand	Sand	Silt loam	Silty clay	Loamy sand
Sand (%)	72.4	89.4	3.6	15.0	68.4
Silt (%)	22.6	10.5	80.8	42.3	19.3
Clay (%)	5.0	0.1	15.6	42.7	12.3
pH					
in H_2O	7.0	5.9	6.0	7.6	7.7
in $CaCl_2$	6.4	5.3	5.8	7.4	6.3

Parameter	Soil				
Soil Designation	**Laacherhof AXXa (0-30cm)**	**Standard Soil 2.1**	**Hoefchen "im Tal"**	**Clay soil LUFA Speyer**	**Laacherhof AXXa (30-60 cm)**
Organic Matter (%) *	3.1	1.2	4.1	1.1	0.5
Organic carbon (%)	1.8	0.7	2.4	0.64	0.3
Cation Exchange Capacity (meq/100 g)	8.0	5.0	10.0	21.1	5.0

* Calculated by multiplying organic carbon content by 1.724 (not reported)

B. Study Design

1. Experimental Conditions

The test system for adsorption and desorption of spiroxamine on four European soils and one European subsoil consisted of Teflon centrifuge tubes with screw caps.

In preliminary tests, the adsorption of the test item to the test system surface, the optimal soil-to-solution ratio, the appropriate adsorption and desorption equilibration times and the stability of the test item in $CaCl_2$ solution (without soil) were determined.

Freundlich isotherm tests were performed with all soils using the indirect and parallel methods. A soil:solution ratio of 1:10, and 24 hours equilibrium time for adsorption and desorption were used for all soils. Solutions of the radiolabelled test substance in acetonitrile were prepared and diluted with 0.01 M $CaCl_2$, such that test substance application achieved concentrations of 4.459-4.867 (two application solutions made for highest concentration as initial solution volume was not sufficient for all soils), 0.441, 0.047 and 0.0097 mg/L. The volume of organic solvent added was not reported (but assumed to be within acceptable limits).

2 g dry weight of soil was transferred into centrifuge tubes and 20 mL of 0.01 M $CaCl_2$ containing test substance were applied to the samples. The samples were then shaken for 24 hours at 20 ± 1°C in the dark. For the desorption step, 20 mL volume of fresh 0.01 M $CaCl_2$ solution were added and the samples were shaken for a further 24 hours under the same conditions. After the adsorption step, a representative supernatant from each soil was subjected to chromatographic analysis by TLC.

Adsorption phase

Parameter		**Description**
Soil condition		Soils were air-dried and sieved to 2 mm
Soil sample weight		2 g (dry weight)
Equilibration solution		No pre-equilibration conducted
Control (preliminary experiment)		No soil (test item in 0.01M $CaCl_2$ only)
Test item concentration	Nominal application rates	0.01, 0.05, 0.5 and 5 mg/L (4 concentrations)
	Analytically (LSC) measured concentrations	Measured concentrations (LSC) in test solution: 4.459-4.867 (two application solutions made for highest concentration as initial solution volume was not sufficient for all soils), 0.441, 0.047 and 0.0097 mg/L
Identity and concentration of co-solvent		Not reported for dosing stock Study media – 0.01M calcium chloride
Soil: Solution ratio		1:10

Parameter		Description
Number of replicates	Control	Duplicate
	Treatments	Duplicate
Equilibration conditions	Time	24 hrs
	Temperature	20±1°C
	Dark	In the dark
	Shaking method	Overhead shaker
Method of separation of supernatant		Centrifugation
Centrifugation	Speed	Not reported for main test, preliminary test centrifuged at >17000 g
	Duration	Not reported for main test, preliminary test centrifuged for 20 mins
	Method of separating supernatant	Supernatant was carefully decanted.

Desorption phase

Parameter		Description
Soil samples from adsorption phase used		Yes
Amount of test item present in the adsorbed state/adsorbed amount (mg a.i./kg soil)		The amounts of test item adsorbed to soil after adsorption ranged from 49.76 to 95.90% (reported for definitive test). See OECD 106 Checklist below for further details for each soil.
Number of desorption cycles		1
Equilibrium solution and quantity used per treatment for desorption		The decanted solution was replaced by fresh aqueous 0.01 M $CaCl_2$ solution
Soil: Solution ratio		1:10
Number of replicates	Control	Duplicate
	Treatments	Duplicate
Desorption Equilibration conditions	Time	24 hrs
	Temperature	20±1°C
	Dark	In the dark
	Shaking method	Overhead shaker
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	Not reported for main test, preliminary test centrifuged at >17000 g
	Duration	Not reported for main test, preliminary test centrifuged for 20 mins
	Method of separating supernatant	Supernatant was carefully decanted.

2. Analytical Procedures

After each adsorption and desorption step the aqueous supernatant was separated from the soil by centrifugation and the amount of spiroxamine in the supernatants was analysed by liquid scintillation counting (LSC). An aliquot of supernatant from the highest concentration (and 0.5 mg/L nominal for Laacherhof AXXa 30-60cm) for each soil was also analysed by normal-phase TLC using a mobile phase of acetonitrile/water/triethylamine (80:18:0.5, v/v/v) or chloroform/methanol/methylethylketone (6:2:1, v/v) and a Bio Imaging analyser, quantified with BioAnalyze Version 3.0.

The partition of the test item in the adsorption and desorption batch equilibrium experiment was determined based on the radioactivity content in the supernatant only (i.e. the indirect method). After the

desorption step, the soil was air-dried and the radioactivity content determined by combustion/LSC to establish the material balance.

Parental mass balance was determined >90% by TLC analysis of the supernatant.

Adsorption and desorption isotherms were calculated by linear regression analysis of the adsorption or desorption data according to the Freundlich equation.

Additional information on stability of spiroxamine in $CaCl_2$ solution (without soil) was determined in the preliminary tests, with >99% of radioactivity confirmed as spiroxamine using the same TLC conditions as described above after 9 days in solution.

The limit of detection (LOD) was not specified in the report; however, the concentrations of the test substance used are sufficient to allow adequately accurate measurements of levels of the test substance.

II. Results and Discussion

A. Results of preliminary tests

Soil:solution ratios of 1:10 were also determined for both adsorption and desorption phases with an equilibration time of 24 hrs.

Shaking an aqueous solution of the test substance in the absence of soil gave a recovery of 94.3 to 97.1% AR indicating that adsorption to the test vessel was not occurring.

Stability of spiroxamine in $CaCl_2$ solution (without soil) was monitored in a preliminary test, giving >99% as unchanged spiroxamine.

B. Transformation of test substance

Stability of spiroxamine during the isotherm determinations was monitored by TLC analysis of the supernatant for all soils, showing >90% AR identified as spiroxamine.

C. Findings

Mass balances ranged from 81.8-98.8% AR. The amount of radioactivity adsorbed on the soil at adsorption equilibrium ranged from 25.0-93.6% AR.

Table CA 7.1.3.1.1-3: Concentrations at adsorption equilibrium and recovery of radioactivity (mean values) for spiroxamine

Soil	Concentration initial (mg/L)	Concentration supernatant (mg/L)	Concentration soil (mg/kg)	Adsorption percentage (%)	Mass balance (% AR) [A]
Laacherhof A XXa, 0-30 cm (Loamy sand)	0.010	0.002	0.080	82.44	87.68
	0.047	0.010	0.365	78.58	94.34
	0.441	0.145	2.960	67.11	98.12
	4.460	2.200	22.595	50.67	**97.88**
Standard soil 2.1 (Sand)	0.010	0.004	0.060	60.70	86.93
	0.047	0.021	0.260	55.29	89.06
	0.441	0.267	1.740	39.45	97.87
	4.460	3.347	11.125	24.95	**98.75**
Hofchen "im Tal" (Silt loam)	0.010	0.001	0.090	93.61	87.27
	0.047	0.004	0.435	92.69	90.82
	0.441	0.051	3.895	88.38	95.74
	4.460	0.809	36.505	81.86	**95.68**
Clay soil LUFA Speyer (Silty clay)	0.010	0.001	0.090	90.38	81.82
	0.047	0.006	0.410	88.22	84.18
	0.441	0.073	3.685	83.56	92.59
	4.460	0.820	36.395	81.61	**91.11**
Laacherhof A XXa, 30-60 cm (Loamy sand)	0.010	0.004	0.065	66.27	88.24
	0.047	0.020	0.270	57.53	94.11
	0.441	0.236	2.045	46.43	98.45
	4.868	2.982	18.855	38.74	**94.51**

Bold values used to calculate the percent loss

A Mass balance (determined as total radioactivity) values quoted are after the desorption step

For the four topsoils (Laacherhof AXXa 0-30cm, Standard soil 2.1, Hofchen "im Tal" and Clay soil LUFA Speyer), Freundlich adsorption coefficients (K_f) ranged from 4.61-44.98 L/kg. When normalised to organic carbon, Freundlich adsorption coefficients (K_{foc}) ranged from 658.8-6417 L/kg, indicating that spiroxamine is likely to exhibit low mobility in soil. The Freundlich exponent 1/n values ranged from 0.768-0.885, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

For the subsoil (Laacherhof AXXa, 30-60 cm), a Freundlich adsorption coefficient of (K_f) 7.25 L/kg was calculated, with Freundlich adsorption coefficient (K_{foc}) of 2415.3 L/kg when normalised to organic carbon. The Freundlich exponent 1/n values of 0.833 indicates that the concentration of the test item affects its adsorption behaviour in the examined concentration range. In comparison to the topsoil equivalent, while overall sorption is lower, it is greater than expected indicating sorption may not be solely related to organic carbon content.

Table CA 7.1.3.1.1-4: Freundlich adsorption coefficients for spiroxamine

Soil	Laacherhof A XX a, 0-30 cm (Loamy sand)	Standard soil 2.1 (Sand)	Hofchen "im Tal" (Silt loam)	Clay soil LUFA Speyer (Silty clay)	Laacherhof A XX a, 30-60 cm (Loamy sand)
OC (%)	1.8	0.7	2.4	0.64	0.3
pH ($CaCl_2$)	6.4	5.3	5.8	7.4	6.3
K_f (L/kg)	12.78	4.61	44.98	41.07	7.25
K_{foc} (L/kg)	709.9	658.8	1874.0	6417.1	2415.3
1/n	0.785	0.768	0.831	0.885	0.833
R^2	1.000	0.999	1.000	1.000	1.000

D. Evaluation of the Data according to EFSA Evaluators Checklist

The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet provided by EFSA (summarised in Table CA 7.1.3.1.1-6). The concentrations in the supernatant and the soil as given in the report were used as input data. Individual replicate concentrations were used in the calculation. Individual replicate concentrations were not available, so mean values as given in the report have been used in the calculation and as the adsorption isotherms were conducted with only 4 concentrations the confidence intervals were calculated by added formulae.

Relevant quality checks were performed to evaluate the acceptability of the study. For all soils these checks confirmed that the mass balance was generally acceptable, as although the lowest concentration samples had mass balances <90% the magnitude of difference from 90% was small and not considered to affect the outcome of the study (full range of mass balance was 81.8-98.8%). The percentage adsorption was acceptable for all soils (24.41 - 93.71%). The LOQ was not reported, however, the analytical method (LSC) ought to have been acceptable assuming reasonable volumes used for counting. The validity of using the indirect method, based on a Kd * soil/solution ratio > 0.3, was confirmed. The degree of sorption was sufficient, and the mass balance at the highest concentration was corrected for stabilityand used to calculate the 'f' value following EFSA (2017).

Table CA 7.1.3.1.1-5: Calculation of 'f' values for checklist

Soil	Mass balance at highest concentration (% AR)	Stability (%)[1]	'f' value for input into checklist (%)
Laacherhof AXXa (0-30 cm)	97.88	90	11.91
Standard soil 2.1	98.75	90	11.12
Hoefchen "im Tal"	95.68	90	13.89
Clay soil LUFA Speyer	99.11	90	18.0
Laacherhof AXXa (30-60 cm)	94.51	90	14.94

[1] As reported from chromatographic analysis of adsorption supernatant

The K_{fE} / K_f ratio ranges give maxima above 1.2 for all soils and the subsoil. However, sorption is high for all tested soils/subsoils, with both δ and K_d x soil/solution ratio passing with good margins. Furthermore, the ratio of 1.2 is only an initial proposal, it is considered that this does not change the overall outcome of the study. The R^2 of the standard linear regressions ranged from 0.998 to 1.000 and the visual fit of both the standard regression and the residual plots were good.

Table CA 7.1.3.1.1-6: Results of the EFSA 106 data evaluation

Soil	Units	Quality criteria	Laacherhof AXXa (0-30cm)	Standard soil 2.1	Hofchen "im Tal"	LUFA Speyer	Laacherhof AXXa (30-60cm)
Adsorption method (direct/indirect)	-		Indirect	Indirect	Indirect	Indirect	Indirect
Soil : solution ratio	g/mL		1:10	1:10	1:10	1:10	1:10
Mass balance of ^{14}C (at all tested concentrations)	%	>90%	87.68-98.12	86.93-98.75	87.27-95.68	81.82-92.59	88.24-98.45
f – due to loss processes*			11.91	11.12	13.89	18.0	14.94
Adsorbed percentage (δ)	%	>20%	50.17-82.21	24.41-60.02	81.03-93.71	83.08-90.45	38.54-66.05
K_d x (soil:solution ratio)		>0.3	1.01-4.62	0.32-1.50	4.27-14.90	4.91-9.47	0.63-1.95

Soil	Units	Quality criteria	Laacher-hof AXXa (0-30cm)	Standard soil 2.1	Hofchen "im Tal"	LUFA Speyer	Laacher-hof AXXa (30-60cm)
K_{fE} / K_f	-	<1.2	1.165-1.307	1.238-1.804	1.172-1.204	1.252-1.276	1.310-1.628
$^{ads}K_F$	L/kg	*	12.803	4.613	45.184	44.702	7.250
(95% confidence interval)**			11.51-14.23	4.12-5.17	39.66-51.48	38.84-51.48	6.63-7.93
$^{ads}1/n$	-	*	0.788	0.773	0.834	0.903	0.837
(95% confidence interval)**			0.76-0.81	0.740-0.805	0.807-0.860	0.872-0.934	0.812-0.862
$^{Ads}R^2$	-	>0.975	0.999	0.998	0.999	0.999	0.999
$^{ads}K_{F,OC}$	L/kg	*	711.3	659	1882	6984.6	2416.5
Visual fit to Freundlich isotherm			Excellent	Excellent	Excellent	Excellent	Excellent
Residual plots randomly distributed			Good	Good	Good	Good	Good

* As no extraction of soil was carried out these values are based on the worst-case total recovered radioactivity recovered (see bold values in Table CA 7.1.3.1.1-3) corrected for stability, see Table CA 7.1.3.1.1-5

** Confidence intervals provided in the EFSA Excel spreadsheet were re-calculated manually as available input data consisted of only 4 concentration levels

Figure 7.1.3.1.1-2: Freundlich Isotherms of spiroxamine on soil Laacher Hof AXXa (0-30cm)

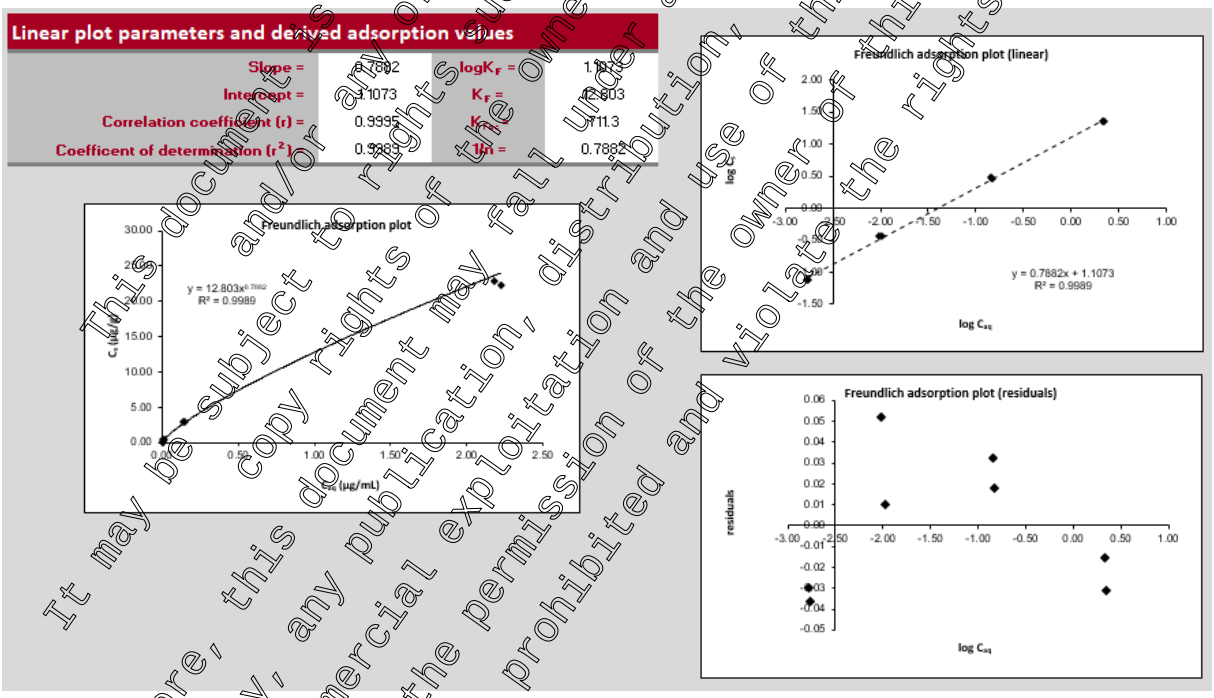


Figure 7.1.3.1.1-3: Freundlich Isotherms of spiroxamine on soil Standard Soil 2.1

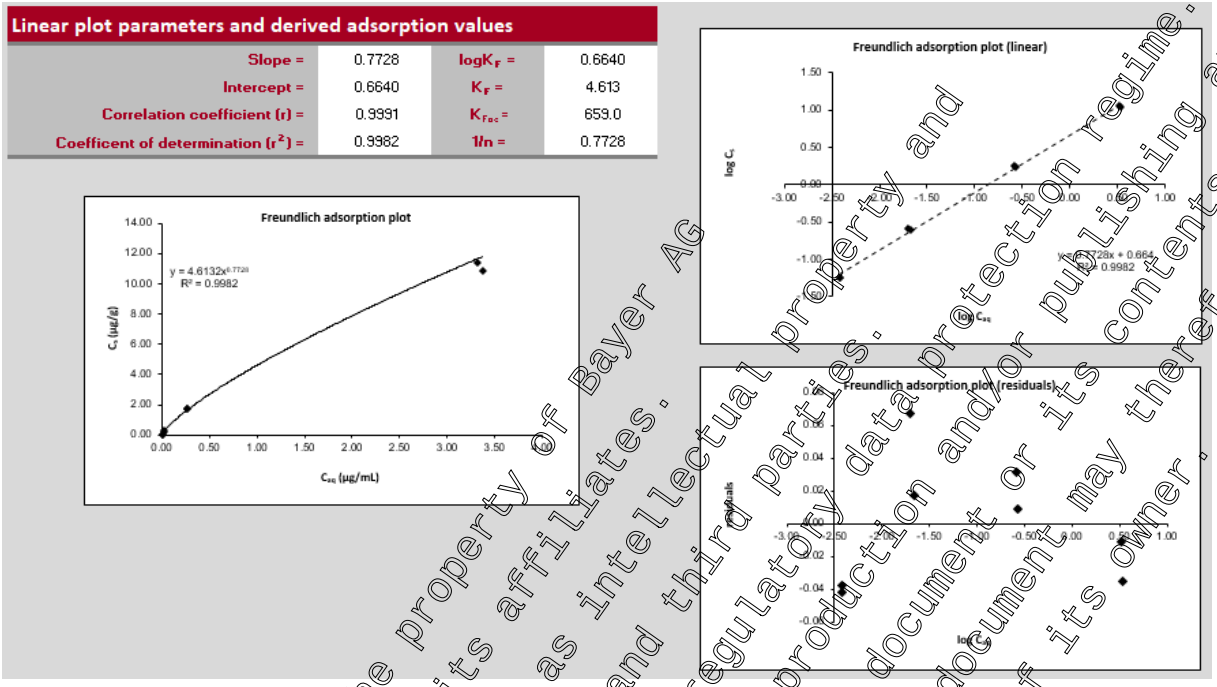


Figure 7.1.3.1.1-4: Freundlich Isotherms of spiroxamine on soil Hoefchen “im Tal”

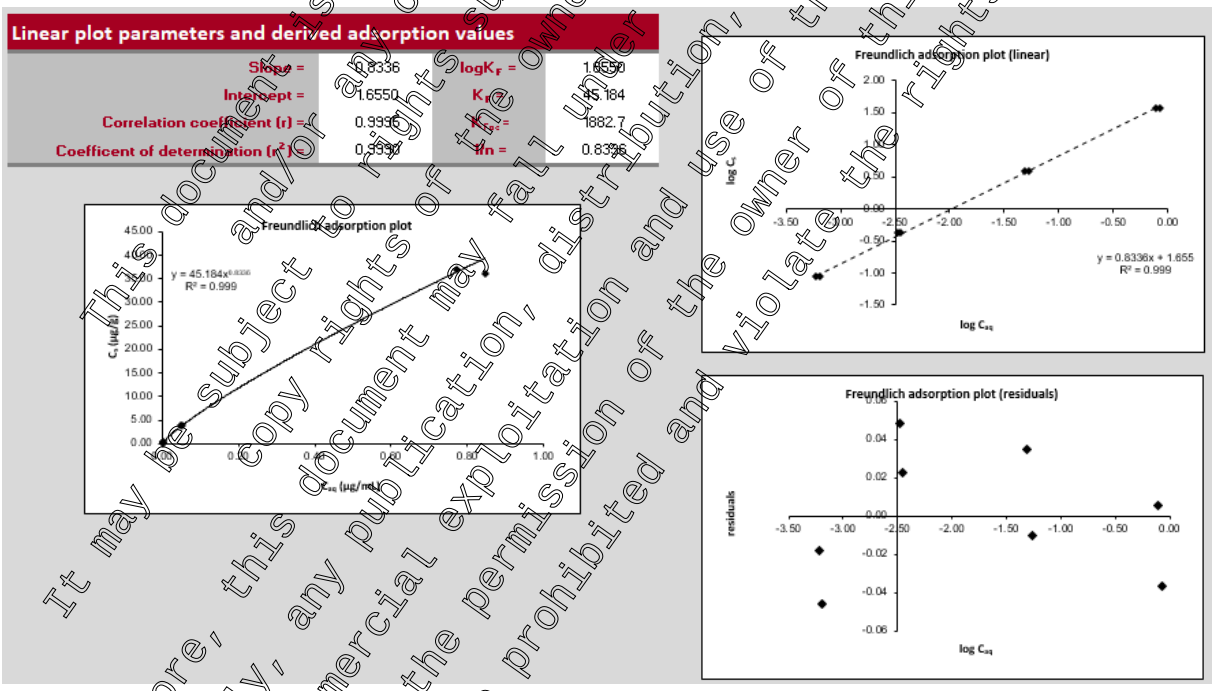


Figure 7.1.3.1.1-5: Freundlich Isotherms of spiroxamine on soil LUFA Speyer

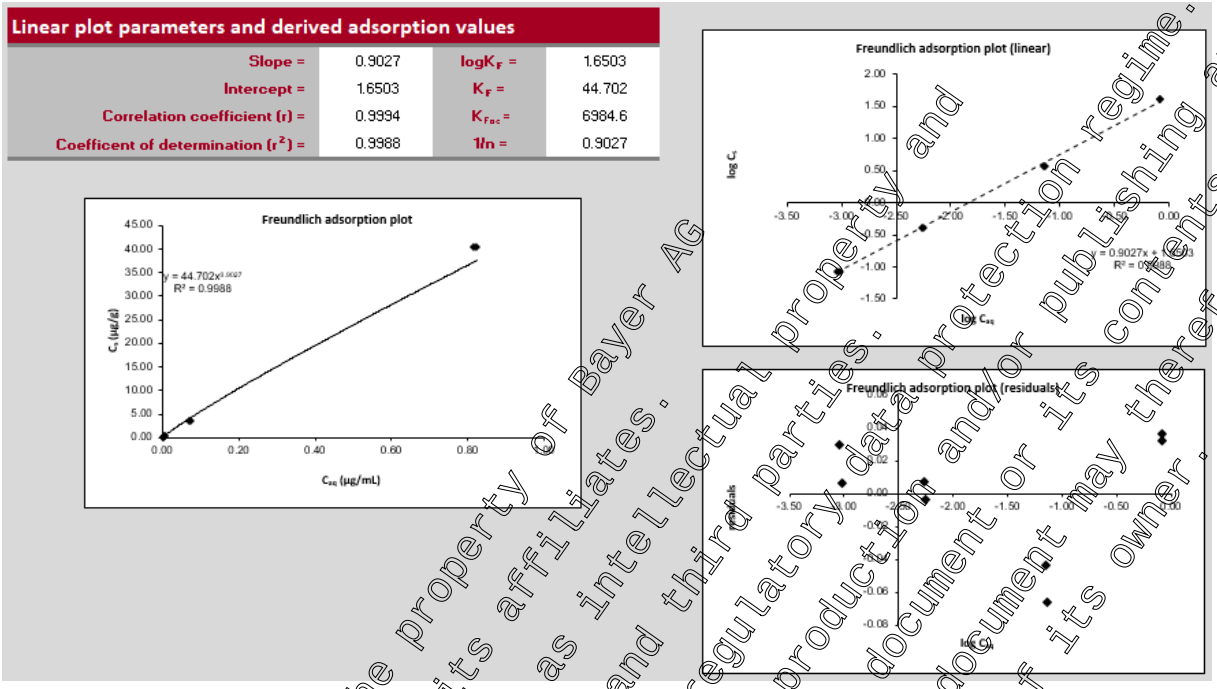
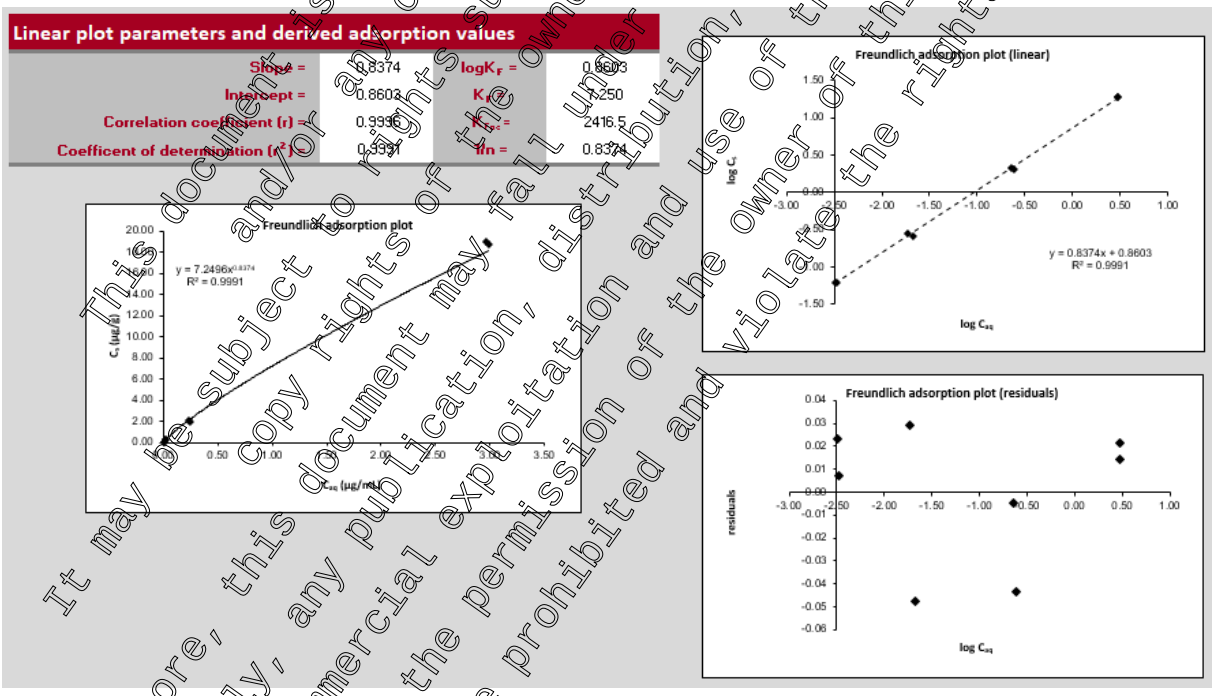


Figure 7.1.3.1.1-6: Freundlich Isotherms of spiroxamine on soil Laacherhof AXXa (30-60cm)



Overall the study has been conducted to a good standard, although certain criteria that might be expected in a modern study have not been reported this does not impact study reliability.

III. Conclusions

Freundlich adsorption coefficients (Kf) ranged from 4.61-44.98 L/kg (n=4). When normalised to organic carbon, Freundlich adsorption coefficients (Kfoc) ranged from 658.8-6417.1 L/kg, indicating that spiroxamine exhibits low mobility in soil according to the McCall mobility classification (McCall et al 1981[7]). The 1/n values ranged from 0.768-0.885, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

Adsorption was shown to be generally correlated with organic carbon content, there was no clear correlation with the pH of the soils.

The OECD 106 Checklist (v2) was used to evaluate the study. The evaluation confirmed acceptability for all soils according to the quality criteria and therefore acceptable for regulatory use. A number of minor differences in the derived K_{FOC} values were noted but this was attributed to rounding errors and for consistency the endpoints determined in the study report are used and considered to have no impact on the risk assessment.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to study guideline(s) OECD 106 (required guideline). Although the study has some deficiencies when compared to the EFSA OECD 106 checklist, the study is considered valid to assess the adsorption and desorption characteristics of the spiroxamine in soil.

Data Point:	KCA 7.1.3.1.1/02
Report Author:	[redacted]
Report Year:	1999
Report Title:	Adsorption/desorption of KWG 4168 on five American soils
Report No:	PF4135
Document No:	M-006186-02-1
Guideline(s) followed in study:	EPA, Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmetal Fate § 163-1
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption and desorption of [cyclohexyl-1-^{14}C]- and [1,3-dioxolan-4-^{14}C]-spiroxamine on five North American soils was studied using the batch equilibrium method with a tiered approach. [cyclohexyl-1-^{14}C]-spiroxamine was used for determination of equilibrium time, whilst all other tests were performed with [1,3-dioxolan-4-^{14}C]-spiroxamine.

Parameter:	**Soil**				
Soil Designation	**Vero Beach (Sand)**	**Grape Vine-yard (Sandy loam)**	**Howe (Sandy loam)**	**Wolf Ranch (Loam)**	**Stanley (Silty clay)**
Textural Classification (USDA)	Sand	Sandy loam	Sandy loam	Loam	Silty clay
pH ($CaCl_2$)	6.7	5.8	6.7	7.8	5.1
Organic carbon (%)	0.2	0.45	1.12	0.97	1.05

Preliminary tests were performed to determine the appropriate soil:solution ratios and equilibrium time. In this study preliminary tests did not investigate adsorption of the test substance to the test vessels or stability of the test substance in calcium chloride solution, these aspects were addressed by reference to the study M-006189-01-2 (see CA 7.1.3.1.1/01).

Freundlich isotherm tests were performed with all soils using the indirect and parallel methods. A soil:solution ratio of 1:20, and 24 hours equilibrium time for adsorption and desorption were used for

all soils. For the adsorption step, appropriate solutions of the test substance were applied to the samples and the samples were shaken for 24 hours at 20 ± 1°C in the dark. Following the adsorption step, the samples were removed from the shaker, centrifuged, and the adsorption supernatants removed and analysed by normal phase TLC and LSC. For the desorption step, a 20 mL volume of fresh 0.01 M $CaCl_2$ solution was added and the samples were shaken for a further 24 hours at 20 ± 1°C in the dark. Following the desorption step, the samples were removed from the shaker, centrifuged, and the desorption supernatants removed. The desorption supernatants were analysed by LSC and the highest concentration solutions of each test substance were also analysed by normal phase TLC. The soil samples were combusted in a sample oxidiser and analysed by LSC to determine the radioactivity present.

Mass balances ranged from 94.8-103.9% AR. The amount of radioactivity adsorbed on the soil at adsorption equilibrium ranged from 23.2-97.8% AR.

Freundlich adsorption coefficients (Kf) ranged from 8.55-892.59 L/kg. When normalised to organic carbon, Freundlich adsorption coefficients (Kfoc) ranged from 1347-85008 L/kg, indicating that spiroxamine exhibits low mobility in soil according to the McCall mobility classification (McCall et al 1981[7]). The 1/n values ranged from 1.01-1.06, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range. The OECD 106 Checklist (v2) was used to evaluate the study, however all final parameters used are taken from the study report. The evaluation confirmed acceptability for all soils according to the quality criteria and therefore acceptable for regulatory use.

Soil	Vero Beach (Sand)	Grape Vineyard (Sandy loam)	Howe (Sandy loam)	Wolf Ranch (Loam)	Stanley (Silty clay)
OC (%)	0.2	0.45	1.12	0.97	1.05
pH ($CaCl_2$)	6.70	5.80	6.70	7.80	5.10
K_f (L/kg)	8.55	14.47	15.09	381.65	892.59
K_{foc} (L/kg)	4276	3216	1347	39346	85008
1/n	1.06	1.05	1.02	1.02	1.01
R^2	0.996	0.998	0.999	1.000	1.000

I. Materials and Methods

A. Materials

1. Test Items

Test substance: [cyclohexyl-1-^{14}C]-spiroxamine

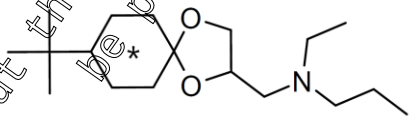
Lot/Batch No.: KMI2216

8686/B, 8686/23, 9569

Specific activity: 3.63 MBq/mg

Radiochemical purity: 98% (HPLC)

Structure:



* Denotes position of [^{14}C]-radiolabel

Test substance: [1,3-dioxolan-4-^{14}C]-spiroxamine

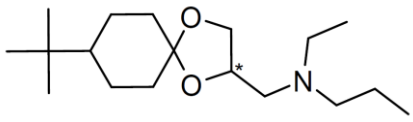
Lot/Batch No.: THS4429

837/1

Specific activity: 4.30 MBq/mg

Radiochemical purity: >99% (HPLC)

Structure:



* Indicates position of radiolabel

2. Test System (soil)

Five North American soils were used. The soils were collected from field sites in Vero Beach (Florida, USA), Grape Vineyard (California, USA), Howe (Indiana, USA), Wolf Ranch (California, USA) and Stanley (Kansas, USA), and varied in organic carbon, pH and clay content. After collection, soils were homogenized, air-dried, and passed through a 2 mm sieve.

Table CA 7.1.3.1.1-7: Physico-chemical properties of test soils

Parameter	Soil				
Soil Designation	**Vero Beach**	**Grape Vine-yard**	**Howe**	**Wolf Ranch**	**Stanley**
Geographic Location City	Vero Beach, Florida	California	Howe, Indiana	California	Stanley, Kansas
Country	USA	USA	USA	USA	USA
Textural Classification (USDA)	Sand	Sandy loam	Sandy loam	Loam	Silty clay
Sand (%)	98.7	64.0	65.7	29.7	17.0
Silt (%)	0.3	30.2	26.4	45.1	41.0
Clay (%)	1.0	5.9	7.9	25.2	42.0
pH in $CaCl_2$	6.7	5.8	6.7	7.8	5.1
Organic Matter (%) *	0.34	0.78	1.93	1.67	8.79
Organic carbon (%)	0.2	0.45	1.12	0.97	1.05
Cation Exchange Capacity (meq/100 g)	3.9	5.0	10.0	19.0	27.0

* Calculated by multiplying organic carbon content by 1.724 (not reported)

B. Study Design

1. Experimental Conditions

The test system for adsorption and desorption of spiroxamine on five North American soils consisted of Teflon centrifuge tubes with screw caps. All vessels used were cleaned with methanol and concentrated sulfuric acid and conditioned with non-radiolabelled test substance in order to minimize any adsorption to the vessels' walls.

In preliminary tests, the optimal soil-to-solution ratio and the appropriate adsorption and desorption equilibration times were determined.

Freundlich isotherm tests were performed with all soils using the indirect and parallel methods. A soil:solution ratio of 1:20, and 24 hours equilibrium time for adsorption and desorption were used for all soils. Solutions of the radiolabelled test substance in acetonitrile were prepared and diluted with 0.01 M $CaCl_2$, such that test substance application achieved concentrations of 5.443, 0.526, 0.055 and 0.012 mg/L. The organic solvent added was ≤1% of the $CaCl_2$ solution (v:v).

1 g dry weight of soil was transferred into centrifuge tubes and 20 mL of 0.01 M $CaCl_2$ containing test substance were applied to the samples. The samples were then shaken for 24 hours at 20 ± 1°C in the dark. For the desorption step, 20 mL volume of fresh 0.01 M $CaCl_2$ solution were added and the samples were shaken for a further 24 hours under the same conditions. Stability of the test substance was monitored during the isotherm determinations by TLC analysis of the supernatant after the adsorption step (each soil, top concentration only).

Adsorption phase

Parameter		Description
Soil condition		Soils were air-dried and sieved to 2 mm
Soil sample weight		1 g (dry weight)
Equilibration solution		No pre-equilibration conducted
Control (preliminary experiment)		Not conducted (reference made to M-006189-01-2, CA 7.1.3.1.1/01, where adsorption of the test substance to the test vessels shown to be minimal)
Test item concentration	Nominal application rates	0.01, 0.05, 0.5 and 5 mg/L (4 concentrations)
	Analytically (LSC) measured concentrations	Measured concentrations (LSC) in test solution: 5.443, 0.526, 0.055 and 0.012 mg/L
Identity and concentration of co-solvent		Dosing stock made up in acetonitrile Study media – 0.01M calcium chloride
Soil: Solution ratio		1:20
Number of replicates	Control	Not conducted
	Treatments	Duplicate
Equilibration conditions	Time	24 hrs
	Temperature	20±1°C
	Dark	In the dark
	Shaking method	Overhead shaker
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	Not reported
	Duration	Not reported
	Method of separating supernatant	Supernatant was carefully decanted.

Desorption phase

Parameter	Description
Soil samples from adsorption phase used	Yes
Amount of test item present in the adsorbed state/adsorbed amount (mg a.i./kg soil)	The amounts of test item adsorbed to soil after adsorption ranged from 23.2 to 97.8% (reported for definitive test). See OECD 106 Checklist below for further details for each soil.
Number of desorption cycles	3 (5 mg/L nominal concentration only) 1 (all other concentrations)
Equilibrium solution and quantity used per treatment for desorption	The decanted solution was replaced by fresh aqueous 0.01 M $CaCl_2$ solution
Soil: Solution ratio	1:20

Parameter		Description
Number of replicates	Control	Not conducted, reference to M-006189-01-2 where stability is demonstrated
	Treatments	Duplicate
Desorption Equilibration conditions	Time	24 hrs
	Temperature	20±1°C
	Dark	In the dark
	Shaking method	Overhead shaker
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	Not reported
	Duration	Not reported
	Method of separating supernatant	Supernatant was carefully decanted.

2. Analytical Procedures

After each adsorption and desorption step the aqueous supernatant was separated from the soil by centrifugation and the amount of spiroxamine in the supernatants was analysed by liquid scintillation counting (LSC). An aliquot of supernatant from the highest concentration for each soil was also analysed by normal-phase TLC using a mobile phase of acetonitrile/water/ammonium hydroxide (80:18:2, v/v/v) and a Bio Imaging analyser, quantified with Tina version 2.08a.

The partition of the test item in the adsorption and desorption batch equilibrium experiment was determined based on the radioactivity content in the supernatant only (i.e. the indirect method). After the desorption step, the soil was air-dried and the radioactivity content determined by combustion/LSC to establish the material balance.

Stability of parent test substance was determined as 97.0, 97.1, 96.6, 80.4 and 61.3% in Vero Beach, Grape Vineyard, Howe, Wolf Ranch and Stanley soils, respectively, by TLC analysis of the supernatant after the adsorption step.

Adsorption and desorption isotherms were calculated by linear regression analysis of the adsorption or desorption data according to the Freundlich equation.

The limit of detection (LOD) was not specified in the report, however, the concentrations of the test substance used are sufficient to allow adequately accurate measurements of levels of the test substance.

H. Results and Discussion

A. Results of preliminary tests

Adsorption of the test substance to the test vessels was not assessed in this study, however, the report made reference to study M-006189-01-2 (see CA 7.1.3.1.1/01), where adsorption of the test substance to the test vessels was shown to be minimal and stability of the test substance in calcium chloride solution (without soil) was shown to be stable over a period of 9 days.

Initially in preliminary tests, a soil-to-solution ratio of 1:10 was selected for the Grape Vineyard, Howe, Wolf Ranch and Stanley soils and a ratio of 1:2 for the Vero Beach soil by reference to the study M-006189-04-2 (see CA 7.1.3.1.1/01). This led to percentages adsorbed of 86.5-94.9, 71.3-94.6, 76.2-95.7, 98.3-99.0 and 99.3-99.6 for the Vero Beach, Grape Vineyard, Howe, Wolf Ranch and Stanley soils, respectively. In light of this, a soil to solution ratio of 1:20 was chosen for all soils. The equilibration time was determined using a soil to solution ratio of 1:20 for all soils and monitoring adsorption over 72 hrs.

B. Transformation of test substance

Stability of the test substance was monitored during the isotherm determinations by TLC analysis of the

supernatant after the adsorption step (each soil, top concentration only). The stability was 97.0, 97.1, 96.6, 80.4 and 61.3% for the Vero beach, Grape Vineyard, Howe, Wolf Ranch and Stanley soils, respectively.

C. Findings

Mass balances ranged from 94.8-103.9% AR. The amount of radioactivity adsorbed on the soil at adsorption equilibrium ranged from 23.2-97.8% AR. For the controls without soil, no sorption to the test vessels was measured.

Table CA 7.1.3.1.1-8: Concentrations at adsorption equilibrium and recovery of radioactivity (mean values) for spiroxamine

Soil	Concentration initial (mg/L)	Concentration supernatant (mg/L)	Concentration soil (mg/kg)	Adsorption percentage (δ)	Mass balance (%)
Vero Beach (Sand)	0.012	0.009	0.060	27.43	97.53
	0.055	0.042	0.260	23.94	99.96
	0.526	0.404	2.440	23.21	103.41
	5.443	3.461	39.640	36.42	**101.57**
Grape Vinyard (Sandy loam)	0.012	0.007	0.090	38.47	96.05
	0.055	0.036	0.390	35.64	100.28
	0.526	0.335	3.820	36.33	103.90
	5.443	2.897	50.910	46.77	**101.64**
Howe (Sandy loam)	0.012	0.007	0.090	40.56	98.28
	0.055	0.032	0.460	42.29	99.27
	0.526	0.327	3.980	37.89	103.18
	5.443	2.928	50.310	46.22	**101.08**
Wolf Ranch (Loam)	0.012	0.0007[A]	0.220	94.20	95.79
	0.055	0.003	1.040	94.50	97.27
	0.526	0.032	9.870	93.95	101.37
	5.443	0.262	103.620	95.19	**99.42**
Stanley (Silty clay)	0.012	0.0003[A]	0.230	97.60	94.79
	0.055	0.0013[A]	1.070	97.66	98.54
	0.526	0.013	10.260	97.66	101.42
	5.443	0.122	106.430	97.77	**102.08**

Bold values used to calculate the percent loss

A Due to rounding errors the ln c (µg/mL) values from Appendix 16/17 of the study report were used to calculate these values

Freundlich adsorption coefficients (K_f) ranged from 8.55-892.59 L/kg. When normalised to organic carbon, Freundlich adsorption coefficients (K_{foc}) ranged from 1347-85008 L/kg, indicating that spiroxamine is likely to exhibit low mobility in soil. The Freundlich exponent 1/n values ranged from 1.01-1.06, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

Table CA 7.1.3.1.1-9: Freundlich adsorption coefficients for spiroxamine

Soil	Vero Beach (Sand)	Grape Vineyard (Sandy loam)	Howe (Sandy loam)	Wolf Ranch (Loam)	Stanley (Silty clay)
OC (%)	0.2	0.45	1.12	0.97	1.05
pH ($CaCl_2$)	6.70	5.80	6.70	7.80	5.10
K_f (L/kg)	8.55	14.47	15.09	381.65	892.59
K_{foc} (L/kg)	4276	3216	1347	39346	85008
1/n	1.06	1.05	1.02	1.02	1.01
R^2	0.996	0.998	0.999	1.000	1.000

D. Evaluation of the Data according to EFSA Evaluators Checklist

The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet provided by EFSA (summarised in Table CA 7.1.3.1.1-11). The concentrations in the supernatant as given in the report were used as input data (note – concentration (µg/mL) calculated from provided LSC data). Individual replicate concentrations were used in the calculation.

The degree of sorption was sufficient, and the mass balance at the highest concentration was corrected for stability andthen used to calculate the 'f' value, following EFSA (2017).

Table CA 7.1.3.1.1-10: Calculation of 'f' values for checklist

Soil	Mass balance at highest concentration (% AR)	Stability (%)[1]	'f' value for input into checklist (%)
Vero beach	101.57	97.0	1.48
Grape vineyard	101.64	97.1	1.31
Howe	101.08	96.6	2.36
Wolf ranch	99.42	80.4	20.07
Stanley	102.08	61.3	37.42

[1]As reported from chromatographic analysis of adsorption supernatant.

The percentage adsorption was acceptable for all soils (23.31 - 97.77%). The LOQ was not reported, however, the analytical method (LSC) ought to have been acceptable assuming reasonable volumes used for counting. The validity of using the indirect method, based on a Kd * soil/solution ratio > 0.3, was confirmed. The degree of sorption was sufficient; however, in order to calculate a conservative "f" value, the worst-case total recovered radioactivity was used. The K_{fE} / K_f ratio ranges give ranges above 1.2 for the Wolf ranch and Stanley soils. However, sorption is high for all tested soils/subsoils, with both δ and K_d x soil/solution ratio passing with good margins. Furthermore, the ratio of 1.2 is only an initial proposal, it is considered that this does not change the overall outcome of the study. It is considered that all soils should be included in the regulatory dataset. The R^2 of the standard linear regressions ranged from 0.989 to 0.999 and the visual fit of both the standard regression and the residual plots were good.

Table CA 7.1.3.1.1-11: Results of the EFSA 106 data evaluation

Soil	Units	Quality criteria	Vero Beach (Sand)	Grape Vineyard (Sandy loam)	Howe (Sandy loam)	Wolf Ranch (Loam)	Stanley (Silty clay)
Adsorption method (direct/indirect)	-		Indirect	Indirect	Indirect	Indirect	Indirect
Soil : solution ratio	g/mL		1:20	1:20	1:20	1:20	1:20
Mass balance of ^{14}C (at all tested concentrations)	%	>90%	97.53-103.41	96.05-103.90	98.28-103.18	95.79-101.37	94.79-102.08
f – due to loss processes*			1.48	1.31	2.36	20.07	37.42
Adsorbed percentage (δ)	%	>20%	22.42-39.71	34.21-46.95	37.03-49.06	93.88-95.20	97.41-97.84
K_d x (soil:solution ratio)		>0.3	0.29-0.66	0.52-0.89	0.59-0.96	15.34-19.84	37.66-45.28
K_{fE} / K_f		<1.2	1.039-1.071	1.029-1.040	1.051-1.068	1.267-1.272	1.619-1.623
$^{ads}K_f$	L/kg	*	8.535	14.442	15.043	377.138	876.665
(95% confidence interval)**			6.12-11.90	11.61-17.96	12.31-18.38	318.84-446.09	743.51-1033.67
$^{ads}1/n$	-	*	1.047	1.043	1.014	1.020	1.008
(95% confidence interval)**			0.935-1.159	0.972-1.114	0.950-1.078	0.986-1.053	0.979-1.036

Soil	Units	Quality criteria	Vero Beach (Sand)	Grape Vineyard (Sandy loam)	Howe (Sandy loam)	Wolf Ranch (Loam)	Stanley (Silty clay)
$^{Ads}R^2$	-	>0.975	0.989	0.995	0.996	0.999	0.999
$^{ads}K_{F,OC}$	L/kg	*	4267.3	3209.3	1343.2	38880.2	83491.9
Visual fit to Freundlich isotherm			Good	Good	Good	Good	Good
Residual plots randomly distributed			Good	Good	Good	Good	Good

* As no extraction of soil was carried out, these values are based on the worst-case total recovered radioactivity recovered (see bold values in Table CA 7.1.3.1.1-8) corrected for stability, see Table CA 7.1.3.1.1-10

** Confidence intervals provided in the EFSA Excel spreadsheet were re-calculated manually as available input data consisted of only 4 concentration levels

Figure 7.1.3.1.1-7: Freundlich Isotherms of spiroxamine on Vero Beach soil

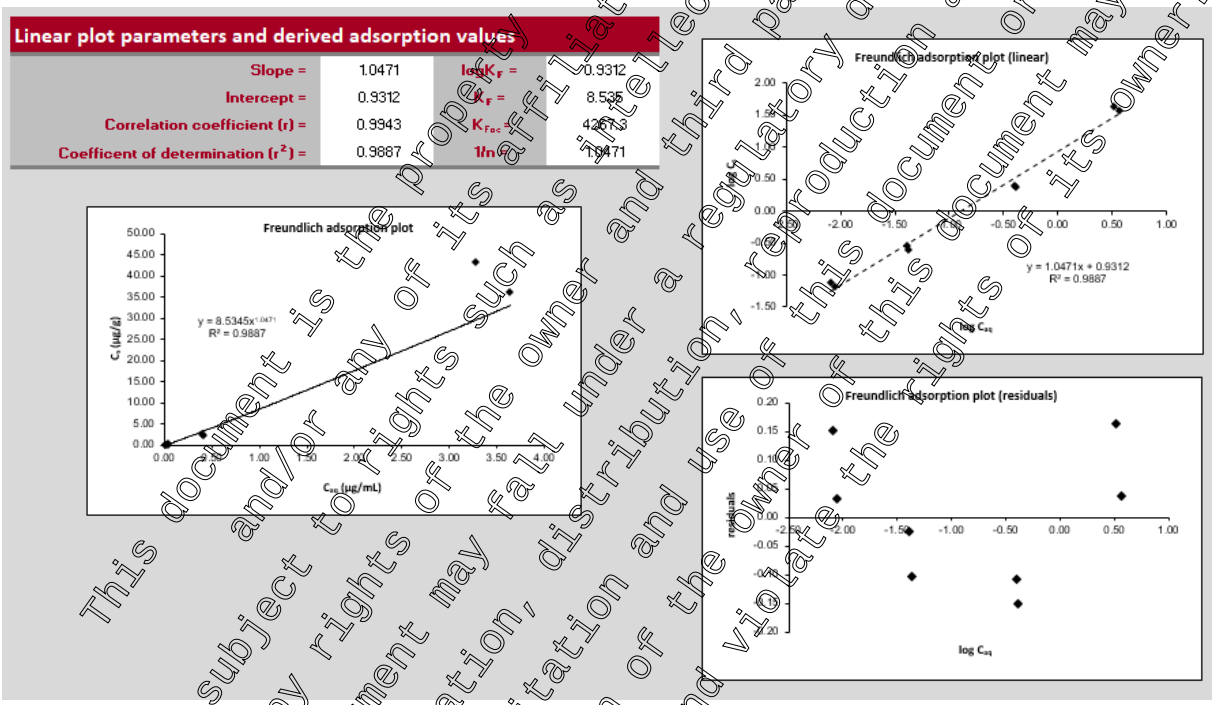


Figure 7.1.3.1.1-8: Freundlich Isotherms of spiroxamine on Grape Vineyard soil

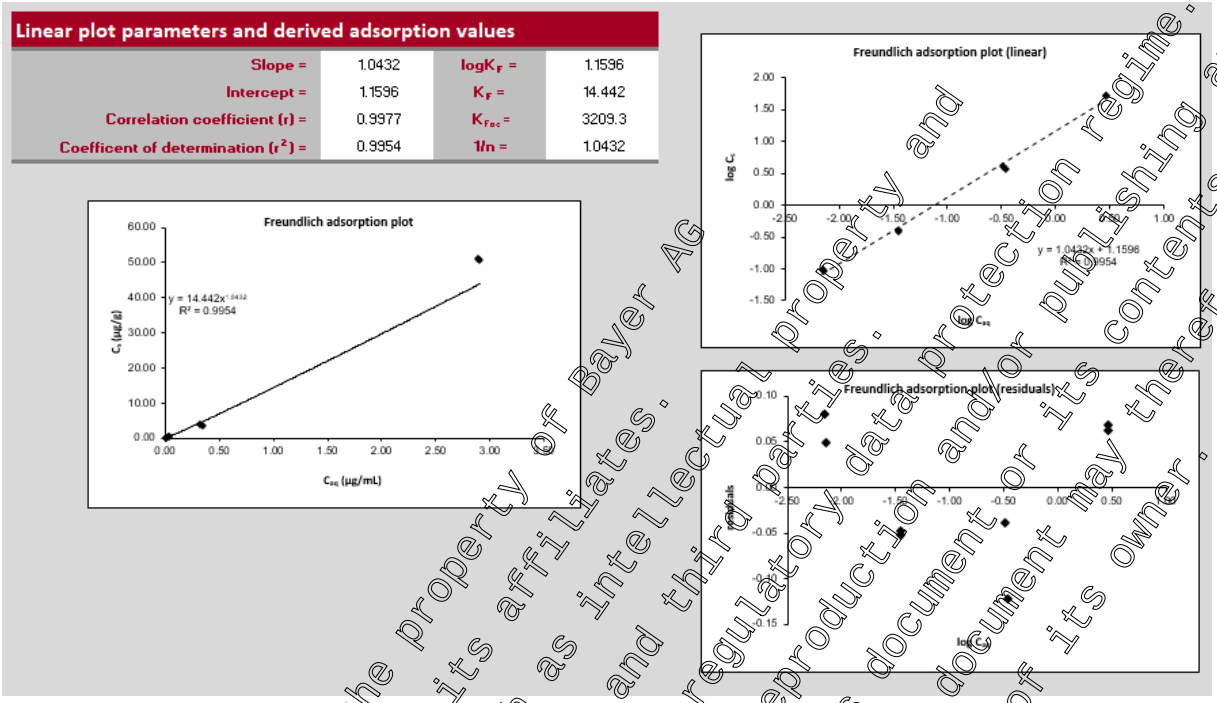


Figure 7.1.3.1.1-9: Freundlich Isotherms of spiroxamine on Howe soil

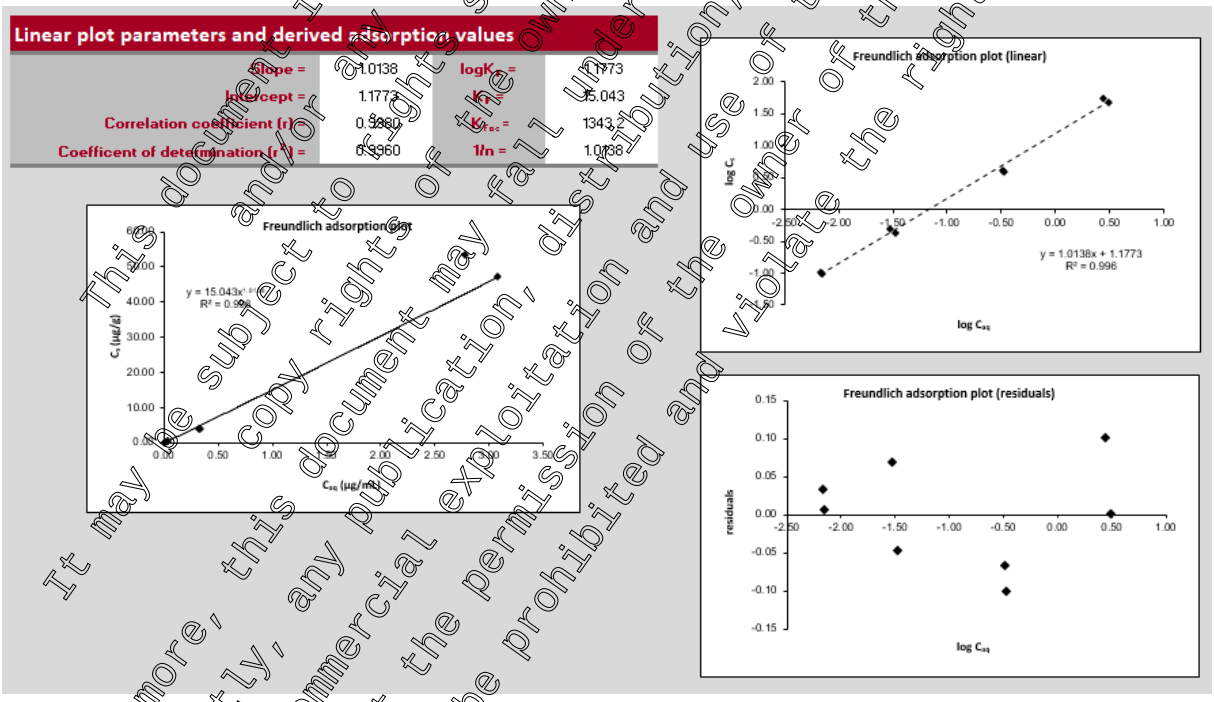


Figure 7.1.3.1.1-10: Freundlich Isotherms of spiroxamine on Wolf Ranch soil

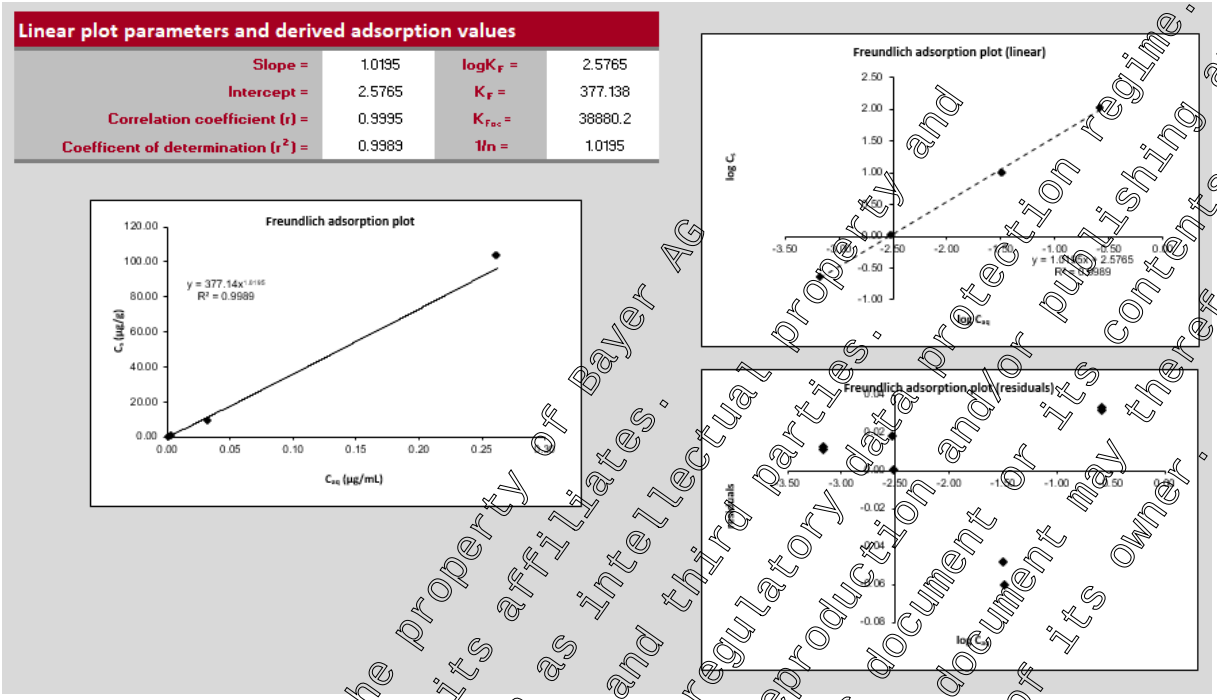
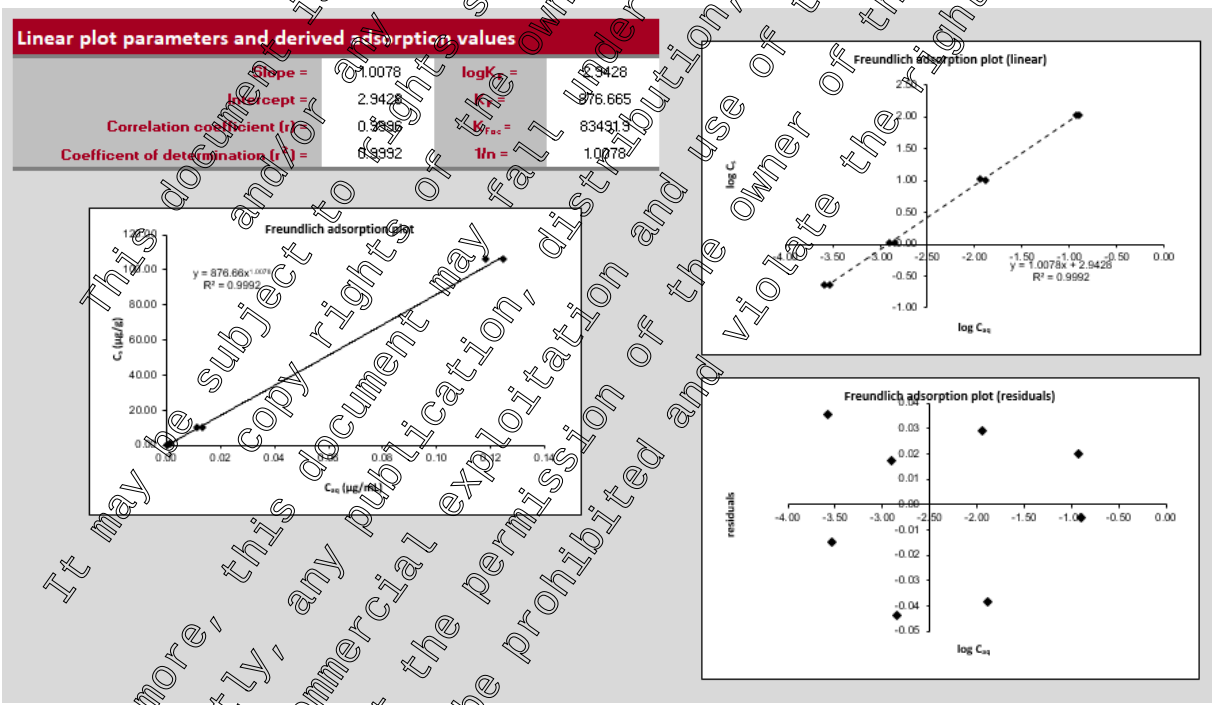


Figure 7.1.3.1.1-11: Freundlich Isotherms of spiroxamine on Stanley soil



Overall, the study was conducted to a reasonable standard and the study conclusions considered reliable.

III. Conclusions

Freundlich adsorption coefficients (Kf) ranged from 8.55-892.59 L/kg. When normalised to organic carbon, Freundlich adsorption coefficients (Kfoc) ranged from 1347-85008 L/kg, indicating that spiroxamine exhibits low mobility in soil according to the McCall mobility classification (McCall et al 1981[7]). The 1/n values ranged from 1.01-1.06, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

Adsorption was shown to be generally correlated with organic carbon content, and there was a weak correlation observed between pH and sorption, where Kfoc was lower as pH increased (r^2=0.153).

The OECD 106 Checklist (v2) was used to evaluate the study. The evaluation confirmed acceptability for all soils according to the quality criteria and therefore acceptable for regulatory use. A number of minor differences in the derived K_{FOC} values were noted but this was attributed to rounding errors and for consistency the endpoints determined in the study report are used and considered to have no impact on the risk assessment.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to study guideline(s) OECD 106 (required guideline). The study is considered valid to assess the adsorption and desorption characteristics of the spiroxamine in soil.

Although the study has a number of deviations from the current version of OECD 106 (2002) it is considered valid to assess the adsorption and desorption characteristics of the spiroxamine in soil.

Data Point:	KCA 7.1.3.1.1/03
Report Author:	[redacted]
Report Year:	2004
Report Title:	Partition coefficients of soil metabolites of spiroxamine
Report No:	MEF 04/370
Document No:	M-085664-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Supportive only

Executive Summary

This study is submitted as supporting information only (it was previously included in the RAR (2010), RAR (2017) and is therefore included for completeness).

The study involved the estimation of the partition coefficients of the main soil metabolites of spiroxamine by QSAR calculations. However, the study is no longer required or has since been superseded (based on real values) and consequently a full summary is not provided.

CA 7.1.3.1.2 Adsorption and desorption of metabolites, breakdown and reaction products

The adsorption and desorption of metabolites of spiroxamine have been investigated in three studies (KCA 7.1.3.1.2/01 to KCA 7.1.3.1.1/03) which were evaluated during the previous EU review. In addition:

- One further study providing supplemental information to the study KCA 7.1.3.1.2/03 (M-086089-01-1)
- One further study is being conducted to provide soil sorption properties for metabolite M06 (spiroxamine-acid) and will be provided as soon as possible.

The applicant considers the adsorption endpoints from batch equilibrium studies are appropriate for use

in risk assessment (in agreement with the previous EFSA conclusion: EFSA Journal (2010);8(10):1719).

Substance	Report reference	Document no.	Comment
Metabolite M01	KCA 7.1.3.1.2/01	M-006084-01-1	Submitted for first renewal of spiroxamine, 2010. Reviewed under UP. Considered valid and acceptable.
Metabolite M02	KCA 7.1.3.1.2/02	M-006086-01-1	
Metabolite M03	KCA 7.1.3.1.2/03	M-006089-01-1	
Metabolite M03	KCA 7.1.3.1.2/04	M-006087-01-1	Previously submitted but not evaluated. Included as supporting information.
Metabolite M06	KCA 7.1.3.1.2/05	TBA	New data not yet reviewed under UP.

The resulting soil sorption parameters for metabolites of spiroxamine are summarised below:

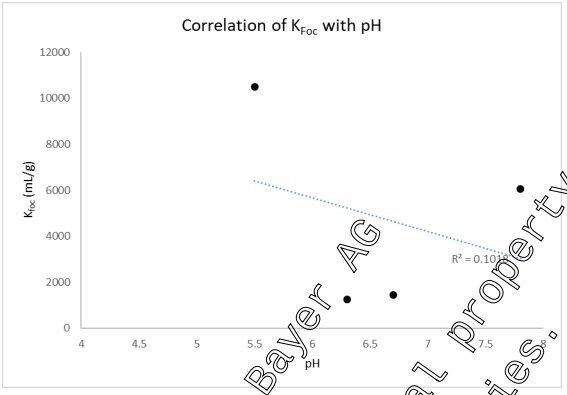
Table CA 7.1.3.1.2-1: Overall summary of Freundlich soil adsorption parameters for metabolites of spiroxamine

Study	Soil name	Soil properties			Soil sorption parameters		
		Texture	pH [A]	OC (%)	K_f (L/kg)	K_{foc} (L/kg)	1/n
Metabolite M01 KCA 7.1.3.1.2/01 M-006084-01-1	Vero Beach	Sand	6.3	0.32	3.96	1237	0.867
	Howe	Sandy loam	6.7	1.12	16.27	1453	0.813
	Wolf Ranch	Loam	7.8	0.97	58.81	6063	0.862
	Stanley	Silty clay	5.5	1.49	156.61	10511	0.852
						3271 (geo-mean, n=4)	**0.848 (mean, n=4)**
Metabolite M02 KCA 7.1.3.1.2/02 M-006086-01-1	Vero Beach	Sand	6.3	0.32	2.93	917	0.876
	Howe	Sandy loam	6.7	1.12	12.79	1142	0.827
	Wolf Ranch	Loam	7.8	0.97	54.39	5607	0.922
	Stanley	Silty clay	5.5	1.49	134.0	8994	0.886
						2695 (geo-mean, n=4)	**0.878 (mean, n=4)**
Metabolite M03 KCA 7.1.3.1.2/03 M-006089-01-1	Vero Beach	Sand	6.3	0.32	1.77	552	0.939
	Howe	Sandy loam	6.7	1.12	3.93	351	0.871
	Wolf Ranch	Loam	7.8	0.97	15.9	1641	0.890
	Stanley	Silty clay	5.5	1.49	371	24893	0.835
						1677 (geo-mean, n=4)	**0.900 (mean, n=4)**

A pH (0.01M $CaCl_2$) unless otherwise stated

The correlation for the spiroxamine metabolite M01 of soil sorption parameter K_{foc} with soil pH is presented below:

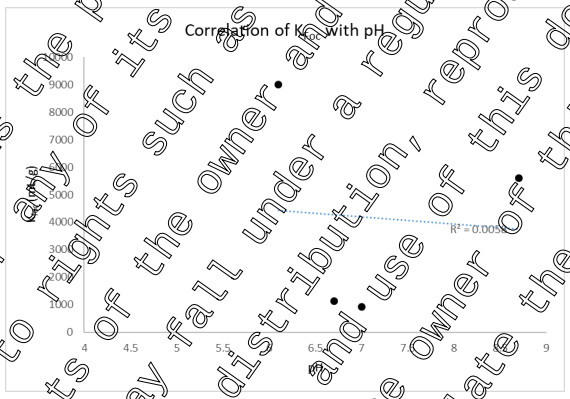
Figure 7.1.3.1.2-1: Correlation of K_{foc} with soil pH for spiroxamine metabolite M01



There was no strong correlation between soil sorption parameter K_{foc} with soil pH for M01 (spiroxamine-desethyl) (R^2=0.102), therefore no pH dependence was concluded.

The correlation for the spiroxamine metabolite M02 of soil sorption parameter K_{foc} with soil pH is presented below:

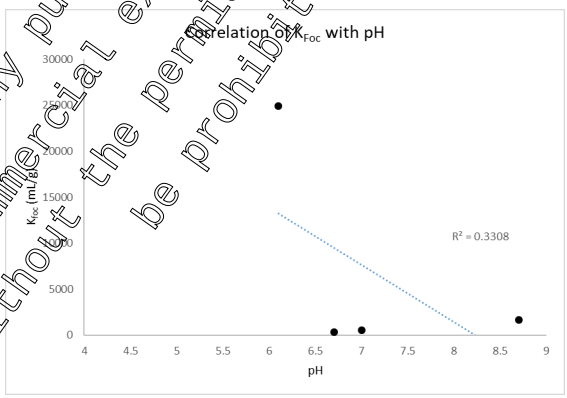
Figure 7.1.3.1.2-2: Correlation of K_{foc} with soil pH for spiroxamine metabolite M02



There was no strong correlation between soil sorption parameter K_{foc} with soil pH for M02 (spiroxamine-despropyl) (R^2=0.005), therefore no pH dependence was concluded.

The correlation for the spiroxamine metabolite M03 of soil sorption parameter K_{foc} with soil pH is presented below:

Figure 7.1.3.1.2-3: Correlation of K_{foc} with soil pH for spiroxamine metabolite M03



There was no strong correlation between soil sorption parameter K_{foc} with soil pH for M03 (spiroxamine -N-oxide) (R^2=0.331), therefore no pH dependence was concluded.

Existing studies, previously evaluated

Data Point:	KCA 7.1.3.1.2/01
Report Author:	[redacted]
Report Year:	1996
Report Title:	Adsorption/desorption of KWG 4557 on four different soils
Report No:	FM760
Document No:	M-006084-01-1
Guideline(s) followed in study:	USEPA (=EPA): Section N, 163-1; EU (=EEC): 95/36/EC of July 1995; OECD Guideline for Testing of Chemicals, No 106
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption and desorption of [cyclohexyl-1-^{14}C]-M01 (spiroxamine-desethyl) on four North American soils was studied using the batch equilibrium method with a tiered approach.

Parameter	Soil			
Soil Designation	**Vero beach**	**Howe**	**Wolf Ranch**	**Stanley**
Textural Classification (USDA)	Sand	Sandy loam	Loam	Silty clay loam
pH ($CaCl_2$)	6.3	6.7	7.8	5.5
Organic carbon (%)	0.32	1.12	0.97	1.49

Preliminary tests were performed to determine the appropriate soil:solution ratios and equilibrium time and to evaluate stability of the test substance in $CaCl_2$ solution with soil (under test conditions), potential for sorption of the test substance to test vessels and any effects of the inclusion of a biocide in solution.

In the preliminary tests, soil:solution ratios of 6:20 (Vero Beach) 3:20 (Howe) and 1:20 (Wolf Ranch and Stanley), equilibrium time for adsorption (1 hour for Vero Beach, Wolf Ranch and Stanley soils and 48 hours for Howe soil) and desorption (1 hour for Vero Beach, Wolf Ranch and Stanley soils and 48 hours for Howe soil) were determined. As part of the equilibrium time determination, the stability of the test substance was monitored and shown to be >99% for Vero Beach, Howe and Wolf Ranch soils after 72 hrs and *ca.* 65% for Stanley soil after 24 hrs. In control samples, shaking the test substance in $CaCl_2$ solution in the absence of soil gave a recovery of 99.1% AR after 72 hrs, indicating that adsorption to the test vessels was not occurring.

Freundlich isotherm tests were performed with all soils using the indirect and parallel methods. For the adsorption step, appropriate solutions of the test substance were applied to the samples and the samples were shaken for 1 or 48 hours at 22 ± 1°C. Following the adsorption step, the samples were removed from the shaker, centrifuged, and the adsorption supernatants removed. Chromatographic analysis of supernatants showed >85% AR present as unchanged test substance in after the adsorption step (i.e. 1 hour for Vero Beach, Wolf Ranch and Stanley soils and 48 hours for Howe soil).

For the desorption step, a 20 mL volume of fresh 0.01 M $CaCl_2$ solution was added and the samples were shaken for a further 1-48 hours at 22 ± 1°C. Following the desorption step, the samples were removed from the shaker, centrifuged, and the desorption supernatants removed. The desorption supernatants were analysed by LSC and the highest concentration solutions of each test substance were also

analysed by HPLC (with radiochemical detection). The soil samples were combusted in a sample oxidiser and analysed by LSC to determine the radioactivity present.

Mass balances ranged from 88.8-103.3% AR. The amount of radioactivity adsorbed on the soil at adsorption equilibrium ranged from 49.8-95.9% AR. After the adsorption phase, 49.76 – 70.81% AR was adsorbed to Vero beach soil, 66.95 – 89.09% AR adsorbed to Howe soil, 74.66 – 88.17% AR to Wolf ranch and 88.94 – 95.90% AR.

Freundlich adsorption coefficients (K_f) ranged from 3.96-156 L/kg. When normalised to organic carbon, Freundlich adsorption coefficients (K_{foc}) ranged from 1237-10511 L/kg, indicating that M01 (spiroxamine-desethyl) is likely to exhibit low mobility in soil. The 1/n values ranged from 0.813-0.867. Adsorption was shown to be correlated with organic carbon content, there was no clear correlation with the pH of the soils. The OECD 106 Checklist (v2) was used to evaluate the study, however all final parameters used are taken from the study report. The evaluation confirmed acceptability for all soils according to the quality criteria and therefore acceptable for regulatory use.

Soil	Vero Beach (Sand)	Howe (Sandy loam)	Wolf Ranch (Loam)	Stanley (Silty clay loam)
OC (%)	0.32	1.12	0.97	1.49
pH ($CaCl_2$)	6.3	6.7	7.8	5.5
K_f (L/kg)	3.96	16.27	58.81	156.61
K_{foc} (L/kg)	1237	1453	6063	10511
1/n	0.867	0.813	0.862	0.852
R^2	1.000	0.999	1.000	1.000

I. Materials and Methods

A. Materials

1. Test Items

Test substance: [cyclohexyl-1-^{14}C]-M01 (spiroxamine-desethyl)

* Indicates position of radiolabel

Specific activity: 1.48 MBq/mg

Radiochemical purity: >98%

Batch number: THS 4354

2. Test Soil

Four North American soils were used. The soils were collected from field sites in Vero Beach (Florida, USA), Howe (Indiana, USA), Wolf Ranch (California, USA) and Stanley (Kansas, USA), and varied in organic carbon, pH and clay content. After collection, soils were homogenized, air-dried, and passed through a 2 mm sieve.

Table CA 7.1.3.1.2-2: Physico-chemical properties of test soil

Parameter	Soil			
Soil Designation	**Vero beach**	**Howe**	**Wolf Ranch**	**Stanley**
Geographic Location				
City	Vero beach, Florida	Howe, Indiana	Fresno, California	Stanley, Kansas
Country	USA	USA	USA	USA
Textural Classification (USDA)	Sand	Sandy loam	Loam	Silty clay loam
Sand (%)	98.7	65.7	29.7	17.0
Silt (%)	0.3	26.4	45.1	41.0
Clay (%)	1.0	7.9	25.2	42.0
pH				
in H_2O	7.0	6.7	8.7	6.1
in $CaCl_2$	6.3	6.7	7.8	5.5
Organic Matter (%) *	0.55	1.93	1.67	2.57
Organic carbon (%)	0.32	1.12	0.97	1.49
Cation Exchange Capacity (meq/100 g)	n.a.	10	19	n.a.
Water Holding Capacity (%)				
maximum	n.a.	25.5	32.0	n.a.
at 1/3 bar	2.8	14.8	16.8	34.0

n.a.: not analysed
* Calculated by multiplying organic carbon content by 1.724 (not reported)

B. Study Design

1. Experimental conditions

The test system for adsorption and desorption of M01 (spiroxamine-desethyl) on four North American soils consisted of borosilicate glass centrifuge tubes with Teflon seals and screw caps.

In preliminary tests, the adsorption of the test item to the test system surface, the optimal soil-to-solution ratio, the appropriate adsorption and desorption equilibration times, the stability of the test item and the effects on inclusion of biocide in the solution were determined.

Freundlich isotherm tests were performed with all soils using the indirect and parallel methods. Soil:solution ratios of 6:20 (Vero Beach), 3:20 (Howe) and 1:20 (Wolf Ranch and Stanley), and equilibrium time for adsorption (1 hour for Vero Beach, Wolf Ranch and Stanley soils and 48 hours for Howe soil) and desorption (1 hour for Vero Beach, Wolf Ranch and Stanley soils and 48 hours for Howe soil) were used. Solutions of the radiolabelled test substance in acetonitrile were prepared and diluted with 0.01 M $CaCl_2$ such that test substance application achieved concentrations of 4.78, 0.47, 0.05 and 0.01 mg/L. Biocide was not included in isotherm solutions as it was demonstrated that this gave no improvement for stability purposes. The volume of organic solvent added was not clearly reported (but assumed to be within acceptable limits).

6 (Vero beach), 3 (Howe) or 1 g (Wolf ranch and Stanley) dry weight of soil were transferred into centrifuge tubes and 20 mL of 0.01 M $CaCl_2$ containing test substance were applied to the samples. The samples were then shaken for 1 (all soils except Howe) or 48 hours (Howe) at 22 ± 1°C in the dark. For the desorption step, 20 mL volume of fresh 0.01 M $CaCl_2$ solution were added and the samples were shaken for a further 1 or 48 hours under the same conditions. After the adsorption step, the supernatant was subjected to chromatographic analysis by HPLC (top concentration only).

Adsorption phase

Parameter		Description
Soil condition		Soils were air-dried and sieved to 2 mm
Soil sample weight		1 g (dry weight) per replicate for Wolf Ranch and Stanley soils 3 g (dry weight) per replicate for Howe soil 6 g (dry weight) per replicate for Vero Beach soil
Equilibration solution		No pre-equilibration conducted
Control (preliminary experiment)		No soil (test item in 0.01M $CaCl_2$ only)
Test item concentration	Nominal application rates	Nominal concentrations were not specified (assumed 0.01 to 5 mg/L)
	Analytically (LSC) measured concentrations	Measured concentrations (LSC) in test solution: 4.78, 0.47, 0.05 and 0.01 mg/L (4 concentrations)
Identity and concentration of co-solvent		Dosing stock made up in acetonitrile Study media - calcium chloride and $HgCl_2$ added as biocidal agent
Soil: Solution ratio		1:20 (Wolf Ranch and Stanley) 3:20 (Howe soil) 6:20 (Vero Beach)
Number of replicates	Control	Not stated
	Treatments	Duplicate
Equilibration conditions	Time	4 hrs (Vero Beach, Wolf Ranch & Stanley soils) 48 hrs (Howe soil)
	Temperature	22±1°C
	Dark	In the dark
	Shaking method	Overhead shaker
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	6000 rpm
	Duration	15 minutes
	Method of separating supernatant	Supernatant was carefully decanted.

Desorption phase

Parameter	Description
Soil samples from adsorption phase used	Yes
Amount of test item present in the adsorbed state/adsorbed amount (mg a.s./kg soil)	The amounts of test item adsorbed to soil after adsorption ranged from 49.76 to 95.90% (reported for definitive test). See OECD 106 Checklist below for further details for each soil.
Number of desorption cycles	1
Equilibrium solution and quantity used per treatment for desorption	The decanted solution was replaced by fresh aqueous 0.01 M $CaCl_2$ + $HgCl_2$ solution. Approximately 43-48 mL was used as equilibration solution.
Soil: Solution ratio	1:20 (Wolf Ranch and Stanley) 3:20 (Howe soil) 6:20 (Vero Beach)

Parameter		Description
Number of replicates	Control	Not stated
	Treatments	Duplicate
Desorption Equilibration conditions	Time	1 hrs (Vero Beach, Wolf Ranch & Stanley soils) 48 hrs (Howe soil)
	Temperature	22±1°C
	Dark	In the dark
	Shaking method	Overhead shaker
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	6000 rpm
	Duration	15 minutes
	Method of separating supernatant	Supernatant was carefully decanted.

2. Analytical Procedures

After each adsorption and desorption step the aqueous supernatant was separated from the soil by centrifugation and the amount of M01 (spiroxamine-desethyl) in the supernatants was quantified by liquid scintillation counting (LSC).

The partition of the test item in the adsorption and desorption batch equilibrium experiment was determined based on the radioactivity content in the supernatant only (i.e. the indirect method). After the desorption step, the soil was air-dried and the radioactivity content determined by combustion/LSC to establish the material balance.

Parental mass balance was determined >85% by HPLC/radio-detection analysis of the supernatant only.

Adsorption and desorption isotherms were calculated by linear regression analysis of the adsorption or desorption data according to the Freundlich equation.

Additional information on stability of M01 (spiroxamine-desethyl) was determined in the preliminary tests. HPLC analysis of the supernatant over duration of 24, 48 and 72 hrs showed for the Vero beach, Wolf ranch and Howe soils >99% AR present as M01 (spiroxamine-desethyl) after 72 hrs and for the Stanley soil *ca.* 65% AR present as M01 (spiroxamine-desethyl) after 24 hrs. The reverse phase High Performance Liquid Chromatography (HPLC) system used a Lichrospher 100 RP-18 column and gradient elution of acetonitrile + 0.5% triethylamine and water + 0.5% triethylamine and radio-detector.

The limit of detection (LOD) was not specified in the report, however, the concentrations of the test substance used are likely sufficient to allow adequately accurate measurements of levels of the test substance.

II. Results and Discussion

A. Results of Preliminary Tests

Soil:solution ratios of 6:20 (Vero Beach) 3:20 (Howe) and 1:20 (Wolf Ranch and Stanley), equilibrium time for adsorption (1 hour for Vero Beach, Wolf Ranch and Stanley soils and 48 hours for Howe soil) and desorption (1 hour for Vero Beach, Wolf Ranch and Stanley soils and 48 hours for Howe soil) were determined. Recovery from control samples without soil were >99.1% AR showing any sorption of the metabolite M01 (spiroxamine-desethyl) to the test vessels was minimal.

As part of the equilibrium time determination, stability of the test substance was monitored and shown to be >99% for Vero Beach, Howe and Wolf Ranch soils after 72 hrs and *ca.* 65% for Stanley soil after 24 hrs.

B. Transformation of test substance

Stability of M01 (spiroxamine-desethyl) was monitored during the isotherm determinations by HPLC

analysis of the supernatant after the adsorption step. The majority of the radioactivity in the supernatant was unchanged metabolite M01 (spiroxamine-desethyl) (only minimal details are provided in the report but a recovery of >85% over all soils was quoted).

C. Findings

Mass balances ranged from 88.8-103.3% AR. The amount of radioactivity adsorbed on the soil at adsorption equilibrium ranged from 49.8-95.9% AR.

Table CA 7.1.3.1.2-3: Concentrations at adsorption equilibrium and recovery of radioactivity (mean values) for M01 (spiroxamine-desethyl)

Soil	Concentration initial (mg/L) [A]	Concentration supernatant (mg/L) [A]	Concentration soil (mg/kg) [A]	Adsorption percentage (%)	Mass balance (% AR) [B]
Vero Beach (Sand)	0.011	0.003	0.027	70.81	103.25
	0.047	0.015	0.105	67.83	101.02
	0.474	0.179	0.983	62.31	100.50
	4.783	2.403	7.933	49.76	**99.76**
Howe (Sandy loam)	0.011	0.001	0.067	89.09	101.33
	0.047	0.007	0.267	85.77	93.84
	0.474	0.084	2.600	82.35	99.16
	4.783	1.581	21.35	66.95	**99.63**
Wolf Ranch (Loam)	0.011	0.002	0.090	88.17	96.91
	0.047	0.007	0.790	85.48	98.10
	0.474	0.097	7.520	79.47	96.01
	4.783	1.212	71.41	74.66	**97.85**
Stanley (Silty clay loam)	0.011	0.001	0.210	95.90	96.30
	0.047	0.003	0.880	95.09	98.08
	0.474	0.030	8.860	93.64	88.82
	4.783	0.529	85.07	88.94	**90.29**

Bold values used to calculate the percent loss

A Note: these values have been calculated from the reported values of µg/20 mL

B Mass balance (determined as total radioactivity) values quoted are after the desorption step

Freundlich adsorption coefficients (K_f) ranged from 3.96-156 L/kg. When normalised to organic carbon, Freundlich adsorption coefficients (K_{foc}) ranged from 1237-10511 L/kg, indicating that spiroxamine is likely to exhibit low mobility in soil. The Freundlich exponent 1/n values ranged from 0.813-0.867, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

Table CA 7.1.3.1.2-4: Freundlich adsorption coefficients for M01 (spiroxamine-desethyl)

Soil	Vero Beach (Sand)	Howe (Sandy loam)	Wolf Ranch (Loam)	Stanley (Silty clay loam)
OC (%)	0.32	1.12	0.97	1.49
pH ($CaCl_2$)	6.3	6.7	7.8	5.5
K_f (L/kg)	3.96	16.27	58.81	156.61
K_{foc} (L/kg)	1237	1453	6063	10511
1/n	0.867	0.813	0.862	0.852
R^2	1.000	0.999	1.000	1.000

D. Evaluation of the data according to EFSA Evaluators Checklist

The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet provided by EFSA (summarised in Table CA 7.1.3.1.2-6). The concentrations in the supernatant and the soil as given in the report were used as input data. Individual replicate concentrations were not available, so mean values as given in the report have been used in the calculation and as the adsorption isotherms were conducted with only 4 concentrations the confidence intervals were calculated by added formulae.

The degree of sorption was sufficient, and the mass balance at the highest concentration, corrected for stability, was used to calculate the 'f' value, following EFSA (2017). For Vero beach, Howe and Wolf ranch soils, ">99%" stability is used as reported from the preliminary test after 72 hrs (which covers the equilibration time used in the isotherm determination). For Stanley soil, the >85% stability as reported from the isotherm determination was used as the best available data.

Table CA 7.1.3.1.2-5: Calculation of 'f' values for checklist

Soil	Mass balance at highest concentration (% AR)	Stability (%)[A]	'f' value for input into checklist (%)
Vero beach	99.76	>99	1.24
Howe	99.63	>99	1.37
Wolf ranch	97.85	>99	3.13
Stanley	90.29	85	23.25

A From preliminary testing for Vero beach, Howe and Wolf ranch soils, and from chromatographic analysis of adsorption supernatant for Stanley soil.

Relevant quality checks were performed to evaluate the acceptability of the study. For the Vero Beach, Howe and Wolf Ranch soils, these checks confirmed that the mass balance (93.9-103.3%) was acceptable and that the percentage adsorption was generally acceptable for all soils (49.76 - 95.45%). The LOQ was not reported, however, the analytical method (LSC) ought to have been acceptable assuming reasonable volumes used for counting. The validity of using the indirect method, based on a Kd * soil/solution ratio > 0.3, was confirmed. The degree of sorption was sufficient, however there were signs of degradation in Stanley soil, the effect of which could not be fully assessed with the limited data available in the report. The R^2 of the standard linear regressions ranged from 0.998 to 0.999 and the visual fit of both the standard regression and the residual plots were good. The K_{fE} / K_f ratio ranges give ranges above 1.2 for Stanley soil. Sorption is high for all tested soils/subsoils, with both δ and K_d x soil/solution ratio passing with good margins. Furthermore, the ratio of 1.2 is only an initial proposal, it is considered that this does not change the overall outcome of the study. Consequently, all soils should be considered as reliable and included in the regulatory dataset.

Table CA 7.1.3.1.2-6: Results of the EFSA 106 data evaluation

Soil	Units	Quality criteria	Vero Beach	Howe	Wolf Ranch	Stanley
Adsorption method	-	-	Indirect	Indirect	Indirect	Indirect
Soil solution ratio	g/mL	-	1:20	1:20	1:20	1:20
Mass balance of ^{14}C	%	>90%	99.8-103.3	93.8-101.2	96.9-98.1	88.8-98.1
f – due to loss processes *	%	-	1.24	1.37	3.13	23.25
Adsorbed percentage (δ)	%	>20%	49.8-72.7	67.0-90.9	74.7-86.4	89.0-95.5
K_d x soil:solution ratio	-	>0.3	0.99-2.67	2.03-10.00	2.95-6.33	8.05-21.00
K_{fE} / K_f	-	<1.2	1.017-1.026	1.015-1.021	1.038-1.044	1.322-1.354
ads K_f	L/kg	-	3.933	15.94	60.26	160.50
95% confidence interval**	-	*	3.10-4.99	9.85-25.79	44.97-80.75	95.70-269.19
ads 1/n	-		0.857	0.794	0.877	0.867

Soil	Units	Quality crite-ria	Vero Beach	Howe	Wolf Ranch	Stanley
95% confi-dence inter-val**	-	*	0.793-0.921	0.686-0.902	0.808-0.945	0.767-0.967
ads R^2	-	>0.975	0.999	0.998	0.999	0.999
ads K_{foc}	L/kg		1229	1423	6206	10766
Visual fit to Freundlich iso-therm	-	-	Good	Good	Good	Good
Residual plots randomly dis-tributed	-	-	Good	Good	Good	Good

* As no extraction of soil was carried out, these values are based on the worst-case total recovered radioactivity recovered (see bold values in Table CA 7.1.3.1.2-3) corrected for stability, see Table CA 7.1.3.1.2-5

** Confidence intervals provided in the EFSA Excel spreadsheet were re-calculated manually as available input data consisted of only 4 concentration levels

Figure 7.1.3.1.2-4: Freundlich Isotherms of M01 (spiroxamine-desethyl) on Vero Beach soil

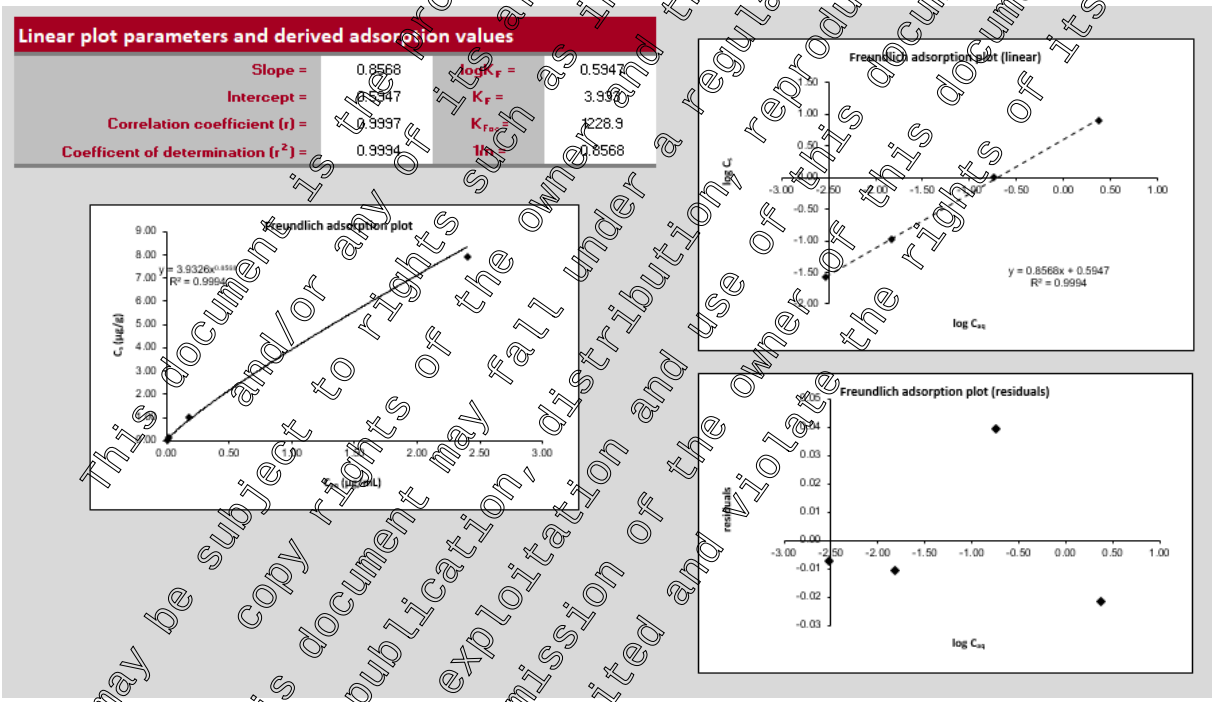


Figure 7.1.3.1.2-5: Freundlich Isotherms of M01 (spiroxamine-desethyl) on Howe soil

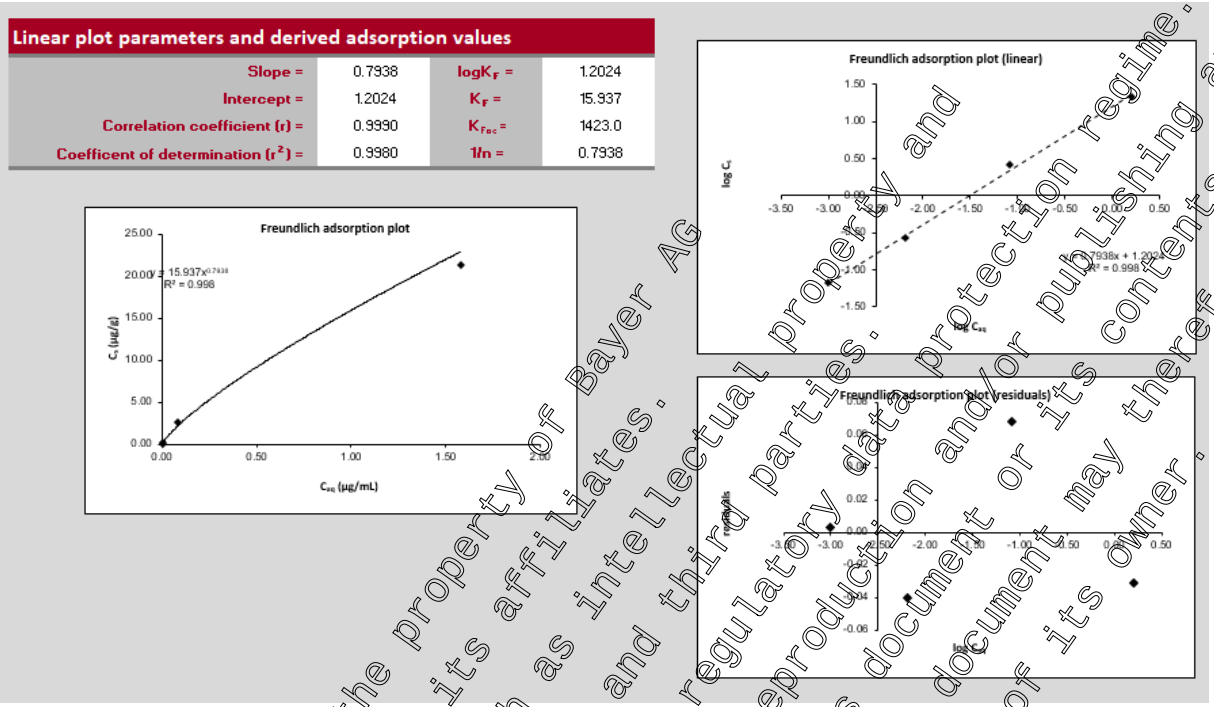


Figure 7.1.3.1.2-6: Freundlich Isotherms of M01 (spiroxamine-desethyl) on Wolf Ranch soil

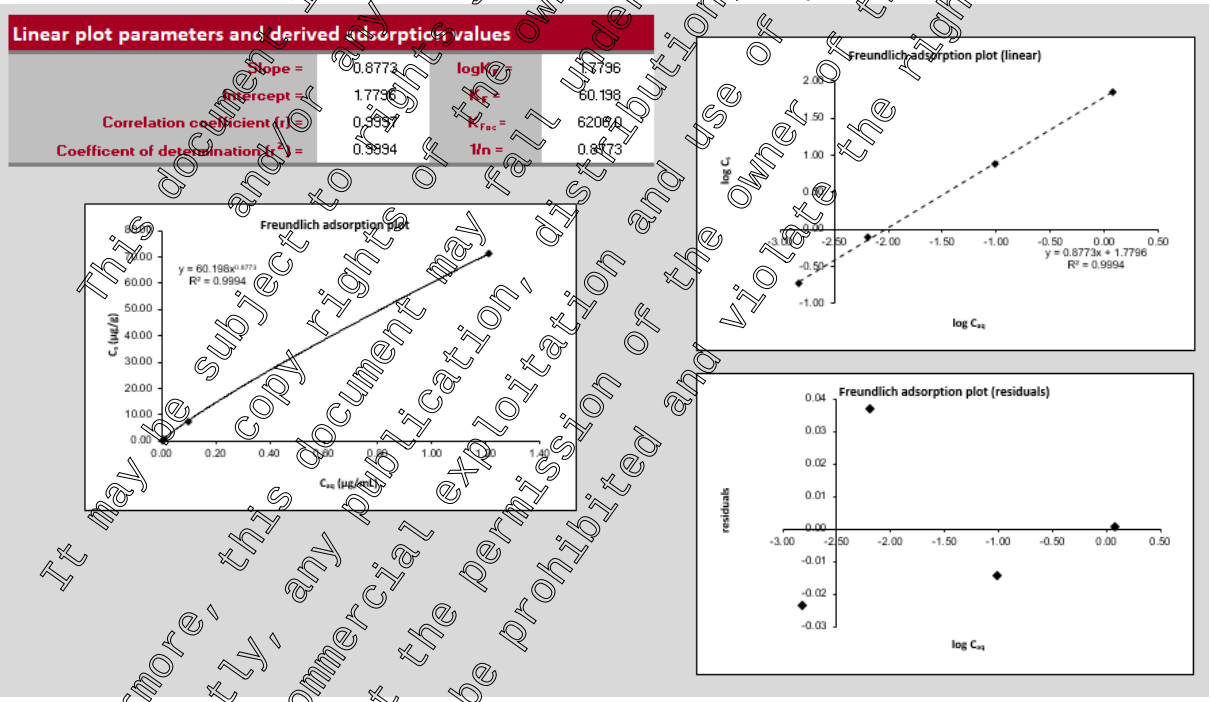
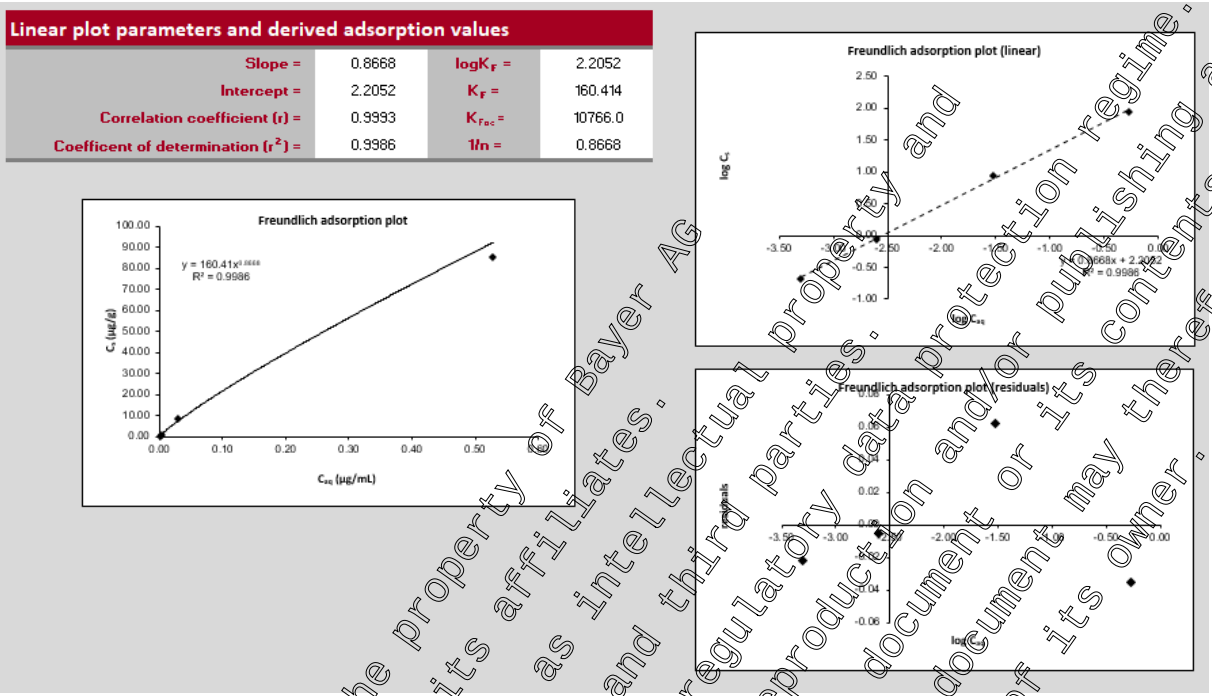


Figure 7.1.3.1.2-7: Freundlich Isotherms of M01 (spiroxamine-desethyl) on Stanley soil



Overall, the study was conducted to a reasonable standard and all soils should be considered as reliable and included in the regulatory dataset.

III. Conclusions

Freundlich adsorption coefficients (K_f) ranged from 3.96-156 L/kg (n=4). When normalised to organic carbon, Freundlich adsorption coefficients (K_{foc}) ranged from 1237-10511 L/kg, indicating that M01 (spiroxamine-desethyl) exhibits medium to immobile mobility in soil according to the McCall mobility classification (McCall et al 1981[7]). The 1/n values ranged from 0.813-0.867, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

Adsorption was shown to be generally correlated with organic carbon content, there was no clear correlation with the pH of the soils.

The OECD 106 Checklist (v2) was used to evaluate the study. The evaluation generally confirmed acceptability according to the quality criteria and therefore acceptable for regulatory use. A number of minor differences in the derived K_{foc} values were noted but this was attributed to the original calculation being performed on averages rather than single timpoints. Since the differences were very minor, for consistency the endpoints determined in the study report are used and considered to have no impact on the risk assessment.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to study guideline(s) OECD 106 (required guideline). The study is considered valid to assess the adsorption and desorption characteristics of the spiroxamine metabolite M01 (spiroxamine-desethyl) in soil.

Data Point:	KCA 7.1.3.1.2/02
Report Author:	[redacted]
Report Year:	1996
Report Title:	Adsorption/Desorption of KWG 4669 on four different soils
Report No:	FM761
Document No:	M-006086-01-1
Guideline(s) followed in study:	EPA, Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate § 163-1; Leaching and Adsorption/Desorption Studies of October 18, 1982 EC, Com-mission Directive 95/36/EC amending Council Directive 91/414/EEC (An-nexes I and II, Fate and Behaviour in the Environment) July 14, 1995 OECD – Guideline for Testing of Chemicals No.: 106 Adsorption /Desorption, May 12, 1981
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recog-nised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption and desorption of [cyclohexyl-1-^{14}C]-M02 (spiroxamine-despropyl) on four North American soils was studied using the batch equilibrium method with a tiered approach.

Parameter	**Soil**			
Soil Designation	**Vero beach**	**Howe**	**Wolf Ranch**	**Stanley**
Textural Classification (USDA)	Sand	Sandy loam	Loam	Silty clay loam
pH ($CaCl_2$)	6.3	6.7	7.8	5.5
Organic carbon (%)	0.32	1.12	0.97	1.49

Preliminary tests were performed to determine the appropriate soil:solution ratios and equilibrium time and to evaluate stability of the test substance in $CaCl_2$ solution with soil (under test conditions), potential for sorption of the test substance to test vessels and any effects of the inclusion of a biocide in solution.

In the preliminary tests, Soil:solution ratios of 1:20 (Vero Beach) 3:20 (Howe) and 1:20 (Wolf Ranch and Stanley), equilibrium time for adsorption (1 hour for Vero Beach, Wolf Ranch and Stanley soils and 48 hours for Howe soil) and desorption (1 hour for Vero Beach, Wolf Ranch and Stanley soils and 48 hours for Howe soil) were determined. As part of the equilibrium time determination, the stability of the test substance was monitored and shown to be >99% for Vero Beach, Howe and Wolf Ranch soils after 72 hrs and *ca.* 81% for Stanley soil after 24 hrs. In control samples, shaking the test substance in $CaCl_2$ solution the absence of soil for 72 hours gave a mean recovery of 97.0% AR indicating that adsorption to the test vessel was not occurring.

Freundlich isotherm tests were performed with all soils using the indirect and parallel methods. For the adsorption step, appropriate solutions of the test substance were applied to the samples and the samples were shaken for 1 or 48 hours at 22 ± 1°C. Following the adsorption step, the samples were removed from the shaker, centrifuged, and the adsorption supernatants removed. Chromatographic analysis of supernatants were reported to show >90% AR present as unchanged test substance in all soils after 1hr (Vero beach, Wolf ranch and Stanley soils) or 48hr (Howe soil).

For the desorption step, a 20 mL volume of fresh 0.01 M $CaCl_2$ solution was added and the samples were shaken for a further 1-48 hours at 22 ± 1°C. Following the desorption step, the samples were removed from the shaker, centrifuged, and the desorption supernatants removed. The desorption supernatants were analysed by LSC and the highest concentration solutions of each test substance were also

analysed by HPLC (with radiochemical detection). The soil samples were combusted in a sample oxidiser and analysed by LSC to determine the radioactivity present.

Mass balances ranged from 90.33-100.25% AR. The amount of radioactivity adsorbed on the soil at adsorption equilibrium ranged from 41.58-93.49% AR. After the adsorption phase, 41.58 – 60.69% AR was adsorbed to Vero beach soil, 61.58 – 82.67% AR adsorbed to Howe soil, 72.99 – 81.43% AR to Wolf ranch and 86.90 – 93.49% AR to Stanley soil.

Freundlich adsorption coefficients (K_f) ranged from 2.93-134.0 L/kg. When normalised to organic carbon, Freundlich adsorption coefficients (K_{foc}) ranged from 916.7-8993.6 L/kg, indicating that M01 (spiroxamine-desethyl) exhibits medium to immobile mobility in soil according to the McCall mobility classification (McCall et al 1981[7]). The 1/n values ranged from 0.827-0.922, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range. Adsorption was shown to be generally correlated with organic carbon content and there was no clear correlation with the pH of the soils. The OECD 106 Checklist (v2) was used to evaluate the study, however all final parameters used are taken from the study report. The evaluation confirmed acceptability for all soils according to the quality criteria and therefore acceptable for regulatory use.

Soil	Vero Beach (Sand)	Howe (Sandy loam)	Wolf Ranch (Loam)	Stanley (Silty clay loam)
OC (%)	0.32	1.12	0.97	1.49
pH ($CaCl_2$)	7.0	6.7	8.7	6.1
K_f (L/kg)	2.93	12.79	54.39	134.0
K_{foc} (L/kg)	916.7	1141.6	5606.8	8993.6
1/n	0.876	0.827	0.922	0.886
R^2	0.999	0.998	1.0000	0.999

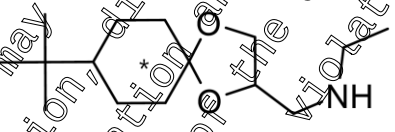
I. Materials and Methods

A. Materials

1. Test Items

Test substance:

[cyclohexyl-1-^{14}C] M02 (spiroxamine-despropyl)



* indicates position of radiolabel

Specific activity: 1.81 MBq/mg

Radiochemical purity: >98%

Batch number THS 4352

2. Test Soil

Four North American soils were used. The soils were collected from field sites in Vero Beach (Florida, USA), Howe (Indiana, USA), Wolf Ranch (California, USA) and Stanley (Kansas, USA), and varied in organic carbon, pH and clay content. After collection, soils were homogenized, air-dried, and passed through a 2 mm sieve.

Table CA 7.1.3.1.2-7: Physico-chemical properties of test soil

Parameter	Soil			
Soil Designation	**Vero Beach**	**Howe**	**Wolf Ranch**	**Stanley**
Geographic Location				
City	Vero beach, Florida	Howe, Indiana	Fresno, California	Stanley, Kansas
Country	USA	USA	USA	USA
Textural Classification (USDA)	Sand	Sandy loam	Loam	Silty clay loam
Sand (%)	98.7	65.7	29.7	17.0
Silt (%)	0.3	26.4	45.1	41.0
Clay (%)	1.0	7.9	25.2	42.0
pH				
in H_2O	7.0	6.7	8.7	6.1
in $CaCl_2$	6.3	6.7	7.8	5.5
Organic Matter (%) *	0.55	1.92	1.67	2.56
Organic carbon (%)	0.32	1.12	0.97	1.49
Cation Exchange Capacity (meq/100 g)	n.a.	10	19	n.a.
Water Holding Capacity (%)				
maximum	n.a.	25.5	32.0	n.a.
at 1/3 bar	2.8	14.8	16.8	34.0

n.a.: not analysed
* Calculated by multiplying organic carbon content by 1.724 (not reported)

B. Study Design

1. Experimental conditions

The test system for adsorption and desorption of M02 (spiroxamine-despropyl) on four North American soils consisted of borosilicate glass centrifuge tubes with Teflon seals and screw caps.

In preliminary tests, the adsorption of the test item to the test system surface, the optimal soil-to-solution ratio, the appropriate adsorption and desorption equilibration times, the stability of the test item and effects of the inclusion of a biocide in solution were determined.

Freundlich isotherm tests were performed with all soils using the indirect and parallel methods. Soil:solution ratios of 6:20 (Vero Beach) 3:20 (Howe) and 1:20 (Wolf Ranch and Stanley), and equilibrium time for adsorption (1 hour for Vero Beach, Wolf Ranch and Stanley soils and 48 hours for Howe soil) and desorption (1 hour for Vero Beach, Wolf Ranch and Stanley soils and 48 hours for Howe soil) were used. Solutions of the radiolabelled test substance in acetonitrile were prepared and diluted with 0.01 M $CaCl_2$ such that test substance application achieved concentrations of 5.27, 0.54, 0.06 and 0.01 mg/L. Biocide was not included in isotherm solutions as it was demonstrated that this gave no improvement for stability purposes. The volume of organic solvent added was not clearly reported (but assumed to be within acceptable limits).

6 (Vero Beach), 3 (Howe) or 1 g (Wolf Ranch and Stanley) dry weight of soil were transferred into centrifuge tubes and 20 mL of 0.01 M $CaCl_2$ containing test substance were applied to the samples. The samples were then shaken for 1 (all soils except Howe) or 48 hours (Howe) at 22 ± 1°C in the dark. For the desorption step, 20 mL volume of fresh 0.01 M $CaCl_2$ solution were added and the samples were shaken for a further 1 or 48 hours under the same conditions. After the adsorption step, the supernatant was subjected to chromatographic analysis by HPLC (top concentration only).

Adsorption phase

Parameter		Description
Soil condition		Soils were air-dried and sieved to 2 mm
Soil sample weight		1 g (dry weight) per replicate for Wolf Ranch and Stanley soils 3 g (dry weight) per replicate for Howe soil 6 g (dry weight) per replicate for Vero Beach soil
Equilibration solution		No pre-equilibration conducted
Control (preliminary experiment)		No soil (test item in 0.01M $CaCl_2$ only)
Test item concentration	Nominal application rates	Nominal concentrations were not specified (assumed 0.01 to 5 mg/L)
	Analytically (LSC) measured concentrations	Measured concentrations (LSC) in test solution: 5.27, 0.54, 0.06 and 0.01 mg/L (4 concentrations)
Identity and concentration of co-solvent		Dosing stock made up in acetonitrile Study media - $CaCl_2$ only
Soil: Solution ratio		1:20 (Wolf Ranch and Stanley) 3:20 (Howe soil) 6:20 (Vero Beach)
Number of replicates	Control	Duplicate
	Treatments	Duplicate
Equilibration conditions	Time	1 hrs (Vero Beach, Wolf Ranch & Stanley soils) 48 hrs (Howe soil)
	Temperature	22±1°C
	Dark	In the dark
	Shaking method	Overhead shaker
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	6000 rpm
	Duration	15 minutes
	Method of separating supernatant	Supernatant was carefully decanted.

Desorption phase

Parameter	Description
Soil samples from adsorption phase used	Yes
Amount of test item present in the adsorbed state/adsorbed amount (mg a.i./kg soil)	The amounts of test item adsorbed to soil after adsorption ranged from 41.58 to 93.49% (reported for definitive test). See OECD 106 Checklist below for further details for each soil.
Number of desorption cycles	1
Equilibrium solution and quantity used per treatment for desorption	The decanted solution was replaced by fresh aqueous 0.01 M $CaCl_2$ solution. Approximately 20 mL was used as equilibration solution.
Soil: Solution ratio	1:20 (Wolf Ranch and Stanley) 3:20 (Howe soil) 6:20 (Vero Beach)

Parameter		Description
Number of replicates	Control	Not stated
	Treatments	Duplicate
Desorption Equilibration conditions	Time	1 hrs (Vero Beach, Wolf Ranch & Stanley soils) 48 hrs (Howe soil)
	Temperature	22±1°C
	Dark	In the dark
	Shaking method	Overhead shaker
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	6000 rpm
	Duration	15 minutes
	Method of separating supernatant	Supernatant was carefully decanted

2. Analytical Procedures

After each adsorption and desorption step the aqueous supernatant was separated from the soil by centrifugation and the amount of M02 (spiroxamine-despropyl) in the supernatants was analysed by liquid scintillation counting (LSC).

The partition of the test item in the adsorption and desorption batch equilibrium experiment was determined based on the radioactivity content in the supernatant only (i.e. the indirect method). After the desorption step, the soil was air-dried and the radioactivity content determined by combustion/LSC to establish the material balance.

Parental mass balance was determined >90% by HPLC/radio-detection analysis of the supernatant only.

Adsorption and desorption isotherms were calculated by linear regression analysis of the adsorption or desorption data according to the Freundlich equation.

Additional information on stability of M02 (spiroxamine-despropyl) was determined in the preliminary tests. HPLC analysis of the supernatant over duration of 24, 48 and 72 hrs showed for the Vero beach, Wolf ranch and Howe soils >99% AR present as M02 (spiroxamine-despropyl) after 72 hrs and for the Stanley soil *ca.* 81% AR present as M02 (spiroxamine-despropyl) after 24 hrs. The reverse phase High Performance Liquid Chromatography (HPLC) system used a Lichrospher 100 RP-18 column and gradient elution of acetonitrile + 0.5% triethylamine and water + 0.5% triethylamine (starting gradient 55:45, v/v) and radio-detector.

The limit of detection (LOD) was not specified in the report, however, the concentrations of the test substance used are likely sufficient to allow adequately accurate measurements of levels of the test substance.

II. Results and Discussion

A. Results of Preliminary Tests

Soil:solution ratios of 1:20 (Vero Beach) 3:20 (Howe) and 1:20 (Wolf Ranch and Stanley), equilibrium time for adsorption (1 hour for Vero Beach, Wolf Ranch and Stanley soils and 48 hours for Howe soil) and desorption (1 hour for Vero Beach, Wolf Ranch and Stanley soils and 48 hours for Howe soil) were determined. Recovery from control samples shaking an aqueous solution of the test substance in the absence of soil for 72 hours gave a mean recovery of 97.0% AR indicating that adsorption to the test vessel was not occurring.

As part of the equilibrium time determination, the stability of the test substance was monitored and shown to be >99% for Vero Beach, Howe and Wolf Ranch soils after 72 hrs and *ca.* 81% for Stanley soil after 24 hrs.

B. Transformation of test substance

Stability of M02 (spiroxamine-despropyl) was monitored during the isotherm determinations by HPLC analysis of the supernatant after the adsorption step. The majority of radioactivity in the supernatant was unchanged metabolite M02 (spiroxamine-despropyl) (only minimal details are provided in the report but a recovery of >90% over all soils was quoted).

C. Findings

Mass balances ranged from 90.33-100.25% AR (based on total radioactivity). The amount of radioactivity adsorbed on the soil at adsorption equilibrium ranged from 41.58-93.49% AR.

Table CA 7.1.3.1.2-8: Concentrations at adsorption equilibrium and recovery of radioactivity (mean values) for M02 (spiroxamine-despropyl)

Soil	Concentration initial (mg/L) [A]	Concentration supernatant (mg/L) [A]	Concentration soil (mg/kg) [A]	Adsorption percentage (%)	Mass balance (% AR) [B]
Vero Beach (Sand)	0.013	0.005	0.025	60.69	98.54
	0.056	0.022	0.112	60.28	95.92
	0.538	0.254	0.957	53.38	96.18
	5.268	3.078	7.300	41.58	**100.25**
Howe (Sandy loam)	0.013	0.002	0.067	81.39	94.32
	0.054	0.009	0.293	82.67	98.62
	0.513	0.136	2.510	73.43	99.34
	5.363	2.060	22.013	61.58	**97.94**
Wolf Ranch (Loam)	0.013	0.003	0.210	81.43	95.77
	0.056	0.012	0.890	79.52	95.08
	0.538	0.132	8.110	75.42	92.94
	5.275	1.423	76.890	72.99	**97.49**
Stanley (Silty clay loam)	0.013	0.001	0.240	93.49	93.78
	0.056	0.004	1.030	92.83	94.21
	0.538	0.048	9.800	91.11	90.33
	5.268	0.690	91.55	86.90	**94.62**

Bold values used to calculate the percent loss

A Note: these values have been calculated from the reported values of µg/20 mL

B Mass balance (determined as total radioactivity) values quoted are after the desorption step

Freundlich adsorption coefficients (K_f) ranged from 2.93-1134.0 L/kg. When normalised to organic carbon, Freundlich adsorption coefficients (K_{foc}) ranged from 916.7-8993.6 L/kg, indicating that M02 (spiroxamine-despropyl) is likely to exhibit low mobility in soil. The Freundlich exponent 1/n values ranged from 0.827-1.000, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

Table CA 7.1.3.1.2-9: Freundlich adsorption coefficients for M02 (spiroxamine-despropyl)

Soil	Vero Beach (Sand)	Howe (Sandy loam)	Wolf Ranch (Loam)	Stanley (Silty clay loam)
OC (%)	0.32	1.12	0.97	1.49
pH ($CaCl_2$)	7.0	6.7	8.7	6.1
K_f (L/kg)	2.93	12.79	54.39	134.0
K_{foc} (L/kg)	916.7	1141.6	5606.8	8993.6
1/n	0.876	0.827	0.922	0.886
R^2	0.999	0.998	1.0000	0.999

D. Evaluation of the data according to EFSA Evaluators Checklist

The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet provided by EFSA (summarised in Table CA 7.1.3.1.2-11). The concentrations in the supernatant and the soil as given in the report were used as input data. Individual replicate concentrations were not available,

so mean values as given in the report have been used in the calculation and as the adsorption isotherms were conducted with only 4 concentrations the confidence intervals were calculated by added formulae.

The degree of sorption was sufficient, and the mass balance at the highest concentration, corrected for stability, was used to calculate the 'f' value, following EFSA (2017). Chromatographic analysis of supernatant during the isotherm test showed a reported ">99%" stability for Vero Beach, Howe and Wolf ranch soils, and ">90%" for Stanley soil. Therefore, 99% and 90% have been used below as appropriate.

Table CA 7.1.3.1.2-10: Calculation of 'f' values for checklist

Soil	Mass balance at highest concentration (% AR)	Stability (%)[1]	'f' value for input into checklist (%)
Vero beach	100.25	99	0.75
Howe	97.94	99	0.04
Wolf ranch	97.49	99	3.48
Stanley	94.62	90	14.84

[1] From chromatographic analysis of adsorption supernatant during isotherm test

Relevant quality checks were performed to evaluate the acceptability of the study. For the Vero Beach, Howe and Wolf Ranch soils, these checks confirmed that the mass balance (90.33-100.25%) was acceptable and that the % adsorption was generally acceptable for all soils (49.76 - 95.45%). The LOQ was not reported, however, the analytical method (LSC) was considered acceptable assuming reasonable volumes used for counting. The validity of using the indirect method, based on a Kd* soil/solution ratio > 0.3, was confirmed. The R^2 of the standard linear regressions ranged from 0.998 to 0.999 and the visual fit of both the standard regression and the residual plots were good. The K_{fE} / K_f ratio ranges give maxima very slightly above 1.2 for Stanley soil only. However, sorption is high for all tested soils/subsoils, with both δ and K_d x soil/solution ratio passing with good margins. Furthermore, the ratio of 1.2 is only an initial proposal, it is considered that this does not change the overall outcome of the study.

Table CA 7.1.3.1.2-11: Results of the EFSA 106 data evaluation

Soil	Units	Quality criteria	Vero Beach	Howe	Wolf Ranch	Stanley
Adsorption method	-	-	Indirect	Indirect	Indirect	Indirect
Soil solution ratio	g/mL		1:20	1:20	1:20	1:20
Mass balance of ^{14}C	%	>90%	95.92-100.25	93.8-101.2	92.94-97.49	90.33-94.62
f – due to loss processes *	%	-	0.75	0.04	3.48	14.84
Adsorbed percentage (δ)	%	≥20%	41.58-61.54	61.59-84.62	73.02-80.77	86.90-92.86
K_d x soil:solution ratio		>0.3	0.71-1.60	1.60-5.50	2.71-4.20	6.63-13.00
K_{fE} / K_f		1.2	1.012-1.018	1.000-1.001	1.045-1.050	1.190-1.206
ads K_f	L/kg	-	2.937	12.594	54.785	138.350
95% confidence interval**	-	*	2.11-4.08	9.22-17.20	49.589-60.525	79.15-241.84
ads 1/n	-		0.871	0.815	0.928	0.903
95% confidence interval**	-	*	0.774-0.968	0.737-0.891	0.902-0.953	0.784-1.203
ads R^2	-	>0.975	0.999	0.999	1.000	0.998

Soil	Units	Quality criteria	Vero Beach	Howe	Wolf Ranch	Stanley
ads K_{foc}	L/kg		917.8	1124.4	5648	92852
Visual fit to Freundlich isotherm	-	-	Good	Good	Good	Good
Residual plots randomly distributed	-	-	Good	Good	Good	Good

* As no extraction of soil was carried out, these values are based on the worst-case total recovered radioactivity recovered (see bold values in Table CA 7.1.3.1.2-8) corrected for stability, see Table CA 7.1.3.1.2-10

** Confidence intervals provided in the EFSA Excel spreadsheet were re-calculated manually as available input data consisted of only 4 concentration levels

Figure 7.1.3.1.2-8: Freundlich Isotherms of M02 (spiroxamine-despropyl) on Vero Beach soil

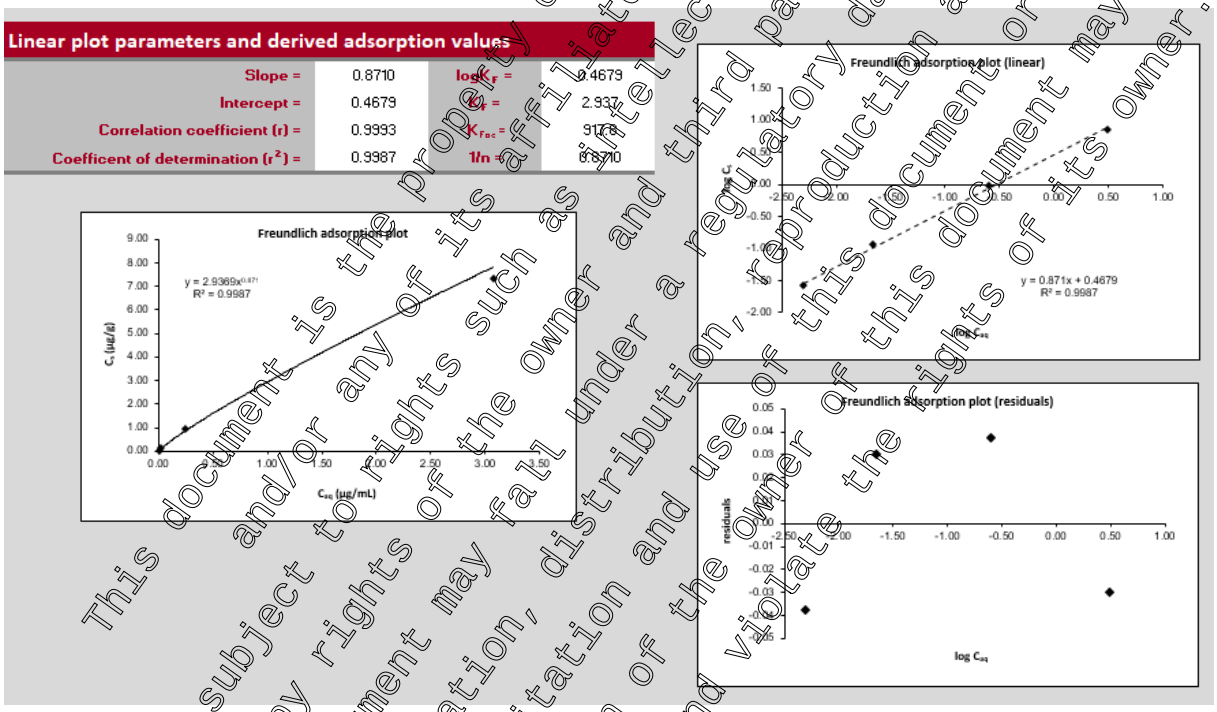


Figure 7.1.3.1.2-9: Freundlich Isotherms of M02 (spiroxamine-despropyl) on Howe soil

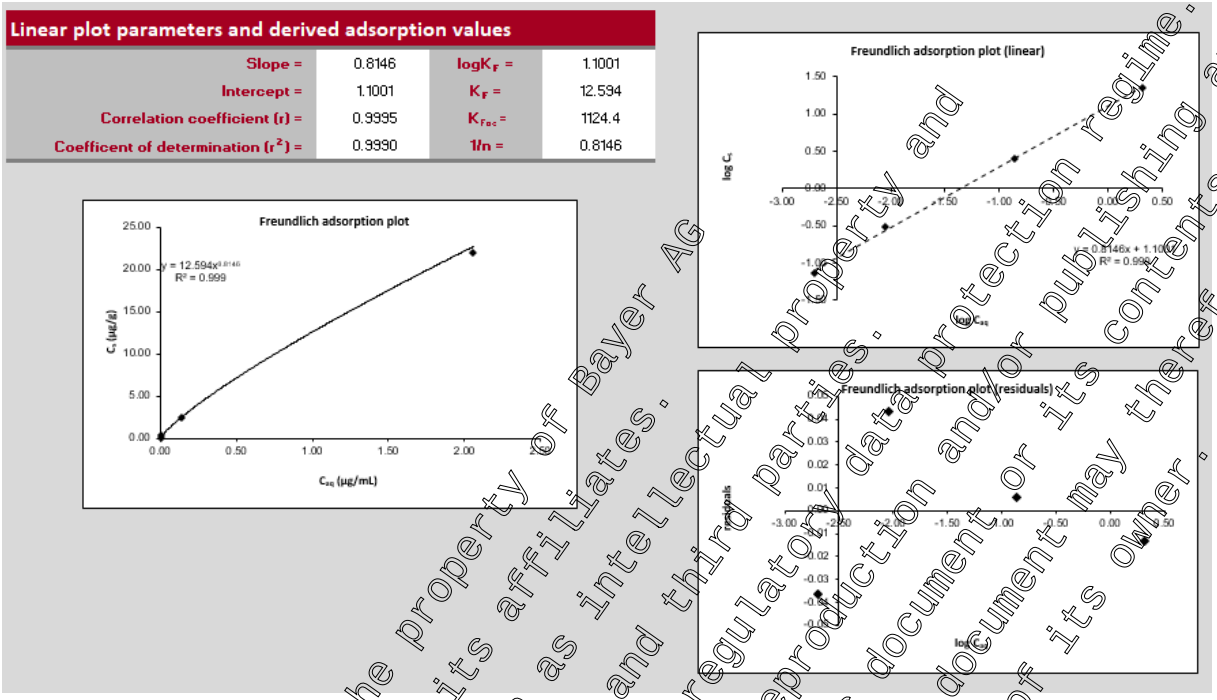


Figure 7.1.3.1.2-10: Freundlich Isotherms of M02 (spiroxamine-despropyl) on Wolf Ranch soil

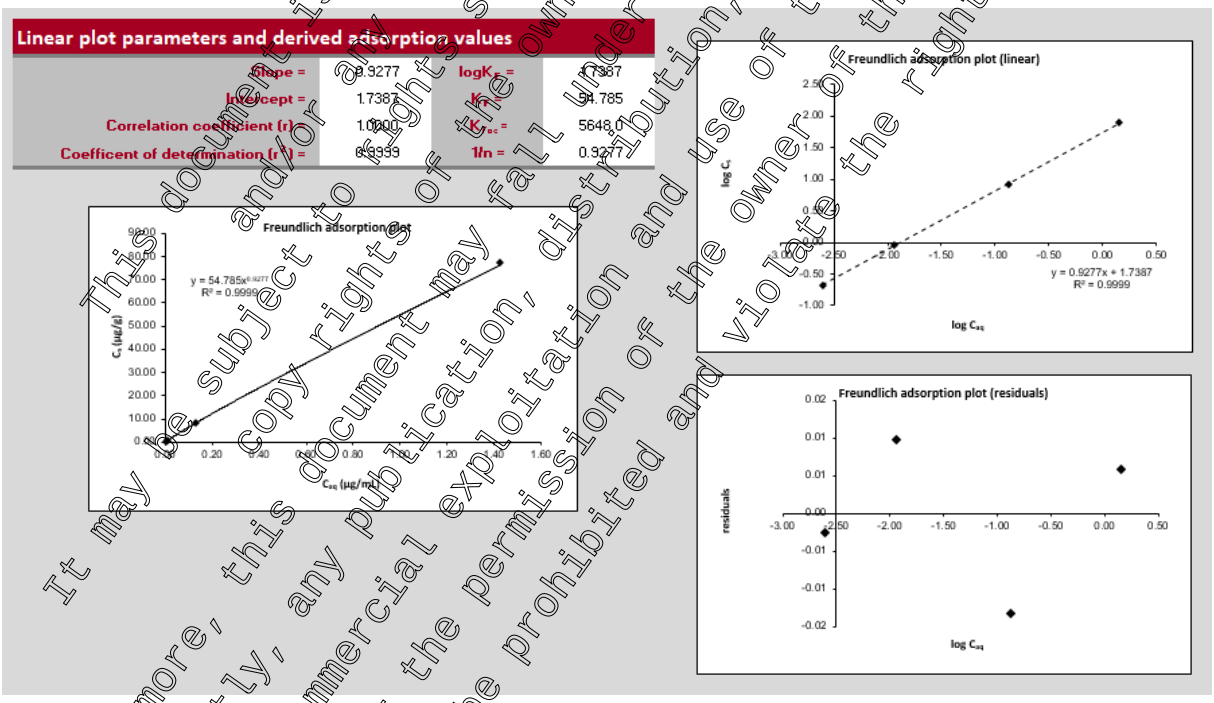
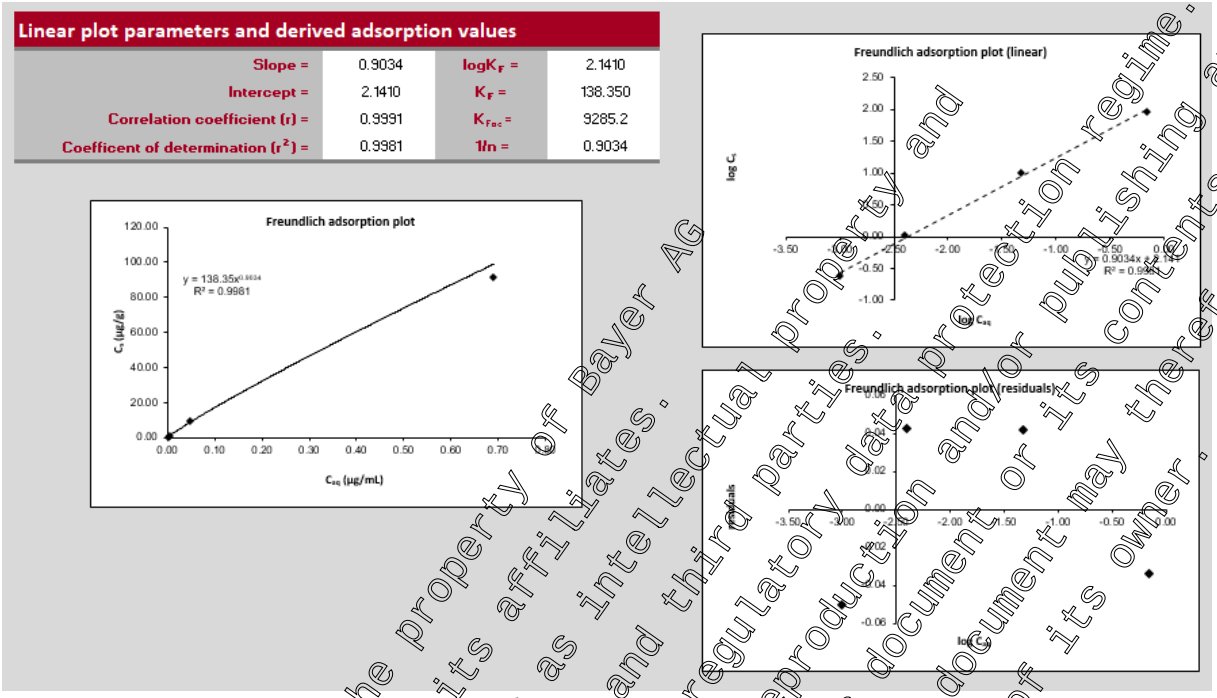


Figure 7.1.3.1.2-11: Freundlich Isotherms of M02 (spiroxamine-despropyl) on Stanley soil



Overall, the study was conducted to a good standard and all soils pass the checklist criteria. The study conclusions can thus be considered reliable.

III. Conclusions

Freundlich adsorption coefficients (K_f) ranged from 2.93-134.0 L/kg (n=4). When normalised to organic carbon, Freundlich adsorption coefficients (K_{foc}) ranged from 916.7-8993.6 L/kg, indicating that M02 (spiroxamine-despropyl) exhibits medium to immobile mobility in soil according to the McCall mobility classification (McCall et al 1981). The 1/n values ranged from 0.827-0.922, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

Adsorption was shown to be generally correlated with organic carbon content and there was no clear correlation with the pH of the soils.

The OECD 106 Checklist (v2) was used to evaluate the study. The evaluation confirmed acceptability according to the quality criteria and therefore acceptable for regulatory use. A number of minor differences in the derived K_{FOC} values were noted but this was attributed to the original calculation being performed on averages rather than single timpoints. Since the differences were very minor, for consistency the endpoints determined in the study report are used and considered to have no impact on the risk assessment.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to study guideline(s) OECD 106 (required guideline). The study is considered valid to assess the adsorption and desorption characteristics of the spiroxamine metabolite M02 (spiroxamine-despropyl) in soil.

Data Point:	KCA 7.1.3.1.2/03
Report Author:	[redacted]
Report Year:	1997
Report Title:	Adsorption/desorption of [cyclohexyl-1-14C] WAK 6301 on four different soils
Report No:	FM763
Document No:	M-006089-01-1
Guideline(s) followed in study:	OECD – Guideline for Testing of Chemicals No.: 106 Adsorption/Desorption, May 12, 1981
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption and desorption of [cyclohexyl-1-^{14}C] M03 (spiroxamine-N-oxide) on four North American soils was studied using the batch equilibrium method with a tiered approach.

Parameter	Soil			
Soil Designation	**Vero beach**	**Howe**	**Wolf Ranch**	**Stanley**
Textural Classification (USDA)	Sand	Sandy loam	Loam	Silty clay loam
pH ($CaCl_2$)	6.3	6.7	7.8	5.5
Organic carbon (%)	0.32	1.02	0.97	1.49

Preliminary tests were performed to determine the appropriate soil:solution ratios and equilibrium time and to evaluate stability of the test substance in $CaCl_2$ solution with soil (under test conditions), potential for sorption of the test substance to test vessels and any effects of the inclusion of a biocide in solution.

Soil:solution ratios of 6:20 (Vero Beach and Howe) 3:20 (Wolf Ranch) and 1:20 (Stanley), and equilibrium time for adsorption (48 hours for Vero Beach, Wolf Ranch and Howe soils and 1 hours for Stanley soil) and desorption (48 hours for Vero Beach, Wolf Ranch and Howe soils and 1 hour for Stanley soil) were determined. As part of the equilibrium time determination, the stability of the test substance was monitored and shown to be >99% for all soils. In control samples, shaking the test substance in $CaCl_2$ solution in the absence of soil gave a recovery of 97.8% AR after 72 hrs, indicating that adsorption to the test vessels was not occurring.

Freundlich isotherm tests were performed with all soils using the indirect and parallel methods. For the adsorption step, appropriate solutions of the test substance were applied to the samples and the samples were shaken for 1 or 48 hours at 22 ± 1°C. Following the adsorption step, the samples were removed from the shaker, centrifuged, and the adsorption supernatants removed. For the desorption step, a 20 mL volume of fresh 0.01 M $CaCl_2$ solution was added and the samples were shaken for a further 1-48 hours at 22 ± 1°C. Following the desorption step, the samples were removed from the shaker, centrifuged, and the desorption supernatants removed. Supernatants were quantified by LSC. Supernatants of the highest concentration solutions of each soil were also analysed by Thin Layer Chromatography (TLC) with radiochemical detection. The soil samples were combusted in a sample oxidiser and quantified by LSC to determine the radioactivity present.

Mass balances ranged from 91.45-101.70% AR. The amount of radioactivity adsorbed on the soil at adsorption equilibrium ranged from 30.10-98.64% AR. After the adsorption phase, 30.10 – 41.72% AR was adsorbed to Vero Beach soil, 48.78 – 98.32% AR adsorbed to Howe soil, 68.89 – 81.49% AR to Wolf ranch and 95.59 – 98.64% AR to Stanley soil.

Freundlich adsorption coefficients (K_f) ranged from 1.767-370.899 L/kg. When normalised to organic

carbon, Freundlich adsorption coefficients (K_{foc}) ranged from 552.3-24892.5 L/kg, indicating that M03 (spiroxamine-N-oxide) exhibits medium to immobile mobility in soil according to the McCall mobility classification (McCall et al 1981[7]). The 1/n values ranged from 0.835-0.939, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range. Adsorption was shown to be generally correlated with organic carbon content and there was no clear correlation with the pH of the soils. The OECD 106 Checklist (v2) was used to evaluate the study; however all final parameters used are taken from the study report. The evaluation confirmed acceptability according to the quality criteria and therefore acceptable for regulatory use.

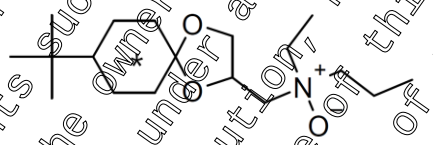
Soil	Vero Beach (Sand)	Howe (Sandy loam)	Wolf Ranch (Loam)	Stanley (Silty clay loam)
OC (%)	0.32	1.12	0.97	1.49
pH ($CaCl_2$)	7.0	6.7	8.7	6.1
K_f (L/kg)	1.7674	39260	15.9163	370.8988
K_{foc} (L/kg)	552.3	350.5	1640.9	24892.5
1/n	0.9388	0.8714	0.8898	0.8348
R^2	0.9981	0.9990	0.9996	0.9991

I. Materials and Methods

A. Materials

1. Test Items

Test substance:



[cyclohexyl-1-^{14}C]-M03 (spiroxamine-N-oxide)

* Indicates position of radiolabel

Specific activity: 3.44 MBq/mg

Radiochemical purity: >99%

Batch number: KML 2352, 575/1

2. Test Soil

Four North American soils were used. The soils were collected from field sites in Vero Beach (Florida, USA), Howe (Indiana, USA), Wolf Ranch (California, USA) and Stanley (Kansas, USA), and varied in organic carbon, pH and clay content. After collection, soils were homogenized, air-dried, and passed through a 2 mm sieve.

Table CA 7.1.3.1.2-12: Physico-chemical properties of test soil

Parameter		Soil			
Soil Designation		Vero Beach	Howe	Wolf Ranch	Stanley
Geographic location					
City		Vero beach, Florida	Howe, Indiana	Fresno, California	Stanley, Kansas
Country		USA	USA	USA	USA
Textural classification (USDA)		Sand	Sandy loam	Loam	Silty clay loam
Sand	(%)	98.7	65.7	29.7	17.0
Silt	(%)	0.3	26.4	45.1	41.0
Clay	(%)	1.0	7.9	25.2	42.0

Parameter	Soil			
Soil Designation	**Vero Beach**	**Howe**	**Wolf Ranch**	**Stanley**
pH				
in H_2O	7.0	6.7	8.7	6.0
in $CaCl_2$	6.3	6.7	7.8	5.5
Organic Matter (%) *	0.55	1.92	1.67	2.56
Organic carbon (%)	0.32	1.12	0.97	1.49
Cation Exchange Capacity (meq/100 g)	n.a.	10	19	n.a.
Water Holding Capacity (%)				
maximum	n.a.	25.5	32.0	n.a.
at 1/3 bar	2.8	14.8	16.8	34.0

n.a.: not analysed
* Calculated by multiplying organic carbon content by 1.724 (not reported)

B. Study Design

1. Experimental conditions

The test system for adsorption and desorption of M03 (spiroxamine-N-oxide) on four North American soils consisted of borosilicate glass centrifuge tubes with Teflon seals and screw caps.

In preliminary tests, the adsorption of the test item to the test system surface, the optimal soil-to-solution ratio, the appropriate adsorption and desorption equilibration times, the stability of the test item and the effects of inclusion of a biocide in the solution were determined.

Freundlich isotherm tests were performed with all soils using the indirect and parallel methods. Soil:solution ratios of 6:20 (Vero Beach and Howe) 3:20 (Wolf Ranch) and 1:20 (Stanley), and equilibrium time for adsorption (48 hours for Vero Beach, Wolf Ranch and Howe soils and 1 hours for Stanley soil) and desorption (48 hours for Vero Beach, Wolf Ranch and Howe soils and 1 hour for Stanley soil) were used. Solutions of the radiolabelled test substance in acetonitrile were prepared and diluted with 0.01 M $CaCl_2$, such that test substance application achieved concentrations of 4.94, 0.49, 0.05 and 0.01 mg/L for solutions applied to Vero Beach and Howe. For Wolf Ranch, concentrations of 4.94, 0.50, 0.05 and 0.01 mg/L were achieved and for Stanley, concentrations of 5.11, 0.50, 0.05 and 0.01 mg/L were achieved. Biocide was not included in isotherm solutions as it was demonstrated that this was not needed for stability purposes. The volume of organic solvent added was not clearly reported (but assumed to be within acceptable limits).

6, 3 or 1 g dry weight of soil (as appropriate) were transferred into centrifuge tubes and 20 mL of 0.01 M $CaCl_2$ containing test substance were applied to the samples. The samples were then shaken for 1 or 48 hours at 22 ± 1°C in the dark. For the desorption step, 20 mL volume of fresh 0.01 M $CaCl_2$ solution were added and the samples were shaken for a further 1 or 48 hours under the same conditions. After the adsorption step, the supernatant was subjected to chromatographic analysis by TLC (top concentration only).

Adsorption phase

Parameter		Description
Soil condition		Soils were air-dried and sieved to 2 mm
Soil sample weight		1 g (dry weight) per replicate for and Stanley soil 3 g (dry weight) per replicate for Wolf Ranch soil 6 g (dry weight) per replicate for Vero Beach and Howe soils
Equilibration solution		No pre-equilibration conducted
Control (preliminary experiment)		No soil (test item in 0.01M $CaCl_2$ only)
Test item concentration	Nominal application rates	Nominal concentrations were not specified (assumed 0.01 to 5 mg/L)
	Analytically (LSC) measured concentrations	Measured concentrations (LSC) in test solution: 4.94, 0.49, 0.05 and 0.01 mg/L for Vero Beach and Howe. 4.94, 0.50, 0.05 and 0.01 mg/L for Wolf Ranch. 5.11, 0.50, 0.05 and 0.01 mg/L for Stanley (4 concentrations)
Identity and concentration of co-solvent		Dosing stock made up in acetonitrile Study media – $CaCl_2$ only
Soil: Solution ratio		1:20 (Stanley) 3:20 (Wolf Ranch) 6:20 (Vero Beach and Howe)
Number of replicates	Control	Duplicate
	Treatments	Duplicate
Equilibration conditions	Time	1 hrs (Stanley soil) 48 hrs (Vero Beach, Wolf Ranch & Howe soil)
	Temperature	22±1°C
	Dark	In the dark
	Shaking method	Overhead shaker
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	6000 rpm
	Duration	15 minutes
	Method of separating supernatant	Supernatant was carefully decanted.

Desorption phase

Parameter	Description
Soil samples from adsorption phase used	Yes
Amount of test item present in the adsorbed state/adsorbed amount (mg a.s./kg soil)	The amounts of test item adsorbed to soil after adsorption ranged from 30.10 to 98.64% (reported for definitive test). See OECD 106 Checklist below for further details for each soil.
Number of desorption cycles	1
Equilibrium solution and quantity used per treatment for desorption	The decanted solution was replaced by fresh aqueous 0.01 M $CaCl_2$ solution. Approximately 20 mL was used as equilibration solution.
Soil: Solution ratio	1:20 (Stanley) 3:20 (Wolf Ranch) 6:20 (Vero Beach and Howe)

Parameter		Description
Number of replicates	Control	Not stated
	Treatments	Duplicate
Desorption Equilibration conditions	Time	1 hrs (Stanley soil) 48 hrs (Vero Beach, Wolf Ranch & Howe soils)
	Temperature	22±1°C
	Dark	In the dark
	Shaking method	Overhead shaker
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	6000 rpm
	Duration	15 minutes
	Method of separating supernatant	Supernatant was carefully decanted

2. Analytical Procedures

After each adsorption and desorption step the aqueous supernatant was separated from the soil by centrifugation and the amount of M03 (spiroxamine-N-oxide) in the supernatants was quantified by liquid scintillation counting (LSC).

The partition of the test item in the adsorption and desorption batch equilibrium experiment was determined based on the radioactivity content in the supernatant only (i.e. the indirect method). After the desorption step, the soil was air-dried and the radioactivity content determined by combustion/LSC to establish the material balance.

Parental mass balance was determined >99% in all soils by reverse phase Thin Layer Chromatography (TLC) analysis of the supernatant only using a mobile phase of acetonitrile/water/25% ammonia (80:18:2, v/v/v).

Adsorption and desorption isotherms were calculated by linear regression analysis of the adsorption or desorption data according to the Freundlich equation.

Additional information on stability of M03 (spiroxamine-N-oxide) was determined in the preliminary tests. Chromatographic analysis of the supernatant after 1 or 48 hrs showed >99% AR present as M03 (spiroxamine-N-oxide) for all soils.

The limit of detection (LOD) was not specified in the report, however, the concentrations of the test substance used are likely sufficient to allow adequately accurate measurements of levels of the test substance.

II. Results and Discussion

A. Results of Preliminary Tests

Soil:solution ratios of 6:20 (Vero Beach and Howe) 3:20 (Wolf Ranch) and 1:20 (Stanley), and equilibrium time for adsorption (48 hours for Vero Beach, Wolf Ranch and Howe soils and 1 hours for Stanley soil) and desorption (48 hours for Vero Beach, Wolf Ranch and Howe soils and 1 hour for Stanley soil) were determined. Shaking an aqueous solution of the test substance in the absence of soil for 72 hours gave a mean recovery of 97.8% AR indicating that adsorption of the test substance to the test vessels was not occurring.

As part of the equilibrium time determination, stability of M03 (spiroxamine-N-oxide) was monitored in the supernatant only and shown to be >99% for all soils after 72 hrs.

B. Transformation of test substance

Stability of M03 (spiroxamine-N-oxide) was monitored during the isotherm determinations by TLC

analysis of the supernatant after the adsorption step. >99% of the radioactivity was unchanged M03 (spiroxamine-N-oxide).

C. Findings

Mass balances ranged from 91.45-101.70% AR. The amount of radioactivity adsorbed on the soil at adsorption equilibrium ranged from 30.10-98.64% AR.

Table CA 7.1.3.1.2-13: Concentrations at adsorption equilibrium and recovery of radioactivity (mean values) for M03 (spiroxamine-N-oxide)

Soil	Concentration initial (mg/L) [A]	Concentration supernatant (mg/L) [A]	Concentration soil (mg/kg) [A]	Adsorption percentage (%)	Mass balance (% AR) [B]
Vero Beach (Sand)	0.011	0.007	0.013	38.65	101.70
	0.048	0.028	0.067	41.72	92.01
	0.494	0.292	0.672	40.80	91.58
	4.944	3.456	4.960	30.10	**96.99**
Howe (Sandy loam)	0.011	0.004	0.025	68.93	100.14
	0.048	0.015	0.108	98.32	93.28
	0.494	0.185	1.027	62.46	91.96
	4.944	2.533	8.038	48.78	**95.17**
Wolf Ranch (Loam)	0.011	0.002	0.057	81.49	98.62
	0.051	0.010	0.277	81.44	95.12
	0.501	0.125	2.510	75.19	98.59
	4.944	1.538	22.707	68.89	**91.45**
Stanley (Silty clay loam)	0.011	0.0001*	0.200	98.64	94.54
	0.051	0.0008	1.00	98.48	95.00
	0.501	0.011	9.800	97.79	95.66
	4.944	0.226	97.640	95.59	**93.42**

Bold values used to calculate the percent less

A Note: these values have been calculated from the reported values of µg/20 mL

B Mass balance (determined as total radioactivity) values quoted are after the desorption step

Freundlich adsorption coefficients (K_f) ranged from 1.767-370.899 L/kg. When normalised to organic carbon, Freundlich adsorption coefficients (K_{foc}) ranged from 916.7-8993.6 L/kg, indicating that M03 (spiroxamine-N-oxide) is likely to exhibit low mobility in soil. The Freundlich exponent 1/n values ranged from 0.8348-0.9388, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

Table CA 7.1.3.1.2-14: Freundlich adsorption coefficients for M03 (spiroxamine-N-oxide)

Soil	Vero Beach (Sand)	Howe (Sandy loam)	Wolf Ranch (Loam)	Stanley (Silty clay loam)
OC (%)	0.32	1.12	0.97	1.49
pH ($CaCl_2$)	7.0	6.7	8.7	6.1
K_f (L/kg)	1.7674	3.9260	15.9163	370.8988
K_{foc} (L/kg)	552.3	350.5	1640.9	24892.5
1/n	0.9388	0.8714	0.8898	0.8348
R^2	0.9981	0.9990	0.9996	0.9991

D. Evaluation of the data according to EFSA Evaluators Checklist

The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet provided by EFSA (summarised in Table CA 7.1.3.1.2-16). The concentrations in the supernatant and the soil as given in the report were used as input data. Individual replicate concentrations were not available, so mean values as given in the report have been used in the calculation and as the adsorption isotherms were conducted with only 4 concentrations the confidence intervals were calculated by added formulae.

The degree of sorption was sufficient, and the mass balance at the highest concentration, corrected for

stability, was used to calculate the 'f' value, following EFSA (2017). Chromatographic analysis of supernatant during the isotherm test showed a reported ">99%" stability for all soils, therefore, 99% has been used below.

Table CA 7.1.3.1.2-15: Calculation of 'f' values for checklist

Soil	Mass balance at highest concentration (% AR)	Stability (%)[A]	'f' value for input into checklist (%)
Vero beach	96.99	99	7.98
Howe	95.17	99	5.78
Wolf ranch	91.45	99	9.46
Stanley	93.42	99	7.51

A From chromatographic analysis of adsorption supernatant during isotherm test

Relevant quality checks were performed to evaluate the acceptability of the study. These checks confirmed that the mass balance (91.45-101.70%) and the % adsorption (30.10-98.64%) was acceptable for all soils. The LOQ was not reported, however, the analytical method (LSC) ought to have been acceptable assuming reasonable volumes used for counting. The validity of using the indirect method, based on a Kd * soil/solution ratio > 0.3, was confirmed. The K_E / K_f ratio ranges are all below 1.2 for all soils. The R^2 of the standard linear regressions ranged from 0.998 to 1.000 and the visual fit of both the standard regression and the residual plots were good.

Table CA 7.1.3.1.2-16: Results of the FFSA 196 data evaluation

Soil	Units	Quality criteria	Vero Beach	Howe	Wolf Ranch	Stanley
Adsorption method	-	-	Indirect	Indirect	Indirect	Indirect
Soil solution ratio	g:mL		6:20	6:20	3:20	1:20
Mass balance of ^{14}C	%	>90%	91.58-101.70	91.96-100.14	91.45-98.62	93.42-95.66
f – due to loss processes *	%	-	3.98	5.78	9.46	7.51
Adsorbed percentage (δ)	%	>20%	30.11-41.05	48.78-68.42	68.89-81.37	95.58-98.61
K_d x soil:solution ratio		>0.3	0.43-0.70	0.95-2.17	2.21-4.37	21.65-71.04
K_{fE} / K_f	-	1.2	1.107-1.152	1.092-1.134	1.132-1.159	1.082-1.085
ads K_f	L/kg	-	1.764	3.952	15.965	373.935
95% confidence interval**			0.99-3.15	2.25-6.94	11.34-22.49	181.39-770.87
ads 1/n	-		0.944	0.882	0.894	0.837
95% confidence interval**			0.763-1.124	0.728-1.037	0.809-0.979	0.720-0.954
ads R^2	-	>0.975	0.996	0.997	0.999	0.998

Soil	Units	Quality criteria	Vero Beach	Howe	Wolf Ranch	Stanley
ads K_{foc}	L/kg		551.2	352.9	1645.9	250963
Visual fit to Freundlich isotherm	-	-	Good	Good	Good	Good
Residual plots randomly distributed	-	-	Good	Good	Good	Good

* As no extraction of soil was carried out, these values are based on the worst-case total recovered radioactivity recovered (see bold values in Table CA 7.1.3.1.2-13) corrected for stability, see Table CA 7.1.3.1.2-15

** Confidence intervals provided in the EFSA Excel spreadsheet were re-calculated manually as available input data consisted of only 4 concentration levels

Figure 7.1.3.1.2-12: Freundlich Isotherms of M03 (spiroxamine-N-oxide) on Vero Beach soil

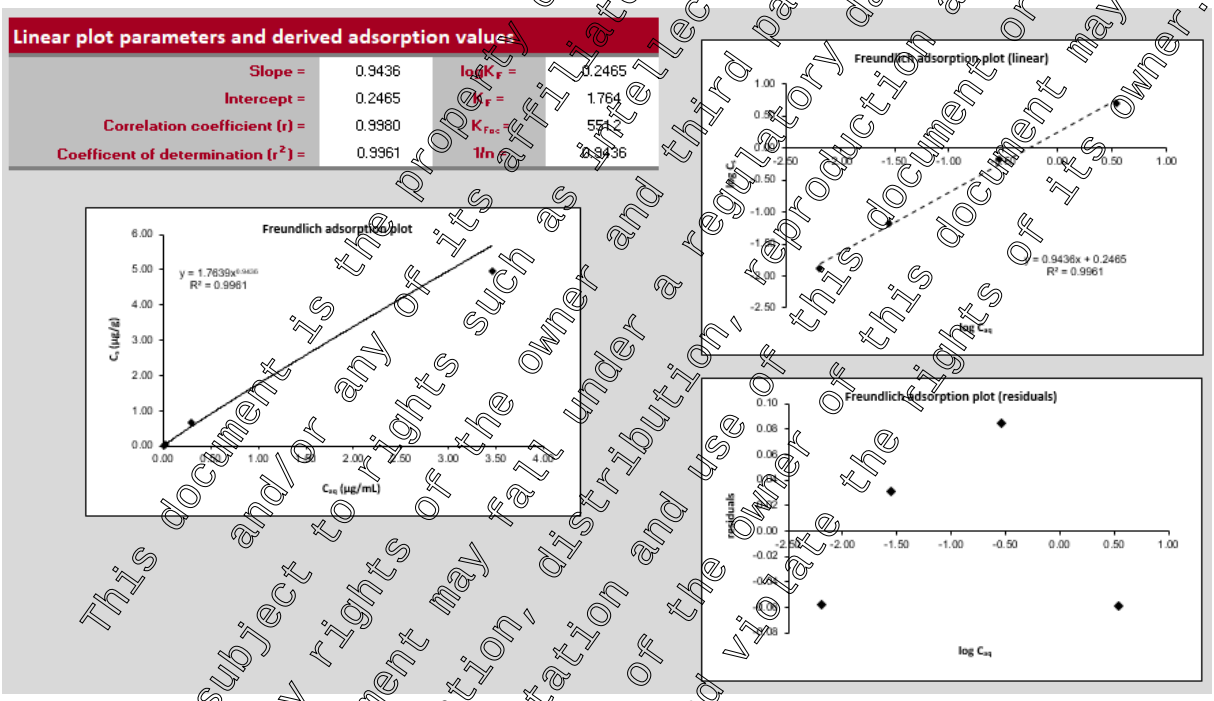


Figure 7.1.3.1.2-13: Freundlich Isotherms of M03 (spiroxamine-N-oxide) on Howe soil

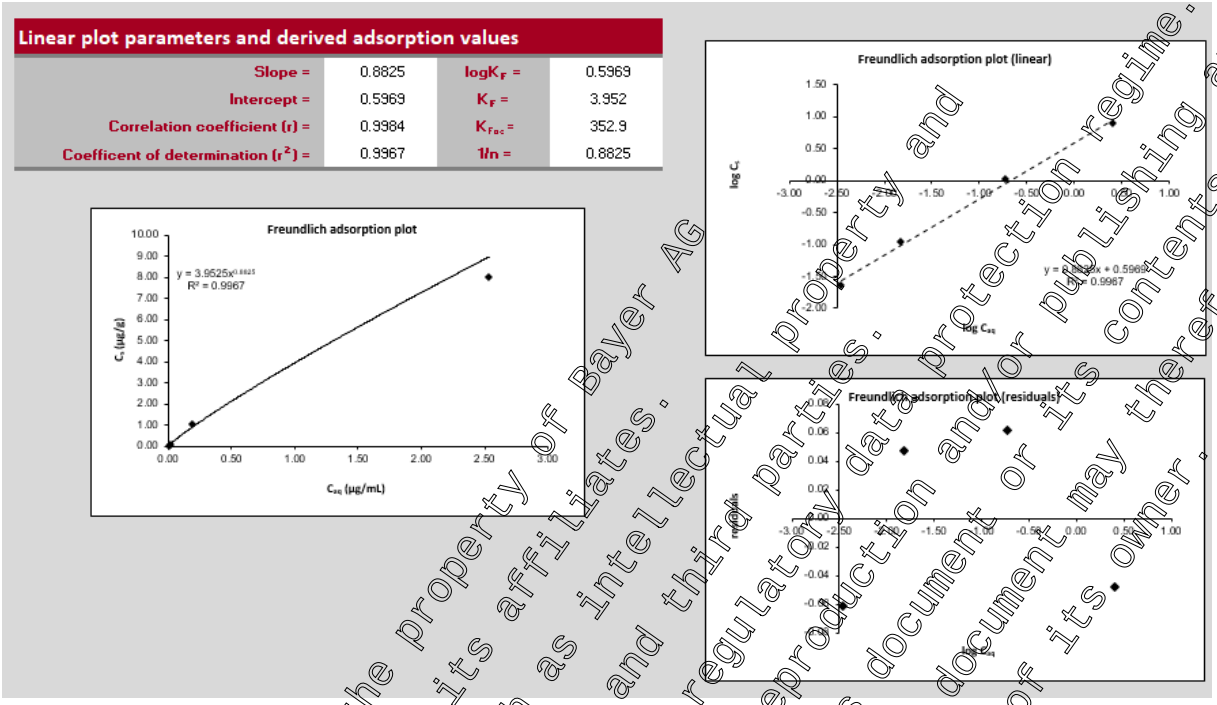


Figure 7.1.3.1.2-14: Freundlich Isotherms of M03 (spiroxamine-N-oxide) on Wolf Ranch soil

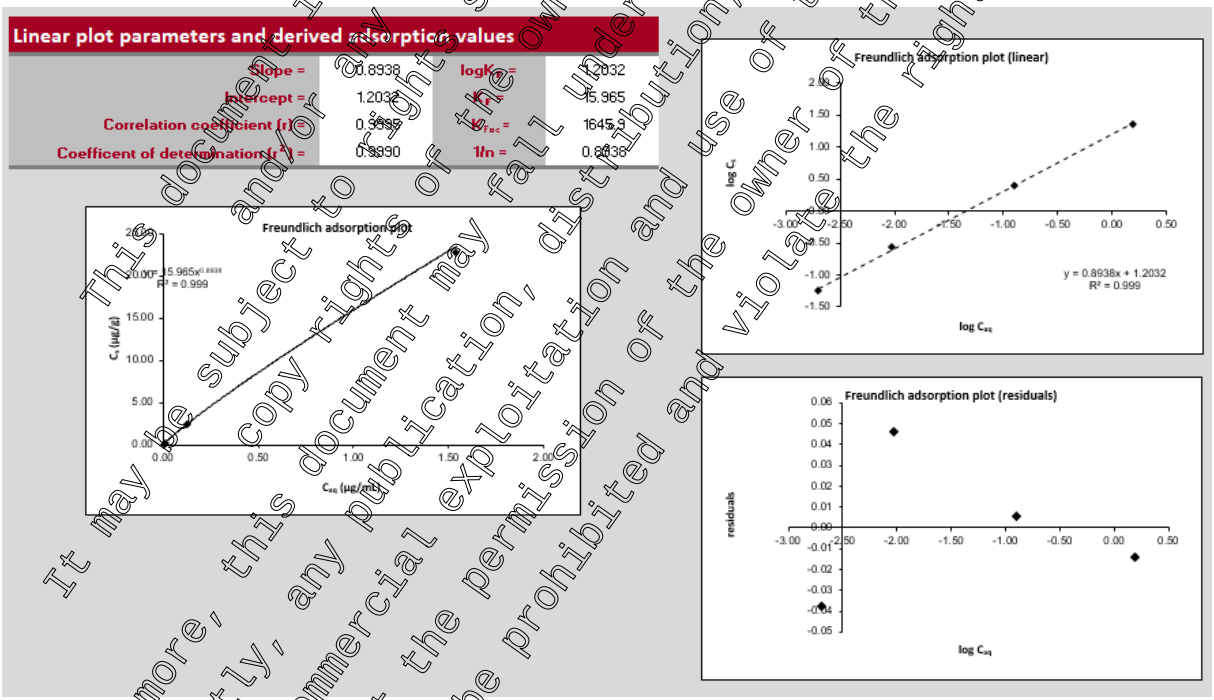
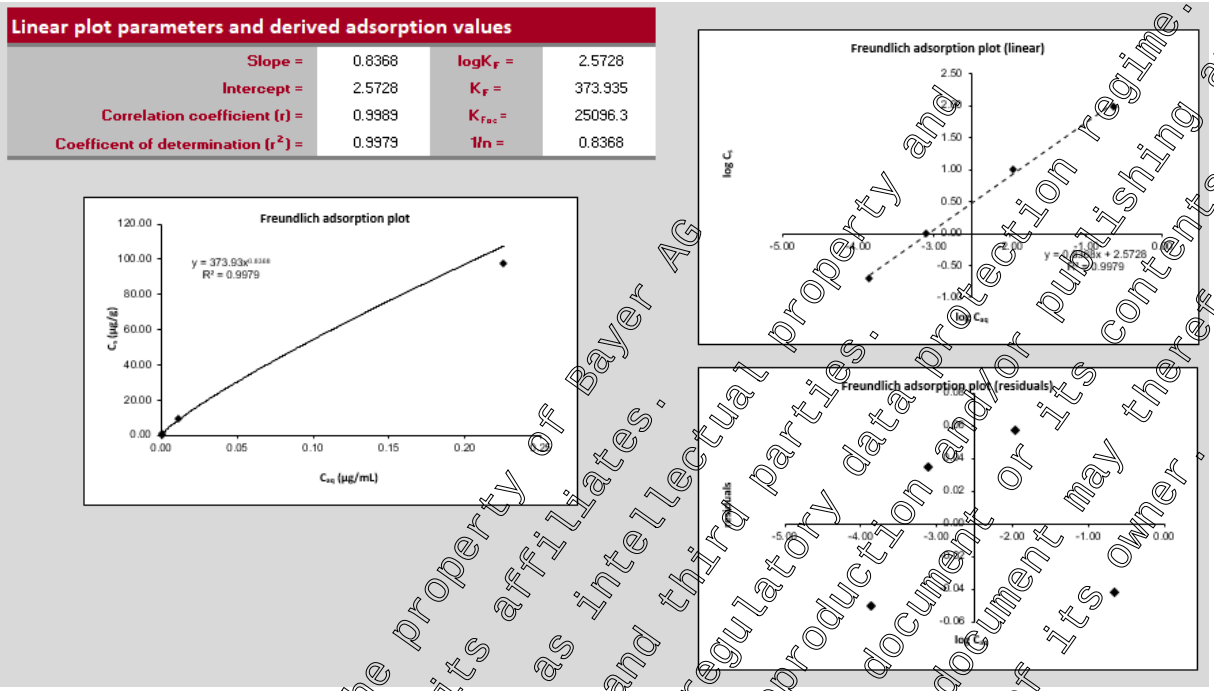


Figure 7.1.3.1.2-15: Freundlich Isotherms of M03 (spiroxamine-N-oxide) on Stanley soil



Overall, the study was conducted to a good standard, and all soils pass the checklist criteria. Overall, the study conclusions can be considered reliable.

III. Conclusions

Freundlich adsorption coefficients (K_f) ranged from 1.767-370.899 L/kg (n=4). When normalised to organic carbon, Freundlich adsorption coefficients (K_{foc}) ranged from 5523-24892.5 L/kg, indicating that M03 (spiroxamine-N-oxide) exhibits medium to immobile mobility in soil according to the McCall mobility classification (McCall et al 1981). The 1/n values ranged from 0.835-0.939, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

Adsorption was shown to be generally correlated with organic carbon content and there was no clear correlation with the pH of the soils.

The OECD 106 Checklist (v2) was used to evaluate the study. The evaluation confirmed acceptability according to the quality criteria and therefore acceptable for regulatory use. A number of minor differences in the derived K_{FOC} values were noted but this was attributed to the original calculation being performed on averages rather than single timpoints. Since the differences were very minor, for consistency the endpoints determined in the study report are used and considered to have no impact on the risk assessment.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to study guideline(s) OECD 106 (required guideline). The study is considered valid to assess the adsorption and desorption characteristics of the spiroxamine metabolite M03 (spiroxamine-N-oxide) in soil.

Data Point:	KCA 7.1.3.1.2/04
Report Author:	[redacted]
Report Year:	1998
Report Title:	Adsorption of [cyclohexyl-1-14C]WAK 6301 on Stanley soil before and after destruction of organic matter
Report No:	MR-598/98
Document No:	M-006087-01-1
Guideline(s) followed in study:	USEPA (=EPA): Section N, 163-1
Deviations from current test guideline:	Yes Only two concentration were investigated in this study and no stability checks were conducted. Therefore, the OECD 106 checklist could not be completed on this study.
Previous evaluation:	No, submitted, not evaluated
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

The adsorption of [cyclohexyl-1-^{14}C]-M03 (spiroxamine-N-oxide) was investigated in this supplementary study on one North American soil, Stanley (silty clay loam, pH 5.5, 1.49 % OC) using the indirect method. Two separate tests were conducted on Stanley soil. One test to determine the adsorption of [cyclohexyl-1-^{14}C]-M03 and a second using hydrogen peroxide to determine the effect of the organic matter of the soil sample. For each system two different concentrations (4.854 and 0.048 mg/L) were investigated. A soil/water ratio of 1:20 was used in both studies, which was determined from a previous study (Fent, 1997, CA 7.1.3.1.2/03). For each study, one gram (dry weight) of soil was combined with 20 mL of $CaCl_2$ solution. The soil solutions were agitated for 1 hour in both the adsorption and hydrogen peroxide test in the dark at 20±1°C.

For the adsorption study no desorption measurements were determined. After the adsorption step the aqueous supernatant was separated from the soil by centrifugation and the amount of M03 (spiroxamine-N-oxide) in the supernatants was determined and quantified by liquid scintillation counting (LSC) and thin layer chromatography (TLC).

The chromatographic analyses of the centrifuged supernatants (without hydrogen peroxide treatment) for the high-test concentration showed that 96% of the measured radioactivity could be assigned to parent. In the case of the H_2O_2-treated soil, the values were lower (*ca*. 85% AR).

Based on a carbon organic content of 1.49%, the K_{oc} value of Stanley was calculated to be 13,441 mL/g. The H_2O_2-treatment of soil was designed to have destroyed the organic carbon of the soil and lower the Kd value. However, the Kd value showed an increased value, resulting in an extremely high K_{oc} value. It was proposed by the study author that the treatment of the soil enhanced the sorption capacity and sorption locations. In this case, it is assumed to be due to the clay minerals (montmorillonit etc.).

Based on the classification system of McCall *et al*. (1980), M03 (spiroxamine-N-oxide) must be considered to be immobile in soils like that tested in this study.

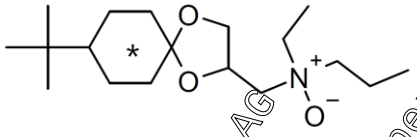
The OECD 106 checklist could not be used on the data presented in this summary as insufficient concentrations were investigated. Therefore, this summary is supportive information only.

I. Materials and Methods

A. Materials

1. Test Items

Test substance:



[cyclohexyl-1-^{14}C]-M03 (spiroxamine-N-oxide)	* Indicates position of radiolabel
Specific activity:	3.44 MBq/mg
Radiochemical purity:	>99% (HPLC)
Batch number	KML 2352

2. Test Soil

The soil used in this study were collected from Stanley (Kansas, USA). After collection, soils were homogenized, air-dried, and passed through a 2 mm sieve. For the adsorption study, a soil moisture of 3.11% (drying at 105°C) was determined for the sieved soil. For the hydrogen peroxide study, a soil moisture of 7.69% (drying at 105°C) was determined.

Table CA 7.1.3.1.2-17: Physico-chemical properties of test soil

Parameter	Soil
Soil Designation	**Stanley**
Geographic Location	
City	Stanley, Kansas
Country	USA
Textural Classification (USDA)	Silty clay loam
Sand (%)	17.0
Silt (%)	41.0
Clay (%)	42.0
pH	
in H_2O	6.1
in $CaCl_2$	5.5
Organic Matter (%) *	2.56
Organic carbon (%)	1.49
Cation Exchange Capacity (meq/100 g)	n.a.
Water Holding Capacity (%)	
maximum	n.a.
at 1/3 bar	34.0

n.a.: not analysed

B. Study Design

1. Experimental conditions

Two separate tests were conducted on Stanley soil. One test to determine the adsorption of [cyclohexyl-1-^{14}C]-M03 and a second using hydrogen peroxide to determine the effect of the organic matter of the

soil sample.

For the hydrogen peroxide study 10 grams of soil was treated with 30 mL of a 30% H_2O_2 solution for five hours. The soil was then boiled for a few minutes before being cooled and filtered. The remaining soil was air-dried.

For application [cyclohexyl-1-^{14}C]-M03 was dissolved in 3 mL of acetonitrile resulting in a stock solution containing 9.537 MBq or 2.773 mg of test substance per 3 mL. From the stock solution two different concentrations (4.854 and 0.048 mg/L) were prepared following dilution with $CaCl_2$ solution.

A soil/water ratio of 1:20 was used in both studies, which was determined from a previous study (Fent, 1997, CA. 7.1.3.1.2/03). For each study, one gram (dry weight) of soil was combined with 20 mL of $CaCl_2$ solution. The soil solutions were agitated for 1 hour in both the adsorption and hydrogen peroxide test.

Adsorption phase

Parameter		Description
Soil condition		Soils were air-dried and sieved to 2 mm
Soil sample weight		1 g (dry weight) for Stanley soil
Equilibration solution		No pre-equilibration conducted
Control (preliminary experiment)		No soil (test substance in 0.01M $CaCl_2$ only)
Concentration	Analytically (LSC) measured concentrations	Measured concentrations (LSC) in test solution: 4.854 and 0.048 mg/L
Identity and concentration of co-solvent		Dosing stock made up in acetonitrile Study media - calcium chloride
Soil: Solution ratio		1:20
Number of replicates	Control	Not stated
	Treatments	Duplicate
Equilibration conditions	Time	1 hrs
	Temperature	20±1°C
	Dark	In the dark
	Shaking method	Overhead shaker
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	5,000 rpm
	Duration	15 minutes
	Method of separating supernatant	Supernatant was decanted.

2. Analytical Procedures

For the adsorption study no desorption measurements were determined. After the adsorption step the aqueous supernatant was separated from the soil by centrifugation and the amount of M03 (spiroxamine-N-oxide) in the supernatants was quantified by LSC.

TLC analysis was used to determine the purity of the test substance and the proportion of any degradation products. Aliquots of 4 to 100 µL were spotted on a SI-60 TLC plate. The components were separated by TLC in a saturated glass tank using the solvent system acetonitrile/H_2O/NH_4OH (80:18:2 v:v:v).

The partition of the test substance in the adsorption equilibrium experiment was determined based on the radioactivity content in the supernatant only (i.e. the indirect method). Adsorption and desorption isotherms were calculated by linear regression analysis of the adsorption or desorption data according

to the Freundlich equation.

The limit of detection (LOD) was not specified in the report, however, the concentrations of the test substance used are likely sufficient to allow adequately accurate measurements of levels of the test substance.

II. Results and Discussion

The equilibrium concentrations of [cyclohexyl-1-^{14}C]-M03 (spiroxamine-N-oxide) is shown in Table CA 7.1.3.1.2-18. The proportion of [cyclohexyl-1-^{14}C]-M03 (spiroxamine-N-oxide) adsorbed onto soil Stanley ranged from 92.4-99.4% AR. The chromatographic analyses of the centrifuged supernatants, after establishment of the equilibrium (without hydrogen peroxide treatment) for the high-test-concentration, showed that 96% of the measured radioactivity could be assigned to parent. In the case of the H_2O_2-treated soil the values were lower (*ca*. 85% AR).

Table CA 7.1.3.1.2-18: Concentrations at adsorption equilibrium for [cyclohexyl-1-^{14}C]-M03 (spiroxamine-N-oxide)

Starting concentration (mg/L)	Replicate	Concentration in aqueous solution (µg/20 mL)		Adsorbed on soil at equilibrium (µg/20 g)
		Initial	At equilibrium	
4.854	A	97.07	7.41 ± 0.05	89.66 ± 0.05
	B	97.07	1.97 ± 0.01	95.11 ± 0.05
0.048	A	0.96	0.03*	0.93*
	B	0.96	0.01 ± 0.00	0.94 ± 0.00

* No duplicate measured.

The constants of the adsorption isotherms according to Freundlich were calculated by linear regression. Freundlich constants and the Kd-values describing the adsorption are given in Table CA 7.1.3.1.2-19.

Table CA 7.1.3.1.2-19: Adsorption isotherms from [cyclohexyl-1-^{14}C]-M03 (spiroxamine-N-oxide)

Soil	1/n	Kd	KOC
Stanley (native)	0.8096	209	13,441
Stanley (H_2O_2 treated)	0.9060	777	→ ∞

Based on a carbon organic content of 1.49%, the K_{oc} value of Stanley was calculated to be 13,441 mL/g. The H_2O_2-treatment of soil was designed to have destroyed the organic carbon of the soil and lower the Kd value. However, the Kd value showed an increased value, resulting in an extremely high K_{oc} value. It was proposed by the study author that the treatment of the soil enhanced the sorption capacity and sorption locations. In this case, it is assumed to be due to the clay minerals (montmorillonit etc.).

Based on the classification system of McCall *et al*. (1980) M03 (spiroxamine-N-oxide) must be considered to be immobile in soils like that tested in this study

III. Conclusions

The adsorption of [cyclohexyl-1-^{14}C]-M03 (spiroxamine-N-oxide) was investigated in a supplementary study on one North American soil, Stanley (silty clay loam, pH 5.5, 1.49 % OC) using the indirect method. Two separate tests were conducted on Stanley soil. One test to determine the adsorption of [cyclohexyl-1-^{14}C]-M03 and a second using hydrogen peroxide to determine the effect of the organic matter of the soil sample. For each system two different concentrations (4.854 and 0.048 mg/L) were investigated.

Based on the classification system of McCall *et al.* (1980) M03 (spiroxamine-N-oxide) must be considered to be immobile in soils. The OECD 106 checklist could not be used on the data presented in this summary as insufficient concentrations were investigated. Therefore, this summary is supportive information only.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to USEPA (=EPA): Section N, 163-1 study guidelines, which were in force at the time of study completion. However, the study does differ significantly to current OECD 106 guidelines and as a result should only be considered as supporting information only.

Data Point:	KCA 7.1.3.1.2/05
Report Author:	TBA
Report Year:	TBA
Report Title:	TBA
Report No:	TBA
Document No:	TBA
Guideline(s) followed in study:	OECD Guideline for Testing of Chemicals, No. 307 Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
Remarks previous evaluation:	Not applicable (New study, not previously submitted)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

**2 A study investigating the soil sorption properties of metabolite M06 is currenly on-going and will be supplied as part of a top up submission (estimate September 2021).

CA 7.1.3.2 Aged sorption

A study investigating the aged sorption properties of the active substance spiroxamine has not been performed (higher tier option).

CA 7.1.4 Mobility in soil

CA 7.1.4.1 Column leaching studies

CA 7.1.4.1.1 Column leaching of the active substance

Adequate soil sorption parameters for the active substance spiroxamine are provided under Point CA 7.1.3.1.1, consequently column leaching studies with the active substance are not required. However, one existing study investigating the leaching behaviour of spiroxamine applied as a formulation (KCA 7.1.4.1.1/03 (M-006196-01-2)) and two existing studies investigating the leaching behaviour of aged residue of spiroxamine in soil are available (KCA KCA 7.1.4.1.1/01 (M-006198-01-1) and KCA 7.1.4.1.1/02 (M-006194-01-1)). These studies have therefore been included for completeness and as supplemental information.

Substance	Report reference	Document no.	Comment
Spiroxamine	KCA 7.1.4.1.1/01	M-006189-01-2	Submitted for first approval of spiroxamine, 1999. Reviewed under UP. Considered valid and acceptable.
Spiroxamine	KCA 7.1.4.1.1/02	M-006194-01-1	
Spiroxamine	KCA 7.1.4.1.1/03	M-006196-01-2	Submitted but not evaluated previously. Included for completeness only.

The mobility of [cyclohexyl-1-^{14}C]-spiroxamine and its degradation products were investigated by column leaching experiments in three soils: BBA 2.1 (#SP149, loamy sand; pH 5.4, 0.54% OC), BBA 2.1 (#SP149, sand; pH 5.7, 0.57% OC) and Laacherhof (silty loam; pH 6.4, 1.08% OC) following ageing for 1 and 3 months under aerobic conditions at 20°C and 40% maximum water holding capacity in the dark.

[Cyclohexyl-1-^{14}C]-spiroxamine was applied to the soils at two nominal application rates of 375 g a.s./ha and 750 g a.s./ha and aged for one and two months. Under the given conditions, the parent compound degraded in the three soil systems relatively quickly. The majority of radioactivity was identified as parent accounting for 54.7% AR, 39.0% AR and 25.7% AR by study termination in BBA 2.1 (SP 149), BBA 2.1 (SP 1121) and Laacherhof soils respectively. M01 (desethyl) was identified as a major metabolite in all three systems reading a maximum of 7.3% AR, 8.5% AR and 8.1% AR by study termination in BBA 2.1 (SP 149), BBA 2.1 (SP 1121) and Laacherhof soils respectively. Other metabolites such as M02 (despropyl), M03 (N-oxide), M06 (acid), M11 (desethyl acid), M12 (despropyl acid) and M15 (ketone) were identified, however, no metabolite was detected at greater that 5% AR at two consistent time points.

The high sorption displayed by spiroxamine and its metabolites is reflected in the outcome of column leaching studies, with only 0.2 %AR being found in the leachate of the aged soils. The major residue in the leachate was found to be M03 (spiroxamine-N-oxide) representing only 0.03% of the applied radioactivity in column leachates. Overall, leaching behaviour of spiroxamine (including individual isomers) or its major soil metabolites is not envisaged.

Existing studies, previously evaluated

Data Point:	KCA 7.1.4.1.1/01
Report Author:	[redacted]
Report Year:	1994
Report Title:	Leaching behaviour of KWG 4168 aged in soils
Report No:	PF4000
Document No:	M-006198-01-1
Guideline(s) followed in study:	BBA Ref., Leaching behaviour of Plant Protection Products (4-2) 1986 Part IV
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The mobility of [cyclohexyl-1-^{14}C]-spiroxamine and its degradation products have been investigated by column leaching experiments in three soils: BBA 2.1 (#SP149, loamy sand; pH 5.4, 0.54% OC), BBA 2.1 (#SP149, sand; pH 5.7, 0.57% OC) and Laacherhof (silty loam; pH 6.4, 1.08% OC) following ageing

for 1 and 3 months under aerobic conditions at 20ºC and 40% maximum water holding capacity in the dark.

[Cyclohexyl-1-^{14}C]-spiroxamine was applied to the soils at two nominal application rates of 375 g a.s./ha and 750 g a.s./ha and aged for one and two months.

Tests were performed in flow through systems consisting of glass flasks each containing 100 g soil (dry weight) attached to a quartz wool trap to collect organic volatiles followed by two calcium hydroxide traps to collect carbon dioxide. Application of [cyclohexyl-1-^{14}C]-spiroxamine in a solvent (acetonitrile:trimethylamine, 100+0.5 v/v) were made to a sub-sample of soil before being thoroughly mixed. Soil samples were lightly agitated after application to aid distribution throughout the soil and to allow solvent evaporation. Samples were aged for 1 or 2 months under aerobic incubation conditions (dark, 20°C and 40% moisture capacity). Soil columns were leached using a peristaltic pump and 393 mL of water over 2 days, which corresponded to 200 mL of rainfall.

Extractable radioactivity from the soil declined in all three soils over the soils, 88.6% AR at DAT 0 to 70.5% AR by DAT 62 in BBA 2.1 (SP 149) soil. Similarly in BBA 2.1 (SP 1121) soil samples extractable radioactivity declined from 88.3% AR at DAT 0 to 59.5% AR by DAT 60. Similarly in Laacherhof soil samples extractable radioactivity declined from 79.4% AR at DAT 0 to 44.0% AR by DAT 62.

Non-extractable residues (NER) increased from 10.5% AR at DAT 0 to 18.3% AR by DAT 62 in BBA 2.1 (SP 149) soil. Similarly in BBA 2.1 (SP 1121) soil samples non-extractable radioactivity increased from 10.4% AR at DAT 0 to 20.8% AR by DAT 60. Similarly in Laacherhof soil samples non-extractable radioactivity increased from 20.0% AR at DAT 0 to 25.4% AR by DAT 62.

Under the given conditions, the parent compound degraded in the three soil systems relatively quickly. The majority of radioactivity was identified as parent accounting for 54.7% AR, 39.0% AR and 25.7% AR by study termination in BBA 2.1 (SP 149), BBA 2.1 (SP 1121) and Laacherhof soils, respectively.

M01 (spiroxamine-desethyl) was identified as a major metabolite in all three systems reading a maximum of 7.3% AR, 8.5% AR and 8.1% AR by study termination in BBA 2.1 (SP 149), BBA 2.1 (SP 1121) and Laacherhof soils respectively. Other metabolites such as M02 (spiroxamine-despropyl), M03 (spiroxamine-N-oxide), M06 (acid), M11 (desethyl acid), M12 (despropyl acid) and M15 (ketone) were identified, however, no metabolite was detected at greater that 5% AR at two consistent time points.

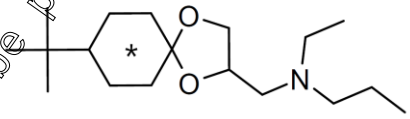
With respect to the leachate, based on the applied radioactivity, the amount of radioactivity measured in the total water was 0.2 - 0.5 %. The different application rates and soil types did not influence the percentage of radioactivity in the leachate, and on the basis of these findings, spiroxamine can be classified as immobile in soil after prior ageing.

1. Materials and Methods

A. Materials

1. Test Items

[cyclohexyl-1-^{14}C]-spiroxamine:



* Denotes position of [^{14}C]-radiolabel

Specific Activity:	2.59 MBq/mg
Radiochemical Purity:	>99%
Isomer A:B	55%: 45%

2. Test System (soil)

The biologically active soil was dried in air to an extent that it could be sieved to a particle size of 1 mm. The study was performed using one test soil as characterised in Table CA 7.1.4.1- 1.

Table CA 7.1.4.1- 1: Physico-chemical properties of test soil

Parameter	Soil		
Soil Designation:	**BBA 2.1**	**BBA 2.1**	**Laacherhof**
Batch	SP 149	SP 1121	
Textural Classification (USDA)	Loamy sand	Sand	Silty loam
Sand [50 - 2000 µm] (%)	85.9	89.4	36.5
Silt [2 – 50 µm] (%)	8.8	10.5	52.5
Clay [< 2 µm] (%)	5.3	0.1	11.0
pH in H_2O (1:1) in 0.01M $CaCl_2$ (1:1)	 6.0 5.4	 6.1 5.7	 7.0 6.4
Organic Matter (%)*	0.93	0.98	1.86
Organic Carbon (%)	0.54	0.57	1.08
Cation Exchange Capacity (meq/100 g)	5	5	10
Moisture 40% of the maximum water holding capacity (g H_2O / 100 g dry soil)	11.0	12.0	13.1
Soil Microbial Biomass (mg C/kg dry soil) Control With active substance	 0 DAT: 81 62 DAT: 62 0 DAT: 114 62 DAT: 65	 0 DAT: 84 60 DAT: 64 0 DAT: - 60 DAT: **	 0 DAT: 310 62 DAT: 230 0 DAT: 418 62 DAT: 299

* Calculated by multiplying organic carbon content by 1.724 (not reported)

** The soil sample originally proposed to determine the microbial biomass at the end of the study was instead used for a leaching experiment.

B. Study Design

1. Experimental Conditions

[Cyclohexyl-1-^{14}C]-spiroxamine was dissolved in acetronitrile:triethylamine (100+0.5 v/v) to make the two nominal application rates of 375 g a.s./ha and 750 g a.s./ha. The parent compound was added to a soil subsample. This soil subsample was air-dried in a porcelain dish and ground. The parent compound was pipetted onto the soil subsample. This sample was thoroughly mixed with a spatula and after evaporation of the solvent this subsample was added to the total amount of soil. The rest radioactivity in the porcelain dish was determined and deducted from the theoretically applied radioactivity.

Tests were performed in flow through systems consisting of glass flasks each containing 100 g soil (dry weight) and attached to an oil-coated quartz wool plug to collect organic volatiles followed by two calcium hydroxide traps to collect carbon dioxide.

Samples were aged for 1 or 2 months under aerobic incubation conditions (dark, 20°C and 40% moisture

capacity). Each column (450 mm length, 50 mm diameter) was packed with aged soil (280 mm) and flooded with water until the surface was reached. The soil columns were leached using a peristaltic pump and 393 mL of water over 2 days, reach corresponded to 200 mL of rainfall.

2. Sampling

Duplicate samples for each soil were removed for analysis after 0, 30 (32) and 60 (62) days after treatment (DAT).

3. Analytical Procedures

The amount of radioactivity in the leachate fractions was determined by means of liquid scintillation counting (LSC). Soil samples were extracted three times at room temperature with acetonitrile by addition of solvent, vigorous mechanical shaking and centrifugation. After each shaking process the supernatant was decanted through a folded filter paper. Radioactivity in extracts was determined by LSC. Degradation products were identified by comparison of the retention times of reference standards by means of two different TLC methods. Confirmatory analysis using an alternative technique was conducted by TLC with co-chromatography against reference items on selected samples. The limit of detection (LOD) in the extract was for a single peak ≥0.2 % AR and in the leachates ≥0.01% AR.

Volatile radioactivity in oil-coated quartz wool plug was extracted with 50 mL of ethyl acetate and quantified by LSC. Carbon dioxide in the calcium hydroxide traps was released by acid precipitation and quantified by LSC. Non-extractable residues (NER) in extracted soils were determined by combustion.

II. Results and Discussion

A. Data

The distribution and characterisation of radioactivity for the test soils incubated at 20°C following application of [cyclohexyl-1-^{14}C]-spiroxamine are summarized in Table CA 7.1.4.1- 2 to Table CA 7.1.4.1- 7.

Table CA 7.1.4.1- 2: Mass balance of [cyclohexyl-1-^{14}C]-spiroxamine at 20°C in BBA 2.1 (SP 149) soil under aerobic conditions [% AR]

Compound	Incubation time (DAT)		
	DAT		
	0	30	62
Volatile	n.d.	7.1	11.9
Soil extracted	88.6	78.2	70.5
Soil bound	10.5	15.7	18.3
Filter paper	2.3	1.9	2.0
Total	99.1	101.0	100.7

n.d.: not detected, DAT: days after treatment

Table CA 7.1.4.1- 3: Mass balance of [cyclohexyl-1-^{14}C]-spiroxamine at 20°C in BBA 2.1 (SP 1121) soil under aerobic conditions [% AR]

Compound	Incubation time (DAT)		
	DAT		
	0	32	60
Volatile	n.d.	12.3	17.8
Soil extracted	88.3	69.1	59.5
Non-extractable residues (NER)	10.5	15.7	18.3
Filter paper	2.6	2.1	2.1
Total	98.7	99.0	98.1

n.d.: not detected, DAT: days after treatment

Table CA 7.1.4.1- 4: Mass balance of [cyclohexyl-1-^{14}C]-spiroxamine at 20°C in Laacherhof soil under aerobic conditions [% AR]

Compound	Incubation time (DAT)		
	DAT		
	0	30	62
Volatile	n.d.	16.8	29.5
Soil extracted	79.4	59.2	44.0
Non-extractable residues (NER)	20.0	23.0	25.4
Filter paper	1.8	1.0	1.2
Total	99.4	99.0	98.9

n.d.: not detected, DAT: days after treatment

Table CA 7.1.4.1- 5: Degradation of [cyclohexyl-1-^{14}C]-spiroxamine at 20°C in BBA 2.1 (SP 149) soil under aerobic conditions [% AR]

Compound	Incubation time (DAT)		
	DAT		
	0	30	60
Spiroxamine	83.5	66.5	54.7
M01 (desethyl)	4.7	5.5	7.3
M02 (despropyl)	1.5	3.5	4.7
M03 (N-oxide)	1.9	1.7	1.7
M06 (acid)	n.d.	0.7	0.9
M11 (desethyl acid)	n.d.	0.1	0.2
M12 (despropyl acid)	n.d.	0.2	0.2
M15 (ketone)	n.d.	n.d.	0.4
Unknown	n.d.	n.d.	0.4

n.d.: not detected, DAT: days after treatment
All values expressed as percentage of applied radioactivity (% AR)

Table CA 7.1.4.1- 6: Degradation of [cyclohexyl-1-^{14}C]-spiroxamine at 20°C in BBA 2.1 (SP 1121) soil under aerobic conditions [% AR]

Compound	Incubation time (DAT)		
	DAT		
	0	32	60
Spiroxamine	81.7	50.1	39.0
M01 (desethyl)	2.7	7.4	8.5
M02 (despropyl)	2.3	4.7	5.5
M03 (N-oxide)	1.2	2.0	2.0
M06 (acid)	n.d.	4.1	3.3
M11 (desethyl acid)	n.d.	0.5	0.6
M12 (despropyl acid)	n.d.	0.3	0.4
M15 (ketone)	0.4	n.d.	n.d.
Unknown	n.d.	n.d.	0.3

n.d.: not detected, DAT: days after treatment
All values expressed as percentage of applied radioactivity (% AR)

Table CA 7.1.4.1- 7: Degradation of [cyclohexyl-1-^{14}C]-spiroxamine at 20°C in Laacherhof soil under aerobic conditions [% AR]

Compound	Incubation time (DAT)		
	DAT		
	0	30	62
Spiroxamine	75.6	42.2	25.7
M01 (desethyl)	1.3	7.4	8.1
M02 (despropyl)	1.1	5.2	5.8
M03 (N-oxide)	1.4	2.3	1.9
M06 (acid)	n.d.	1.7	1.0
M11 (desethyl acid)	n.d.	0.3	0.2
M12 (despropyl acid)	n.d.	0.2	0.2
M15 (ketone)	n.d.	n.d.	0.7
Unknown	n.d.	n.d.	0.4

n.d.: not detected, DAT: days after treatment
All values expressed as percentage of applied radioactivity (% AR)

B. Material Balance

Mass balances ranged from 99.1-101.0 % AR for BBA 2.1 (SP 149) soil, 98.1-99.0 % AR for BBA 2.1 (SP 1121) soil and 98.9-99.4 % AR for Laacherhof soil.

C. Extractable and Non-Extractable Residues

Extractable radioactivity from the soil declined in all three soils over the soils, 88.6% AR at DAT 0 to 70.5% AR by DAT 62 in BBA 2.1 (SP 149) soil. Similarly in BBA 2.1 (SP 1121) soil samples extractable radioactivity declined from 88.3% AR at DAT 0 to 59.5% AR by DAT 60. Similarly in Laacherhof soil samples extractable radioactivity declined from 79.4% AR at DAT 0 to 44.0% AR by DAT 62.

Non-extractable residues (NER) increased from 10.5% AR at DAT 0 to 18.3% AR by DAT 62 in BBA 2.1 (SP 149) soil. Similarly in BBA 2.1 (SP 1121) soil samples non-extractable radioactivity increased from 10.4% AR at DAT 0 to 20.8% AR by DAT 60. Similarly in Laacherhof soil samples non-extractable radioactivity increased from 20.0% AR at DAT 0 to 25.4% AR by DAT 62.

D. Volatile Radioactivity

Significant levels of volatile degradates evolved throughout the study period in all soils sampled. $^{14}CO_2$, reached a maximum levels of 11.9% AR, 17.8% AR and 29.5% AR by study termination in BBA 2.1

(SP 149), BBA 2.1 (SP 1121) and Laacherhof soils respectively.

E. Degradation of Parent Compound

Under the given conditions, the parent compound degraded in the three soil systems relatively quickly. The majority of radioactivity was identified as parent accounting for 54.7% AR, 39.0% AR and 25.7% AR by study termination in BBA 2.1 (SP 149), BBA 2.1 (SP 1121) and Laacherhof soils respectively.

M01 (desethyl) was identified as a major metabolite in all three systems reading a maximum of 7.3% AR, 8.5% AR and 8.1% AR by study termination in BBA 2.1 (SP 149), BBA 2.1 (SP 1121) and Laacherhof soils respectively. Other metabolites such as M02 (despropyl), M03 (N-oxide), M06 (acid), M11 (desethyl acid), M12 (despropyl acid) and M15 (ketone) were identified, however, no metabolite was detected at greater that 5% AR at two consistent time points.

F. Leachate

With respect to the leachate, based on the applied radioactivity, the amount of radioactivity measured in the total water was 0.2 - 0.5 %. The different application rates and soil types did not influence the percentage of radioactivity in the leachate, and on the basis of these findings, spiroxamine can be classified as immobile in soil after prior ageing.

III. Conclusions

The mobility of [cyclohexyl-1-^{14}C]-spiroxamine and its degradation products were investigated by column leaching experiments in three soils: BBA 2.1 (#SP149, loamy sand; pH 5.4, 0.54% OC), BBA 2.1 (#SP149, sand; pH 5.7, 0.57% OC) and Laacherhof (silty loam; pH 6.4, 1.08% OC) following ageing for 1 and 3 months under aerobic conditions at 20°C and 40% maximum water holding capacity in the dark.

[Cyclohexyl-1-^{14}C]-spiroxamine was applied to the soils at two nominal application rates of 375 g a.s./ha and 750 g a.s./ha and aged for one and two months.

Under the given conditions, the parent compound degraded in the three soil systems relatively quickly. The majority of radioactivity was identified as parent accounting for 54.7% AR, 39.0% AR and 25.7% AR by study termination in BBA 2.1 (SP 149), BBA 2.1 (SP 1121) and Laacherhof soils respectively.

M01 (desethyl) was identified as a major metabolite in all three systems reading a maximum of 7.3% AR, 8.5% AR and 8.1% AR by study termination in BBA 2.1 (SP 149), BBA 2.1 (SP 1121) and Laacherhof soils respectively. Other metabolites such as M02 (despropyl), M03 (N-oxide), M06 (acid), M11 (desethyl acid), M12 (despropyl acid) and M15 (ketone) were identified, however, no metabolite was detected at greater that 5% AR at two consistent time points.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to BBA guidelines. The study is considered valid to assess the leaching behaviour of [cyclohexyl-1-^{14}C]-spiroxamine in soil.

Data Point:	KCA 7.1.4.1.1/02
Report Author:	[redacted]
Report Year:	1998
Report Title:	Leaching behaviour of KWG 4168 after aging in soil (aged leaching) in accordance with EPA requirements
Report No:	PF4349
Document No:	M-006194-01-1
Guideline(s) followed in study:	EPA, Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate § 163-1, Leaching and Adsorption/Desorption Studies of October 18, 1982
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The mobility of [cyclohexyl-1-^{14}C]-spiroxamine and its degradation products have been investigated by a column leaching experiment in one soil: Wolf Ranch (loam; pH 7.8, 0.97% OC) following ageing for 30 days under aerobic conditions at 20°C and 75% water content (1/3 bar) in the dark. [Cyclohexyl-1-^{14}C]-spiroxamine was applied to the soil at a nominal application rate of 500 g a.s./ha (equivalent to 1.0 mg/kg).

Soil containing the aged residues were applied in a layer to the top of two 30 cm soil columns of the same soil type. The columns were irrigated for 5 days with 0.01 M $CaCl_2$ solution correspond to an amount of rainfall of 50.8 cm. The leachate was collected in 10 fractions of 100 ml each. Volatile compounds formed in the headspace of the columns were purged with air into the attached calcium hydroxide traps.

After draining the columns were put in a freezer and the frozen soil was pushed out of the glass tubes. The frozen soil column was cut into a top section of aged soil and 5 segments of about even size constituting the untreated soil. All samples were extracted three times at room temperature using acetonitrile and then a harsher extraction using methanol at 190°C. Characterisation of soils extracts was determined using thin layer chromatography (TLC).

The total recovery of radioactivity ranged from 94.4% to 96.3% AR for the two columns. A total of 26 – 69 % AR was attributed to spiroxamine, which was found in the soil samples at day 0 until the end of the ageing period of 30 days. The corresponding mineralisation rate to carbon dioxide was 1.4 % of the AR after the ageing and leaching procedure.

The parent compound and its metabolites showed a very low potential of leaching. Nearly all the radioactivity applied to the soil columns via the aged soil remained in the upper soil segment (aged soil segment) after leaching. Only 4.4 to 7.5% of the applied radioactivity was translocated into the segments 1 to 5 (0-30 cm).

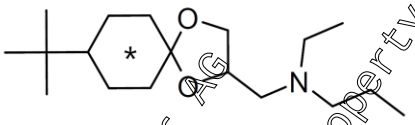
Based on the applied radioactivity, the amount of radioactivity measured in the total leachate was 0.17% for both columns. The amount of unchanged parent compound was determined to be <0.01% in column 1 and 0.02% of the applied radioactivity in column 2. More than eight metabolites were detected in the leachate extracts. The major residue was found to be M03 (N-oxide) representing 0.03% of the applied radioactivity in both column leachates. As a result, [cyclohexyl-1-^{14}C]-spiroxamine was classified as being immobile in soil after aging.

I. Materials and Methods

A. Materials

1. Test Items

[cyclohexyl-1-^{14}C]-spiroxamine



* Denotes position of [^{14}C]-radiolabel

Specific Activity:	3.63 MBq/mg
Radiochemical Purity:	>98%
Isomer A:B	53%: 46%

2. Test System (soil)

The biologically active soil was dried in air to an extent that it could be sieved to a particle size of ≤2 mm. The study was performed using one test soil as characterised in Table CA 7.1.4.1- 8.

Table CA 7.1.4.1- 8: Physico-chemical properties of test soil

Parameter		**Soil**
Soil Designation:		**Wolf Ranch**
Location		**Frensno, CA, USA**
Textural Classification (USDA)		Loam
Sand [50 - 2000 µm]	(%)	29.7
Silt [2 – 50 µm]	(%)	45.1
Clay [< 2 µm]	(%)	25.2
pH in water		8.7
pH in 0.01M $CaCl_2$ (1:1)		7.8
Organic Matter (%)*		1.67
Organic Carbon (%)		0.97
Cation Exchange Capacity (meq/100 g)		19
Water content at 75% of the 1/3 bar moisture (g/100 g dry soil)		15.2
Soil Microbial Biomass (mg C/kg dry soil) Start of study (control)		317

* Calculated by multiplying organic carbon content by 1.724 (not reported)

B. Study Design

1. Experimental Conditions

Corresponding to the intended application rate a defined amount of test substance was added to aliquots of soil (100 g of dry weight). The amount of soil corresponded to a soil layer of 5 cm taking into account a surface area of 19.6 cm^2 in the leaching column. The soil was air dried and sieved to a particle size of ≤ 2 mm. An aliquot of the radiolabelled substance was diluted in acetonitrile and added to a soil sub-sample. After mixing and evaporation of the solvent this subsample was added to the total amount of soil and the total sample was thoroughly mixed in a tumbling mixer.

After mixing the whole amount of soil, two batches were processed (extracted and analysed) directly

after the application (day 0). Five batches were filled into incubation flasks. The soil moisture was adjusted to 75 % of the water content at a pressure of 1/3 bar and the flasks were closed with a trap attachment filled with calcium hydroxide for collecting volatile organic compounds and carbon dioxide. The flasks were incubated for 30 days under dark aerobic conditions at a temperature of 20 °C ± 2°C.

After the incubation period, two batches were analysed (day 30). Two soil samples were transferred to the top of two soil columns, which had been packed over a thin bottom layer of sand and quartz wool to a height of 30 cm with untreated soil. Considering the cross section of the column and a simulated rainfall of 508 mm, the flow rate of 0.1 M calcium chloride solution was adjusted to 200 ml per day. A total volume of 996 ml was passed through the columns and collected in 10 fractions of equal volume and stored at low temperature until processing. Volatile compounds formed in the headspace of the columns were purged with air into the attached traps.

After draining the columns were put in a freezer and the frozen soil was pushed out of the glass tubes. The frozen soil column was cut into a top section of aged soil and 5 segments of about even size constituting the untreated soil. The deepest soil segment contained also the bottom layer of sea sand.

2. Analytical Procedures

Aliquots of the soil extracts were investigated by TLC on silica gel using one solvent system and on RP-silica gel using two solvent systems. Components were visualised by autoradiography, radioactivity scanning with quantification and in the case of unlabelled reference substances by reaction on the TLC plate in an iodine chamber or by spraying with cobalt-II-thiocyanate.

All extracts were radioassayed. Carbon dioxide trapped in the calcium hydroxide of the trap attachment was released with hydrochloric acid in a closed apparatus, and absorbed in a scintillation cocktail for the determination by LSC. Volatiles trapped in the polyurethane plug were extracted with ethanol and determined by LSC. Soil samples were air-dried and milled. The amount of radioactivity was determined by combustion of homogenised aliquots.

II. Results and Discussion

A. Data

The distribution of radioactive in the two columns can be seen in Table CA 7.1.4.1- 9. The characterisation of radioactivity in the aged soil and soil columns can be seen in Table CA 7.1.4.1- 10.

Table CA 7.1.4.1- 9: Distribution of [cyclohexyl-1-^{14}C]-spiroxamine in Wolf Ranch soil under anaerobic conditions [% AR]

[cyclohexyl-1-^{14}C]-spiroxamine		Column 1	Column 2
Volatiles ($^{14}CO_2$)	During incubation	1.4	1.4
	During leaching	<0.01	<0.01
	Other volatiles	<0.01	<0.01
Soil extracted	Aged soil segment	68.1	71.9
	0-6 cm	4.5	2.4
	6-12 cm	0.2	0.2
	12-18 cm	0.1	0.1
	18-24 cm	<0.1	<0.1
	24-30 cm	<0.1	<0.1
	Subtotal	92.9	74.7
Soil bound	Aged soil segment	17.3	18.5
	0-6 cm	2.3	1.3
	6-12 cm	0.2	0.1
	12-18 cm	0.1	0.1
	18-24 cm	<0.1	<0.1
	24-30 cm	<0.1	<0.1
	Subtotal	19.9	20.1
Leachate		0.2	0.2
Total		**94.4**	**96.3**

Table CA 7.1.4.1- 10: Ageing of [cyclohexyl-1-^{14}C]-spiroxamine and quantitation of compounds in the top soil layer, soil segments and leachate [% AR]

Compound	% Applied Radioactivity							
	Day 0	Day 30	Column 1			Column 2		
	Soil sample***		Aged soil*	0-6 cm**	Leach-ate	Aged soil*	0-6 cm**	Leach-ate
Volatile ($^{14}CO_2$)	n.d.	1.4		1.4			1.4	
Spiroxamine	39.9	65.2	66.2	3.6	0.004	69.9	1.6	0.017
M01 (desethyl)	n.d.	1.2	0.7	0.1	n.d.	0.8	0.1	n.d.
M02 (despropyl)	2.0	0.8	0.5	0.1	n.d.	0.6	0.1	n.d.
M03 (N-oxide)	35.9	1.1	0.6	0.5	0.028	0.5	0.6	0.029
Unknown	0.2	n.d.	n.d.	<0.1	n.d.	n.d.	<0.1	n.d.
Diffuse	0.5	n.d.	n.d.	0.1	n.d.	n.d.	n.d.	n.d.
Extracted/identified	78.5	68.3	68.1	4.5	0.05	71.9	2.4	0.14
Non-extracted	17.6	28.5	17.3	2.3	0.12	18.5	1.3	0.03
Filter	n.d.	0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Aggravated cond.	n.d.	1.3	0.81	0.08	n.d.	0.93	0.06	n.d.
Total Recovery	**95.8**	**95.7**	**94.4**			**96.3**		

n.d. No data
* Top soil layer
** First soil segment
*** Value presented as mean of the applied radioactivity.

B. Material Balance

The total recovery of radioactivity ranged from 94.4% to 96.3% AR.

B. Volatile Radioactivity

[Cyclohexyl-1-^{14}C]-spiroxamine was slowly mineralised to CO_2. During the incubation period of 30 days the portion which was degraded to $^{14}CO_2$ was measured to be about 1.4% of the applied radioactivity. The formation of $^{14}CO_2$ during the leaching period was <0.1 %. Volatile organic compounds were not detected (<0.1%).

C. Aged Soil prior to leaching

A total of *ca*. 65% of unchanged parent compound was found in the soil after an aging period of 30 days. In addition, three degradation products were detected in the organic extracts which chromatographically agreed with the reference substances M01 (desethyl), M02 (despropyl) and M03 (N-oxide). Each of the metabolites amounted about 1% of the applied radioactivity in the soil batches incubated for 30 days. At termination of the aging period, 1.4% of the applied radioactivity was mineralized to $^{14}CO_2$.

D. Leachate

Based on the applied radioactivity, the amount of radioactivity measured in the total water was 0.17% for both columns included in the leaching experiment. The amount of unchanged parent compound was determined to be <0.01% in column 1 and 0.02% of the applied radioactivity in column 2. The major residue was found to be M03 (N-oxide) representing 0.03% of the applied radioactivity in both column leachates.

E. Leaching soil column

After termination of the overhead irrigation 92.9 and 74.7% of the applied radioactivity were recovered from the aged soil segment of column 1 and 2, respectively. The total amount of radioactivity translocated into segments 1 to 5 (0-30 cm) was measured to be 4.4 to 7.5% only.

Only those segment extracts containing >1% of the applied radioactivity were analysed by TLC, i.e. the aged soil segment and segment 1 (0-6 cm) of both columns. Parent compound was the major residue detected in the extracts. The metabolite pattern was identical to the organic soil extracts of the day 30 incubation batches.

III. Conclusions

The mobility of [cyclohexyl-1-^{14}C]-spiroxamine and its degradation products was investigated by a column leaching experiment in one soil: Wolf Ranch (loam; pH 7.8, 0.97% OC) following ageing for 30 days under aerobic conditions at 20°C and 75% water content (1/3 bar) in the dark. [Cyclohexyl-1-^{14}C]-spiroxamine was applied to the soil at a nominal application rate of 500 g a.s./ha (equivalent to 1.0 mg/kg).

A total of 26 – 69 % of unchanged spiroxamine was found in the soil samples at day 0 until the end of the ageing period of 30 days. The corresponding mineralisation rate to carbon dioxide was 1.4 % of the AR after the ageing and leaching procedure.

Based on the applied radioactivity, the amount of radioactivity measured in the total leachate was 0.17% for both columns. The amount of unchanged parent compound was determined to be <0.01% in column 1 and 0.02% of the applied radioactivity in column 2. More than eight metabolites were detected in the leachate extracts. The major residue was found to be M03 (N-oxide) representing 0.03% of the applied radioactivity in both column leachates. As a result, [cyclohexyl-1-^{14}C]-spiroxamine is classified as being immobile in soil after aging.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to guidance which was acceptable at the time of. The study is considered valid to assess the mobilityof [cyclohexyl-1-^{14}C]-spiroxamine in soil.

Data Point:	KCA 7.1.4.1.1/03
Report Author:	[redacted]
Report Year:	1995
Report Title:	Versickerungsverhalten (ohne Alterung) einer KWG 4168 haltigen Pflanzenschutzmittelformulierung im Boden gemaess BBA-Anforderungen
Report No:	M-006196-01-2
Document No:	M-006196-01-2
Guideline(s) followed in study:	BBA Ref.: Leaching Behaviour of Plant Protection Products (4-2) 1986 Part IV
Deviations from current test guideline:	None
Previous evaluation:	No, submitted, not evaluated
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The leaching behaviour of formulated [cyclohexyl-1-^{14}C]-spiroxamine (EC 500) was investigated without ageing in three different soils: Speyer 2.1 (sand, pH 5.3, 0.57% OC), Speyer 2.2 (loamy sand, pH 6.3, 2.48% OC) and Speyer 2.3 (sandy loam, pH 6.6, 1.44% OC). Leaching behaviour was investigated under aerobic conditions at 20-25°C and 40% moisture holding capacity in the dark. [Cyclohexyl-1-^{14}C]-spiroxamine was applied to the soil at a nominal application rate of 750 g a.s./ha.

The columns (length = 30 cm) were constantly irrigated with about 393 ml water each over a period of 48 hours and were protected against light influence, as was the leaching water. The leaching fractions were analysed by means of liquid scintillation measurement.

Soil samples were shown to be biologically viable at the start of the study.

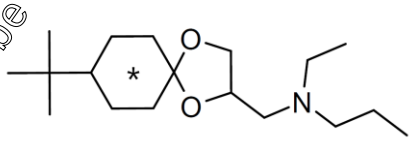
Because only traces in a range of 0.01% to 0.09% of the applied radioactivity could be determined in the leaching water, no further analysis of the leachate was performed. According to the classification spiroxamine (as EC 500) was classified as "immobile".

I. Materials and Methods

A. Materials

1. Test Items

[cyclohexyl-1-^{14}C]-spiroxamine



* Denotes position of [^{14}C]-radiolabel

Specific Activity:	3.63 MBq/mg
Radiochemical Purity:	>98%

Isomer A:B 56%: 44%

2. Test System (soil)

The soils were taken from wooden containers (which in the warm season are kept in the open and in the winter in the greenhouse) before the start of the experiment, where the soils are stored under plant cover. The field-fresh soils were dried in air and sieved through a 1 mm sieve. The study was performed using one test soil as characterised in Table CA 7.1.4.1- 11.

Table CA 7.1.4.1- 11: Physico-chemical properties of test soil

Parameter	**Soil**		
Soil Designation:	**Speyer 2.1**	**Speyer 2.2**	**Speyer 2.3**
Location	**Germany**	**Germany**	**Germany**
Batch	Sp 1121	F 23994	Sp 3121
Sampled for the study	01.03.95	01.03.95	01.03.95
Textural Classification (DIN 19682)	Sand	Loamy sand	Sandy loam
Sand [63 - 2000 µm] (%)	88.5	79.7	64.3
Silt [2 – 63µm] (%)	11.4	13.1	24.9
Clay [< 2 µm] (%)	0.1	7.2	10.8
pH in water	5.3	6.3	6.6
Organic Matter (%)*	0.98	4.28	2.48
Organic Carbon (%)	0.57	2.48	1.44
Cation Exchange Capacity (meq/100 g)	5.0	10	12.5
40% of max. water capacity (g/100 g dry soil)	11.95	17.9	n.d.
Soil Microbial Biomass (mg C/kg dry soil) Start of study	140	324	312

n.d. Not determined
* Calculated by multiplying organic carbon content by 1.724 (not reported)

B. Study Design

1. Experimental Conditions

A formulation (EC 500) was used that contained spiroxamine. The (theoretical) active ingredient application rate was 750 g a.s./ha. With an active substance content in the formulation of approx. 50 % this resulted in a formulated product application rate of 1.5 kg/ha per application. Each experimental batch was based on a soil area of 19.6 cm^2 (diameter of the leachate column = 5 cm). This gave an added quantity per soil column of *ca.* 0.15 mg and was diluted with water. To determine the homogeneity of the application solution before, during and after the addition of the application solution to the soil columns, in each case the corresponding quantities (39.5 µl) were taken and measured by LSC counter after dilution. The mean value determined here of 0.527 MBq/soil column (corresponding to 742 g a.s./ha) defines the active ingredient quantity applied per soil column.

The leachate columns consisted of a glass column approx. 45 cm long and with an internal diameter of 5 cm. The columns (2 columns for each soil) were first closed at the bottom end with quartz wool. This

was followed by a sand layer to which fresh, biologically active, air-dried soil sieved through a 1 mm sieve was added to the column in portions and filled to a height of 30 cm with gentle vibration. Soil mass added ranged from 662.7-829.9 grams of soil. After a glass frit had been added, the soils were saturated with demineralised water.

The application solution was added dropwise and the column was protected from light (black cardboard) and irrigation was started. This water quantity dripped slowly and evenly onto the column over a period of 48 h (393 ml/48 h corresponds to approx. 0.137 ml/min, the water was pumped with a peristaltic pump).

The total leachate per column was collected in one fraction. After irrigation had finished, the column was left to drip (dripping time: approx. 5 hours). This fraction was also collected in a narrow-necked glass vessel and added to the leachate fraction.

2. Analytical Procedures

The leachates were first centrifuged for 15 minutes at a speed of 2000 rpm. For this, the entire leachate was transferred for each sample quantitatively into a centrifuge beaker. The collection vessel was then rinsed 3 times with *ca.* 3 mL water each time. As slight turbidity was still present after centrifugation, the entire solution was filtered through a folded filter. The centrifuge beaker was rinsed 3 times with *ca.* 3 mL water each time.

After stirring, the radioactivity of the leachates (without centrifugation/filtration) was determined using an LSC under alkaline and acid conditions.

II. Results and Discussion

A. Data

The analysis data showed that only very small quantities of radioactivity were bonded to soil particles in the leachate as shown in Table CA 7.1.4.1- 12. Soil samples were shown to be biologically viable at the start of the study.

Table CA 7.1.4.1- 12 Radioactivity in leachates after filtration [% AR]

Soil	Mean value of % of radioactivity applied
Speyer 2.1	0.04
Speyer 2.2	0.01
Speyer 2.3	0.09

III. Conclusions

The leaching behaviour of formulated [cyclohexyl-1-^{14}C]-spiroxamine (EC 500) was investigated without ageing in three different soils: Speyer 2.1 (sand, pH 5.3, 0.57% OC), Speyer 2.2 (loamy sand, pH 6.3, 2.48% OC) and Speyer 2.3 (sandy loam, pH 6.6, 1.44% OC). Leaching behaviour was investigated under aerobic conditions at 20-25°C and 40% moisture holding capacity in the dark. [Cyclohexyl-1-^{14}C]-spiroxamine was applied to the soil at a nominal application rate of 750 g a.s./ha.

The columns (length = 30 cm) were constantly irrigated with about 393 ml water each over a period of 48 hours and were protected against light influence, as was the leaching water. The leaching fractions were analysed by means of liquid scintillation measurement.

Soil samples were shown to be biologically viable at the start of the study.

Because only traces in a range of 0.01% to 0.09% of the applied radioactivity could be determined in the leaching water, no further analysis of the leachate was performed. According to the classification l spiroxamine (as EC 500) was classified as “immobile”.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to BBA guidelines. The study is considered valid to assess the mobility of [cyclohexyl-1-^{14}C]-spiroxamine in soil.

CA 7.1.4.1.2 Column leaching of metabolites, breakdown and reaction products

Adequate soil sorption parameters for all major soil metabolites of the active substance spiroxamine (as defined under Point CA 7.4.1) are provided under Point CA 7.1.3.1.2, consequently column leaching studies with any metabolites are not required.

CA 7.1.4.2 Lysimeter studies

Adequate soil sorption parameters for the active substance spiroxamine and all major soil metabolites (as defined under Point CA 7.4.1) are provided under Points CA 7.1.3.1.1 and CA 7.1.3.1.2. Furthermore, determination of the predicted environmental concentration in groundwater conducted under Point CP 9.2.4 do not indicate groundwater concentrations exceeding the relevant trigger levels, consequently lysimeter and/or field leaching studies with the active substance or any metabolites are not required.

CA 7.1.4.3 Field leaching studies

Adequate soil sorption parameters for the active substance spiroxamine and all major soil metabolites (as defined under Point CA 7.4.1) are provided under Points CA 7.1.3.1.1 and CA 7.1.3.1.2. Furthermore, determination of the predicted environmental concentration in groundwater conducted under Point CP 9.2.4 do not indicate groundwater concentrations exceeding the relevant trigger levels, consequently lysimeter and/or field leaching studies with the active substance or any metabolites are not required.

However, three existing studies investigating the dissipation of spiroxamine under field conditions have previously been included under this data point and therefore these studies are included for completeness and as supplemental information.

Substance	Report reference	Document no.	Comment
Spiroxamine	KCA 7.1.4.3/01	M-006116-01-1	Submitted for first approval of spiroxamine, 1999. Reviewed under UP. Considered valid and acceptable.
Spiroxamine	KCA 7.1.4.3/02	M-006126-01-1	
Spiroxamine	KCA 7.1.4.3/03	M-006127-01-1	

Existing studies, previously evaluated

Data Point:	KCA 7.1.4.3/01
Report Author:	[redacted]
Report Year:	1995
Report Title:	Dissipation of KWG 4168 in soils under field conditions (Germany and Great Britain)
Report No:	RA-2078/93
Document No:	M-006116-01-1
Guideline(s) followed in study:	BBA Guideline IV-4.1 (1986)
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The details of this study are fully summarised under point KCA 7.1.2.2.1/01.

Data Point:	KCA 7.1.4.3/02
Report Author:	[redacted]
Report Year:	1995
Report Title:	Dissipation of KWG 4168 in soils under field conditions
Report No:	RA-2002/94
Document No:	M-006126-01-1
Guideline(s) followed in study:	BBA Guideline IV-4.1 (1986)
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The details of this study are fully summarised under point KCA 7.1.2.2.1/02.

Data Point:	KCA 7.1.4.3/03
Report Author:	[redacted]
Report Year:	1995
Report Title:	Dissipation of KWG 4168 in soils under field conditions (Great Britain and France)
Report No:	RA-2132/94
Document No:	M-006127-01-1
Guideline(s) followed in study:	BBA Guideline IV-4.1 (1986)
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The details of this study are fully summarised under point KCA 7.1.2.1/03.

CA 7.2 Fate and behaviour in water and sediment

Use of the active substance can potentially lead to the compound reaching surface water during treatments or *via* soil run-off. The fate and behaviour of the active substance in the aquatic environment has been investigated in laboratory studies according to the data requirements laid down in EC Regulation 283/2013.

Overview:

Behaviour in the Aquatic Environment

Degradation of spiroxamine has been assessed in the aquatic environment through standard OECD guideline tests which allow an investigation of the route and rate of abiotic and biotic processes.

Two hydrolysis studies, which have been previously evaluated, are presented and are considered reliable to address the hydrolytic behaviour of spiroxamine. It was found that in a single radiolabelled study investigating hydrolysis in pH 5, 7 and 9 buffers at 25°C, spiroxamine was stable to hydrolysis at acidic and neutral pH. At pH9, limited evidence of degradation was presented with observation of M01 (spiroxamine desethyl), M02 (spiroxamine despropyl) and M03 (spiroxamine-N-oxide) reaching a maximum of 1.6, 2.7 and 4.08 %AR respectively at study end (KCA 7.2.1.1/01 (M-006003-01-1)). It should be noted that the study was impacted by poor mass balance at pH 9, raising a number of questions about the reliability of this study. As such, a second study was performed to fully elucidate potential hydrolysis processes.

Furthermore, evaluation of isomer specific hydrolytic behaviour was also investigated in KCA 7.2.1.1/02 (M-006002-01-1). At 30°C it was found that there was some limited potential for isomer specific behaviour but, due to the slow rate of hydrolysis, these processes are not considered relevant for the overall understanding of spiroxamine behaviour in the aquatic environment. Investigations into potential preferential degradation of spiroxamine in the OECD 308 and OECD 309 systems are being conducted in order to provide a thorough understanding on potential isomer-specific behaviour and how this impacts the risk assessment in terms of use of an isomer Uncertainty Factor (UF) in the risk assessment (EFSA, 2019).

The molar absorbance coefficient of spiroxamine is <10 L/mol/cm at wavelength 295 nm (ref CA 2.4), therefore photolysis in aqueous solution is not expected to be a significant pathway. In the studies available, the half-life of spiroxamine in light exposed samples was calculated to be equivalent to 200 days midday sunlight at 40°N (KCA 7.2.1.2/01 (M-006004-01-1)). No significant metabolites were observed.

The calculated quantum yield for spiroxamine is 6.42 x 10^{-4} molecules per photon and it can be concluded that photolysis is not a significant degradation mechanism for spiroxamine. Overall, abiotic processes represented by hydrolysis and photolysis are not considered to be the primary mechanism of spiroxamine degradation in the aquatic environment.

In order to investigate biological degradation of spiroxamine, three water/sediment studies have been performed, of which two were previously evaluated during the last spiroxamine renewal. In KCA 7.2.2.3/01 (M-006015-01-1), the route of radiolabelled spiroxamine was investigated in two water sediment systems (Hoenniger water and Stilwell) at 20°C for up to 100 days. Spiroxamine rapidly dissipated from the water phase to the sediment phase, with no major metabolites formed in the Hoenniger water sediment system. In the Stilwell system, spiroxamine dissipated rapidly from the water phase with the formation of M03 (spiroxamine-N-oxide) as a major metabolite, notably observed at 11.3%AR at 0 DAT in only the Stilwell system. Since M03 (spiroxamine-N-oxide) was not observed as a major metabolite in any of the other 5 water sediment test systems, and was only observed above trigger levels at 0-2 DAT in the Stilwell system, this is considered to be a study artefact and is not presented in the proposed route of degradation in aquatic systems (Figure 7.2-1).

In a second study (KCA 7.2.2.3/04 (M-303324-01-1)), radiolabelled spiroxamine was incubated in Anglerweiher and Hoenniger water sediment systems at 20°C for 118 days. Consistent with the previous study, spiroxamine rapidly dissipated from the water phase with the observation of M06 (spiroxamine acid) as the only major metabolite reaching 25.6%AR in the water phase and 7.9%AR in the sediment phase of the Anglerweiher system. Similar patterns of metabolism were observed in the Hoenniger water system.

In order to address new data requirements on isomer behaviour (FOCUS, 2019), a new water sediment study was conducted in Emperor Lake and Calwich Abbey water sediment systems (KCA 7.2.2.3/07 (M-763128-01-1)). In this study, the degradation of individual isomers of radiolabelled spiroxamine was studied in addition to standard achiral analysis. As the chiral analysis was constrained by analytical challenges, the chiral analysis is currently being finalised and is not available to submit at this time but will be provided to the RMS upon completion. The achiral analysis demonstrated similar findings as reported by KCA 7.2.2.3/04 (M-303324-01-1). Spiroxamine dissipated rapidly from the water phase to the sediment phase where it degraded to the major metabolite M06 (spiroxamine-acid) at 28.2 %AR in the water phase and 16.3%AR in the sediment phase of the Calwich Abbey system (KCA 7.2.2.3/07 (M-763128-01-1)). Degradation in the Calwich Abbey system followed a similar profile to Emperor Lake but with less overall degradation. For Calwich Abbey, significant replicate variability was observed at 14 and 30 DAT which impacted the reliability of kinetics fits. The proposed route of degradation in aquatic systems is presented in Figure 7.2-1. Degradation of spiroxamine in water/sediment systems also led to the formation of the following minor metabolites: M01 (spiroxamine-desethyl, maximum 4.3% AR) and M02 (spiroxamine-despropyl, maximum 3.2% AR). Although other minor metabolites were sometimes observed, none exceeded levels of 3% AR at any time. Notably, M03 was not observed confirming the hypothesis that M03 formation in KCA 7.2.2.3/01 (M-006015-01-1) was not reproducible and is a study artefact.

In order to establish relevant endpoints for risk assessment, the water sediment data was assessed following the FOCUS kinetics guidance (2014). The spiroxamine persistence DT_{50} values in the total system ranged from 1.4-118 days and DT_{90} values ranged from 83.7-628 days. Modelling DT_{50} values for spiroxamine ranged from 72.6-1,000 days, with a geometric mean of 157.9 days in the total system established for use in risk assessment. The 1,000 days default DT_{50} was applied to the total system Calwich Abbey as no reliable kinetic fit could be established as a consequence of the significant replicate variability at 14 and 30 DAT preventing the identification of the breakpoint for hockey stick kinetics. As such, the geomean DT_{50} can be considered to be very conservative.

The spiroxamine persistence $DissT_{50}$ values in the surface water were very short with rapid dissipation of spiroxamine from the water to phase to the sediment phase. $DissT_{50}$ ranged from 0.27 – 7.69 days and $DissT_{90}$ values ranged from 2.5-36.4 days. Modelling $DissT_{50}$ values ranged from 0.47-8.35 days, with a geometric mean of 1.52 days in the surface water.

Dissipation of spiroxamine in sediment could not be accurately fitted to a kinetic model in three of the six test systems resulting in the use of a number of FOCUS default endpoints. As such, the spiroxamine persistence $DissT_{50}$ values in the sediment phase ranged from 24.3-1,000 days and $DissT_{90}$ values ranged from 191-3,320 days. Modelling $DissT_{50}$ values ranged from 72.0-1,000 days, with a geometric mean of 269.9 days in the sediment.

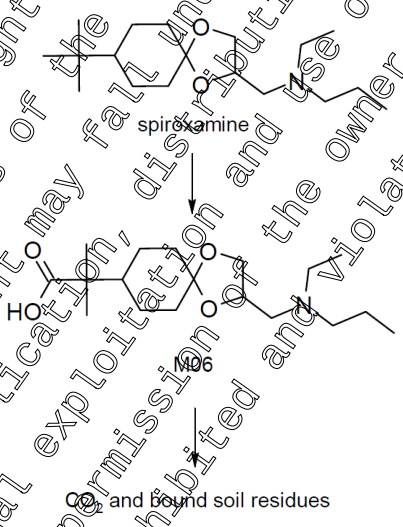
For the M06 (spiroxamine-acid) persistence values in the total system, an acceptable fit could only be determined for one of the test systems (as a consequence of a lack of a decline phase) resulting in a number of presented FOCUS default endpoints. The total system gave persistence DT_{50} values of 47 – 1,000 days and DT_{90} values ranged from 156 – 3,320 days. M06 (spiroxamine-acid) modelling total system gave a geomean DT_{50} value of 293.6 days (f.f = 0.453) for use in risk assessments.

For M06 (spiroxamine-acid) persistence in surface water DissT50 values could only be established in two of four trials, resulting in a number of presented FOCUS default endpoints, and ranged from 89.1-1,000 days and DissT90 values ranged from 156-3,320 days. M06 (spiroxamine-acid) modelling water phase also gave two established DissT50 values from the four soils, resulting in a number of presented FOCUS default endpoints, ranging from 32.8-1,000 days.

For M06 (spiroxamine-acid) persistence values in the sediment phase, an acceptable fit could only be determined for one of the four test systems resulting in a number of presented FOCUS default endpoints. The sediment phase gave a range of $DissT_{50}$ values of 89.2 – 1,000 days and a range of $DissT_{90}$ value of 296 – 3,320 days. M06 (spiroxamine-acid) modelling sediment phase gave a range of $DissT_{50}$ values from 89.2 – 1,000 days.

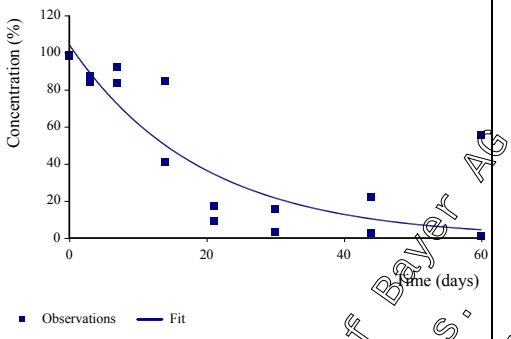
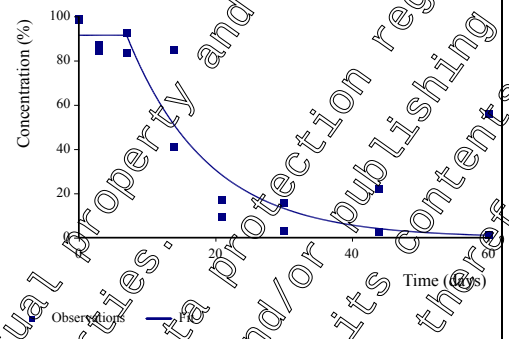
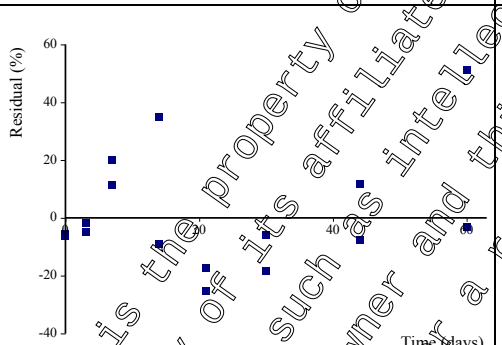
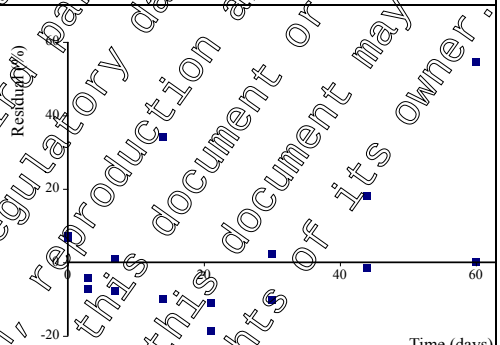
The proposed route of degradation in aquatic systems is presented in Figure 7.2-1.

Figure 7.2-1: Degradation of spiroxamine in the water/sediment systems



The behaviour of spiroxamine has also been investigated at two concentrations in pelagic water systems, with both achiral and chiral analysis performed (KCA 7.2.2.2/01 (M-763130-01-1)). At the time of submission, only the achiral results were available for submission, and this demonstrated that spiroxamine can degrade rapidly in pelagic water systems, with both concentrations degrading with a DT_{50} of approximately 10 days. Of note, the 100 µg/L system showed evidence of an 8 days lag phase prior to entering the decay phase (see Table CA 7.2-1) indicating the presence of a microbial population capable of up-regulating enzymes to degrade spiroxamine. This mechanism was also attributed to the significant data scatter seen in the 100 µg/L system. No degradation was observed in sterile controls. The major metabolites observed were found to be M01 (6.7% AR; desethyl), M02 (7.9% AR, despropyl), M03 (38.4% AR, N-oxide), M05 (9.2% AR, hydroxy), M06 (42.5% AR, acid), desamino-M06 (9.9% AR, acid), M11 (5.9% AR, desethyl acid) and M12 (9% AR, despropyl acid).

Table CA 7.2-1: Evidence of a lag phase for degradation of spiroxamine at high concentrations in pelagic systems

	SFO	HS
Plot		
Residuals		
Visual fit	Poor, data shows potential residuals	Good, residuals show no systematic error
χ^2 error (%)	22.9	20.4
t-test	k: $p < 0.05$	k_2: $p < 0.05$
DT_{50} (days)	13.2	15.3
DT_{90} (days)	43.8	34.4
Assessment	Fit not acceptable. Data shows potential trend, χ2 is high. Rate parameter differs from zero.	Fit acceptable. Data shows considerable scatter, χ2 improved with HS fitting. Rate parameter is statistically different to zero.
Discussion	HS is the best fit on the basis of χ2 and goodness of fit	

The pattern of metabolism in the OECD309 study was found to be significantly more extensive than the OECD308 study, indicating that in the presence of sediment spiroxamine degrades more slowly as a consequence of partitioning rapidly out of the aqueous phase to the sediment layer and a reduction in bioavailability. In the absence of sediment, the DT_{50} of spiroxamine degradation was significantly <40 days indicating that spiroxamine should not be considered persistent in aquatic systems but also demonstrating the importance of the sediment layer in the fate and behaviour of spiroxamine in the aquatic environment. Chiral analysis of spiroxamine in the OECD309 study are on-going at the time of submission but will be included at a later date, including a discussion on any potential UF.

CA 7.2.1 Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

CA 7.2.1.1 Hydrolytic degradation

The hydrolysis of spiroxamine has been investigated in two studies (KCA 7.2.1.1/01 and KCA 7.2.1.1/02) which were evaluated during the previous EU review.

Substance	Report reference	Document no.	Test material used	Comment
Spiroxamine	KCA 7.2.1.1/01	M-006003-01-1	[cyclohexyl-1-^{14}C]-spiroxamine	Submitted for first approval of spiroxamine, 1999. Reviewed under UP. Considered valid and acceptable.
Spiroxamine	KCA 7.1.3.1.1/02	M-006002-01-1	Non radiolabelled spiroxamine	Submitted for first renewal of spiroxamine, 2010. Reviewed under UP. Considered valid and acceptable.

Two hydrolysis studies which were previously evaluated in the last renewal are presented. It was found that in a single radiolabelled study investigating hydrolysis in pH 5, 7 and 9 buffers at 25°C, that spiroxamine was stable to hydrolysis at acidic and neutral pH. At pH9, limited evidence of degradation was presented with observation of M01 (spiroxamine desethyl), M02 (spiroxamine despropyl) and M03 (spiroxamine-N-oxide) as metabolites reaching a maximum of 1.6, 2.7 and 4.08 %AR respectively at study end (KCA 7.2.1.1/01 (M-006003-01-1)). Of note, the study was impacted by poor mass balance at pH 9 which raises a number of questions about the reliability of this study. As such, a second study was performed to fully elucidate potential hydrolysis processes.

In a second study, hydrolysis of non-radiolabelled spiroxamine was investigated in pH4, 7 and 9 buffers at 50°C for 8 days (Tier 1 test). By contrast, in this study spiroxamine was found to be stable to hydrolysis at pH 7 and 9 but showed some hydrolytic degradation at pH 4, with the Tier 2 test at pH4 at environmentally relevant temperatures demonstrating that the hydrolysis rate was limited. Under acidic conditions and at elevated temperatures (50°C) metabolites M15 (spiroxamine-ketone) and M28 (spiroxamine-aminodiol) are observed, however these are not expected to form at significant concentrations at environmentally relevant temperatures (KCA 7.2.1.1/02 (M-006002-01-1)). Overall, the two studies highlight potentially different hydrolytic behaviours of spiroxamine with the observations of KCA 7.2.1.1/01 (M-006003-01-1) for pH9 potentially unreliable given the poor recovery. The outcome of KCA 7.2.1.1/02 (M-006002-01-1) demonstrated potential hydrolytic behaviour at pH 4 forming metabolites M15 and M28 which notably aligns with the outcome of the high temperature hydrolysis study (CA 6.5.1/01 M-444801-01-1). Overall, this suggests that the outcome of KCA 7.2.1.1/02 (M-006002-01-1) is reliable and that spiroxamine slowly hydrolyses at pH4 forming M15 and M28.

Further evaluation of isomer specific hydrolytic behaviour was also investigated in KCA 7.2.1.1/02 (M-006002-01-1). At 30°C, it was found that there was potential for isomer specific behaviour, but due to the slow rate of hydrolysis, these processes are not considered relevant for the overall understanding of spiroxamine behaviour in the aquatic environment.

Data Point:	KCA 7.2.1.1/01
Report Author:	[redacted]
Report Year:	1995
Report Title:	Hydrolysis of KWG 4168 in sterile aqueous buffer solutions
Report No:	PF4074
Document No:	M-006003-01-1
Guideline(s) followed in study:	EPA Pesticide Assessment Guidelines, Subdivision N - Chemistry: Environmental Fate §161-1 Hydrolysis Studies
Deviations from current test guideline:	Yes. Hydrolysis conditions not in accordance with OECD 507
Previous evaluation:	yes, evaluated and classified DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

The hydrolysis of [cyclohexyl-1-^{14}C]-labelled spiroxamine was investigated at 25 ± 1°C at pH 5, 7 and 9, in the dark, under sterile conditions. [^{14}C]-labelled spiroxamine was dissolved in sterile buffer at a nominal concentration of 1 mg/L.

Samples were removed immediately after treatment and after 1, 3, 7, 14, 20, 24 and 30 days. Sterility of test samples was confirmed at the beginning and end of the 25°C test. Due to low mass balance recoveries at some time points in pH 9 buffer, a second experiment using this buffer was conducted, with samples removed at 1, 7, 14, 22 and 30 days.

Radiochemical balances were quantitative in all samples. Buffer samples were analysed by TLC to identify the compounds present, based on comparison of retention times with known concentration of test substance and analytical standards. Multiple TLC techniques were used as confirmatory methods.

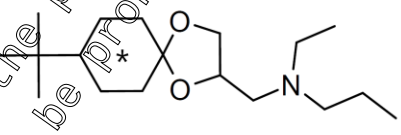
Spiroxamine did not degrade significantly in the sterile pH 5 and 7 buffer solutions after 30 days at 25°C, however decline of approximately 18% was seen after 30 days in the pH 9 buffer solution in the second experiment. This indicates that spiroxamine is hydrolytically stable under acidic and neutral conditions, and hydrolyses slowly under basic conditions. M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl) and M03 (spiroxamine-N-oxide) were detected in small amounts in the second experiment in pH 9.0 buffer, reaching maxima of 1.60, 2.27 and 4.08% AR respectively at the end of the study.

I. Materials and Methods

A. Materials

1. Test Items

[Cyclohexyl-1-^{14}C]-spiroxamine



* Denotes position of [^{14}C]-radiolabel

Specific activity:	2.59 MBq/mg
Batch number:	KML 2039
Radiochemical purity:	97.7% (TLC analysis)

2. Sterile Buffers

The buffers used were: pH 5 (0.02M acetate), pH 7 (0.02M phosphate) and pH 9 (0.02M borate) and were prepared as follows:

pH 5 – 0.04 M sodium acetate solution brought to a volume of 250 mL with water and adjusted to pH 5.0 using 0.04 M sodium hydroxide or acetic acid.

pH 7 – 74 mL of 0.04 M sodium hydroxide was added to 125 mL of 0.04 M potassium dihydrogen phosphate solution, brought to 250 mL with water. pH is adjusted to 7.0 using 0.04 M sodium hydroxide or phosphoric acid.

pH 9 – 0.04 moles of boric acid was dissolved in 1 L of 0.04 M potassium chloride solution. To 125 mL of this solution, 53 mL of 0.04 M sodium hydroxide was added and brought to 250 mL with water. The pH was adjusted to 9.0 with 0.04 M sodium hydroxide or boric acid.

All buffer solutions used filtered Milli-Q water were diluted to 0.01 M and sterilized prior to use by autoclaving.

B. Study Design

1. Experimental Conditions

The hydrolysis of [cyclohexyl-1-^{14}C]-labelled spiroxamine was investigated at 25 ± 1 °C, at pH 5, 7 and 9, in the dark, under sterile conditions. [cyclohexyl-1-^{14}C]-labelled spiroxamine was dissolved in sterile buffer at a nominal concentration of 1 mg/L. The aqueous solubility of spiroxamine was above the test concentration (see Point CA 2.5).

Due to poor material balances at 30 days after treatment, an additional experiment at pH 9.0 was conducted, also using 0.02M borate buffer made in the same way as described above.

2. Sampling

Duplicate aliquots of samples were removed immediately after treatment and after 1, 3, 7, 14, 20, 24 and 30 days. Sterility and pH of test samples was confirmed using additional samples from 0, 14 and 30 days after treatment.

The additional experiment at pH 9.0 was sampled in duplicate after 1, 7, 14, 22 and 30 days, with additional samples at 14 and 30 days for sterility testing.

3. Analytical Procedures

Aliquots were removed and directly analysed by LSC. Samples were analysed by TLC without concentration was used to identify compounds present, based on comparison of retention times with known concentration of test substance and, in some cases, analytical standards (for example metabolites). Three different TLC systems were used for the main experiment:

1) Normal phase, using acetonitrile/water/triethylamine (80/18/0.5, v/v/v) solvent system

2) Normal phase, using dichloromethane/methanol/methylethylketone (60/20/10, v/v/v) solvent system

3) Normal phase, using chloroform/methanol/methylethylketone (100/10/20, v/v/v) solvent system

Two further TLC systems were used for the supplementary experiment at pH 9.0:

1) Normal phase, using acetonitrile/water/ammonia (25%) (80:18:2, v/v/v) solvent system

2) Reverse phase, using n-hexane/dichloromethane/2-propanol/ammonia (30/70/10/2, v/v/v) solvent system on the first run, and chloroform/ethanol (50/50, v/v) on the second run.

Radioactive zones were analysed by a Bio-Imaging analyser, and the limit of detection (LOD) was for a single peak ≥1% of applied radioactivity (corresponding to 0.008 mg a.s./L). There are metabolite values below 1% AR given for the additional experiment at pH 9.0, however as none of the values are approaching 10% (max individual value of 5.70%) they are simply given as reported.

Identity of spiroxamine was also confirmed by concentrating, running on TLC system 2) above twice (the second time using material eluted with methanol from the main radioactive zone on the TLC plate from the first run), and then using GC/MS.

4. Determination of degradation kinetics

The reported hydrolysis half-life of spiroxamine in pH 5, 7 and 9 aqueous buffers was calculated based on a regression analysis of spiroxamine residues as determined by TLC.

II. Results and Discussion

A. Data

The results of the HPLC analysis of aqueous buffer solution are summarised in Table CA 7.2.1.1-1 to Table CA 7.2.1.1-4.

Table CA 7.2.1.1-1: Characterisation of radioactivity in pH 5.0 aqueous buffer solution [% AR]

Compound	Replicate	Incubation time (DAT)							
		0	1	3	7	14	20	24	30
Spiroxamine	A	96.43	99.81	100.14	101.06	100.37	101.68	100.75	99.57
	B	100.92	101.68	102.74	99.06	101.63	99.67	100.75	100.96
	Mean	**98.68**	**100.75**	**101.44**	**100.06**	**101.0**	**100.68**	**100.75**	**100.27**
Total radioactivity	A	97.96	101.20	101.52	102.37	99.65	102.85	101.57	100.78
	B	102.04	102.99	103.94	100.29	101.81	100.70	102.21	101.91
	C	-	-	-	-	102.64	-	-	105.63
	Mean	**100.0**	**102.09**	**102.73**	**101.33**	**101.36**	**101.78**	**101.89**	**102.77**

DAT: days after treatment
All values expressed as mean percentage of applied radioactivity (% AR)

Table CA 7.2.1.1-2: Characterisation of radioactivity in pH 7.0 aqueous buffer solution [% AR]

Compound	Replicate	Incubation time (DAT)							
		0	1	3	7	14	20	24	30
Spiroxamine	A	97.98	97.40	99.04	97.78	101.09	99.93	102.94	100.42
	B	101.37	99.96	102.0	100.26	97.33	98.85	104.96	103.14
	Mean	**99.68**	**98.68**	**100.52**	**99.02**	**99.21**	**99.39**	**103.95**	**101.78**
Total radioactivity	A	98.63	98.09	99.69	98.25	101.60	100.56	104.04	103.09
	B	101.37	100.63	102.0	100.26	101.86	99.46	106.33	105.96
	C	-	-	-	-	97.86	-	-	102.02
	Mean	**100.0**	**99.36**	**100.85**	**99.26**	**100.44**	**100.01**	**105.19**	**103.69**

DAT: days after treatment
All values expressed as mean percentage of applied radioactivity (% AR)

Table CA 7.2.1.1-3: Characterisation of radioactivity in pH 9.0 aqueous buffer solution – first experiment [% AR]

Compound	Replicate	Incubation time (DAT)							
		0	1	3	7	14	20	24	30
Spiroxamine	A	90.88	98.12	89.93	104.0	101.20	99.61	101.67	71.33
	B	100.78	101.54	88.18	103.79	99.40	99.05	106.64	72.35
	Mean	**95.83**	**99.83**	**89.06**	**103.90**	**100.30**	**99.33**	**104.16**	**71.84**
Total radioactivity	A	94.70	102.13	89.93	105.04	101.04	100.07	102.68	72.63
	B	105.30	105.66	90.05	105.43	102.20	99.55	107.59	72.71
	C	-	-	-	-	100.04	-	-	73.07
	Mean	**100.0**	**103.90**	**89.99**	**105.23**	**101.09**	**99.81**	**105.14**	**72.81**

DAT: days after treatment
All values expressed as mean percentage of applied radioactivity (% AR)

Table CA 7.2.1.1-4: Characterisation of radioactivity in pH 9.0 aqueous buffer solution – second experiment [% AR]

Compound	Replicate	Incubation time (DAT)					
		0	1	7	14	22	30
Spiroxamine	A	93.36	88.03	89.79	83.71	80.60	82.40
	B	101.62	91.58	91.64	89.90	87.11	87.82
	Mean	**97.49**	**89.81**	**90.72**	**86.81**	**83.86**	**85.11**
M01 (spiroxamine-desethyl)	A	0.59	0.33	n.d.	0.82	0.51	1.29
	B	0.65	0.44	n.d.	0.70	0.72	1.90
	Mean	**0.62**	**0.39**	**-**	**0.76**	**0.62**	**1.60**
M02 (spiroxamine-despropyl)	A	0.40	0.58	n.d.	1.43	1.40	2.12
	B	0.44	0.35	n.d.	1.21	1.06	2.42
	Mean	**0.42**	**0.47**	**-**	**1.32**	**1.23**	**2.27**
M03 (spiroxamine-N-oxide)	A	1.29	2.60	4.51	3.19	3.70	3.83
	B	1.41	2.40	3.22	3.78	2.40	4.32
	Mean	**1.35**	**2.50**	**3.87**	**3.49**	**4.05**	**4.08**
Diffuse radioactivity	A	0.08	n.d.	n.d.	1.06	2.98	1.24
	B	0.12	<0.01	0.46	n.d.	0.15	n.d.
	Mean	**0.1**	**-**	**0.46**	**1.06**	**1.57**	**1.24**
Total radioactivity	A	95.76	91.53	94.25	90.21	91.19	90.88
	B	104.24	94.77	95.32	95.48	91.44	96.46
	Mean	**100.0**	**93.15**	**94.79**	**92.85**	**91.32**	**93.67**

n.d.: not detected, DAT: days after treatment
All values expressed as percentage of applied radioactivity (% AR)

B. Material Balance

Good radiochemical balances were achieved with a mean recovery of applied radioactivity of 100.0 to 102.77% AR for pH 5, 99.26 to 105.19% AR for pH 7, 72.81 to 105.23% AR for pH 9 samples from the first experiment. Due to the mass balance falling below values recommended in the guideline, data from this first experiment using pH 9 buffer has not been considered further. From the second experiment in pH 9.0 buffer, recoveries of 91.32 to 100.0% AR were achieved. The pH values and sterility of samples were maintained throughout the study.

C. Transformation of the Test Substance

Spiroxamine was stable to hydrolysis at pH 5 and 7. Due to poor mass balance, values from the first experiment using pH 9 buffer were not considered, however decline of *ca.* 15% spiroxamine was seen using pH 9 buffer in a second experiment. TLC analysis confirmed that spiroxamine was the major compound detected. M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl) and M03 (spiroxamine-N-oxide) were detected in small amounts in the second experiment in pH 9.0 buffer, reaching maxima of 1.60, 2.27 and 4.08% AR respectively at the end of the study.

D. Degradation Kinetics

In the report, hydrolysis half-lives in pH 5, 7 and 9 buffers were calculated by extrapolation using single-exponential first-order kinetics. The reported degradation rates are sufficient to demonstrate that spiroxamine is stable to hydrolysis at pH values of 5 and 7 and is only slowly hydrolysed at pH value 9.

III. Conclusions

The hydrolysis of [^{14}C]-spiroxamine was studied using sterile aqueous buffer solutions at pH 5, 7 and 9 incubated at 25°C for a 30 day period.

Spiroxamine did not degrade significantly in the sterile pH 5 and 7 buffer solutions after 30 days at 25°C, however, decline of approximately 15% was seen after 30 days in the pH 9 buffer solution in the second experiment. This indicates that spiroxamine is hydrolytically stable under acidic and neutral conditions, and hydrolyses slowly under basic conditions. M01 (spiroxamine-desethyl), M02 (spirox-

amine-despropyl) and M03 (spiroxamine-N-oxide) were detected in small amounts in the second experiment in pH 9.0 buffer, reaching maxima of 1.60, 2.27 and 4.08% AR respectively at the end of the study.

Assessment and conclusion by applicant:

Study does not fully meet the current guidance and the requirements in 283/2013.

The study was conducted to study guideline(s) USEPA (=EPA): N, 161-1 (similar to required guideline, minor differences). The study is considered supplemental to assess the hydrolysis of spiroxamine as a function of pH as a consequence of poor mass balance at pH 9.

Data Point:	KCA 7.2.1.1/02
Report Author:	[redacted]
Report Year:	1997
Report Title:	Hydrolysis of KWG 4168 (Spiroxamine, proposed) as a function of pH
Report No:	145000922
Document No:	M-006002-01-1
Guideline(s) followed in study:	OECD guideline for the testing of chemicals No. 111: Hydrolysis Studies
Deviations from current test guideline:	Yes Residues: Hydrolysis conditions not in accordance with OECD 507. Fate: Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The hydrolysis of non-labelled spiroxamine was investigated at 50 ± 0.5°C at pH 4, 7 and 9, over 8 days in the dark, under sterile conditions. A tier II test was also conducted at pH 4 and 50°C (over 22 days) and 30°C (over 102 days). Non-labelled spiroxamine was dissolved in sterile buffer at a nominal concentration of 1 mg/L.

Samples were removed immediately after treatment (*ca.* 12 hours) and after 8 days in the preliminary test at 50°C. In the tier II test, duplicate aliquots from the 50°C test were removed *ca.* 12 hours after treatment and after 0.8, 1, 1.25, 2, 3.29, 6, 7.29, 15 and 22 days. From the 30°C test, duplicate aliquots were removed *ca.* 12 hours after treatment and after 7, 15, 22, 33, 75 and 102 days. Temperature and pH were monitored throughout, and sterility of test samples was confirmed at 0, 15 and 22 days incubated at 50°C, and 0, 15, 22, 33, 75 and 102 days incubated at 30°C.

Buffer samples were analysed by GC-MS to identify the compounds present, based on comparison of retention times with known concentration of test substance. Breakdown products were identified using HPLC-MS.

Spiroxamine did not degrade significantly in the sterile pH 7 and 9 buffer solutions after 8 days at 50°C (corresponding to a half-life of >1 year at 25°C). In a tier II experiment in pH 4 buffer solution, decline of approximately 80% was seen after 22 days at 50°C, and decline of approximately 50% was seen after 102 days at 30°C. This indicates that spiroxamine hydrolyses slowly under environmentally relevant conditions (temperature and pH). At low pH and at high temperature spiroxamine hydrolysed under acidic conditions. Metabolites M15 (spiroxamine-ketone) or 4-tert-butylcyclohexanone in the report and M28 (spiroxamine-aminodiol) or N-ethyl-N-propyl-3-aminopropane-(1,2)/aminodiol in the report were detected in pH 4 buffer, although they were only quantified at 22 DAT after incubation at 50°C, at 3.95

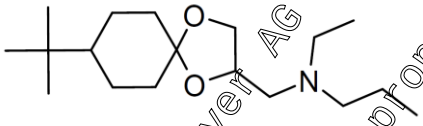
and 4.35 mg/L respectively.

I. Materials and Methods

A. Materials

1. Test Items

Non labelled spiroxamine



Batch number:	920522ELB01
Chemical purity:	99.0% (GLC analysis, isomer A 53.0% and isomer B 46.0%)

2. Sterile Buffers

The buffers used were: pH 4 (0.01M citrate), pH 7 (0.01M phosphate) and pH 9 (0.01M borate) as follows:

pH 4 – 2.105 g/L citric acid, adjusted to pH 4.0 using sodium hydroxide.

pH 7 – 3.5831 g/L disodium phosphate, adjusted to pH 7.0 using phosphoric acid.

pH 9 – 3.8118 g/L sodium tetraborate, adjusted to pH 9.0 using phosphoric acid.

All buffer solutions used filtered Milli-Q water and were sterilized at 90°C prior to use.

B. Study Design

1. Experimental Conditions

In a preliminary test, the hydrolysis of non-labelled spiroxamine was investigated at 50 ± 0.5°C, at pH 4, 7 and 9, in the dark, under sterile conditions. Spiroxamine was dissolved in sterile buffer at a nominal concentration of 10 mg/L. According to the report, the aqueous solubility of spiroxamine isomer A is 14 mg/L and isomer B is 10 mg/L at 20°C and pH 9.0, with solubility rising steeply with decreasing pH.

A tier II test was conducted at pH 4 only, temperatures of 50 ± 0.5 and 30 ± 0.5°C and all other conditions the same the preliminary test.

2. Sampling

In the preliminary test, duplicate aliquots of samples were removed *ca.* 12 hours after treatment and after 8 days. Temperature and pH were monitored throughout, and sterility was confirmed from aliquots taken at the same times as quantification samples.

The tier II test, duplicate aliquots from the 50°C test were removed *ca.* 12 hours after treatment and 0.8, 1, 1.25, 2, 3.29, 6, 7.29, 15 and 22 days was sampled in duplicate. From the 30°C test, duplicate aliquots were removed *ca.* 12 hours after treatment and after 5, 15, 22, 33, 75 and 102 days. Temperature and pH were monitored throughout, and sterility was confirmed at 0, 15 and 22 days incubated at 50°C, and 0, 15, 22, 33, 75 and 102 days incubated at 30°C.

3. Analytical Procedures

Aliquots were removed and analysed by GC-MS without concentration, based on comparison of retention times with known concentration of test substance. The carrying gas was helium and column was a 15m+4m fused silica closed coupling, I.D. 0.25 mm.

In order to identify breakdown products, reverse-phase HPLC-MS using a LiChrospher 100 Diol column, mobile phase of water+25% NH_4OH (8/2, v/v) and acetonitrile (starting gradient of 10/90, v/v).

This was reported for samples from 22 DAT at 50°C only.

4. Determination of degradation kinetics

The reported hydrolysis half-life of spiroxamine in pH 4 aqueous buffers was calculated based on linear regression of spiroxamine residues as determined by GLC using the software StatgraphicsPlus v3.0.

II. Results and Discussion

A. Data

The results from the preliminary test are summarised in Table CA 7.2.1.1-5 to Table CA 7.2.1.1-7. Results from the Tier II test are summarise in Table CA 7.2.1.1-8 to Table CA 7.2.1.1-9.

Table CA 7.2.1.1-5: Residues in preliminary test at 50°C in pH 4.0 aqueous buffer solution [mg/L]

Compound	Incubation time (DAT)	
	0	8
Spiroxamine [A]	9.62	3.31
Spiroxamine (isomer A)	5.22	2.39
Spiroxamine (isomer B)	4.40	0.92

DAT: days after treatment
All values expressed as mg/L
A Isomer A + Isomer B

Table CA 7.2.1.1-6: Residues in preliminary test at 50°C in pH 7.0 aqueous buffer solution [mg/L]

Compound	Incubation time (DAT)	
	0	8
Spiroxamine [A]	8.97	8.27
Spiroxamine (isomer A)	4.85	4.49
Spiroxamine (isomer B)	4.19	3.78

DAT: days after treatment
All values expressed as mg/L
A Isomer A + Isomer B

Table CA 7.2.1.1-7: Residues in preliminary test at 50°C in pH 9.0 aqueous buffer solution [mg/L]

Compound	Incubation time (DAT)	
	0	8
Spiroxamine [A]	4.88	5.05
Spiroxamine (isomer A)	2.71	2.80
Spiroxamine (isomer B)	2.17	2.25

DAT: days after treatment
All values expressed as mg/L
A Isomer A + Isomer B

Table CA 7.2.1.1-8: Residues in Tier II test at 30°C in pH 4.0 aqueous buffer solution [mg/L]

Compound	Incubation time (DAT)						
	0	5	15	22	33	75	102
Spiroxamine (isomer A)	5.55	5.60	6.38	5.16	5.39	4.97	3.83
Spiroxamine (isomer B)	4.94	4.92	4.67	3.98	3.05	1.60	0.87
Spiroxamine [A]	10.49	10.52	11.05	9.14	8.44	6.57	4.70

DAT: days after treatment
All values expressed as mg/L
A Isomer A + Isomer B

Table CA 7.2.1.1-9: Residues in Tier II test at 50°C in pH 4.0 aqueous buffer solution [mg/L]

Compound	Incubation time (DAT)									
	0	0.8	1	1.25	2	3.29	6	7.29	15	22
Spiroxamine (isomer A)	7.50	6.45	6.63	5.91	5.84	4.69	4.84	4.06	3.16	1.92
Spiroxamine (isomer B)	6.34	5.34	4.97	4.35	4.04	2.93	2.45	1.86	0.76	0.34
Spiroxamine [A]	13.84	11.79	11.60	10.26	9.88	7.62	7.29	5.92	3.92	2.26
M15 (ketone)	-	-	-	-	-	-	-	-	-	3.95
M28 (aminodiol	-	-	-	-	-	-	-	-	-	4.35

DAT: days after treatment
All values expressed as mg/L
A Isomer A + Isomer B

B. Transformation of the Test Substance

Spiroxamine was stable to hydrolysis at pH 7 and 9. There was a discrepancy between the applied test substance and that recovered by analysis at pH 9 in the preliminary test, however, the report explains that this loss was due to absorption to the filter. At pH 4, losses of around 66% of test substance were observed after 8 days at 50°C during a tier II test, metabolites M15 (spiroxamine-ketone) and M28 (spiroxamine-aminodiol) were detected in pH 4.0 buffer, although they were only quantified at 22 DAT after incubation at 50°C (3.95 and 4.35 mg/L respectively). The report considers the concentrations of these two metabolites correspond to a degradation of spiroxamine of 80% by the end of the study. At 30°C in pH 4 buffer, recovery of 4.70 mg/L total spiroxamine after 102 days incubation indicates a degradation of around 50%.

C. Degradation Kinetics

Spiroxamine was stable (i.e. half-life >1 year at 25°C) to hydrolysis at pH 7 and 9. At pH 4, half-lives at 20°C for isomer A and B of 790 and 120 days, respectively were calculated in the report. The reported degradation rates are sufficient to demonstrate that spiroxamine is only slowly hydrolysed.

III. Conclusions

The hydrolysis of [^{14}C]-spiroxamine was studied using sterile aqueous buffer solutions at pH 5, 7 and 9.

Spiroxamine did not degrade significantly in the sterile pH 7 and 9 buffer solutions after 8 days at 50°C (corresponding to a half-life of >1 year at 25°C). In a tier II experiment in pH 4 buffer solution, decline of approximately 80% was seen after 22 days at 50°C, and decline of approximately 50% was seen after 102 days at 30°C. This indicates that spiroxamine hydrolyses slowly under acidic conditions. Metabolites M15 (spiroxamine-ketone) and M28 (spiroxamine-aminodiol) were detected in pH 4 buffer, although they were only quantified at 22 DAT after incubation at 50°C, at 3.95 and 4.35 mg/L respectively.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to study guideline(s) OECD 111 (required guideline). The study is considered valid to assess the hydrolysis of non-radiolabelled spiroxamine as a function of pH.

CA 7.2.1.2 Direct photochemical degradation

The molar absorbance coefficient of the active substance spiroxamine is <10 L/mol/cm at wavelength 295 nm (ref CA 2.4), therefore studies investigating the direct photolysis of the active substance in aqueous solution are not needed according to the data requirements laid down in Commission Regulation (EU) No 283/2013. However, two existing studies are available and have therefore been included for completeness. One study (KCA 7.2.1.2/01) investigates the direct photolysis of the active substance in aqueous solution to address the data point. A further study (KCA 7.2.1.2/02) calculates the quantum yield for photo-degradation as supporting data. Both studies were evaluated during the previous EU review.

Substance	Report reference	Document no.	Comment
Spiroxamine	KCA 7.2.1.2/01	M-006004-01-1	Submitted for first approval of spiroxamine, 1999. Reviewed under VP. Considered valid and acceptable.
Spiroxamine	KCA 7.2.1.2/02	M-006008-01-1	

In accordance with the data requirements defined by EC Regulation 283/2013, for aqueous photolysis studies included under Point CA 7.2.1.2, metabolites are considered major if they exceed 10% AR, otherwise metabolites are considered minor.

Data Point:	KCA 7.2.1.2/01
Report Author:	[redacted]
Report Year:	1995
Report Title:	Photolysis of KWG 4168 in aqueous solution
Report No:	PF 4075
Document No:	M-006004-01-1
Guideline(s) followed in study:	USEPA (=EPA): Sec. N 162-2;
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The aqueous photolysis of [cyclohexyl-1-^{14}C]-spiroxamine was investigated after exposure to artificial light in sterile 0.01M phosphate buffer solution at pH 7 for up to 15 days continuous irradiation. [Cyclohexyl-1-^{14}C]-spiroxamine was dissolved in buffer at a concentration of 1.33 mg/L with 0.1% acetonitrile present as a co-solvent.

Quartz sample tubes were irradiated in a Heraeus Suntest apparatus equipped with a Xenon lamp with filters to block infrared light and irradiation below 280 nm. Light exposed samples were incubated at 25 ± 1°C with continuous temperature monitoring. Dark control samples were run concurrently for 15 days under the same conditions in quartz vessels wrapped in aluminium foil to prevent irradiation.

Irradiated samples were removed after 0, 2, 5, 7, 12 and 15 days after treatment, and dark controls were removed at 15 days after treatment.

Triplicate aliquots were taken for LSC to quantify the radioactivity present in solution. Buffer solutions were analysed by Thin Layer Chromatography (TLC) using three separate methods run against reference standards to confirm quantification and identity.

Light intensity was measured by radiometer and uranyl oxalate actinometer. The actinometer is prepared using 0.01M uranyl nitrate and 0.05M oxalic acid, with samples irradiated for 10 minutes and analysed via titration with 0.05 N $KMnO_4$ solution.

Recovery of applied radioactivity was 94.29 to 100.0% for light exposed samples, and 97.82% for dark control samples.

Spiroxamine exhibited slow degradation in light exposed samples and represented 78.22% of applied radioactivity after 15 days continuous irradiation. No significant degradation was detected in the dark control samples, with spiroxamine accounting for 95.73% of applied radioactivity at the end of the incubation period.

Several separate peaks below 10% were detected in irradiated samples and were identified. M03 (spiroxamine-N-oxide) reached a maximum of 4.01% AR at 5 DAT. M02 (spiroxamine-despropyl) reached a maximum of 4.47% AR at 15 DAT, still rising at the end of the study. M01 (spiroxamine-desethyl) reached a maximum of 4.53% AR at 12 DAT, though recoveries also include isomer A of M05 (spiroxamine-hydroxy). M05 (spiroxamine-hydroxy) reached a maximum of 3.13% AR at 12 DAT, though it is noted that this corresponds to isomer B of this metabolite only.

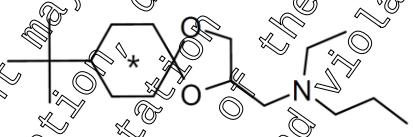
The DT_{50} of [^{14}C]-spiroxamine in light exposed samples was calculated to be equivalent to 236 days midday sunlight at 40°N. Dark incubations did not show an appreciable decline.

I. Materials and Methods

A. Materials

1. Test Items

[cyclohexyl-1-^{14}C]-spiroxamine



* Denotes position of [^{14}C]-radiolabel

Specific activity	3.63 MBq/mg
Batch number:	THS 4319
Purity:	98% (TLC analysis)

2. Sterile Buffer

The study was carried out in 0.01M phosphate buffer pH 7.0, with an electrical conductivity of 0.6 mS.

B. Study Design

1. Experimental Conditions

The aqueous photolysis of [cyclohexyl-1-^{14}C]-spiroxamine, was exposed to artificial light in sterile 0.01M phosphate buffer solution at pH 7 for up to 15 days continuous irradiation. [cyclohexyl-1-^{14}C]-spiroxamine, was dissolved in buffer at a nominal concentration of 1.33 mg/L with 0.1% acetonitrile present as a co-solvent. This application rate is based on the maximum rate of 2 kg a.s./ha,

distributed in an assumed water layer of 15 cm (standard assumption is a 30 cm water layer, which would correspond to 0.667 mg/L). Application solution was made up and dispensed into 20 quartz vessels, 12 of which were irradiated in a Heraeus Suntest apparatus equipped with a Xenon lamp with filters to block infrared light and irradiation below 280 nm. The average intensity of this lamp over the study period is 11.1 mW/cm^2. Light exposed samples were incubated at 25 ± 1°C and the temperature was monitored continuously. Dark control samples were run concurrently in two of the quartz vessels, wrapped in aluminium foil to prevent irradiation and subject to the same conditions as irradiated samples. The remaining vessels were used for sterility checks, measurement of pH, or retained in case of losses or the need to identify metabolites.

2. Sampling

Irradiated samples were removed after 0, 2, 5, 7, 12 and 15 days after treatment, and dark controls were removed at 15 days after treatment. At each time point the pH and temperature were measured.

Sterility of test samples was confirmed at the beginning and end of the study period.

3. Analytical Procedures

Triplicate aliquots were taken for LSC at each sampling point to quantify the radioactivity present in solution.

Buffer solutions were analysed using three different TLC methods without concentration, and chromatographed against reference standards to confirm results and identify metabolites:

A) Normal phase using an acetonitrile/water/25% ammonia (80/18/2, v/v/v) solvent system

B) Normal phase using a chloroform/methanol/25% ammonia (65/28/8, v/v/v) solvent system

C) Reverse phase using an n-hexane/dichloromethane/2-propanol/ammonia (30/70/10/2, v/v/v/v) solvent system for the first run, and a chloroform/ethanol (50/50, v/v) solvent system for the second run.

Radioactive zones on the TLC plates were measured using a Bio-imaging Analyser. The detection limit for a single peak in the solutions was ≥0.6% of applied radioactivity.

One isomer of M05 (spiroxamine-hydroxy) had the same chromatographic behaviour as M01 (spiroxamine-desethyl) with TLC method A). For simplification of the evaluation it was assumed that the isomers were formed in a ratio of 50:50 similar to the ratio of the diastereoisomers of the applied [^{14}C]-Spiroxamine. To validate the assumed ratio the ratio of the isomers of metabolite M02 (spiroxamine-despropyl) was determined from DAT 15 and found to be 45:55. This indicates that for M05 (spiroxamine-hydroxy) a possible error of quantification by using a ratio of 50:50 is regarded as negligible. Therefore metabolite M01 (spiroxamine-desethyl) was quantified by subtracting the amount corresponding to 50% of M05 (spiroxamine-hydroxy) (amount of separate isomer) from the total peak (sum of M01 and 50% of M05).

Light intensity was measured by radiometer and uranyl oxalate actinometer. The actinometer is prepared using 0.01M uranyl nitrate and 0.05M oxalic acid with samples irradiated for 10 minutes and analysed via titration with 0.05 N $KMnO_4$ solution.

4. Determination of degradation kinetics

The reported half-life of spiroxamine in light exposed samples was calculated by regression using mean values for each time point. The light intensity of the lamp was compared to reference values for midday summer sunlight at 40°N in order to correct to the period of equivalent full 12 hour days of natural irradiation.

II. Results and Discussion

A. Data

The recovery and distribution of radioactivity in light exposed and dark control samples is presented in Table CA 7.2.1.2-1 and Table CA 7.2.1.2-2.

Table CA 7.2.1.2-1: Distribution of radioactivity in irradiated samples incubated at pH 7 and 25°C [% AR]

Compound	Replicate	Incubation time (DAT)					
		0	2	5	7	12	15
Spiroxamine	A	98.90	89.46	79.73	87.98	81.13	81.78
	B	97.40	90.59	85.22	86.30	75.10	74.67
	Mean	**98.15**	**90.02**	**82.48**	**87.14**	**78.11**	**78.22**
M03 (spiroxamine-N-oxide)	A	1.40	1.00	5.00	1.63	3.58	2.67
	B	1.11	0.83	3.02	2.05	4.40	3.77
	Mean	**1.26**	**0.91**	**4.01**	**1.84**	**3.99**	**3.22**
M02 (spiroxamine-despropyl)	A	0.12	1.95	2.43	3.01	3.62	3.64
	B	0.12	1.31	2.11	3.51	4.60	5.29
	Mean	**0.12**	**1.63**	**2.27**	**3.26**	**4.11**	**4.47**
M01 (spiroxamine-desethyl)	A	0.22	1.76	2.48	3.43	4.63	3.84
	B	0.20	1.47	2.29	3.46	4.43	4.93
	Mean	**0.21**	**1.61**	**2.38**	**3.44**	**4.53**	**4.39**
M05 (spiroxamine-hydroxy)	A	n.d.	2.31	1.53	2.08	1.69	2.38
	B	n.d.	1.29	1.34	2.20	4.56	2.62
	Mean	-	**1.80**	**1.43**	**2.14**	**3.13**	**2.50**
Diffuse radioactivity	A	0.40	2.25	2.03	1.86	2.65	3.39
	B	0.12	1.13	1.41	2.47	4.82	6.39
	Mean	**0.26**	**1.69**	**1.72**	**2.16**	**3.73**	**4.89**
Total radioactivity	A	101.04	98.73	93.19	99.99	97.30	97.70
	B	98.95	96.60	95.39	99.99	97.91	97.67
	Mean	**100.0**	**97.67**	**94.29**	**99.99**	**97.61**	**97.69**

n.d.: not detected, DAT: days after treatment
All values expressed as mean percentage of applied radioactivity (% AR)

Table CA 7.2.1.2-2: Distribution of radioactivity in dark control samples incubated at pH 7 and 25°C [% AR]

Compound	Replicate	Incubation time (DAT)
		15
Spiroxamine	A	94.49
	B	96.98
	Mean	**95.73**
M03 (spiroxamine-N-oxide)	A	2.05
	B	1.15
	Mean	**1.60**
M02 (spiroxamine-despropyl)	A	n.d.
	B	n.d.
	Mean	-
M01 (spiroxamine-desethyl)	A	n.d.
	B	n.d.
	Mean	-
M05 (spiroxamine-hydroxy)	A	n.d.
	B	n.d.
	Mean	-
Diffuse radioactivity	A	0.50
	B	0.46
	Mean	**0.48**
Total radioactivity	A	97.04
	B	98.59
	Mean	**97.82**

n.d.: not detected, DAT: days after treatment
All values expressed as mean percentage of applied radioactivity (% AR)

B. Material Balance

Recovery of applied radioactivity was 94.29 to 100.0% for light exposed samples, and 97.82% for dark control samples.

C. Degradation of Parent Compound

Spiroxamine exhibited slow degradation in light exposed samples and represented 78.22% AR (mean) after 15 days continuous irradiation. No significant degradation was detected in the dark control samples, with spiroxamine accounting for 95.73% of AR (mean) at the end of the incubation period.

Several separate peaks below 10% were detected in irradiated samples and were identified. M03 (spiroxamine-N-oxide) reached a maximum of 4.01% AR (mean) at 5 DAT. M02 (spiroxamine-despropyl) reached a maximum of 4.47% AR (mean) at 15 DAT, still rising at the end of the study. M01 (spiroxamine-desethyl) reached a maximum of 4.53% AR (mean) at 12 DAT, though recoveries also include isomer A of M05 (spiroxamine-hydroxy). M05 (spiroxamine-hydroxy) reached a maximum of 3.13% AR (mean) at 12 DAT, though it is noted that this corresponds to isomer B of this metabolite only. None of the metabolites were observed in significant amounts.

D. Degradation Kinetics

The DT_{50} of [^{14}C]-spiroxamine in light exposed samples was calculated to be 50.5 days irradiation to the test conditions, equivalent to 236 days midday sunlight at 40°N. Dark incubations did not show an appreciable decline, therefore DT_{50} was not calculated for dark controls. DT_{50} values have not been recalculated as they sufficiently show that photolysis in aqueous solution is slow.

III. Conclusions

The results of this study indicate that aqueous photolysis is not a major mode of dissipation of [^{14}C]-spiroxamine under the test conditions employed. The DT_{50} of [^{14}C]-spiroxamine in light exposed samples was calculated to be equivalent to 236 days midday sunlight at 40°N. No significant metabolites were observed.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to study guideline(s): USEPA (=EPA): N, 162-2 (similar to required guideline). The study is considered valid to assess the direct photolysis of [cyclohexyl-1-^{14}C]-spiroxamine in aqueous solutions.

Data Point:	KCA 7.2.1.2/02
Report Author:	[redacted]
Report Year:	1994
Report Title:	Determination of the quantum yield and assessment of the environmental half-life of the direct photodegradation of KWG 4168 in water (buffer pH 7)
Report No:	PF4001
Document No:	M-006008-01-1
Guideline(s) followed in study:	Phototransofrmation of Chemicals in Water, Part A, Direct Phototransfor-mation, UBA (1992)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recog-nised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The aqueous photolysis of [cyclohexyl-1-^{14}C]-spiroxamine was investigated in conjunction with the actinometer uranyl oxalate after exposure to artificial light in aqueous solution at 25 °C. The test was performed in either 0.01M acetate (pH 4), phosphate (pH 7) and borate (pH 9) buffer solutions for UV-VIS absorption spectrum data, or phosphate buffer (pH 7) for photolysis and quantum yield measurements. Test item was incubated for up to 500 minutes continuous irradiation, whilst the actinometer was incubated for 10 minutes continuous irradiation before or after the test item. [^{14}C]-spiroxamine was dissolved in deionised water at a nominal concentration of 4.20 mg/L in acetonitrile.

Quartz vessels (one per time point) were irradiated on a merry-go-round apparatus equipped with a UV emission lamp (filtered to block infrared light and irradiation below 295 nm) maintained at 25±1 °C. No dark control samples were included.

Irradiated samples containing test item were removed after 0, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 minutes of continuous irradiation. Actinometer solutions were irradiated separately for 10 minutes either prior to, or after, test item solution irradiation.

UV-VIS absorption spectra of test solutions in buffers were analysed directly using a UV-VIS-spectrophotometer. Test solutions in water were analysed directly by reverse phase HPLC without concentration. Actinometer solutions were analysed by titration.

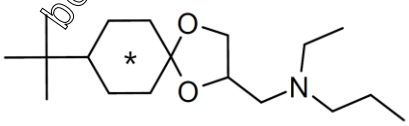
The calculated quantum yield for spiroxamine is 6.42 x 10^{-4} molecules per photon under the test conditions employed. The estimated range of environmental DT$_{50}$ under summer sunlight, estimated in GC SOLAR and the Frank and Klöpffer models is 247 days – > 1 year.

I. Materials and Methods

A. Materials

1. Test Items

[cyclohexyl-1-^{14}C]-spiroxamine



* Denotes position of [^{14}C]-radiolabel

Specific activity	2.59 MBq/mg
Batch number:	KML 2039

Purity: >99% (HPLC analysis)

2. Test solutions

The UV-Vis absorption spectrum experiment was performed using:

- 62 mg/L spiroxamine in 0.02M acetate buffer (pH 5.0)
- 31.0 mg/L spiroxamine in 0.02M acetate buffer (pH 5.0)/acetonitrile (1+1, v/v)
- 12.4 mg/L spiroxaimine in 0.02M phosphate buffer (pH 7.0)
- 155 mg/L spiroxaimine in 0.02M phosphate buffer (pH 7.0)/acetonitrile (1/1, v/v)
- 12.4 mg/L spiroxamine in 0.02M borate buffer (pH 9.0)/acetonitrile (1/1, v/v)
- 12.4 mg spiroxamine in water/acetonitrile (1/1, v/v)
- 31.0 mg spiroxamine in water/acetonitrile (1/1, v/v)
- 62.0 mg/L spiromxaine in water/acetonitrile (7/3, v/v).

The photodegradation experiment was carried out using a nominal 4.20 mg/L spiroxamine, dissolved in acetonitrile, in 0.02M phosphate buffer pH 7.0. Actinometer solution contained 0.01M uranyl ions and 0.05M oxalic acid in water.

B. Study Design

1. Experimental Conditions

The UV-Vis sorption spectra, photolysis and quantum yield of [cyclohexyl-1-^{14}C]-spiroxamine, was investigated by comparison to a chemical actinometer when exposed to artificial light in sterile buffer solutions for up to 500 minutes continuous irradiation. Quartz vessels with a 1 cm optical path length were irradiated on a merry-go-round apparatus with a UV lamp (filtered to block infrared light and irradiation below 295 nm) maintained at 25±1 °C. For photolysis and quantum yield, 22 vessels with test item solution were used, in two separate experiments.

2. Sampling

Irradiated samples were removed after 0, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 minutes irradiation. Actinometer solutions were irradiated separately for 10 minutes either prior to, or after, test item solution irradiation.

3. Analytical Procedures

UV-VIS absorption spectra were analysed using a DMS 90 spectrophotometer. Test item was quantified using reverse-phase HPLC with a Lichrosorb RP Select B column, gradient elution of water/acetonitrile/phosphoric acid (95/5/0.2, v/v/v) and acetonitrile (starting gradient 90/10, v/v) with RAMONA 4 radio-detector. The Limit of Detection (LOD) was not reported.

Samples with actinometer were analysed by titration with manganese sulphate*H_2O in (1 M) sulphuric acid and potassium permanganate (0.01M) solution.

Light intensity was measured by radiometer and uranyl oxalate actinometer. The actinometer is prepared using 0.01M uranyl nitrate and 0.05M oxalic acid, with samples irradiated for 10 minutes and analysed via titration with 0.05 M $KMnO_4$ solution.

4. Determination of degradation kinetics

Environmental half-life of spiroxamine was estimated using two models - GC-SOLAR and an arithmetic model developed by Frank and Klöpffer. Model versions were not given in the study report. Within GC-SOLAR, direct photo-transformation at the surface of pure water was assumed at 30°N, 40°N, 50°N and 60°N latitude with clear sky and typical ozone concentrations. The Frank and Klöpffer model calculates

environmental half-lives for conditions in central Europe (corresponding to 50°N latitude).

II. Results and Discussion

A. Data

The recovery of radioactivity in irradiated samples is presented in Table CA 7.2.1.2-3.

Table CA 7.2.1.2-3: Distribution of radioactivity in irradiated samples incubated at pH 7 and 25°C

	Irradiation time (min)										
	0	**50**	**100**	**150**	**200**	**250**	**300**	**350**	**400**	**450**	**500**
Experiment 1 (mg/L)	4.20	3.94	3.91	3.93	3.82	3.95	3.71	3.86	4.03	3.84	4.03
Experiment 2 (mg/L)	4.19	4.19	4.15	3.98	4.10	4.11	3.99	3.95	4.06	3.88	4.10
Experiment 1 (% applied)[A]	100.0	97.1	99.5	97.4	98.2	98.1	97.6	98.1	98.2	97.3	98.7
Experiment 2 (% applied)[A]	100.0	100.4	100.1	99.6	100.1	99.3	97.2	98.9	99.5	99.6	99.1
Mean	**100.0**	**98.8**	**99.8**	**98.5**	**99.2**	**98.7**	**97.4**	**98.5**	**98.9**	**98.5**	**98.9**

[A]In these cases '% applied' refers to recoveries compared to 0 DAT

B. UV-VIS absorption properties of test item

All the UV-Vis absorption spectra of spiroxamine in different aqueous solutions (water/acetonitrile; buffers pH 5, 7 and 9) showed one comparatively low absorption maximum in the low range of UV at about 190 to 200 nm (ε-values from 180 to max. 8 392 L/mole cm; band width to max. 215 nm).

The absorption properties indicate that direct interactions of spiroxamine in aqueous solution with the sunlight in the troposphere are possible, but only to a very low extent.

C. Quantum yield

The quantum yield of spiroxamine is calculated as 6.42×10^{-4} molecules degraded per photon.

D. Environmental half-life

The full range of half-lives of spiroxamine from GC-SOLAR and the Frank and Klöpffer models is 247 days - >1 year.

III. Conclusions

The calculated quantum yield for spiroxamine is 6.42×10^{-4} molecules per photon under the test conditions employed. The estimated range of environmental DT_{50} under summer sunlight, estimated in GC SOLAR and the Frank and Klöpffer models is 247 days – > 1 year.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study is supporting information.

The study was conducted to determine the quantum yield of the active substance using the ECETOC method. The study is considered valid to assess the quantum yield of the active substance [cyclohexyl-1-^{14}C]-spiroxamine.

CA 7.2.1.3 Indirect photochemical degradation

Data to address the data requirement for indirect photochemical degradation are not required since the molar absorption coefficient ε is < 10 M/mol/cm.

CA 7.2.2 Route and rate of biological degradation in aquatic systems

Use of plant protection products containing the active substance spiroxamine may result in contact with aquatic systems, therefore the route and rate of biological degradation in aquatic systems has been investigated in laboratory studies according to the data requirements laid down in EC Regulation 283/2013.

In accordance with the data requirements defined by EC Regulation 283/2013, for studies investigating the route and rate of biological degradation in aquatic systems included under Point CA 7.2.2, metabolites are considered major if they exceed 10% AR or exceed 5% on consecutive sampling intervals or exceed 5% and are rising at the end of the study, otherwise metabolites are considered minor.

CA 7.2.2.1 "Ready biodegradability"

A study investigating the ready biodegradability of the active substance spiroxamine in standardised laboratory studies has not been performed, instead the fate and behaviour of the active substance in aquatic systems is investigated in studies performed under Point CA 7.2.2.3 and a classification or "not readily biodegradable" is assumed.

CA 7.2.2.2 Aerobic mineralisation in surface water

Use of plant protection products containing the active substance spiroxamine may result in contact with aquatic systems, therefore the aerobic mineralisation in surface water has been investigated in laboratory studies according to the data requirements laid down in EC Regulation 283/2013.

No existing studies are available investigating the aerobic mineralisation in surface water of spiroxamine and therefore one new study was conducted (KCA 7.2.2.2/01). The study was primarily to fulfil the data requirement but also to address the new requirements of EFSA 2019[1]

Substance	Report reference	Document no	Test material used	Comment
Spiroxamine	KCA 7.2.2.2/01	M-763130-01-1	[cyclohexyl-1-^{14}C]-spiroxamine	New data not yet reviewed under UP.

The aerobic mineralisation in surface water of spiroxamine had not been previously investigated. The new study was conducted using [cyclohexyl-1-^{14}C]-spiroxamine only, however as the two ring moieties remained intact and together in all the degradation products observed this is considered sufficient to fully investigate the degradation pathway.

Data Point:	KCA 7.2.2.2/01
Report Author:	[redacted]
Report Year:	2021
Report Title:	[14C]-spiroxamine: Aerobic mineralisation in surface water - Final interim report
Report No:	VC/19/056
Document No:	M-763130-01-1
Guideline(s) followed in study:	**(EU) No. 283/2013** **(EC) No. 1107/2009** **OECD 309**
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

**1,3 The study was still ongoing at time of dossier submission therefore an interim report has been submitted and summarised. The main delays relate to 1) the identification of the component tentatively named desamino-spiroxamine-acid for which an authentic reference standard is being synthesised but not available yet (once this reference standard is available some elements of the confirmatory analysis in a secondary system will need to be completed, i.e. TLC and MS) ii) data regarding chirality are still ongoing. This work will be submitted in the final report for the study (estimate August 2021).

The aerobic mineralisation of [cyclohexyl-1-^{14}C]-spiroxamine was investigated in a "pelagic" test system (natural fresh water) at 20 ± 2˚C, for a period of 59 days. The study was carried out using water from Carsington Water, a reservoir that stores water that is pumped from the River Derwent. Spiroxamine was dosed at nominal concentrations of 10 and 100 µg/L.

Natural water (100 mL) was added to individual 250 mL glass conical flasks immediately after arrival at the test site (approximately 1 day after collection). Each flask was attached to a flow through system with volatile traps attached, the water was stirred constantly and treated with the [cyclohexyl-1-^{14}C] spiroxamine test item. The flasks were treated as soon as possible after filling to minimise any decline in biological activity relative to the natural system. The application rates averaged 10.4 and 103.4 µg/L. For each of the two test concentrations, duplicate flasks and their associated traps were removed at 0, 3, 7, 14, 21, 30, 45 and 59 days.

The redox potential, pH and dissolved oxygen content of the water in the reference flasks dosed with the non-labelled form of the test item was measured at regular intervals during the incubation. Single sterile control samples were taken for analysis at 29 and 58 days (100 µg/L level only).

The positive and solvent control samples showed rapid mineralisation of the [^{14}C]-benzoic acid. The total mean recoveries of radioactivity at the end of the incubation at 59 days were 80.7% and 72.9% AR for the positive and solvent controls, respectively. This demonstrated that the level of biological activity in the test system was sufficient. There was minimal degradation seen in the sterile control water phases with spiroxamine accounting for 90.9% AR and 93.2% AR in the day 29 and 58 day samples, respectively.

The overall material balances were good for both test concentrations: 96.7% and 94.5% AR for 10 and 100 µg/L, respectively. The total radioactivity in volatile traps at 59 days was: 2.9% and 2.7% AR for 10 and 100 µg/L, respectively.

At both dose levels mean recoveries of spiroxamine declined rapidly from 94.3 % AR (mean, 10 µg/L) and 98.4% AR (mean, 100 µg/L) reaching 0.2% AR for the lower dose concentration system at 59 DAT. For the 100 µg/L dose level spiroxamine levels decreased from 98.4% AR (mean) (100 µg/L) at the start, however, were variable for the remainder of the study with large differences between replicates from the same sampling interval, with this variability resulting in an apparent rise in mean levels at the

last sampling point to 28.4% AR (mean).

The metabolite M03 reached a maximum (mean) value of 38.4% AR on day 21 at the 100 µg/L dose level and similarly reached a mean of 35.6% AR on day 45 at the 10 µg/L dose level.

The metabolite M06 reached a maximum of 42.5% AR after 30 days at the 10 µg/L dose level before declining to 28.6% AR by the end of the study. At the 100 µg/L dose level the maximum value of 14.8% was reached after 59 days.

Mean levels of the despropyl and desethyl acid metabolites M12 and M11 never exceeded 5% AR for the 100 µg/L dose level, but at the 10 µg/L dose level both exceeded 5% AR for the last two sampling intervals at days 45 and 59.

DT_{50} and DT_{90} values for the degradation of spiroxamine in the biotic samples were calculated according to the FOCUS guidance document on degradation kinetics. An input data set was derived from the individual data for each time point and each concentration. SFO model was selected as the best fit kinetic in both cases.

Table CA 7.2.2.2-1: Summary of DegT_{50} and DegT_{90} Values

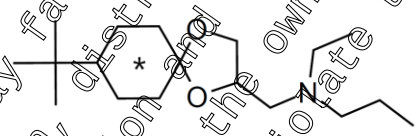
System	Kinetic Model	DT_{50} (days)	DT_{90} (days)	χ^2 error (%)	R^2	Prob >t
10 µg/L	SFO	11.3	37.6	11.4	0.948	4.02E-07
100 µg/L	SFO	13.4	44.4	23.0	0.737	5.34E-04

I. Materials and Methods

A. Materials

1. Test Items

[cyclohexyl-1-^{14}C]-spiroxamine



* Denotes position of [^{14}C]-radiolabel

Specific Activity: 4.26 MBq/mg (34.4 mCi/mmol, 1,273 MBq/mmol)

Radiochemical Purity: 98.3% (HPLC, as determined in study at the time of application)

2. Test System (water system)

The study was performed using one surface water system as characterised in Table CA 7.2.2.2-2. The study was carried out using water from Carsington Water, a reservoir that stores water that is pumped from the River Derwent. Water was collected by bucket and filtered through 100 µm filter into a 20 L container.

Table CA 7.2.2.2-2: Physico-chemical properties of the surface water

Parameter	Water system
Surface water system designation:	**Carsington water**
Geographic Location	A 300 ha hectare reservoir that stores water that is pumped from the River Derwent at Ambergate and smaller quantities of water draining off grassland surround the reservoir.
City	Carsington Water, Millfields
Country	UK
Geographical co-ordinates	SK 24813 49995
Water characteristics	
pH, at sampling depth	8.56
Dissolved oxygen concentration, at the time of treatment (mg/L)	25
Total organic carbon (ppm)	2.6
Hardness (mg equiv $CaCO_3$/L)	131
Nitrate content (mg/L)	0.7
Phosphorus content (mg/L)	0.4

B. Study Design

1. Experimental Conditions

The aerobic mineralisation of [cyclohexyl-1-^{14}C]-spiroxamine was investigated under aerobic conditions in a pelagic surface water system (Carsington water) over a period of 59 days. [Cylohexyl-1-^{14}C]-spiroxamine was dosed at nominal concentrations of 10 and 100 µg/L.

Natural water (100 mL) was added to individual 250 mL glass conical flasks immediately after arrival at the test site (approximately 1 day after collection). Each flask was attached to a flow through system with volatile traps attached (KOH and PU foam bung), the water was stirred constantly and treated with the [cyclohexyl-1-^{14}C] spiroxamine test item. The flasks were treated as soon as possible after filling to minimise any decline in biological activity relative to the natural system. The actual application rates averaged 10.4 and 103.4 µg/L.

Sterile test systems were prepared to determine influences on the degradation of the test substance induced by hydrolysis. Therefore, the respective test systems (surface water, test vessels and trap attachments) were sterilized by autoclaving for 30 minutes at 120°C.

Positive control flasks treated with [^{14}C]-benzoic acid at a nominal dose rate of 10 µg/L were used as control items to verify that the test water showed a good level of biological activity as it is known to mineralize rapidly in most natural waters. Solvent control flasks were treated to show the effects of the addition of organic solvent into the test system.

2. Sampling

Duplicate flasks and their associated traps were removed at each sampling interval. Samples were taken at zero time and following 3, 7, 14, 21, 30, 45 and 59 days incubation. The volatile traping solutions were removed at each sampling point and analysed.

A series of control flasks were treated and incubated:

- Sterile Controls: Single samples containing sterilised water were treated at the 100 µg/L level and incubated under sterile conditions for 29 and 58 days prior to analysis. These controls were used to enable differentiation between biotic and abiotic degradation of the test item.
- Positive Controls: Satisfactory biological activity of the water was demonstrated using flasks

treated with [phenyl-U-^{14}C]-benzoic acid at a nominal dose rate of 10 µg/L. The samples were incubated for 59 days, with their associated traps being changed after 1, 2, 6, 9, 13, 15, 21, 28, 45 and 52 days.

•Solvent controls: The solvent control samples were prepared, incubated and sampled as for the positive controls. These were additionally dosed with the same volume of organic solvent (50 µL acetonitrile) as used for the treatment of the spiroxamine dosed flasks.

The redox potential, pH and dissolved oxygen content of the water in the reference flasks dosed with the non-labelled form of the test item was measured at regular intervals during the incubation.

3. Analytical Procedures

The analysis of water samples to determine the total radioactivity present was determined LSC. Flask washes were performed on day 45 and day 59 samples (including day 58 sterile and day 59 positive and solvent control flasks) by adding acetonitrile to the conical flasks and agitating. The acetonitrile was decanted and radioactivity analysed by LSC analysis.

The 10 µg/L water samples were concentrated and analysed by high performance liquid chromatography (HPLC) and the 100 µg/L water samples were analysed neat by high performance liquid chromatography. Selected samples were also analysed by Liquid Chromatography Mass Spectrometry (LC-MS) to confirm the presence of spiroxamine and the main metabolites.

Trap solutions were removed for analysis at each sampling time and activity quantified by LSC. The identity of the radioactivity in the potassium hydroxide traps was characterised by the addition of barium chloride to representative trap samples and LSC analysis. The PUF bungs was extracted with acetonitrile and supernatant analysed by LSC.

The key analytical techniques and equipment used in this study are summarised below:

Analytical Method	Key Instrumentation
LSC	Packard TriCarb Liquid Scintillation Counters (dpm automatically calculated per quench curve)
HPLC	Agilent 1260 infinity HPLC system; X-Bridge Shield C18 250 x 4.6 mm column
HPLC	Agilent 1260 infinity HPLC system; Phenomenex LUX-AMP 150×4.6mm 3µm particles
TLC	Merck Silicagel 60 F_{254}; (Normal phase)
LC-MS	Thermo Q-Exactive Obitrap Mass Spectrometer Heated Electrospray/Atmospheric Pressure Chemical Ionisation Source (HESI/APcl)

4. Determination of degradation kinetics

DT_{50} and DT_{90} values for the degradation of spiroxamine in the natural water were determined following the recommendations of the FOCUS work group, with calculations performed according to the FOCUS guidance document on degradation kinetics. The kinetic evaluations and the statistical calculations were determined using the software package CAKE (v3.3).

The goodness of fit of the estimated data to the measured data was assessed by visual inspection and based on a scaled error, based on a chi-square (χ^2) test. The significance of the estimated parameters was also confirmed by a single-sided t-test. A scaled error (chi^{2}Err%) of less than 15% is generally considered a good fit whilst a t-test probability of < 0.05 (> 95% parameter significance) is considered sufficiently small.

II. Results and Discussion

A. Data

The oxygen content of the water in the flasks averaged 99.95% saturation (range 98.90% to 100.20%), while the redox potentials relative to the standard silver/silver chloride/4M KCl electrode averaged +250.82 mV (range +215.60 to +271.50 mV). The pH of the water in the reference flasks averaged 8.42 (range 8.33 to 8.55). These results show that the test water remained aerobic and within the environmentally relevant pH range throughout the study.

The distribution and characterisation of radioactivity for each water system incubated at 20°C following application of [cyclohexyl-1-^{14}C]-spiroxamine are summarised in Table CA 7.2.2.2-3 to Table CA 7.2.2.2-6.

Table CA 7.2.2.2-3: Degradation of [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions in Carsington water surface water system – low dose (10 µg/L) [mean % AR]

	Incubation time (DAT)							
	0	**3**	**7**	**14**	**21**	**30**	**45**	**59**
Water phase	99.5	96.2	94.0	91.5	92.2	95.9	93.4	93.0
Flask wash	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	1.3	1.4
PU bung extract	0.0	0.2	0.2	0.2	0.3	0.3	1.0	0.9
Ethylene glycol trap	0.0	0.1	0.1	0.1	0.0	0.0	0.2	0.3
2M KOH trap 1	0.7	0.7	0.7	0.7	0.7	0.8	1.0	1.1
2M KOH trap 2	0.5	0.6	0.6	0.6	0.7	0.6	0.7	0.7
Total volatiles	1.2	1.6	1.6	1.6	1.7	1.8	3.0	3.0
Total % AR	100.7	97.7	95.6	93.1	93.9	97.7	97.6	97.5
Overall mean ± SD	**96.7 ± 2.6%**							

n.p.: not performed

Table CA 7.2.2.2-4: Degradation of [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions in Carsington water surface water system – high dose (100 µg/L) [mean % AR]

	Incubation time (DAT)									
	0	**3**	**7**	**14**	**21**	**29 sterile**	**30**	**45**	**58 sterile**	**59**
Water phase	98.8	94.4	94.4	92.4	90.8	94.9	91.4	90.1	89.9	88.4
Flask wash	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	3.7	6.0	2.2
PU bung extract	0.0	0.2	0.3	0.6	0.9	0.0	0.7	1.2	0.1	2.1
Ethylene glycol trap	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.2	0.1	0.2
2M KOH trap 1	0.1	0.1	0.1	0.2	0.3	0.1	0.3	0.3	0.2	0.4
2M KOH trap 2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Total volatiles	0.1	0.4	0.5	0.8	1.4	0.2	1.3	1.8	0.3	2.8
Total % AR	99.0	94.8	94.9	93.3	92.2	95.1	92.6	95.5	96.1	93.2
Overall mean ± SD	**94.5 ± 2.3%***									

n.p. not performed
* Excluding sterile samples.

Table CA 7.2.2.2-5: Characterisation of [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions in Carsington water surface water system – low dose (10 µg/L). [% AR]

Compound	Replicate	Incubation time (DAT)							
		0	3	7	14	21	30	45	59
Spiroxamine	A	99.0	76.6	54.3	45.3	41.7	4.9	<0.1	0.5
	B	89.5	78.8	69.8	33.6	39.5	9.4	0.9	0.1
	Mean	**94.3**	**78.9**	**63.1**	**40.1**	**40.6**	**7.0**	**0.4**	**0.2**
M12 (spiroxamine-despropyl acid)	A	<0.1	<0.1	<0.1	<0.1	<0.1	2.1	15.2	4.7
	B	<0.1	<0.1	<0.1	<0.1	<0.1	3.0	2.8	5.4
	Mean	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**2.6**	**9.0**	**5.0**
M11 (spiroxamine-desethyl acid)	A	<0.1	<0.1	<0.1	<0.1	<0.1	2.7	5.5	6.5
	B	<0.1	<0.1	<0.1	<0.1	<0.1	1.7	6.0	5.3
	Mean	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**2.2**	**5.7**	**5.9**
M06 (spiroxamine-acid)	A	<0.1	<0.1	1.7	6.7	26.0	51.1	4.5	32.8
	B	<0.1	<0.1	<0.1	5.5	20.3	53.8	36.3	24.4
	Mean	**<0.1**	**<0.1**	**0.9**	**6.1**	**23.2**	**42.5**	**20.4**	**28.6**
Desamino-M06* (desamino-spiroxamine-acid)	A	<0.1	2.3	2.3	5.1	4.4	2.0	7.3	10.4
	B	<0.1	2.3	2.3	3.3	5.9	2.8	5.1	6.0
	Mean	**<0.1**	**2.3**	**2.3**	**4.2**	**5.2**	**2.4**	**6.2**	**8.2**
M05 (spiroxamine-hydroxy)	A	<0.1	<0.1	0.6	3.5	6.0	3.0	0.9	1.1
	B	<0.1	<0.1	<0.1	5.6	12.3	9.8	<0.1	0.9
	Mean	**<0.1**	**<0.1**	**0.3**	**4.5**	**9.2**	**6.4**	**0.4**	**1.0**
M03** (spiroxamine-N-oxide)	A	<0.1	7.2	17.6	16.7	4.2	25.7	38.3	30.0
	B	<0.1	6.8	11.1	23.2	5.0	2.6	33.0	37.0
	Mean	**<0.1**	**7.0**	**14.4**	**19.9**	**4.6**	**14.2**	**35.6**	**33.5**
M02 (spiroxamine-despropyl)	A	<0.1	<0.1	1.5	3.1	6.2	10.1	<0.1	0.8
	B	<0.1	<0.1	<0.1	4.0	6.8	5.4	1.9	<0.1
	Mean	**<0.1**	**<0.1**	**0.8**	**3.5**	**6.5**	**7.7**	**0.9**	**0.4**
M01 (spiroxamine-desethyl)	A	2.3	6.5	7.3	6.4	2.1	2.4	<0.1	<0.1
	B	2.4	5.7	5.9	6.1	3.8	2.6	1.1	<0.1
	Mean	**2.3**	**6.1**	**6.6**	**6.3**	**2.9**	**2.5**	**0.5**	**<0.1**
Unknown	A	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	5.7	<0.1	<0.1	<0.1	<0.1	1.2	<0.1	0.3
	Mean	**2.9**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**0.6**	**<0.1**	**0.2**
Minor metabolites***	A	<0.1	0.4	<0.1	5	0.7	1.2	12.2	8.7
	B	5.7	1.0	<0.1	0.9	<0.1	2.3	2.8	10.0
	Mean	**2.9**	**1.2**	**<0.1**	**1.2**	**0.4**	**1.7**	**7.5**	**9.3**
Total % AR	A	101.4	96.8	94.2	92.4	91.2	94.1	93.7	94.2
	B	97.6	94.6	90.1	84.6	90.5	95.7	93.0	91.9
	Mean	**99.5**	**94.4**	**89.2**	**86.1**	**89.8**	**92.9**	**93.4**	**93.0**

* Tentative ID by LC-MS confirmation against reference standard pending
** Sum of three regions
*** None individually exceeding 4.1%

Table CA 7.2.2.2-6: Characterisation of [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions in Carsington water surface water system – high dose (100 µg/L) [% AR]

Compound	Replicate	Incubation time (DAT)									
		0	**3**	**7**	**14**	**21**	**30**	**29 sterile**	**45**	**58 sterile**	**59**
Spiroxamine	A	98.1	84.2	92.3	84.8	9.4	3.1	90.0 [b)]	2.6 [a)]	93.2 [b)]	55.7 [a)]
	B	98.7	87.2	83.5	40.8	17.2	15.5	n/a	22.2 [a)]	n/a	7.2 [a)]
	Mean	**98.4**	**85.7**	**87.9**	**62.8**	**13.3**	**9.3**	**90.0[b)]**	**12.4[a)]**	**90.7 [b]**	**28.4[a)]**
M12 (spiroxamine-despropyl acid)	A	<0.1	<0.1	<0.1	<0.1	1.7	1.5	<0.1 [b)]	7.7 [b]	<0.1 [b]	0.1
	B	<0.1	<0.1	<0.1	<0.1	<0.1	1.1	n/a	0.5	n/a	2.4
	Mean	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**0.9**	**1.3**	**<0.1[b)]**	**4.1**	**<0.1[b)]**	**1.2**
M11 (spiroxamine-de-sethyl acid)	A	<0.1	<0.1	<0.1	<0.1	2.1	1.7	<0.1 [b]	6.6	<0.1 [b]	0.5
	B	<0.1	<0.1	<0.1	<0.1	<0.1	1.5	n/a	0.4	n/a	3.6
	Mean	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**1.0**	**1.6**	**<0.1[b)]**	**3.5**	**<0.1[b)]**	**2.1**
M06 (spiroxamine-acid)	A	<0.1	<0.1	<0.1	0.4	7.6	8.7	<0.1 [b]	18.0	<0.1 [b]	15.0
	B	<0.1	<0.1	<0.1	1.0	1.5	13.7	n/a	8.8	n/a	14.6
	Mean	**<0.1**	**<0.1**	**<0.1**	**0.7**	**4.6**	**11.2**	**<0.1[b)]**	**13.4**	**<0.1[b)]**	**14.8**
Desamino-M06* (desamino-spiroxamine-acid)	A	<0.1	1.9	<0.1	0.7	9.4	10.3	<0.1 [b]	8.9	<0.1 [b]	2.8
	B	<0.1	1.2	1.4	4.6	10.5	9.2	n/a	5.2	n/a	9.2
	Mean	**<0.1**	**1.6**	**0.7**	**2.6**	**9.9**	**9.7**	**<0.1[b)]**	**7.0**	**<0.1[b)]**	**6.0**
M05 (spiroxamine-hydroxy)	A	<0.1	<0.1	<0.1	<0.1	0.7	2.0	1.1 [b]	3.1	0.1 [b]	1.9
	B	<0.1	<0.1	<0.1	<0.1	1.0	0.8	n/a	0.4	n/a	<0.1
	Mean	**<0.1**	**<0.1**	**<0.1**	**0.6**	**0.8**	**1.4**	**1.1[b)]**	**1.7**	**0.1[b)]**	**0.5**
M03** (spiroxamine-N-oxide)	A	<0.1	3.9	<0.1	1.3	40.0	43.9	<0.1 [b]	35.8	1.0 [b]	5.2
	B	0.3	2.4	4.8	28.1	36.7	28.2	n/a	36.2	n/a	46.2
	Mean	**0.1**	**3.2**	**2.4**	**14.7**	**38.4**	**36.1**	**<0.1[b)]**	**36.0**	**1.0[b)]**	**25.2**
M02 (spiroxamine-despropyl)	A	<0.1	1.1	<0.1	1.1	6.5	7.3	0.4 [b]	2.3	0.4 [b]	1.7
	B	<0.1	0.7	1.9	6.7	8.4	8.6	n/a	7.3	n/a	3.2
	Mean	**<0.1**	**0.9**	**0.4**	**3.9**	**7.4**	**7.9**	**0.4[b)]**	**4.8**	**0.4[b)]**	**3.0**
M04 (spiroxamine-)	A	<0.1	<0.1	0.8	0.7	1.9	3.4	0.7 [b]	1.1	0.9 [b]	<0.1
	B	<0.1	<0.1	<0.1	1.5	4.2	1.9	n/a	2.3	n/a	3.3
	Mean	**<0.1**	**<0.1**	**0.4**	**1.1**	**3.1**	**2.6**	**0.7[b)]**	**2.2**	**0.9[b)]**	**1.6**

Compound	Replicate	Incubation time (DAT)									
		0	3	7	14	21	30	29 sterile	45	58 sterile	59
M01 (spiroxamine-de-sethyl)	A	0.6	3.0	1.5	2.6	5.7	3.8	2.7 [b]	<0.1	1.9 [b]	2.7
	B	<0.1	3.0	2.6	8.6	7.0	5.4	n/a	6.6	n/a	1.3
	Mean	**0.3**	**3.0**	**1.3**	**4.3**	**6.4**	**4.6**	**2.7 [b)]**	**3.3**	**1.9 [b)]**	**2.0**
Minor metabolites***	A	<0.1	<0.1	<0.1	<0.1	5.8	6.3	<0.1 [b]	8.8	<0.1	4.5
	B	<0.1	<0.1	<0.1	0.8	4.2	5.0	n/a	2.9	n/a	5.9
	Mean	**0.3**	**<0.1**	**0.8**	**1.7**	**5.0**	**5.7**	**<0.1 [b)]**	**5.4**	**0.2 [b)]**	**5.6**
Total % AR	A	98.7	94.2	94.6	92.7	90.9	91.9	94.9 [b]	97.5 [a)]	95.1 [b]	93.2 [a)]
	B	99.0	94.7	94.3	92.2	90.6	90.8	n/a	97.5 [a)]	n/a	92.2 [a)]
	Mean	**98.8**	**94.4**	**93.8**	**92.4**	**90.8**	**91.4**	**94.9 [b)]**	**97.5 [a)]**	**95.1 [b)]**	**92.7 [a)]**

a) Includes activity from flask wash samples, which was assigned as Spiroxamine based on representative HPLC analyses.
b) Sterile sample, no duplicate sample collected
n/a Not applicable
* Tentative ID by LC-MS confirmation against reference standard pending
** Sum of three regions
*** None indivi dually exceeding 3.0%

B. Material Balance

The overall material balances were 96.7% and 94.5% for the 10 (range 93.1 to 100.7%) and 100 µg/L (range 92.2 to 99.0%) samples, respectively. The sterile control samples yielded a mean recovery of 95.1% and 96.1% AR at day 29 and day 58, respectively. The positive and solvent control samples showed a mean recovery of 76.8% AR after work up at day 59.

C. Mineralisation

Mineralisation to CO_2 was low in spiroxamine treated samples from both dose levels (1.7 and 0.5% AR-maximum at the 10 and 100 µg/L levels, respectively). Formation of volatile organic compounds (VOC) was insignificant as demonstrated by values of ≤0.3% AR at all sampling intervals for both concentrations in degradation test systems as well as in sterile test systems. An additional small amount of volatile radioactivity (maximum 1.0 and 2.1% AR for the 10 and 100 µg/L levels, respectively) was recovered in the PU bungs (assumed related to parent spiroxamine).

Rapid mineralisation in the positive control flasks (treated with ^{14}C-benzoic acid) demonstrated that the test water had acceptable levels of biological activity for the test.

D. Degradation of Parent Compound

For both test-item dose levels and all intervals the vast majority of the activity was recovered in the water phase with 88.4% AR (mean) being the lowest proportion recovered in the water phase.

At both dose levels mean recoveries of spiroxamine declined rapidly from 94.3 % AR (mean) (10 µg/L) and 98.4% AR (mean) (100 µg/L) reaching 0.2% AR for the lower dose concentration system. For the 100 µg/L dose level spiroxamine levels decreased from 98.4% AR (mean) (100 µg/L) at the start, however, were variable for the remainder of the study with large differences between replicates from the same sampling interval, with this variability resulting in an apparent rise in mean levels at the last sampling point to 28.4% AR (mean). As this is only an interim report, clarity may be provided once the study reaches termination.

The metabolite M05 reached a maximum (mean, n=2) value of 38.4% AR on day 21 at the 100 µg/L dose level and similarly reached a mean of 35.6% AR on day 45 at the 10 µg/L dose level.

The metabolite M06 reached a maximum of 42.5% AR after 30 days at the 10 µg/L dose level before declining to 28.6% AR by the end of the study. At the 100 µg/L dose level the maximum value of 14.8% was reached after 59 days.

Mean levels of the despropyl and desethyl acid degradates M12 and M11 never exceeded 5% AR for the 100 µg/L dose level, but at the 10 µg/L dose level both exceeded 5% AR for the last two sampling intervals at days 45 and 59.

There was minimal degradation seen in the sterile control water phases with spiroxamine accounting for 90.0% AR and 93.2% AR in the day 29 and 58 day samples, respectively.

**3 Additional work is being conducted on the confirmatory analysis (including synthesis of a authentic reference standard to support the tentative identity of the desamino-spiroxamine-acid component; TLC confirmation in a secondary analytical system and MS confirmation).

E. Degradation Kinetics

The results of the kinetic fitting are summarised Table CA 7.2.2.2-1. For both dose levels, the SFO model gave a good fit for the data, with good r-square and chi-square values and a low prob >t. DT_{50} values of 11.5 and 13.4 days were determined for the low and high dose-level, respectively.

F. Isomers of Parent Compound

**1 Additional work is currently being conducted on the chiral analysis of the samples for parent spiroxamine. This work will be submitted in the final report for the study (currently report included is an interim report).

III. Conclusions

The aerobic mineralisation of [cyclohexyl-1-^{14}C]-spiroxamine was investigated in a "pelagic" test system (natural fresh water) at 20 ± 2 °C for a period of 59 days.

Mineralisation to CO_2 was low from both dose levels.

Spiroxamine was degraded extensively. At the lower concentration, spiroxamine was degraded with a DT_{50} value or 11.5 days (SFO) and comprised <1% after DAT 45. At the higher concentration, there was a lag phase to the degradation and more variation observed between replicates (average levels ranged between 9.3 and 28.4% AR over the period 21 to 59 days.

The metabolite M03 reached a maximum (mean, n=2) value of 38.4% AR on day 21 at the 100 µg/L dose level and similarly reached a mean of 35.6% AR on day 45 at the 10 µg/L dose level. The metabolite M06 reached a maximum of 42.5% AR after 30 days at the 10 µg/L dose level before declining to 28.6% AR by the end of the study. At the 100 µg/L dose level the maximum value of 14.8% was reached after 59 days. Mean levels of the despropyl and desethyl acid metabolites M12 and M11 never exceeded 5% AR for the 100 µg/L dose level, but at the 10 µg/L dose level both exceeded 5% AR for the last two sampling intervals at days 45 and 59.

DT_{50} values of 11.3 and 13.4 days were determined for the low and high dose-level, respectively using SFO kinetics.

Very little hydrolysis was observed in the sterile samples (>90% spiroxamine after DAT 59, most prominent degradation product was metabolite M01 (spiroxamine-desethyl, max 1.8% AR)).

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to study guideline(s) OECD 309 (required guideline). The study is considered valid to assess the aerobic mineralisation of [cyclohexyl-1-^{14}C]-spiroxamine in surface water.

CA 7.2.2.3 Water/sediment study

Use of plant protection products containing the active substance spiroxamine may result in contact with aquatic systems, therefore the fate and behaviour of the active substance in water/sediment systems has been investigated in laboratory studies according to the data requirements laid down in EC Regulation

283/2013.

The fate and behaviour of the active substance in water/sediment systems was investigated in six studies (KCA 7.2.2.3/01 to KCA 7.2.2.3/06) which were evaluated during the previous EU review. In addition, two new studies are available for renewal, (KCA 7.2.2.3/07) conducted to address the new data requirement since the previous evaluation and also to address the new requirements of EFSA, 2019[1] and (KCA 7.2.2.3/08) has been conducted to provide an up-to-date kinetic assessment of degradation rates observed in all water/sediment studies to modern requirements (FOCUS 2014[2]).

Substance	Report reference	Document no.	Test material used	Comment
Spiroxamine	KCA 7.2.2.3/01	M-006015-01-1	[cyclohexyl-1-^{14}C]-spiroxamine	Submitted for first approval of spiroxamine, 1999. Reviewed under UP. Considered valid and acceptable.
M03 (spiroxamine-N-oxide)	KCA 7.2.2.3/02	M-006094-01-1	[cyclohexyl-1-^{14}C]-spiroxamine	
M03 (spiroxamine-N-oxide)	KCA 7.2.2.3/03	M-032874-01-1	n.a.	
Spiroxamine	KCA 7.2.2.3/04	M-303324-01-1	[1,3-dioxolane-4-^{14}C]-spiroxamine	Submitted for first renewal of spiroxamine, 2010. Reviewed under UP. Considered valid and acceptable.
Spiroxamine	KCA 7.2.2.3/05	M-304099-01-1	n.a.	
Spiroxamine	KCA 7.2.2.3/06	M-006010-02-1	[cyclohexyl-1-^{14}C]-spiroxamine	
Spiroxamine	KCA 7.2.2.3/07	M-763128-01-1 (interim rpt)	[cyclohexyl-1-^{14}C]-spiroxamine	New data not yet reviewed under UP.
Spiroxamine	KCA 7.2.2.3/08	M-763141-01-1	n.a.	

n.a. not applicable

The fate and behaviour of the active substance spiroxamine in water/sediment systems has been investigated in a total of six systems. Previously evaluated studies have been conducted using both [cyclohexyl-1-^{14}C]-spiroxamine and [1,3-dioxolane-4-^{14}C]-spiroxamine and show a consistent degradation pathway for the duration of the studies and very little to no separation of the two possible radiolabelling positions, therefore the new water/sediment study was sufficiently conducted using [cyclohexyl-1-^{14}C]-spiroxamine only.

New studies, not previously evaluated

Data Point:	KCA 7.2.2.3/07
Report Author:	[redacted]; [redacted]
Report Year:	2020
Report Title:	[14C]-spiroxamine: Route and rate of degradation in aquatic sediment systems under aerobic conditions at 20°C - Interim report
Report No:	VC/19/057
Document No:	M-763128-01-1
Guideline(s) followed in study:	Commission Regulation (EU) No. 283/2013 in accordance with Regulation (EC) No. 1107/2009 OECD 308
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The route and rate of degradation of spiroxamine was investigated in two different water/sediment systems (Calwich Abbey, silt loam; Emperor Lake, sandy loam both from the United Kingdom) under laboratory aerobic conditions at 20°C. The test system was designed to provide a laboratory representation of a "worst case" scenario resulting from direct overspray or run-off of the test substance into a stationary body of water. Cylindrical metabolism flasks of 6 cm inner diameter were filled with a layer of sediment to a depth of 3 cm (50 g dry weight minimum) and overlying water (*ca.* 225 to 250 ml) added to a depth of 12 cm above the sediment surface giving a ratio of approximately 1:4 v/v.

[Cyclohexyl-1-^{14}C]-spiroxamine (radiochemical purity 99.6%) was applied to the surface of the water overlying sediment at a target rate of 84.8 µg of a.s. per flask, which was equivalent to a field application rate of 750 g/ha (assuming a water body depth of 30 cm).

Humidified air was passed through each treated flask continuously to maintain aerobic conditions (the air inlet tube in each flask was just under the water layer surface). Effluent gas was then passed through traps containing ethylene glycol and aqueous potassium hydroxide for the collection of volatile organic components and carbon dioxide, respectively. A further trap consisting of a polyurethane foam bung, was placed in the head of the vessels.

Duplicate treated samples of each water/sediment system were removed for analysis after 0, 1, 3, 7, 14, 30, 42, 62, 76 and 100 days of incubation.

Overlying water was carefully decanted into a glass vessel containing appropriate amounts of acetonitrile and the remaining sediment was transferred to a suitable container for extraction by rinsing with portions of the extraction solvent. Sediment samples were extracted four times at room temperature with acetonitrile/water/35% ammonia solution 80/20/1 v/v/v.

The overall recoveries of applied radioactivity ranged from 92.7 to 103.0% AR for the two test systems. The overall mean material balance was 97.1 and 99.5% AR for the Calwich Abbey and Emperor Lake water/sediment systems, respectively.

The mean proportion of total radioactivity extracted from sediment samples and in the overlying water declined slowly over time from 100.9% AR at 0 DAT to 78.1% at 100 DAT in the Calwich Abbey system. The Emperor Lake system declined similarly, from 100.1% at 0 DAT to 79.5% at 100 DAT. Unextracted residues (bound residues) increased slowly throughout the incubation, reaching maximum levels of 10.7% AR in Calwich Abbey at 100 DAT and 12.4% AR in Emperor Lake at 76 DAT.

Significant radioactivity was detected in the PU bungs in both systems from 30 DAT, reaching maximum levels of 9.5% AR in Calwich Abbey at 30 DAT and 5.6% AR in Emperor Lake by 100 DAT. Subsequently, selected samples of PU bung extracts from both systems were analysed by HPLC and the volatile radioactivity was confirmed to be volatile ^{14}C-spiroxamine. The level of applied radioactivity recovered in the volatile traps was ≤4.9% AR for both water/sediment systems over all sampling intervals and was detected in the KOH trap and was assumed to be carbon dioxide (not confirmed as <5% AR).

Following application of [cyclohexyl-1-^{14}C]-spiroxamine, spiroxamine showed rapid dissipation in the water phase of both systems, declining from 79.9 – 83.9% AR (mean of duplicate samples) at 0 DAT to <1.0% by 76 DAT. In the Calwich Abbey system, the amount of spiroxamine in the sediment increased to 45.4% AR after 7 DAT and subsequently decreased to 30.0% AR after 100 DAT. In the Emperor Lake system, the amount of spiroxamine in the sediment increased to 69.2% AR after 42 DAT and subsequently decreased to 58.6% AR after 100 DAT.

Degradation of [cyclohexyl-1-^{14}C]-spiroxamine was accompanied by the formation of one major degradation product M06 (spiroxamine-acid: max 44.5% AR at 42 DAT) and several other minor metabolites M01 (spiroxamine-desethyl: max 4.3% AR at 100 DAT), M02 (spiroxamine-despropyl: max 3.2% AR at 30 DAT), M03 (spiroxamine-N-oxide: max 2.3% AR at 7 DAT), M11 (spiroxamine-desethyl acid: max 3.3% AR at 76 DAT) and M12 (spiroxamine- despropyl acid: max 3.0% AR at 62 DAT). Some other minor unidentified metabolites were observed but none of which exceeded a total of 3.0% AR at any sampling interval.

DT_{50} values for the degradation of spiroxamine were calculated in the report, however a re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (FOCUS 2014), was performed in the report presented under point KCA 7.2.2.3.08 (M-763141-01-1).

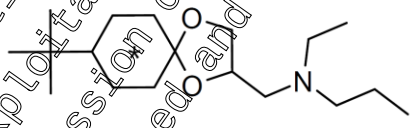
**1 Additional work is currently being conducted on the chiral analysis of the samples for parent spiroxamine. This work will be submitted in the final report for the study (currently report included is an interim report) and will be supplied as part of a top up submission (estimate August 2021).

1. Materials and Methods

A. Materials

1. Test Items

[cyclohexyl-1-^{14}C]-spiroxamine



* Denotes position of [^{14}C]-radiolabel

Specific Activity: 4.26 MBq/mg (34.4 mCi/mmol, 1273 MBq/mmol)

Radiochemical Purity: 99.1% (HPLC, as determined in study at the time of application)

2. Test System (water/sediment system)

The study was performed using the two water/sediment systems as characterised in Table CA 7.2.2.3-1.

Table CA 7.2.2.3-1: Physico-chemical properties of the water sediment systems

Parameter		Water/sediment system	
Water/sediment system designation:		**Calwich Abbey**	**Emperor Lake**
Geographic Location			
City		Calwich, Staffordshire [A]	Chatsworth, Derbyshire [A]
Country		UK	UK
Sediment characteristics			
Textural Classification (USDA)		Silt loam	Sandy loam
Sand [50 - 2000 µm]	(%)	20	72
Silt [2 – 50 µm]	(%)	71	11
Clay [< 2 µm]	(%)	9	17
pH			
in H2O (1:1)		7.2	5.2
in 0.01M CaCl2 (1:1)		7.1	4.9
Organic Matter (%)*		8.1*	3.1*
Organic Carbon (%)		4.7	1.8
Cation Exchange Capacity (meq/100 g)		10.5	6.7
Sediment moisture content (w/w %)		180.5	59.7
Soil Microbial Biomass (mg org C/100 g sediment)			
Initial, DAT 0		75.9	15.4
Final, 100 DAT 120		34.1	17.7
Water characteristics			
pH, at the time of treatment		8.5	7.5
Dissolved oxygen concentration, at the time of treatment (mg/L)		5.5	4.7
Total organic carbon (ppm)		3.0	6.7
Hardness (mg equiv CaCO3/L)		243	33
Nitrate content (mg/L)		2.5	0.6
Phosphorus content (mg/L)		1.2	1.7

* Calculated by multiplying organic carbon content by 1.724 (not reported)
A Location known free of pesticide use for at least 5 yrs and used within 17 days from sampling.

The test soils were handled in accordance with ISO 5667-12 and 5667-15 prior to use.

B. Study Design

1. Experimental Conditions

The behaviour of [cyclohexyl-1-^{14}C]spiroxamine was investigated in two contrasting sediment water systems ('Calwich Abbey' and 'Emperor Lake') over a period of 100 days.

The test system was designed to provide a laboratory representation of a "worst case" scenario resulting from direct overspray of run-off of the test substance into a stationary body of water and then subjected to aerobic conditions. Tests were performed in flow through systems consisting of glass vessels (500 mL, *ca.* 6 cm internal diameter) each containing a sediment layer (3 cm depth) and an overlying water layer (12 cm) i.e. a water to sediment ratio of 4:1 v/v and attached to an ethylene glycol trap to collect organic volatiles followed by two potassium hydroxide traps (2M) to collect carbon dioxide. A further trap consisting of a polyurethane foam bung, was placed in the head of the vessels.

Water/sediment system samples were pre-acclimatised to the incubation conditions (dark, 20°C) for 8-10 days before application of the test substance (within 17 days of sampling).

[Cyclohexyl-1-^{14}C]-spiroxamine was applied to the surface of the water overlying sediment at a target rate of 84.8 µg of a.s. per flask (in a small volume of solvent), which was equivalent to a field application rate of 750 g/ha (assuming a water body depth of 30 cm).

Additional samples for each soil were treated with an equivalent amount of blank solvent only to monitor microbial activity at the beginning and end of the incubation period.

Additionally sediment extracts and surface water samples were separately analysed using a chiral HPLC method. This work is currently on going and will be submitted in the final report for the study (currently report is interim) as soon as possible

2. Sampling

Duplicate samples for each water/sediment system were removed for analysis after 0, 1, 3, 7, 14, 30, 42, 62, 76 and 100 days of incubation. Untreated samples were analysed for biomass at the beginning and end of the experiment.

3. Analytical Procedures

Overlying water was carefully decanted into a glass vessel containing appropriate amounts of acetonitrile, and the remaining sediment was transferred to a suitable container for extraction by rinsing with portions of the extraction solvent. Sediment samples were extracted four times at room temperature with acetonitrile/water/35% ammonia solution 80/20/1 v/v/v, by addition of solvent, vigorous mechanical shaking and centrifugation. Radioactivity in extracts was determined by liquid scintillation counting (LSC). Aqueous samples and combined sediment extracts were mainly analysed without concentration (exceptions were some 1 and 3 DAT sediment samples containing low amounts of radioactivity) by direct injection by reverse phase HPLC with radio-detection. Degradation products were identified by comparison of the retention times of reference standards. The LOD/LOQ of the HPLC method was estimated to be 0.28/0.90% AR. Confirmatory analysis using an alternative technique was conducted by TLC with co-chromatography against reference items on selected samples. Degradation products were also confirmed using LC-MS on selected samples.

Volatile radioactivity in volatile traps was quantified by LSC. Any radioactivity in the polyurethane foam bung was extracted using acetonitrile and quantified by LSC. Carbon dioxide in the potassium hydroxide traps was not confirmed as <5% AR.

Following homogenisation, non-extractable residues (NER) in extracted sediments were determined by combustion.

4. Determination of degradation kinetics

The degradation kinetics of data within this report are subject of a separate modelling study. DT_{50} and DT_{90} values for the degradation of spiroxamine and its metabolites have been re-calculated from the reported data following the recommendations of the FOCUS work group (FOCUS 2014[2]). Full details are provided in the report under point KCA 7.2.2.3.08 (M-763141-01-1).

II. Results and Discussion

A. Data

The distribution of radioactivity for each water/sediment system incubated at 20°C following application of [cyclohexyl-1-^{14}C]-spiroxamine are summarised (as overall means) in Table CA 7.2.2.3-2 and Table CA 7.2.2.3-3.

The characterisation of radioactivity for each water/sediment are summarised (as overall means) in Table CA 7.2.2.3-4 and Table CA 7.2.2.3-8. The characterisation of radioactivity for each separate layer for each water/sediment are summarised (as individual replicates) in Table CA 7.2.2.3-5 to Table CA 7.2.2.3-7 for the Calwich Abbey system and in Table CA 7.2.2.3-9to Table CA 7.2.2.3-11 for the Emperor Lake system.

Table CA 7.2.2.3-2: Recovery and distribution of radioactivity following treatment of Calwich Abbey system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions [mean % AR]

Compound	Incubation time (DAT)									
	0	1	3	7	14	30	42	62	76	100
Overlying water	83.9	86.9	83.8	48.5	44.8	42.1	32.4	26.6	30.9	27.8
Sediment extract	17.1	11.3	15.7	46.9	47.2	37.6	48.0	52.0	50.2	50.3
(sub-total)	100.9	98.2	99.6	95.3	92	79.7	80.4	78.6	81.1	78.1
PU bung*	n.a.	n.a.	n.a.	1.0	1.8	9.5	4.2	6.0	3.2	4.7
Volatile traps**	n/a	n.d.	n.d.	n.d.	n.d.	0.4	1.5	3.6	4.9	1.7
Non-extractable radioactivity	0.4	0.4	0.5	2.5	3.9	3.7	6.5	7.5	8.6	10.7
Total AR	101.4	98.6	100.0	98.8	97.8	93.2	92.7	95.7	97.7	95.2

n.a.: not analysed, n.d.: not detected, DAT: days after treatment
* shown to comprise parent spiroxamine. ** radioactivity associated with first carbon dioxide trap
Overall total recovery = 97.1% AR
All values expressed as mean percentage of applied radioactivity (% AR)

Table CA 7.2.2.3-3: Recovery and distribution of radioactivity following treatment of Emperor Lake system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions [mean % AR]

Compound	Incubation time (DAT)									
	0	1	3	7	14	30	42	62	76	100
Overlying water	79.9	88.0	97.6	47.9	36.0	25.4	15.1	25.7	9.8	9.0
Sediment extract	20.3	11.9	4.6	46.3	56.5	61.9	73.2	60.4	75.9	70.5
(sub-total)	100.2	99.9	102.3	94.2	92.4	87.4	88.3	86.1	85.7	79.5
PU bung*	n.a.	n.a.	n.a.	0.2	1.2	2.0	2.0	4.8	2.2	5.6
Volatile traps**	n/a	n.d.	n.d.	0.1	0.1	0.4	0.8	0.8	2.7	2.1
Non-extractable radioactivity	0.9	1.0	0.0	4.2	5.2	6.0	7.8	6.4	12.4	10.1
Total AR	101.1	100.9	102.3	98.6	99.0	95.8	99.0	98.0	103.0	97.3

n.a.: not analysed, n.d.: not detected, DAT: days after treatment
* shown to comprise parent spiroxamine. ** radioactivity associated with first carbon dioxide trap
Overall total recovery = 99.5% AR
All values expressed as mean percentage of applied radioactivity (% AR)

Table CA 7.2.2.3-4: Characterisation of radioactivity following treatment of Calwich Abbey system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions [mean % AR] - Overview of means

Compound		Incubation time (DAT)									
		0	1	3	7	14	30	42	62	76	100
Total	w	83.9	86.9	83.9	48.5	44.8	42.1	32.4	26.6	30.9	27.8
	s	17.0	11.3	15.7	46.8	47.2	37.6	48.0	52.0	50.1	50.3
Spirox-amine	w	83.9	85.6	81.8	39.5	11.1	12.8	0.4	0.5	0.2	n.d.
	s	17.0	11.3	15.7	45.4	42.6	28.8	29.6	36.5	31.7	30.0
M01 (desethyl)	w	n.d.	0.3	0.3	0.6	0.2	0.9	n.d.	n.d.	n.d.	n.d.
	s	n.d.	n.d.	n.d.	1.4	1.0	0.6	1.3	2.5	2.2	2.4
M02 (despropyl)	w	n.d.	0.4	0.3	0.6	0.5	1.1	n.d.	0.1	n.d.	n.d.
	s	n.d.	n.d.	n.d.	n.d.	1.5	1.6	0.4	1.6	1.2	1.8
M05 (hydroxy)	w	n.d.	n.d.	0.6	0.8	0.2	n.d.	n.d.	n.d.	n.d.	n.d.
	s	n.d.	n.d.	n.d.	n.d.	0.5	n.d.	n.d.	n.d.	n.d.	n.d.
M03 (N-oxide)	w	n.d.	0.1	n.d.	2.3	0.7	1.4	n.d.	n.d.	n.d.	0.2
	s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M06 (acid)	w	n.d.	n.d.	0.1	3.1	26.5	21.9	28.2	21.2	27.0	23.8
	s	n.d.	n.d.	n.d.	n.d.	0.9	6.5	16.3	9.9	13.2	16.0

Compound		Incubation time (DAT)									
		0	1	3	7	14	30	42	62	76	100
M11 (des-ethyl acid)	w	n.d.	n.d.	n.d.	n.d.	1.2	0.6	1.2	1.6	1.5	1.1
	s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.8	n.d.
M12 (des-Propyl acid)	w	n.d.	n.d.	n.d.	n.d.	0.5	0.7	1.3	1.6	1.3	1.4
	s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5	1.4	n.d.	n.d.
Other unknowns	w	n.d.	0.4	0.7	1.6	3.9	2.7	1.3	1.6	1.0	1.2
	s	n.d.	n.d.	n.d.	n.d.	0.7	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected, DAT: days after treatment
All values expressed as mean percentage of applied radioactivity (% AR)

Table CA 7.2.2.3-5: Characterisation of radioactivity following treatment of Calwich Abbey system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions – water system [% AR]

Compound	Replicate	Incubation time (DAT)									
		0	1	3	7	14	30	42	62	76	100
Total	A	82.6	76.3	72.1	42.6	39.1	45.2	34.1	29.6	32.9	32.7
	B	85.3	97.5	95.6	54.4	50.5	39.0	30.7	23.5	29.0	22.9
	Mean	**83.9**	**86.9**	**83.9**	**48.5**	**44.8**	**42.1**	**32.4**	**26.6**	**30.9**	**27.8**
Spiroxamine	A	82.6	75.1	70.1	36.4	20.8	24.8	0.3	n.d.	0.3	n.d.
	B	85.3	96.1	93.6	43.6	1.4	0.8	0.5	0.9	n.d.	n.d.
	Mean	**83.9**	**85.6**	**81.8**	**39.5**	**11.1**	**12.8**	**0.4**	**0.5**	**0.5**	**n.d.**
M01 (desethyl)	A	n.d.	0.3	0.3	0.5	0.5	1.6	n.d.	n.d.	n.d.	n.d.
	B	n.d.	0.3	0.3	0.7	n.d.	0.2	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**0.3**	**0.3**	**0.6**	**0.2**	**0.9**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
M02 (despropyl)	A	n.d.	0.5	0.3	0.5	0.9	2.3	n.d.	0.0	n.d.	n.d.
	B	n.d.	0.3	0.3	0.8	n.d.	n.d.	n.d.	0.3	n.d.	n.d.
	Mean	**n.d.**	**0.4**	**0.3**	**0.6**	**0.0**	**1.1**	**n.d.**	**0.1**	**n.d.**	**n.d.**
M05 (hydroxy)	A	n.d.	n.d.	n.d.	1.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	0.6	0.5	0.4	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**0.3**	**0.8**	**0.2**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
M03 (N-oxide)	A	n.d.	n.d.	0.3	0.6	0.8	2.6	n.d.	n.d.	n.d.	0.5
	B	n.d.	0.3	n.d.	4.0	0.6	0.2	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**0.1**	**0.3**	**2.3**	**0.7**	**1.4**	**n.d.**	**n.d.**	**n.d.**	**0.2**
M06 (acid)	A	n.d.	n.d.	0.2	2.1	13.9	8.8	29.6	24.9	28.6	28.5
	B	n.d.	n.d.	n.d.	4.1	39.2	34.9	26.8	17.6	25.5	19.2
	Mean	**n.d.**	**n.d.**	**0.1**	**3.1**	**26.5**	**21.9**	**28.2**	**21.2**	**27.0**	**23.8**
M11 (des-ethyl acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	0.6	1.3	1.3	1.6	1.1
	B	n.d.	n.d.	n.d.	n.d.	2.4	0.5	1.1	1.8	1.3	1.1
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**1.2**	**0.6**	**1.2**	**1.6**	**1.5**	**1.1**
M12 (des-Propyl acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	0.3	1.6	2.0	1.3	1.2
	B	n.d.	n.d.	n.d.	n.d.	1.1	1.1	0.9	1.2	1.3	1.6
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.5**	**0.7**	**1.3**	**1.6**	**1.3**	**1.4**
Other unknowns	A	n.d.	0.3	0.6	1.3	2.3	4.2	1.3	1.5	1.0	1.4
	B	n.d.	0.5	0.7	1.8	5.5	1.2	1.3	1.7	0.9	1.1
	Mean	**n.d.**	**0.4**	**0.7**	**1.6**	**3.9**	**2.7**	**1.3**	**1.6**	**1.0**	**1.2**

n.d.: not detected, DAT: days after treatment

Table CA 7.2.2.3-6: Characterisation of radioactivity following treatment of Calwich Abbey system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions – Sediment system [% AR]

Compound	Replicate	Incubation time (DAT)									
		0	1	3	7	14	30	42	62	76	100
Total	A	17.1	18.4	26.7	52.0	53.5	31.2	46.7	52.0	52.7	49.4
	B	17.0	4.3	4.7	41.7	40.9	43.9	49.4	52.0	47.6	51.2
	Mean	**17.0**	**11.3**	**15.7**	**46.8**	**47.2**	**37.6**	**48.0**	**52.0**	**50.1**	**50.3**
Spirox-amine	A	17.1	18.4	26.7	50.8	50.2	29.0	27.2	34.9	33.7	31.7
	B	17.0	4.3	4.6	40.1	35.1	28.6	32.0	28.2	29.7	28.4
	Mean	**17.0**	**11.3**	**15.7**	**45.4**	**42.6**	**28.8**	**29.6**	**36.5**	**31.7**	**30.0**
M01 (desethyl)	A	n.d.	n.d.	n.d.	1.1	0.8	n.d.	1.1	2.0	2.1	2.4
	B	n.d.	n.d.	0.1	1.6	1.2	1.2	1.5	3.0	2.3	2.4
	Mean	**n.d.**	**n.d.**	**n.d.**	**1.4**	**1.0**	**0.6**	**1.3**	**2.5**	**2.2**	**2.4**
M02 (despropyl)	A	n.d.	n.d.	n.d.	n.d.	1.6	2.2	0.8	1.2	n.d.	2.2
	B	n.d.	n.d.	n.d.	n.d.	1.3	1.1	n.d.	2.0	2.3	1.4
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**1.5**	**1.6**	**0.4**	**1.6**	**1.2**	**1.8**
M05 (hydroxy)	A	n.d.	n.d.	n.d.	n.d.	0.9	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.5**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
M03 (N-oxide)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
M06 (acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	16.6	14.0	13.3	13.1
	B	n.d.	n.d.	n.d.	n.d.	1.8	13.1	15.9	5.9	13.1	19.0
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.9**	**6.5**	**16.3**	**9.9**	**13.2**	**16.0**
M11 (des-ethyl acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.6	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**1.8**	**n.d.**
M12 (des-Propyl acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.0	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.9	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.5**	**1.4**	**n.d.**
Other unknowns	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	1.4	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.7**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**

n.d.: not detected, DAT: days after treatment

Table CA 7.2.2.3-7: Characterisation of radioactivity following treatment of Calwich Abbey system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions – Total system [% AR]

Compound	Replicate	Incubation time (DAT)									
		0	1	3	7	14	30	42	62	76	100
Total	A	99.7	94.6	98.8	94.6	92.6	76.5	80.8	81.6	85.5	82.0
	B	102.2	101.8	100.3	96.1	91.5	82.9	80.1	75.6	76.6	74.2
	Mean	**100.9**	**98.2**	**99.6**	**95.3**	**92.0**	**79.7**	**80.4**	**78.6**	**81.1**	**78.1**
Spirox-amine	A	99.7	93.5	96.8	87.2	70.9	53.8	27.5	34.9	34.1	31.7
	B	102.2	100.4	98.2	82.7	36.5	29.4	32.5	39.1	29.7	28.4
	Mean	**100.9**	**97.0**	**97.5**	**84.9**	**53.7**	**41.6**	**30.0**	**37.0**	**31.9**	**30.0**
M01 (desethyl)	A	n.d.	0.3	0.3	1.6	1.3	1.6	1.1	2.0	2.1	2.4
	B	n.d.	0.3	0.4	2.3	1.2	1.3	1.5	3.0	2.3	2.4
	Mean	**n.d.**	**0.3**	**0.3**	**2.0**	**1.3**	**1.5**	**1.3**	**2.5**	**2.2**	**2.4**

Compound	Replicate	Incubation time (DAT)									
		0	1	3	7	14	30	42	62	76	100
M02 (despropyl)	A	n.d.	0.5	0.3	0.5	2.5	4.5	0.8	1.2	n.d.	1.2
	B	n.d.	0.3	0.3	0.8	1.3	1.1	n.d.	2.3	2.5	1.4
	Mean	**n.d.**	**0.4**	**0.3**	**0.6**	**1.9**	**2.8**	**0.4**	**1.7**	**1.2**	**1.8**
M05 (hydroxy)	A	n.d.	n.d.	0.5	1.1	0.9	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	0.6	0.5	0.4	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**0.6**	**0.8**	**0.6**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
M03 (N-oxide)	A	n.d.	n.d.	n.d.	0.6	0.8	2.6	n.d.	n.d.	n.d.	0.5
	B	n.d.	0.3	n.d.	4.0	0.6	0.2	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**0.1**	**n.d.**	**2.3**	**0.7**	**1.4**	**n.d.**	**n.d.**	**n.d.**	**0.2**
M06 (acid)	A	n.d.	n.d.	0.2	2.1	13.9	48.8	46.3	38.8	41.9	41.6
	B	n.d.	n.d.	n.d.	4.1	41.0	48.0	42.7	23.5	38.6	38.1
	Mean	**n.d.**	**n.d.**	**0.1**	**3.1**	**27.4**	**28.4**	**44.5**	**31.2**	**40.3**	**39.8**
M11 (des-ethyl acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	0.6	1.3	1.3	5.2	1.1
	B	n.d.	n.d.	n.d.	n.d.	2.4	0.5	1.1	1.8	1.3	1.1
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**1.2**	**0.6**	**1.2**	**1.6**	**3.3**	**1.1**
M12 (des-Propyl acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	0.3	2.6	2.0	1.3	1.2
	B	n.d.	n.d.	n.d.	n.d.	1.1	1.1	0.9	4.1	1.3	1.6
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.5**	**0.7**	**1.8**	**3.0**	**1.3**	**1.4**
Other unknowns	A	n.d.	0.3	0.6	1.3	2.3	4.5	1.3	1.5	1.0	1.4
	B	n.d.	0.5	0.7	1.8	7.0	1.2	1.3	1.7	0.9	1.1
	Mean	**n.d.**	**0.4**	**0.7**	**1.6**	**4.6**	**2.7**	**1.3**	**1.6**	**1.0**	**1.2**

n.d.: not detected, DAT: days after treatment

Table CA 7.2.2.3-8: Characterisation of radioactivity following treatment of Emperor Lake system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions [mean % AR] - Overview of means

Compound		Incubation time (DAT)									
		0	1	3	7	14	30	42	62	76	100
Total	w	79.9	88.0	97.7	47.9	36.0	25.4	15.1	25.7	9.8	9.0
	s	20.3	12.0	4.7	46.2	56.4	62.0	73.2	60.4	75.9	70.5
Spirox-amine	w	79.9	87.2	96.6	45.8	25.0	18.6	6.0	14.0	0.3	0.9
	s	20.3	10.5	4.1	41.9	53.4	56.7	69.2	55.4	48.0	58.6
M01 (desethyl)	w	n.d.	0.3	0.3	0.4	0.5	1.0	0.5	1.2	n.d.	0.4
	s	n.d.	n.d.	0.2	2.2	1.3	3.0	3.0	1.8	2.1	3.9
M02 (despropyl)	w	n.d.	0.3	0.3	0.6	0.6	0.9	0.7	1.7	0.7	0.3
	s	n.d.	n.d.	0.3	2.1	1.3	2.3	1.1	n.d.	0.8	2.1
M05 (hydroxy)	w	n.d.	0.1	0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M03 (N-oxide)	w	n.d.	n.d.	n.d.	n.d.	n.d.	0.2	0.3	0.5	n.d.	0.3
	s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M06 (acid)	w	n.d.	n.d.	n.d.	0.5	8.1	3.3	6.2	5.8	7.0	3.8
	s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.2	23.0	5.8
M11 (des-ethyl acid)	w	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	0.2	0.3	0.3	0.4
	s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5	n.d.
M12 (des-Propyl acid)	w	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2	0.8	0.8
	s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Other unknowns	w	n.d.	0.1	0.3	0.6	1.9	1.3	1.2	1.9	0.6	2.2
	s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.5	n.d.

n.d.: not detected, DAT: days after treatment
All values expressed as mean percentage of applied radioactivity (% AR)

Table CA 7.2.2.3-9: Characterisation of radioactivity following treatment of Emperor system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions – water system [% AR]

Compound	Replicate	Incubation time (DAT)									
		0	1	3	7	14	30	42	62	76	100
Total	A	81.2	98.7	99.8	56.6	36.5	18.4	18.9	9.2	5.2	15.7
	B	78.5	77.2	95.5	39.2	35.5	32.5	32.5	42.1	14.5	2.2
	Mean	**79.9**	**88.0**	**97.7**	**47.9**	**36.0**	**25.4**	**25.7**	**25.7**	**9.8**	**9.0**
Spirox-amine	A	81.2	98.1	98.8	53.8	27.4	9.8	5.8	0.7	n.d.	1.7
	B	78.5	76.3	94.3	37.8	22.6	23.3	6.1	27.3	0.7	n.d.
	Mean	**79.9**	**87.2**	**96.6**	**45.8**	**25.0**	**16.6**	**6.0**	**14.0**	**0.3**	**0.9**
M01 (desethyl)	A	n.d.	0.2	0.3	0.4	0.4	1.0	0.5	0.2	n.d.	0.8
	B	n.d.	0.3	0.4	0.4	0.5	1.1	0.5	2.1	n.d.	n.d.
	Mean	**n.d.**	**0.3**	**0.3**	**0.4**	**0.5**	**1.0**	**0.5**	**1.2**	**n.d.**	**0.4**
M02 (despropyl)	A	n.d.	0.4	0.2	0.7	0.6	1.1	0.8	1.0	0.8	0.4
	B	n.d.	0.2	0.3	0.5	0.6	0.6	0.6	2.3	0.7	0.2
	Mean	**n.d.**	**0.3**	**0.3**	**0.6**	**0.6**	**0.9**	**0.7**	**1.7**	**0.7**	**0.3**
M05 (hydroxy)	A	n.d.	n.d.	0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	0.2	0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**0.1**	**0.2**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
M03 (N-oxide)	A	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	0.6	n.d.	n.d.	0.6
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.0	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.2**	**0.3**	**0.5**	**n.d.**	**0.3**
M06 (acid)	A	n.d.	n.d.	n.d.	0.9	5.8	4.3	9.8	5.2	3.3	6.2
	B	n.d.	n.d.	n.d.	0.2	10.3	2.2	2.6	6.5	10.8	1.4
	Mean	**n.d.**	**n.d.**	**n.d.**	**0.5**	**8.1**	**3.3**	**6.2**	**5.8**	**7.0**	**3.8**
M11 (des-ethyl acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	0.3	0.5	0.4	n.d.	0.7
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3	0.5	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.1**	**0.2**	**0.3**	**0.3**	**0.4**
M12 (des-Propyl acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5	0.6	1.1
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.9	0.4
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.2**	**0.8**	**0.8**
Other unknowns	A	n.d.	n.d.	0.3	0.9	2.2	1.2	0.9	1.3	0.5	4.2
	B	n.d.	0.2	0.3	0.4	1.5	1.3	1.4	2.5	0.8	0.2
	Mean	**n.d.**	**0.1**	**0.3**	**0.6**	**1.9**	**1.3**	**1.2**	**1.9**	**0.6**	**2.2**

n.d.: not detected, DAT: days after treatment

Table CA 7.2.2.3-10: Characterisation of radioactivity following treatment of Emperor system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions – Sediment system [% AR]

Compound	Replicate	Incubation time (DAT)									
		0	1	3	7	14	30	42	62	76	100
Total	A	21.1	2.9	3.3	38.0	55.7	69.5	68.6	73.9	80.8	62.7
	B	19.5	21.0	6.0	54.5	57.1	54.5	77.8	46.9	70.9	78.3
	Mean	**20.3**	**12.0**	**4.7**	**46.2**	**56.4**	**62.0**	**73.2**	**60.4**	**75.9**	**70.5**
Spirox-amine	A	21.1	n.d.	2.9	34.9	53.2	62.6	64.4	65.3	54.5	52.0
	B	19.5	21.0	5.4	48.9	53.5	50.7	73.9	45.5	41.5	65.2
	Mean	**20.3**	**10.5**	**4.1**	**41.9**	**53.4**	**56.7**	**69.2**	**55.4**	**48.0**	**58.6**
M01 (desethyl)	A	n.d.	n.d.	0.2	1.4	1.2	4.1	3.2	2.1	2.6	4.4
	B	n.d.	n.d.	0.3	3.0	1.4	1.9	2.8	1.4	1.5	3.5
	Mean	**n.d.**	**n.d.**	**0.2**	**2.2**	**1.3**	**3.0**	**3.0**	**1.8**	**2.1**	**3.9**

Compound	Replicate	Incubation time (DAT)									
		0	1	3	7	14	30	42	62	76	100
M02 (despropyl)	A	n.d.	n.d.	0.2	1.6	1.2	2.8	1.0	n.d.	n.d.	n.d.
	B	n.d.	n.d.	0.4	2.6	1.3	1.8	1.1	n.d.	1.6	n.d.
	Mean	**n.d.**	**n.d.**	**0.2**	**2.1**	**1.3**	**2.3**	**1.1**	**n.d.**	**0.8**	**2.1**
M05 (hydroxy)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
M03 (N-oxide)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	0.8	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.4**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
M06 (acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.5	22.7	2.0
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	23.3	9.6
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**3.2**	**23.0**	**5.8**
M11 (des-ethyl acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.0	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.5**	**n.d.**
M12 (des-Propyl acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
Other unknowns	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.0	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**1.5**	**n.d.**

n.d.: not detected, DAT: days after treatment

Table CA 7.2.2.3-11: Characterisation of radioactivity following treatment of Emperor system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions – Total system [% AR]

Compound	Replicate	Incubation time (DAT)									
		0	1	3	7	14	30	42	62	76	100
Total	A	102.3	101.6	103.1	94.6	92.2	87.8	87.5	83.1	86.0	78.4
	B	98.0	98.2	101.5	93.7	92.6	87.0	89.0	89.0	85.4	80.6
	Mean	**100.1**	**99.9**	**102.3**	**94.2**	**92.4**	**87.4**	**88.3**	**86.1**	**85.7**	**79.5**
Spirox-amine	A	102.3	98.1	101.7	88.7	80.7	72.4	70.2	66.0	54.5	53.7
	B	98.0	97.3	99.7	86.8	76.1	78.1	80.0	72.8	42.2	65.2
	Mean	**100.1**	**97.7**	**100.7**	**87.7**	**78.4**	**75.2**	**75.1**	**69.4**	**48.3**	**59.4**
M01 (desethyl)	A	n.d.	0.2	0.5	1.9	1.7	5.1	3.7	2.3	2.6	5.2
	B	n.d.	0.3	0.6	3.4	1.9	3.0	3.3	3.5	1.5	3.5
	Mean	**n.d.**	**0.3**	**0.6**	**2.6**	**1.8**	**4.1**	**3.5**	**2.9**	**2.1**	**4.4**
M02 (despropyl)	A	n.d.	0.4	0.4	2.3	1.8	3.9	1.8	1.0	0.8	4.6
	B	n.d.	0.2	0.7	3.0	2.0	2.4	1.7	2.3	2.3	0.2
	Mean	**n.d.**	**0.3**	**0.6**	**2.7**	**1.9**	**3.2**	**1.8**	**1.7**	**1.5**	**2.4**
M05 (hydroxy)	A	n.d.	n.d.	0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	0.2	0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**0.2**	**0.2**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
M03 (N-oxide)	A	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	0.6	n.d.	n.d.	0.6
	B	n.d.	n.d.	n.d.	n.d.	0.8	n.d.	n.d.	1.0	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.4**	**0.2**	**0.3**	**0.5**	**n.d.**	**0.3**
M06 (acid)	A	n.d.	n.d.	n.d.	0.9	5.8	4.5	9.8	11.6	26.0	8.2
	B	n.d.	n.d.	n.d.	0.2	10.3	2.2	2.6	6.5	34.1	11.1
	Mean	**n.d.**	**n.d.**	**n.d.**	**0.5**	**8.1**	**3.3**	**6.2**	**9.1**	**30.0**	**9.6**

Compound	Replicate	Incubation time (DAT)									
		0	1	3	7	14	30	42	62	76	100
M11 (des-ethyl acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	0.3	0.5	0.4	1.0	0.7
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3	0.5	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.1**	**0.2**	**0.3**	**0.8**	**0.4**
M12 (des-Propyl acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5	0.6	1.1
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.9	0.4
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.2**	**0.8**	**0.8**
Other unknowns	A	n.d.	n.d.	0.3	0.9	2.2	1.2	0.9	1.3	0.5	4.2
	B	n.d.	0.2	0.3	0.4	1.5	1.3	1.4	2.5	3.8	0.2
	Mean	**n.d.**	**0.1**	**0.3**	**0.6**	**1.9**	**1.3**	**1.2**	**1.9**	**2.1**	**2.2**

n.d.: not detected, DAT: days after treatment

B. Material Balance

The material balance (mean) at each sampling interval ranged from 92.7 to 103.0% AR for the two test systems. The overall mean material balance was 97.1 and 99.5% AR for the Calwich Abbey and Emperor Lake water/sediment systems, respectively.

C. Extractable and Non-Extractable Residues

The mean proportion of total radioactivity extracted from sediment samples and in the overlying water declined slowly over time from 100.9% AR at 0 DAT to 78.1% at 100 DAT in the Calwich Abbey system. The Emperor Lake system declined similarly, from 100.1% at 0 DAT to 79.5% at 100 DAT. Unextracted residues (bound residues) increased slowly throughout the incubation, reaching maximum levels of 10.7% AR in Calwich Abbey at 100 DAT and 12.4% AR in Emperor Lake at 76 DAT.

D. Volatile Radioactivity

Significant radioactivity was detected in the PU bungs in both systems from 30 DAT, reaching maximum levels of 9.5% AR in Calwich Abbey at 30 DAT and 5.6% AR in Emperor Lake by 100 DAT. Subsequently, selected samples of PU bung extracts from both systems were analysed by HPLC and the volatile radioactivity was confirmed to be volatile ^{14}C-spiroxamine.

The level of applied radioactivity recovered in the volatile traps was ≤4.9% AR for both water/sediment systems over all sampling intervals and was detected in the KOH trap and was assumed to be carbon dioxide (not confirmed as <5% AR).

E. Degradation of Parent Compound

Following application of [cyclohexyl-1-^{14}C]-spiroxamine, spiroxamine showed rapid dissipation in the water phase of both systems, declining from 79.9 – 83.9% AR (mean of duplicate samples) at 0 DAT to <1.0% by 76 DAT. In the Calwich Abbey system, the amount of spiroxamine in the sediment increased to 45.4% AR after 7 DAT and subsequently decreased to 30.0% AR after 100 DAT. In the Emperor Lake system, the amount of spiroxamine in the sediment increased to 69.2% AR after 42 DAT and subsequently decreased to 58.6% AR after 100 DAT.

Degradation of [cyclohexyl-^{14}C]-spiroxamine was accompanied by the formation of one major degradation product, M06 (spiroxamine-acid: max 44.5% AR at 42 DAT) and several other minor metabolites M01 (spiroxamine-desethyl: max 4.3% AR at 100 DAT), M02 (spiroxamine-despropyl: max 3.2% AR at 30 DAT), M03 (spiroxamine-N-oxide: max 2.3% AR at 7 DAT), M11 (spiroxamine-desethyl acid: max 3.3% AR at 76 DAT) and M12 (spiroxamine- despropyl acid: max 3.0% AR at 62 DAT). Some other minor unidentified metabolites were observed, none of which exceeded a total of 3.0% AR at any sampling interval.

F. Degradation Kinetics

Degradation rates determined in the report are superseded by the report presented under point KCA 7.2.2.3/08 (M-763141-01-1).

G. Isomers of Parent Compound

Additional work is being conducted on the chiral analysis of the samples for parent spiroxamine. This work will provide the quantified amounts of each individual isomer of spiroxamine in all samples. This work will be submitted in the final report for the study as soon as possible.

III. Conclusions

Spiroxamine degraded in water/sediment systems under aerobic conditions (20°C) with 30.0-59.4% of applied radioactivity remaining as parent compound after 100 DAT in the Calwich Abbey and Emperor Lake systems, respectively. Some volatility of parent spiroxamine was observed (max 9.5%) in addition to low amounts (<4.9% AR) of other volatiles (assumed to be carbon dioxide). The amounts of unextractable radioactivity were <12.4% AR (mainly humin). The major metabolic pathways involved oxidation of a methyl group to form metabolite M06 (spiroxamine-acid, maximum 44.5% AR).

A re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) is performed in the report presented under point KCA 7.2.2.3/08 (M-763141-01-1).

Assessment and conclusion by applicant:
Study meets the current guidance and the requirements in 283/2013.
The study was conducted to study guideline(s) OECD 308 (required guideline). The study is considered valid to assess the aerobic degradation of [cyclohexyl-1-^{14}C]-spiroxamine in aerobic water/sediment systems.

Existing studies, previously evaluated

Data Point:	KCA 7.2.2.3/01
Report Author:	[redacted]
Report Year:	1995
Report Title:	Aerobic metabolism of KWG4168 in an aquatic model ecosystem
Report No:	PF4629
Document No:	M-006015-01-1
Guideline(s) followed in study:	BBA Ref.: Degradability and Fate of Plant Protection Products in Water/sediment System 5-1 (Dec. 1990)
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The route and rate of degradation of [cyclohexyl-1-^{14}C]-spiroxamine was investigated in two different water/sediment systems (Hoenniger[8], Germany and Stilwell, USA) under laboratory aerobic conditions in the dark at 20°C for up to 100 days. The sediments and the water were collected from an artificial damned pond (Hoenniger, silt loam, 4.4%OC, pH 5.7) and from a man made pond (Stilwell, silt clay loam, 1.6%OC, pH 6.8). [Cyclohexyl-1-^{14}C]-spiroxamine (radiochemical purity 99.6%) was applied to

[8] For consistence with other studies, spelling of Hoenniger may differ to that reported in the study.

the surface of the water overlying sediment at a target rate of 0.25 mg a.s./L, which was equivalent to a field application rate of 750 g/ha (assuming a water body depth of 30 cm). Test systems were filled with a layer of sediment to a depth of 2-2.5 cm (98 g dry weight Hoenniger and 166 g dry weigh Stilwell) and overlying water to a depth of 6 cm; giving a ratio of approximately 1:3 v/v.

Each test vessel was attached to volatile traps of i) oil-coated (2% parrafin oil in hexane) quartz glass wool for sorption of organic volatiles and, ii) soda lime to collect carbon dioxide. A control system containing no test substance was used for the determination of biological activity.

Duplicate treated samples of each water/sediment system were removed for analysis after 0, 0.25, 1, 2, 7, 14, 33, 56 and 100 days of incubation.

Overlying water was carefully decanted into a glass vessel containing appropriate amounts of acetonitrile. Sediment samples were extracted three times at room temperature with acetonitrile, while later time points were refluxed with methanol for 6 hours. All samples were analysed by TLC methods, with the identification of the metabolite M06 determined by GC/MS. The LOD for a single peak was ≥0.1% of the applied radioactivity.

The overall mean material balance was 102.1% and 100.9% AR for the Hoenniger and Stilwell water/sediment systems, respectively.

The mean proportion of total radioactivity extracted from sediment samples increased slowly over the duration of the study from 7.5% AR (mean) at 0 DAT to 49.3% AR (mean) at 100 DAT in the Hoenniger system. The Stilwell system increased similarly, from 1.2% AR (mean) at 0 DAT to 79.5% AR (mean) at 100 DAT. The mean proportion of total radioactivity in the overlying water declined slowly over time from 87.0% AR (mean) at 0 DAT to 2.1% AR (mean) at 100 DAT in the Hoenniger system. The Stilwell system declined similarly, from 92.4% AR (mean) at 0 DAT, to 6.9% AR (mean) at 100 DAT. Non-extracted residues (bound residues) increased slowly throughout the incubation, reaching maximum levels of 44.0% AR (mean) in Hoenniger at 100 DAT and 48.9% AR (mean) in Stilwell at 76 DAT.

Following application of [cyclohexyl-1-^{14}C]-spiroxamine, spiroxamine showed rapid dissipation in the water phase of both systems, declining from 83.7% AR (mean) at 0 DAT to 0.6% by 100 DAT in the Hoenniger systems. Similarly, in the Stilwell system spiroxamine showed rapid dissipation in the water phase of both systems, declining from 80.0% AR (mean) at 0 DAT to 0.1% by 100 DAT. In the Hoenniger system, the amount of spiroxamine in the sediment increased to a maximum of 52.0% AR (mean) after 14 DAT and subsequently decreased to 42.7% AR (mean) after 100 DAT. In the Stilwell system, the amount of spiroxamine in the sediment increased to a maximum of 23.6% AR (mean) after 100 DAT.

Degradation of [cyclohexyl-1-^{14}C]-spiroxamine in the total system was accompanied by the formation of one major degradation product M06 (spiroxamine-acid: max 7.7% AR (mean) at 56 DAT) which was determined to be >5% AR for more than two consecutive time points. Additionally, several other minor metabolites M01 (spiroxamine-desethyl: max 1.9% AR (mean) at 33 DAT), M02 (spiroxamine-despropyl: max 1.9% AR (mean) at 33 DAT), M03 (spiroxamine-N-oxide: max 9.3% AR (mean) at 2 DAT), M11 (spiroxamine-desethyl acid: max 0.5% AR (mean) at 33 DAT) and M05 (spiroxamine-hydroxy max 2.3% AR (mean) at 14 DAT). Some other minor unidentified metabolites were observed but none of which exceeded a total of 2.5% AR (mean) at any sampling interval.

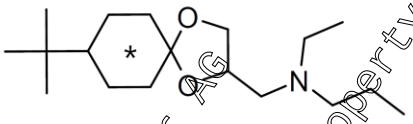
DT_{50} values for the degradation of spiroxamine were calculated in the report, however, a re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014[2]) was performed in the report presented under point KCA 7.2.2.3/08 (M-763141-01-1).

I. Materials and Methods

A. Materials

1. Test Items

[cyclohexyl-1-^{14}C]-spiroxamine



* Denotes position of [^{14}C]-radiolabel

Specific Activity:	2.59 MBq/mg
Isomer A:B ratio	55:45
Radiochemical Purity:	>99%

2. Test System (water/sediment system)

The study was performed using the two water/sediment systems as characterised in Table CA 7.2.2.3-12.

Table CA 7.2.2.3-12: Physico-chemical properties of the water sediment systems

Parameter	Water/sediment system	
Water/sediment system designation:	**Hoenniger**	**Stilwell**
Geographic Location		
City	Wipperfürth	Kansas
Country	Germany	USA
Sediment characteristics		
Textural Classification (USDA)	Silt loam	Silty clay loam
Sand [50 - 2000 µm] (%)	32.3	4.3
Silt [2 - 50 µm] (%)	51.3	57.9
Clay [< 2 µm] (%)	16.4	37.9
pH		
in H_2O start/end	6.2 / 6.0	7.8 / 7.8
in KCl start/end	5.6 / 5.7	6.8 / 6.8
Organic Matter (%)*	7.6*	2.8*
Organic Carbon (%)	4.4	1.6
$N_{(total)}$ (mg/kg) start/end	4000/4000	2000/3000
$P_{(total)}$ (mg/kg) start/end	1030/1000	700/710
Biological (respiration) activity (mg CO_2/h/kg Sediment TS)		
Initial, 0 DAT	21	12
Final, 100 DAT	12	7
Total organic carbon (mg/L)	4 / 7	4 / 6
Hardness (mg equiv CaCO$_3$/L) start/end	8.7	13.2

Parameter	Water/sediment system	
Water/sediment system designation:	**Hoenniger**	**Stilwell**
Nitrate content (mg/L) start/end	77.3/2	80.9/2
Phosphorus content (mg/L) start/end	<0.03/<1	0.20/<1
Water characteristics		
Total organic carbon (mg/L)	4 / 7	4 / 6
Hardness (mg equiv $CaCO_3$/L) start/end	8.7	13.2
Nitrate content (mg/L) start/end	77.3/2	80.9/2
Phosphorus content (mg/L) start/end	<0.03/<1	0.20/<1

* Calculated by multiplying organic carbon content by 1.724 (not reported)

The sediments and the overlying water were collected from an artificial dammed pond (Hoenniger, Germany) and from a man made pond (Stilwell, US). No details provided on test system history. Prior to study initiation the aqueous sediment was passed through a 2 mm mesh sieve and thoroughly mixed before the dry weight was determined. The water samples were filtered through paper filters.

B. Study Design

1. Experimental Conditions

The behaviour of [cyclohexyl-1-^{14}C]-spiroxamine was investigated in two contrasting sediment water systems (Hoenniger and Stilwell) over a period of 100 days.

Each test vessel containing a sediment layer (2-2.5 cm depth; 98 g dry weight Hoenniger and 166 g dry weigh Stilwell) and an overlying water layer (6 cm) i.e. a water to sediment ratio of *ca.* 1:3 v/v. The water layer was gently agitated (25 rpm) by use of a magnetic stirrer. Each test vessel was attached to volatile traps of i) oil-coated (2% parrafin oil in hexane) quartz glass wool for sorption of organic volatiles and, ii) soda lime to collect carbon dioxide. Samples were incubated in the dark at a temperature of 20±2°C.

Water/sediment system samples were pre-acclimatised to the incubation conditions (20°C, dark) for 47 days before application of the test substance. Measurements of oxygen content, pH and redox potential were recorded.

[Cyclohexyl-1-^{14}C]-spiroxamine was applied to the surface of the water overlying sediment using a glass syringe at a target rate of 0.25 mg a.s./L, which was equivalent to a field application rate of 750 g/ha (assuming a water body depth of 30 cm).

A total of 20 batches (for each water-sediment system) were treated with the application solution. Two additional batches without addition of the spiroxamine was used for the determination of biological activity.

2. Sampling

Duplicate treated samples of each water/sediment system were removed for analysis after 0, 0.25, 1, 2, 7, 14, 33, 56 and 100 days of incubation. Untreated samples were analysed for biomass at the beginning and end of the experiment.

3. Analytical Procedures

For supernatant water 50 mL was drained off into a Erlenmeyer flask containing 1N NaOH (1 mL) for later processing (determination of CO_2 and/or carbonates). The remaining water was centrifuged and filtrated with any precipitate formed were combined together with the sediment sample to be extracted.

The volume and radioactivity content of the supernatant water was determined, before samples were analysed using TLC methods.

Sediment sample were extracted three times with acetonitrile at room temperature. The combined acetonitrile extracts were analysed using TLC methods. Aliquots of the extracted sediment of the day 2-100 samples were further extracted with methanol under reflux for 6 hours. After centrifugation and

filtration, the supernatant was analysed by TLC methods.

Volatile radioactivity collected in volatile traps was quantified by LSC. Any radioactivity in the polyurethane foam bung was extracted using ethyl acetate and quantified by LSC. Carbon dioxide in the calcium hydroxide traps determined using 18% hydrochloric acid and quantified by LSC

Following homogenisation, non-extractable residues (NER) in extracted sediments were determined by combustion.

Characterisation of radioactivity was carried out using relevant reference compound by co-chromatography. For identification of the metabolite M06 gas-chromatography was also conducted. The LOD for a single peak was ≥0.1% of the applied radioactivity.

4. Determination of degradation kinetics

The DT_{50} and DT_{90} of spiroxamine determined in the study was reported for each site. However, a re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (FOCUS 2014[2]) was performed in the report presented under point KCA 7.2.2.3.08 (M-763141-01-1).

II. Results and Discussion

A. Data

The distribution of radioactivity for each water/sediment system incubated at 20°C following application of [cyclohexyl-1-^{14}C]-spiroxamine are summarised in Table CA 7.2.2.3-13 and Table CA 7.2.2.3-14.

The characterisation of radioactivity for each water/sediment are summarised (as overall means) in Table CA 7.2.2.3-15 and Table CA 7.2.2.3-19. The characterisation of radioactivity for each separate layer for each water/sediment are summarised (as individual replicates) in Table CA 7.2.2.3-16 to Table CA 7.2.2.3-18 for the Hoeniger system and in Table CA 7.2.2.3-20 to Table CA 7.2.2.3-22 for the Stilwell system.

Table CA 7.2.2.3-13: Recovery and distribution of radioactivity following treatment of Hoeniger system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions [mean % AR]

Compound	Incubation time (DAT)								
	0	0.25	1	2	7	14	33	56	100
Supernatant water	87.0	52.4	37.4	15.2	3.9	2.4	2.3	3.2	2.1
Sediment extract	7.5	24.6	44.7	52.9	52.6	55.1	54.7	46.2	49.3
Non-extractable radioactivity	5.6	20.3	26.9	33.7	44.2	48.6	43.3	44.5	44.0
CO_2*	n.d.	1.4	2.1	1.4	1.8	2.0	3.3	6.6	6.8
Total	100.0	98.6	101.0	103.2	102.4	108.0	103.5	100.4	102.2

n.d.: not detected, DAT: days after treatment
* Includes volatile organic compounds <0.1% AR at any time.
Overall total recovery = 102.1% AR
All values expressed as percentage mean of applied radioactivity (% AR). Mean values determined outside the study report.

Table CA 7.2.2.3-14: Recovery and distribution of radioactivity following treatment of Stilwell system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions [mean % AR]

Compound	Incubation time (DAT)								
	0	0.25	1	2	7	14	33	56	100
Supernatant water	92.4	62.9	49.0	36.3	15.2	13.4	9.0	8.7	6.9
Sediment extract	7.7	36.2	47.2	61.1	82.1	87.7	90.6	88.0	88.9
Non-extractable radioactivity	6.5[A]	22.8	29.7	42.0	52.9	60.7	65.2	67.7	48.9
CO_2*	n.d.	1.4	2.2	2.0	1.5	2.3	3.6	5.5	16.8
Total	100.0	100.4	98.3	99.3	98.8	103.6	103.1	102.2	102.6

n.a.: not analysed, n.d.: not detected, DAT: days after treatment
* Includes the CO_2-part of the supernatant water. Volatile organic compounds <0.1% AR, at any time.
A Distribution of radioactivity of the membrane filter (M03 and non-extracted portion)
Overall total recovery = 100.9% AR
All values expressed as percentage of applied radioactivity (% AR). Mean values determined outside the study report.

Table CA 7.2.2.3-15: Characterisation of radioactivity following treatment of Hoenniger system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions [mean % AR] - Overview of means

Compound		Incubation time (DAT)								
		0	0.25	1	2	7	14	33	56	100
Spiroxamine	w	83.7	51.5	26.2	13.6	1.1	0.8	0.7	0.6	0.6
	s	7.5	24.0	43.3	50.7	48.8	52.0	49.2	40.4	42.7
M01 (desethyl)	w	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2	n.d.	0.1
	s	n.d.	0.3	0.6	1.3	1.1	0.2	1.7	1.2	1.5
M02 (despropyl)	w	0.8	n.d.	n.d.	n.d.	n.d.	n.d.	0.3	0.1	0.1
	s	n.d.	0.1	0.3	0.5	0.8	0.9	1.6	1.5	1.8
M03 (N-oxide)	w	2.1	0.7	1.0	0.3	n.d.	n.d.	0.3	0.1	0.1
	s	n.d.	0.2	0.1	0.5	0.5	0.3	0.2	0.4	0.9
M06 (acid)	w	n.d.	n.d.	n.d.	0.7	2.2	1.1	0.3	2.2	0.7
	s	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	1.3	1.9	1.0
M05 (hydroxy)	w	0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	s	n.d.	0.1	0.4	n.d.	0.9	1.5	0.7	0.8	0.7
Other unknowns	w	n.d.	0.2	0.2	0.3	0.6	0.6	0.5	0.5	0.8
	s	n.d.	n.d.	0.1	n.d.	0.5	n.d.	0.1	0.2	0.9

n.d.: not detected, DAT: days after treatment
All values expressed as percentage of mean applied radioactivity (% AR). Mean values determined outside the study report.

Table CA 7.2.2.3-16: Characterisation of radioactivity following treatment of Hoenniger system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions – Water layer [% AR]

Compound	Replicate	Incubation time (DAT)								
		0	**0.25**	**1**	**2**	**7**	**14**	**33**	**56**	**100**
Spiroxamine	A	95.1	52.1	23.1	7.8	1.2	0.7	0.9	0.7	0.4
	B	75.2	50.9	29.3	19.4	1.0	0.8	0.5	0.4	0.7
	Mean	**85.2**	**51.5**	**26.2**	**13.6**	**1.1**	**0.8**	**0.7**	**0.6**	**0.6**
M01 (desethyl)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	n.d.	0.1
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.2**	**n.d.**	**n.d.**
M02 (despropyl)	A	1.1	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	n.d.	n.d.
	B	0.4	n.d.	n.d.	n.d.	n.d.	n.d.	0.2	0.1	0.1
	Mean	**0.8**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.3**	**n.d.**	**n.d.**
M03 (N-oxide)	A	2.1	0.8	0.5	n.d.	n.d.	n.d.	0.4	n.d.	n.d.
	B	2.1	0.6	1.4	1.4	n.d.	n.d.	0.2	0.1	0.1
	Mean	**2.1**	**0.7**	**1.0**	**0.7**	**n.d.**	**n.d.**	**0.2**	**n.d.**	**n.d.**
M06 (acid)	A	n.d.	n.d.	n.d.	n.d.	3.7	1.3	0.5	0.7	0.5
	B	n.d.	n.d.	n.d.	1.3	0.7	0.8	0.1	3.6	0.8
	Mean	**n.d.**	**n.d.**	**n.d.**	**0.7**	**2.2**	**1.1**	**0.3**	**2.2**	**0.7**
M05 (hydroxy)	A	0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**0.5**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
Other unknowns	A	n.d.	n.d.	0.2	n.d.	0.7	0.8	0.6	0.2	1.5
	B	n.d.	0.3	0.2	0.5	0.4	0.3	0.4	0.7	0.1
	Mean	**n.d.**	**0.2**	**0.2**	**0.2**	**0.6**	**0.6**	**0.5**	**0.5**	**0.8**

n.d.: not detected, DAT: days after treatment
Mean values determined outside the study report

Table CA 7.2.2.3-17: Characterisation of radioactivity following treatment of Hoenniger system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions – Sediment layer [% AR]

Compound	Replicate	Incubation time (DAT)								
		0	**0.25**	**1**	**2**	**7**	**14**	**33**	**56**	**100**
Spiroxamine	A	4.0	22.0	44.6	56.1	47.1	53.9	49.4	49.4	42.6
	B	10.9	25.9	41.9	45.2	50.5	50.0	49.0	31.3	42.7
	Mean	**7.5**	**24.0**	**43.3**	**50.7**	**48.8**	**52.0**	**49.2**	**40.4**	**42.7**
M01 (desethyl)	A	n.d.	0.4	0.6	1.3	1.2	0.2	2.8	0.8	1.4
	B	n.d.	0.2	0.5	1.3	1.1	0.2	0.5	1.6	1.6
	Mean	**n.d.**	**0.3**	**0.6**	**1.3**	**1.2**	**0.2**	**1.7**	**1.2**	**1.5**
M02 (despropyl)	A	n.d.	0.1	0.3	0.5	0.9	0.9	2.2	1.0	1.7
	B	n.d.	0.1	0.3	0.4	0.6	0.8	0.9	1.9	1.8
	Mean	**n.d.**	**0.1**	**0.3**	**0.4**	**0.8**	**0.9**	**1.6**	**1.5**	**1.8**
M03 (N-oxide)	A	n.d.	0.2	0.1	0.6	0.5	0.3	0.2	0.2	1.4
	B	n.d.	0.1	n.d.	0.4	0.5	0.2	0.2	0.5	0.5
	Mean	**n.d.**	**0.2**	**n.d.**	**0.5**	**0.5**	**0.3**	**0.2**	**0.4**	**0.9**
M06 (acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	2.3	0.3	0.5
	B	n.d.	n.d.	n.d.	n.d.	n.d.	0.3	0.3	3.4	1.4
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.4**	**1.3**	**1.9**	**1.0**
M05 (hydroxy)	A	n.d.	n.d.	0.4	n.d.	1.0	1.7	n.d.	0.6	0.7
	B	n.d.	0.2	0.4	n.d.	0.7	1.2	1.3	1.0	0.6
	Mean	**n.d.**	**0.1**	**0.4**	**n.d.**	**0.9**	**1.5**	**0.7**	**0.8**	**0.7**
Other unknowns	A	n.d.	n.d.	0.2	n.d.	0.7	n.d.	0.2	n.d.	1.7
	B	n.d.	n.d.	n.d.	n.d.	0.3	n.d.	n.d.	0.3	0.1
	Mean	**n.d.**	**n.d.**	**0.1**	**n.d.**	**0.5**	**n.d.**	**0.1**	**0.2**	**0.8**

n.d.: not detected, DAT: days after treatment
Mean values determined outside the study report

Table CA 7.2.2.3-18: Characterisation of radioactivity following treatment of Hoenniger system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions – Total system [% AR]

Compound	Replicate	Incubation time (DAT)								
		0	**0.25**	**1**	**2**	**7**	**14**	**33**	**56**	**100**
Spiroxamine	A	99.1	74.1	67.7	63.9	48.3	54.6	50.3	50.1	43.0
	B	83.1	76.8	71.2	64.6	51.5	50.8	49.5	31.7	43.4
	Mean	**91.1**	**75.5**	**69.5**	**64.3**	**49.9**	**52.8**	**49.9**	**41.0**	**43.3**
M01 (desethyl)	A	n.d.	0.4	0.6	1.3	1.2	0.2	3.1	0.8	1.4
	B	n.d.	0.2	0.5	1.3	1.1	0.2	0.6	1.6	1.7
	Mean	**n.d.**	**0.3**	**0.6**	**1.3**	**1.2**	**0.2**	**1.9**	**1.2**	**1.5**
M02 (despropyl)	A	1.1	0.1	0.3	0.5	0.9	0.9	2.6	1.0	1.7
	B	0.4	0.1	0.3	0.4	0.6	0.8	1.1	2.0	1.9
	Mean	**0.8**	**0.1**	**0.3**	**0.4**	**0.8**	**0.9**	**1.9**	**1.5**	**1.8**
M03 (N-oxide)	A	2.1	1.0	0.6	0.6	0.5	0.3	0.5	0.2	1.4
	B	2.1	0.7	1.4	1.8	0.5	0.2	0.4	0.5	0.5
	Mean	**2.1**	**0.9**	**1.0**	**1.2**	**0.5**	**0.3**	**0.4**	**0.4**	**0.9**
M06 (acid)	A	n.d.	n.d.	n.d.	n.d.	3.7	1.7	2.8	1.0	1.0
	B	n.d.	n.d.	n.d.	1.3	0.7	1.1	0.4	7.0	2.2
	Mean	**n.d.**	**n.d.**	**n.d.**	**0.7**	**2.2**	**1.5**	**1.6**	**4.1**	**1.7**
M05 (hydroxy)	A	0.5	0	0.4	n.d.	1	1.7	0	0.6	0.7
	B	0.5	0.2	0.4	n.d.	0.7	1.2	1.3	1.0	0.6
	Mean	**0.5**	**0.1**	**0.4**	**n.d.**	**0.9**	**1.5**	**0.7**	**0.8**	**0.7**
Other unknowns	A	n.d.	n.d.	0.4	0	1.4	0.8	0.8	0.2	3.2
	B	n.d.	0.3	0.2	0.5	0.7	0.3	0.4	1.0	0.2
	Mean	**n.d.**	**0.2**	**0.3**	**0.2**	**1.1**	**0.6**	**0.6**	**0.7**	**1.6**

n.d.: not detected, DAT: days after treatment
Mean values determined outside the study report

Table CA 7.2.2.3-19: Characterisation of radioactivity following treatment of Stilwell system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions [mean % AR] - Overview of means

Compound		Incubation time (DAT)								
		0	**0.25**	**1**	**2**	**7**	**14**	**33**	**56**	**100**
Spiroxamine	w	80.0	56.4	42.9	26.5	9.5	2.2	0.4	0.1	n.d.
	s	1.2	12.3	14.9	16.2	22.9	21.8	20.8	16.5	23.6
M01 (desethyl)	w	0.4	n.d.	0.2	0.5	0.4	0.4	n.d.	n.d.	n.d.
	s	n.d.	0.8	n.d.	0.7	1.0	n.d.	0.1	0.3	0.6
M02 (despropyl)	w	0.7	0.3	n.d.	0.7	0.2	n.d.	0.2	n.d.	n.d.
	s	n.d.	0.3	0.5	0.5	0.9	1.1	0.6	0.5	1.3
M03 (N-oxide)	w	11.3	6.7	5.9	3.7	2.7	2.9	0.9	0.3	0.5
	s	n.d.	0.1	0.4	0.6	1.5	1.0	0.7	0.6	0.7
M06 (acid)	w	n.d.	n.d.	n.d.	n.d.	1.7	6.7	4.8	6.0	4.0
	s	n.d.	n.d.	n.d.	n.d.	n.d.	1.0	1.6	1.8	2.3
M11 (desethyl acid)	w	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	0.5	0.4	0.4
	s	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M05 (hydroxy)	w	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2	n.d.	n.d.
	s	n.d.	n.d.	1.3	0.7	1.5	2.3	1.4	0.5	1.2
Other unknowns	w	n.d.	0.5	n.d.	n.d.	0.8	1.0	2.1	2.0	2.2
	s	n.d.	n.d.	0.5	0.6	1.5	0.1	0.4	0.3	0.5

n.d.: not detected, DAT: days after treatment
All values expressed as percentage of mean applied radioactivity (% AR). Mean values determined outside the study report.

Table CA 7.2.2.3-20: Characterisation of radioactivity following treatment of Stilwell system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions – Water layer [% AR]

Com-pound	Repli-cate	Incubation time (DAT)								
		0	**0.25**	**1**	**2**	**7**	**14**	**33**	**56**	**100**
Spirox-amine	A	87.1	56.3	48.7	27.0	9.1	2.1	0.4	n.d.	n.d.
	B	72.9	54.3	37.1	26.0	9.9	2.3	0.3	0.2	n.d.
	Mean	**80.0**	**55.4**	**42.9**	**26.5**	**9.5**	**2.2**	**0.4**	**0.1**	**n.d.**
M01 (de-sethyl)	A	0.2	n.d.	n.d.	0.4	0.3	0.5	n.d.	n.d.	n.d.
	B	0.5	n.d.	0.3	0.5	0.4	0.3	n.d.	n.d.	n.d.
	Mean	**0.4**	**n.d.**	**0.2**	**0.5**	**0.4**	**0.4**	**n.d.**	**n.d.**	**n.d.**
M02 (despro-pyl)	A	0.7	0.6	n.d.	0.8	0.2	n.d.	0.2	n.d.	n.d.
	B	0.7	n.d.	n.d.	0.8	0.2	n.d.	0.2	n.d.	n.d.
	Mean	**0.7**	**0.3**	**n.d.**	**0.7**	**0.2**	**n.d.**	**0.2**	**n.d.**	**n.d.**
M03 (N-ox-ide)	A	10.8	8.2	6.4	8.2	2.4	2.7	0.9	0.4	0.5
	B	11.8	5.2	5.4	9.2	3.0	3.0	0.9	0.2	0.4
	Mean	**11.3**	**6.7**	**5.9**	**8.7**	**2.7**	**2.9**	**0.9**	**0.3**	**0.5**
M06 (acid)	A	n.d.	n.d.	n.d.	n.d.	1.8	3.8	4.6	4.2	4.5
	B	n.d.	n.d.	n.d.	n.d.	1.6	9.5	4.9	7.7	3.4
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**1.7**	**6.7**	**4.8**	**6.0**	**4.0**
M11 (de-sethyl acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3	0.3	0.3
	B	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	0.6	0.5	0.4
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.4**	**0.5**	**0.4**	**0.4**
M05 (hy-droxy)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.2**	**n.d.**	**n.d.**
Other un-knowns	A	n.d.	0.6	n.d.	n.d.	0.7	1.2	1.9	1.5	1.8
	B	n.d.	0.3	n.d.	n.d.	0.8	0.7	2.3	2.4	2.5
	Mean	**n.d.**	**0.5**	**n.d.**	**n.d.**	**0.8**	**1.0**	**2.1**	**2.0**	**2.2**

n.d.: not detected, DAT: days after treatment
Mean values determined outside the study report

Table CA 7.2.2.3-21: Characterisation of radioactivity following treatment of Stilwell system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions – Sediment layer [% AR]

Compound	Replicate	Incubation time (DAT)								
		0	**0.25**	**1**	**2**	**7**	**14**	**33**	**56**	**100**
Spiroxamine	A	0.7	17.3	12.3	15.8	22.3	22.7	22.7	17.5	26.3
	B	1.7	7.2	17.4	16.5	23.5	20.8	18.8	15.4	20.9
	Mean	**1.2**	**12.3**	**14.9**	**16.2**	**22.9**	**21.8**	**20.8**	**16.5**	**23.6**
M01 (desethyl)	A	n.d.	1.1	n.d.	0.6	1.0	n.d.	0.1	0.2	0.8
	B	n.d.	0.4	n.d.	0.7	0.9	n.d.	0.1	0.3	0.7
	Mean	**n.d.**	**0.8**	**n.d.**	**0.7**	**1.0**	**n.d.**	**0.1**	**0.3**	**0.6**
M02 (despropyl)	A	n.d.	0.4	0.5	0.5	0.8	1.1	0.6	0.5	1.1
	B	n.d.	0.2	0.4	0.4	1.0	1.0	0.6	0.5	1.4
	Mean	**n.d.**	**0.3**	**0.5**	**0.5**	**0.9**	**1.1**	**0.6**	**0.5**	**1.3**
M03 (N-oxide)	A	n.d.	0.2	0.4	0.4	1.4	1.0	0.6	0.5	0.6
	B	n.d.	n.d.	0.4	0.8	1.5	0.9	0.7	0.6	0.7
	Mean	**n.d.**	**0.1**	**0.4**	**0.6**	**1.5**	**1.0**	**0.7**	**0.6**	**0.7**
M06 (acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	0.6	1.5	1.4	2.6
	B	n.d.	n.d.	n.d.	n.d.	n.d.	1.3	1.6	2.1	1.9
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**1.0**	**1.6**	**1.8**	**2.3**
M11 (desethyl acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
M05 (hydroxy)	A	n.d.	n.d.	1.2	0.7	1.4	2.4	1.4	0.5	1.1
	B	n.d.	n.d.	1.4	0.7	1.5	2.2	1.4	0.5	1.3
	Mean	**n.d.**	**n.d.**	**1.3**	**0.7**	**1.5**	**2.3**	**1.4**	**0.5**	**1.2**
Other unknowns	A	n.d.	n.d.	1.0	0.7	1.3	0.1	0.3	0.2	0.5
	B	n.d.	n.d.	n.d.	0.4	1.7	n.d.	0.4	0.4	0.4
	Mean	**n.d.**	**n.d.**	**0.5**	**0.6**	**1.5**	**0.1**	**0.4**	**0.3**	**0.5**

n.d.: not detected, DAT: days after treatment
Mean values determined outside the study report.

Table CA 7.2.2.3-22: Characterisation of radioactivity following treatment of Stilwell system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under aerobic conditions – Total system [% AR]

Compound	Replicate	Incubation time (DAT)								
		0	0.25	1	2	7	14	33	56	100
Spiroxamine	A	87.8	73.6	61.0	42.8	31.4	24.8	23.1	17.5	26.3
	B	74.6	61.7	54.5	42.5	33.4	23.1	19.1	15.6	20.9
	Mean	**81.2**	**67.7**	**57.8**	**42.7**	**32.4**	**24**	**21.2**	**16.6**	**23.6**
M01 (desethyl)	A	0.2	1.1	n.d.	1.0	1.3	0.5	0.1	0.2	0.8
	B	0.5	0.4	0.3	1.2	1.3	0.3	0.1	0.3	0.7
	Mean	**0.4**	**0.8**	**0.2**	**1.2**	**1.4**	**0.4**	**0.1**	**0.3**	**0.6**
M02 (despropyl)	A	0.7	1.0	0.5	1.0	1.0	1.1	0.8	0.5	1.1
	B	0.7	0.2	0.4	1.2	1.2	1.0	0.8	0.5	1.4
	Mean	**0.7**	**0.6**	**0.5**	**1.1**	**1.1**	**1.1**	**0.8**	**0.5**	**1.3**
M03 (N-oxide)	A	10.8	8.4	6.8	8.6	3.8	3.7	1.5	0.9	1.1
	B	11.8	5.2	5.8	10.0	4.5	3.9	1.6	0.8	1.3
	Mean	**11.3**	**6.8**	**6.3**	**9.3**	**4.2**	**3.9**	**1.6**	**0.9**	**1.2**
M06 (acid)	A	n.d.	n.d.	n.d.	n.d.	1.8	4.4	6.1	5.6	7.1
	B	n.d.	n.d.	n.d.	n.d.	1.6	10.8	6.5	9.8	5.3
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**1.7**	**7.7**	**6.4**	**7.8**	**6.3**
M11 (desethyl acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3	0.3	0.3
	B	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	0.6	0.5	0.4
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.4**	**0.5**	**0.4**	**0.4**
M05 (hydroxy)	A	n.d.	n.d.	1.2	0.7	1.4	2.4	1.6	0.5	1.1
	B	n.d.	n.d.	1.4	0.7	1.5	2.2	1.6	0.5	1.3
	Mean	**n.d.**	**n.d.**	**1.3**	**0.7**	**1.5**	**2.3**	**1.6**	**0.5**	**1.2**
Other unknowns	A	n.d.	0.6	1.0	0.7	2.0	1.3	2.2	1.7	2.3
	B	n.d.	0.3	n.d.	0.4	2.5	0.7	2.7	2.8	2.9
	Mean	**n.d.**	**0.5**	**0.5**	**0.6**	**2.3**	**1.1**	**2.5**	**2.3**	**2.7**

n.d.: not detected, DAT: days after treatment
Mean values determined outside the study report.

B. Material Balance

The material balance (mean) at each sampling interval ranged from 98.3 to 108.0% AR for the two test systems. The overall mean material balance was 102.1 and 100.9% AR for the Hoenniger and Stilwell water/sediment systems, respectively.

C. Extractable and Non-Extractable Residues

The mean proportion of total radioactivity extracted from sediment samples increased slowly over time from 7.5% AR (mean) at 0 DAT to 49.3% AR (mean) at 100 DAT in the Hoenniger system. The Stilwell system increased similarly, from 7.2% AR (mean) at 0 DAT to 79.5% AR (mean) at 100 DAT. The mean proportion of total radioactivity in the overlying water declined slowly over time from 87.0% AR (mean) at 0 DAT to 2.1% AR (mean) at 100 DAT in the Hoenniger system. The Stilwell system declined similarly, from 92.4% AR (mean) at 0 DAT to 6.9% AR (mean) at 100 DAT. Non-extracted residues (bound residues) increased slowly throughout the incubation, reaching maximum levels of 44.0% AR (mean) in Hoenniger at 100 DAT and 48.9% AR (mean) in Stilwell at 76 DAT.

D. Volatile Radioactivity

Significant radioactivity carbon dioxide was in both systems from 30 DAT, reaching maximum levels of 6.8% AR in Hoenniger at 100 DAT and 16.8% AR in Stilwell by 100 DAT. Subsequently, selected samples of PU bung extracts from both systems were analysed by HPLC and the volatile radioactivity was confirmed to be volatile ^{14}C-spiroxamine.

The level of applied radioactivity recovered as volatile organic material ≤0.1% AR for all systems measured.

E. Degradation of Parent Compound

Following application of [cyclohexyl-1-^{14}C]-spiroxamine, spiroxamine showed rapid dissipation in the water phase of both systems, declining from 83.7% AR (mean) at 0 DAT to 0.6% by 100 DAT in the Hoenniger systems. Similarly, in the Stilwell system spiroxamine showed rapid dissipation in the water phase of both systems, declining from 80.0% AR (mean) at 0 DAT to ≥0.1% by 100 DAT. In the Hoenniger system, the amount of spiroxamine in the sediment increased to a maximum of 52.0% AR (mean) after 14 DAT and subsequently decreased to 42.7% AR (mean) after 100 DAT. In the Stilwell system, the amount of spiroxamine in the sediment increased to a maximum of 23.6% AR (mean) after 100 DAT.

Degradation of [cyclohexyl-1-^{14}C]-spiroxamine in the total system was accompanied by the formation of one major degradation product M06 (spiroxamine-acid: max 7.7% AR (mean) at 56 DAT) which was determined to be >5% AR for more than two consecutive time points. Additionally, several other minor metabolites M01 (spiroxamine-desethyl: max 1.9% AR (mean) at 33 DAT), M02 (spiroxamine-despropyl: max 1.9% AR (mean) at 33 DAT), M03 (spiroxamine-N-oxide: max 9.3% AR (mean) at 2 DAT), M11 (spiroxamine-desethyl acid: max 0.5% AR (mean) at 33 DAT) and M05 (spiroxamine- hydroxy max 2.3% AR (mean) at 14 DAT). Some other minor unidentified metabolites were observed but none of which exceeded a total of 2.5% AR (mean) at any sampling interval.

F. Degradation Kinetics

Degradation rates determined in the report are superseded by the report presented under point KCA 7.2.2.3/08 (M-763141-01-1).

III. Conclusions

[Cyclohexyl-1-^{14}C]-spiroxamine degraded in water/sediment systems under aerobic conditions (20°C, dark) with 0.6-≥0.1% of applied radioactivity remaining as parent compound after 100 DAT in the Hoenniger and Stilwell systems, respectively. Carbon dioxide was a major degradation project (max 16.8% at 100 DAT), while no volatile organic compound were observed. The amounts of non-extractable radioactivity were 44.0% and 48.9% AR (mean) at the end of the study for Hoenniger and Stilwell systems, respectively. The major metabolic pathways involved oxidation of a methyl group to form M06 (spiroxamine-acid, maximum 7.7% AR (mean)).

A re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) is performed in the report presented under point KCA 7.2.2.3/08 (M-763141-01-1).

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.
The study was conducted in accordance with study guideline(s) BBA Ref.: Degradability and Fate of Plant Protection Products in Water sediment Systems 5-1 (Dec. 1990) (similar to required guideline, minor differences).
The study is considered valid to assess fate and behaviour of [cyclohexyl-1-^{14}C]-spiroxamine in aerobic water sediment systems.

Data Point:	KCA 7.2.2.3/02
Report Author:	[redacted]
Report Year:	1996
Report Title:	Aerobic metabolism of KWG 4168-N-Oxide in an aquatic model ecosystem
Report No:	PF4180
Document No:	M-006094-01-1
Guideline(s) followed in study:	BBA Ref.: Degradability and Fate of Plant Protection Products in Water sediment System 5-1 (Dec. 1990)
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

The route and rate of degradation of [cyclohexyl-1-^{14}C]-spiroxamine N-oxide (M03) was investigated in one water/sediment systems (Hoenniger, silt loam, 4.4% OC, pH 5.7) under laboratory aerobic conditions in the dark at 20°C for up to 6 days. The purpose of this study was to investigate the metabolite M03 which was detected in a previous water sediment study at low concentrations (see KCA 7.2.2.3/01 (M-006015-01-1)) and was not intended to be a full standalone study. The study was intended to simulate a use rate of 750 a.s./ha; therefore considering a transformation of 50% of the parent spiroxamine into spiroxamine N-oxide (M03) and a water depth of 30 cm the application rate corresponds to 0.066 mg/500 mL water. Test systems were filled with a layer of sediment to a depth of 1.7 cm (31.7 g dry weight) and overlying water (500 mL).

Two vessels were treated with application solution, closed with an oxygen permeable trap attachment and kept in the dark at 20± 2°C. At the first sampling dates (day 1 and 3), only water samples were taken, whilst from day 6 onwards complete batches were analysed.

After decantation the sediment was extracted with acetonitrile at room temperature three times. The combined filtered extracts were investigated by TLC. The radioactivity still present in the material of the filter was determined by combined combustion and LSC of trapped combustion gases. The stability of [cyclohexyl-1-^{14}C]-spiroxamine N-oxide (M03) under the condition of processing was demonstrated by adding the test substance to an aliquot of moist sediment and by processing as described above.

All liquid extracts were radioassayed. Radioactive residues bound in the sediment were determined by combined combustion and LSC of trapped combustion gases. Aliquots of the sediments extracts or supernatant water were investigated by TLC on silica gel with one solvent systems. Components were visualised by autoradiography, radioactivity scanning and in the case of unlabelled reference substances by reaction in an iodine chamber.

The overall mean material balance was 109.2% AR for the Hoenniger water/sediment systems.

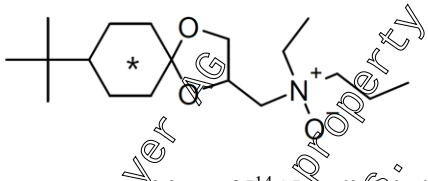
A total amount of 22% of the test substance [cyclohexyl-1-^{14}C]-spiroxamine N-oxide (M03) was detected in the supernatant water after 6 days. 40.9% AR was extracted in the form of the transformation product spiroxamine, with 37.2% AR found in the sediment and 3.7% AR from the aqueous phase. Another portion of 20.3% AR constituted non-extractable sediment residues.

I. Materials and Methods

A. Materials

1. Test Items

[cyclohexyl-1-^{14}C]-spiroxamine N-oxide (M03)



* Denotes position of [^{14}C]-radiolabel

Specific Activity: 3.44 MBq/mg

Radiochemical Purity: >98%

2. Test System (water/sediment system)

The study was performed using the water/sediment system as characterised in Table CA 7.2.2.3-23.

Table CA 7.2.2.3-23: Physico-chemical properties of the water sediment systems

Parameter	Water/sediment system
Water/sediment system designation:	**Hoenniger**
Geographic Location City Country	 Wipperfürth Germany
Sediment characteristics	
Textural Classification (USDA)	Silt loam
Sand [50 - 2000 µm] (%)	32.3
Silt [2 – 50 µm] (%)	51.3
Clay [< 2 µm] (%)	16.4
pH in H_2O start/end in KCl start/end	 6.2 / 6.0 5.6 / 5.7
Organic Matter (%)*	7.6*
Organic Carbon (%)	4.4
$N_{(total)}$ (mg/kg) start/end	4000/4000
$P_{(total)}$ (mg/kg) start/end	1030/1000
Biological (respiration) activity (mg CO_2/h/kg Sediment TS) Initial, DAT 0 Final, 100 DAT 120	 21 12
Total organic carbon (mg/L)	4 / 7
Hardness (mg equiv $CaCO_3$/L) start/end	8.7

Parameter	Water/sediment system
Water/sediment system designation:	**Hoenniger**
Nitrate content (mg/L) start/end	77.3/2
Phosphorus content (mg/L) start/end	<0.03/<1
Water characteristics	
Total organic carbon (mg/L)	4 / 7
Hardness (mg equiv $CaCO_3$/L) start/end	8.7
Nitrate content (mg/L) start/end	77.3/2
Phosphorus content (mg/L) start/end	<0.03/<1

* Calculated by multiplying organic carbon content by 1.724 (not reported)

A Although location not specified, the report does state that the source of the test system was from the same location as used for study KCA 7.2.2.3/01 (M-006015-01-1)

The sediments and the water were collected from an artificial dammed pond (Hoenniger). No details provided on test system history. Prior to study initiation, the aqueous sediment was passed through a 2 mm mesh sieve and thoroughly mixed before the dry weight was determined. The water samples were filtered through paper filters.

B. Study Design

1. Experimental Conditions

The behaviour of [cyclohexyl-1-^{14}C]-spiroxamine N-oxide (M03) was investigated in one sediment/water systems (Hoenniger) over a period of 6 days. The purpose of this study was to investigate the metabolite M03 which was detected in a previous water sediment study at low concentrations, see KCA 7.2.2.3/01 (M-006015-01-1).

Test systems were filled with a layer of sediment to a depth of 1.7 cm (31.7 g dry weight) and overlying water (500 mL).The water layer was gently agitated (30 rpm) by use of a magnetic stirrer. Each test vessel was attached to a calcium hydroxide volatile trap with quartz wool plugs and polyurethane foam bungs to collect carbon dioxide. Samples were incubated in the dark at a temperature of 20±2°C. The study was intended to simulate a use rate of 750 g s./ha, therefore considering a transformation of 50% of the parent spiroxamine into spiroxamine N-oxide (M03) and a water depth of 30 cm the application rate corresponds to 0.066 mg/500 mL water.

2. Sampling

Duplicate treated samples of each water/sediment system were removed for analysis after 1, 3 and 6 days of incubation. At the first sampling dates (day 1 and 3), only water samples were taken, whilst on day 6 complete batches were analysed.

3. Analytical Procedures

After decantation, the sediment was extracted with acetonitrile at room temperature three times. The combined filtered extracts were investigated by TLC. The radioactivity still present in the material of the filter was determined by combined combustion and LSC of trapped combustion gases. The stability of [cyclohexyl-1-^{14}C]-spiroxamine N-oxide (M03) under the condition of processing was demonstrated by adding the test substance to an aliquot of moist sediment and by processing as described above.

All liquid extracts were radioassayed. Radioactive residues bound in the sediment were determined by combined combustion and LSC of trapped combustion gases. Aliquots of the sediments extracts or supernatant water were investigated by TLC on silica gel with one solvent systems. The limit of detection (LOD) for a single peak was ≥0.1% of the applied radioactivity. Components were visualised by autoradiography, radioactivity scanning and in the case of unlabelled reference substances by reaction in an iodine chamber.

II. Results and Discussion

A. Data

The distribution and characterisation of radioactivity for the water/sediment system incubated at 20°C following application of [cyclohexyl-1-^{14}C]-spiroxamine N-oxide (M03) are summarised in Table CA 7.2.2.3-24 and Table CA 7.2.2.3-25.

Table CA 7.2.2.3-24: Recovery and distribution of radioactivity following treatment of Hoenniger system with [cyclohexyl-1-^{14}C]-spiroxamine N-oxide (M03) under aerobic conditions [mean % AR]

Compound	Incubation time (DAT)		
	1	3	6
Supernatant water	80.0	61.4	33.2
Sediment	n.a.	n.a.	76.0
Non-extractable radioactivity	n.a.	n.a.	20.1
Total	n.a.	n.a.	109.2

n.a.: not analysed, DAT: days after treatment
Overall mean radioactive from the whole system was 109.2% AR at 6 DAT
All values expressed as mean percentage of applied radioactivity (% AR)

Table CA 7.2.2.3-25: Characterisation of radioactivity following treatment of Hoenniger system with [cyclohexyl-1-^{14}C]-spiroxamine N-oxide (M03) under aerobic conditions [% AR]

Compound		Incubation time (DAT)					
		1		3		6	
		Replicate A	Replicate B	Replicate A	Replicate B	Replicate A	Replicate B
Supernatant water	Spiroxamine	0.3	0.3	2.3	3.6	1.9	3.4
	M01 (desethyl)	0.5	0.8	1.9	2.4	2.2	3.8
	M02 (despropyl)	0.6	0.8	2.1	2.3	2.9	3.1
	M03 (N-oxide)	76.3	80.3	52.4	52.6	23.5	20.5
	M06 (acid)	n.d.	n.d.	0.8	0.9	0.8	1.2
	M11 (desethyl acid)	n.d.	n.d.	n.d.	n.d.	0.4	0.5
	M12 (despropyl acid)	n.d.	n.d.	n.d.	n.d.	n.d.	<0.1
	M15 (ketone)	n.d.	n.d.	0.5	0.9	n.d.	n.d.
	Total	**77.7**	**82.2**	**60.1**	**62.7**	**31.7**	**34.7**
Sediment	Spiroxamine	n.a.	n.a.	n.a.	n.a.	37.4	37.0
	M01 (desethyl)	n.a.	n.a.	n.a.	n.a.	7.5	7.0
	M02 (despropyl)	n.a.	n.a.	n.a.	n.a.	5.1	4.7
	M03 (N-oxide)	n.a.	n.a.	n.a.	n.a.	3.0	1.8
	M15 (ketone)	n.a.	n.a.	n.a.	n.a.	3.6	3.1
	Unknown	n.a.	n.a.	n.a.	n.a.	0.8	3.1
	Unextracted portion*	n.a.	n.a.	n.a.	n.a.	19.8	21.1
	Total	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**76.8**	**75.2**
Total		**n.a.**	**n.a.**	**n.a.**	**n.a.**	**108.5**	**109.9**
Total system	Spiroxamine	n.a.	n.a.	n.a.	n.a.	39.3	42.4
	M01 (desethyl)	n.a.	n.a.	n.a.	n.a.	9.7	10.8
	M02 (despropyl)	n.a.	n.a.	n.a.	n.a.	8.0	8.0
	M03 (N-oxide)	n.a.	n.a.	n.a.	n.a.	26.5	22.3
	M06 (acid)	n.a.	n.a.	n.a.	n.a.	0.8	1.2
	M11 (desethyl acid)	n.a.	n.a.	n.a.	n.a.	0.4	0.5
	M12 (despropyl acid)	n.a.	n.a.	n.a.	n.a.	n.d.	<0.1
	M15 (ketone)	n.a.	n.a.	n.a.	n.a.	3.6	3.1
	Unknown	n.a.	n.a.	n.a.	n.a.	0.8	0.5
	Total	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**89.1**	**88.9**

n.a.: not analysed, n.d.: not detected, DAT: days after treatment
Overall mean radioactive from the whole system was 109.2% AR at 6 DAT.

B. Material Balance

The material balance was 109.2% AR at the one sampling interval where measured (6 day).

C. Degradation of Parent Compound

A total amount of 22% of the test substance [cyclohexyl-1-^{14}C]-spiroxamine N-oxide (M03) was detected in the supernatant water after 6 days. 37.2% AR were extracted in the form of the transformation product spiroxamine from the sediment and a further 3.7% AR from the aqueous phase. Another portion of 20.3% constituted non-extractable sediment residues. The authors of the study concluded that the outcome of the experiment cannot be explained by degradation of the test compound during processing of the sediment, as it had been proved by recovery experiments with [cyclohexyl-1-^{14}C]-spiroxamine N-oxide (M03) that it remains intact under the processing conditions.

III. Conclusions

The behaviour of [cyclohexyl-1-^{14}C]-spiroxamine N-oxide (M03) was investigated in one sediment/water systems (Hoenniger) over a period of 6 days in a small scale investigation. The purpose of this study was to investigate the fate of the metabolite M03 which was detected in a previous water sediment study at low concentrations (KCA 7.2.2.3/01 (M-006015-01-1)). A total amount of 22% of the test substance [cyclohexyl-1-^{14}C]-spiroxamine N-oxide (M03) was detected in the supernatant water after 6 days. 37.2% was extracted in the form of the transformation product spiroxamine from the sediment and 3.7% from the aqueous phase. Another portion of 20.3% constituted non-extractable sediment residues.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was not conducted to study guideline(s) OECD 308 (required guideline) in entirety. However, the study is considered supplementary information to assess fate and behaviour of [cyclohexyl-1-^{14}C]-spiroxamine N-oxide (M03) in aerobic water sediment systems.

Data Point:	KCA 7.2.2.3/03
Report Author:	[redacted]
Report Year:	1996
Report Title:	Metabolism of KWG 4168 and its N-oxide in an aquatic model ecosystem and the ecotoxicological relevance of the N-oxide
Report No:	M9937
Document No:	M-032874-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Supportive only

Executive Summary

This study was previously considered during the evaluation of spiroxamine (DAR (1997), RAR (2010), RAR (2017)) and is therefore included again for completeness. The study reviews the fate and behaviour of the metabolite M03 (spiroxamine-N-oxide) observed in the studies KCA 7.2.2.3/01 (M-006015-01-1) and KCA 7.2.2.3/02 (M-006094-01-1) and contains no primary data. Full summaries of the referenced studies are provided under their respectively locations.

Assessment and conclusion by applicant:

The study and its data are considered as supplementary data with no use in risk assessment.

Data Point:	KCA 7.2.2.3/04
Report Author:	[redacted]; [redacted]
Report Year:	2008
Report Title:	[1,3-Dioxolane-4-14C]spiroxamine: Aerobic aquatic metabolism
Report No:	MEF-07/483
Document No:	M-303324-01-1
Guideline(s) followed in study:	OECD 308; 95/36/EC amending 91/414/EEC, Annexes II and III; SETAC Procedures, March 1995
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The route and rate of degradation of [1,3-dioxolane-4-^{14}C]-spiroxamine was investigated in two different water/sediment systems (Hoenniger and Anglerweiher, Germany) under laboratory aerobic conditions in the dark at 20°C for up to 118 days.

The sediments and the water samples were collected from an artificial dammed pond (Hoenniger, sandy loam, 3.8% OC, pH 5.2) and from a man made lake (Anglerweiher, sand, 0.8% OC, pH 6.6). [1,3-dioxolane-4-^{14}C]-spiroxamine (radiochemical purity >98%) was applied to the surface of the water overlying sediment at a target rate of 0.25 mg a.s./L, which was equivalent to a field application rate of 750 g/ha (assuming a water body depth of 30 cm). The water to sediment ratio was 3:1 (v/v). Sediment volumes of 175 mL (height *ca.* 2 cm) were transferred to the incubation vessels and water volumes of 520 mL (height *ca.* 6 cm) were added.

Prior to application of the test item, the systems were maintained under test conditions for the purpose of equilibration for 7 days. The flasks were incubated in the dark at 20°C. Aerobic conditions were maintained by the flow of air through an inlet. The laboratory microcosm flasks were connected with traps to collect CO_2 (using calcium hydroxide) and volatile organic compounds (using polyurethane foam). The redox potential as well as the pH and the dissolved oxygen content were determined before processing of water and sediment samples. Duplicate treated samples of each water/sediment system were removed for analysis after 0, 0.25, 1, 2, 7, 14, 30, 61 and 118 days of incubation.

The water samples were analysed without any processing. The sediment samples were extracted with acetonitrile at ambient temperature, followed by a hot extraction for an hour using acetonitrile and by a hot extraction for 6 hours with methanol. Spiroxamine residues were analysed by HPLC. Identification of the test item was achieved by co-chromatography with reference item as well as by HPLC-MS/MS and HPLC-^{1}H-NMR.

The total material balance of the two water/sediment systems was 94.0% of the applied radioactivity (AR), calculated as mean value of both systems and two parallel experiments for each system.

The radioactivity in the Anglerweiher water decreased steadily from 64.6% AR (mean) at 0 DAT to 10.9% AR (mean) at study end. The radioactivity in the Hoenniger water decreased from 62.5% of the AR (mean) at 0 DAT to 4.9% AR (mean) at study termination. Extractable ^{14}C residues in the Anglerweiher sediment increased from 25.5% AR (mean) at 0 DAT, to a maximum of 63.4% AR (mean) at 2 DAT before decreasing to 18.9% AR (mean) at study termination. Extractable ^{14}C residues in the Hoenniger sediment increased from 20.7% (mean) at 0 DAT, to a maximum of 46.6% (mean) at 2 DAT before decreasing to 32.8% AR (mean) at study end. The maximum of non-extractable ^{14}C residues in the two sediments was 33.2% AR and 40.7% AR in Anglerweiher and Hoenniger, respectively (mean values).

At the end of the study, 27.1% (mean, Anglerweiher) and 7.6% (mean, Hoenniger) of the AR was present as CO_2. Organic volatile compounds amounted to less than 0.1% of the AR in both systems.

Following application of [1,3-dioxolane-4-^{14}C]-spiroxamine, spiroxamine showed rapid dissipation in the water phase of both systems, declining from 62.5% AR (mean) at 0 DAT to below the LOD by 118 DAT in the Hoenniger systems. Similarly, in the Anglerweiher system spiroxamine showed rapid dissipation in the water phase, declining from 64.6% AR (mean) at 0 DAT to below the LOD by 118 DAT.

In the Hoenniger system, the amount of spiroxamine in the sediment increased to a maximum of 54.2% AR (mean) after 2 DAT and subsequently decreased to 29.3% AR (mean) after 118 DAT. In the Anglerweiher system, the amount of spiroxamine in the sediment increased to a maximum of 60.1% AR (mean) after 2 DAT, and subsequently decreased to 14.9% AR (mean) after 118 DAT.

Degradation of [1,3-dioxolane-4-^{14}C]-spiroxamine in the total system was accompanied by the formation of one major degradation product M06 (spiroxamine-acid). In the water phase it amounted to a maximum of 25.6% of the AR (Anglerweiher) and 13.6% of the AR (Hoenniger Weiher). In the sediment extracts M06 accounted for only 7.9% and 8.9% of the AR, respectively. Additionally several other minor unknown metabolites were observed but not identified in the report, with the sum of minor metabolites reached a maximum of 13.5% AR (61 DAT) and 4.2% AR (61 DAT) in the total system for the Anglerweiher and Hoenniger system respectively. Nevertheless, each unknown metabolite peak did not exceed 3% (mean) of the AR.

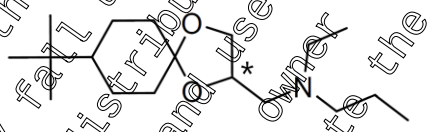
DT_{50} values for the degradation of spiroxamine were calculated in the report, however a re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014), was performed in the report presented under point KCA 7.2.2.3/08 (M-763141-01-1).

I. Materials and Methods

A. Materials

1. Test Items

[1,3-dioxolane-4-^{14}C]-spiroxamine



* Denotes position of [^{14}C]-radiolabel

Specific Activity: 4.09 MBq/mg

Radiochemical Purity: >98% (TLC)

2. Test System (water/sediment system)

The study was performed using the two water/sediment systems as characterised in Table CA 7.2.2.3-26.

Table CA 7.2.2.3-26: Physico-chemical properties of the water sediment systems

Parameter		Water/sediment system	
Water/sediment system designation:		**Anglerweiher**	**Hoenniger Weiher**
Geographic Location City Country		Leverkusen, North Rhine-Westphalia Germany	Wasserfuhr, close to Wipperfuerth, North Rhine-Westphalia, Germany
Sediment characteristics			
Textural Classification (USDA)		Sand	Sandy loam
Sand [50 - 2000 µm]	(%)	95.7	50.1
Silt [2 – 50 µm]	(%)	3.5	39.2
Clay [< 2 µm]	(%)	0.8	10.7
pH in H_2O in $CaCl_2$		 7.2 6.6	 5.2 5.5
Organic Matter (%)		1.3	6.5
Organic Carbon (%)		0.8	3.8
$N_{(total)}$ (%)		0.06	0.26
$P_{(total)}$ (mg P/kg)		121	493
Microbial Activity [mg CO_2 /hr/kg sediment (dry wt)] Initial, DAT 0 Final, 118 DAT		 9.6 3.8	 32.9 13.1
Cation Exchange Capacity (meq./100 g)		3.3	13.9
Water characteristics			
Temperature		8.5	6.0
pH		7.1	7.2
Total organic carbon (mg/L)		Initial: <2 DAT 118: 6	Initial: <2 DAT 118: 18
Hardness (°dH) "Deutsche Härte", German scale for hardness of water indicating [mg/L CaCO3]		3.1	9.8
Oxygen Concentration (saturation)		81%	87%
Total Nitrogen (mg/L)		5.1	3.8
Redox Potential E_h (mV) at sampling		282	300

The sediments and the water were collected from an artificial dammed pond (Hoenniger) and from a small lake from a reclaimed gravel pit (Anglerweiher). Samples were taken for both the Hoenniger and Anglerweiher site on the 27th November 2007. The pesticide history of the two sites shows no pesticides have been used. The sediment was collected from the top 20-50 cm and was passed through a 2 mm mesh sieve prior to use.

B. Study Design

1. Experimental Conditions

The behaviour of [1,3-dioxolane-4-^{14}C]-spiroxamine was investigated in two contrasting sediment water systems (Hoenniger and Anglerweiher) over a period of 118 days.

The water to sediment ratio was 3:1 (v/v). Sediment volumes of 175 mL (height *ca.* 2 cm) were transferred to the incubation vessels, and water volumes of 520 mL (height *ca.* 6 cm) was added. Prior to

application the systems were maintained under the test conditions for an equilibration period of 7 days. The laboratory microcosm flasks were connected with traps to collect CO_2 (using soda lime) and volatile organic compounds (using polyurethane foam).

The test item [1,3-dioxolane-4-^{14}C]-spiroxamine was applied onto the water surface at a rate of about 175 µg/batch, corresponding to about 250 µg/L water. The flasks were incubated in the dark at 20°C with the water layer gently agitated using a magnetic stirrer. Aerobic conditions were maintained by passive exchange of air through the solid traps.

2. Sampling

The redox potential as well as the pH and the dissolved oxygen content were determined before processing of water and sediment samples. Duplicate treated samples of each water/sediment system as well as volatile traps were removed for analysis after 0, 0.25, 1, 2, 7, 14, 30, 61 and 118 days of incubation.

3. Analytical Procedures

The supernatant water was decanted from the sediment and centrifuged. Aliquots of water were investigated by HPLC without concentration. Representative aliquots were also investigated by HP-TLC at optional sampling dates to confirm the HPLC results. An aliquot of 50 mL of the centrifuged supernatant water was adjusted to alkaline pH and used for the determination of dissolved $^{14}CO_2$.

The moist sediment was extracted twice with acetonitrile for 60 min using a shaker. Then, the sediment was extracted with acetonitrile under reflux for 60 min. After each extraction step the samples were centrifuged and the supernatants were decanted. All extracts were combined for analysis.

Finally, the sediment was extracted once under harsh conditions (methanol, 6 hour under reflux). The amount of radioactivity was determined by liquid scintillation counting (LSC) of appropriate aliquots. Aliquots of the sediment extracts were concentrated and analysed by HPLC. The extracted sediment residue was air-dried, weighed and homogenised in a planet mill. The amount of radioactivity in the non-extractable residue (NER) was detected by combustion/LSC.

The non-extractable (bound) residue remaining after this extraction procedure was representatively characterised for both sediments at day 118. The portion of humin, humic acid and fulvic acid was determined using a sequence of treatments with NaOH, HCl, centrifugation, and washing.

Spiroxamine and the M06 were identified by co-chromatography with reference compounds as well as by HPLC-MS/MS and HPLC-^{1}H-NMR. The LOQ for a single peak in the sediment extracts and in water was 2.3% AR and 2.8% AR, respectively.

The radioactivity absorbed by the soda lime ($^{14}CO_2$) was liberated by 18% aqueous HCl and purged with nitrogen into cooled LSC cocktails. The absorbed radioactivity in the scintillation cocktails was measured by LSC. The amount of $^{14}CO_2$ in the sediment was determined at the last sampling interval for both systems. $^{14}CO_2$ was quantified in the same manner as described for the calcium hydroxide.

The chemical identity of $^{14}CO_2$ trapped in the calcium hydroxide was shown for a water/sediment sample of the Anglerweiher system at day 118. The carbon dioxide was re-liberated from the LSC cocktail by slow addition of glacial acetic acid and the RA was absorbed in a series of three connected trap vessels filled with aqueous NaOH. After RA determination of aliquots by LSC, aqueous Na_2CO_3 solution and aqueous $BaCl_2$ solution were added to the solution of the first trap. After centrifugation, aliquots of the supernatant solution were radioassayed by LSC. The observed inclusion of 99.2% of the radioactivity of the NaOH solution into the [^{14}C]$BaCO_3$ precipitate was considered as a chemical proof for the identity of ^{14}C-carbon dioxide.

Volatile organic compounds possibly contained in the PU foam plugs were extracted with ethyl acetate and aliquots were radio-assayed by LSC. Due to the low amount (<0.1% AR) they were not further analysed.

4. Determination of degradation kinetics

The DT_{50} and DT_{90} of spiroxamine determined in the study was reported for each site. However, a re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) and the EFSA guidance on deriving $DegT_{50}$ values from laboratory and field dissipation studies (EFSA 2014), was performed in the report presented under point KCA 7.2.2.3/08 (M-763141-01-1).

II. Results and Discussion

A. Data

The redox potential in the supernatant water of both test systems after acclimation remained at high positive mV-values (225 to 272 mV). The redox potential in the sediment was between 140 mV and 279 mV. Some variations between different vessels were observed. In general, sufficient oxygen (approximately 72 - 98% of saturation) was available in the individual vessels at each sampling time. The data showed that the supernatant water was aerobic during the entire incubation period.

The microbial activity indicated that the systems were biologically active during the entire period of the test. In the Anglerweiher system as well as in the Hoenniger system, a reduction of the microbial activity was observed during the course of the experiment. This is often observed for laboratory experiments due to the gradual depletion of nutrients in the sediment and the lack of further usable organic matter as a source of energy. The total organic carbon content (TOC) of the water phase was in a range of <2 to 18 mg/L in both systems.

The extraction efficiency of the method was tested for sediment samples of day 0 (approximately 2 h post-application). A total of 91.1% and 86.4% AR was extracted from sediment of Anglerweiher and Hoenniger Weiher, respectively, using three times acetonitrile (two extractions at ambient temperature and one extraction under reflux), and one extraction under aggravated conditions with methanol (Table B.8.4-7 and Table B.8.4-8). This procedure left a non-extractable residue of only 3.3% and 7.7% of the AR for the two sediments. The results indicated that [1,3-dioxolane-4-^{14}C]spiroxamine was rapidly bound to the sediment and that the extraction method was suitable to extract the applied test item from the sediment matrix.

The distribution each water/sediment system incubated at 20°C following application of [1,3-dioxolane-4-^{14}C]-spiroxamine are summarised in Table CA 7.2.2.3-27 and Table CA 7.2.2.3-28. The characterisation of radioactivity for each water/sediment are summarised (as overall means) in Table CA 7.2.2.3-29 and Table CA 7.2.2.3-33. The characterisation of radioactivity for each separate layer for each water/sediment are summarised (as individual replicates) in Table CA 7.2.2.3-30 to Table CA 7.2.2.3-32 for the Anglerweiher system and in Table CA 7.2.2.3-34 to Table CA 7.2.2.3-36 for the Hoenniger Weiher system.

Table CA 7.2.2.3-27: Recovery and distribution of radioactivity following treatment of Anglerweiher system with [1,3-dioxolane-4-^{14}C]-spiroxamine under aerobic conditions (mean % AR)

Compound	Incubation time (DAT)								
	0	0.25	1	2	7	14	30	61	118
Overlying water	61.8	78.0	46.8	23.1	28.0	33.7	27.4	25.7	11.4
Sediment extract	26.5	20.2	41.1	64.3	52.9	45.6	43.3	29.4	20.2
(sub-total)	91.1	95.7	87.7	88.2	80.9	76.5	70.0	51.9	31.0
Non-extractable radioactivity	3.3	2.1	6.0	7.2	11.7	16.4	17.0	25.8	33.2
CO_2*	n.a.	<0.1	0.2	0.3	0.9	1.5	6.6	13.4	27.1
Volatile organic	n.a.	<0.1	<0.1	<0.1	0.1	0.1	<0.1	<0.1	<0.1
Total	94.5	97.9	93.9	95.8	93.5	94.4	93.6	91.2	91.3

n.a.: not analysed, DAT: days after treatment
Overall total recovery = 94.0% AR
All values expressed as mean percentage of applied radioactivity (% AR)

Table CA 7.2.2.3-28: Recovery and distribution of radioactivity following treatment of Hoeniger Weiher system with [1,3-dioxolane-4-^{14}C]-spiroxamine under aerobic conditions [mean % AR]

Compound	Incubation time (DAT)								
	0	0.25	1	2	7	14	30	61	118
Overlying water	62.5	64.7	38.2	21.5	16.0	16.0	6.9	4.2	4.9
Sediment extract	23.9	22.8	40.9	54.4	45.4	39.0	48.4	44.4	38.1
(sub-total)	86.4	87.5	79.1	75.9	58.5	55.0	55.3	48.5	43.1
Non-extractable radioactivity	7.2	8.5	15.2	20.3	34.4	38.0	38.2	40.2	40.7
CO_2*	n.a.	<0.1	0.1	0.1	0.2	0.4	0.8	3.8	7.6
Volatile organic	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total	94.1	96.1	94.3	96.2	93.1	93.4	94.4	92.5	91.4

n.a.: not analysed, DAT: days after treatment
Overall total recovery = 94.0% AR
All values expressed as mean percentage of applied radioactivity (% AR)

Table CA 7.2.2.3-29: Characterisation of radioactivity following treatment of Anglerweiher system in the total water-sediment system with [1,3-dioxolane-4-^{14}C]-spiroxamine under aerobic conditions [mean % AR] – overview of means

Compound		Incubation time (DAT)								
		0	0.25	1	2	7	14	30	61	118
Spiroxamine	w	64.6	75.5	40.7	15.5	14.3	1.9	n.d.	n.d.	n.d.
	s	25.5	19.8	39.8	60.1	43.9	35.2	31.3	17.1	14.9
M06 (acid)	w	n.d.	n.d.	2.1	4.7	9.4	25.6	23.4	15.1	3.3
	s	n.d.	n.d.	n.d.	1.8	3.0	4.0	7.9	6.3	4.0
Minor metabolites*	w	n.d.	n.d.	3.8	3.7	4.3	3.5	2.8	7.5	7.5
	s	1.0	n.d.	1.3	2.3	6.0	6.3	4.1	6.0	1.3

n.d.: not detected, DAT: days after treatment
All values expressed as mean percentage of applied radioactivity (% AR)
* Each individual metabolite ≤3% of the AR.
** Due to an application error at 7 DAT, only one replicate was used for calculation of the values for 7 DAT.

Table CA 7.2.2.3-30: Recovery and distribution of radioactivity following treatment of Anglerweiher system with [1,3-dioxolane-4-^{14}C]-spiroxamine under aerobic conditions in the water layer [% AR]

Compound	Replicate	Incubation time (DAT)								
		0	0.25	1	2	7	14	30	61	118
Spiroxamine	A	61.8	78.0	41.4	10.6	14.3	2.2	n.d.	n.d.	n.d.
	B	67.4	73.0	40.0	20.4	7.2**	1.6	n.d.	n.d.	n.d.
	Mean	**64.6**	**75.5**	**40.7**	**15.5**	**14.3**	**1.9**	**n.d.**	**n.d.**	**n.d.**
M06 (acid)	A	n.d.	n.d.	1.7	7.5	9.4	28.9	23.9	18.0	4.1
	B	n.d.	n.d.	2.5	1.8	4.3**	22.4	22.8	12.1	2.5
	Mean	**n.d.**	**n.d.**	**2.1**	**4.7**	**9.4**	**25.6**	**23.4**	**15.1**	**3.3**
Minor metabolites*	A	n.d.	n.d.	3.7	5.0	4.3	2.7	3.1	7.7	7.3
	B	n.d.	n.d.	4.0	2.4	0.9**	4.2	2.5	7.2	7.8
	Mean	**n.d.**	**n.d.**	**3.8**	**3.7**	**4.3**	**3.5**	**2.8**	**7.5**	**7.5**

n.d.: not detected, DAT: days after treatment
All values expressed as percentage of applied radioactivity (% AR)
* Each metabolite ≤3% of the AR.
** due to an application error, only sample A was used for calculation

Table CA 7.2.2.3-31: Recovery and distribution of radioactivity following treatment of Anglerweiher system with [1,3-dioxolane-4-^{14}C]-spiroxamine under aerobic conditions in the sediment layer [% AR]

Compound	Replicate	Incubation time (DAT)								
		0	0.25	1	2	7	14	30	61	118
Spiroxamine	A	27.6	18.5	37.7	51.3	43.9	35.6	28.8	18.9	16.5
	B	23.5	21.1	41.8	68.9	15.8**	34.8	33.8	15.3	13.1
	Mean	**25.5**	**19.8**	**39.8**	**60.1**	**43.9**	**35.2**	**31.3**	**17.1**	**14.9**
M06 (acid)	A	n.d.	n.d.	n.d.	1.0	3.0	3.5	9.4	6.9	4.7
	B	n.d.	n.d.	n.d.	2.6	2.0**	4.6	6.5	5.1	3.3
	Mean	**n.d.**	**n.d.**	**n.d.**	**1.8**	**3.0**	**4.0**	**7.9**	**6.3**	**4.0**
Minor metabolites*	A	1.0	n.d.	1.6	0.0	6.0	5.1	6.5	5.1	1.2
	B	1.0	n.d.	1.0	4.6	2.3**	7.5	1.7	7.0	1.3
	Mean	**1.0**	**n.d.**	**1.3**	**2.3**	**6.0**	**6.3**	**4.1**	**6.0**	**1.3**

n.d.: not detected, DAT: days after treatment
All values expressed as percentage of applied radioactivity (% AR)
* Each metabolite ≤3% of the AR.
** due to an application error, only sample A was used for calculation

Table CA 7.2.2.3-32: Recovery and distribution of radioactivity following treatment of Anglerweiher system with [1,3-dioxolane-4-^{14}C]-spiroxamine under aerobic conditions in the total water-sediment system [% AR]

Compound	Replicate	Incubation time (DAT)								
		0	0.25	1	2	7	14	30	61	118
Spiroxamine	A	89.4	96.5	78.8	61.9	58.2	37.8	28.8	18.9	16.5
	B	90.9	94.1	81.8	89.3	-	36.4	33.8	15.3	13.3
	Mean	**90.1**	**95.3**	**80.5**	**75.6**	**58.2**	**37.1**	**31.3**	**17.1**	**14.9**
M06 (acid)	A	n.d.	n.d.	1.7	8.5	12.4	32.4	33.3	24.9	8.8
	B	n.d.	n.d.	2.5	4.4	-	27.0	29.3	17.8	5.8
	Mean	**n.d.**	**n.d.**	**2.1**	**6.5**	**12.4**	**29.6**	**31.3**	**21.4**	**7.3**
Minor metabolites*	A	1.0	n.d.	5.3	5.0	10.3	7.8	9.6	12.8	5.5
	B	1.0	n.d.	5.0	7.0	-	11.7	4.2	14.2	9.1
	Mean	**1.0**	**n.d.**	**5.1**	**6.0**	**10.3**	**9.8**	**6.9**	**13.5**	**8.5**

n.d.: not detected, DAT: days after treatment
- due to an application error, only sample A was used for calculation
All values expressed as percentage of applied radioactivity (% AR)
* Each metabolite ≤3% of the AR.

Table CA 7.2.2.3-33: Characterisation of radioactivity following treatment of Hoenniger Weiher system in the total water-sediment system with [1,3-dioxolane-4-^{14}C]-spiroxamine under aerobic conditions [mean % AR] – overview of means

Compound		Incubation time (DAT)								
		0	0.25	1	2	7**	14	30	61	118
Spiroxamine	w	62.5	64.7	35.5	16.3	3.6	1.5	n.d.	n.d.	n.d.
	s	23.7	22.8	40.5	54.2	45.2	37.8	48.4	35.1	29.3
M06 (acid)	w	n.d.	n.d.	n.d.	2.0	7.9	13.6	6.3	3.8	4.7
	s	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	n.d.	5.0	8.9
Minor metabolites*	w	n.d.	n.d.	2.0	3.3	1.7	0.9	n.d.	n.d.	0.2
	s	n.d.	n.d.	n.d.	n.d.	n.d.	0.5	n.d.	4.2	n.d.

n.d.: not detected, DAT: days after treatment
All values expressed as mean percentage of applied radioactivity (% AR)
* Each individual metabolite ≤3% of the AR.
** Due to an application error at 7 DAT, only one replicate was used for calculation of the values for 7 DAT.

Table CA 7.2.2.3-34: Recovery and distribution of radioactivity following treatment of Hoeniger system with [1,3-dioxolane-4-^{14}C]-spiroxamine under aerobic conditions in the water layer [% AR]

Compound	Replicate	Incubation time (DAT)								
		0	0.25	1	2	7	14	30	61	118
Spiroxamine	A	61.9	64.4	34.1	20.0	1.9	1.8	n.d.	n.d.	n.d.
	B	63.2	65.1	36.9	12.5	5.3	1.1	n.d.	n.d.	n.d.
	Mean	**62.5**	**64.7**	**35.5**	**16.3**	**3.6**	**1.5**	**n.d.**	**n.d.**	**n.d.**
M06 (acid)	A	n.d.	n.d.	n.d.	1.7	11.1	12.9	6.5	4.2	3.2
	B	n.d.	n.d.	n.d.	2.2	4.8	15.3	6.1	3.3	6.1
	Mean	**n.d.**	**n.d.**	**n.d.**	**2.0**	**7.9**	**13.6**	**6.3**	**3.8**	**4.7**
Minor metabolites*	A	n.d.	n.d.	2.8	3.3	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	1.2	3.2	3.3	1.8	n.d.	n.d.	0.4
	Mean	**n.d.**	**n.d.**	**2.0**	**3.3**	**1.7**	**0.9**	**n.d.**	**n.d.**	**0.2**

n.d.: not detected, DAT: days after treatment
All values expressed as percentage of applied radioactivity (% AR)
* Each metabolite ≤3% of the AR.

Table CA 7.2.2.3-35: Recovery and distribution of radioactivity following treatment of Hoeniger system with [1,3-dioxolane-4-^{14}C]-spiroxamine under aerobic conditions in the sediment layer [% AR]

Compound	Replicate	Incubation time (DAT)								
		0	0.25	1	2	7	14	30	61	118
Spiroxamine	A	25.4	22.9	41.5	50.2	44.1	38.4	45.8	29.5	24.3
	B	22.0	22.6	39.5	58.2	46.2	37.1	50.9	40.8	34.3
	Mean	**23.7**	**22.8**	**40.5**	**54.2**	**45.2**	**37.8**	**48.4**	**35.1**	**29.3**
M06 (acid)	A	n.d.	n.d.	n.d.	n.d.	n.d.	1.4	n.d.	7.1	11.4
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.9	6.4
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.7**	**n.d.**	**5.0**	**8.9**
Minor metabolites*	A	n.d.	n.d.	n.d.	n.d.	n.d.	1.0	n.d.	6.7	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.7	n.d.
	Mean	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.5**	**n.d.**	**4.2**	**n.d.**

n.d.: not detected, DAT: days after treatment
All values expressed as percentage of applied radioactivity (% AR)
* Each metabolite ≤3% of the AR.

Table CA 7.2.2.3-36: Recovery and distribution of radioactivity following treatment of Hoeniger system with [1,3-dioxolane-4-^{14}C]-spiroxamine under aerobic conditions in the total water-sediment system [% AR]

Compound	Replicate	Incubation time (DAT)								
		0	0.25	1	2	7	14	30	61	118
Spiroxamine	A	87.3	87.3	75.6	70.2	46.0	40.2	45.8	29.5	24.3
	B	85.2	87.8	76.4	70.7	51.5	38.2	50.9	40.8	34.3
	Mean	**86.2**	**87.5**	**76.0**	**70.5**	**48.8**	**39.3**	**48.4**	**35.1**	**29.3**
M06 (acid)	A	n.d.	n.d.	n.d.	1.7	11.1	13.3	6.5	11.3	14.7
	B	n.d.	n.d.	n.d.	2.2	4.8	15.3	6.1	6.2	12.5
	Mean	**n.d.**	**n.d.**	**n.d.**	**2.0**	**7.9**	**14.3**	**6.3**	**8.8**	**13.6**
Minor metabolites*	A	n.d.	n.d.	2.8	3.3	n.d.	1.0	n.d.	6.7	n.d.
	B	n.d.	n.d.	1.2	3.2	3.3	1.8	n.d.	1.7	0.4
	Mean	**n.d.**	**n.d.**	**2.0**	**3.3**	**1.7**	**1.4**	**n.d.**	**4.2**	**0.2**

n.d.: not detected, DAT: days after treatment
All values expressed as percentage of applied radioactivity (% AR)
* Each metabolite ≤3% of the AR.

B. Material Balance

The total material balance of the two water/sediment systems was 94.0% of the applied radioactivity (AR) calculated as mean value of both systems and two parallel experiments for each system. The complete material balance found at all sampling intervals demonstrated that no significant portion of radioactivity dissipated from the vessels or was lost during processing.

C. Extractable and Non-Extractable Residues

The radioactivity in the Anglerweiher water decreased steadily from 64.6% AR (mean) at 0 DAT to 10.9% AR (mean) at study end. The radioactivity in the Hoenniger Weiher water decreased from 62.5% of the AR (mean) at 0 DAT to 4.9% AR (mean) at study termination. Extractable ^{14}C residues in the Anglerweiher sediment increased from 25.5% AR (mean) at 0 DAT, to a maximum of 63.4% AR (mean) at 2 DAT before decreasing to 18.9% AR (mean) at study termination. Extractable ^{14}C residues in the Hoenniger sediment increased from 20.7% (mean) at 0 DAT, to a maximum of 46.6% (mean) at 2 DAT before decreasing to 32.8% AR (mean) at study end. The maximum of non-extractable ^{14}C residues in the two sediments was 33.2% AR and 40.7% AR in Anglerweiher and Hoenniger, respectively (mean values).

The non-extractable (bound) residue remaining after the general extraction procedure was characterised at the end of the study (118 DAT) for both sediments. The results indicated that *ca* 15% AR could be assigned to the humic acid fraction, 52-55% AR to the fulvic acid fraction and *ca* 37% AR to the humin fraction.

D. Volatile Radioactivity

The radioactivity found in the PU traps amounted to <0.1% of the AR for both systems. Therefore, only a negligible amount of radioactivity was assigned to volatile organic compounds. At the end of the study, 27.1% AR (mean, Anglerweiher) and 7.6% AR (mean, Hoenniger) was present as CO_2.

E. Degradation of Parent Compound

Following application of [1,3-dioxolane-4-^{14}C]-spiroxamine, spiroxamine showed rapid dissipation in the water phase of both systems, declining from 62.5% AR (mean) at 0 DAT to below the LOD by 118 DAT in the Hoenniger Weiher system. Similarly, in the Anglerweiher system spiroxamine showed rapid dissipation in the water phase, declining from 64.6% AR (mean) at 0 DAT to below the LOD by 118 DAT.

In the Hoenniger Weiher system, the amount of spiroxamine in the sediment increased to a maximum of 54.2% AR (mean) after 2 DAT and subsequently decreased to 29.3% AR (mean) after 118 DAT. In the Anglerweiher system, the amount of spiroxamine in the sediment increased to a maximum of 60.1% AR (mean) after 2 DAT, and subsequently decreased to 14.9% AR (mean) after 118 DAT.

Degradation of [1,3-dioxolane-4-^{14}C]-spiroxamine in the total system was accompanied by the formation of one major degradation product M06 (spiroxamine-acid). In the water phase, it amounted to a maximum of 25.6% of the AR (Anglerweiher) and 13.6% of the AR (Hoenniger Weiher). In the sediment extracts, M06 accounted for only 7.9% and 8.9% of the AR, respectively. Additionally, several other minor unknown metabolites were observed but not identified in the report, with the sum of minor metabolites reached a maximum of 13.5% AR (61 DAT) and 4.2% AR (61 DAT) in the total system for the Anglerweiher and Hoenniger Weiher system respectively. Nevertheless, each zone did not exceed 3% (mean) of the AR.

F. Degradation Kinetics

Degradation rates determined in the report are superseded by the report presented under point KCA 7.2.2.3/08 (M-763141-01-1).

III. Conclusions

The distribution of radioactivity following application of [1,3-dioxolane-4-^{14}C]-spiroxamine showed that spiroxamine was rapidly translocated from the water phase to the sediment in both systems. Spiroxamine was degraded under the test conditions. The main metabolite in water and sediment was M06 (maximum formation: 31.3%). The sum of minor metabolites (not defined in the report) reached a maximum of 13.5%. $^{14}CO_2$ was formed indicating mineralisation of the parent compound and its intermediate degradation products.

A re-evaluation of the degradation kinetics in accordance with FOCUS guidance document on degradation kinetics (2014) was performed and is presented in the report under point KCA 7.2.2.3/08 (M-763141-01-1).

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to study guideline(s) OECD 308 (required guideline). The study is considered valid to assess the aerobic degradation of [1,3-dioxolane-4-^{14}C]-spiroxamine in aerobic water/sediment systems.

Data Point:	KCA 7.2.2.3/05
Report Author:	[redacted]
Report Year:	2008
Report Title:	Kinetic modelling evaluations of data from water sediment studies to derive modelling endpoints
Report No:	VC/08/029
Document No:	M-304099-01-1
Guideline(s) followed in study:	91/414/EEC, 95/36/EC of July 1995, Section 5, Subsection 7.2.1.3
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

This study was previous considered during the evaluation of spiroxamine (RAR (2010), RAR (2017)) and is therefore included again for completeness. The study performs a kinetic evaluation according to FOCUS 2006[5] of water/sediment systems reported in studies KCA 7.2.2.3/04 (M-303324-01-1) and KCA 7.2.2.3/06 (M-006040-02-1). The kinetic evaluation reported in this study is superseded by the new kinetic evaluation performed in study KCA 7.2.2.3/08 (M-763141-01-1) on all water/sediment systems from relevant studies.

Assessment and conclusion by applicant:

The study and its data are considered as supplementary data with no use in risk assessment.

Data Point:	KCA 7.2.2.3/06
Report Author:	[redacted]
Report Year:	1998
Report Title:	Anaerobic aquatic metabolism of the active ingredient KWG 4168
Report No:	PF4288
Document No:	M-006010-02-1
Guideline(s) followed in study:	US EPA Pesticide Assessment Guidelines, Subdivision N, Chemistry Environmental Fate, § 162-3: Anaerobic Aquatic Metabolism Studies
Deviations from current test guideline:	Yes (refer below) Some minor deviation(s) not relevant for the reliability of the study (described in study summary)
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The route and rate of degradation of [cyclohexyl-1-^{14}C]-spiroxamine was investigated in a water/sediment systems (Stilwell, USA, silty clay loam, 1.22% OC, pH 7.4) under laboratory anaerobic conditions at 20°C in the dark over a period of 360 days. As such, the study is not relevant under the data requirements in EC Regulation 283/2013. However, the study was previously considered during the evaluation of spiroxamine (RAR (2010), RAR (2017)) and is therefore included again for completeness but as supplemental information only.

Incubation vessels were filled with 200 mL of the sediment (285 g moist weight = 130 g dry weight) and filled up to a total volume of 500 mL with water giving a ratio of approximately 1:2.5 (v/v).

[Cyclohexyl-1-^{14}C]-spiroxamine (radiochemical purity >99%) was applied to the surface of the water overlying sediment at a target rate of 38 µg of a.s/500 mL, which was equivalent to an annual application of three applications of 1.5 kg a.s./ ha; on a pond of 2 m depth.

Before application of the active substance, the system was pre-incubated under anaerobic conditions (N_2, 200 mL/min) and additives were supplied in order to get a negative redox potential. The incubation vessels were first closed with a bubble counter attachment in order to led CO_2 escape developed from the added sucrose. When the system became anaerobic, this attachment was replaced by an 'air sample bag' which fed into two calcium hydroxide volatile trap to collect carbon dioxide and other volatile compounds. Each vessel was incubated in the dark at 20°C while being gently agitated by mechanical shaker.

To determine influence of the biomass on the degradation of [cyclohexyl-1-^{14}C]-spiroxamine during incubation four samples were sterilised. The samples were autoclaves three times for 20 minutes at 121°C. Under sterile condition, 5.8 g of mercuric chloride was applied to the supernatant along with the test substance. The application solution was sterilised by filtration through a sterile filter.

Overlying water was carefully decanted into a glass vessel containing appropriate amounts of acetonitrile. Sediment samples were extracted three times at room temperature with acetonitrile, while later time points were refluxed with methanol for 6 hours. All samples were analysed by TLC methods.

The material balance (mean) at each sampling interval ranged from 92.4 to 102.8% AR. The overall mean material balance was 98.5% AR for the Stilwell water/sediment system.

The mean proportion of total radioactivity extracted from sediment samples and in the supernatant water remained consistent over the 360 day incubation; starting from 89.5% AR at 0 DAT and remaining at 72.0% AR at 360 DAT. Non-extracted residues (bound residues) increased slowly throughout the incubation, reaching maximum levels of 20.4% AR at 250 DAT.

During the incubation period of 360 days an amount equivalent to ≤ 2.8% of the applied radioactivity

was degraded to carbon dioxide. Only trace amounts of organic volatile compounds were detected during the incubation time (< 0.1% AR).

[Cyclohexyl-1-^{14}C]-spiroxamine showed rapid dissipation in the water phase, declining from 47.9 AR (mean of duplicate samples) at 0 DAT to 0.2% by 360 DAT. The amounts of non-extractable radioactivity increased slowly throughout the incubation, reaching maximum levels of 20.4% AR at 250 DAT. The major metabolic pathways involved the formation of M01 (spiroxamine-desethyl: max 8.8% AR at 14 DAT, total system) which was observed at two consecutive time-points at greater than 5% AR. Several other minor metabolites were also observed.

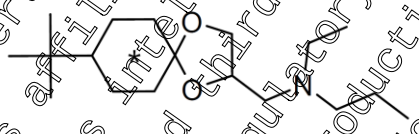
The whole system DT_{50} value for spiroxamine under anaerobic conditions was 279 days.

I. Materials and Methods

A. Materials

1. Test Items

[cyclohexyl-1-^{14}C]-spiroxamine



* Denotes position of [^{14}C]-radiolabel

Specific Activity: 3.63 MBq/mg

Radiochemical Purity: >99% (TLC)

2. Test System (water/sediment system)

The study was performed using the one water/sediment systems as characterised in Table CA 7.2.2.3-37.

Table CA 7.2.2.3-37: Physico-chemical properties of the water sediment systems

Parameter		Water/sediment system
Water/sediment system designation:		**Stillwell**
Geographic Location		
City		MRP Pond Stilwell, Kansas
Country		USA
Sediment characteristics		
Textural Classification (USDA)		Silty clay loam
Sand [50 - 2000 µm]	(%)	6.8
Silt [2 – 50 µm]	(%)	56.5
Clay [< 2 µm]	(%)	36.7
pH in H_2O (1:1) in 0.01M $CaCl_2$ (1:1)		 8.0 7.4
Organic Matter (%)*		2.1*
Organic Carbon (%)		1.22
Cation Exchange Capacity (meq/100 g)		23

Parameter	Water/sediment system
Water/sediment system designation:	**Stillwell**
Biological (respiration) activity [mg CO_2/h/kg sediment (dry weight)]	
Initial, DAT 0	22
Final, DAT 360 (average)	10
Water characteristics	
Dissolved oxygen concentration, at the time of treatment (mg/L)	5
Total organic carbon (mg/L)	10
Hardness (degree DH)	17.8
N(total) (mg/kg dry weight)	1300
P(total) (mg/kg dry weight)	700

* Calculated by multiplying organic carbon content by 1.724 (not reported)

Water and sediment were freshly sampled from a pond in Stilwell on the 23rd of March 1994. The aqueous sediment was passed through a 2 mm mesh sieve and was thoroughly mixed before use. No pesticide history was given for previous years before the trial for either site.

B. Study Design

1. Experimental Conditions

The behaviour of [cyclohexyl-1-^{14}C]-spiroxamine was investigated in one sediment water systems (Stillwell) over a period of 360 days.

Incubation vessels were filled with 200 mL of the sediment (285 g moist weight = 130 g dry weight) and filled up to a total volume of 500 mL with water giving a ratio of approximately 1:2.5 (v/v). Before application of the active substance, the system was pre-incubated under anaerobic conditions (N_2, 200 mL/min) and additives (sucrose) were supplied in order to get a negative redox potential. The incubation vessels were first closed with a bubble counter attachment in order to led CO_2 escape developed from the added sucrose. When the system became anaerobic this attachment was replaced by an 'air sample bag'.

The test application rate was intended to simulate a use rate with three applications of 1.5 kg a.s./ha. This rate corresponded to a concentration of 38 µg a.s./500 mL considering a water layer of 200 cm. The application solution was applied to the surface of the water overlying sediment and the system. After application of the test solution each vessel was purged with nitrogen below the air sample bag was connect to a calcium hydroxide trap for the collection of carbon dioxide and other volatile compounds. Each vessel was incubated in the dark at 20°C while being gently agitated by mechanical shaker (30 rpm).

The biological activity was determined in the sediment before application. To check the influence of the biomass on the degradation of [cyclohexyl-1-^{14}C]-spiroxamine during incubation four samples were sterilised. The samples were autoclaves three times for 20 minutes at 121°C. Under sterile condition, 5.8 g of mercuric chloride was applied to the supernatant along with the test substance. The application solution was sterilised by filtration through a sterile filter.

The biological activity was determined in the sediment before application. Two vessels were incubated with and without spiroxamine to determine the influence of the incubation and the test substance on the biological system. The redox potential was negative throughout the entire study; therefore, no further procedures were necessary to keep the systems anaerobic.

2. Sampling

Duplicate samples for each water/sediment system were removed for analysis after 0 (2 hour), 3, 7, 14, 31, 61, 122, 250 and 360 DAT (days after treatment). Sterile samples were sample taken in duplicate

from the sterile water/sediment system at 61 and 360 DAT.

3. Analytical Procedures

Before opening of the vessels, volatiles possibly present in the head-space of the flasks were purged into the trap attachment by means of a stream of nitrogen. The radioactivity in the calcium hydroxide was determined by LSC after having added hydrochloric acid in a closed apparatus and after having trapped the released carbon dioxide. The pH-value and oxygen content of the solution, and the redox potential of water and sediment were determined. An aliquot of the supernatant water was added with sodium hydroxide in order to keep possibly dissolved carbon dioxide in solution. After centrifugation the water phase was radio-assayed. In order to measure dissolved carbon dioxide, the water was then transferred into a closed apparatus, amended with hydrochloric acid and the released carbon dioxide was trapped and measured by LSC. The precipitate was added to the remainder of the sediment.

The main amount of the aqueous phase was centrifuged and then filtered. The precipitates resulting from the centrifugation steps were extracted three times with acetonitrile at room temperature. The filtered extracts were combined after each centrifugation. An aliquot of the sediment was mixed with quartz sand and extracted once with methanol under reflux (2 hours). All extracts were investigated by LSC and TLC. The remaining non-extractable radioactivity was determined by combustion and LSC analysis of trapped combustion gases.

Water samples and sediment extracts were investigated by TLC with four solvent systems on silica gel and RP-silica gel plates. Components were visualised by autoradiography, radioactivity scanning and in the case of unlabelled reference substances by reaction in an iodine chamber or by spraying with cobalt-II-thiocyanate. Identification of the metabolites was performed by mass spectroscopy after isolation of radioactive zones obtained by TLC. The detection limit for a single peak was >0.1% of the applied radioactivity.

4. Determination of degradation kinetics

Degradation kinetics were determined in the report using first order kinetics. The degradation rates presented in the report have not been re-calculated to current guidance (i.e. FOCUS 2014[2]) as the study is supplied as supporting information only.

II. Results and Discussion

A. Data

It was confirmed that the water sediment system was biologically active during the entire test period and that it was anaerobic. The oxygen saturation was measured to be < 3% and the redox potential between –120 and –296 mV confirming that the test systems were anaerobic during the study.

The distribution and characterisation of radioactivity for each water/sediment system incubated at 20°C under anaerobic conditions following application of [cyclohexyl-1-^{14}C]-spiroxamine are summarised in Table CA 7.2.2.3-38 to Table CA 7.2.2.3-39.

Table CA 7.2.2.3-38: Recovery and distribution of radioactivity following treatment of Stilwell system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under anaerobic conditions [mean % AR]

Compound	Incubation time (DAT)										
	0*	3	7	14	31	61	122	250	360	Sterile	
										61	360
Supernatant water	51.1	42.1	25.8	15.6	9.5	8.4	9.5	6.7	5.2	2.3	1.9
Sediment extract	38.4	37.6	62.5	68.0	73.1	73.0	68.7	64.1	66.8	88.1	85.3
(sub-total)	89.5	79.7	88.3	83.6	82.6	81.4	78.2	70.8	72.0	90.4	87.2
CO_2	0.1	0.1	0.1	0.3	0.1	0.5	0.6	2.8	1.9	0.1	<0.1
Non-extractable radioactivity	8.0	21.1	11.4	13.7	16.6	15.3	17.5	20.4	18.3	11.2	14.2
Folded filter	2.5	0.4	0.2	0.2	0.3	0.3	0.7	0.2	0.4	1.1	0.8
Total AR	100.0	101.2	99.9	97.7	99.5	97.4	96.8	94.1	92.4	102.8	102.1

DAT: days after treatment
* Sample taken at 2 hours is representative of zero time point.
Overall total recovery = 98.5% AR
All values expressed as mean percentage of applied radioactivity (% AR)

Table CA 7.2.2.3-39: Characterisation of radioactivity following treatment of Stilwell system with [cyclohexyl-1-^{14}C]-spiroxamine at 20°C under anaerobic conditions [mean % AR]

Compound		Incubation time (DAT)										
		0	3	7	14	31	61	122	250	360	sterile	
											61	100
Total	w	51.2	42.3	26.0	15.7	9.6	8.7	9.6	6.9	5.4	2.4	2.0
	s	38.5	31.3	48.9	52.0	59.7	53.8	56.2	47.3	52.6	81.9	71.8
Spiroxamine	w	47.9	37.1	18.7	9.6	3.9	1.3	0.3	0.2	0.2	1.6	0.6
	s	32.7	31.3	48.9	52.0	59.7	53.8	56.2	47.3	52.6	81.9	71.8
M01 (desethyl)	w	1.1	1.2	2.0	1.9	0.4	0.4	0.4	0.3	0.1	n.d.	0.2
	s	2.4	2.5	5.9	6.9	5.7	8.0	4.7	6.7	7.1	2.5	5.2
M02 (despropyl)	w	0.3	0.9	1.3	0.7	0.9	0.3	0.1	0.1	0.1	0.2	0.2
	s	0.8	1.0	2.5	3.3	3.1	3.9	3.2	3.2	2.0	2.4	4.5
M03 (N-oxide)	w	1.2	0.8	1.4	1.0	0.5	0.6	0.2	0.4	n.d.	0.2	0.9
	s	0.4	0.6	0.6	0.9	0.7	1.2	0.5	2.2	0.5	0.7	0.8
M06 (acid)	w	n.d.	0.5	0.6	1.4	2.9	3.7	5.0	1.1	3.2	0.1	n.d.
	s	0.2	0.1	0.3	0.6	0.9	1.3	2.5	1.5	1.9	n.d.	n.d.
M11 (desethyl acid)	w	n.d.	0.2	0.2	0.3	0.4	0.9	1.4	1.5	0.6	<0.1	n.d.
	s	n.d.	n.d.	n.d.	n.d.	0.2	0.5	n.d.	0.2	0.2	n.d.	n.d.
M12 (despropyl acid)	w	n.d.	n.d.	n.d.	n.d.	0.1	0.9	1.8	1.6	0.4	n.d.	n.d.
	s	n.d.	n.d.	n.d.	n.d.	n.d.	0.3	n.d.	0.3	n.d.	n.d.	n.d.
M15 (Ketone)	w	n.d.	n.d.	0.4	n.d.	0.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	s	1.3	1.6	2.8	2.5	1.9	2.8	1.1	2.4	2.1	0.7	0.9
Other unknowns	w	0.7	0.6	0.8	0.8	0.4	0.6	0.4	1.7	0.8	0.2	0.1
	s	0.7	0.8	1.6	1.9	1.0	1.5	0.7	0.5	0.5	0.1	0.3

n.d.: not detected, DAT: days after treatment
* Sample taken at 2 hours is representative of zero time point.
Overall total recovery = 98.5% AR
All values expressed as mean percentage of applied radioactivity (% AR)

B. Material Balance

The material balance (mean) at each sampling interval ranged from 92.4 to 102.8% AR. The overall mean material balance was 98.5% AR for the Stilwell water/sediment system.

C. Extractable and Non-Extractable Residues

The mean proportion of total radioactivity extracted from sediment samples and in the supernatant water remained consistent over the 360 day incubation; starting from 89.5% AR at 0 DAT and remaining at 72.0% AR at 360 DAT. Non-extracted residues (bound residues) increased slowly throughout the incubation, reaching maximum levels of 20.4% AR at 250 DAT.

D. Volatile Radioactivity

During the incubation period of 360 days, an amount equivalent to ≤ 2.8% of the applied radioactivity was degraded to carbon dioxide. Only trace amounts of organic volatile compounds were detected during the incubation time (< 0.1% e.g. methane).

E. Degradation of Parent Compound

Following application of [cyclohexyl-1-^{14}C]-spiroxamine, spiroxamine showed rapid dissipation in the water phase, declining from 47.9 AR (mean of duplicate samples) at 0 DAT to 0.2% by 360 DAT. The amount of spiroxamine in the sediment increased 59.7% AR after 31 DAT and subsequently decreased to 52.6% AR after 360 DAT.

Degradation of [cyclohexyl-1-^{14}C]-spiroxamine was accompanied by the formation of one major degradation product M01 (spiroxamine-desethyl: max 8.8% AR at 14 DAT, total system) which was observed at two consecutive time-points at greater than 5% AR. In addition, several other minor metabolites M06 (spiroxamine-acid: max 5.0% AR at 122 DAT), M02 (spiroxamine-despropyl: max 3.9% AR at 61 DAT), M03 (spiroxamine-N-oxide: max 2.2% AR at 250 DAT), M11 (spiroxamine-desethyl acid: max 1.5% AR at 250 DAT), M12 (spiroxamine-despropyl acid: max 4.8% AR at 122 DAT) and M15 (spiroxamine- ketone: max 2.8% AR at 7 DAT). Some other minor unidentified metabolites were observed but none of which exceeded a total of 2.8% AR at any sampling interval.

F. Degradation Kinetics

The whole system DT_{50} value for spiroxamine was 279 days.

III. Conclusions

Spiroxamine degraded in water/sediment systems under anaerobic conditions (20°C) with 52.8% of AR (mean) remaining as parent compound after 360 DAT in the Stilwell system. Low amounts of carbon dioxide accumulation was observed (max 2.8% AR, mean) in addition to low amounts (0.1% AR) of other volatiles. The amounts of non-extractable radioactivity increased slowly throughout the incubation, reaching maximum levels of 20.4% AR at 250 DAT. The major metabolic pathways involved the formation of M01 (spiroxamine-desethyl: max 8.8% AR at 14 DAT, total system) which was observed at two consecutive time-points at greater than 5% AR. Several other minor metabolites were also observed.

The whole system DT_{50} value for spiroxamine under anaerobic conditions was 279 days.

Assessment and conclusion by applicant:

The study was conducted to study guideline(s) USEPA (=EPA): N, 162-3 (**not** equivalent to required guideline). The study is not considered valid to assess the aerobic degradation of [cyclohexyl-1-^{14}C]-spiroxamine in aerobic water/sediment systems.

The study and its data are considered as supplementary data with no use in risk assessment.

Data Point:	KCA 7.2.2.3/08
Report Author:	[redacted]
Report Year:	2021
Report Title:	Spiroxamine: Kinetic assessment of water/sediment studies
Report No:	0471836-KIN4
Document No:	M-763141-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The degradation/dissipation behaviour of either [cyclohexyl-1-^{14}C]-spiroxamine or [1,3-dioxolane-4-^{14}C]-spiroxamine was investigated in three laboratory studies (KCA 7.2.2.3/01 (M-006015-01-1), KCA 7.2.2.3/04 (M-303324-01-1) and KCA 7.2.2.3/07 (M-763128-01-1)) involving six water/sediment systems.

The data from these studies have been used to determine the degradation/dissipation DT$_{50}$'s of spiroxamine in water/sediment. The data were considered appropriate for calculation of both persistence and modelling endpoints. In the KCA 7.2.2.3/01 (M-006015-01-1) study the metabolite M06 was detected at >5% applied radioactivity (AR) in the Stilwell total system only. In the KCA 7.2.2.3/04 (M-303324-01-1) study the metabolite M06 was detected at >5% applied radioactivity (AR) in the supernatant water and in the sediment extracts of both water/sediment systems. Where metabolites were detected at >5% AR and showed a clear decline in the total system, a kinetics assessment were performed. In the KCA 7.2.2.3/07 (M-763128-01-1) study the metabolite M06 was detected at >5% applied radioactivity (AR) in the supernatant water and in the sediment extracts of both water/sediment systems. Where metabolites were detected at >5% AR and showed a clear decline in the total system, a kinetics assessment were performed. The data from these studies were analysed using the CAKE v3.4 (2020) software package according to guidance provided by FOCUS (2014) based on Level P-1 and M-1 kinetics (single compartment kinetics). DT$_{50}$ and DT$_{90}$ values were calculated for comparison with relevant study triggers and persistence criteria and separate DT$_{90}$ values were calculated for use as modelling endpoints. The FOCUS (2014) flowcharts for calculating persistence and modelling endpoints have been followed. Each compartment of the water/sediment systems has been considered following the steps in the flowcharts.

The persistence and modelling endpoints for spiroxamine and metabolite M06 are summarised in Table CA 7.2.2.3-40 and Table CA 7.2.2.3-41, respectively.

I. Materials and Methods

The degradation/dissipation behaviour of either [cyclohexyl-1-^{14}C]-spiroxamine or [1,3-dioxolane-4-^{14}C]-spiroxamine was investigated in three laboratory studies (KCA 7.2.2.3/01 (M-006015-01-1), KCA 7.2.2.3/04 (M-303324-01-1) and KCA 7.2.2.3/07 (M-763128-01-1)) involving six water/sediment systems.

For the KCA 7.2.2.3/01 (M-006015-01-1) study the data were sufficient to allow for the calculation of kinetic endpoints for spiroxamine. However, for the Stilwell test system kinetics could not be fitted for the sediment phase as no decline was determined. The limit of detection (LOD) for thin layer chromatography (TLC) was reported as 0.1% of the applied radioactivity. The limit of quantification (LOQ) was not reported, therefore for the purposes of data generation, it was assumed that the LOD is nominally 1/3 LOQ (LOQ assumed as 0.3% AR).

For the KCA 7.2.2.3/04 (M-303324-01-1) study the data were sufficient to allow for the calculation of kinetic endpoints for spiroxamine. For the metabolite M06, data was not sufficient to determine kinetics for the sediment phase for the Hoenninger system. The LOQ for the HP-TLC methods was determined for a single peak in the water and sediment extracts to be in the range of 0.1% AR. The LOD was determined to be 0.77% AR.

For the KCA 7.2.2.3/07 (M-763128-01-1) study the data were sufficient to allow for the calculation of kinetic endpoints for spiroxamine. For the metabolite M06, data was not sufficient to determine kinetics for either the Calwich Abbey or Emperor Lake systems. The LOQ for the HPLC methods was determined for a single peak in the water and sediment extracts to be in the range of 0.9% AR. The LOD was determined to be 0.3% AR.

Full details of the water/sediment systems are provided in the study report.

A. Data handling

Input data were generated according to the data handling recommendations made in the FOCUS guidance for degradation kinetics (FOCUS, 2014). True duplicate samples were analysed in all three studies, so these individual values were used in the kinetic assessment. The handling of values below the limit of detection (LOD) and limit of quantification (LOQ) was performed according to the procedure recommended by (FOCUS, 2014) as follows:

- All values between LOD and LOQ were set to the actual measured value. If the actual measured concentration was not reported, 0.5 × (LOQ + LOD) was used.
- Set sample < LOD just after detection amount to 0.5 × LOD.
- All samples after the first non-detect (<LOD) were omitted unless positive detections above LOQ were made later in the experiment. In that case, samples were included up to the first non-detect (<LOD) which is NOT followed by later positive samples above LOQ.

The initial percent recovery of spiroxamine, M_0, in the total system and the water phase was set equivalent to the initial mass balance value for the total system. The initial percent recovery for spiroxamine in the sediment was selected based on the individual maximum values that provided the average maximum value in sediment.

In all three studies, M_0, in the total system and the water phase were corrected for radiochemical purity.

Where metabolites were detected at >5% AR and showed a clear decline in the total system, a kinetic assessment was performed. Where the concentrations of a metabolite in the total system were still increasing at the end of the study a kinetics assessment could not be performed. The initial amounts of the metabolites were set to 0%.

B. Input data

The data input to the kinetic models for each water/sediment system is provided in detail in the study report (see Table 3-4 to Table 3-9, pages 16-21).

C. Kinetic modelling

The kinetic modelling was conducted using CAKE version 3.4 (2020). In the first instance, the data were directly fitted, un-weighted, with the complete usable data set and unconstrained initial concentration (M_0).

The acceptability of kinetic fits was judged both visually and according to the χ^2 error and the t-test functions as recommended by FOCUS (2014). The visual assessment is recommended as the main tool for assessing goodness of fit. However, it is also recommended that a χ^2 error of less than 15% and a t-test probability of greater than 95% ($p < 0.05$) / 90% ($p < 0.1$) for estimated degradation rate constants indicate an acceptable fit. The χ^2 error was not considered as an absolute cut-off criterion as FOCUS guidance indicates that there will be cases where the error is higher than 15%, but the fit still represents

a reasonable description of the degradation behaviour. This particularly the case for field data and metabolites. In such situations examination of plots of residuals for systematic error is considered important. The t-test assesses whether degradation rate constants differs significantly from zero (i.e. no degradation). Alternatively, confidence intervals can be examined. In this assessment, the t-test was chosen for assessing confidence in rate constants. [The t-test was also not considered as an absolute cut-off criterion as FOCUS guidance indicates that where p is between 0.05 and 0.1, the parameter may still be considered acceptable. In such situations the visual quality of the fit was considered important.]

When fitting the FOMC model, FOCUS guidance indicates that the t-test is not appropriate as a measure of confidence for the gamma-distribution parameters α and β (smaller values of β indicate more rapid degradation, and α only indicates the shape of the curve and has nothing to do with the rate of degradation). Therefore, if a FOMC fit indicated slow degradation, confidence intervals for β were examined to determine if they were high compared to the parameter estimate, which would indicate that the parameter estimate was not reliable.

When calculating modelling endpoints for a metabolite, it was considered important to derive a formation fraction wherever possible. In the FOCUS flowsheets, if the SFO fit for a metabolite is not considered acceptable, a case-by-case decision is required. The first option given is to assess the decline of the metabolite after its maximum ('top-down' method). However, this method does not allow formation fraction assessment. The second option given is to fix the formation fraction to a worst-case value (usually 1) and use this in combination with a worst-case DT_{50} (usually 1000 days). However, this method almost always results in a clear overestimation of observed metabolite residues. The final option given is to use alternative – but conservative – estimates that describe the observed patterns. In this assessment, alternative – but conservative – estimates were chosen. These were implemented by fixing the formation fraction to the fitted value and using this in combination with a conservative DT_{50}, or vice versa. The parameter to amend and its value were chosen based on expert judgement and iterative amendments to give a conservative description of the observed metabolite residue pattern. In general, however, if residues throughout the study were underestimated, the formation fraction was conservatively amended, and if only residues late in the study were underestimated, the DT_{50} was conservatively amended. On rare occasions adjusting both parameters is necessary to obtain a conservative estimate that describes the observed pattern.

The FOCUS (2014) Level P-I flowcharts for calculating persistence and modelling endpoints for spiroxamine and Level M-I flowcharts calculating persistence and modelling endpoints based on metabolite formation and degradation have been followed. Each water/sediment compartment has been considered following the steps in the flowchart and the considerations are discussed in detail on the following pages. The full CAKE outputs from all kinetic fits (including the initial settings) are presented in the study report (see Appendix 2 for persistence fits and Appendix 3 for modelling fits.

II. Results and Discussion

A. Persistence/trigger endpoints

The kinetic evaluation was conducted using CAKE version 3.4 (2020) following the FOCUS (2014) Level P-I decision flowchart for persistence endpoints for spiroxamine. A summary of the fits achieved and decisions taken is provided for each test system in Appendix 5.1: and for each system in Appendix 5.1.1 to Appendix 5.1.6.

The resulting persistence or best-fit endpoints are presented in Table CA 7.2.2.3-40.

The spiroxamine persistence DT_{50} values in the total system ranged from 1.4-118 days and DT_{90} values ranged from 83.7-628 days.

The spiroxamine persistence DT_{50} values in the surface water ranged from 0.27 – 7.69 days and DT_{90} values ranged from 2.5-36.4 days.

Dissipation of spiroxamine in sediment could not be accurately fit to a kinetic model in three of the six test systems resulting in a number of presented FOCUS default endpoints. The spiroxamine persistence DT_{50} values in the sediment phase ranged from 24.3-1,000 days and DT_{90} values ranged from 191-3,320

days.

For the M06 persistence values in the total system, an acceptable fit could only be determined for one of the test systems resulting in a number of presented FOCUS default endpoints. The total system gave persistence DT_{50} values of 47 – 1,000 days and DT_{90} values ranged from 156 – 3,320 days.

For M06 persistence in surface water DT_{50} values could only be established in two of four trials, resulting in a number of presented FOCUS default endpoints, and ranged from 8.31-1,000 days and DT_{90} values ranged from 156-3,320 days.

For M06 persistence values in the sediment phase, an acceptable fit could only be determined for one of the four test systems resulting in a number of presented FOCUS default endpoints. The sediment phase gave a range of DT_{50} values of 89.2 – 1,000 days and a range of DT_{90} value of 296 – 3,320 days.

B. Modelling endpoints

The kinetic evaluation was conducted using CAKE version 3.4 (2020) following the FOCUS (2014) decision flowchart for modelling endpoints. A summary of the fits achieved and decisions taken is provided for each test system in Appendix 5.2 and for each system in Appendix 5.2.1 to Appendix 5.2.6.

The resulting modelling endpoints are presented in Table CA 7.2.2.2-41.

The spiroxamine modelling (DT_{50MOD}) values in the total system ranged from 42.8-1,000 days, with a geometric mean of 157.9 days in the total system. The spiroxamine modelling (DT_{50MOD}) values in the surface water ranged from 0.47-8.35 days, with a geometric mean of 1.52 days in the surface water. Dissipation of spiroxamine in sediment could not be accurately fit to a kinetic model in three of the six test systems resulting in a number of presented FOCUS default endpoints. The spiroxamine modelling (DT_{50MOD}) values in the sediment phase ranged from 72.0-1,000 days, with a geometric mean of 269.9 days in the sediment.

For the M06 persistence values in the total system, an acceptable fit could only be determined for one of the test systems resulting in a number of presented FOCUS default endpoints. M06 modelling total system gave a geomean DT_{50MOD} value of 293.6 days (f_t = 0.453).

For M06 modelling water phase gave two established DT_{50MOD} values from the four soils, resulting in a number of presented FOCUS default endpoints, ranging from 32.8-1,000 days.

For M06 modelling values in the sediment phase, an acceptable fit could only be determined for one of the four test systems resulting in a number of presented FOCUS default endpoints. M06 modelling sediment phase gave a range of DT_{50MOD} values from 89.2 – 1,000 days.

Table CA 7.2.2.3-40: Summary of persistence endpoints for spiroxamine and metabolite M06

Water/sediment system			Spiroxamine							M06						
System name	pH (H_2O)		Total system		Water		Sediment		Kinetics (TS / Water / Sed)	Total system		Water		Sediment		Kinetics (TS / Water / Sed)
	water	sed.	DT_{50}/ DT_{90} (days)	χ^2 error (%)	DT_{50}/ DT_{90} (days)	χ^2 error (%)	DT_{50}/ DT_{90} (days)	χ^2 error (%)		DT_{50}/ DT_{90} (days)	χ^2 error (%)	DT_{50}/ DT_{90} (days)	χ^2 error (%)	DT_{50}/ DT_{90} (days)	χ^2 error (%)	
CA 7.2.2.3/01 (M-006015-01-1) temp 20°C																
Hoenniger water	ND	5.6	51.4/ 628[1]	6.8	0.274/ 2.51	1.96	262[1]/ 871[1]	5.08	DFOP/ DFOP/ SFO*	Not observed						
Stilwell	ND	6.8	1.37/ 83.7	10.3	0.401/ 5.89	6.03	1,000 / 3,320	NA	DFOP/ DFOP/ Def.	1,000 /3,320	NA	Not observed >5%				Def./ NA/ NA
CA 7.2.2.3/04 (M-303324-01-1) temp 20°C																
Anglerweiher	7.1	7.2	9.47/ 237[1]	4.44	0.814/ 7.08	5.84	24.3/ 191[1]	6.71	FOMC/HS/ DFOP*	47/156	26.7	46.9/ 156	9.26	89.2/ 296	0.393	SFO/ SFO*/ SFO*/
Hoenniger water	7.2	5.5	9.82/ 429[1]	4.79	0.596/ 3.6	3.77	117/ 388[1]	5.9	DFOP/ FOMC/ SFO*	1,000 / 3,320	NA	8.31/ >1,000	9.07	1,000 / 3,320	NA	Def/ FOMC*/ Def
CA 7.2.2.3/07 (M-763128-01-1) temp 20°C																
Calwich Abbey	8.2	7.2	220/ 453[1]	8.7	5.61/ 18.6	12.9	1,000[1]/ 3,320	NA	FOMC/ SFO/ Def.	1,000 / 3,320	NA	1,000 / 3,320	NA	1,000 / 3,320	NA	Def/ Def/ Def
Emperor Lake	6.9	5.2	118[1]/ 474[1]	5.68	7.69/ 36.4	18.0	1,000[1]/ 3,320[1]	NA	HS/ FOMC/ SFO*	1,000 / 3,320	NA	1,000 / 3,320	NA	1,000 / 3,320	NA	Def/ Def/ Def
	Worst-case :		118/ 628**		7.69/ 36.4		1,000/ 3,320			1,000/ 3,320		1,000/ 3,320		1,000/ 3,320		

TS total system. ND not determined, NA Not applicable. * Decline after max [top-down]. ** Worst-cases from different systems. Def. default
[1] Interpret with care – extrapolated beyond experimental period

Table CA 7.2.2.3-41: Summary of modelling endpoints for spiroxamine and metabolite M06

Water/sediment system			Spiroxamine							M06								
System name	pH (H_2O)		Total system		Water		Sediment		Kinetics (TS/ Water/ Sed)	Total system			Water		Sediment		Kinetics (TS/ Water/ Sed)
	water	sed.	DT_{50}/ DT_{90} (days)	χ^2 error (%)	DT_{50}/ DT_{90} (days)	χ^2 error (%)	DT_{50}/ DT_{90} (days)	χ^2 error (%)		DT_{50}/ DT_{90} (days)	f.f.	χ^2 error (%)	DT_{50}/ DT_{90} (days)	χ^2 error (%)	DT_{50}/ DT_{90} (days)	χ^2 error (%)	
CA 7.2.2.3/01 (M-006015-01-1) temp 20°C																	
Hoenniger water	ND	5.6	249	6.8	0.468	21.5	262	5.98	DFOP/ SFO/ SFO*	Not observed							
Stilwell	ND	6.8	42.8	10.3	0.965	24.1	1,000	NA	DFOP/ SFO/ Def	1,000	0.15	69.9	Not observed				SFO/ ND/ ND
CA 7.2.2.3/04 (M-303324-01-1) temp 20°C																	
An-glerweiher	7.1	7.2	72.6	5.37	0.81	12.4	72.0	6.71	DFOP/ SFO/ DFOP*	45.1	0.7412	26.2	46.9	9.26	89.2	0.393	SFO/ SFO*/ SFO*
Hoenniger water	7.2	5.5	184	4.79	0.728	8.18	117	5.9	DFOP/ SFO/ SFO*	1,000	0.3	38.4	32.8	25.4	1,000	ND	Def/ SFO*/ Def
CA 7.2.2.3/07 (M-763128-01-1) temp 20°C																	
Calwich Ab-bey	8.2	7.2	1,000	NA	5.61	12.9	175	10.7	Def/ SFO/ SFO*	1,000	0.65	20.1	1,000	NA	1,000	NA	SFO/ Def/ Def
Emperor Lake	6.9	5.2	109	6.39	8.35	18.3	1,000	NA	SFO/ SFO/ Def	48.4	0.4251	34.7	1,000	NA	1,000	NA	SFO/ Def/ Def
Geometric mean :			157.9		1.52		269.9			293.6	-		198.0		546.5		
Arithmetic mean :											0.453						

III. Conclusions

Persistence/trigger and modelling endpoints (DT_{50} and DT_{90}) representing the degradation rate of spiroxamine in water/sediment studies were calculated in accordance with FOCUS (2014) guidance.

The spiroxamine persistence DT_{50} values in the total system ranged from 1.4-118 days and DT_{90} values ranged from 83.7-628 days. Modelling (DT_{50MOD}) values for spiroxamine ranged from 42.8-1,000 days, with a geometric mean of 157.9 days in the total system. The spiroxamine persistence DT_{50} values in the surface water ranged from 0.27 – 7.69 days and DT_{90} values ranged from 2.5-36.4 days. Modelling (DT_{50MOD}) values ranged from 0.47-8.35 days, with a geometric mean of 1.52 days in the surface water. Dissipation of spiroxamine in sediment could not be accurately fit to a kinetic model in three of the six test systems resulting in a number of presented FOCUS default endpoints. The spiroxamine persistence DT_{50} values in the sediment phase ranged from 24.3-1,000 days and DT_{90} values ranged from 191-3,320 days. Modelling (DT_{50MOD}) values ranged from 72.0-1,000 days, with a geometric mean of 269.9 days in the sediment.

For the M06 persistence values in the total system, an acceptable fit could only be determined for one of the test systems resulting in a number of presented FOCUS default endpoints. The total system gave persistence DT_{50} values of 47 – 1,000 days and DT_{90} values ranged from 156 – 3,320 days. M06 Modelling total system gave a geomean DT_{50MOD} value of 293.6 days (f.f = 0.453). For M06 persistence in surface water DT_{50} values could only be established in two of four trials, resulting in a number of presented FOCUS default endpoints, and ranged from 8.31-1,000 days and DT_{90} values ranged from 156-3,320 days. M06 modelling water phase also gave two established DT_{50MOD} values from the four soils, resulting in a number of presented FOCUS default endpoints, ranging from 32.8-1,000 days. For M06 persistence values in the sediment phase, an acceptable fit could only be determined for one of the four test systems resulting in a number of presented FOCUS default endpoints. The sediment phase gave a range of DT_{50} values of 89.2 – 1,000 days and a range of DT_{90} value of 296 – 3,320 days. M06 modelling sediment phase gave a range of DT_{50MOD} values from 89.2 – 1,000 days.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study was conducted to guideline(s) FOCUS 2006, 2014 (required guideline). The study is considered valid to assess best fit and modelling DT_{50} values for spiroxamine and associated metabolites in water/sediment studies.

CA 7.2.2.4 Irradiated water/sediment study

A study investigating the degradation of the active substance in water/sediment systems under artificial light has not been performed (higher tier option).

CA 7.2.3 Degradation in the saturated zone

Based on the information described under Point CP 9.2.4, following use of the formulated product, the active substance and any of the major degradation product as defined under Point CA 7.4.1, is not expected to leach through the soil profile to the saturated zone in significant quantities. Therefore, no further studies have been carried out.

CA 7.3 Fate and behaviour in air

CA 7.3.1 Route and rate of degradation in air

The fate and behaviour of spiroxamine in air was assessed in the previous EU evaluation and no new data or assessment is provided here. The studies considered are one study calculating the expected half-

life of the active substance in the atmosphere (KCA 7.3.1/01 (M-006025-02-1) and two studies investigating volatility of the active substance under simulated usage conditions:

Substance	Report reference	Document no.	Comment
Spiroxamine	KCA 7.3.1/01	M-006025-02-1	Submitted for first approval of spiroxamine, 1999. Reviewed under UP, Considered valid and acceptable.
Spiroxamine	KCA 7.3.1/02	M-006029-01-1	
Spiroxamine	KCA 7.3.1/03	M-006028-01-1	Submitted for first renewal of spiroxamine, 2010. Reviewed under UP, Considered valid and acceptable.

Overview:

Based on an overall vapour pressure value for the whole active substance (i.e. combined A and B isomers) of 4.7 x 10^{-3} Pa (20°) and individual vapour pressure values of 3.0 x 10^{-3} and 6.0 x 10^{-3} Pa (20°C) for the A and B diastereoisomers (see Point CA 2.2), respectively and calculated Henry's law constant for the whole active substance of 4 x 10^{-3} Pa m^3/mol (pH7, 20°C) and individual Henry's law constants of 2.5 x 10^{-3} and 5.0 x 10^{-3} Pa m^3/mol (pH7, 20°C) for the A and B diastereoisomers (see Point CA 2.2), respectively, spiroxamine is semi-volatile and may have a potential to volatilise from plant, soil and water surfaces. However, experimentally in studies investigating the amount of active substance volatilised under field conditions, it was shown that the amount volatilised was ca. 2% after 24 hrs. Any volatilisation of the active substance from the laboratory soil studies under Point CA 7.1.1. was also very low (<1% AR), although some volatilisation was observed from water surfaces in the water/sediment study (under Point CA 7.2.2.3). However, the estimated photochemical oxidative degradation half-life (using the Atkinson equation) in air of the active substance spiroxamine is <3 hours and therefore, if present, spiroxamine will not persist in the atmosphere.

Data Point:	KCA 7.3.1/01
Report Author:	[redacted]
Report Year:	1994
Report Title:	Calculation of the chemical lifetime of KWG 4168 in the troposphere
Report No:	PF4002
Document No:	M-006025-02-1
Guideline(s) followed in study:	Federal Biological Institute for Agriculture and Forestry, Braunschweig, FRG, Part IV, 6-1 (July 1990)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The decomposition rate constant of spiroxamine in the atmosphere is estimated according to the Atkinson's method using the Atmospheric Oxidation Program (version 1.51, US EPA).

The reaction rate constant of spiroxamine with hydroxyl radical was calculated to be 162.4455 x 10^{-12} cm^3 molecules cm^3. Assuming that a 12-hour daytime hydroxyl radical concentration is 1.5 x 10^6 molecules cm^{-3}, the corresponding DT_{50} rate was calculated to be 0.790 hours (0.066 days). Such a short degradation rate indicates spiroxamine is not persistent in the atmosphere.

I. Material and Methods

The rate constant of spiroxamine, in the reaction with hydroxyl radical or ozone in air was calculated

based on the Atkinson's method by using the Atmospheric Oxidation Program (version 1.51, US EPA). The calculation is based on an annual and global averaged 12-hour daytime hydroxyl radical concentration of 1.5 x 10^6 molecules cm^{-3} and 24-hour daytime ozone concentration of 7 x 10^{11} molecules cm^{-3}.

II. Results

The reaction rate constant of spiroxamine with hydroxyl radical was calculated to be 162.4455 x 10^{-42} cm^3 molecules cm^3.

III. Conclusions

The reaction rate constant of spiroxamine with hydroxyl radical was calculated to be 162.4455 x 10^{-12} cm^3 molecules cm^3 according to the Atkinson's method, using the Atmospheric Oxidation Program (version 1.51, US EPA). Assuming that a 12-hour daytime hydroxyl radical concentration is 1.5 x 10^6 molecules cm^{-3}, the corresponding DT_{50} rate was calculated to be 0.790 hours (0.066 days). Such a short degradation rate indicates spiroxamine is not persistent in the atmosphere.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study is an existing study that was previously assessed in the DAR (1997) and the RAR (2010), RAR (2017) and accepted by the RMS.

Data Point:	KCA 7.3.1/02
Report Author:	[redacted]
Report Year:	1994
Report Title:	Determination of the volatilization behaviour of KWG 4168 EC 500 in a field trial
Report No:	PF4023
Document No:	M-006029-01-1
Guideline(s) followed in study:	BBA-Guidelines for the Testing of Plant Products in Registration Procedures, Part IV, 6-1 (July 1990)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The volatilization behaviour of the active ingredient spiroxamine formulated as EC 500 was investigated in a registration study in accordance with the BBA-Guideline IV, 6-1. Three experiments were carried out under field conditions in containers, with a surface area of 1 m^2, sown with winter wheat (variety Orestis) at a sowing density of 500 seeds per m^2. Applications occurred a growth stages BBCH 37 – 39.

The applications using formulated [Cyclohexyl-l-^{14}C] spiroxamine were made in May 1994. The application equipment consisted of a mobile steel frame to which a vertically adjustable platform with the arrangement of spay nozzles was attached. The space between the plant container and nozzle platform could be sealed in order to avoid contamination of the environment during application. Practice-relevant application was simulated by spraying with a set of 3 individual nozzles at a distance of 50 cm each. The spray boom was driven by an electronically pulsed motor at a speed of 6 km/h over a distance of 100 cm (total container). During the entire test period of 24 hours, the following climatic data were recorded. The total amount of formulated active ingredient reaching the target area (initial deposit) was 83-94% on plants and 17-6% on soil (including the rim of the vessels).

The 5 vessels in the center were used for sampling. Within 2 min of the application, one of these vessels was harvested for determination of the initial spray deposit. Further vessels were taken after 1, 3, 6 and 24 hours for processing. The sequence of removing plant vessels was (1) 2, 3, 4 and 5:

At sampling, the plants were cut off above the ground and transferred into acetonitrile. Subsequently the roots were removed from the soil, rinsed with acetonitrile and added to the plants. This acetonitrile solution and the rinsed soil were added to the first soil extraction. The plants were extracted three times with acetonitrile, the acetonitrile phases combined and investigated by two TLC methods.

For soil, the top 2 cm was scraped off with a spoon and extracted three times with acetonitrile. Subsequently, the radioactivity and the content of active ingredient and metabolites were determined. The first extraction steps were carried out immediately in order to minimize volatilization effects during processing. The measured values immediately after application (vessel 1) were defined as the applied radioactivity or applied amount of active ingredient for the further discussion.

The quantification of spiroxamine and relevant metabolites was made by evaluation of the thin-layer chromatograms developed with system I. System II was used to confirm the qualitative results obtained. The isomers A and B of spiroxamine were nominally separated by TLC but determined together. Plants and soil were extracted immediately after sampling. Extracts that were not analyzed directly were deep frozen and stored up to 2 months at approximately -20°C until analysis.

A total of 83-94% of radioactivity applied onto the simulated field segment was detected on the plants and 17-6% on the ground (soil and rim of the vessels). Normalising the radioactivity measured on the plants immediately after application to 100% allowed the calculation of losses due to volatilization from the plant surfaces during the course of the trial in percent of the initial deposit. After the maximum test duration of 24 hours, 74% (mean of three experiments) of the radioactivity having reached the plant stand was recovered.

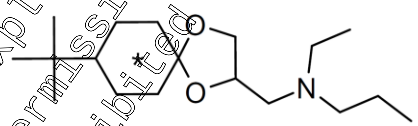
On average, 26% of the radioactivity applied to the plants had volatilized (disappeared) from the plant surfaces after 24 hours. The analyses of the plant extracts indicated that the recovered radioactivity was predominantly unchanged parent compound. The volatilization rate from the simulated winter wheat field, including soil, with different weather scenarios was on average 24% within the measuring period of 24 hours.

I. Materials and Methods

A. Materials

1. Test Items

[cyclohexyl-1-^{14}C]-spiroxamine

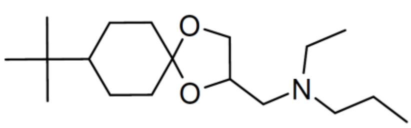


* Denotes position of [^{14}C]-radiolabel

Specific Activity: 3.63 MBq/mg

Radiochemical Purity: >98%

Unlabeled Spiroxamine:



Batch Number:	920522 ELB01
Radiochemical Purity:	99%

For the trial, radioactively labelled and non-labelled KWG 4168 were formulated as EC 500. [Cyclohexyl-1-14C]-spiroxamine and unlabelled spiroxamine were combined in acetonitrile. After evaporating the solvent, spiroxamine was thoroughly mixed with the blank formulation (formulation without active substance) and emulsified in 160 ml water by ultrasonication. Purity and content (radioactivity) of active ingredient were checked in the formulation before and after application (see Table CA 7.3.1-1).

Table CA 7.3.1-1: Composition of spray application solution

Formulation without A.S.	^{14}C-A.S.	^{12}C-A.S.	Water	New specific radiochem
300 mg	6.2 mg; 22.6 MBq	295 mg	160 ml	75 KBq/mg

2. Test Soil

Fresh soil was collected from a single field site and placed into containers for the conduct of the volatilisation experiment. The soil characterisation details can be found in Table CA 7.3.1-2.

Table CA 7.3.1-2: Physico-chemical properties of test soil

Parameter		Soil
Geographic Location City Country		 Langenfield (Rhineland) Germany
Textural Classification (USDA)		Loamy sand
Sand [50 - 2000 µm]	(%)	77.3
Silt [2 – 50 µm]	(%)	17.5
Clay [< 2 µm]	(%)	5.2
pH in $CaCl_2$		 5.9
Organic Matter (%) *		2.41
Organic Carbon (%)		1.4
Water Holding Capacity (g H_2O/100g soil dry weight) 40%		 10.6

* Calculated by multiplying organic carbon content by 1.72

B. Study Design

The objective of the present study was to determine the volatilization behaviour of the active ingredient in a practice-relevant trial, i.e. under field conditions, in compliance with the valid guideline. The study was carried out in the experimental station and laboratories of Bayer AG, Monheim in accordance with GLP regulations.

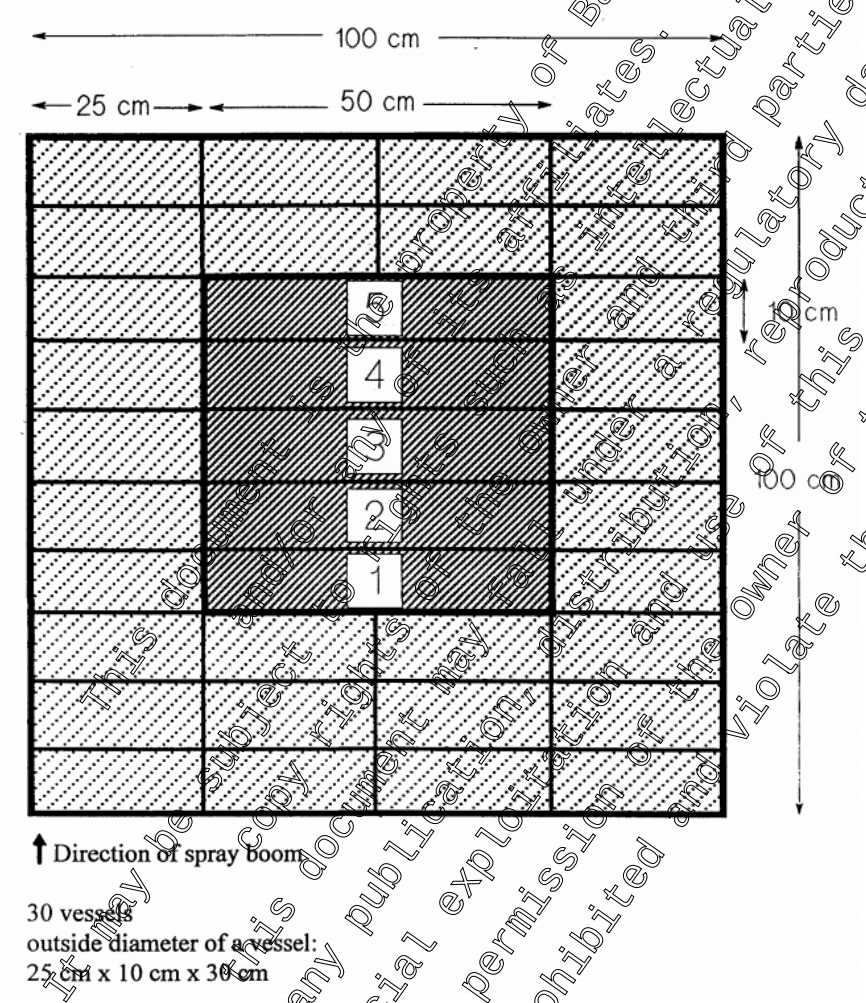
The use of radioactive labelled spiroxamine allowed the quantitative determination of both non-volatile degradation products as well as metabolites and parts of parent compound bound to plant and soil matrices. These would not be detectable without the use of a tracer and hence would otherwise be included in the overall balance as seemingly volatilized spiroxamine. Since the use of radioactive isotopes in the free field is not permitted in Germany, for radiation protection and health reasons, the trials were carried out in plant containers under simulated field conditions in a controlled area. The crop was sprayed with

the formulated spiroxamine in a manner reflecting common practice i.e. using a set of nozzles. During the subsequent waiting period of up to 24 hours, the climatic conditions prevailing during the test-period were recorded.

1. Experimental Conditions

Fresh soil collected from the field was placed in containers for the purposes of the study conduct. Each PVC plant container of dimensions 1 m x 1 m was composed of 35 individual vessels. The actual sampling area consisted of 5 individual vessels of dimensions 50 cm x 10 cm x 30 cm (depth). These 5 vessels were then surrounded by the remaining 30 vessels (25 cm x 10 cm x 30 cm) to simulate a close plant stand and minimize margin effects. See Figure 7.3.1-1:

Figure 7.3.1-1: Outline of trial design in pots



Winter wheat (variety Orestis) was sown on November 23, 1993 in three plant containers in the field at a sowing density of 500 seeds per m^2. Applications were made during May 16-24. The development stages of winter wheat were:

- BBCH37: flag leaf just visible, but still rolled.

- BBCH39: ligule of flag leaf just visible, flag leaf fully unrolled.

The application equipment consisted of a mobile steel frame to which a vertically adjustable platform with the arrangement of spay nozzles was attached. The space between the plant container and nozzle platform could be sealed in order to avoid contamination of the environment during application. Practice-relevant application was simulated by spraying with a set of 3 individual nozzles at a distance of 50

cm each. The spray boom was driven by an electronically pulsed motor at a speed of 6 km/h, the acceleration and the stopping distances were 10 cm each. The spray boom was moved at a constant speed over a distance of 100 cm. Nozzles, hoses, valves and the supply vessel with the application solution were attached to the spray boom. For the spraying process the supply vessel was put under pressure by means of pressurized air. Before and during application the solution was kept in motion by means of a magnetic stirrer in order to prevent inhomogeneity of spray solution in the supply vessel. The spraying process was started by putting the supply vessel under pressure and turning on the magnetic stirrer. This was followed by opening magnetic valves fixed in front of the nozzles. Only once a uniform spray pattern was achieved was the spray boom moved over the plant stand at the predetermined speed. Application details are provided in Table CA 7.3.1-3:

Table CA 7.3.1-3: Application details

Number of sprays	Spray boom height	Number of nozzles	Distance between nozzles	Nozzle type	Pressure at nozzle	Travel speed
1	50 cm	3	50 cm	Lechler LU 120-04	3.6 bar	6 km/h

Two min after application a lift truck was used to move the plant container from the application equipment without vibration.

The spray pattern was checked in pre-tests (i.e. distribution of the spray solution and reproducibility). This was done using a radioactive compound with very low volatilization which was sprayed with the application equipment on filter paper. Five pieces, corresponding to the area of the vessels, were cut out and the amount of the radioactivity of these filter paper pieces was determined.

The study was composed of 3 individual experiments. The applications were made in the morning in each case. Each trial lasted for 24 hours. See Table CA 7.3.1-4:

Table CA 7.3.1-4: Application dates

Experiment	Start (hour 0)	Trial dates
1	10:00 am	May 16-17
2	9:08 am	May 18-19
3	11:19 am	May 24-25

During the entire test period of 24 hours the following climatic data were recorded:

a) At the level of the plant stand:

- -air temperature
- -humidity
- -wind velocity
- -rainfall

b) At 2 m above ground:

- -prevailing wind direction
- -wind velocity
- -duration of sunshine
- -intensity of sunshine.

The listed weather data were recorded with a weather station of Lambrecht Co. The data for wind, temperature and humidity were measured at one second intervals and averaged over periods of 10 min each.

2. Sampling

The 5 vessels in the center were used for sampling. Within 2 min of the application, one of these vessels was harvested for determination of the initial spray deposit. Further vessels were taken after 1, 3, 6 and 24 hours for processing. The sequence of removing plant vessels was (1) 2, 3, 4 and 5:

3. Analytical Procedures

The plants were cut off above the ground and transferred into acetonitrile. Subsequently the roots were removed from the soil, rinsed with acetonitrile and added to the plants. This acetonitrile solution and the rinsed soil were added to the first soil extraction. The plants were extracted three times with acetonitrile, the acetonitrile phases combined and investigated by two TLC methods:

> System I: Silica F-254 Merck 60 gel plates. Solvent system of acetonitrile:water:ammonia (25%) 80:18:2 composition.
>
> System II: Silica RP-18 F-254 S Merck plates. First solvent system of n-hexane: dichloromethane: 2-propyl alcohol: ammonia (25%) of 30:70:10:2 (v/v). The second solvent system was chloroform:ethyl alcohol 50:50 (v/v).

The top 2 cm of the soil was scraped off with a spoon and extracted three times with acetonitrile. The first extraction steps were carried out immediately in order to minimize volatilization effects during processing. Subsequently, the radioactivity and the content of active ingredient and metabolites were determined The measured values immediately after application (vessel 1) were defined as applied radioactivity or applied amount of active ingredient for the further discussion.

The quantification of spiroxamine and relevant metabolites was made by evaluation of the thin-layer chromatograms developed with system I. System II was used to confirm the qualitative results obtained. The isomers A and B of spiroxamine were nominally separated by TLC but determined together. Plants and soil were extracted immediately after sampling. Extracts that were not analyzed directly were deep frozen and stored up to 2 months at approximately -20°C until analysis. Parent compound and relevant metabolites were stable under those storage conditions. This was proven by analysis of a sample that had been stored for 6 months at -20°C. The detection limit for a single metabolite was >0.5% of the applied radioactivity.

II. Results and Discussion

A. Data

The results of the measurement of the radioactivity distribution between soil and plants immediately after application to the target area are given in Table CA 7.3.1-5.

Table CA 7.3.1-5: Distribution of radioactivity post-application

Experiment	Plants	Soil and rim
1: May 16,1994	94%	6%
2: May 18, 1994	89%	11%
3: May 24, 1994	83%	17%

A total of 83-94% of radioactivity applied onto the simulated field was detected on the plants and 17-6% on the ground (soil and rim of the vessels).

Normalising the radioactivity measured on the plants immediately after application to 100% allowed the calculation of losses due to volatilization from the plant surfaces during the course of the trial in percent of the initial deposit. After the maximum test duration of 24 hours, 74% (mean of three experiments) of the radioactivity having reached the plant stand was recovered (Table CA 7.3.1-6).

Table CA 7.3.1-6: Volatilisation of spiroxamine from plant surfaces

Experiment	Initial deposit on plants	Recovery in % after:			
		1 hr	3h	6h	24 h
1	100%	61.1	67.9	63.6	70.4
2	100%	93.3	78.0	88.1	64.9
3	100%	93.5	108.0	84.7	87.6
Mean	100%	82.6	84.7	78.8	74.3

Analyses of the plant extracts indicated degradation of the parent compound during the incubation period of 24 hours. After 24 hours, 48% of the radioactivity applied on the plants was parent compound (mean value). See Table CA 7.3.1-7:

Table CA 7.3.1-7: Composition of plant extracts by TLC (% applied radioactivity)

Experiment	1		2		3	
	3 h	24 h	3 h	24 h	3 h	24 h
Extracted portion	59.8	59.1	67.2	55.6	96.6	72.8
Spiroxamine	47.1	44.2	58.1	47.4	73.0	51.8
M01 (spiroxamine-de-sethyl)	4.5	5.6	2.7	2.9	8.5	7.0
M02 (spiroxamine-despropyl)	4.4	5.3	2.8	2.7	8.1	6.9
M03(spiroxamine-N-oxide)	2.7	2.7	2.6	1.8	3.8	4.8
Unknown (≥1)	1.7	1.3	1.0	0.8	3.2	2.3
Unextracted portion:	8.1	11.3	10.8	9.3	11.5	14.8
Total	67.9	70.4	78.0	64.9	108.1	87.6

Only 6 to 17% of the total radioactivity applied, reached the soil (including the rim of the vessels) due to the high coverage of the soil by the target plants. Recovery rates of this radioactivity showed a relatively high variation during the test period. During experiment 1, the radioactivity increased in the soil presumably due to rainfall. See Table CA 7.3.1-8:

Table CA 7.3.1-8: Volatilisation of spiroxamine from soil surfaces

Experiment	Initial deposit on soil	Recovery in % after:			
		1 hr	3h	6h	24 h
1	100%	113.6	151.2	151.7	192.5
2	100%	97.7	71.4	93.4	42.8
3	100%	95.1	149.5	68.2	93.5
Mean	100%	102.1	124.0	104.4	109.6

The analyses of the soil extracts demonstrated that degradation of spiroxamine had occurred from TLC analysis. After 24 hours, 28% of the radioactivity applied on soil was parent compound (see Table CA 7.3.1-9).

Table CA 7.3.1-9: Composition of soil extracts by TLC (% applied radioactivity)

Experiment	1		2		3	
	3 h	24 h	3 h	24 h	3 h	24 h
Extracted portion	50.2	31.1	38.7	16.3	128.1	57.6
Spiroxamine	44.2	24.5	35.5	12.9	105.7	46.3
M01 (spiroxamine-de-sethyl)	1.2	1.0	0.5	0.5	5.1	1.8
M02 (spiroxamine-despropyl)	1.0	0.8	0.8	0.5	4.7	2.0
M03(spiroxamine-N-oxide)	3.8	1.8	1.9	2.4	9.8	4.9
Unknown (≥1)	<0.1	3.0	<0.1	<0.1	2.8	2.6
Unextracted portion:	101.0	161.4	32.7	26.5	21.4	35.9
Total	151.2	192.5	71.4	42.8	149.5	93.5

Despite different weather scenarios, the active ingredient showed a relatively uniform volatilization behaviour in the complete field segment consisting of target plants and accompanying soil. The fact that the radioactivity contents did not uniformly decline as the trial progressed can be explained by the heterogeneity of the plant stands in the individual vessels. Processing of aliquot target areas carried out after 24 hours yielded recovery rates of 63-89%. The total rate of volatilization from the field segment plants and soil as parent compound or metabolite averaged 24%. See Table CA 7.3.1-10:

Table CA 7.3.1-10: Volatilisation of spiroxamine from the total system

Experiment	Initial deposit on soil	Recovery in % after:			
		1 hr	3h	6h	24 h
1	100%	64.0	72.5	68.4	77.1
2	100%	93.7	77.3	88.6	62.7
3	100%	93.8	115.0	82.0	88.6
Mean	100%	83.8	88.3	79.7	76.1

III. Conclusion

The complete ecosystem especially the time of application, the target crop and the degree of soil coverage by plants should always be taken into consideration for an environmentally relevant assessment of the volatilization behaviour of plant protectants. The application in this trial was made onto an almost closed stand of winter wheat (flag leaf stage 37-39). Due to the high coverage of soil by plants only 6 to 17% of the total radioactivity applied reached the ground (soil and rim of vessels) immediately after spraying. The application of spiroxamine EC 500 was performed under normal weather conditions and the volatilization rate of parent compound including possible volatile metabolites was determined to be 24%. Predominantly unchanged parent compound was recovered from the plant and soil extracts.

An assessment of the photochemical oxidative degradability of spiroxamine in air was carried out. Based on calculations according to Atkinson the average chemical life was in the range of one to three hours.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study provides supplementary information on the volatile behaviour of spiroxamine but is not based on a guideline compliant study design.

Data Point:	KCA 7.3.1/03
Report Author:	[redacted]
Report Year:	1998
Report Title:	Volatilisation behaviour of KWG 4168 EC 500 in a field trial, treatment of bare soil
Report No:	MR-643/98
Document No:	M-006028-01-1
Guideline(s) followed in study:	BBA-Guidelines for the Testing of Plant Products in Registration Procedures, Part IV, 6-1 (July 1990)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The volatilisation behaviour of the active ingredient spiroxamine formulated as EC 500 was investigated in a registration study in accordance with the BBA-Guideline IV, 6-1. Field soil collected from Lagenfield, Germany, was used to fill a number of plant containers consisting of 42 individual chambers for the conduct of the study. The experiment was carried out under field conditions with a target area of 1 m². Bare soil was treated.

Upon receipt of the 14C-spiroxamine, TLC analysis found that the test item was only 60% pure necessitating a preliminary clean-up step using TLC, giving a final radiochem purity of >99%. The spray application using [cyclohexyl-1-^{14}C] formulated spiroxamine was made in June 16, 1998. The application equipment consisted of a mobile steel frame to which a vertically adjustable platform with the arrangement of spay nozzles was attached. The space between the plant container and nozzle platform could be sealed in order to avoid contamination of the environment during application. Practice-relevant application was simulated by spraying with a set of 3 individual nozzles at a distance of 50 cm each. The spray boom was driven by an electronically pulsed motor at a speed of 6 km/h over a distance of 100 cm (total container). During the entire test period of 24 hours, the local climatic conditions were recorded.

The 12 vessels in the center were sampled. Within 3 min after application of the test item, two of these vessels (A 3.1 and A 3.2) were taken for determination of the initial spray deposit. The sequence of removing vessels was:

- 0 h: vessels A 3.1 and A 3.2
- 1 h: vessels A 5.1 and A 5.2
- 3 h: vessels A 1.1 and A 1.2
- 6 h: vessels A 2.1 and A 4.2
- 24 h: vessels A 4.1; A 2.2; A 6.1 and A 6.2

The top 2-3 cm of the soil was scraped off with a spoon and extracted two times with acetonitrile (250 ml, 150 ml). Subsequently, the radioactivity and the content of active ingredient and metabolites were determined by two TLC methods. The quantification of a.i. and relevant metabolites was made by evaluation of the thin layer chromatograms developed with method I. Method II was used to confirm the qualitative results obtained. The isomers A and B of spiroxamine were normally separated by TLC but calculated (quantified) together. The soil was extracted immediately after sampling. Extracts were analysed directly within 6 days (the detection limit for a single metabolite was ≥ 0.5% of the applied radioactivity). The radioactivity in the extracted soil was determined by combustion. The measured radio-

active value immediately after application (mean value of vessels A 3.1 and A 3.2) was defined as applied radioactivity or applied amount of active ingredient for the further discussions.

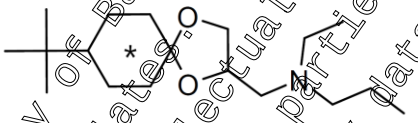
The volatilisation of spiroxamine from bare soil was very low (2% of the applied compound after 24 hours). The analyses of the soil extracts indicated that the recovered radioactivity was predominantly unchanged parent compound after the period of 24 hours.

I. Materials and Methods

A. Materials

1. Test Items

[cyclohexyl-1-^{14}C]-spiroxamine:

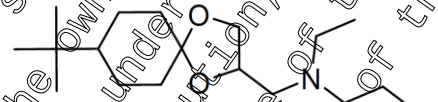


* Denotes position of [^{14}C]-radiolabel

Specific Activity: 3.68 MBq/mg

Radiochemical Purity: >99% following a TLC purification step

Non radiolabelled spiroxamine:



Radiochemical Purity: 98.3%

^{14}C-spiroxamine was dissolved in 2 ml acetonitrile and analysed with TLC method I. Due to the radiochemical purity of only 60% a.i. (hypothesized to be caused by auto-radiolysis due to the high concentration of the test substance during storage) the compound was purified with TLC method I prior to use of the material in the study. The purification was conducted by scraping the silica gel associated the radioactive zone corresponding to spiroxamine from the TLC plate and extracting the 14C-spiroxamine with 2 ml acetonitrile (stock solution). This purification led to a radiochemical purity of > 99% [with a ratio of the diastereomers A: B = 53%: 47%].

For the trial, radioactivity labelled and non-labelled spiroxamine were formulated as EC 500. 1.3 ml of the stock solution (1.9 mg 14C-spiroxamine equivalent to 7,057 kBq) and 297 mg of unlabelled spiroxamine were combined in acetonitrile (total volume 4.3 ml). The new specific radioactivity was 23.6 kBq/mg. After evaporating the solvent, spiroxamine was thoroughly mixed with 298.9 mg blank formulation (formulation without a.i.). The formulation was then emulsified in 159.4 ml water with ultrasonication. Purity and content (radioactivity) of active ingredient was checked in the formulation before and after application. Please refer to Table CA 7.3.1-11:

Table CA 7.3.1-11: Composition of spray application solution

Formulation without A.S.	14C-A.S.	12C-A.S.	Water	New specific radiochem
298.9 mg	1.9 mg; 7,057 kBq	297 mg	159.4 ml	23.6 kBq/ml

2. Test Soil

Fresh soil was taken from a soil stack and used to fill a number of plant containers for the experiment. The soil characterisation details can be found in Table CA 7.3.1-12.

Table CA 7.3.1-12: Physico-chemical properties of test soil

Parameter		Soil
Geographic Location		
City		Langenfield (Rhineland)
Country		Germany
Textural Classification (USDA)		Loamy sand
Sand [50 - 2000 µm]	(%)	77.3
Silt [2 – 50 µm]	(%)	17.5
Clay [< 2 µm]	(%)	5.2
pH in $CaCl_2$		5.9
Organic Matter (%) *		2.41
Organic Carbon (%)		1.4
Cation Exchange Capacity (meq/100 g)		5
Water Holding Capacity 40% (g water/ 100 g dry weight soil)		10.6

* Calculated by multiplying organic carbon content by 1.72

B. Study Design

The objective of the present study was to determine the volatilization behaviour of the active ingredient in a practice-relevant trial, i.e. under field conditions, in compliance with the valid guideline. The study was carried out in the experimental station and laboratories of Bayer AG, Monheim in accordance with GLP regulations.

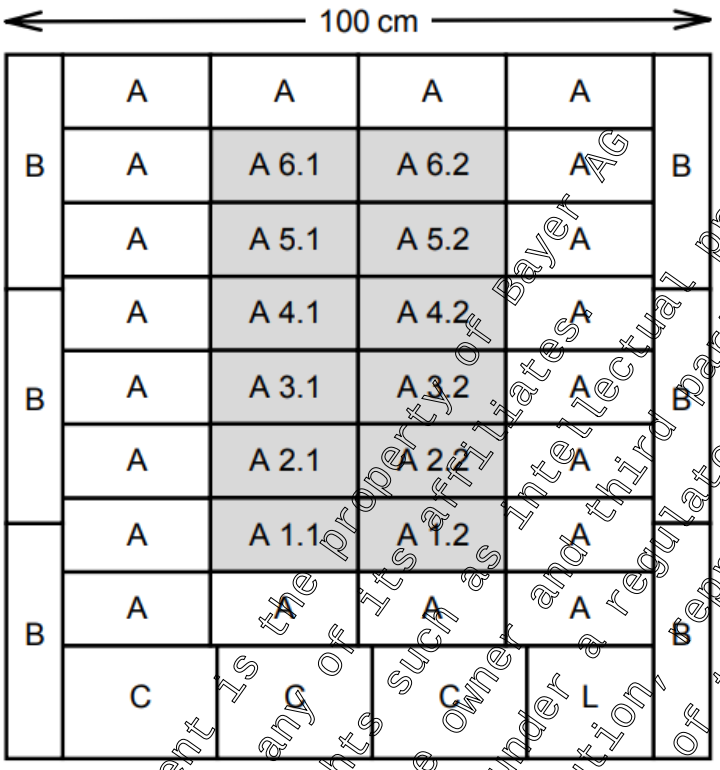
The use of radioactive labelled a.i. allowed the quantitative determination of both nonvolatile degradation products as well as metabolites and parts of parent compound bound to soil matrices. These would not be detectable without the use of a tracer and hence would otherwise be included in the overall balance as seemingly volatilised a.i. Since the use of radioactive isotopes in the free field is not permitted in Germany, for radiation protection and health reasons, the trial was carried out in a container under simulated field conditions in a controlled area. The bare soil was sprayed with the formulated a.i. in a manner reflecting common practice, i.e. using a set of nozzles. During the subsequent waiting period of up to 24 hours, the climatic conditions prevailing during the test-period were recorded.

1. Experimental Conditions.

Fresh soil collected from the field was placed in containers for the purposes of the study conduct. Each PVC plant container of dimensions 1 m x 1 m. The actual sampling space consists of 12 individual vessels with internal dimensions of 204 mm x 99 mm x 77 mm depth. These “sampling vessels” are surrounded by vessels simulating a field plot to minimise marginal effects. Please see Figure 7.3.1-2 for details.

Figure 7.3.1-2: Outline of trial design in pots

The test vessels are marked as A 1.1 - A 6.2



- Direction of spray boom run: from the bottom to the top
- Total number of containers (including "L"): 42
- Material: Hostalit Z ® (3 mm thick)
- Overall exterior dimension (in mm): Type A: 210 x 105 x 80 (depth)
  Type B: 333 x 80 x 80 (depth)
  Type C: 220 x 160 x 80 (depth)
  L: empty space (sealed with foil)

The application equipment consisted of a mobile steel frame to which a vertically adjustable platform with the arrangement of spay nozzles was attached. The space between the plant container and nozzle platform could be sealed in order to avoid contamination of the environment during application. The application (at agricultural relevant rates) was simulated by spraying with a set of 3 individual nozzles at a spacing of 50 cm each. The spray boom was driven by an electronically pulsed motor at a speed of 6 km/h over a distance of 100 cm (total container). Before and during application, the solution was kept in motion by means of a magnetic stirrer in order to prevent inhomogeneity of spray solution in the supply vessel.

Re-calculations indicated, that about 30 ml spray solution was applied to the container. This spray solution was less than needed for the application of the total amount of spiroxamine. However, since spiroxamine is not sprayed directly onto bare soil, a reduced application rate for soil is appropriate (good agricultural practice). No additional pre-test was performed with the intended formulation to determine the exact amount of spray solution per m².

Application details are provided in Table CA 7.3.1-13:

Table CA 7.3.1-13: Application details

Number of sprays	Spray boom height	Number of nozzles	Distance between nozzles	Nozzle type	Pressure at nozzle	Travel speed
1	50 cm	3	50 cm	Lechler LU 120-04	3.5 bar	6 km/h

During the entire test period of 24 hours the following climatic data were recorded using a Lambrecht Co. weather station:

a) At the level of the plant stand:

- -air temperature
- -humidity
- -wind velocity
- -rainfall

b) At 2 m above ground:

- -prevailing wind direction
- -wind velocity
- -duration of sunshine
- -intensity of sunshine

The data for wind, temperature and humidity were measured at one second intervals and averaged over periods of 10 min each.

2. Sampling

The 12 vessels in the center were used for sampling. Within 3 min after application of the test item, two of these vessels (A 3.1 and A 3.2) were taken for determination of the initial spray deposit. The sequence of removing vessels was:

- 0 h: vessels A 3.1 and A 3.2
- 1 h: vessels A 5.1 and A 5.2
- 3 h: vessels A 1.1 and A 1.2
- 6 h: vessels A 2.1 and A 4.2
- 24 h: vessels A 4.1, A 2.2, A 6.1 and A 6.2

3. Analytical Procedures

When sampling the soil in the container, the top 2-3 cm of the soil was scraped off with a spoon and extracted two times with acetonitrile (1 x 250 ml and 1 x 150 ml). Subsequently, the radioactivity and the content of active ingredient and metabolites were determined by TLC using two methods:

Method I: Silica F-254 Merck 60 gel plates. Solvent system of acetonitrile:water:ammonia (25%) 80:18:2 composition.

Method II: Silica RP-18 F-254 S Merck plates. First solvent system of n-hexane: dichloromethane: 2-propyl alcohol: ammonia (25%) of 30:70:10:2 (v/v).

The quantification of a.i. and relevant metabolites was made by evaluation of the thin layer chromatograms developed with method I. Method II was used to confirm the qualitative results obtained. The

isomers A and B of spiroxamine were separated in the TLC system but calculated (quantified) together. The soil was extracted immediately after sampling. Extracts were analysed directly within 6 days (the detection limit for a single metabolite was ≥ 0.5% of the applied radioactivity).

The radioactivity in the extracted soil was determined by combustion. The measured radioactive value immediately after application (mean value of vessels A 3.1 and A 3.2) was defined as applied radioactivity or applied amount of active ingredient for the further discussions.

The electro-spray ionisation mass spectra (ESI) via syringe were obtained with a TSQ 7000 instrument (Finnigan).

II. Results and Discussion

A. Data

The application was made on June 16, 1998 at 10:40 (a.m.) and the experiment lasted for 24 hours. The identity of the test substance 14C-spiroxamine was verified by TLC and MS. Normalising the radioactivity measured on the soil after application (0 h) to 100% allowed the calculation of losses due to volatilisation from the soil surface during the course of the trial in percent of the initial deposit. After the maximum test duration of 24 hours the volatilisation rate was very low (2%, see Table CA 7.3.1-14):

Table CA 7.3.1-14: Volatilisation of spiroxamine from plant surfaces

Initial deposit on soil (0 hrs)	Recovery in % after:			
	1 hr	3h	6h	24 h
100%	106.9	96.8	103.0	98.2

Approximately 20% of the applied radioactivity was not extracted with acetonitrile from the soil, proving the good binding of spiroxamine to soil (see Table CA 7.3.1-15). Re-calculations indicate that an amount, corresponding to 358 g a.i./ha was sprayed onto bare soil. This represents about 50% of the maximum use rate (750 g a.i./ha). Under the rules of good agricultural practice by far less than 50% of the application rate will reach the soil due to interception by the crop covering the soil. After 24 hours 68% of the applied radioactivity was found to be parent compound with all detected metabolites were each below 10% of the applied radioactivity (see Table CA 7.3.1-15).

Table CA 7.3.1-15: Composition of soil extracts by TLC

Sampling Date	0 h	1 h	3 h	6 h	24 h
Extracted portion	85.2	87.0	75.5	84.9	79.4
Spiroxamine	n.d.	n.d.	72.2	n.d.	68.1
M01 (spiroxamine-de-sethyl)	n.d.	n.d.	1.1	n.d.	2.4
M02 (spiroxamine-desopropyl)	n.d.	n.d.	0.7	n.d.	1.8
M03(spiroxamine-N-oxide)	n.d.	n.d.	1.6	n.d.	5.5
Unknown (≤1)	n.d.	n.d.	n.d.	n.d.	1.6
Unextracted portion	14.8	19.9	21.3	18.2	18.8
TOTAL	100.0	106.9	96.8	103.1	98.2

III. Conclusion

The spray application of spiroxamine EC 500 was performed under normal weather conditions onto bare soil. The volatilisation of parent compound including possible volatile metabolites from bare soil was very low (2% after 24 hours). Predominantly unchanged parent compound was recovered from the soil extracts.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study provides supplementary information on the volatile behaviour of spiroxamine but is not based on a guideline compliant study design.

CA 7.3.2 Transport via air

Due to low volatility and degradation of spiroxamine residues in air by OH radicals, transport via air is expected to be negligible.

CA 7.3.3 Local and global effects

Due to low volatility and degradation of spiroxamine residues in air by OH radicals, transport via air is expected to be negligible.

CA 7.4 Definition of the residue

CA 7.4.1 Definition of the residue for risk assessment

Based on the route of degradation studies presented under Point CA 7.1.1 (for soil) and Point CA 7.2 (for the aquatic environment) and the overview of metabolites observed in the various studies presented in Table CA 7.4.1-1, the following residue definitions for risk assessment are proposed for soil, groundwater, surface water, sediment and air:

Soil: Spiroxamine and metabolites M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl), M03 (spiroxamine-N-oxide) and M06 (spiroxamine-acid)

Groundwater: Spiroxamine and metabolites M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl), M03 (spiroxamine-N-oxide) and M06 (spiroxamine-acid)

Surface water: Spiroxamine and metabolites M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl), M03 (spiroxamine-N-oxide) and M06 (spiroxamine-acid)

Sediment: Spiroxamine and metabolite M06 (spiroxamine-acid)

Air: Spiroxamine only

Table CA 7.4.1-1: Overview of maximum levels of spiroxamine and metabolite observed in environmental fate studies [% AR]

Component	Maximum level observed (% AR)					
	Soil studies [C]			Aquatic studies [C]		
	Aerobic soil [A]	Anaerobic soil [A]	Soil photo-degradation [A]	Photolysis [B]	W/S layers [A]	Whole system [A]
Spiroxamine	-	-	-	-	- water 69.2 sed.	-
M01	12.0 [8.8]	7.6 [- [E]]	9.1 [9.1]	4.5 [4.5]	1.2 water [n.a.] 3.9 sed. [n.a.]	4.3 [<10[G]]

Component	Maximum level observed (% AR)					
	Soil studies [C]			Aquatic studies [C]		
	Aerobic soil [A]	Anaerobic soil [A]	Soil photo-degradation [A]	Photolysis [B]	W/S layers [A]	Whole system [A]
M02	9.2 [9.2]	5.8 [- [E]]	6.1 [6.1]	4.5 [4.5]	1.7 water [n.a.] 2.3 sed. [n.a.]	3.2 [<10 [G]]
M03	7.9 [7.9]	3.3 [- [E]]	6.2 [6.2]	4.0 [4.0]	11.3 water [11.3] 1.5 sed. [1.5]	11.3 [n.a. [H]]
M06	5.3 [D] [3.5 [F]]	9.9 [- [E]]	not observed [not observed]	not observed [not observed]	28.2 water [25.6] 23.0 sed. [8.9]	44.5 [n.a. [I]]

n.a. not available; sed. - sediment

A Major metabolites are defined as >10% AR; >5% AR at consecutive timepoints; or >5% AR and increasing at end of study

B Major metabolites are defined as >10% AR

C Proposed new LoEP for renewal [existing LoEP DAR 2017]

D Maximum level observed at last sampling interval in study

E Existing LoEP (RAR 2017) does not proposed endpoints for degradation of spiroxamine under anaerobic conditions

F Existing LoEP (RAR 2017) does not provide a max observed value for metabolite M06 and the default minimum (0.0001) is used for PECsw calculation. However, in the existing study KCA 7.1.1.1/01 (M-006135-01-1) metabolite M06 is observed at a maximum of 3.5% AR

G Existing LoEP (RAR 2017) quotes "<10% AR" as maximum observed value for metabolites M01 and M02 and the default minimum (0.0001) is used for PECsw calculation. However, in the existing studies KCA 7.2.2.3/01 (M-006015-01-1) and KCA 7.2.2.3/04 (M-303324-01-1) the maximum observed amount in the whole system of metabolites M01 and M02 is 1.9 and 1.9% AR, respectively

H Existing LoEP (RAR 2017) does not quote a whole system maximum observed amount of metabolite M03. However, in the existing studies KCA 7.2.2.3/01 (M-006015-01-1) and KCA 7.2.2.3/04 (M-303324-01-1) the maximum observed amount in the whole system of metabolite M03 is 11.3% AR

I Existing LoEP (RAR 2017) does not quote a whole system maximum observed amount of metabolite M06. However, in the existing studies KCA 7.2.2.3/01 (M-006015-01-1) and KCA 7.2.2.3/04 (M-303324-01-1) the maximum observed amount in the whole system of metabolite M03 is 31.3% AR

CA 7.4.2 Definition of the residue for monitoring

Based on the information considered for the definition of the residue for risk assessment and the persistence and relative toxicity of the components involved, the following residue definitions for monitoring purposes proposed for soil, groundwater, surface water, sediment and air.

Soil: Spiroxamine only

(metabolites M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl), M03 (spiroxamine-N-oxide) and M06 (spiroxamine-acid) can be concluded as sufficiently less toxic than parent spiroxamine in the terrestrial environment, see CP 10.4.1.

Groundwater: Spiroxamine only

(metabolites M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl), M03 (spiroxamine-N-oxide) and M06 (spiroxamine-acid) can be concluded as sufficiently less toxic than parent spiroxamine in the terrestrial environment, see CP 10.4.1., and the aquatic environment, see CP 10.2.

Surface water: Spiroxamine only

(metabolites M01 (spiroxamine-desethyl), M02 (spiroxamine-despropyl), M03

	(spiroxamine-N-oxide) and M06 (spiroxamine-acid) can be concluded as sufficiently less toxic than parent spiroxamine in the aquatic environment, see CP 10.2.
Sediment:	Spiroxamine only
	(metabolite M06 (spiroxamine-acid) can be concluded as sufficiently less toxic than parent spiroxamine in the aquatic environment, see CP 10.2.
Air:	Spiroxamine only

CA 7.5 Monitoring data

Monitoring data from databases in the EU

No central EU databases are available for monitoring data in surface water, groundwater, soil and air for plant protection active substances. Therefore, an unsystematic database search was conducted by searching monitoring databases or monitoring reports available from e.g. environmental agencies of several Member States to conclude on available monitoring data.

Data Point:	KCA 7.5/02
Report Author:	[redacted]; [redacted]
Report Year:	2021
Report Title:	Spiroxamine and metabolites: Public environmental monitoring data (groundwater, surface water, drinking water, soil, air and sediment)
Report No:	EnSa-21-0063
Document No:	M-762915-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Searches for public monitoring data were performed to provide an overview of residue concentrations of spiroxamine (SPX, parent) and its metabolites spiroxamine-desethyl (M01), spiroxamine-despropyl (M02), spiroxamine-N-oxide (M03) and spiroxamine-acid (M06) in the environmental compartments groundwater, surface water, drinking water, soil, air and sediment in the frame of the EU approval renewal of spiroxamine according to 1107/2009 and 2020/1740. The search was predominantly based on internet sources identifying databases and reports which are publicly accessible e.g. from the Environmental Agencies of European countries. The parent SPX as well as its metabolites were the subject of this overview of monitoring results. Available monitoring data was analysed for exceedance of regulatory thresholds appropriate for the compartment and the results summarised. The search revealed that monitoring information is not generally publicly available in all European countries and not necessarily available at a national level, while in some cases being available at federal state/region/province level. In terms of concentration thresholds, this report facilitates analysis within the context of the Water Framework Directive (2000/60/EC) and the associated Groundwater Directive (2006/118/EC), the Drinking Water Directive (1998/83/EC) and the Plant Protection Products Directive (1107/2009/EC). Key Regulatory Acceptable Concentrations (RACs) for SPX are considered for surface water (0.188 µg/L) and for sediment (712 µg/kg). For groundwater, a value of 0.1 µg/L applies for SPX. As part of

the EFSA conclusion (2010) for SPX, SPX-desethyl (M01), SPX-despropyl (M02) and SPXN-oxide (M03) are classified as a non-relevant metabolites. Metabolite SPX-acid (M06) is tentatively regarded as relevant for water unless the relevant algae study is submitted and demonstrates nonrelevance. This conclusion was derived by applying the guidance document for the assessment of the relevance of metabolites in groundwater within the EU (Sanco/221/2000 rev. 10 Feb 2003) as currently valid guidance document. No monitoring data are reported for the metabolites SPX-desethyl (M01), SPX-despropyl (M02), SPX-N-oxide (M03) and SPX-acid (M06) in any database/report under consideration. Monitoring data for SPX was found in databases (and reports) of Austria, Denmark, France, Germany, Italy, the Netherlands and Sweden, plus the Danube river database. The groundwater monitoring data search resulted in a compliance rate of 99.99% with the threshold of 0.1 µg/L (19 analyses of 130,633 analyses in total), with a maximum concentration of 20.76 µg/L. The surface water monitoring data search resulted in a compliance rate of 100% with the Tier 1-RACSW of 0.188 µg/L (5 analyses of 1,004,326 analyses in total were reported above the RAC), with a maximum concentration 7.2 µg/L. For the air compartment, 792 analyses with SPX concentrations above the LOQ (0.0009-0.0.14 ng/m³ as stated in the databases) were found (14.0% of 5,677 analyses). The median value of the quantified analyses amounted to 0.36 ng/m^3. The reported maximum residue concentration amounted to 65.93 ng/m^3. For the sediment compartment, the 435 analyses found reported SPX concentrations under LOQ (0.3- 5.0 µg/kg d.w. as stated in the databases). Monitoring of SPX is not documented for drinking water and soil. It cannot be discounted that some of the detections identified in groundwater, surface water, air and sediment are erroneous given they originate from non-GLP monitoring networks and programmes of unknown quality. Overall, it can be concluded from assessment of readily available public monitoring datasets that spiroxamine (SPX) does not pose a concern for the investigated environmental compartments.

I. Materials and Methods

A. Materials

1. Test Items

This study reports the findings of a review of National member state databases on residues of spiroxamine and metabolites in environmental compartments. All applications of spiroxamine related to environmental monitoring are resultant from applications of commercially available formulations in the field to good agricultural practice.

2. A. Study Design

In accordance with Commission Regulation (EU) 2020/1740, Bayer AG, Crop Science Division will submit a dossier to support the renewal of the approval of the active substance spiroxamine (SPX, parent). At present, there is no guidance to indicate what attention should be paid to publicly available monitoring data, if any, and how the validity of this data should be assessed for such submissions. In order to ensure a complete data package is submitted and demonstrate the safety of this active substance, an overview of potential findings of parent SPX and its metabolites in databases reflecting 'public' monitoring programs is provided. This search included the following European countries with monitoring programs in place: Austria, Czech Republic, Denmark, France, Germany, Greece, Ireland, Italy, the Netherlands, Portugal, Romania, Slovenia, Spain, Sweden, Switzerland, and United Kingdom. The search also included 'supra national' i.e. EU and river basin databases, for which Public Monitoring Programs and their data accessible via a link on the internet. Evaluation of these data should be undertaken with caution as they are collected from monitoring points of unknown quality and to unknown quality standards and experience from using these data suggests that they contain false positive findings. Monitoring data for SPX was found in databases (and reports) of Austria, Denmark, France, Germany, Italy, the Netherlands and Sweden, plus the Danube river database.

2. B. Data Collection

Public monitoring databases and associated monitoring reports were investigated for several countries covering groundwater and/or surface water and to a lesser extent drinking water, soil, sediment and air. The information in the databases and/or reports was collated and/or evaluated for the European country's

agency/organisation on the search list. An overview of the monitoring situation is compiled in Table 3.1. For the sake of completeness, countries with public monitoring program but where no relevant public monitoring data on SPX were available are also listed in Table 3.1, but not further mentioned in the results. For databases not reported in English language and/or some key words in drop down menus not translated, these terms were translated using 'Google' translate functions, i.e. for Denmark, Germany, Italy, the Netherlands, Czech Republic, Romania, Slovenia and Sweden. See Table CA 7.5-1.

Table CA 7.5-1: Overview of European Monitoring Data Sources and Outcome.

Country	Data Source/Organisation	Monitoring Data Available (yes/no)
Europe	NORMAN – EMPODAT Database	No monitoring data reported
	Danube River Basin Water Quality Database	Yes
	European Environment Agency – Status and quality of Europe's water database (Waterbase)	Not considered due to replication of individual MS data
Austria	Austrian Federal Ministry for Sustainability and Tourism – H2O Water Database	No monitoring data reported
	Austrian Federal Environment Agency and Ministry for Sustainability and Tourism – Several reports	Yes
Czech Republic	IS Arrow – Assessment and Reference of water monitoring	No monitoring data reported
Denmark	Geological Survey of Denmark and Greenland – Groundwater database (Jupiter)	Yes
	"Danish Pesticide Leaching Assessment Programme"	No monitoring data reported
France	ADES – National Groundwater Quality	Yes
	Portal Naïades – National Surface Water Quality Data Portal	Yes
	ATMO – National network of associations for the air quality	Yes
	CNEP - National pesticide monitoring plan in air	Yes
Germany	Sachsen Environment Agency – Surface water/Groundwater Quality Portal (IDA) and report	Yes
	Rheinland Pfalz - GeoPortal	Yes
	Rheinland Pfalz Environmental Agency – Annual reports on water quality monitoring	Yes
	Rheinland Pfalz Environmental Agency – Annual reports of stations for water investigation	Yes
	Baden-Württemberg State Institute for the Environment – Interactive data and map service (UDO)	No monitoring data reported
	German Federal agency for hydrology – Rhine river basin community	No monitoring data reported
	Elbe River Basin - Data information system	Yes
	LAWA - Working Group on water issues of the Federal States and the Federal Government	No monitoring data reported
	Bavarian Environmental Agency – Environment atlas	Yes
	Schleswig-Holstein – Agency for Agriculture, Environment and Rural Areas	Yes
Greece	Greek Ministry of Energy and climate Change – Report on quality of surface and groundwater of the country	No monitoring data reported
Ireland	Irish Environmental Protection Agency – Annual reports on Water Quality in Ireland	No monitoring data reported
Italy	Italian National Institute for Environmental Protection and Research (ISPRA) – Pesticide Portal and national and regional annual reports	Yes

Country	Data Source/Organisation	Monitoring Data Available [yes/no]
Netherlands	Dutch Institute of Environmental Sciences - Atlas for pesticides in surface water	Yes
	Water Quality Data portal of the Netherlands	Yes
	Ground Water Quality Reports	No monitoring data reported
	"Research on exposure of residents to pesticides in the Netherlands"	No monitoring data reported
	Groundwater Atlas for Pesticides – pesticide model	No monitoring data reported
Portugal	Portuguese Environment Agency – Report on state of environment	No monitoring data reported
Romania	Romanian National Administration "Apele Române" – Several reports	No monitoring data reported
Slovenia	Slovenia Environment Agency – Several reports and raw data	No monitoring data reported
Spain	"Ríos hormonados Amplia presencia de plaguicidas disruptores endocrinos en los ríos españoles"	No monitoring data reported
	Spanish Water framework Junta de Andalucia	No monitoring data reported
	Hydrographic confederation of Ebro	No monitoring data reported
	Hydrographic confederation of Guadalquivir	No monitoring data reported
	Hydrographic confederation of Júcar	No monitoring data reported
	Hydrographic confederation of Segura	No monitoring data reported
	Basque Water agency	No monitoring data reported
Sweden	Swedish University of Agricultural Sciences –Pesticide Database	Yes
	"Long-term Data from the Swedish National Environmental Monitoring Program of Pesticides in Surface Waters"	No monitoring data reported
Switzerland	Swiss National Environment Agency – Several reports	No monitoring data reported
	Regional Environment Agency of Canton Solothurn – Several raw data	No monitoring data reported
United Kingdom	England Environment Agency - Water quality archive	No monitoring data reported
	UK Water Quality Sampling Harmonised Monitoring	No monitoring data reported
	Drinking Water Inspectorate of England and Wales – Chief Inspector Annual Reports and regional overviews	No monitoring data reported
	Pesticide Monitoring Bulletin	No monitoring data reported

II. Results and Discussion

Spiroxamine was barely quantified (ca. 99.99% of the analyses showed residue levels below LOQ) in groundwater and surface water analyses retrieved from readily available public monitoring databases spanning 2005 – 2020 for seven European countries, namely Austria, Denmark, France, Germany, Italy, the Netherlands and Sweden, plus the Danube river database. In the cases where it was quantified with residue levels above the LOQ, there were only rarely findings above the applied thresholds.

Groundwater:

For SPX, a total of 130,633 groundwater analyses were investigated, of which 119 analyses quantified SPX (with residue levels at or above LOQ). A compliance rate of 99.99% with the regulatory threshold was observed with 19 analyses exceeding the regulatory threshold of 0.1 µg/L. These exceedances were only found in analyses from France where they represent 0.015% of all analyses. The maximum concentration was reported as 20.76 µg/L (in France). See Table CA 7.5- 2.

Table CA 7.5- 2: Summary of public monitoring results for spiroxamine (SPX) in groundwater

Country	Data source/ Organisation	Monitoring Period	Analyses	Quantifications* (≥LOQ)	≥0.1 µg/L	≥0.1 µg/L (%)
Denmark	Groundwater database (Jupiter)	2016	39	0	0	0
France	ADES – National Groundwater Quality Portal	2006 – 2020	125,422	113	19	0.015
	Sachsen Environment Agency – Water Quality Portal (IDA)	2012-2020	1,885	5	0	0
	Rheinland Pfalz – Geoportal	2007 – 2016	438	0	0	0
	Rheinland Pfalz Environmental Agency – Annual reports	2011-2012	120	0	0	0
	Bavarian Environmental Agency – Environment atlas	2013-2020	2,191	1	0	0
Italy	Italian National Institute for Environmental Protection and Research (ISPRA)	2011-2016 2018	13,8211	911	01	0
Sweden	Swedish University of Agricultural Sciences – Pesticide Database	2010-2018	538	0	0	0

* Quantifications represent the number of analyses with residue levels ≥ LOQ

[1] In the reports from Italy, a total number of 16,420 analyses were investigated in 2011-2018. For the years 2011-2016 and 2018 (total analyses: 13,821), no analyses were shown to be ≥ 0.1 µg/L. For 2017 (2,599 analyses, 69 analyses above LOQ), a maximum value of 0.1 µg/L was indicated, but it could not be determined how many of the analyses were = 0.1µg/L. Therefore, the year 2017 has been excluded from the statistics.

Surface Water

A total of 1,004,326 analyses of surface water were investigated (excluding Germany – Rheinland Pfalz reports and the Netherlands – Atlas for pesticides in surface water), of which 595 analyses quantified SPX (with residue levels at or above LOQ). A compliance rate of 100% with the Tier 1-RACSW of 0.188 µg/L was observed, with 5 analyses exceeding of the regulatory acceptable concentration (Tier 1-RAC-SW) of 0.188 µg/L. These exceedances were found in analyses from Germany and Italy where they represent 0.04% and 0.004% of all analyses, respectively. The maximum surface water concentration was reported as 7.2 µg/L (in Germany). See Table CA 7.5- 3.

Table CA 7.5- 3: Summary of public monitoring results for Spiroxamine (SPX) in surface water

Country	Data source/ Organisation	Monitor-ing Period	Analyses	Quantifica-tions* (≥LOQ)	≥RAC	≥RAC (%)
EU	Danube River Basin Water Quality Database	2013	211	0	0	0
Austria	Austrian Federal Environ-ment Agency – reports	2013-2015	844,0861	0	0	0
France	Naïades – National Surface Water Quality Data Portal	2005-2019	121,638	0	0	0
Germany	Sachsen Environment Agency – Water Quality Por-tal (IDA)	2012 - 2020	10,028	274	4	0.04
	Elbe River Basin - Data in-formation system	2012-2019	294	10	0	0.0
	Rheinland Pfalz Environ-mental Agency – Annual re-ports	2006-2010	526	2	-2	-2
	Schleswig-Holstein – Agency for Agriculture, En-vironment and Rural Areas	2018-2020	224	1	0	0.0
Italy	Italian National Institute for Environmental Protection and Research (ISPRA)	2005-2018	25,243	305	1	0.004
Netherlands	Dutch Institute of Environ-mental Sciences - Atlas for pesticides in surface water	2006-2019	1,803	-3	-3	-3
	Water Quality Data portal of the Netherlands	2006-2018	1,589	2	0	0.0
Sweden	Swedish University of Agri-cultural Sciences Pesticide Database	2010-2019	1,168	2	0	0.0

* Quantifications represent the number of analyses with residue levels ≥ LOQ (neither "zero" nor ""<LOQ"). The value of the LOQ depends on the analytical method specified and is therefore variable (LOQ ranged from 0.002 to 0.15 µg/L and in some databases LOQ value is not specified)

** RAC value for SPX: 0.188 µg/L

[1] In the Danube river Database one analysis was done from the measuring site "Oberloiben", a measuring point found also in the reports from the Austrian Federal Environment Agency. In order to avoid redundancy, this analysis was excluded from the Danube River Database.

[2] In the annual reports of the German Rheinland Pfalz Environmental Agency, surface water data were only compared to 0.1 µg/L, therefore no statistics are possible (14 analyses ≥ 0.1 µg/L).

[3] In the database from the Dutch Institute of Environmental Sciences - Atlas for pesticides in surface water, surface water data were only presented as "average per year", all ≥ LOQ (LOQ: 0.002 µg/L). No data about "maximum per year" were available on the website.

Drinking Water

Analyses of spiroxamine in drinking water are not documented

Soil

Analyses of spiroxamine in soil are not documented

Air

A total of 5,677 analyses of air were investigated, of which 792 analyses quantified SPX (with residue levels at or above LOQ, with LOQ ranging from 0.0001 to 0.14 ng/m^3 as stated in the databases). See Table CA 7.5- 4.

Table CA 7.5- 4: Summary of public monitoring data for Spiroxamine (SPX) in air

Country	Data source/ Organisation	Monitoring Period	Analyses	Quantifications* (≥LOQ)	Quantifications* (%)
France	ATMO – National network of associations for the air quality	2005-20171	4,2931	728	17.0
	CNEP - National pesticide monitoring plan in air	2018-2019	1,348	54	4.0
Sweden	Swedish University of Agricultural Sciences – Pesticide Database	2017-2018	36	10	27.8

* Quantifications represent the number of analyses with residue levels ≥ LOQ (neither "zero" nor ""<LOQ"). The value of the LOQ depends on the analytical method specified and is therefore variable (LOQ ranged from 0.0001 to 0.14 ng/m³)
[1]In the ATMO database, a total number of 6,714 analyses were investigated in 2005-2019. For the years 2018-2019, an overlap was observed with the analyses presented in the CNEP database and therefore the analyses from ATMO database for these years are excluded from the statistics.

Sediment

A total of 435 analyses of sediments were investigated (see Table 4.13), of which no analyses quantified SPX (with residue levels at or above LOQ, 0.3-5.0 µg/kg d.w. as stated in the databases). See Table CA 7.5- 5.

Table CA 7.5- 5: Summary of public monitoring data for spiroxamine (SPX) in sediment

Country	Data source/ Organisation	Monitoring Period	Analyses	Quantifications* (≥LOQ)	≥RAC**	≥RAC** (%)
France	Naïades – National Surface Water Quality Data Portal	2015-2018	405	0	0	0.0
Sweden	Swedish University of Agricultural Sciences – Pesticide Database	2003-2018	30	0	0	0.0

* Quantifications represent the number of analyses with residue levels ≥ LOQ (neither "zero" nor ""<LOQ"). The value of the LOQ depends on the analytical method specified and is therefore variable (LOQ ranged from 0.0001 to 0.14 ng/m³)
** RAC value for SPX: 712 µg/kg d.w.

Detailed results can be found in the original report.

III. Conclusion

The groundwater monitoring data search resulted in a compliance rate of 99.99% with the threshold of 0.1 µg/L (19 analyses of 130,639 analyses in total), with a maximum concentration of 20.76 µg/L. The surface water monitoring data search resulted in a compliance rate of 100% with the Tier 1-RAC_{SW} of 0.188 µg/L (5 analyses of 1,004,326 analyses in total were reported above the RAC), with a maximum concentration 7.2 µg/L. For the air compartment, 792 analyses with SPX concentrations above the LOQ (0.0001-00.14 ng/m³ as stated in the databases) were found (14.0% of 5,677 analyses). The median value of the quantified analyses amounted to 0.36 ng/m³. The reported maximum residue concentration amounted to 65.93 ng/m³. For the sediment compartment, the 435 analyses found reported SPX concentrations under LOQ (0.3-5.0 µg/kg d.w. as stated in the databases). Monitoring of SPX is not documented for drinking water and soil. It cannot be discounted that some of the detections identified in groundwater, surface water, air and sediment are erroneous given they originate from non-GLP monitoring networks and programmes of unknown quality. Overall, it can be concluded from assessment of readily available public monitoring datasets that spiroxamine (SPX) does not pose a concern for the investigated environmental compartments.

Assessment and conclusion by applicant:

Study meets the current guidance and the requirements in 283/2013.

The study addresses the requirement to review Member State data on spiroxamine monitoring in the environment. The study is considered valid.

Data Point:	KCA 7.5/01
Report Author:	[redacted]
Report Year:	1996
Report Title:	Storage stability of KWG 4168 in water
Report No:	MR-690/96
Document No:	M-006018-01-1
Guideline(s) followed in study:	None quoted
Deviations from current test guideline:	None
Previous evaluation:	No, submitted, not evaluated RAR (2010), RAR (2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The storage stability of spiroxamine in water was investigated in samples which were refrigerated (5°C) and frozen (<-18°C) for a maximum storage duration of 77 weeks. The purpose of the study was to determine the storage stability of spiroxamine from aquatic toxicity tests when stored under refrigerated and deep frozen conditions. Water used in the study was prepared by adding salt stock solutions to demineralised water (conductivity <0.2 µmho/cm) with a pH of between 7.0-7.5.

The test was performed with at two concentration (11.1 µg/L and 1.11 µg/L). For each test solution 21 bottles we prepared. Ten boutles were stored in a freezing chamber (<-18°C) and ten bottles were stored in cold storage (5°C). One bottle of each test solution was used to determine the actual concentration of spiroxamine.

Samples were taken from refrigerated and deep frozen conditions at study initiation, 1, 2, 4, 8, 21, 32, and 77 weeks after study initiation. Analysis of the samples was carried out in accordance to method 00252 M001. The method describes the determination of spiroxamine in water using gas chromatography and mass spectrometry (GC-MS) analysis.

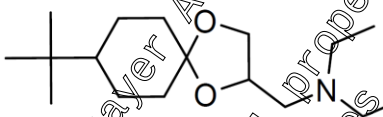
The method was validated by recoveries, which were performed with each set of samples. The recoveries were between 80 and 116%. The results are not corrected by the recoveries. For both concentrations no significant degradation of spiroxamine was observed during the storage by refrigeration or deep frozen.

I. Materials and Methods

A. Materials

1. Test Items

Spiroxamine



Batch	920522EL.B01
Purity:	99.0 %

2. Test System

Water used in the study was prepared by adding salt stock solutions to demineralised water (conductivity <0.2 µmho/cm) with a pH of between 7.0-7.5. The prepared media was representative of that used in aquatic toxicity studies.

B. Study Design

1. Experimental Conditions

The test was performed with at two concentration (111.1 µg/L and 1.11 µg/L). The test solutions were prepared by adding defined volumes of the stock solution to the test water. For each test solution 21 bottles we prepared. Ten bottles were stored in a freezing chamber (<-18°C) and ten bottles were stored in cold storage (5°C). One bottle of each test solution was used to determine the actual concentration of spiroxamine.

2. Sampling

Samples were taken from refrigerated and deep frozen conditions at study initiation, 1, 2, 4, 8, 21, 32, and 77 weeks after study initiation.

3. Analytical Procedures

Analysis of the samples was carried out in accordance to method 00252 M001 (M-00849002-2). The method describes the determination of spiroxamine in water using gas chromatography and mass spectrometry (GC-MS) analysis.

Prior to the analyses the samples were extracted by solid phase extraction. For this purpose C18 cartridges were conditioned with volumes of 20 ml of methanol and 20 ml milli-Q-water. Then different volumes (100 ml respectively 10 ml depending on the nominal concentration) of the samples were sucked through the preconditioned cartridges. After drying for one hour the cartridges were eluted with a volume of 10 ml of methanol and ammonia solution (990:10). After evaporating to dryness the residue is dissolved in different volumes (depending on the nominal concentrations) of a mixture of 50% n-butyl-acetate 49.5% methanol and 0.5% ammonia solution (25%).

The method was validated concurrently with the test solution analyses. The relative standard deviations were between 0.04 and 0.2 for the peak areas and between < 0.001 and 0.006 for the retention times of both isomers.

II. Results and Discussion

A. Data

Full details and acceptable validation data to support this method (Method 00252 M001 are presented in Document M-CA 4, Section 4.1.2 (M-00849002-2). The method complies with the EU regulatory requirements outlined within SANCO/3029/99 rev. 4 and is suitable for the determination of spiroxamine and its metabolites in soil samples by HPLC/MS/MS.

The method was validated by recoveries, which were performed with each set of samples. The recoveries, which were performed during the study are given in Table CA 7.5-6. The recoveries were between 80 and 116%. The results are not corrected by the recoveries.

Table CA 7.5-7 shows the stability of spiroxamine. The data show no significant decline in the concentration of KWG 4168 during the storage for 77 weeks when stored deep frozen (<-18 °C) or refrigerated (5°C).

Table CA 7.5-6: Recovery of spiroxamine, results shown as average (%)

Sample Time (week)	Nominal concentration (µg/L)	Recovery (Average %)
0	1.11	83
	111.1	89
1	1.11	109
	111.1	90
2	1.11	115
	111.1	88
4	1.11	100
	111.1	89
8	1.11	111
	111.1	83
21	1.084	108
	108.4	91
32	0.993	97
	99.3	84
77	0.9999	94
	99.99	96

Table CA 7.5-7: Results of spiroxamine storage stability conducted (results shown as average, µg/L)

Test system	Sample Time (week)	Average (µg/L) for 1.11 µg/L	Average (µg/L) for 111.1 µg/L
*	0	0.921	91.8
Refrigerator	1	1.30	98.6
	2	1.19	109
	4	0.989	90.0
	8	1.12	100
	21	1.13	95.0
	32	0.978	92.0
	77	1.08	86.2
Deep Frozen	1	1.24	107
	2	1.20	95.2
	4	1.29	98.6
	8	0.936	95.9
	21	1.05	106
	32	1.06	108
	77	1.04	91.2

* Sample of 0 day, no storage.

III. Conclusions

The storage stability of spiroxamine in water was investigated in samples, which were stored in a refrigerated (5°C) and frozen (-18°C) for a maximum storage duration of 77 weeks. For both concentrations no significant degradation of spiroxamine was observed during the storage by refrigeration or deep frozen.

Assessment and conclusion by applicant:

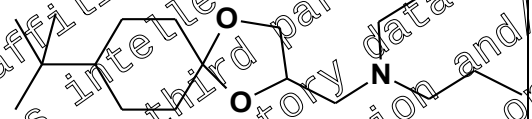
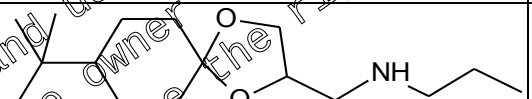
Study meets the current guidance and the requirements in 283/2013.

The study was not conducted to any study guidelines. The study is considered supportive information only to demonstrate the stability of spiroxamine in water samples.

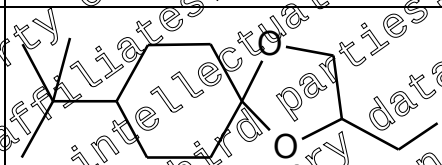
APPENDICES

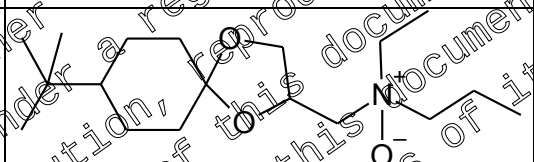
Appendix 1: Substances and metabolites: structure, codes, synonyms

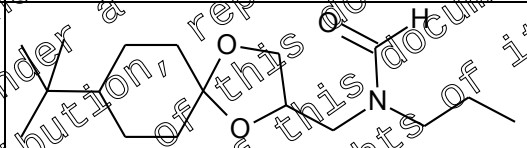
The information from the N3 document (Substances and metabolites: structure, codes, synonyms) is reproduced here for information only.

Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
spiroxamine KWG 4168 AE 1344293	IUPAC name: N-[(8-tert-butyl-1,4-dioxaspiro[4.5]dec-2-yl)methyl]-N-ethylpropan-1-amine (IUPAC) 8-(1,1-dimethylethyl)-N-ethyl-N-propyl-1,4-dioxaspiro[4.5]decane-2-methanamine SMILES notation: CC(C)(C)C1CCC2(CC1)OCC(CN(CC)CCC)O2 InChiKe: 1S/C18H35NO2/c1-6-12-19(7-2)13-16-14-20-18(21-16)10-8-15(9-11-18)17(3,4)5/h15-16H,6-14H2,1-5H3		All compartments
M01 Spiroxamine-desethyl KWG 4168-desethyl FHW 0104H KWG 4557	IUPAC name: N-[(8-tert-butyl-1,4-dioxaspiro[4.5]dec-2-yl) methyl]propan-1-amine (IUPAC) SMILES notation: CC(C)(C)C1CCC2(CC1)OCC(CNCCC)O2 InChiKe: 1S/C16H31NO2/c1-5-10-17-11-14-12-18-16(19-14)8-6-13(7-9-16)15(2,3)4/h13-14,17H,5-12H2,1-4H3	 GROUP A	Present in *in vitro* incubations with rat, dog and human hepatocytes **Livestock** Eggs hen (11.5% TRR) Muscle hen (9.3% TRR) Sub-cutaneous fat hen (8.4% TRR) Liver hen (21.3% TRR) **Plants** Banana pulp (0.9% TRR) Banana pulp (1.1% TRR) Grapes (1.1% TRR) Grapes (2.1% TRR)

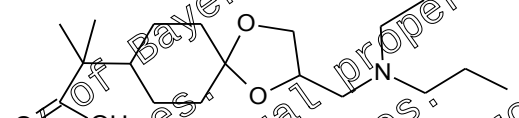
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
			Spring wheat straw (2% TRR) Spring wheat grain (0.5% TRR) Spring wheat forage (3.2-5.1% TRR- M01 not resolved from M05) Winter wheat straw (5.2% TRR- M01 not resolved from M05) Winter wheat forage (4.8-7.1% TRR- M01 not resolved from M05) **Confined rotational crops** Swiss chard leaves (11.6-12.1% TRR- M01 not resolved from M05) Wheat straw (2.4-4.5% TRR- M01 not resolved from M05) Turnip roots (3.7-4.4% TRR- M01 not resolved from M05) Turnip root (11.14.8% TRR- M01 not resolved from M05) Swiss chard immature leaves (3.1-12.6% TRR) Swiss chard mature leaves (4.0-12.3% TRR) Wheat forage (9.3-20.0% TRR) Wheat hay (7.4-10.4 % TRR) Wheat grain (2.9% TRR) Wheat straw (5.6-15.1% TRR) Turnip roots (1.5% TRR) Turnip tops (3.1-9.3% TRR) **Soil** Soil, aerobic (12% AR) Soil, anaerobic (7.6% AR) Soil, photo-degradation (9.1% AR)

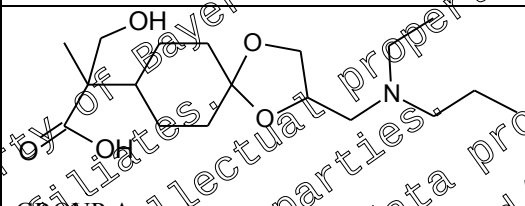
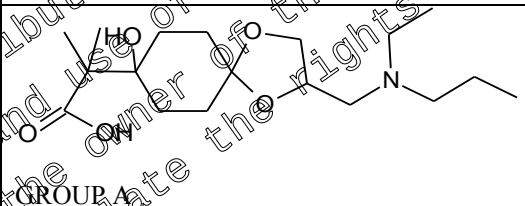
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
			Water: Photolysis water (4.5% AR) Water/sediment, aerobic (4.3% AR)
M02 Spiroxamine-despro-pyl KWG 4168-despropyl KWG 4669 WAK 6174 Despropyl-KWG	IUPAC name: N-[(8-tert-butyl-1,4-dioxaspiro[4.5]dec-2-yl) methyl]eth-anamine SMILES notation: CC(C)(C)C1CCC2(CC1)OCC(CNCC)O2 InChiKe: 1S/C15H29NO2/c1-5-16-10-13-11-17-15(18-13)8-6-12(7-9-15)14(2,3)4/h12-13,16H,5-11H2,1-4H3	 GROUP A	**Livestock** Eggs hen (10.2% TRR) Muscle hen (11.3% TRR) Sub cutaneous fat hen (3.4% TRR) Liver hen (21.7% TRR) **Plants** Banana pulp (0.4% TRR) Banana pulp (0.5% TRR) Grapes (0.5% TRR) Grapes (1.5% TRR) Spring wheat forage (4.3-4.6% TRR) Spring wheat straw (3.2% TRR) Spring wheat grain (3.0% TRR) Winter wheat forage (3.3-4.6 % TRR) Winter wheat straw (4.2% TRR) **Rotational crops** Swiss chard leaves (7.5-14.2% TRR) Wheat straw (2.9-3.5% TRR) Turnip roots (2.6-3.3% TRR) Turnip tops (16.7-17.7% TRR) Swiss chard immature leave (11.0-50.0% TRR) Swiss chard mature leave (19.0-51.2% TRR) Wheat forage (23.4-46.6% TRR) Wheat hay (15.2-30.9% TRR)

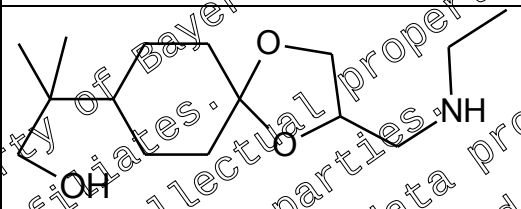
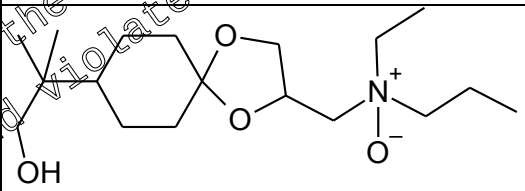
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
			Wheat grain (4.8% TRR) Wheat straw (14.3-17.4% TRR) Turnip roots (2.9% TRR) Turnip tops (14.5-21.1% TRR) **Soil** Soil, aerobic (9.2% AR) Soil, anaerobic (5.8% AR) Soil, photodegradation (6.1% AR) **Water** Photolysis water (4.5% AR) Water/sediment, aerobic (3.2% AR)
M03 Spiroxamine-N-oxide KWG 4168-N-oxide WAK 6301 WAK 6301/1 KWG-N-oxide	IUPAC name: [(8-tert-butyl-1,4-dioxaspiro[4.5]dec-2-yl) methyl]ethyl(propyl) amine oxide SMILES notation: CC(C)(C)C1CCC2(CC1)OCC(C[N+]([O-])(CC)CCC)O2 InChiKe: 1S/C18H35NO3/c1-6-12-19(20,7-3)13-16-14-21-18(22-16)10-8-15(9-11-18)17(3,4)5/h15-16H,6-14H2,1-5H3	 GROUDA	**Rat** Liver (0.12 mg/kg) **Livestock** Not reported **Plants** Banana pulp (1.2% TRR) Banana pulp (0.8% TRR) Grapes (2.9% TRR) Grapes (4.7% TRR) Spring wheat forage (8.0-9.0% TRR) Spring wheat straw (22.0% TRR) Spring wheat grain (17.8% TRR) Winter wheat forage (11.1-12.7% TRR) Winter wheat straw (20.9% TRR) Winter wheat grain (1.2% TRR) **Rotational crops** Swiss chard leaves (6.1-14.2% TRR) Wheat straw (12.1-12.7% TRR) Turnip roots (2.8-3.5% TRR)

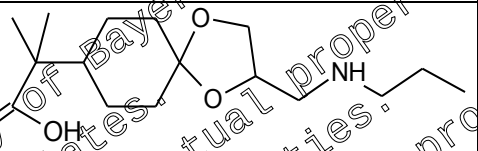
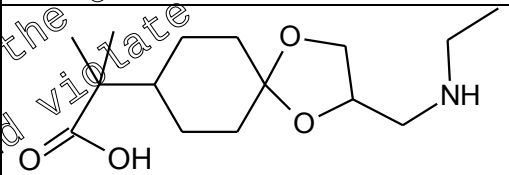
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
			Turnip tops (4.8-9.9% TRR) Wheat hay (1.7-2.1% TRR) Wheat grain (4.3% TRR) Wheat straw (1.4-5.0% TRR) **Soil** Soil, aerobic (7.9% AR) Soil, anaerobic (3.3% AR) Soil, photodegradation (6.2% AR) **Water** Photolysis water (4.0% AR) Water/sediment, aerobic (11.3% AR)
M04 Spiroxamine-N-formyl-desethyl KWG 4168-N-formyl-desethyl WAK 6782 N-formyl-desethyl-KWG	IUPAC name: N-[(8-tert-butyl-1,4-dioxaspiro[4.5]dec-2-yl)methyl]-N-propylformamide SMILES notation: CC(C)(C)C1CCC2(CC1)OCC(CN(C=O)CCC)O2 InChiKe: 1S/C17H31NO3/c1-5-10-18(13-19)11-15-12-20-17(21-15)8-6-14(7-9-17)16(2,3)4/h13-15H,5-12H2,1-4H3	 GROUP A	**Livestock** Not found **Plants** Spring wheat forage (2.1-5.3% TRR) Spring wheat straw (7.5% TRR) Spring wheat grain (6.9% TRR) Winter wheat forage (4.6-5.8% TRR) Winter wheat straw (9.7% TRR) **Rotational crops** Wheat straw (7.5-9.2% TRR) Wheat hay (1.5% TRR) Wheat grain (1.5% TRR) Wheat straw (2.8-6.4% TRR) **Soil** not found **Water** not found

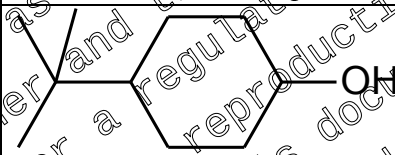
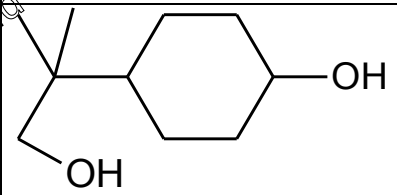
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
M05 Spiroxamine-hydroxy KWG 4168-hydroxyl WAK 5868 WAK 5868/2 Hydroxy-KWG	IUPAC name: 2-(2-{[ethyl(propyl)amino]methyl}-1,4-dioxaspiro[4.5]dec-8-yl)-2-methylpropan-1-ol SMILES notation: CC(C)(CO)C1CCC2(CC1)OCC(CN(CC)CCC)O2 InChiKe: 1S/C18H35NO3/c1-5-11-19(6-2)12-16-13-21-18(22-16)9-7-15(8-10-18)17(3,4)14-20/h15-16,20H,5-14H2,1-4H3	 GROUP A	**Rat** Proposed intermediate in formation of M06 **Livestock** Not found **Plants** Grapes (0.3% TRR) Spring wheat straw (2.4% TRR) Spring wheat grain (1.6% TRR) **Rotational crops** Swiss chard leaves (11.6-12.1% TRR) Wheat straw (2.4-4.5% TRR) Turnip roots (3.7-4.4% TRR) Turnip tops (11.0-14.8% TRR) Swiss chard immature leave (1.9-17.2% TRR) Swiss chard mature leave (1.5-12.8% TRR) Wheat forage (1.3% TRR) Wheat hay (2.5% TRR) Turnip roots (0.8% TRR) Turnip tops (3.6% TRR) **Soil** not found **Water** not found

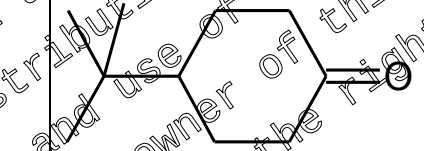
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
M06 Spiroxamine-acid KWG 4168-acid WAK 5708 WAK 5708/P ECW 80511 ECW 8046[a] KWG 4168-carboxylic acid	IUPAC name: 2-(2-{[ethyl(propyl)amino]methyl}-1,4-dioxaspiro[4.5]dec-8-yl)-2-methylpropanoic acid SMILES notation: O=C(O)C(C)(C)C1CCC2(CC1)OCC(CN(CC)CCC)O2 InChiKe: 1S/C18H33NO4/c1-5-11-19(6-2)12-15-13-22-18(23-15)9-7-14(8-10-18)17(3,4)16(20)21/h14-15H,5-13H2,1-4H3,(H,20,21)	 GROUP A	Present in *in vitro* incubations with mouse, rat, dog and human hepatocytes **Rat** Excreta (12.7-30.5% AD) **Livestock** Milk goat (53% TRR) Liver goat (19.6% TRR) Kidney goat (10.4% TRR) Muscle goat (48.3% TRR) Fat goat (30.5% TRR) Eggs hen (37.4% TRR) Muscle laying (37.3% TRR) Sub cutaneous fat hen (1.7% TRR) Liver hen (8.5% TRR) [M06 = aglycon of M19] **Rotational crops** Free M06 not detected [M06 = aglycon of M44] **Soil** Soil, aerobic (5.3% AR) Soil, anaerobic (9.9% AR) Soil, photo-degradation (not observed) **Water** Photolysis water (not observed) Water/sediment, aerobic (44.5% AR)

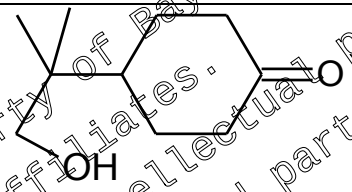
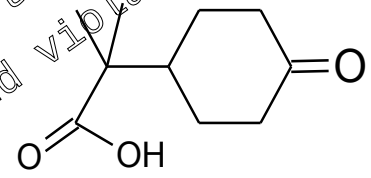
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
M07 Spiroxamine-hydroxy acid KWG 4168-hydroxy acid KNO 2212	IUPAC name: 2-(2-{[ethyl(propyl)amino]methyl}-1,4-dioxaspiro[4.5]dec-8-yl)-3-hydroxy-2-methylpropanoic acid SMILES notation: O=C(O)C(C)(CO)C1CCC2(CC1)OCC(CN(CC)CCC)O2 InChiKe: 1S/C18H33NO5/c1-4-10-19(5-2)11-15-12-23-18(24-15)8-6-14(7-9-18)17(3,13-20)16(21)22/h14-15,20H,4-13H2,1-3H3,(H,21,22)	 GROUP A	Present in *in vitro* incubations with mouse, rat, dog and human hepatocytes **Livestock** Milk goat (10.9% TRR) Liver goat (1.7% TRR) Kidney goat (16.0% TRR) Muscle goat (10.3% TRR) Fat goat (9.7% TRR) **Plants** not found **Soil** not found **Water** not found
M08 Spiroxamine-8-hydroxy acid KWG 4168-8-hydroxy acid ECW 8096 ECW 9291A	IUPAC name: 2-(2-{[ethyl(propyl)amino]methyl}-8-hydroxy-1,4-dioxaspiro[4.5] dec-8-yl)-2-methylpropanoic acid SMILES notation: O=C(O)C(C)(C)C1(O)CCC2(CC1)OCC(CN(CC)CCC)O2 InChiKe: 1S/C18H33NO5/c1-5-11-19(6-2)12-14-13-23-18(24-14)9-7-17(22,8-10-18)16(3,4)15(20)21/h14,22H,5-13H2,1-4H3,(H,20,21)	 GROUP A	**Rat** Excreta (0.6-12.0% AD) **Livestock** Liver goat (1.2% TRR) Kidney goat (2.3% TRR) **Plants** not found **Soil** not found **Water** not found

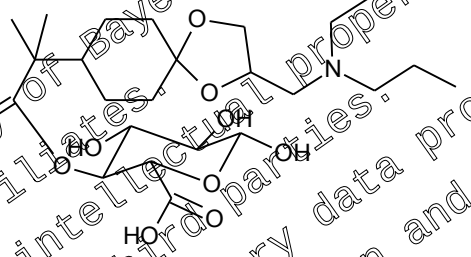
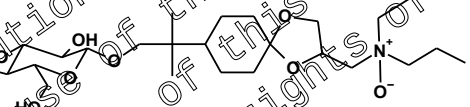
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
M09 Spiroxamine-hydroxy-despropyl KWG 4168-hydroxy-despropyl WAK 6079/1 WAK 6079-1 Hydroxy-despropyl KWG	IUPAC name: 2-{2-[(ethylamino)methyl]-1,4-dioxaspiro [4.5]dec-8-yl}-2-methylpropan-1-ol SMILES notation: CC(C)(CO)C1CCC2(CC1)OCC(CNCC)O2 InChiKe: 1S/C15H29NO3/c1-4-16-9-13-10-18-15(19-13)7-5-12(6-8-15)14(2,3)11-17/h12-13,16-17H,4-11H2,1-3H3	 GROUP A	**Livestock** not found **Plants** Spring wheat straw (0.3% TRR) Spring wheat forage (1.6% TRR – M09 not resolved from unknown metabolite 9) Winter wheat forage (0.2-0.6% TRR-M09 not resolved from unknown metabolite 9) Winter wheat straw (0.4% TRR-M09 not resolved from unknown metabolite 9) **Rotational crops** Free M09 not detected [M09 = aglycon of M39] **Soil** not found **Water** not found
M10 Spiroxamine-hydroxy-N-oxide KWG 4168-hydroxy-N-oxide	IUPAC name: 2-(2-{ethyl(propyl)nitrony}methyl}-1,4-dioxaspiro[4.5]dec-8-yl)-2-methylpropan-1-ol SMILES notation: CC.CC(C)(CO)C1CCC2(CC1)OCC(C[N+]([O-])(CC)CCC)O2 InChiKe: 1S/C18H35NO4.C2H6/c1-5-11-19(21,6-2)12-16-13-22-18(23-16)9-7-15(8-10-18)17(3,4)14-20;1-2/h15-16,20H,5-14H2,1-4H3;1-2H3	 GROUP A	**Livestock** not found **Plants** Spring wheat- Free M10 not detected [M10 = aglycon of M20 and M21] **Soil** not found **Water** - not found

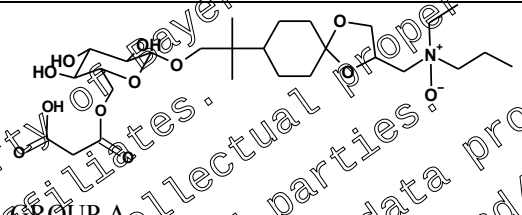
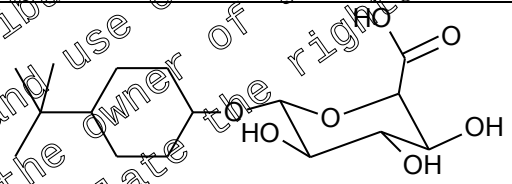
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
M11 Spiroxamine-desethyl acid KWG 4168-desethyl acid ECW 8044 ECW 8044A ECW 8045 ECW 8045A KNO 2222 KNO 22243 WAK 5756 WAK 5756B WAK 5756P	IUPAC name: 2-methyl-2-{2-[(propylamino)methyl]-1,4-dioxaspiro[4.5]dec-8-yl}propanoic acid SMILES notation: O=C(O)C(C)(C)C1CCC2(CC1)OCC(CNCCC)O2 InChiKe: 1S/C16H29NO4/c1-4-9-17-10-13-11-20-16(21-13)7-5-12(6-8-16)15(2,3)14(18)19/h12-13,17H,4-11H2,1-3H3,(H,18,19)	 GROUP A	Present in *in vitro* incubations with mouse and rat hepatocytes **Rat** Excreta (3.4-13% AD) **Livestock** Milk goat (5.5% TRR) Liver goat (3.8% TRR) Kidney goat (5.8% TRR) Muscle goat (6.4% TRR) Fat goat (4.3% TRR) **Plants** not found **Rotational crops** Free M11 not detected [M11 = aglycon of M43] **Soil** not found **Water** not found
M12 Spiroxamine-despropyl acid KWG 4168-despropyl acid BNF 5534 BNF 5534A ECW 8042 ECW 8042A KNO 2218A KNO 2218B	IUPAC name: SMILES notation: O=C(O)C(C)(C)C1CCC2(CC1)OCC(CNCC)O2 InChiKe: 1S/C15H27NO4/c1-4-16-9-12-10-19-15(20-12)7-5-11(6-8-15)14(2,3)13(17)18/h11-12,16H,4-10H2,1-3H3,(H,17,18)	 GROUP A	Present in *in vitro* incubations with mouse and rat hepatocytes **Rat** Excreta (2.1-13.2%) **Livestock** Liver goat (3.5% TRR) Kidney goat (9.0% TRR) Muscle goat (6.8% TRR) Fat goat (6.2% TRR) **Plants**

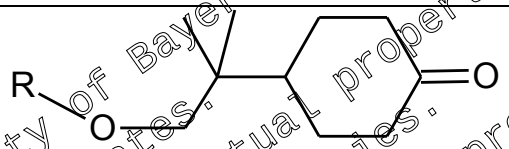
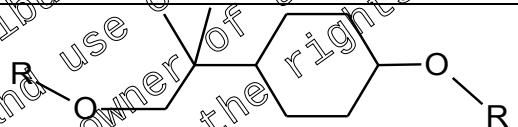
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
			not found **Rotational crops** Free M12 not detected [M12 = aglycon of M45] **Soil** not found **Water** not found
M13 Spiroxamine-cyclo-hexanol KWG 4168-cyclohex-anol BNF 5550A WAK 6850-3A Cyclohexanol	IUPAC name: 4-tert-butylcyclohexanol SMILES notation: CC(C)(C)C1CCC(O)CC1 InChiKe: 1S/C10H20O/c1-10(2,3)8-4-6-9(11)7-5-8/h8-9,11H,4-7H2,1-3H3	 GROUP 3	**Rat** Free M13 not detected [M13 = agly-con of M22] **Livestock** Goat- Free M13 not detected [M13 = aglycon of M22] **Plants** Grapes- Free M13 not detected [M13 = aglycon of M32, M33, M34, M35 and M36] **Soil** not found **Water** not found
M14 Spiroxamine-diol KWG 4168 diol WAK 6482-4 Diol	IUPAC name: 4-(2-hydroxy-1,1-dimethylethyl) cyclohexanol SMILES notation: OC1CCC(CC1)CC(C)(C)CO InChiKe: 1S/C10H20O2/c1-10(2,7-11)8-3-5-9(12)6-4-8/h8-9,11-12H,3-7H2,1-2H3		**Livestock** Not found **Plants** Banana pulp (12.8% TRR- hydroly-sis product) Grapes (13.0% TRR- hydrolysis product)

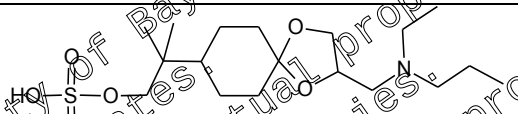
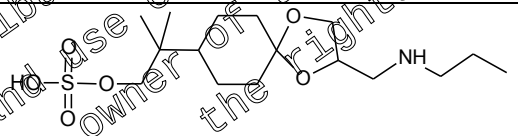
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
		GROUP B	Spring wheat straw (0.2% TRR- hydrolysis product) Spring wheat grain (2.6% TRR- hydrolysis product) **Rotational crops** Swiss chard leaves (8.8-13.2% TRR- hydrolysis product) Wheat straw (3.5-4% TRR- hydrolysis product) Turnip tops (4.4-13.0% TRR- hydrolysis product) **Soil** not found **Water** not found
M15 Spiroxamine-ketone KWG 4168-ketone WAK 5428 Cyclohexanone	IUPAC name: 4-tert-butylcyclohexanone SMILES notation: O=C1CCC(CC1)C(C)(C)C InChiKe: 1S/C10H18O/c1-10(2,3)8-4-6-9(11)7-5-8/h8H,4-7H2,1-3H3	 GROUP B	**Rat** Present as an artefact of sample work up in rat excreta **Livestock** Not found **Plants** Grapes (1.3% TRR- hydrolysis product) Spring wheat straw (5.5% TRR- hydrolysis product) Spring wheat grain (4.6% TRR- hydrolysis product) **Soil** not found **Water**

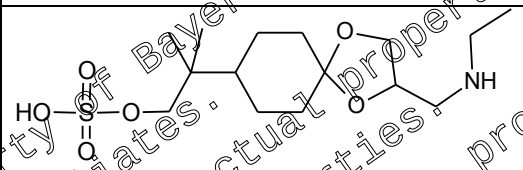
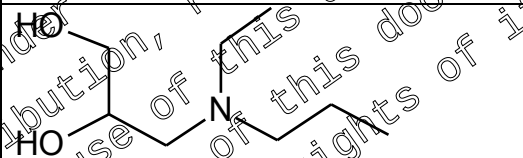
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
			not found
M16 Spiroxamine-hydroxy-ketone KWG 4168-hydroxy-ketone BNF 5569B BNF 5544B Hydroxyketone	IUPAC name: 4-(2-hydroxy-1,1-dimethylethyl)cyclohexanone SMILES notation: OCC(C)(C)C1CCC(=O)CC1 InChiKe: 1S/C10H18O2/c1-10(2,7-11)8-3-5-9(12)6-4-8/h8,11H,3-7H2,1-2H3	 GROUP B	**Livestock** Not found **Plants** Grapes (0.5% TRR- hydrolysis product) Spring wheat straw (1.0% TRR- hydrolysis product) Spring wheat grain (7.6% TRR- hydrolysis product) **Rotational crops** Swiss chard leaves (15.6-29.3% TRR- hydrolysis product) Wheat straw (8.9-11.6% TRR- hydrolysis product) Turnip tops (11.7-37.3% TRR- hydrolysis product) **Soil** not found **Water** not found
M17 Spiroxamine-ketone acid KWG 4168-ketone acid WAK 6131-2-7	IUPAC name: 2-methyl-2-(4-oxocyclohexyl)propanoic acid SMILES notation: O=C1CCC(CC1)C(C)(C)C(=O)O InChiKe: InChI=1S/C10H16O3/c1-10(2,9(12)13)7-3-5-8(11)6-4-7/h7H,3-6H2,1-2H3,(H,12,13)	 GROUP B	**Livestock** Not found **Plants** not found **Soil** not found **Water** not found

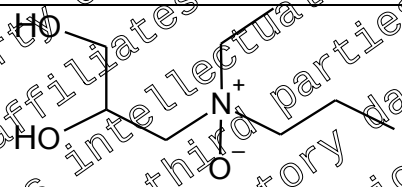
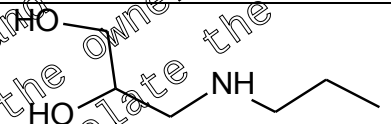
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
M19 Spiroxamine-acid glucuronide KWG 4168-acid glucuronide ECW 80802 KNO 1634 KNO 1634/A	IUPAC name: 1-O-[2-(2-{[ethyl(propyl)amino]methyl}-1,4-dioxaspiro[4.5]dec-8-yl)-2-methylpropanoyl]hexopyranuronic acid SMILES notation: O=C(O)C1OC(OC(=O)C(C)(C)C2CCC3(CC2)OCC(CN(CC)CCC)O3)C(O)C(O)C1O InChiKe: 1S/C24H41NO10/c1-5-11-25(6-2)12-15-13-32-24(35-15)9-7-14(8-10-24)23(3,4)22(31)34-21-18(28)16(26)17(27)19(33-21)20(29)30/h14-19,21,26-28H,5-13H2,1-4H3,(H,29,30)	 GROUP A	**Rat** Excreta (0.4-1.7% AD) **Livestock** Liver goat (3.5% TRR) Kidney goat (9.0% TRR) Muscle goat (6.8% TRR) Fat goat (6.2% TRR) **Plants** not found **Soil** not found **Water** not found
M20 Spiroxamine-hydroxy-N-oxide glucoside KWG 4168-hydroxy-N-oxide glucoside glucoside of hydroxy-N-oxide	IUPAC name: 2-(2-{[ethyl(propyl)nitroryl]methyl}-1,4-dioxaspiro[4.5]dec-8-yl)-2-methylpropyl hexopyranoside SMILES notation: OC1C(O)C(O)C(CO)OC1OCC(C)(C)C1CCC2(CC1)OCC(C[N+]([O-])(CC)CCC)O2 InChiKe: 1S/C24H45NO9/c1-5-11-25(30,6-2)12-17-14-32-24(34-17)9-7-16(8-10-24)23(3,4)15-31-22-21(29)20(28)19(27)18(13-26)33-22/h16-22,26-29H,5-15H2,1-4H3	 GROUP W	**Livestock** Not found **Plants** Spring wheat forage (0.7% TRR) Spring wheat straw (2.0% TRR) **Rotational crops** Swiss chard leaves (2.5% TRR) Wheat straw (2.1-2.6% TRR) Turnip tops (8.4-10.4% TRR) **Soil** not found **Water** not found

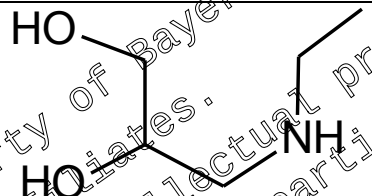
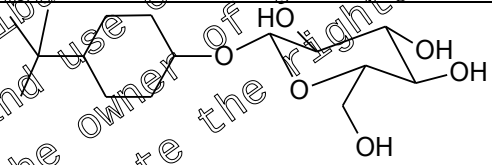
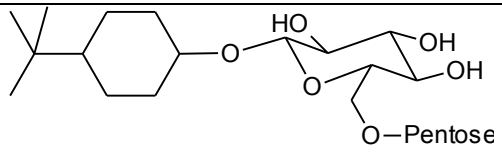
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
M21 Spiroxamine-hydroxy-N-oxide malonyl glucoside KWG 4168-hydroxy-N-oxide malonyl glucoside	IUPAC name: 2-(2-{[ethyl(propyl)nitroryl]methyl}-1,4-dioxaspiro[4.5]dec-8-yl)-2-methylpropyl 6-O-(carboxyacetyl)hexopyranoside SMILES notation: O=C(O)CC(=O)OCC1OC(OCC(C)(C)C2CCC3(CC2)OCC(C[N+]([O-])(CC)CCC)O3)C(O)C(O)C1O InChiKe: 1S/C27H47NO12/c1-5-11-28(35,6-2)13-18-14-38-27(40-18)9-7-17(8-10-27)26(3,4)16-37-25-24(34)23(33)22(32)19(39-25)15-36-21(31)12-20(29)30/h17-19,22-25,32-34H,5-16H2,1-4H3,(H,29,30)	 GROUP A	**Livestock** not found **Plants** Spring wheat forage (0.2-2.0% TRR) Spring wheat straw (1.9% TRR) Winter wheat forage (0.2-0.9% TRR) Winter wheat straw (3.1% TRR) **Rotational crops** Swiss chard leaves (1.6% TRR) Wheat straw (4.4% TRR) Turnip tops (1.7-3.7% TRR) **Soil** not found **Water** not found
M22 Spiroxamine-cyclohexanol-glucuronide KWG 4168-cyclohexanol-glucuronide ECW 8081	IUPAC name: 4-tert-butylcyclohexyl hexopyranosiduronic acid SMILES notation: CC(C)(C)C1CCC(CC1)OC1OC(C(O)C(O)C1O)C(=O)O InChiKe: 1S/C16H28O7/c1-16(2,3)8-4-6-9(7-5-8)22-15-12(19)10(17)11(18)13(23-15)14(20)21/h8-13,15,17-19H,4-7H2,1-3H3,(H,20,21)	 GROUP B	**Rat** Excreta (0.6-1.7% AD) **Livestock** Kidney goat (0.4% TRR) **Plants** not found **Soil** not found **Water** not found

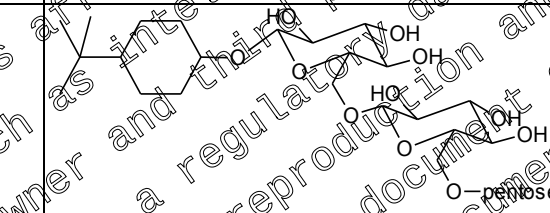
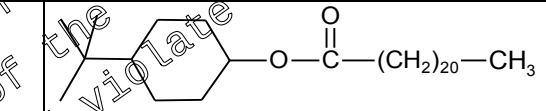
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
M23 Spiroxamine-hydroxy-ketone-conjugate KWG 4168-hydroxy-ketone-conjugate Conjugate of hydroxy-ketone	IUPAC name: Not available SMILES notation: CC(C)(CO[*])C1CCC(=O)CC1 InChiKe: Not available	 GROUP B	**Livestock** not found **Plants** Spring wheat forage and straw (tentative identification after hydrolysis; 1.0 – 1.8% TRR) **Rotational crops** Swiss chard leaves (2.2% TRR) Wheat straw (1.5-2.4% TRR) Turnip roots (8.1% TRR) Turnip tops (3.8-7.9% TRR) **Soil** not found **Water** not found
M24 Spiroxamine-diol-di-glycoside KWG 4168-diol-di-glycoside Diol-diglycoside (grapes) Diconjugated Diol Conjugate of diol (CRC, R was unspecified)	IUPAC name: SMILES notation: [*]OC1CCC(CC1)C(C)(C)CO[*] InChiKe: Not available	 GROUP B	**Livestock** not found **Plants** Banana (9.2% TRR) Grapes (14.8% TRR – main component of metabolite group 12) **Rotational crops** Swiss chard leaves (3.0% TRR) Wheat straw (1.9-2.2% TRR- Turnip roots (7.8% TRR) Turnip tops (2.0-4.3% TRR) **Soil** not found **Water**

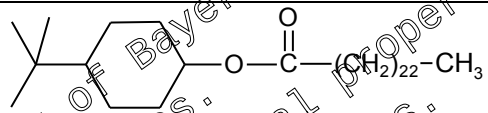
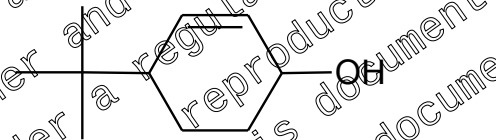
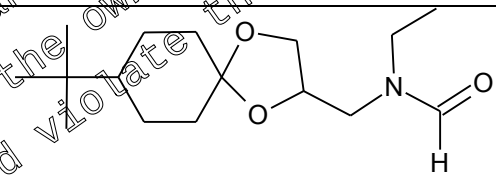
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
			not found
M25 Spiroxamine-sulfate KWG 4168-sulfate ECW 8076 ECW 80772 KNO 22302	IUPAC name: 2-(2-{[ethyl(propyl)amino]methyl}-1,4-dioxaspiro[4.5]dec-8-yl)-2-methylpropyl hydrogen sulfate SMILES notation: O=S(=O)(O)OCC(C)(C)C1CCC2(CC1)OCC(CN(CC)CCC)O2 InChiKe1S/C18H35NO6S/c1-5-11-19(6-2)12-16-13-23-18(25-16)9-7-15(8-10-18)17(3,4)14-24-26(20,21)22/h15-16H,5-14H2,1-4H3,(H,20,21,22)	 GROUP A	Present in *in vitro* incubations with mouse, rat and human hepatocytes **Rat** Excreta (1.0-32.5% AD) **Livestock** Milk goat (8.1% TRR) Liver goat (2.2% TRR) Kidney goat (1.6% TRR) **Plants** not found **Soil** not found **Water** not found
M26 Spiroxamine-desethyl-sulfate KWG 4168-desethyl-sulfate ECW 8079 ECW 80822 KNO 2226	IUPAC name: 2-methyl-2-{2-[(propylamino)methyl]-1,4-dioxaspiro[4.5]dec-8-yl}propyl hydrogen sulfate SMILES notation: O=S(=O)(O)OCC(C)(C)C1CCC2(CC1)OCC(CNCCC)O2 InChiKe: 1S/C16H31NO6S/c1-4-9-17-10-14-11-21-16(23-14)7-5-13(6-8-16)15(2,3)12-22-24(18,19)20/h13-14,17H,4-12H2,1-3H3,(H,18,19,20)	 GROUP A	**Rat** Excreta (0.7-14.6% AD) **Livestock** Liver goat (1.9% TRR) Kidney goat (3.2% TRR) Fat goat (3.5% TRR) **Plants** not found **Soil** not found **Water** not found

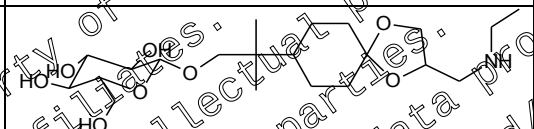
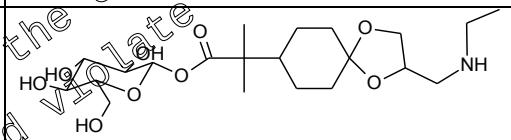
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
M27 Spiroxamine-despropyl-sulfate KWG 4168-despropyl-sulfate ECW 80862 ECW 80873 KNO 2233B	IUPAC name: 2-{2-[(ethylamino)methyl]-1,4-dioxaspiro[4.5]dec-8-yl}-2-methylpropyl hydrogen sulfate SMILES notation: O=S(=O)(O)OCC(C)(C)C1CCC2(CC1)OCC(CNCC)O2 InChiKe: 1S/C15H29NO6S/c1-4-16-9-13-10-20-15(22-13)7-5-12(6-8-15)14(2,3)11-21-23(17,18)19/h12-13,16H,4-11H2,1-3H3,(H,17,18,19)	 GROUP A	**Rat** Excreta (0.3-11.3% AD) **Livestock** Liver goat (4.7% TRR) Kidney goat (3.8% TRR) Fat goat (3.5% TRR) **Plants** not found **Soil** not found **Water** not found
M28 Spiroxamine-aminodiol KWG 4168-aminodiol KNO 1458/1 WAK 5427 KML 2202 Aminodiol	IUPAC name: 3-[ethyl(propyl)amino]propane-1,2-diol SMILES notation: CCCN(CC(O)CO)CC InChiKe: 1S/C8H19NO2/c1-3-5-9(4-2)6-8(11)7-10/h8,10-11H,3-7H2,1-2H3	 GROUP C	**Rat** Excreta (2.2-5.6% AD) **Livestock** not found **Plants** Banana pulp (31.2% TRR) Grapes (37.5% TRR) **Rotational crops** Swiss chard immature leave (1.8-2.4% TRR) Swiss chard mature leave (3.9% TRR) Wheat hay (0.3-0.6% TRR) Wheat straw (0.2% TRR) Turnip roots (4.9% TRR) Turnip tops (0.9-3.3% TRR) **Soil**

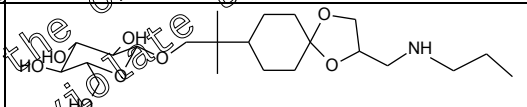
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
			not found **Water** not found
M29 Spiroxamine-amino-diol-N-oxide KWG 4168-amino-diol-N-oxide WAK 6885 Aminodiol-N-oxide	IUPAC name: N-ethyl-2,3-dihydroxy-N-propylpropan-1-amine N-oxide SMILES notation: CCC[N+]([O-])(CC(O)CO)CC InChiKe: 1S/C8H19NO3/c1-3-5-9(12,4-2)6-8(11)7-10/h8,10-11H,3-7H2,1-2H3	 GROUP C	**Livestock** not found **Plants** Grapes (0.1% TRR) **Rotational crops** Swiss chard immature leave (1.0-5.2% TRR) Swiss chard mature leave (0.8% TRR) Turnip roots (4.7-4.8% TRR) **Soil** not found **Water** not found
M30 Spiroxamine-desethyl-aminodiol KWG 4168-desethyl-aminodiol WAK 6894 Desethyl-aminodiol	IUPAC name: 3-(propylamino)propane-1,2-diol SMILES notation: CCCNCC(O)CO InChiKe: InChI=1S/C6H15NO2/c1-2-3-7-4-6(9)5-8/h6-9H,2-5H2,1H3	 GROUP C	**Livestock** not found **Plants** Banana pulp (0.6% TRR) Grapes (1.1% TRR) **Soil** not found **Water** not found

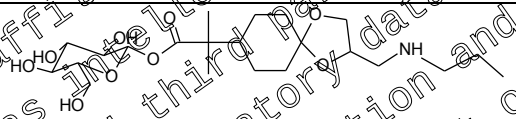
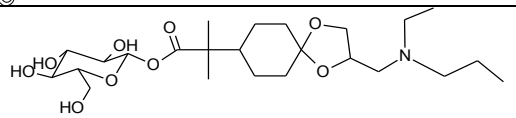
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
M31 Spiroxamine-despropyl-aminodiol KWG 4168-despropyl-aminodiol WAK 6893 Despropyl-aminodiol	IUPAC name: 3-(ethylamino)propane-1,2-diol SMILES notation: CCNCC(O)CO InChiKe: 1S/C5H13NO2/c1-2-6-3-5(8)4-7/h5-8H,2-4H2,1H3	 GROUP C	**Livestock** not found **Plants** Banana pulp (0.6% TRR) Grapes (1.2% TRR) **Rotational crops** Wheat forage (1.6% TRR) Wheat hay (1.4-1.7% TRR) Wheat straw (1.4-1.8% TRR) Turnip roots (6.1% TRR) Turnip tops (0.4% TRR) **Soil** not found **Water** not found
M32 Spiroxamine-cyclohexanol glucoside KWG 4168-cyclohexanol glucoside Cyclohexanol Glucoside	IUPAC name: 4-tert-butylcyclohexyl hexopyranoside SMILES notation: CC(C)(C)C1CCC(CC1)OC1OC(CO)C(O)C(O)C1O InChiKe: 1S/C16H30O6/c1-16(2,3)9-4-6-10(7-5-9)21-15-14(20)13(19)12(18)11(8-17)22-15/h9-15,17-20H,4-8H2,1-3H3	 GROUP B	**Livestock** not found **Plants** not found [transient] **Soil** not found **Water** not found
M33 Spiroxamine-cyclohexanol-glucopyranosyl-pentose	IUPAC name: Not available SMILES notation: Not available InChiKe: Not available		**Livestock** not found **Plants** Grapes (19.1% TRR) **Soil**

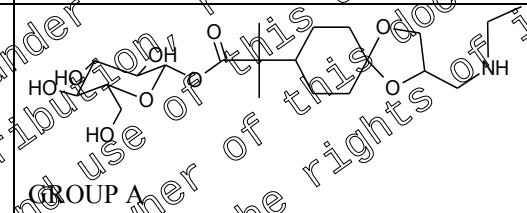
Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
KWG 4168-cyclohexanol-glucopyranosyl-pentose Cyclohexanol-glucopyranosyl-pentose Conjugate of cyclohexanol		GROUP B (position of pentose not specified)	not found **Water** not found
M34 Spiroxamine-cyclohexanol-glucopyranosyl-glucopyranosyl-pentose KWG 4168-cyclohexanol-glucopyranosyl-glucopyranosyl-pentose Cyclohexanol-glucopyranosyl-glucopyranosyl-pentose Conjugate of cyclohexanol	IUPAC name: Not available SMILES notation: Not available InChiKe: Not available	 GROUP B (position of glucopyranosyl-pentose not specified)	**Livestock** not found **Plants** Grapes (3.5% TRR) **Soil** not found **Water** not found
M35 Spiroxamine-docosanoic acid ester KWG 4168-docosanoic acid ester PA1345 Docosanoic acid ester	IUPAC name: 4-tert-butylcyclohexyl docosanoate SMILES notation: Not available InChiKe: Not available	 GROUP B	**Livestock** not found **Plants** Grapes (13.0% TRR) **Soil** not found **Water** not found

Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
M36 Spiroxamine-tetracosanoic acid ester KWG 4168-tetracosanoic acid ester PA1344 Tetracosanoic acid ester	IUPAC name: 4-tert-butylcyclohexyl tetracosanoate SMILES notation: Not available InChiKe: Not available	 GROUP B	**Livestock** not found **Plants** Grapes (4.2% TRR) **Soil** not found **Water** not found
M37 Spiroxamine-cyclohexenol KWG 4168-cyclohexenol	IUPAC name: 4-tert-butylcyclohexen-1-ol SMILES notation: CC(C)(C)C1C=CC(O)CC1 InChiKe: 1S/C10H18O/c1-10(2,3)8-4-6-9(11)7-5-8/h4,6,8-9,11H,5,7H2,1-3H3	 or isomer GROUP B	**Livestock** not found **Plants** Grapes (3.2% TRR- hydrolysis product) **Soil** not found **Water** not found
M38 Spiroxamine-N-formyl-despropyl KWG 4168-N-formyl-despropyl	IUPAC name: N-[(8-tert-butyl-1,4-dioxaspiro[4.5]dec-2-yl)methyl]-N-ethylformamide SMILES notation: CC(C)(C)C1CCC2(CC1)OCC(CN(C=O)CC)O2 InChiKe: 1S/C16H29NO3/c1-5-17(12-18)10-14-11-19-16(20-14)8-6-13(7-9-16)15(2,3)4/h12-14H,5-11H2,1-4H3	 GROUP A	**Livestock** not found **Plants** not found **Rotational crops** Wheat hay (4.8-6.8% TRR) Wheat grain (2.7% TRR) Wheat straw (3.4-7.6% TRR) **Soil** not found

Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
			Water not found
M39 Spiroxamine-hydroxy-despropyl glycoside KWG 4168-hydroxy-despropyl glycoside	IUPAC name: glycoside of 2-{2-[(ethylamino)methyl]-1,4-diox-aspiro[4.5]dec-8-yl}-2-methyl propan-1-ol SMILES notation: OC1C(O)C(O)C(CO)OC1OCC(C)(C)C1CCC2(CC1)OCC(CNCC)O2 InChiKe: 1S/C21H39NO8/c1-4-22-9-14-11-28-21(30-14)7-5-13(6-8-21)20(2,3)12-27-19-18(26)17(25)16(24)15(10-23)29-19/h13-19,22-26H,4-12H2,1-3H3	 GROUP A	**Livestock** not found **Plants** not found **Rotational crops** Swiss chard immature leave (2.0% TRR) Swiss chard mature leave (2.8% TRR) Wheat forage (1.6-2.1% TRR) Wheat hay (1.6-4.4% TRR) Wheat straw (0.5-1.6% TRR) Turnip tops (3.1-21.3% TRR) **Soil** not found **Water** not found
M40 Spiroxamine-hydroxy glycoside KWG 4168-hydroxy glycoside	IUPAC name: glycoside of 2-(2-{[ethyl(propyl) amino]methyl}-1,4-dioxaspiro[4.5]dec-8-yl)-2-methylpropan-1-ol SMILES notation: OC1C(O)C(O)C(CO)OC1OCC(C)(C)C1CCC2(CC1)OCC(CN(CCC)CC)O2 InChiKe: 1S/C24H45NO8/c1-5-11-25(6-2)12-17-14-31-24(33-17)9-7-16(8-10-24)23(3,4)15-30-22-21(29)20(28)19(27)18(13-26)32-22/h16-22,26-29H,5-15H2,1-4H3	 GROUP A	**Livestock** not found **Plants** not found **Rotational crops** Swiss chard immature leave (0.8-4.7% TRR) Swiss chard mature leave (0.8-3.8% TRR) Wheat forage (1.3-1.5% TRR)

Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
			Wheat hay (1.0-2.9% TRR) Wheat straw (0.9-12.6% TRR) Turnip tops (1.0-7.6% TRR) **Soil** not found **Water** not found
M41 Spiroxamine-hydroxy-desethyl KWG 4168-hydroxy-desethyl WAK 6084/1	IUPAC name: 2-methyl-2-{2-[(propylamino)methyl]-1,4-dioxaspiro[4.5]dec-8-yl}propan-1-ol SMILES notation: CC(C)(CO)C1CCC2(CC1)OCC(CNCCC)O2 InChiKe: 1S/C16H31NO3/c1-4-9-17-10-14-11-19-16(20-14)7-5-13(6-8-16)15(2,3)12-18/h13-14,17-18H,4-12H2,1-3H3	 GROUP A	**Livestock** not found **Plants** not found **Rotational crops** No free M41 detected [aglycon of M42] **Soil** not found **Water** not found
M42 Spiroxamine-hydroxy-desethyl glycoside KWG 4168-hydroxy-desethyl glycoside	IUPAC name: glycoside of 2-{2-[(propylamino)methyl]-1,4-dioxaspiro[4.5]dec-8-yl}-2-methylpropan-1-ol SMILES notation: OC1C(O)C(OC(CO)C1O)CC(C)(C)C1CCC2(CC1)OCC(CNCCC)O2 InChiKe: 1S/C22H41NO8/c1-4-9-23-10-15-12-29-22(31-15)7-5-14(6-8-22)21(2,3)13-28-20-19(27)18(26)17(25)16(11-24)30-20/h14-20,23-27H,4-13H2,1-3H3	 GROUP A	**Livestock** not found **Plants** not found **Rotational crops** Swiss chard mature leave (0.4-1.6% TRR) Wheat forage (1.6-3.4% TRR) Wheat hay (2.7-6.5% TRR) Wheat straw (0.8-4.7% TRR) Turnip roots (2.8% TRR)

Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
			Turnip tops (3.2-14.6% TRR) **Soil** not found **Water** not found
M43 Spiroxamine-desethyl acid glycoside KWG 4168-desethyl acid glycoside	IUPAC name: glycoside of 2-methyl-2-{2-[(propylamino)-methyl]-1,4-di-oxaspiro[4.5]dec-8-yl}propanoic acid SMILES notation: OC1C(O)C(O)C(CO)OC1OC(=O)C(C)(C)C1CCC2(CC1)OCC(CNCCC)O2 InChiKe: 1S/C22H39NO9/c1-4-9-23-10-14-12-29-22(32-14)7-5-13(6-8-22)21(2,3)20(28)31-19-18(27)17(26)16(25)15(11-24)30-19/h13-19,23-27H,4-12H2,1-3H3	 GROUP A	**Livestock** not found **Plants** not found **Rotational crops** Swiss chard immature leave (1.8% TRR) Swiss chard mature leave (0.6% TRR) Wheat forage (0.6-2.6% TRR) Wheat hay (1.3-3.3% TRR) Wheat straw (0.5-3.4% TRR) Turnip roots (2.6% TRR) Turnip tops (5.7% TRR) **Soil** not found **Water** not found
M44 Spiroxamine-acid glycoside KWG 4168-acid glycoside	IUPAC name: glycoside of 2-(2-{[ethyl(propyl)amino]methyl}-1,4-dioxaspiro[4.5]dec-8-yl)-2-methylpropanoic acid SMILES notation: OC1C(O)C(O)C(CO)OC1OC(=O)C(C)(C)C1CCC2(CC1)OCC(CN(CC)CCC)O2	 GROUP A	**Livestock** not found **Plants** not found **Rotational crops**

Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
	InChiKe: 1S/C24H43NO9/c1-5-11-25(6-2)12-16-14-31-24(34-16)9-7-15(8-10-24)23(3,4)22(30)33-21-20(29)19(28)18(27)17(13-26)32-21/h15-21,26-29H,5-14H2,1-4H3		Swiss chard immature leave (2.2-4.6% TRR) Swiss chard mature leaves (2.4-3.1% TRR) Wheat forage (0.9-2.3% TRR) Wheat hay (1.6-6.4% TRR) Wheat straw (1.0-10.5% TRR) Turnip roots (1.9% TRR) Turnip tops (6.0-11.6% TRR) **Soil** not found **Water** not found
M45 Spiroxamine-despropyl acid glycoside KWG 4168-despropyl acid glycoside	IUPAC name: glycoside of 2-{2-[(ethylamino)methyl]-1,4-dioxaspiro[4.5]dec-8-yl}-2-ethylpropanoic acid SMILES notation: OC1C(O)C(O)C(CO)OC1OC(=O)C(C)(C)C1CCC2(CC1)OCC(CNCC)O2 InChiKe: InChI=1S/C21H37NO9/c1-4-22-9-13-11-28-21(31-13)7-5-12(6-8-21)20(2,3)19(27)30-18-17(26)16(25)15(24)14(10-23)29-18/h12-18,22-26H,4-11H2,1-3H3	 GROUP A	**Livestock** not found **Plants** not found **Rotational crops** Swiss chard immature leaves (2.6-6.0% TRR) Swiss chard mature leave (1.8-5.5% TRR) Wheat forage (1.3-2.5% TRR) Wheat hay (1.5-3.7% TRR) Wheat straw (0.6-2.8% TRR) Turnip roots (3.7-9.1% TRR) Turnip tops (1.7-3.6% TRR) **Soil** not found **Water**

Code Number (Synonyms)	Description	Structural formula	Compound found in (level):
			not found

* no need to include all metabolites found in rat in case not found in the other matrices.
** levels should be expressed as % of applied radioactivity (AR) or total radioactive residue (TRR) for environmental compartments and plant/animal residues, respectively

Appendix 2: Estimation of soil sorption Koc value based on Log Pow

The soil sorption Koc values of the separate A and B isomers was estimated from the corresponding Log Pow values using KocWin, v2.00 (part of EPI suite).

i) Estimation if Koc for A isomer based on Log Pow value of 2.79 (pH 7)

```
SMILES : C1CC(C(C)(C)C)CCC12(OCC(CN(CC)CCC)O2)
CHEM   : KWG 4168
MOL FOR: C18 H35 N1 O2
MOL WT : 297.49
---------------------------- KOCWIN v2.00 Results ----------------------------

 Koc Estimate from MCI:
 ----------------------
      First Order Molecular Connectivity Index ..... 9.883
      Non-Corrected Log Koc (0.5213 MCI + 0.60) ..... 5.7519
      Fragment Correction(s):
           3   Nitrogen to Carbon (aliphatic) (-N-C)..  : -0.6382
           2   Ether, aliphatic  (-C-O-C-) ..... : -1.7432
      Corrected Log Koc ..... :  3.3705

                Estimated Koc:  2347  L/kg  <==========

 Koc Estimate from Log Kow:
 --------------------------
      Log Kow  (User entered  ) ..... :  2.79
      Non-Corrected Log Koc (0.55313 logKow + 0.9251)  .. 2.4683
      Fragment Correction(s):
           3   Nitrogen to Carbon (aliphatic) (-N-C)..  : -0.9654
           2   Ether, aliphatic  (-C-O-C-) ..... : -0.1812
      Corrected Log Koc ..... :  2.2218

                Estimated Koc:  166.6  L/kg  <==========
```

ii) Estimation if Koc for B isomer based on Log Pow value of 2.98 (pH 7)

```
SMILES : C1CC(C(C)(C)C)CCC12(OCC(CN(CC)CCC)O2)
CHEM   : KWG 4168
MOL FOR: C18 H35 N1 O2
MOL WT : 297.49
---------------------------- KOCWIN v2.00 Results ----------------------------

 Koc Estimate from MCI:
 ----------------------
      First Order Molecular Connectivity Index ..... :  9.883
      Non-Corrected Log Koc (0.5213 MCI + 0.60) ..... :  5.7519
      Fragment Correction(s):
           3   Nitrogen to Carbon (aliphatic) (-N-C)..  : -0.6382
           2   Ether, aliphatic  (-C-O-C-) ..... : -1.7432
      Corrected Log Koc ..... :  3.3705

                Estimated Koc:  2347  L/kg  <==========

 Koc Estimate from Log Kow:
 --------------------------
```

Log Kow (User entered) ..... : 2.98
Non-Corrected Log Koc (0.55313 logKow + 0.9251) .... : 2.5734
Fragment Correction(s):
3 Nitrogen to Carbon (aliphatic) (-N-C).. : -0.0654
2 Ether, aliphatic (-C-O-C-) ..... : -0.1812
Corrected Log Koc ..... : 2.3269

Estimated Koc: 212.3 L/kg <==========

Summary table of estimated Koc values for spiroxamine isomers

id.	CAS no.	SMILES code	Log Pow	Estimated Koc (L/kg)
A	118134-30-8	C1CC(C(C)(C)C)CCC12(OCC(CN2CC)CCC)O2)	2.79	167
B			2.98	212

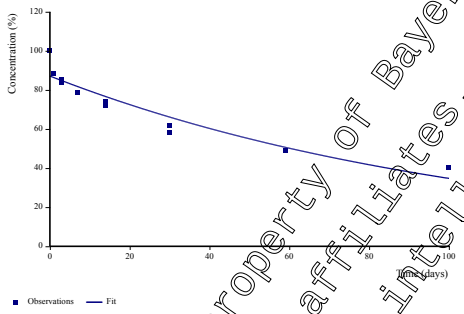
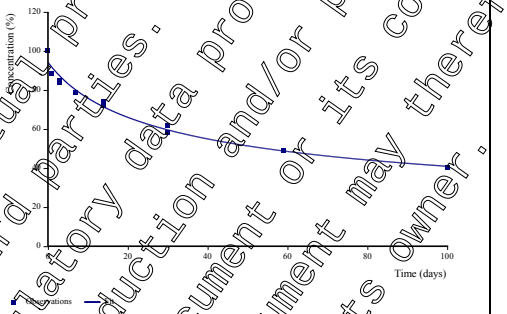
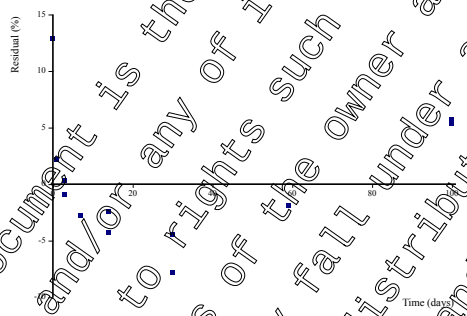
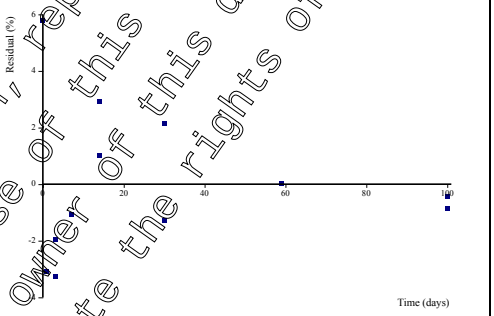
Appendix 3: Kinetic evaluation of laboratory soil studies

Appendix 3.1: Kinetic evaluation for persistence/trigger endpoints

Appendix 3.1.1. Degradation of 14C-cyclohexyl- labelled spiroxamine, M01, M02 and M03 in BBA 2.2/Speyer 2.2 soil (KCA 7.1.1.1/01 (M-006135-01-1))

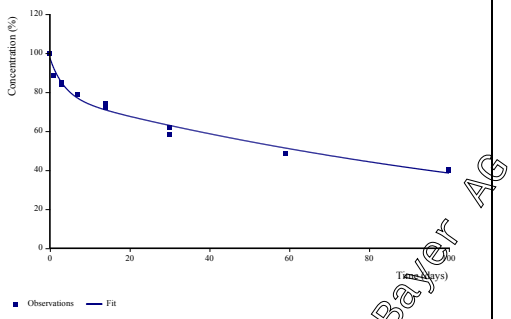
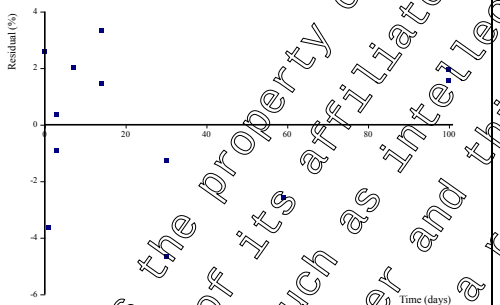
Appendix 3.1.1.1. Spiroxamine kinetics

Step 1: Run SFO, FOMC. SFO more appropriate than FOMC and gives acceptable fit?

	SFO	FOMC
Plot		
Residuals		
Visual fit	Intermediate, residuals show systematic error and final data point is underestimated	Excellent, residuals show no systematic error
χ^2 error (%)	6.4	3.1
t-test	$p < 0.05$	NA
DT_{50} (days)	75.4	65.8
DT_{90} (days)	251[1]	4870[1]
Assessment	Fit not acceptable χ2 error is low and rate parameter differs significantly from zero, however, visual fit is intermediate (residuals show systematic error and final data point is underestimated)	Fit potentially acceptable Visual fit is excellent and χ^2 error is low. Significant extrapolation from study period to DT90[2].
Discussion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not considered acceptable. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No Go to step 2 below	

[1] Interpret with care – extrapolated beyond experimental period
[2] EFSA (2009)

Step 2: Run DFOP. Determine which of the models (FOMC, DFOP) is best.

	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 error (%)	3.2
t-test	k_1: p < 0.1 k_2: p < 0.05
DT_{50} (days)	66.6
DT_{90} (days)	296[1]
Assessment	Fit acceptable Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero
Discussion	iv) Determine which of the models (FOMC, DFOP) is best. Both FOMC and DFOP give acceptable fits according to FOCUS (2014). The use of FOMC for extrapolation beyond the experimental period is not recommended (EFSA 2009). On the basis of study data encompassing 100 days, the FOMC fit drastically extrapolates a DT_{90} of 4 870 days. Visually, the FOMC and DFOP fits are extremely similar, with a difference of only 0.1% in χ^2 error. Therefore, it is considered that the DFOP fit reflects the study data most accurately. v) Does the best-fit model give an acceptable description of the data? Yes DFOP should be used for persistence endpoints

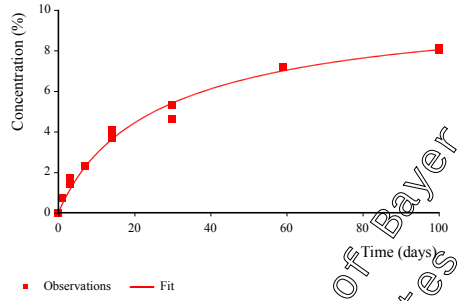
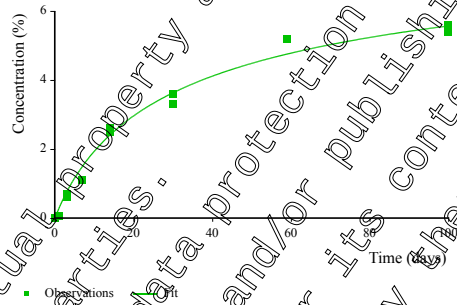
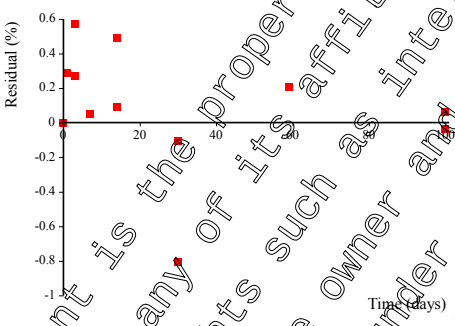
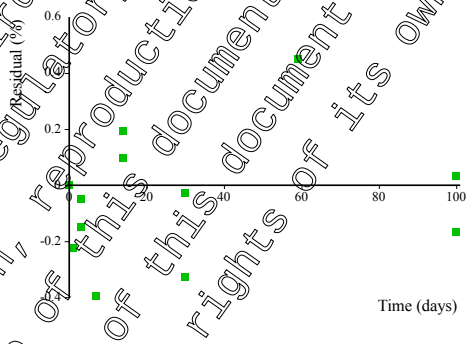
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For parent spiroxamine use DFOP. DT_{50} = 66.6 days, DT_{90} = 296 days.

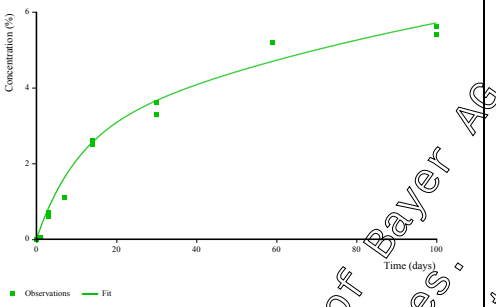
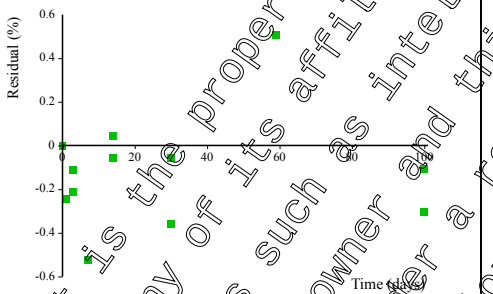
Appendix 3.1.1.2. Metabolite kinetics (M01 and M02)

Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error	Excellent, residuals show no systematic error
χ^2 error (%)	5.6	9.5
t-test	*k*: p > 0.1	*k*: p > 0.1
DT_{50} (days)	555[1]	>10,000[1]
DT_{90} (days)	1,840[1]	>10,000[1]
Assessment	Fit acceptable Visual fit is excellent and χ^2 error is low, however, rate parameter does not differ significantly from zero	Fit not acceptable Visual fit is excellent and χ^2 error is low, however, rate parameter does not differ significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable due to excellent description of the data. Rate parameter not differing significantly from zero is considered to be the result of the lack of decline phase in the data.	iii) SFO fit for metabolite acceptable? SFO is not considered acceptable. Therefore, DFOP or FOMC should be assessed. However, these models will not improve the fit. Thereafter, decline after max should be assessed. However, this was not possible as there is limited decline data to clearly establish the dissipation pattern. Based on the study data encompassing 100 days, predicted endpoints of 10,000 days is a drastic extrapolation and is considered unreliable given the data. Therefore, fit using a default DT_{50} of 1,000 days performed to demonstrate a reasonable worst case.

[1] Interpret with care – extrapolated beyond experimental period

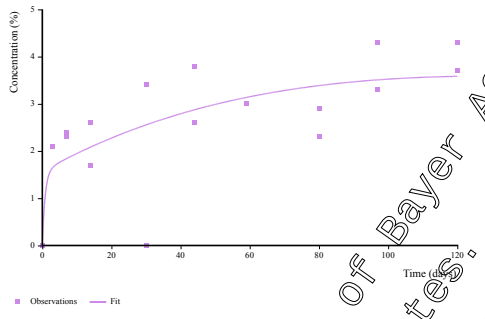
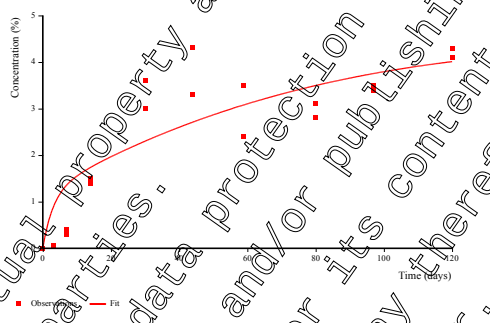
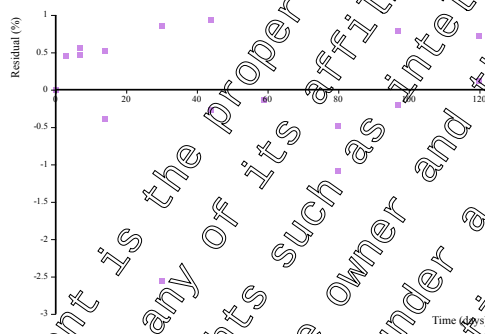
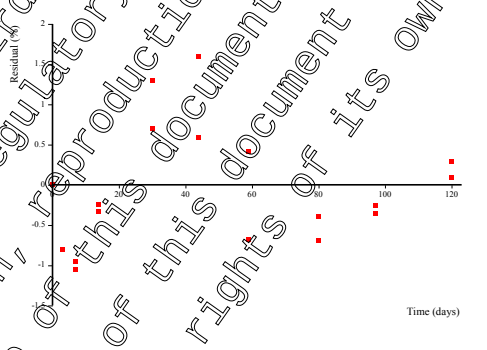
Step 2: Run parent best-fit and metabolite SFO, DT_{50} fixed to default value. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO M02
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error
χ^2 error (%)	8.46
t-test	N/A
DT_{50} (days)	1,000[1]
DT_{90} (days)	3,320[1]
Assessment	Fit acceptable Visual fit is excellent and χ^2 error is low. Fixing DT50 to 1,000 days provides an excellent fit to study data and is a reasonable worst case.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable.

[1] Fixed to FOCUS conservative default

Appendix 3.1.1.3. Metabolite kinetics (M03 and M06)

Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M03	M06
Plot		
Residuals		
Visual fit	Good, fit is conservative, residuals show no systematic error	Intermediate, residuals show no systematic error
χ^2 error (%)	14.3	23.5
t-test	k_2 p < 0.05	k_2 p > 0.05 (p value equals 0.1)
DT_{50} (days)	79	191[1]
DT_{90} (days)	262[1]	635[1]
Assessment	Fit acceptable Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero	Fit acceptable Visual fit is intermediate and χ^2 error is slightly high, however rate parameter does not differ significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints	iii) SFO fit for metabolite acceptable? SFO is considered acceptable. General fit to data is intermediate without systematic error. Although rate parameter does not differ from zero, this is due to a lack of decline data and p = 0.1. The fit is acceptable.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

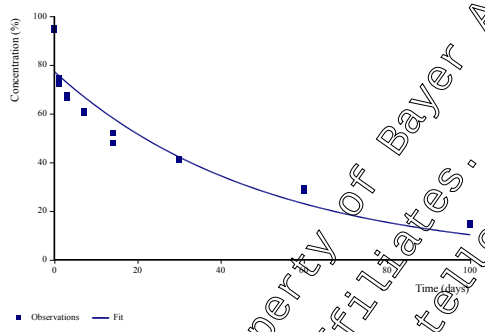
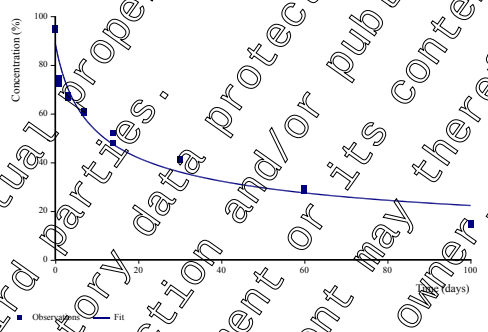
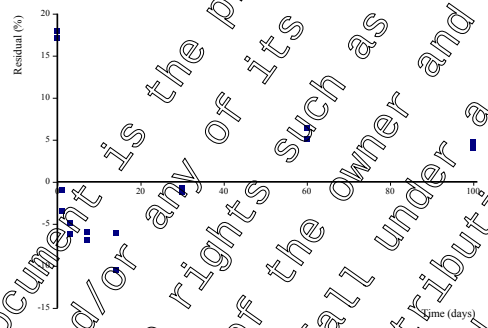
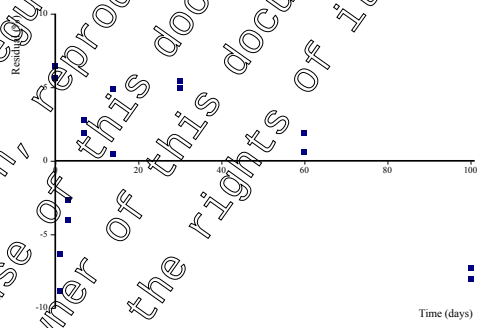
For M01 use DFOP/SFO. DT_{50} = 70.7 days, DT_{90} = 235 days.
For M02 use DFOP/SFO. DT_{50} = 65.2 daysDT_{90} = 217 days.
For M03 use DFOP/SFO. DT_{50} = 79 days, DT_{90} = 262 days.

For M06 use DFOP/SFO DT_{50} = 191 days, DT90 = 635 days

Appendix 3.1.2. Degradation of ^{14}C-cyclohexyl labelled spiroxamine, M01, M02 and M03 in Laacherhof soil (KCA 7.1.1.1/02 (M-006141-01-1))

Appendix 3.1.2.1. Spiroxamine kinetics

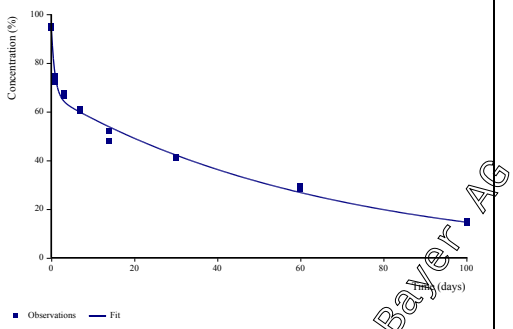
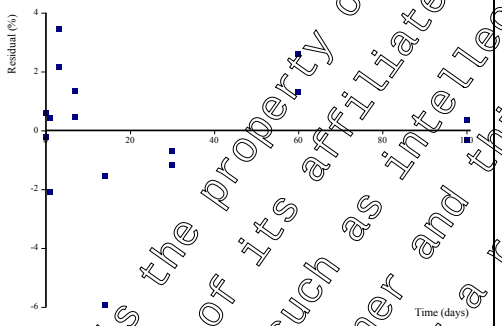
Step 1: Run SFO, FOMC. SFO more appropriate than FOMC and gives acceptable fit?

	SFO	FOMC
Plot		
Residuals		
Visual fit	Intermediate, residuals show systematic error and fit not conservative	Good, residuals show no systematic error and fit is conservative
χ^2 error (%)	11.9	8.6
t-test	*k*: $p < 0.05$	NA
DT_{50} (days)	34.8	17.2
DT_{90} (days)	116	908[1]
Assessment	Fit not acceptable χ^2 error is acceptable and rate parameter differs significantly from zero, however, visual fit is intermediate (residuals show systematic error and fit not conservative)	Fit acceptable Visual fit is good and χ^2 error is low. Significant extrapolation from study period to DT90[2].
Discussion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Deviation from SFO is not due to outliers or experimental artefacts iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No Go to step 2 below	

[1] Interpret with care – extrapolated beyond experimental period

[2] EFSA (2009)

Step 2: Run DFOP. Determine which of the models (FOMC, DFOP) is best.

	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 error (%)	3.3
t-test	*k*: p < 0.05 *k*: p < 0.05
DT_{50} (days)	22.5
DT_{90} (days)	128[1]
Assessment	Fit acceptable Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero
Discussion	iv) Determine which of the models (FOMC, DFOP) is best. Both FOMC and DFOP give acceptable fits. DFOP selected (lower χ^2 error). v) Does the best-fit model give an acceptable description of the data? Yes DFOP should be used for persistence end-points

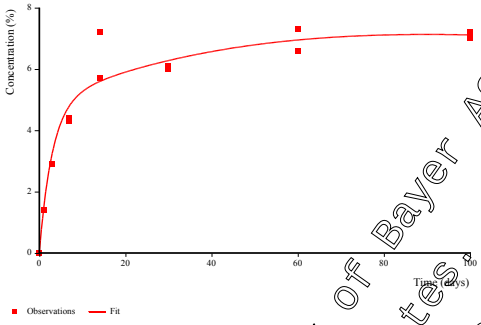
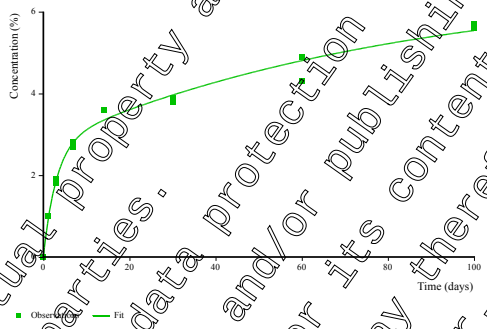
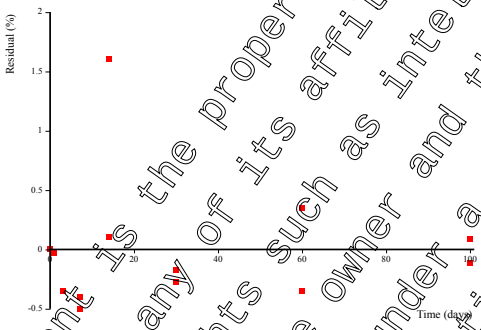
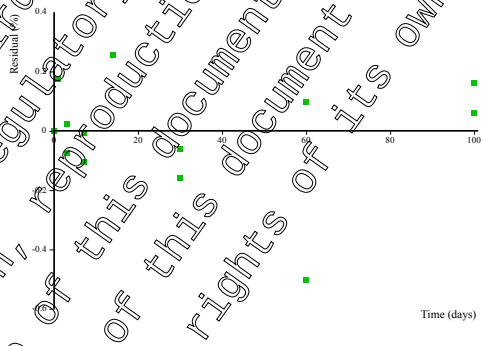
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use DFOP. DT_{50} = 22.5 days, DT_{90} = 128 days.

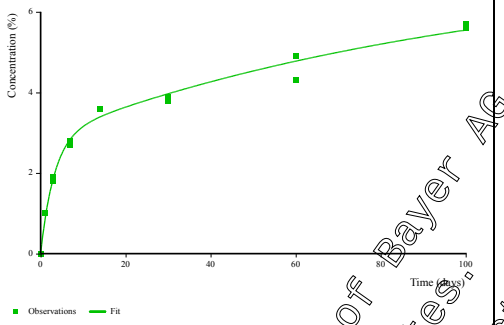
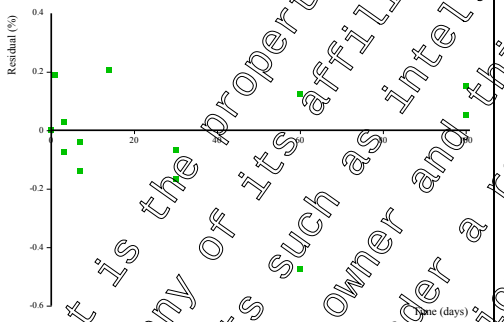
Appendix 3.1.2.2. Metabolite kinetics (M01 and M02)

Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error	Excellent, residuals show no systematic error
χ^2 error (%)	6.2	3.5
t-test	*k*: p < 0.05	*k*: p > 0.1
DT_{50} (days)	167[1]	4,860[1]
DT_{90} (days)	554[1]	>10,000[1]
Assessment	Fit acceptable Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero	Fit not acceptable Visual fit is excellent and χ^2 error is low, however, rate parameter does not differ significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints	iii) SFO fit for metabolite acceptable? SFO is not considered acceptable. Therefore, DFOP or FOMC should be assessed. However, these models will not improve the fit. Thereafter, decline after max. should be assessed. However, this was not possible as there is limited decline data to clearly establish the dissipation pattern. Based on the study data encompassing 100 days, predicted endpoints of 10,000 days is a drastic extrapolation and is considered unreliable given the data. Therefore, fit using a default DT_{50} of 1,000 days performed to demonstrate a reasonable worst case.

[1] Interpret with care – extrapolated beyond experimental period

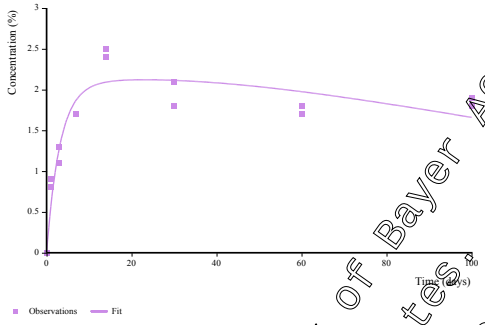
Step 2: Run parent best-fit and metabolite SFO, DT_{50} fixed to default value. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO M02
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error
χ^2 error (%)	3.19
t-test	NA
DT_{50} (days)	1,000[1]
DT_{90} (days)	3,320[1]
Assessment	Fit acceptable Visual fit is excellent and χ^2 error is low. Fixing DT50 to 1,000 days provides an excellent fit to study data and is a reasonable worst case.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable.

[1] Fixed to FOCUS conservative default

Appendix 3.1.2.3. Metabolite kinetics (M03)

Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO
	M03
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error
χ^2 error (%)	10.7
t-test	*k*: p < 0.05
DT_{50} (days)	58.1
DT_{90} (days)	193[1]
Assessment	Fit acceptable Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero
Discussion	If SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints

[1] Interpret with care – extrapolated beyond experimental period

Summary

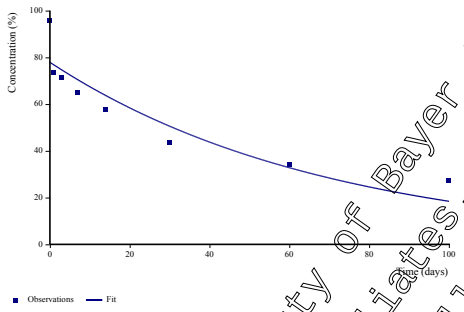
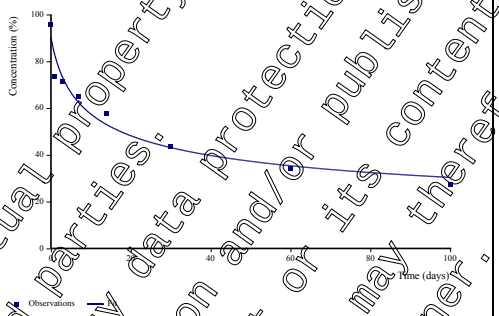
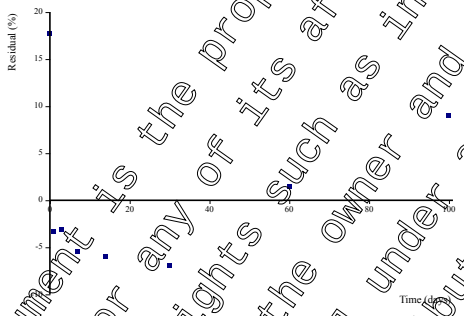
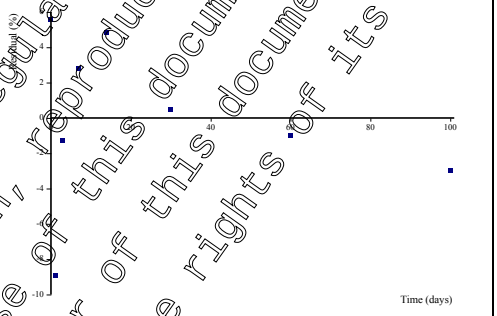
M01 use DFOP/SFO, DT_{50} = 167 days, DT_{90} = 554 days.
M02 use conservative default DT_{50} = 1,000 days, DT90 = 3,320 days.

M03 use DFOP/SFO, DT_{50} = 58.1 days, DT_{90} = 193 days.

Appendix 3.1.3. Degradation of ^{14}C-cyclohexyl labelled spiroxamine, M01, M02 and M03 in Monheim 3 soil (KCA 7.1.1.1/02 (M-006141-01-1))

Appendix 3.1.3.1. Spiroxamine kinetics

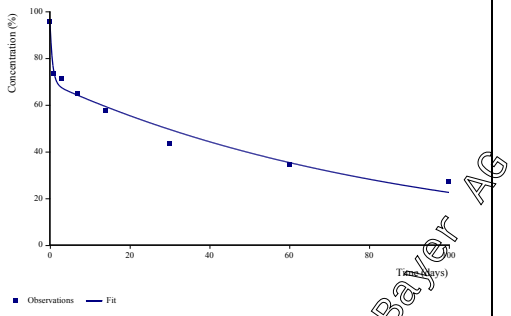
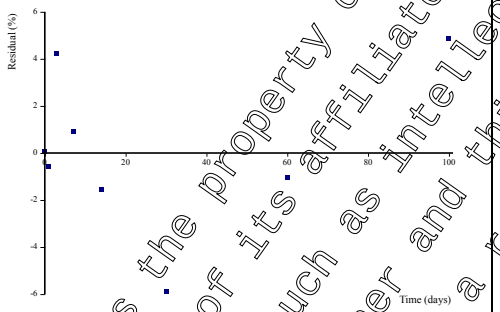
Step 1: Run SFO, FOMC. SFO more appropriate than FOMC and gives acceptable fit?

	SFO	FOMC
Plot		
Residuals		
Visual fit	Intermediate, residuals show systematic error and fit not conservative	Excellent, residuals show no systematic error
χ^2 error (%)	11.1	6.4
t-test	ns. $p < 0.05$	NA
DT_{50} (days)	48.1	25.7
DT_{90} (days)	160[1]	5,340[1]
Assessment	Fit not acceptable χ^2 error is acceptable and rate parameter differs significantly from zero, however visual fit is intermediate (residuals show systematic error and fit not conservative)	Fit acceptable Visual fit is excellent and χ^2 error is low. Significant extrapolation from study period to DT90[2].
Discussion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not considered acceptable (intermediate visual fit). ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No Go to step 2 below	

[1] Interpret with care – extrapolated beyond experimental period

2 EFSA (2009)

Step 2: Run DFOP. Determine which of the models (FOMC, DFOP) is best.

	DFOP
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error
χ^2 error (%)	5.0
t-test	k_1: p 0.05-0.1 k_2: p < 0.05
DT_{50} (days)	33.2
DT_{90} (days)	176[1]
Assessment	Visual fit is good and χ^2 error is low, however, k_1 rate parameter does not differ significantly from zero at a 5% level but this only describes a small percentage of the decline
Discussion	iv) Determine which of the models (FOMC, DFOP) is best. DFOP gives the best acceptable fit on χ^2. v) Does the best-fit model give an acceptable description of the data? Yes DFOP should be used for persistence endpoints

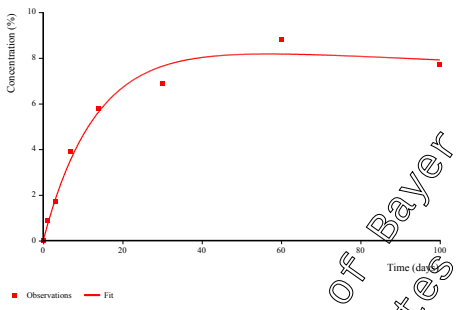
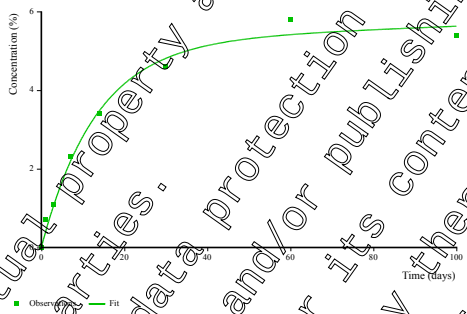
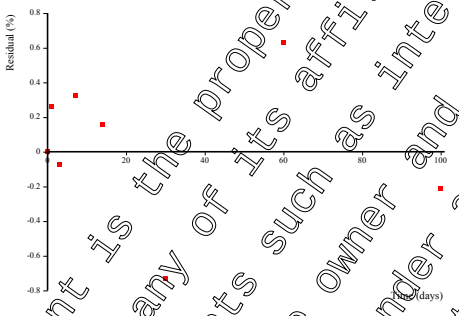
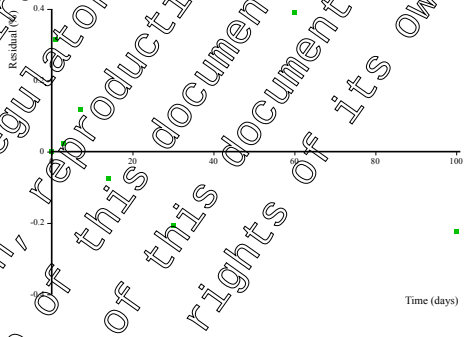
[1]Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use DFOP. DT_{50} = 33.2 days, DT_{90} = 176 days.

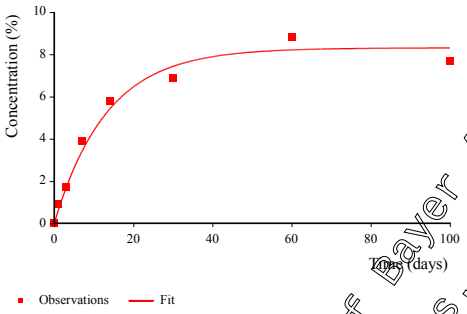
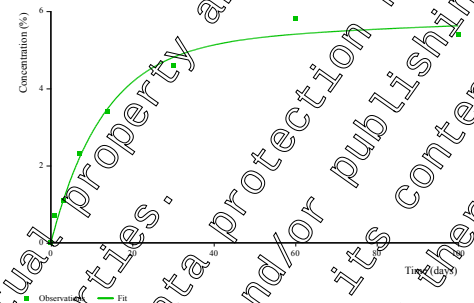
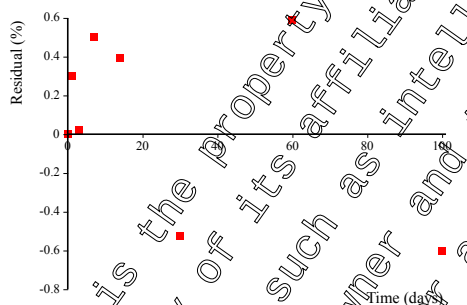
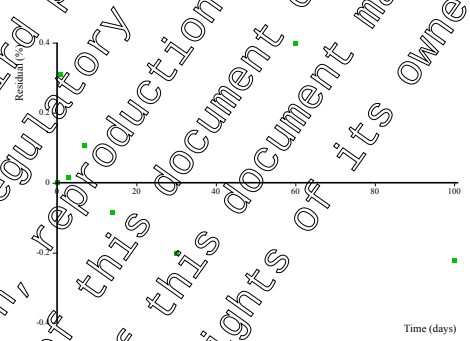
Appendix 3.1.3.2. Metabolite kinetics (M01 and M02)

Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Intermediate, fit slightly under estimates the peak formation of the metabolite.	Excellent, residuals show no systematic error
χ^2 error (%)	6.41	5.45
t-test	k: p > 0.1	k: p= 0.5
DT_{50} (days)	366[1]	>10,000[1]
DT_{90} (days)	1,220[1]	>10,000[1]
Assessment	Visual fit is intermediate with a high χ^2 value. Rate parameter is not statistically different to zero. However, this is considered to be driven by the lack of a decline phase as opposed to data quality.	Visual fit is intermediate with a high χ^2 value. Rate parameter is not statistically different to zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable. In order to evaluate potential application of the FOCUS default, a fit with M01 DT50 set to 1,000 days performed.	iii) SFO fit for metabolite acceptable? No. Based on the study data encompassing 100 days, predicted endpoints of 10,000 days is a drastic extrapolation and is considered unreliable given the data. Therefore, fit using a default DT_{50} of 1,000 days performed to demonstrate a reasonable worst case. Fit with a DT_{50} of 1,000 days (default).

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Run parent best-fit and metabolite SFO, DT_{50} M02 fixed to default value. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	Parent DFOP, metabolites SFO
	M01	M02
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error	Excellent, residuals show no systematic error
χ^2 error (%)	6.74	5.12
t-test	N/A	N/A
DT_{50} (days)	1,000[1]	1,000[1]
DT_{90} (days)	3,320[1]	3,320[1]
Assessment	Fit not acceptable. Use of the 1,000 days results in increased Chi² versus previous modelling.	Fit acceptable Visual fit is excellent and χ^2 error is low.
Discussion	iii) SFO fit for metabolite acceptable? SFO with default DT50 is not considered acceptable as it increases Chi² versus previous estimate.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable.

[1] Fixed to FOCUS conservative default

Appendix 3.1.3.3. Metabolite kinetics (M03)

Metabolite M03 was not observed in this soil.

Summary:

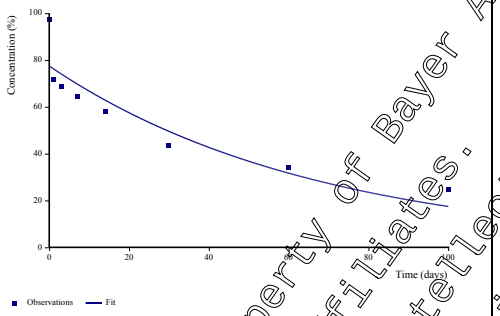
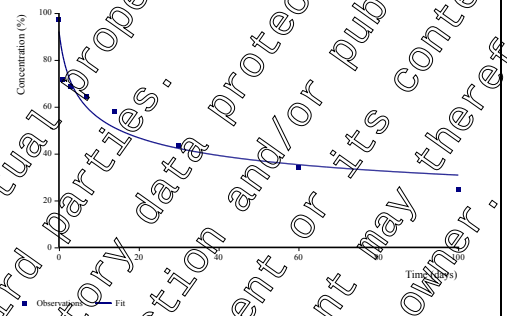
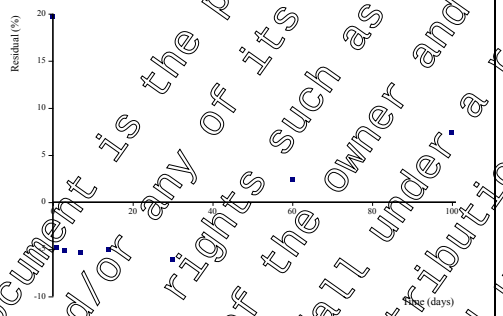
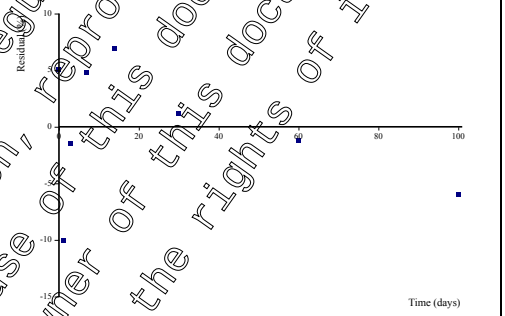
For M01 use DFOP/SFO DT_{50} = 366 days, DT_{90} = 1,220 days.
For M02 use conservative default DT_{50} = 1,000 days.

M03 Not observed.

Appendix 3.1.4. Degradation of ^{14}C-cyclohexyl labelled spiroxamine, M01, M02 and M03 in Howe soil (KCA 7.1.1.1/02 (M-006141-01-1))

Appendix 3.1.4.1. Spiroxamine kinetics

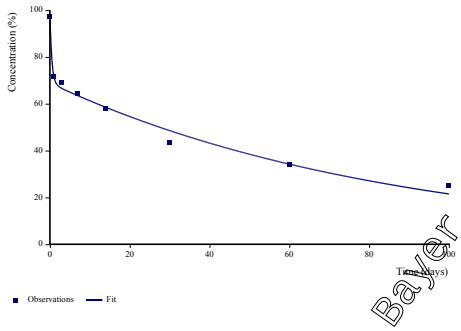
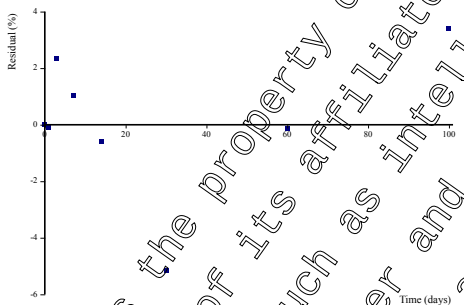
Step 1: Run SFO, FOMC. SFO more appropriate than FOMC and gives acceptable fit?

	SFO	FOMC
Plot		
Residuals		
Visual fit	Intermediate, residuals show systematic error and fit not conservative	Good, residuals show no systematic error and fit is conservative
χ^2 error (%)	11.9	8.0
t-test	*k*: $p < 0.05$	NA
DT_{50} (days)	46.5	21.2
DT_{90} (days)	155	9,110[1]
Assessment	Fit not acceptable χ^2 error is acceptable and rate parameter differs significantly from zero, however, visual fit is intermediate (residuals show systematic error and fit not conservative)	Fit potentially acceptable Visual fit is good and χ^2 error is low. Significant extrapolation from study period to DT90[2].
Discussion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Deviation from SFO is not due to outliers or experimental artefacts iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No Go to step 2 below	

[1] Interpret with care – extrapolated beyond experimental period

[2] EFSA (2009)

Step 2: Run DFOP. Determine which of the models (FOMC, DFOP) is best.

	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 error (%)	3.8
t-test	k_1 p < 0.1 k_2 p < 0.05
DT_{50} (days)	29.9
DT_{90} (days)	168[1]
Assessment	Fit acceptable Visual fit is excellent, χ^2 error is low and rate parameters differ significantly from zero
Discussion	iv) Determine which of the models (FOMC, DFOP) is best. Both FOMC and DFOP give acceptable fits. DFOP selected (lower χ^2 error). v) Does the best-fit model give an acceptable description of the data? Yes DFOP should be used for persistence endpoints

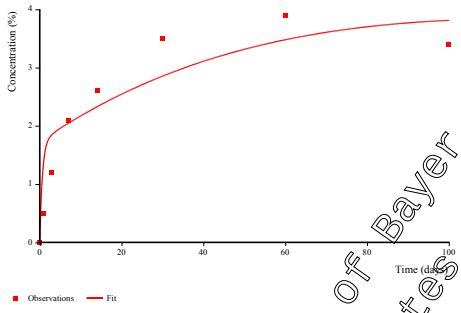
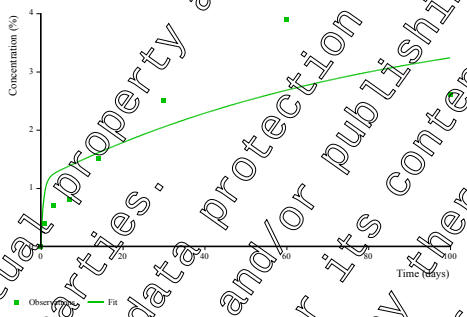
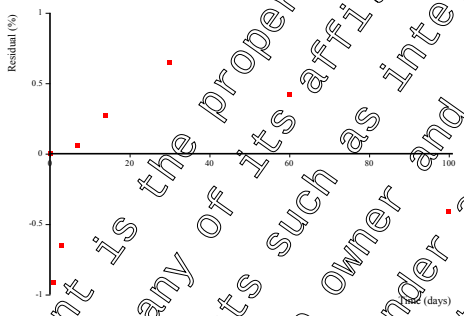
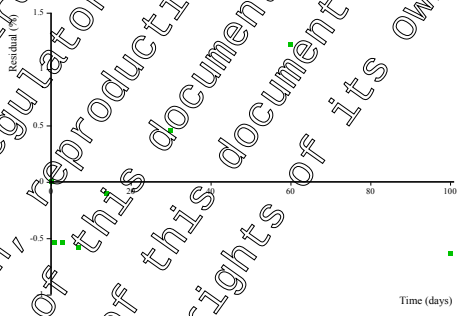
[1]Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use DFOP. DT_{50} = 29.9 days, DT_{90} = 168 days.

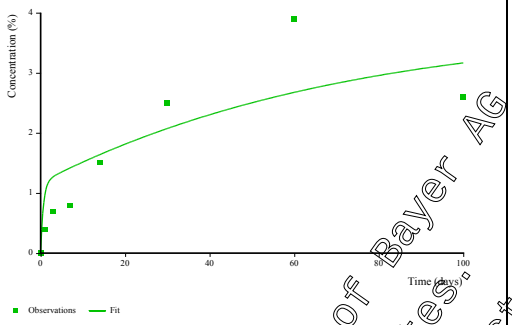
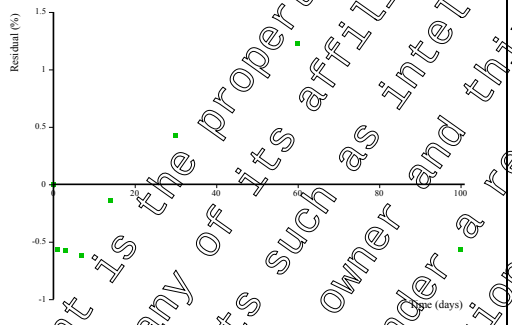
Appendix 3.1.4.2. Metabolite kinetics (M01 and M02)

Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show systematic error	Intermediate, residuals show systematic error
χ^2 error (%)	17.7	29.5
t-test	*k*: p > 0.1	*k*: p > 0.1
DT_{50} (days)	202[1]	>10,000[1]
DT_{90} (days)	672[1]	>10,000[1]
Assessment	Fit potentially acceptable Visual fit is intermediate, χ^2 error is slightly high but rate parameter does not differ significantly from zero.	Fit potentially acceptable Visual fit is intermediate, χ^2 error is high and rate parameter does not differ significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable. χ^2 error is slightly high but the formation of M01 is well described. Rate parameter is not significantly different to zero but this is a consequence of limited decline data to clearly establish the dissipation pattern.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable. χ^2 error is high but the formation of M02 is well described. Rate parameter is not significantly different to zero but this is a consequence of limited decline data to clearly establish the dissipation pattern. Based on the study data encompassing 100 days, predicted endpoints of 10,000 days is a drastic extrapolation and is considered unreliable given the data. Therefore, fit using a default DT_{50} of 1,000 days performed to demonstrate a reasonable worst case.

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Run parent best-fit and metabolite SFO, DT_{50} fixed to default value. SFO fit for metabolite acceptable?

	Parent DFOP, metabolite SFO M02
Plot	
Residuals	
Visual fit	Intermediate, residuals show systematic error
χ^2 error (%)	27.8
t-test	N/A
DT_{50} (days)	1,000[1]
DT_{90} (days)	3,320[1]
Assessment	Fit acceptable Visual fit is intermediate and χ^2 error is low. Use of 1,000 day default provides a good description of the study data and is a reasonable worst case.
Discussion	iii) SFO fit for metabolite acceptable? SFO using default DT_{50} is considered acceptable residuals are small.

[1] Fixed to FOCUS conservative default

Appendix 3.1.4.3. Metabolite kinetics (M03)

Metabolite M03 was not observed in this soil.

Summary:

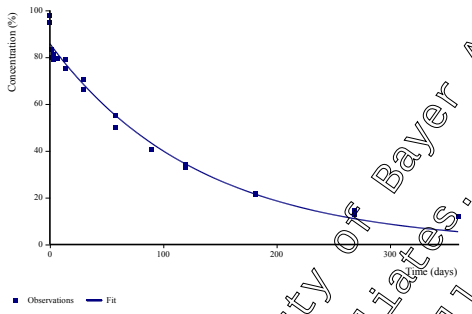
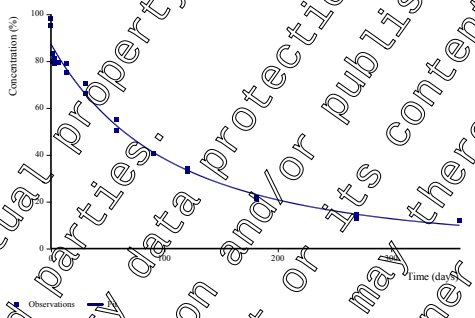
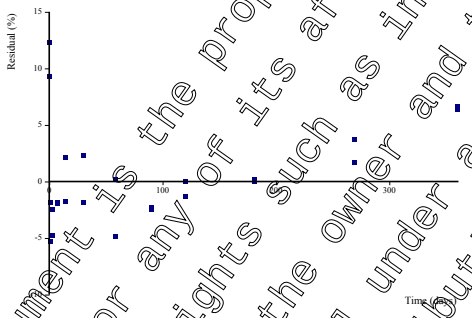
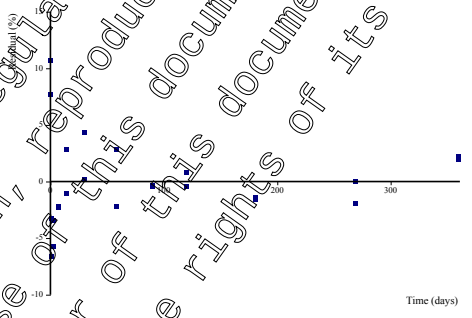
For M01 use conservative default DT_{50} = 202 days.
For M02 DT_{50} / DT_{90} use conservative default DT_{50} = 1,000 days.

M03 Not observed.

Appendix 3.1.5. Degradation of ^{14}C-cyclohexyl labelled spiroxamine, M01, M02 and M03 in Wolf Ranch soil (KCA 7.1.1.1/04 (M-006148-01-1))

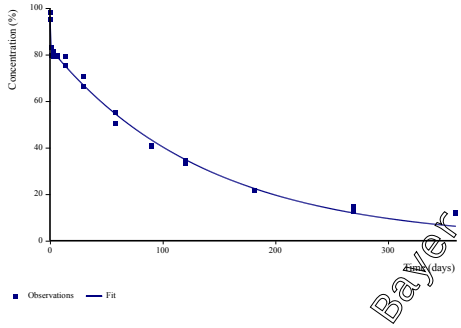
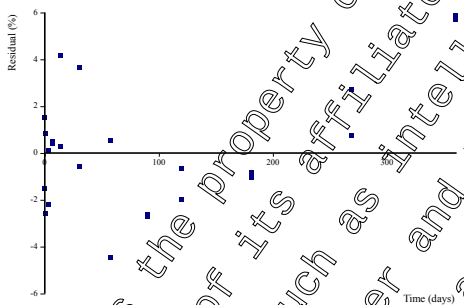
Appendix 3.1.5.1. Spiroxamine kinetics

Step 1: Run SFO, FOMC. SFO more appropriate than FOMC and gives acceptable fit?

	SFO	FOMC
Plot		
Residuals		
Visual fit	Good, however residuals show systematic error and fit not conservative	Excellent, residuals show no systematic error
χ^2 error (%)	6.1	5.4
t-test	n. $p < 0.05$	NA
DT_{50} (days)	90.8	81.9
DT_{90} (days)	302[1]	393[1]
Assessment	χ^2 error is low and rate parameter differs significantly from zero, however visual fit although good the residuals show systematic error and fit not conservative	Visual fit is excellent and χ^2 error is low
Discussion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not considered acceptable (intermediate visual fit). ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No Go to step 2 below	

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Run DFOP. Determine which of the models (FOMC, DFOP) is best.

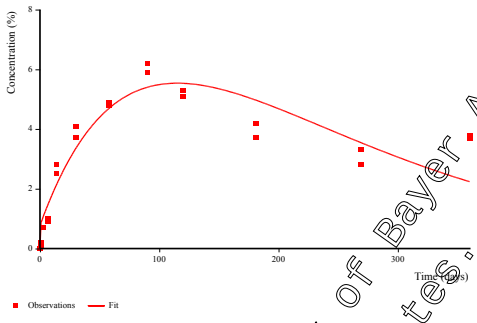
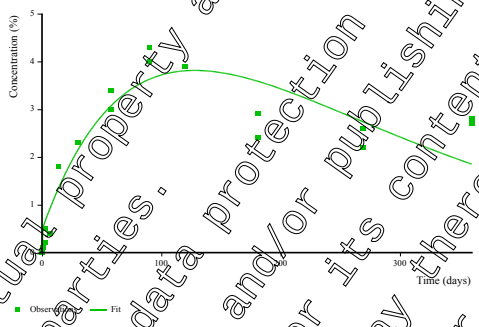
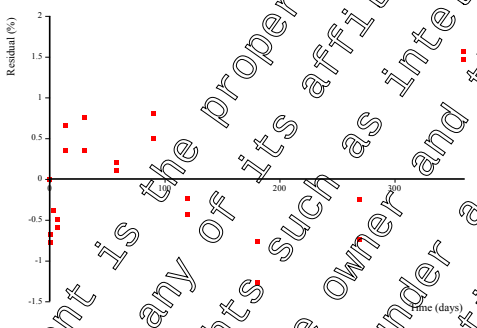
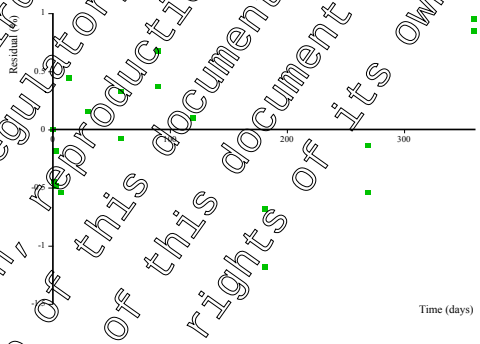
	DFOP
Plot	
Residuals	
Visual fit	Good, residuals show systematic error
χ^2 error (%)	3.6
t-test	k_1 p >0.1 k_2 < 0.05
DT_{50} (days)	75
DT_{90} (days)	299
Assessment	Good, residuals show no systematic error and fit conservative.
Discussion	iv) Determine which of the models (FOMC, DFOP) is best. DFOP selected (lower χ^2). v) Does the best-fit model give an acceptable description of the data? Yes DFOP should be used for persistence end-points

Summary:

For spiroxamine use DFOP. DT_{50} = 75 days, DT_{90} = 299 days.

Appendix 3.1.5.2. Metabolite kinetics (M01 and M02)

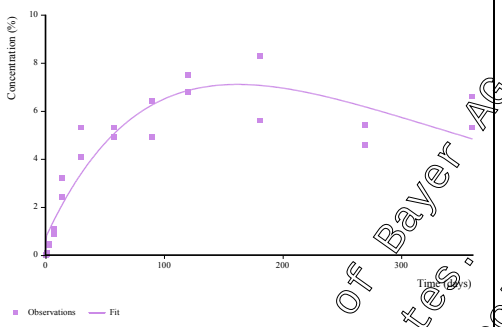
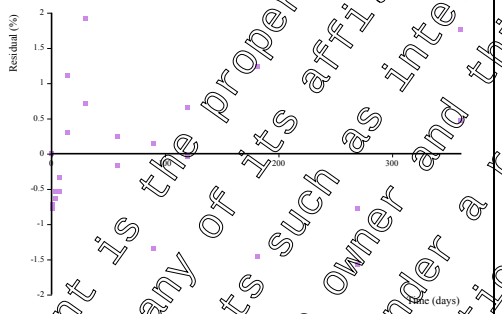
Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 error (%)	18.1	19.0
t-test	*k*: $p < 0.05$	*k*: $p < 0.05$
DT_{50} (days)	78.2	95.4
DT_{90} (days)	260[1]	317[1]
Assessment	Visual fit is good, χ^2 error is slightly high and rate parameter differs significantly from zero	Visual fit is good, χ^2 error is slightly high and rate parameter differs significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints

[1] Interpret with care – extrapolated beyond experimental period

Appendix 3.1.5.3. Metabolite kinetics (M03)

Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO
	M03
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error
χ^2 error (%)	15.1
t-test	*k*: p < 0.05
DT_{50} (days)	152
DT_{90} (days)	507[1]
Assessment	Visual fit is good, χ^2 error is slightly high and rate parameter differs significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints

[1] Interpret with care – extrapolated beyond experimental period

Summary:

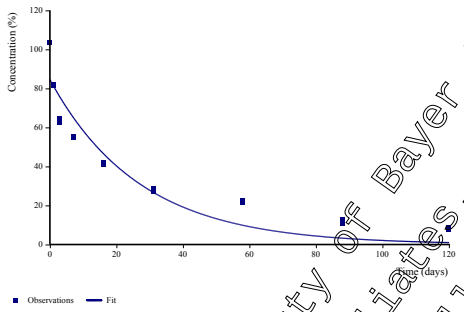
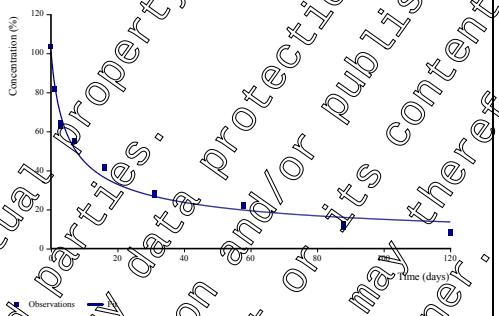
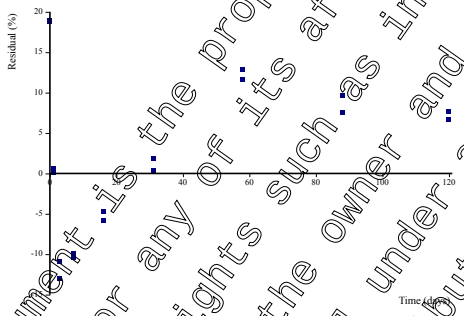
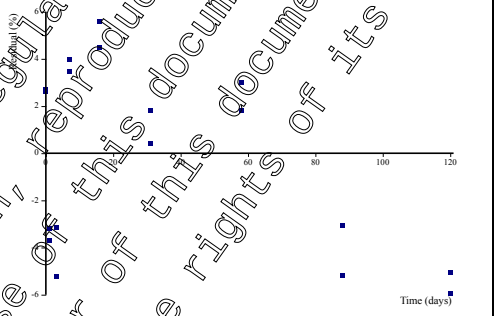
For M01 use DFOP/SFO. DT_{50} = 78.2 days, DT_{90} = 260 days.
For M02 use DFOP/SFO. DT_{50} = 95.4 days, DT_{90} = 317 days.

For M03 use DFOP/SFO. DT_{50} = 152 days, DT_{90} = 507 days.

Appendix 3.1.6. Degradation of ^{14}C-dioxolane labelled spiroxamine, M01, M02 and M03 in Hoefchen am Hohenseh soil (KCA 7.1.1.1/05 (M-303803-01-1))

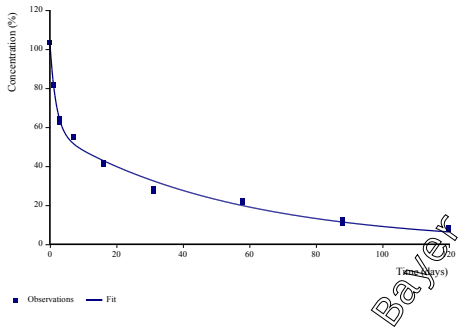
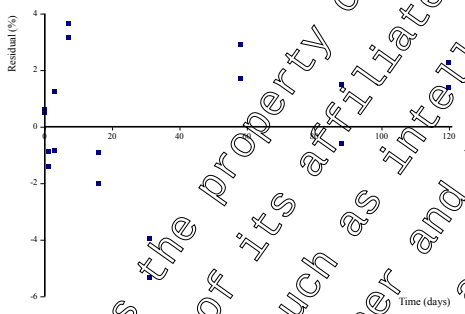
Appendix 3.1.6.1. Spiroxamine kinetics

Step 1: Run SFO, FOMC. SFO more appropriate than FOMC and gives acceptable fit?

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor, residuals show systematic error and fit not conservative	Excellent, residuals show no systematic error and fit is conservative
χ^2 error (%)	17.4	7.0
t-test	*, $p < 0.05$	NA
DT_{50} (days)	18.7	7.4
DT_{90} (days)	62.2	217[1]
Assessment	Fit not acceptable χ^2 error is reasonable and rate parameter differs significantly from zero, however visual fit is poor (residuals show systematic error and fit not conservative)	Fit acceptable Visual fit is excellent and χ^2 error is low
Discussion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC (higher χ^2 error). ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Deviation from SFO is not due to outliers or experimental artefacts iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No Go to step 2 below	

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Run DFOP. Determine which of the models (FOMC, DFOP) is best.

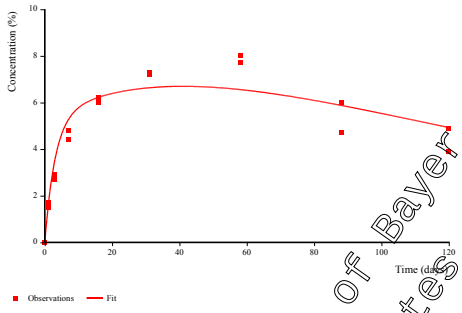
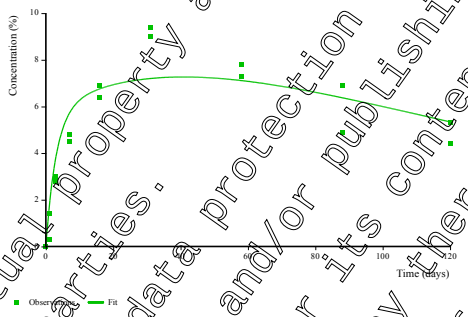
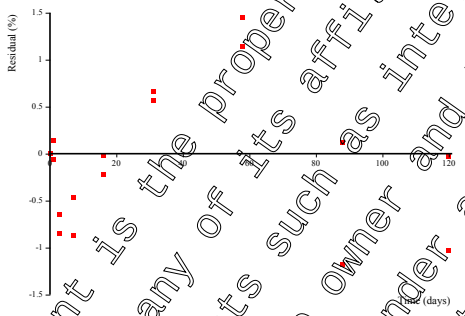
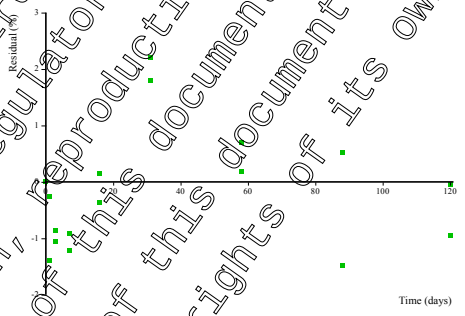
	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 error (%)	4.4
t-test	k_1: $p < 0.05$ k_2: $p < 0.05$
DT_{50} (days)	7.2
DT_{90} (days)	93.6
Assessment	Fit acceptable Visual fit is excellent, χ^2 error is low and rate parameters differ significantly from zero
Discussion	iv) Determine which of the models (FOMC, DFOP) is best. Both FOMC and DFOP give acceptable fits. DFOP selected (lower χ^2 error). v) Does the best-fit model give an acceptable description of the data? Yes DFOP should be used for persistence end-points

Summary:

For spiroxamine use DFOP, DT_{50} = 7.2 days, DT_{90} = 93.6 days.

Appendix 3.1.6.2. Metabolite kinetics (M01 and M02)

Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 error (%)	10.9	14.8
t-test	*k*: $p < 0.05$	*k*: $p < 0.05$
DT_{50} (days)	78.7	78.8
DT_{90} (days)	261[1]	262[1]
Assessment	Fit acceptable Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero	Fit acceptable Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero
Discussion	ii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints	ii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints

[1] Interpret with care – extrapolated beyond experimental period

Appendix 3.1.6.3. Metabolite kinetics (M03)

M03 was not observed in this soil

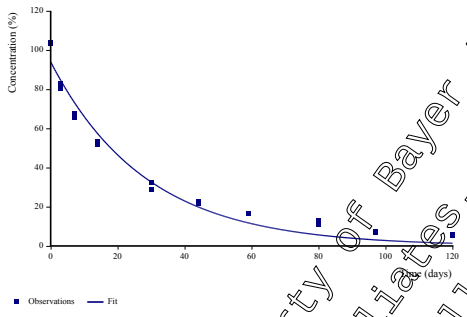
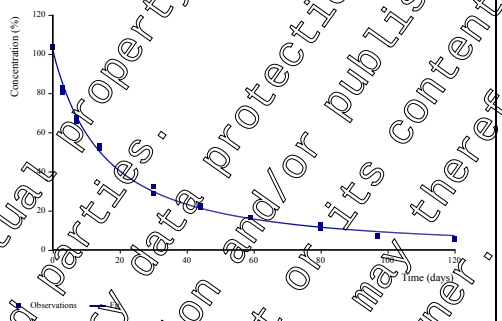
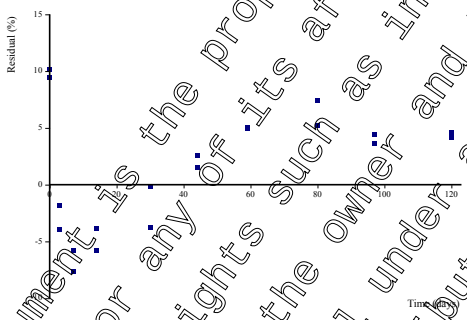
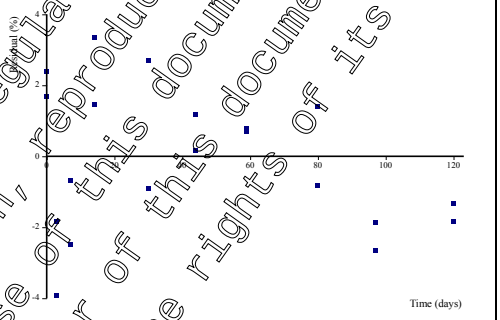
Summary:

For M01 use DFOP/SFO. DT_{50} = 78.7 days, DT_{90} = 261 days.
For M02 use DFOP/SFO. DT_{50} = 78.8 days, DT_{90} = 262 days.
M03 was not observed in this soil.

Appendix 3.1.7. Degradation of ^{14}C-cyclohexyl labelled spiroxamine, M01, M02, M03 and M06 in Longwoods soil (KCA 7.1.1.1/06 (M-762349-01-1))

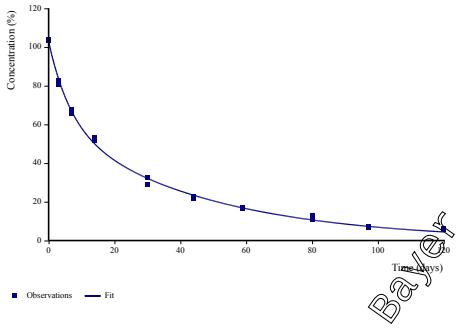
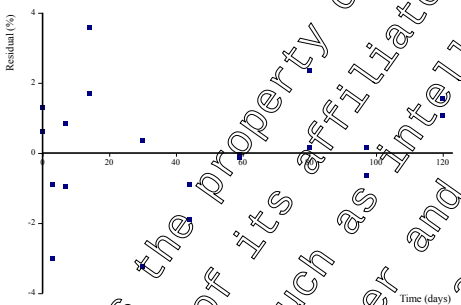
Appendix 3.1.7.1. Spiroxamine kinetics

Step 1: Run SFO, FOMC. SFO more appropriate than FOMC and gives acceptable fit?

	SFO	FOMC
Plot		
Residuals		
Visual fit	Intermediate, residuals show systematic error and fit is not conservative	Excellent, residuals show no systematic error and fit is conservative
χ^2 error (%)	10.8	3.8
t-test	t: $p < 0.05$	NA
DT_{50} (days)	19.6	13.5
DT_{90} (days)	65.4	90.2
Assessment	Fit not acceptable χ^2 error is acceptable and rate parameter differs significantly from zero, however, visual fit is intermediate (residuals show systematic error and fit not conservative)	Fit acceptable Visual fit is excellent and χ^2 error is low
Discussion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Deviation from SFO is not due to outliers or experimental artefacts iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No Go to step 2 below	

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Run DFOP. Determine which of the models (FOMC, DFOP) is best.

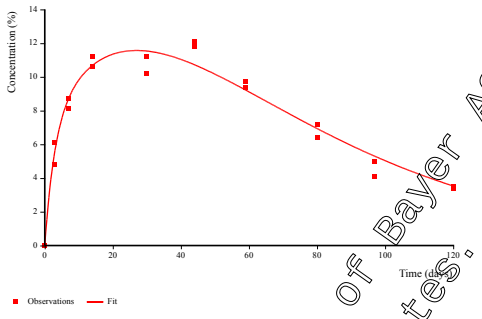
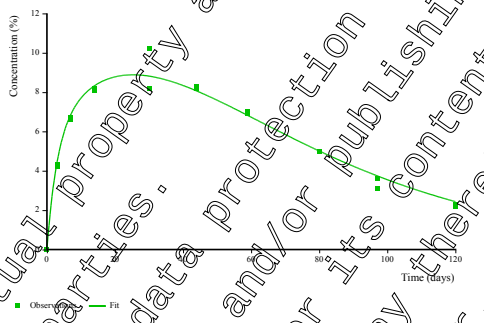
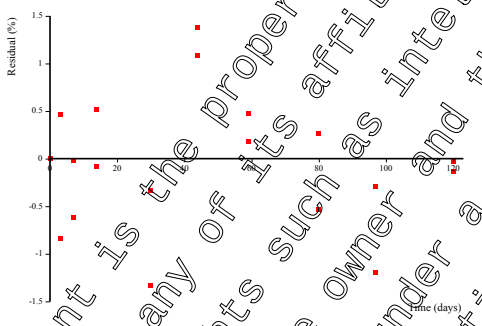
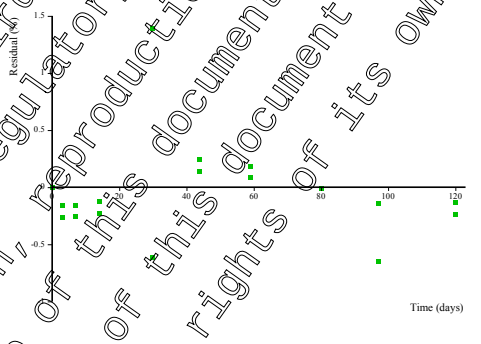
	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 error (%)	3.2
t-test	k_1: $p < 0.05$ k_2: $p < 0.05$
DT_{50} (days)	13.1
DT_{90} (days)	81.2
Assessment	Fit acceptable Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero
Discussion	iv) Determine which of the models (FOMC, DFOP) is best. Both FOMC and DFOP give acceptable fits. DFOP selected (lower χ^2 error). v) Does the best-fit model give an acceptable description of the data? Yes DFOP should be used for persistence endpoints

Summary:

For spiroxamine use DFOP, DT_{50} = 13.1 days, DT_{90} = 81.2 days.

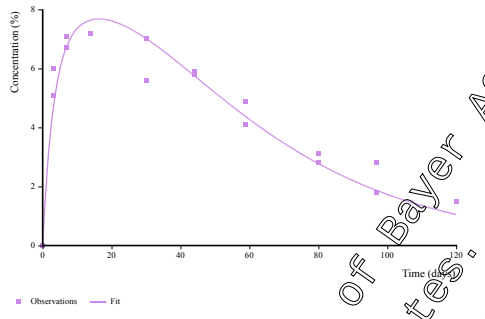
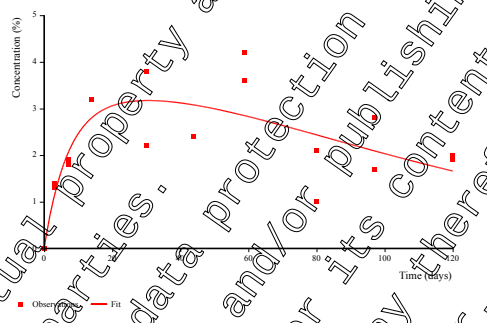
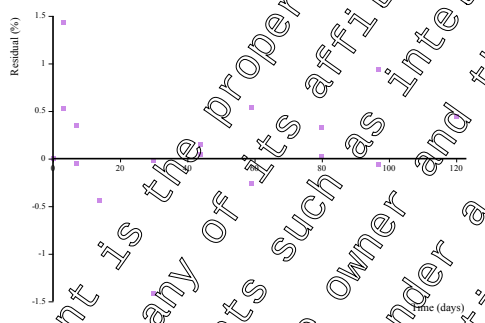
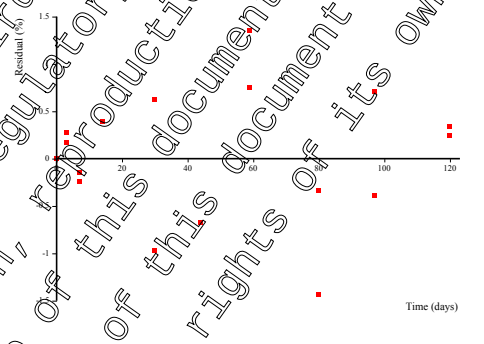
Appendix 3.1.7.2. Metabolite kinetics (M01 and M02)

Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 error (%)	5.9	3.3
t-test	*k*: $p < 0.05$	*k*: $p < 0.05$
DT_{50} (days)	28.9	26.6
DT_{90} (days)	95.8	88.2
Assessment	Fit acceptable Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero	Fit acceptable Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero
Discussion	ii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints

Appendix 3.1.7.3. Metabolite kinetics (M03 and M06)

Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M03	M06
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Intermediate, residuals show no systematic error
χ^2 error (%)	8.1	18.5
t-test	*k*: p < 0.05	*k*: p < 0.05
DT_{50} (days)	16.7	49.6
DT_{90} (days)	55.4	165
Assessment	Fit acceptable Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero	Fit acceptable Visual fit is good, χ^2 error is slightly high and rate parameter differs significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints

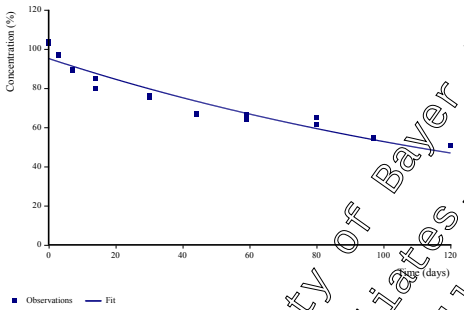
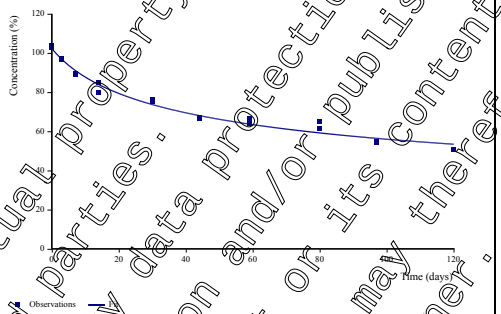
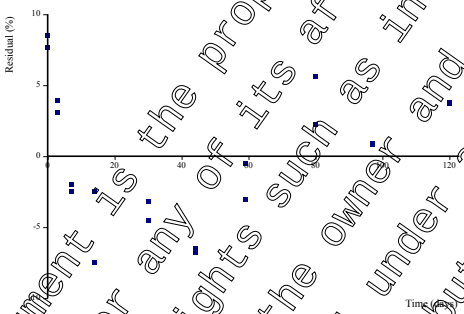
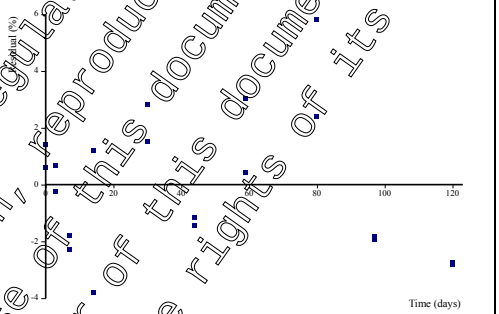
Summary:

For M01 use DFOP/SFO. DT_{50} = 28.9 days, DT_{90} = 95.8 days.
For M02 use DFOP/SFO. DT_{50} = 26.6 days, DT_{90} = 88.2 days.
For M03 use DFOP/SFO. DT_{50} = 16.7 days, DT_{90} = 55.4 days.
For M06 use DFOP/SFO. DT_{50} = 49.6 days, DT_{90} = 165 days.

Appendix 3.1.8. Degradation of ^{14}C-cyclohexyl labelled spiroxamine, M01, M02, M03 and M06 in Refesol 02-A soil (KCA 7.1.1.1/06 (M-762349-01-1))

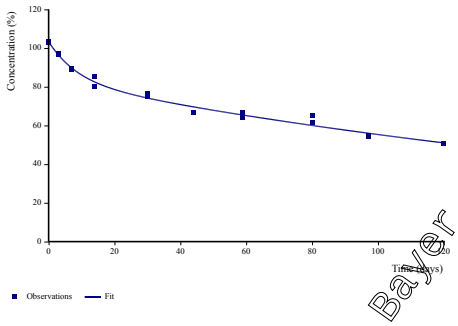
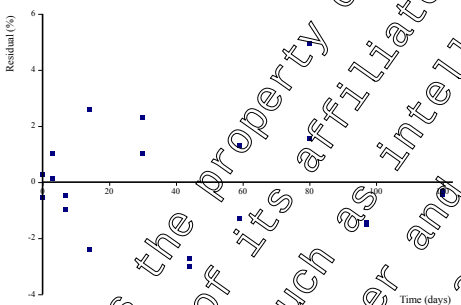
Appendix 3.1.8.1. Spiroxamine kinetics

Step 1: Run SFO, FOMC. SFO more appropriate than FOMC and gives acceptable fit?

	SFO	FOMC
Plot		
Residuals		
Visual fit	Intermediate, residuals show no systematic error, but fit is not conservative	Good, residuals show no systematic error and fit is conservative
χ^2 error (%)	4.9	2.4
t-test	*, $p < 0.05$	NA
DT_{50} (days)	117	141[1]
DT_{90} (days)	389[1]	>10,000[1]
Assessment	Fit not acceptable Visual fit is intermediate, χ^2 error is low and rate parameter differs significantly from zero	Fit acceptable Visual fit is good and χ^2 error is low. Significant extrapolation from study period to DT90[2].
Discussion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC (higher χ^2 error and visual fit improved by FOMC). ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Deviation from SFO is not due to outliers or experimental artefacts iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No Go to step 2 below	

[1] Interpret with care – extrapolated beyond experimental period
[2] EFSA (2009)

Step 2: Run DFOP. Determine which of the models (FOMC, DFOP) is best.

	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error
χ^2 error (%)	1.9
t-test	k_1: $p < 0.05$ k_2: $p < 0.05$
DT_{50} (days)	116
DT_{90} (days)	510[1]
Assessment	Fit acceptable Visual fit is excellent, χ^2 error is low and rate parameters differ significantly from zero
Discussion	iv) Determine which of the models (FOMC, DFOP) is best. Both FOMC and DFOP give acceptable fits. DFOP selected (lower χ^2 error and better visual fit) v) Does the best-fit model give an acceptable description of the data? Yes DFOP should be used for persistence end-points

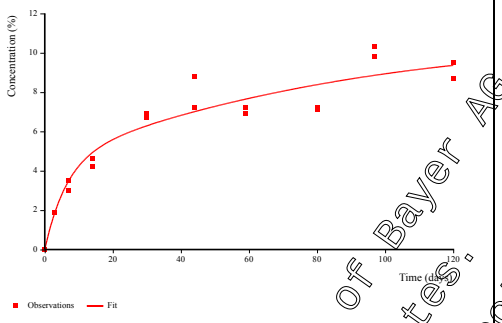
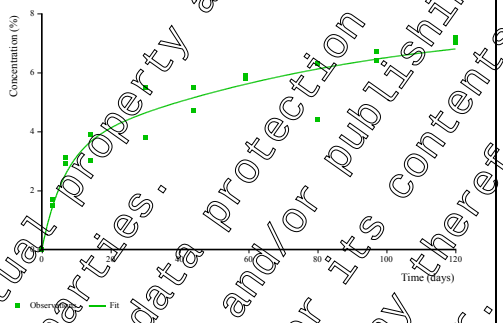
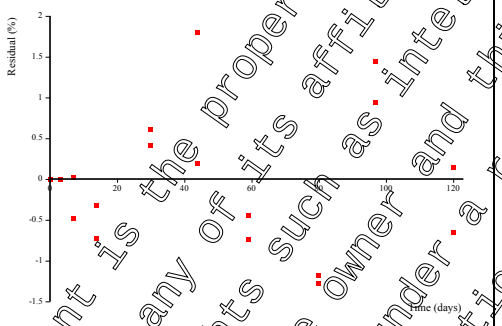
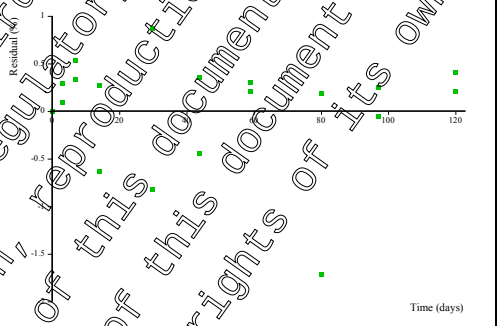
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use DFOP. DT_{50} = 116 days, DT_{90} = 510 days.

Appendix 3.1.8.2. Metabolite kinetics (M01 and M02)

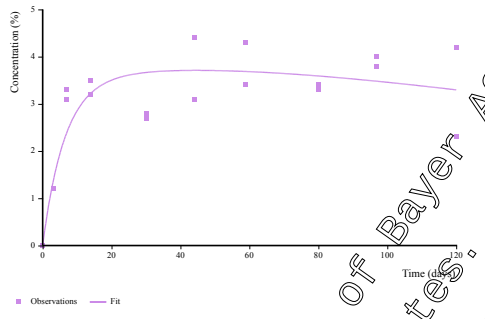
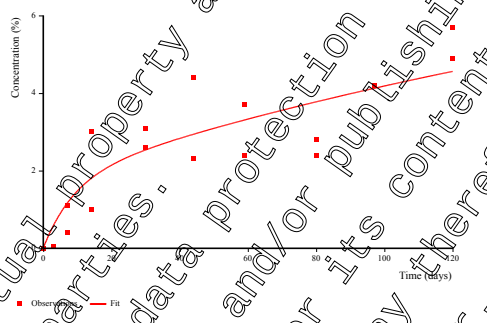
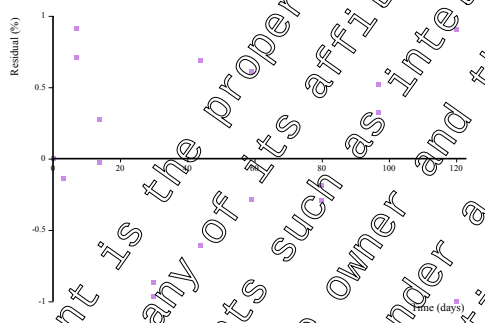
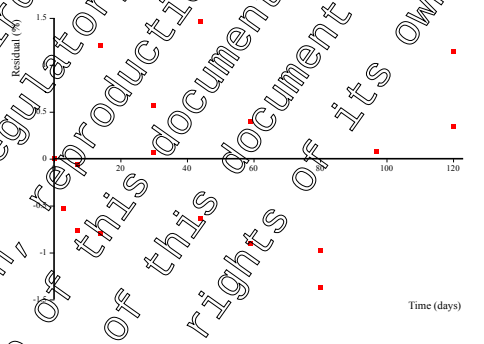
Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Intermediate, residuals show no systematic error	Intermediate, residuals show no systematic error
χ^2 error (%)	9.3	5.6
t-test	*k*: p < 0.05	k: p < 0.05
DT_{50} (days)	219[1]	204[1]
DT_{90} (days)	727[1]	678[1]
Assessment	Fit acceptable Visual fit is intermediate, χ^2 error is acceptable, and rate parameter differs significantly from zero at a 5% level.	Fit acceptable Visual fit is intermediate, χ^2 error is acceptable and rate parameter differs significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints

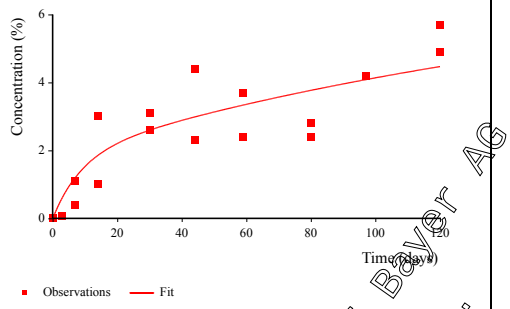
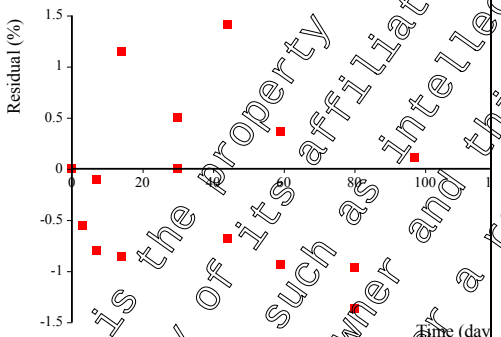
[1] Interpret with care – extrapolated beyond experimental period

Appendix 3.1.8.3. Metabolite kinetics (M03 and M06)

Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M03	M06
Plot		
Residuals		
Visual fit	Intermediate, residuals show no systematic error	Intermediate, residuals show no systematic error
χ^2 error (%)	10.2	16.6
t-test	*k*: p < 0.05	*k*: p > 0.1
DT_{50} (days)	53.1	>10,000
DT_{90} (days)	176[1]	>10,000
Assessment	Fit acceptable Visual fit is acceptable, χ^2 error is acceptable and rate parameter differs significantly from zero	Fit potentially acceptable Visual fit is intermediate and χ^2 error is only slightly high, however rate parameter does not differ significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints	iii) SFO fit for metabolite acceptable? SFO is potentially acceptable. Based on the study data encompassing 120 days, predicted end-points of 10,000 days is a drastic extrapola-tion and is considered unreliablegiven the data. Therefore, fit using a default DT_{50} of 1,000 days performed to demonstrate a good fit to study data and establish a reasonable worst case.

[1] Interpret with care – extrapolated beyond experimental period

	Parent DFOP, metabolites SFO M06
Plot	
Residuals	
Visual fit	Visual fit is intermediate
χ^2 error (%)	16.1
t-test	N/A
DT_{50} (days)	1,000
DT_{90} (days)	3,320
Assessment	SFO using default DT_{50} is acceptable. Modelling with the FOCUS default rate constant provides an acceptable fit.
Discussion	SFO is acceptable.

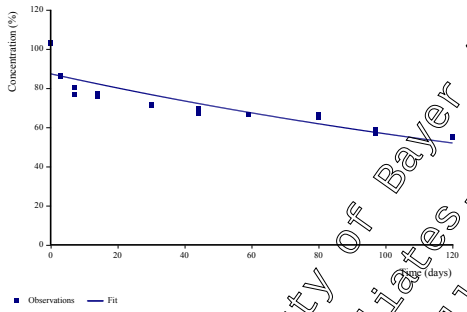
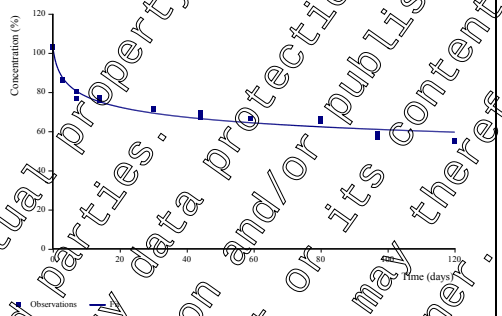
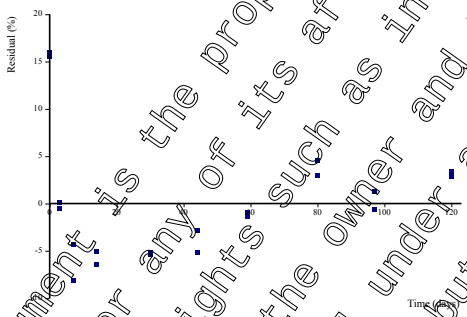
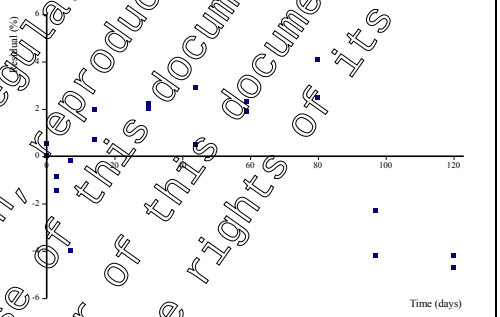
Summary:

For M01 use DFOP/SFO. DT_{50} = 219 days, DT_{90} = 727 days.
For M02 use DFOP/SFO. DT_{50} = 204 days, DT_{90} = 678 days.
For M03 use DFOP/SFO. DT_{50} = 53.1 days, DT_{90} = 176 days.
For M06 use DFOP/SFO DT_{50} = 1,000 days (FOCUS default).

Appendix 3.1.9. Degradation of ^{14}C-cyclohexyl labelled spiroxamine, M01, M02, M03 and M06 in Refesol 03-G soil (KCA 7.1.1.1/06 (M-762349-01-1))

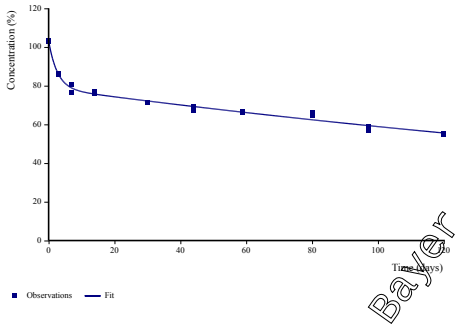
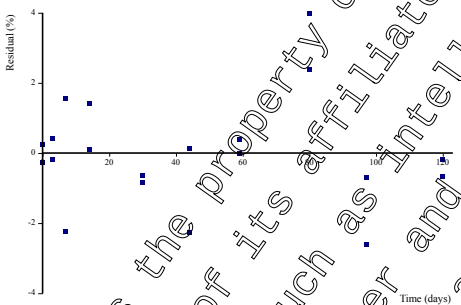
Appendix 3.1.9.1. Spiroxamine kinetics

Step 1: Run SFO, FOMC. SFO more appropriate than FOMC and gives acceptable fit?

	SFO	FOMC
Plot		
Residuals		
Visual fit	Intermediate, residuals show systematic error	Good, however residuals show systematic error. Fit is conservative
χ^2 error (%)	7.2	2.9
t-test	*, $p < 0.05$	NA
DT_{50} (days)	159[1]	423[1]
DT_{90} (days)	527[1]	>10,000[1]
Assessment	Fit not acceptable. χ^2 error is low and rate parameter differs significantly from zero, however visual fit is intermediate (residuals show systematic error)	Fit acceptable Visual fit is good and χ^2 error is low. Significant extrapolation from study period to DT90[2].
Discussion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Deviation from SFO is not due to outliers or experimental artefacts iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No Go to step 2 below	

[1] Interpret with care – extrapolated beyond experimental period
[2] EFSA (2009)

Step 2: Run DFOP. Determine which of the models (FOMC, DFOP) is best.

	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 error (%)	1.5
t-test	k_1: $p < 0.05$ k_2: $p < 0.05$
DT_{50} (days)	142[1]
DT_{90} (days)	696[1]
Assessment	Fit acceptable Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero
Discussion	iv) Determine which of the models (FOMC, DFOP) is best. Both FOMC and DFOP give acceptable fits. DFOP selected (lower χ^2 error). v) Does the best-fit model give an acceptable description of the data? Yes DFOP should be used for persistence endpoints

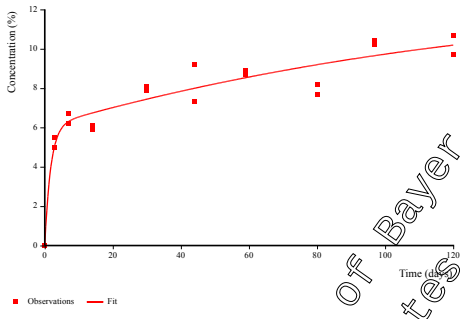
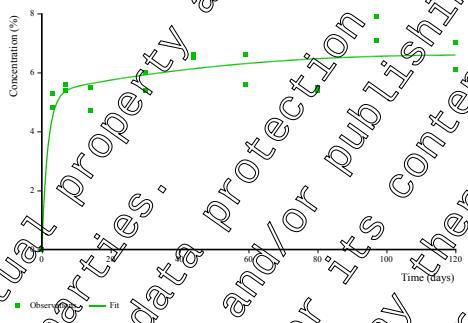
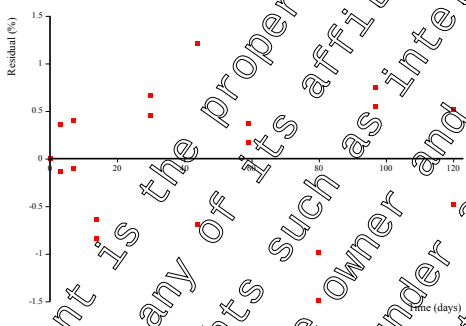
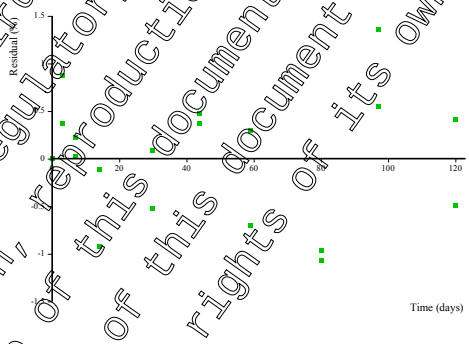
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use DFOP. DT_{50} = 142 days, DT_{90} = 696 days.

Appendix 3.1.9.2. Metabolite kinetics (M01 and M02)

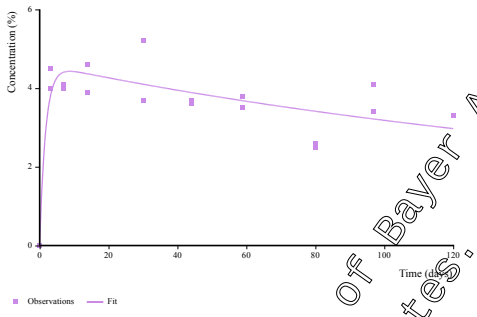
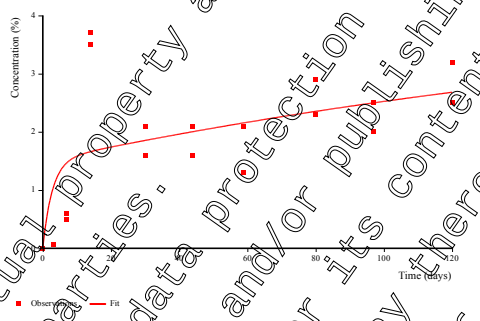
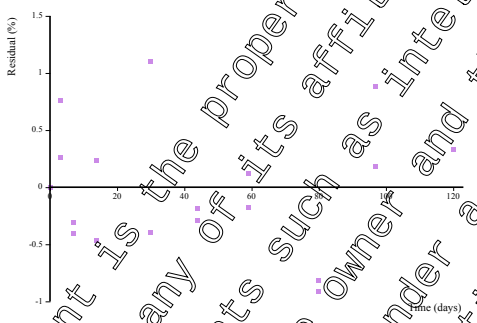
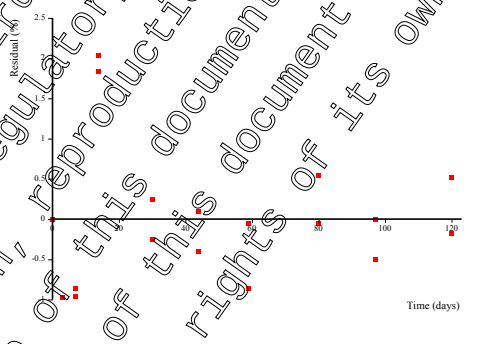
Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 error (%)	5.8	7.6
t-test	*k*: $p < 0.05$	*k*: $p < 0.05$
DT_{50} (days)	350[1]	128[1]
DT_{90} (days)	1,160[1]	426[1]
Assessment	Fit acceptable Visual fit is good, χ^2 error is low, and rate parameter differs significantly from zero	Fit acceptable Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero
Discussion	ii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints

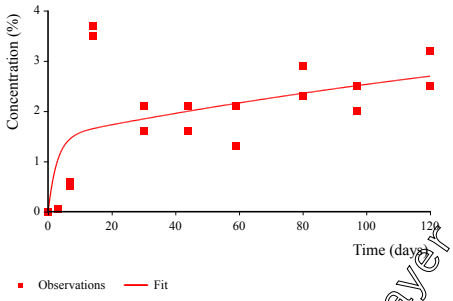
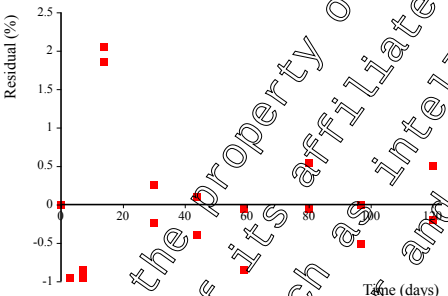
[1] Interpret with care – extrapolated beyond experimental period

Appendix 3.1.9.3. Metabolite kinetics (M03 and M06)

Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M03	M06
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Intermediate, residuals show no systematic error
χ^2 error (%)	9.3	33.9
t-test	*k*: p < 0.05	*k*: p > 0.1
DT_{50} (days)	49.9	1,380[1]
DT_{90} (days)	166[1]	4,570[1]
Assessment	Fit acceptable Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero	Fit not acceptable Visual fit is intermediate, χ^2 error is high and rate parameter does not differ significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints	iii) SFO fit for metabolite acceptable? SFO is not considered acceptable. Use of DFOP and FOMC do not improve the fit and not possible to fit to decline phase. Therefore, fit using a default DT_{50} of 1,000 days performed to demonstrate a reasonable worst case..

[1] Interpret with care - extrapolated beyond experimental period

	Parent DFOP, metabolites SFO M06
Plot	
Residuals	
Visual fit	Visual fit is excellent, no evidence of systematic error in residuals.
χ^2 error (%)	32.1
t-test	N/A
DT_{50} (days)	1,000[1]
DT_{90} (days)	3,320[1]
Assessment	SFO is acceptable when considering a fixed DT_{50}. Although χ^2 is high the metabolite fit presented adequately described the study data and represents a reasonable worst case.
Discussion	SFO is acceptable. Use 1,000 day FOCUS default.

[1] FOCUS default

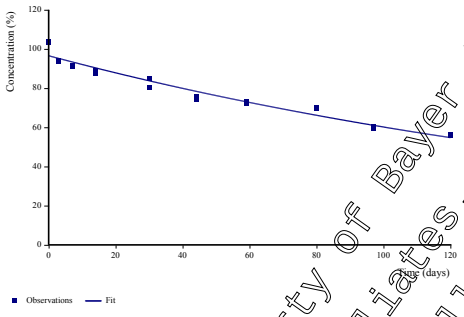
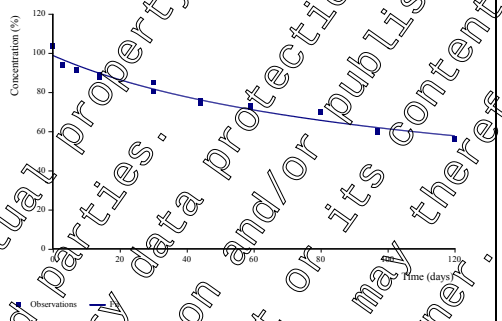
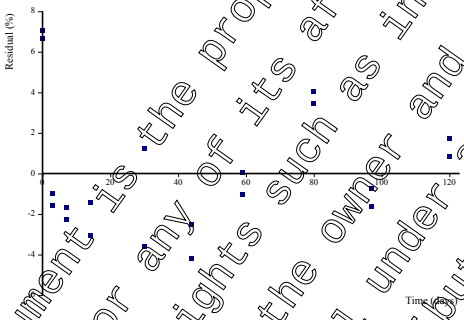
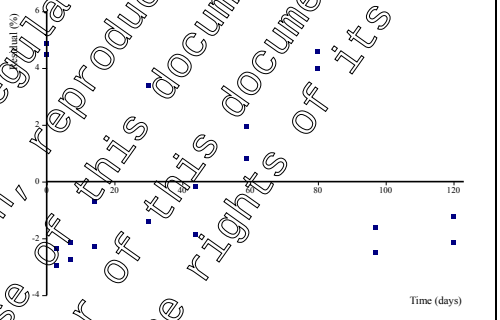
Summary:

For M01 use DFOP/SFO. DT_{50} = 350 days, DT_{90} = 1,160 days.
For M02 use DFOP/SFO. DT_{50} = 128 days, DT_{90} = 426 days.
For M03 use DFOP/SFO. DT_{50} = 49.9 days, DT_{90} = 166 days.
For M06 use DFOP/SFO DT_{50} = 1,000 days (FOCUS default).

Appendix 3.1.10. Degradation of ^{14}C-cyclohexyl labelled spiroxamine, M01, M02, M03 and M06 in Speyer 6S soil (KCA 7.1.1.1/06 (M-762349-01-1))

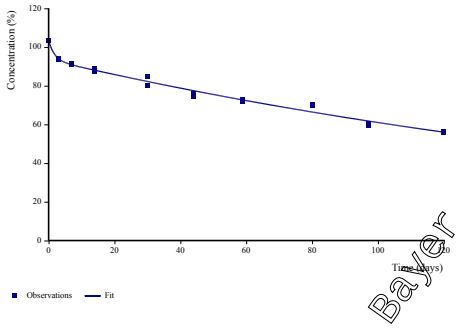
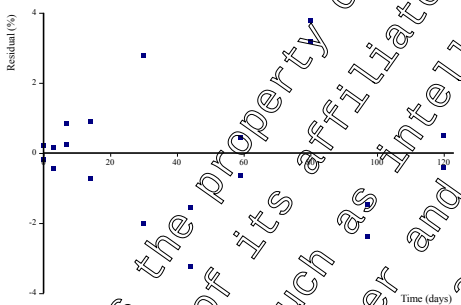
Appendix 3.1.10.1. Spiroxamine kinetics

Step 1: Run SFO, FOMC. SFO more appropriate than FOMC and gives acceptable fit?

	SFO	FOMC
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error and fit is conservative	Excellent, residuals show no systematic error
χ^2 error (%)	3.2	2.9
t-test	*. $p < 0.05$	NA
DT_{50} (days)	146[1]	186[1]
DT_{90} (days)	484[1]	6,340[1]
Assessment	Fit acceptable Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero	Fit acceptable Visual fit is excellent and χ^2 error is low. Significant extrapolation from study period to DT90[2].
Discussion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Deviation from SFO is not due to outliers or experimental artefacts iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No Go to step 2 below	

[1] Interpret with care – extrapolated beyond experimental period
[2] EFSA (2009)

Step 2: Run DFOP. Determine which of the models (FOMC, DFOP) is best.

	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 error (%)	1.7
t-test	k_1: $p < 0.05$ k_2: $p < 0.05$
DT_{50} (days)	137[1]
DT_{90} (days)	514[1]
Assessment	Fit acceptable Visual fit is excellent and χ^2 error is low, and rate parameters differ significantly from zero at a 5% level.
Discussion	iv) Determine which of the models (FOMC, DFOP) is best. DFOP gives the best fit (lower χ^2). v) Does the best-fit model give an acceptable description of the data? Yes DFOP should be used for persistence end-points

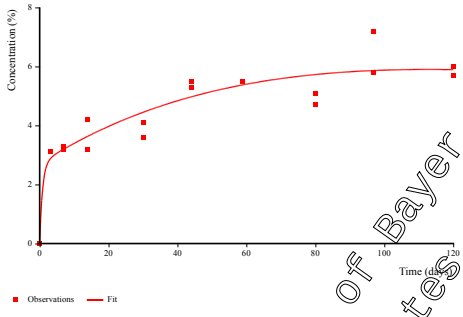
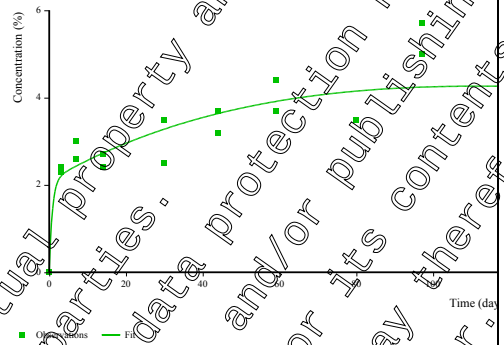
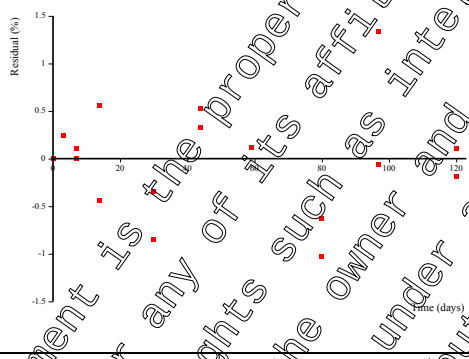
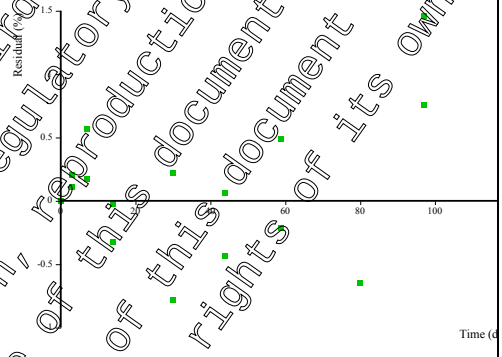
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use DFOP: DT_{50} = 137 days, DT_{90} = 514 days.

Appendix 3.1.10.2.Metabolite kinetics (M01 and M02)

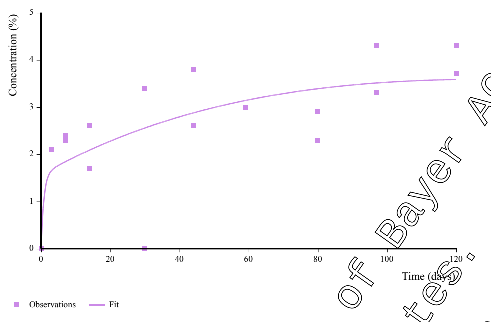
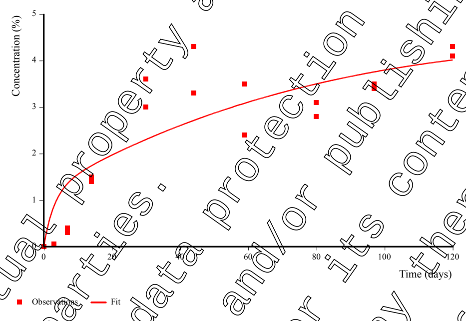
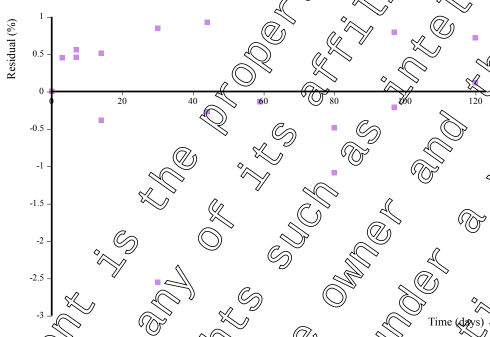
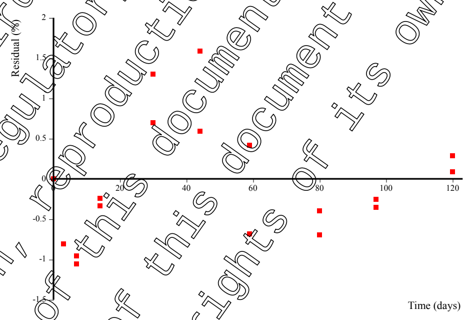
Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 error (%)	7.4	11.4
t-test	*k*: $p < 0.05$	*k*: $p < 0.05$
DT_{50} (days)	70.7	65.2
DT_{90} (days)	235[1]	217[1]
Assessment	Fit acceptable Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero	Fit acceptable Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero
Discussion	ii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints

[1] Interpret with care – extrapolated beyond experimental period

Appendix 3.1.10.3. Metabolite kinetics (M03 and M06)

Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M03	M06
Plot		
Residuals		
Visual fit	Good, fit is conservative, residuals show no systematic error	Intermediate, residuals show no systematic error
χ^2 error (%)	14.3	23.5
t-test	*k*: $p < 0.05$	*k*: $p > 0.05$ (p value equals 0.1)
DT_{50} (days)	79	191[1]
DT_{90} (days)	262[1]	635[1]
Assessment	Fit acceptable Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero	Fit acceptable Visual fit is intermediate and χ^2 error is slightly high, however rate parameter does not differ significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints	iii) SFO fit for metabolite acceptable? SFO is considered acceptable. General fit to data is intermediate without systematic error. Although rate parameter does not differ from zero, this is due to a lack of decline data and p = 0.1. The fit is acceptable.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

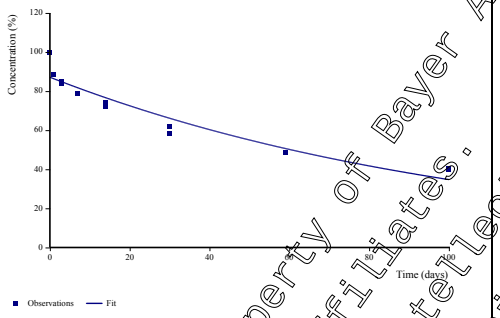
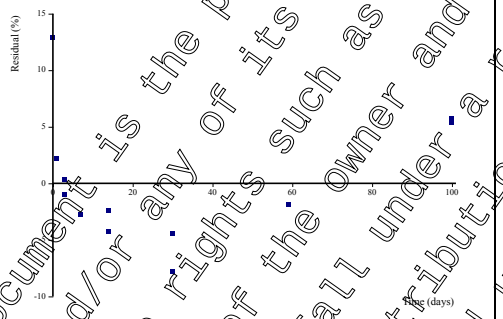
For M01 use DFOP/SFO. DT_{50} = 70.7 days, DT_{90} = 235 days.
For M02 use DFOP/SFO. DT_{50} = 65.2 days DT_{90} = 217 days.
For M03 use DFOP/SFO. DT_{50} = 79 days, DT_{90} = 262 days.
For M06 use DFOP/SFO DT_{50} = 191 days, DT90 = 635 days

Appendix 3.2: Kinetic evaluation for modelling endpoints

Appendix 3.2.1. Degradation of ^{14}C-cyclohexyl labelled spiroxamine, M01, M02 and M03 in BBA 2.2/Speyer 2.2 soil (KCA 7.1.1.1/01 (M-006135-01-1))

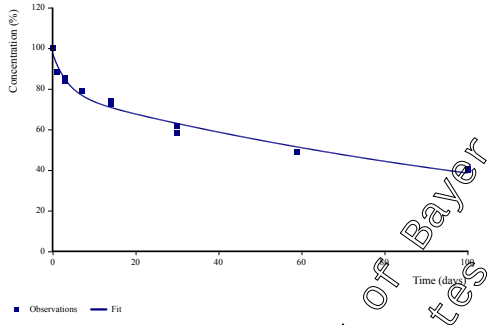
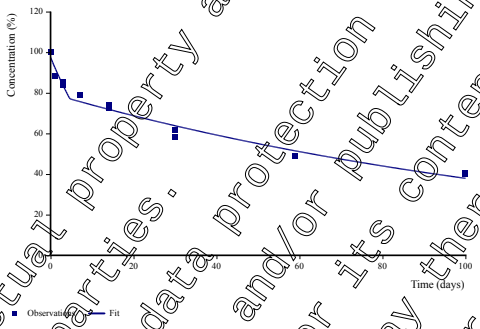
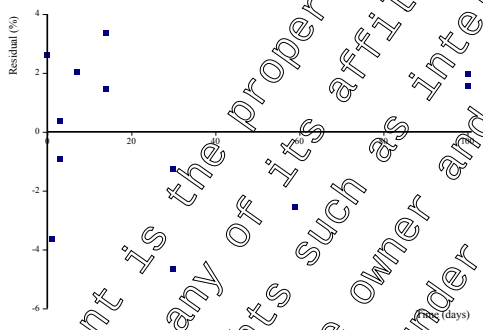
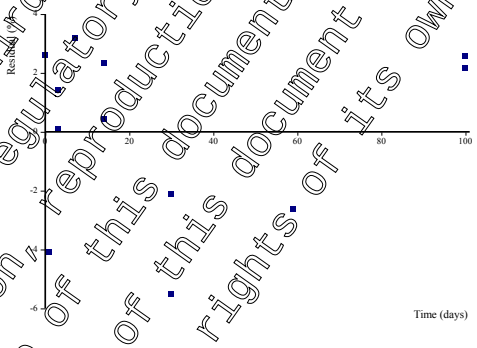
Appendix 3.2.1.1. Spiroxamine kinetics

Step 1: Run SFO. SFO statistically and visually acceptable?

	SFO
Plot	
Residuals	
Visual fit	Intermediate, residuals show systematic error and fit not conservative
χ^2 error (%)	6.4
t-test	*k*: $p < 0.05$
DT_{50} (days)	75.4
DT_{90} (days)	251
Modelling DT_{50} (days)	75.4
Assessment	Fit not acceptable χ^2 error is low and rate parameter differs significantly from zero, however visual fit is intermediate (residuals show systematic error and fit not conservative)
Discussion	i) SFO statistically and visually acceptable? No. ii) Run modified fitting. SFO statistically and visually acceptable? Deviation from SFO is not due to outliers or experimental artefacts Step 2: Correction procedure: iii) Bi-phasic pattern. Yes. iv) Aim: modelling fate of parent only? Yes. v) 10% initially measured concentration reached within experimental period? No Go to step 2 below:

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Correction procedure (continued). Run HS or DFOP. vi) HS or DFOP statistically and visually acceptable?

	DFOP	HS
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error	Excellent, residuals show no systematic error
$\chi 2$ error (%)	3.2	3.6
t-test	k_1: $p < 0.05$ k_2: $p < 0.05$	k_1: $p < 0.05$ k_2: $p < 0.05$
DT_{50} (days)	66.6	66.3
DT_{90} (days)	296[1]	283[1]
Modelling DT_{50} (days)	k_2 DT_{50} = 98.9	k_2 DT_{50} = 93.2
Assessment	Fit acceptable Visual fit is excellent, $\chi 2$ error is low and rate parameter differs significantly from zero	Fit acceptable Visual fit is excellent, $\chi 2$ error is low and rate parameter differs significantly from zero
Discussion	vi) HS or DFOP statistically and visually acceptable? Both DFOP and HS are considered acceptable. DFOP should be used for modelling endpoints (lower $\chi 2$ error) Use DT50 from slow phase of DFOP model for fate modelling	

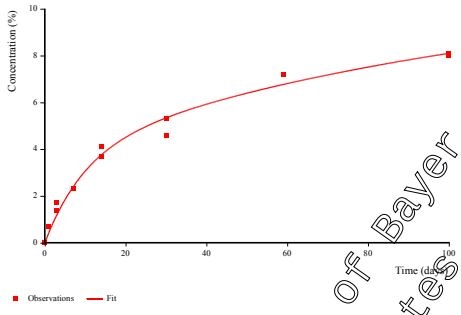
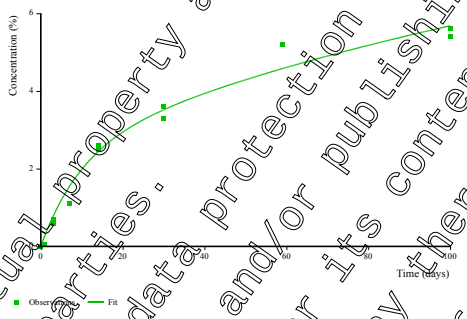
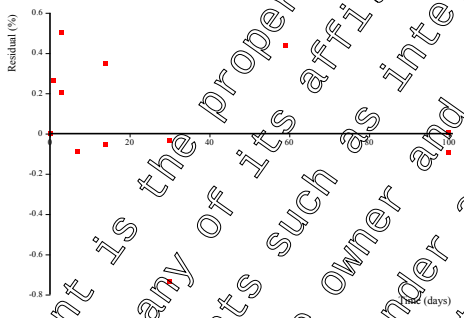
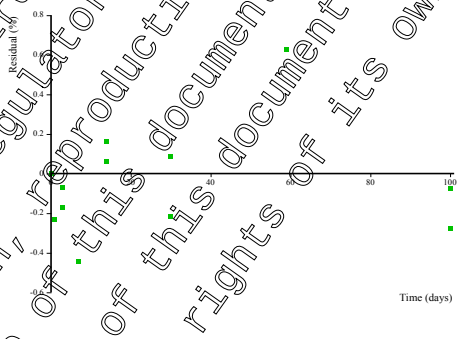
[1]Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use DFOP. $DT_{50\ mod}$ = 98.9 days (based on k_2 DFOP).

Appendix 3.2.1.2. Metabolite kinetics (M01 and M02)

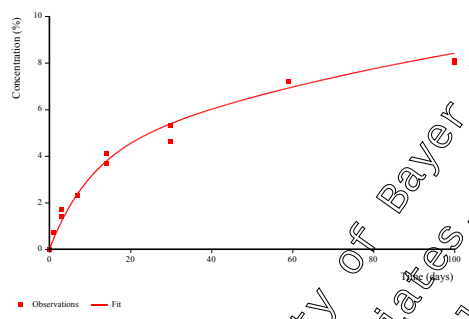
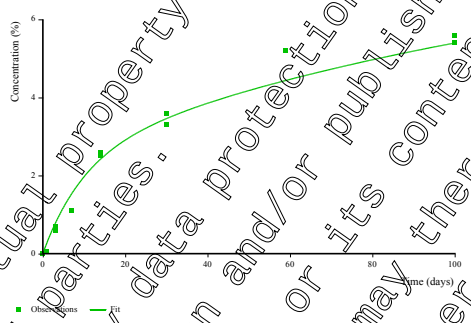
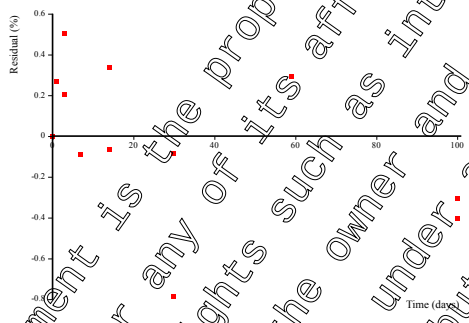
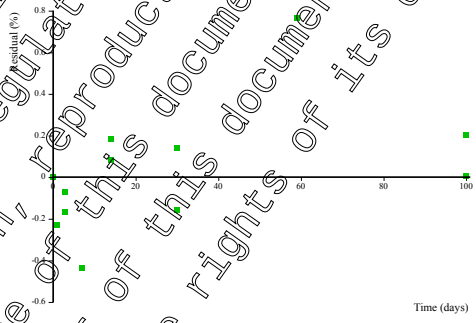
Step 1: Run parent and metabolites all SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error	Excellent, residuals show no systematic error
χ^2 error (%)	5.6	9.5
t-test	*k*: p > 0.1	*k*: p > 0.1
DT_{50} (days)	555[1]	>10,000[1]
DT_{90} (days)	1,840[1]	>10,000[1]
Modelling DT_{50} (days)	555[1]	>10,000[1]
Formation fraction	0.162	0.104
Assessment	Fit not acceptable Visual fit is excellent and χ^2 error is low, however, rate parameter does not differ significantly from zero	Fit not acceptable Visual fit is excellent and χ^2 error is low, however, rate parameter does not differ significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is not considered acceptable. Therefore, DFOP or FOMC should be assessed. However, these models are not appropriate for metabolites which are formed gradually. Thereafter, decline after max should be assessed. However, this was not possible as there is limited decline data to clearly establish the dissipation pattern. Therefore, use of default DT_{50} and modelled formation fraction of 0.162 investigated.	iii) SFO fit for metabolite acceptable? SFO is not considered acceptable. Therefore, DFOP or FOMC should be assessed. However, these models are not appropriate for metabolites which are formed gradually. Thereafter, decline after max should be assessed. However, this was not possible as there is limited decline data to clearly establish the dissipation pattern. Therefore, use of default DT_{50} and modelled formation fraction of 0.104 investigated.

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Run parent DFOP and metabolite SFO with DT_{50} of 1,000 days and modelled formation fractions (conservative estimate).

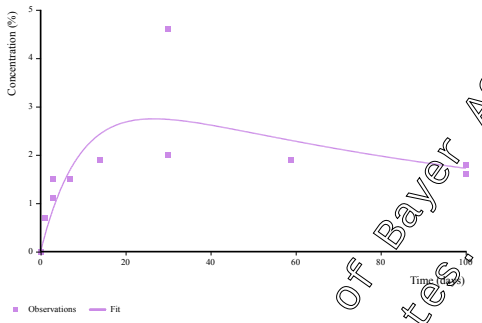
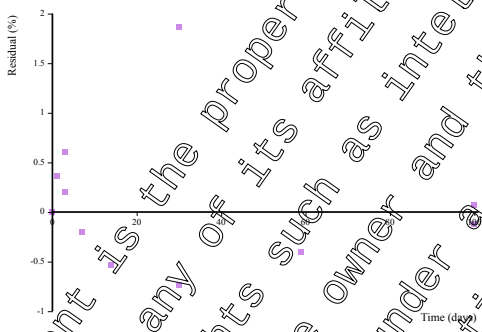
SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error	Excellent, residuals show no systematic error
χ^2 error (%)	5.14	9.39
t-test	NA	NA
DT_{50} (days)	1,000[1]	1,000[1]
DT_{90} (days)	3,320[1]	3,320[1]
Modelling DT_{50} (days)	1,000[1]	1,000[1]
Formation fraction	0.162	0.104
Assessment	Fit acceptable Visual fit is excellent and χ^2 error is low.	Fit acceptable Visual fit is excellent and χ^2 error is low.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable. Fixing formation fraction to a higher value (modelled formation fraction x 1.5) yields worse fit (see appendix), supporting fit presented here.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable. Fixing formation fraction to a higher value (modelled formation fraction x 1.5) yields worse fit (see appendix), supporting fit presented here.

[1] Fixed to FOCUS conservative default

Appendix 3.2.1.3. Metabolite kinetics (M03)

Step 1: Run parent and metabolites all SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO M03
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error
χ^2 error (%)	18
t-test	*k*: p < 0.05
DT_{50} (days)	29.8
DT_{90} (days)	98.8
Modelling DT_{50} (days)	29.8
Formation fraction	0.125
Assessment	Visual fit is good, χ^2 error is slightly high and rate parameter differs significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints

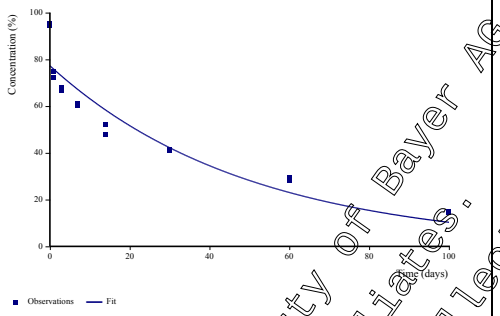
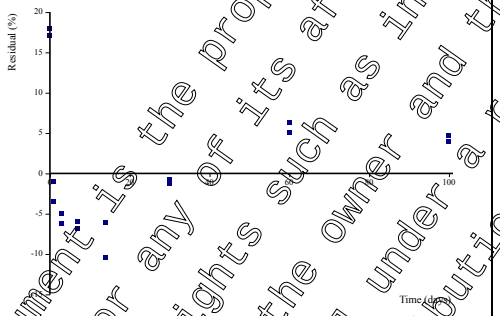
Summary:

For M01 use DFOP/SFO (using conservative parameters). $DT_{50\ mod}$ = 1,000 (conservative default), $f.f._{from\ parent}$ = 0.162.
For M02 use DFOP/SFO (using conservative parameters). $DT_{50\ mod}$ = 1,000 (conservative default), $f.f._{from\ parent}$ = 0.104.
For M03 use DFOP/SFO. DT_{50mod} = 29.8 days, $f.f._{from\ parent}$ = 0.125.

Appendix 3.2.2. Degradation of ^{14}C-cyclohexyl labelled spiroxamine, M01, M02 and M03 in Laacherhof soil (KCA 7.1.1.1/02 (M-006141-01-1))

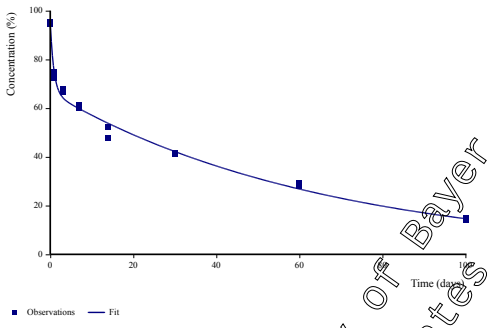
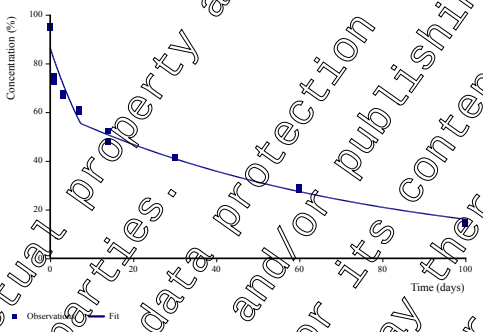
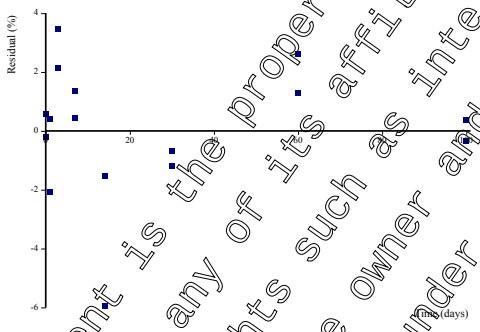
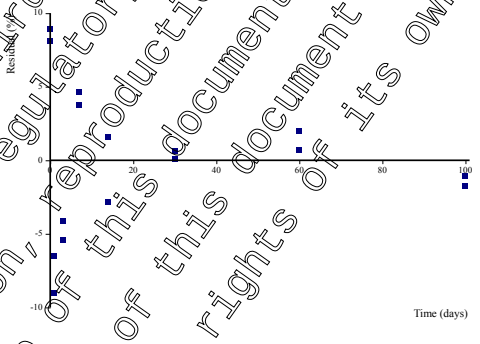
Appendix 3.2.2.1. Spiroxamine kinetics

Step 1: Run SFO. SFO statistically and visually acceptable?

	SFO
Plot	
Residuals	
Visual fit	Intermediate, residuals show systematic error and fit not conservative
χ^2 error (%)	11.9
t-test	ns. $p < 0.05$
DT_{50} (days)	34.8
DT_{90} (days)	116[1]
Modelling DT_{50} (days)	21.5[2]
Assessment	Fit not acceptable χ^2 error is acceptable and rate parameter differs significantly from zero, however, visual fit is intermediate (residuals show systematic error and fit not conservative)
Discussion	i) SFO statistically and visually acceptable? No ii) Run modified fitting. SFO statistically and visually acceptable? Deviation from SFO is not due to outliers or experimental artefacts Step 2: Correction procedure: iii) Bi-phasic pattern. Yes. iv) Aim: modelling fate of parent only? Yes. v) 10% initially measured concentration reached within experimental period? No Go to step 2 below:

[1] Interpret with care – extrapolated beyond experimental period
[2] Normalised to pF 2.0 soil moisture

Step 2: Correction procedure (continued). Run HS or DFOP. vi) HS or DFOP statistically and visually acceptable?

	DFOP	HS
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error	Excellent, residuals show no systematic error
$\chi 2$ error (%)	3.3	8.7
t-test	k_1: $p < 0.05$ k_2: $p < 0.05$	k_1: $p < 0.05$ k_2: $p < 0.05$
DT_{50} (days)	22.5	26.6
DT_{90} (days)	328[1]	147[1]
Model-ling DT_{50} (days)	k_2 DT_{50} = 28.7[2]	k_2 DT_{50} = 32.9[2]
Assess-ment	Fit acceptable Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero	Fit acceptable Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero
Discus-sion	vi) HS or DFOP statistically and visually acceptable? Both DFOP and HS are acceptable. DFOP should be used for modelling endpoints (lower χ^2 error) Use DT50 from slow phase of DFOP model for fate modelling	

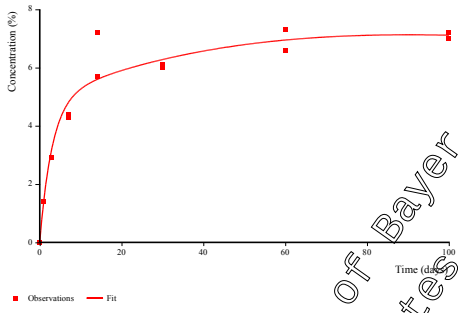
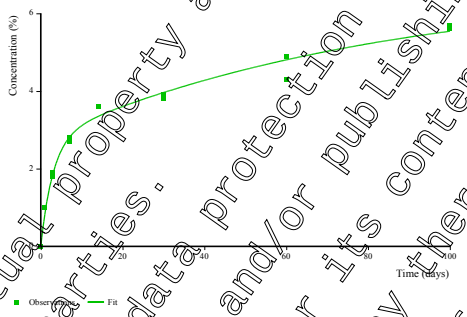
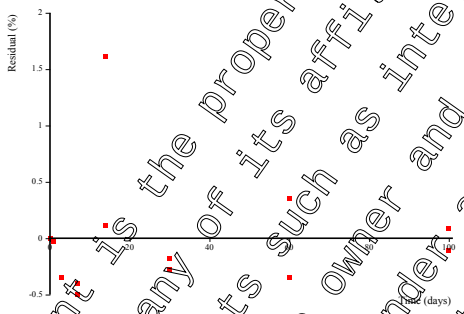
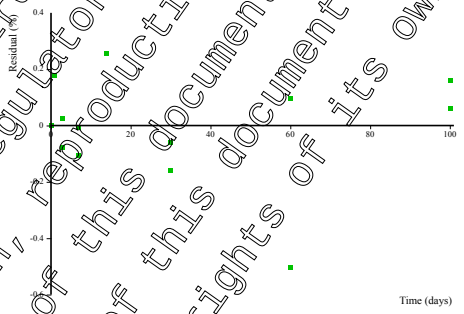
[1] Interpret with care – extrapolated beyond experimental period
[2] Normalised to pF 2.0 soil moisture

Summary:

For spiroxamine use DFOP. $DT_{50\ mod}$ = 28.7 days (based on k_2 DFOP of 45.4 normalised to pF 2.0).

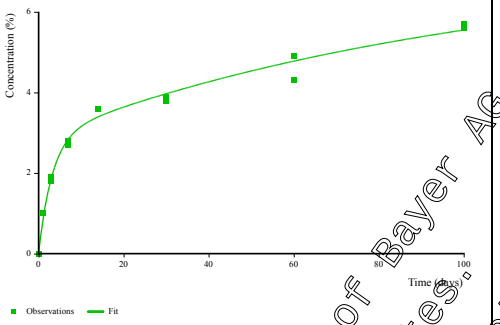
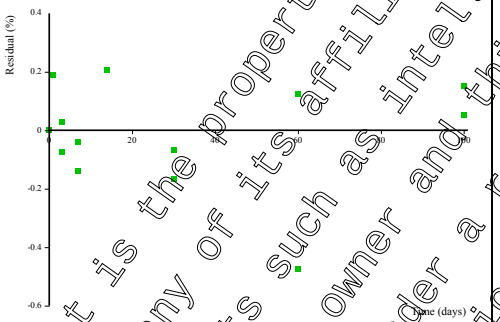
Appendix 3.2.2.2. Metabolite kinetics (M01 and M02)

Step 1: Run parent and metabolites all SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error	Excellent, residuals show no systematic error
χ^2 error (%)	6.2	3.5
t-test	*k*: p < 0.05	*k*: p > 0.1
DT_{50} (days)	167[1]	4,860[1]
DT_{90} (days)	554[1]	>10,000[1]
Modelling DT_{50} (days)	105.5[2]	n.a.
Formation fraction	0.137	0.08
Assessment	Fit acceptable Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero	Fit not acceptable Visual fit is excellent and χ^2 error is low, however, rate parameter does not differ significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints	iii) SFO fit for metabolite acceptable? SFO is not considered acceptable. Therefore, DFOP or FOMC should be assessed. However, these models will not improve the fit.. Thereafter, decline after max should be assessed. However, this was not possible as there is limited decline data to clearly establish the dissipation pattern. Therefore, use of default DT_{50} and modelled formation fraction of 0.08 as a realistic worst case investigated.

[1] Interpret with care – extrapolated beyond experimental period
[2] Normalised to pF 2.0 soil moisture

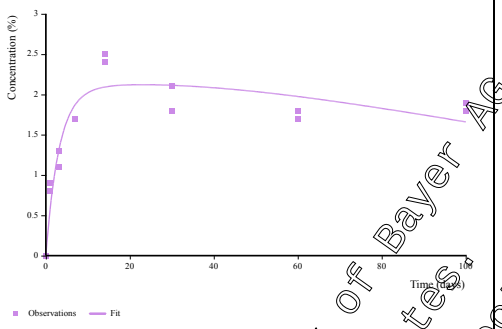
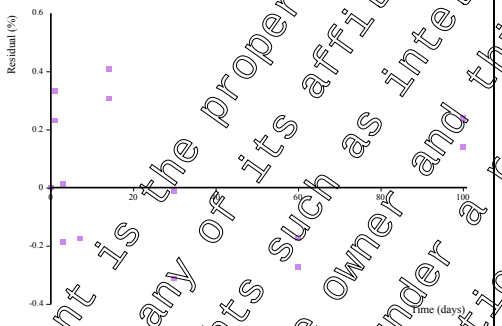
Step 2: Run parent best-fit and metabolite SFO, DT50 fixed to default value. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO M02
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error
χ^2 error (%)	3.36
t-test	NA
DT_{50} (days)	1000 (conservative default)
DT_{90} (days)	3,320[1]
Modelling DT_{50} (days)	1000
Formation fraction	0.08
Assessment	Fit acceptable Visual fit is excellent and χ^2 error is low iii) SFO fit for metabolite acceptable? SFO is considered acceptable
Discussion	Fixing formation fraction to a higher value (modelled formation fraction of 2.0) yields worse fit (see appendix), supporting fit presented here.

[1] Fixed to FOCUS conservative default

Appendix 3.2.2.3. Metabolite kinetics (M03)

Step 1: Run parent and metabolites all SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO
	M03
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error
χ^2 error (%)	10.7
t-test	*k*: p < 0.05
DT_{50} (days)	58.1
DT_{90} (days)	193[1]
Modelling DT_{50} (days)	36.7[2]
Formation fraction	0.06
Assessment	Fit acceptable Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints

[1] Interpret with care – extrapolated beyond experimental period
[2] Normalised to pF 2.0 soil moisture

Summary:

For M01 use DFOP/SFO $DT_{50\ mod}$ = 105.5 (from a non-normalised DT50 of 167 days), $f.f._{from\ parent}$ = 0.137.

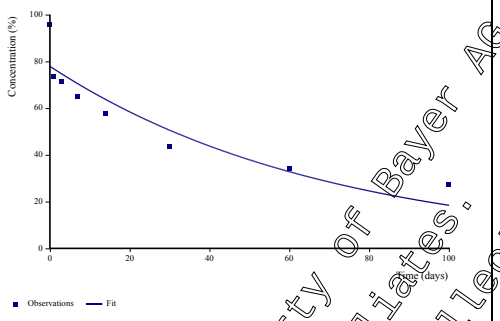
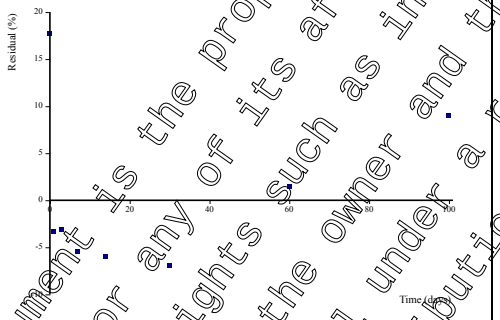
For M02 use DFOP/SFO (using conservative parameters). $DT_{50\ mod}$ = 1000 days, $f.f._{from\ parent}$ = 0.08.

For M03 use DFOP/SFO. DT_{50mod} = 36.7 days (from a non-normalised DT50 of 58.1 days), $f.f._{from\ parent}$ = 0.06.

Appendix 3.2.3. Degradation of ^{14}C-cyclohexyl labelled spiroxamine, M01, M02 and M03 in Monheim 3 soil (KCA 7.1.1.1/02 (M-006141-01-1))

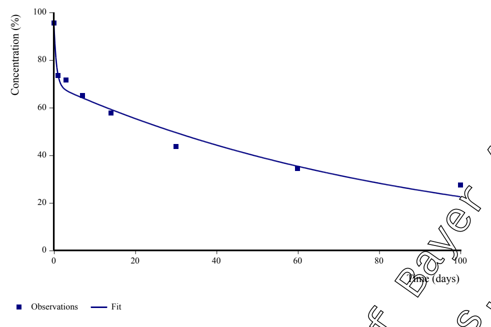
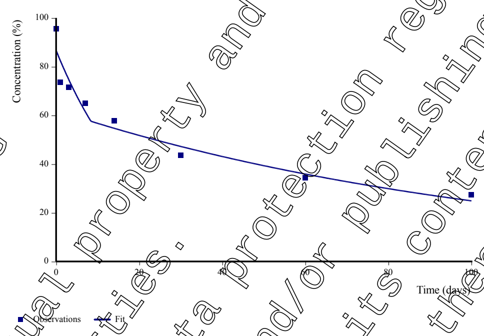
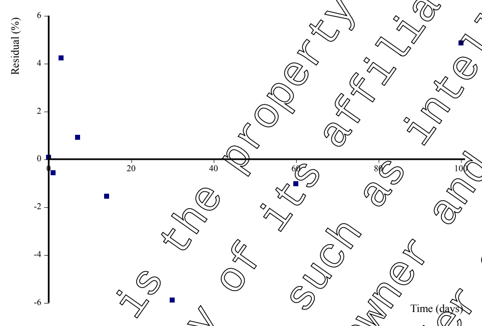
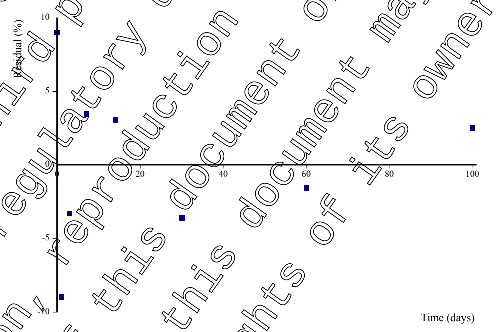
Appendix 3.2.3.1. Spiroxamine kinetics

Step 1: Run SFO. SFO statistically and visually acceptable?

	SFO
Plot	
Residuals	
Visual fit	Intermediate, residuals show systematic error and fit not conservative
χ^2 error (%)	11.1
t-test	✓, $p < 0.05$
DT_{50} (days)	48.1
DT_{90} (days)	160[1]
Modelling DT_{50} (days)	43.8[2]
Assessment	Fit not acceptable χ^2 error is acceptable and rate parameter differs significantly from zero, however, visual fit is intermediate (residuals show systematic error and fit not conservative)
Discussion	i) SFO statistically and visually acceptable? No ii) Run modified fitting. SFO statistically and visually acceptable? Deviation from SFO is not due to outliers or experimental artefacts Step 2: Correction procedure: iii) Bi-phasic pattern. Yes. iv) Aim: modelling fate of parent only? Yes. v) 10% initially measured concentration reached within experimental period? No Go to step 2 below:

[1] Interpret with care – extrapolated beyond experimental period
[2] Normalised to pF 2.0 soil moisture

Step 2: Correction procedure (continued). Run HS or DFOP. vi) HS or DFOP statistically and visually acceptable?

	DFOP	HS
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error	Good, residuals show no systematic error
χ2 error (%)	5.0	8.1
t-test	k_1: p 0.05-0.1; $k_2 < 0.05$	k_1: $p < 0.05$ k_2: $p < 0.05$
DT_{50} (days)	33.2	39.5
DT_{90} (days)	176[1]	215[1]
Modelling DT_{50} (days)	k_2 $DT_{50} = 56.1$[2]	k_2 $DT_{50} = 69.0$[2]
Assessment	Fit acceptable Visual fit is excellent and χ^2 error is acceptable. k_1 p value above 0.05, however, it is below 0.1 (due to small no. of data points). k_2 parameter is significantly different to zero, however, and as this is used for modelling endpoint, fit considered acceptable.	Fit acceptable Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero
Discussion	vi) HS or DFOP statistically and visually acceptable? Both DFOP and HS are considered acceptable. DFOP should be used for modelling endpoints due to lower χ^2 Use DT50 from slow phase of DFOP model for fate modelling	

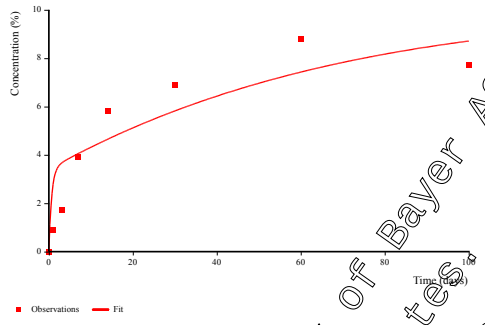
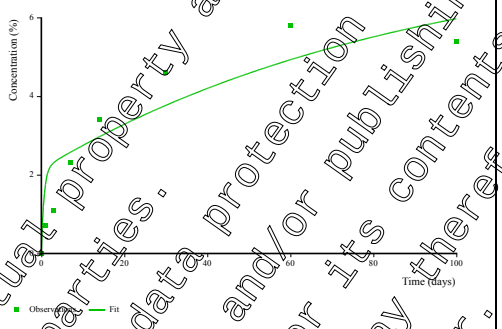
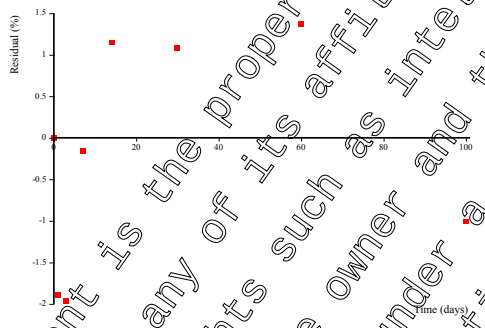
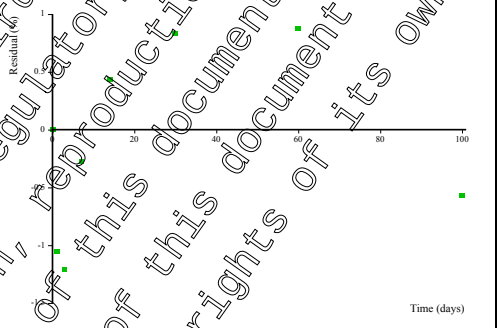
[1] Interpret with care – extrapolated beyond experimental period
[2] Normalised to pF 2.0 soil moisture

Summary:

For spiroxamine use DFOP. $DT_{50\ mod} = 56.1$ days (based on k_2 DFOP 61.7 days normalised to pF 2.0).

Appendix 3.2.3.2. Metabolite kinetics (M01 and M02)

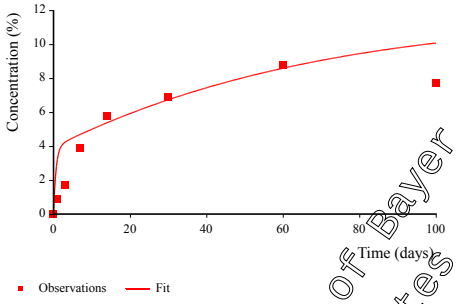
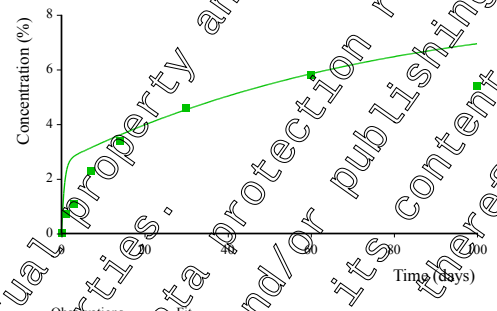
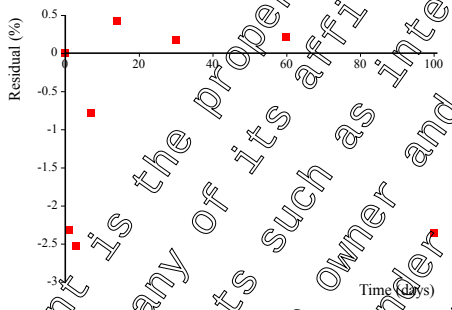
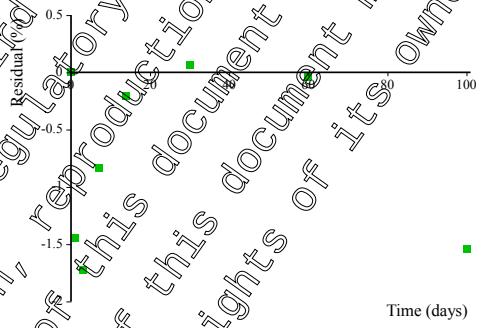
Step 1: Run parent and metabolites all SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Intermediate, residuals show no systematic error	Intermediate, residuals show no systematic error
χ^2 error (%)	20.1	19.4
t-test	*k*: p > 0.1	*k*: p > 0.1
DT_{50} (days)	597[1]	>10,000[1]
DT_{90} (days)	1,980[1]	>10,000[1]
Modelling DT_{50} (days)	543[2]	>10,000[1]
Formation fraction	0.13	0.081
Assessment	Fit not acceptable Visual fit is intermediate, χ^2 error is high and rate parameter does not differ significantly from zero	Fit not acceptable Visual fit is intermediate, χ^2 error is high and rate parameter does not differ significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is not considered acceptable. Therefore, DFOP or FOMC should be assessed. However, these models will not improve the fit. Thereafter, decline after max should be assessed. However, this was not possible as there is limited decline data to clearly establish the dissipation pattern. Therefore, use of default DT_{50} and an increased formation fraction of 0.15 investigated as a realistic worst case.	iii) SFO fit for metabolite acceptable?. SFO is not considered acceptable. Therefore, DFOP or FOMC should be assessed. However, these models will not improve the fit.. Thereafter, decline after max should be assessed. However, this was not possible as there is limited decline data to clearly establish the dissipation pattern. Therefore, use of default DT_{50} and formation fraction of 0.1 inestigated as a realistic worst case.

[1] Interpret with care – extrapolated beyond experimental period
[2] Normalised to pF 2.0 soil moisture

Step 2: Case-by-case decision (alternative – but conservative – estimates).

SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Visual fit is conservative.	Visual fit is conservative.
χ^2 error (%)	23.4	22.9
t-test	N/A	N/A
DT_{50} (days)	1,000	1,000
DT_{90} (days)	3,320	3,320
Modelling DT_{50} (days)	1,000	1,000
Formation fraction	0.15	0.1
Assessment	Visual fit is conservative	Visual fit is conservative
Discussion	iii) SFO is considered conservative and should be used for modelling endpoints. Fixing formation fraction to a higher value (modelled formation fraction x 1.5) yields worse fit (see appendix), supporting fit presented here.	iii) SFO is considered conservative and should be used for modelling endpoints. Fixing formation fraction to a higher value (modelled formation fraction x 1.5) yields worse fit (see appendix), supporting fit presented here.

Appendix 3.2.3.3 Metabolite kinetics (M03)

Metabolite M03 was not observed for this soil.

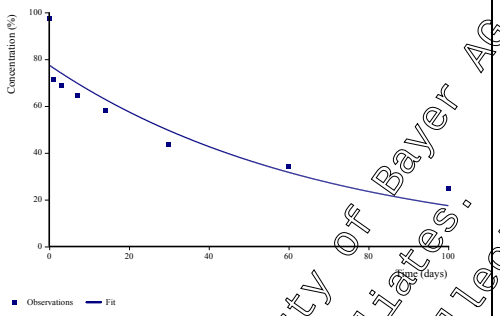
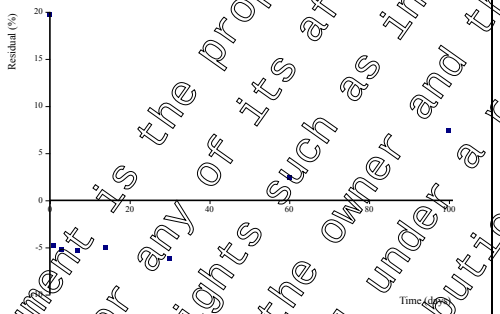
Summary

For M01 use DFOP/SFO (using conservative parameters). $DT_{50\ mod}$ = 1,000 days, $f.f._{from\ parent}$ = 0.15.
For M02 use DFOP/SFO (using conservative parameters). $DT_{50\ mod}$ = 1,000 days, $f.f._{from\ parent}$ = 0.1.
M03 was not observed for this soil.

Appendix 3.2.4. Degradation of ^{14}C-cyclohexyl labelled spiroxamine, M01, M02 and M03 in Howe soil (KCA 7.1.1.1/02 (M-006141-01-1))

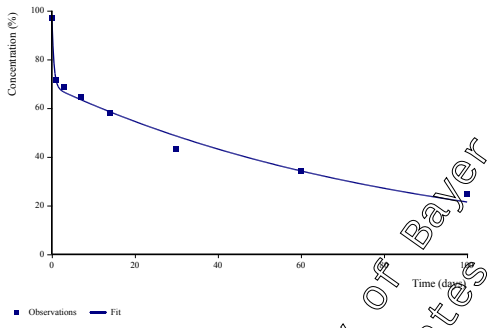
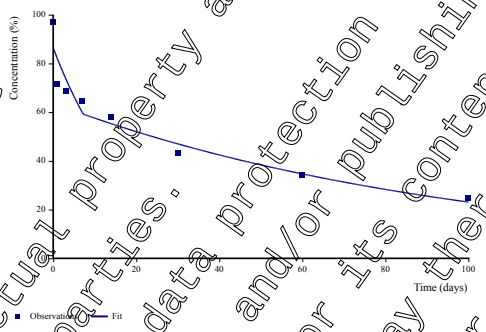
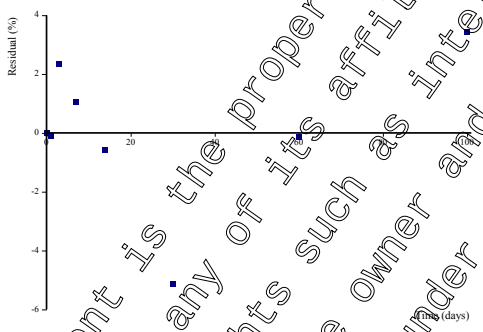
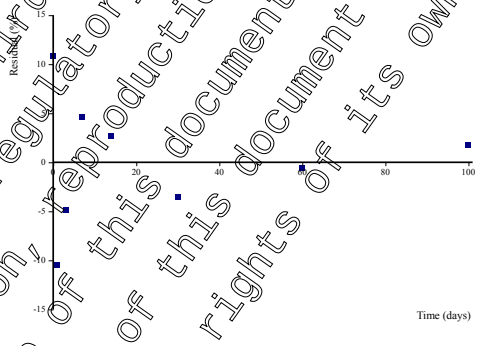
Appendix 3.2.4.1. Spiroxamine kinetics

Step 1: Run SFO. SFO statistically and visually acceptable?

	SFO
Plot	
Residuals	
Visual fit	Intermediate, residuals show systematic error and fit not conservative
χ^2 error (%)	11.9
t-test	✓, $p < 0.05$
DT_{50} (days)	46.5
DT_{90} (days)	155[1]
Modelling DT_{50} (days)	37.0[2]
Assessment	Fit not acceptable χ^2 error is acceptable and rate parameter differs significantly from zero, however, visual fit is intermediate (residuals show systematic error and fit not conservative)
Discussion	i) SFO statistically and visually acceptable? No ii) Run modified fitting. SFO statistically and visually acceptable? Deviation from SFO is not due to outliers or experimental artefacts Step 2: Correction procedure: iii) Bi-phasic pattern. Yes. iv) Aim: modelling fate of parent only? Yes. v) 10% initially measured concentration reached within experimental period? No Go to step 2 below:

[1] Interpret with care – extrapolated beyond experimental period
[2] Normalised to pF 2.0 soil moisture

Step 2: Correction procedure (continued). Run HS or DFOP. vi) HS or DFOP statistically and visually acceptable?

	DFOP	HS
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error	Excellent, residuals show no systematic error
$\chi 2$ error (%)	3.8	9.7
t-test	k_1: p = 0.05-0.1 k_2: p < 0.05	k_1: p < 0.05 k_2: p < 0.05
DT_{50} (days)	29.9	38.4
DT_{90} (days)	368[1]	197[1]
Modelling DT_{50} (days)	k_2 DT_{50} = 47.0[2]	k_2 DT_{50} = 54.3[2]
Assessment	Fit acceptable Visual fit is excellent, χ^2 error is low and rate parameters differ significantly from zero	Fit acceptable Visual fit is excellent, χ^2 error is low and rate parameters differ significantly from zero
Discussion	vi) HS or DFOP statistically and visually acceptable? Both DFOP and HS are considered acceptable. DFOP should be used for modelling endpoints (lower χ^2 error) Use DT50 from slow phase of DFOP model for fate modelling	

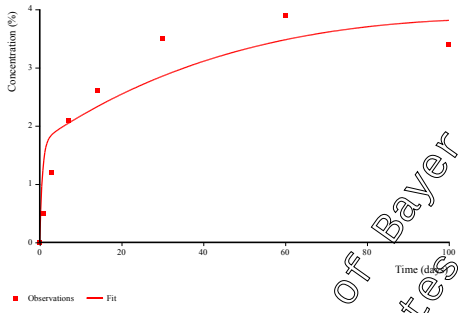
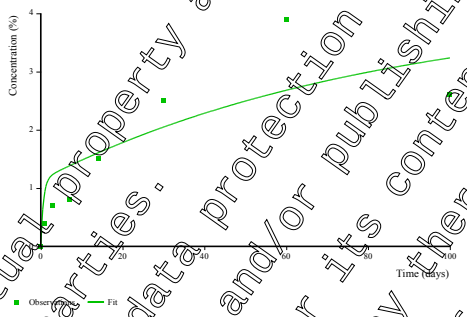
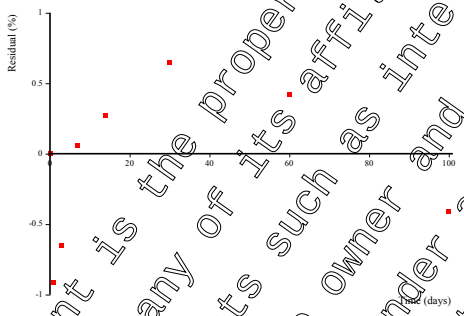
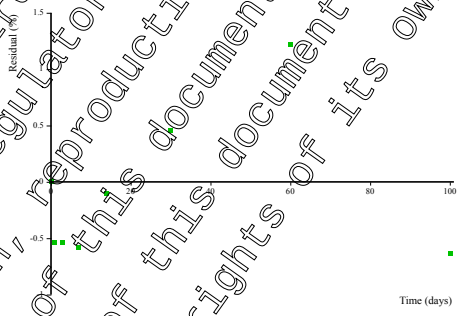
[1] Interpret with care – extrapolated beyond experimental period
[2] Normalised to pF 2.0 soil moisture

Summary:

For spiroxamine use DFOP, $DT_{50\,mod}$ = 47.0 days (based on k_2 DFOP 59.5 days normalised to pF 2.0).

Appendix 3.2.4.2. Metabolite kinetics (M01 and M02)

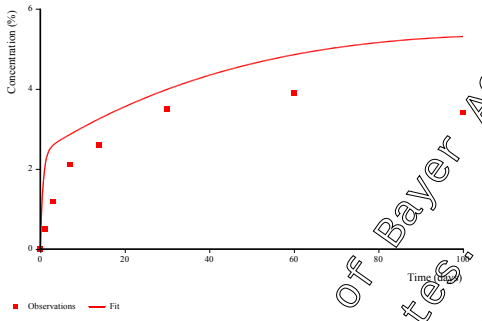
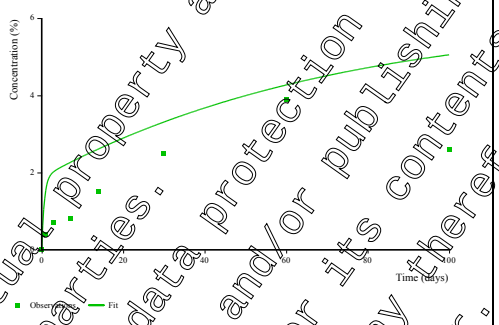
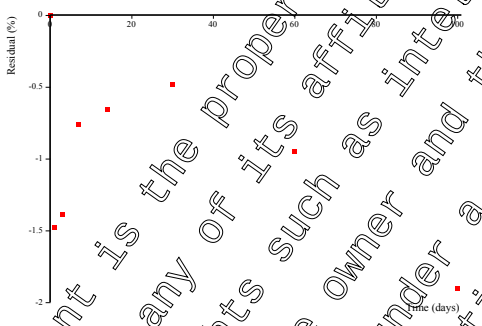
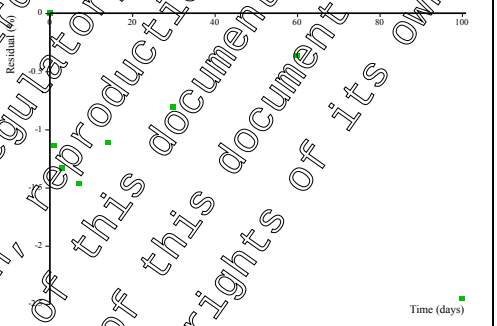
Step 1: Run parent and metabolites all SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Intermediate, residuals show systematic error	Poor, residuals show systematic error
χ^2 error (%)	17.7	29.5
t-test	*k*: p > 0.1	*k*: p > 0.1
DT_{50} (days)	202[1]	>10,000[1]
DT_{90} (days)	672[1]	>10,000[1]
Modelling DT_{50} (days)	159.6[2]	>10,000[1]
Formation fraction	0.065	0.043
Assessment	Fit not acceptable Visual fit is intermediate, χ^2 error is slightly high and rate parameter do not differ significantly from zero	Fit not acceptable Visual fit is poor, χ^2 error is high and rate parameters do not differ significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is not considered acceptable. Therefore, DFOP or FOMC should be assessed. However, these models are not appropriate for metabolites which are formed gradually. Thereafter, decline after max should be assessed. However, this was not possible as there is limited decline data to clearly establish the dissipation pattern. Therefore, use of the estimated DT_{50} and a refined formation fraction of 0.09 investigated as a realistic worst case.	iii) SFO fit for metabolite acceptable? SFO is not considered acceptable. Therefore, DFOP or FOMC should be assessed. However, these models are not appropriate for metabolites which are formed gradually. Thereafter, decline after max should be assessed. However, this was not possible as there is limited decline data to clearly establish the dissipation pattern. Therefore, use of default DT_{50} and formation fraction of 0.07 proposed as a worst case.

[1] Interpret with care – extrapolated beyond experimental period
[2] Normalised to pF 2.0 soil moisture

Step 2: Case-by-case decision (alternative – but conservative – estimates).

SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Conservative	Conservative
χ^2 error (%)	NA	NA
t-test	NA	NA
DT_{50} (days)	202[1]	1000[1](conservative default)
DT_{90} (days)	671[1]	3,320[1]
Modelling DT_{50} (days)	159.6[1,2]	1000[1]
Formation fraction	0.09	0.07
Assessment	Visual fit is conservative	Visual fit is conservative
Discussion	iii) SFO is considered conservative and should be used for modelling endpoints. In this case the predicted DT_{50} of 202 days with a modified formation fraction results in a more realistic worst case degradation pattern.	iii) SFO is considered conservative and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period
[2] Normalised to pF 2.0 soil moisture

Appendix 3.2.4.3. Metabolite kinetics (M03)

Metabolite M03 was not observed for this soil.

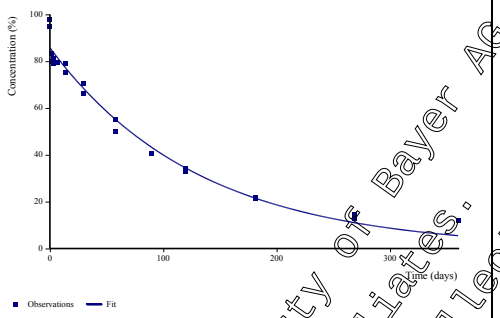
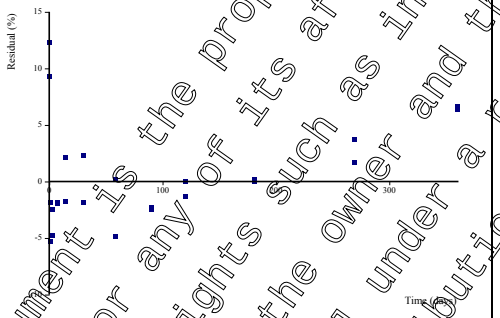
Summary:

M01 use DFOP/SFO (using conservative parameters). $DT_{50\ mod}$ =159.6 days (based on a non-normalised DT50 of 202), $f.f._{from\ parent}$ = 0.09.
M02 use DFOP/SFO (using conservative parameters). $DT_{50\ mod}$ = 1000 days, $f.f._{from\ parent}$ = 0.07.
M03 was not observed for this soil.

Appendix 3.2.5. Degradation of ^{14}C-cyclohexyl labelled spiroxamine, M01, M02 and M03 in Wolf Ranch soil (KCA 7.1.1.1/04 (M-006148-01-1))

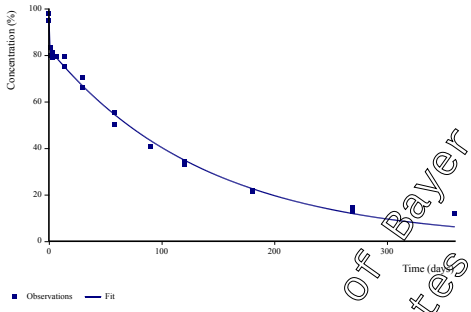
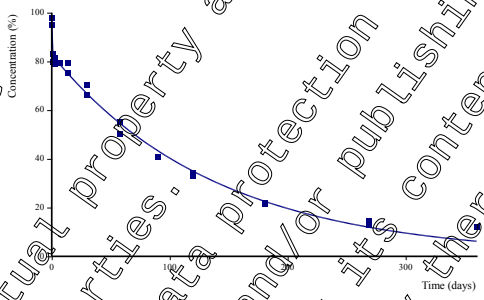
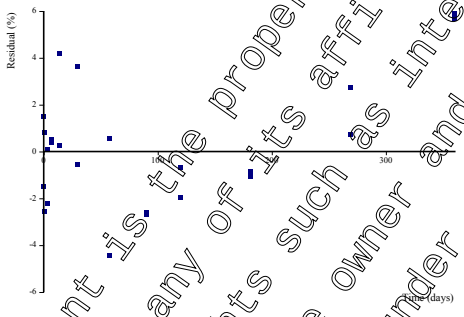
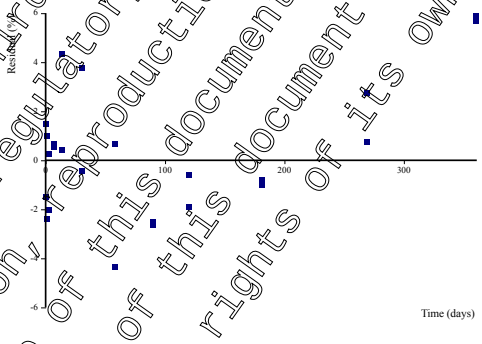
Appendix 3.2.5.1. Spiroxamine kinetics

Step 1: Run SFO. SFO statistically and visually acceptable?

	SFO
Plot	
Residuals	
Visual fit	Good but residuals show systematic error and fit not conservative
χ^2 error (%)	6.1
t-test	ns. $p < 0.05$
DT_{50} (days)	90.8
DT_{90} (days)	302[1]
Modelling DT_{50} (days)	64.1[2]
Assessment	Fit not acceptable χ^2 error is low and rate parameter differs significantly from zero, however, visual fit although good the residuals show systematic error and fit not conservative
Discussion	i) SFO statistically and visually acceptable? No ii) Run modified fitting. SFO statistically and visually acceptable? Deviation from SFO is not due to outliers or experimental artefacts Step 2: Correction procedure: iii) Bi-phasic pattern. Yes. iv) Aim: modelling fate of parent only? Yes. v) 10% initially measured concentration reached within experimental period? No Go to step 2 below:

[1] Interpret with care – extrapolated beyond experimental period
[2] Normalised to pF 2.0 soil moisture

Step 2: Correction procedure (continued). Run HS or DFOP. vi) HS or DFOP statistically and visually acceptable?

	DFOP	HS
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error and fit not conservative	Good and residuals show no systematic error, however, fit not conservative
$\chi 2$ error (%)	3.6	3.6
t-test	k_1: p >0.1 k_2 < 0.05	k_1: p >0.1 k_2 < 0.05
DT_{50} (days)	75	75
DT_{90} (days)	299[1]	299[1]
Modelling DT_{50} (days)	k_2 DT_{50} = 68.0[2]	k_2 DT_{50} = 68.0[2]
Assessment	Fit acceptable Visual fit is good and χ^2 error is low. k_1 rate parameter does not differ significantly from zero, however, k_2 parameter is significantly different to zero, and as this is used for modelling endpoint, fit is considered acceptable.	Fit acceptable Visual fit is good and χ^2 error is low. k_1 rate parameter does not differ significantly from zero, however, k_2 parameter is significantly different to zero, and as this is used for modelling endpoint, fit is considered acceptable..
Discussion	vi) HS or DFOP statistically and visually acceptable? Both DFOP and HS are considered acceptable. DFOP should be used for modelling endpoints Use DT50 from slow phase of DFOP model for fate modelling	

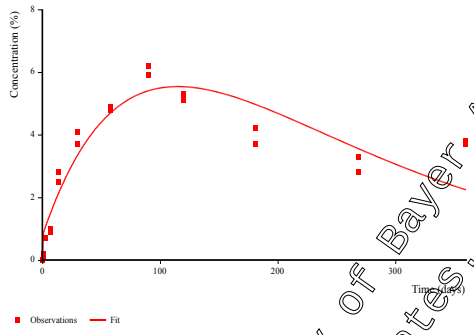
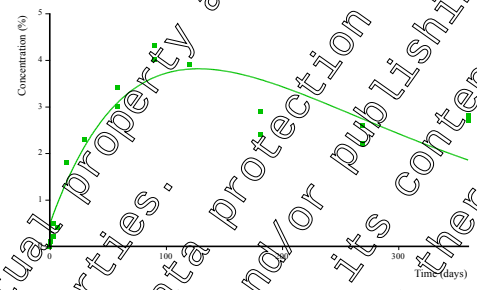
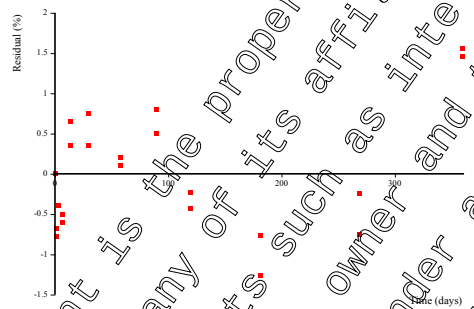
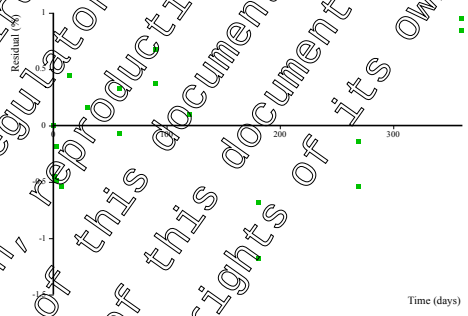
[1] Interpret with care – extrapolated beyond experimental period
[2] Normalised to pF 2.0 soil moisture

Summary:

For spiroxamine use DFOP. $DT_{50\,mod}$ = 68.0 days (based on k_2 DFOP 96.3 days normalised to pF 2.0).

Appendix 3.2.5.2. Metabolite kinetics (M01 and M02)

Step 1: Run parent and metabolites all SFO. SFO fit for metabolite acceptable?

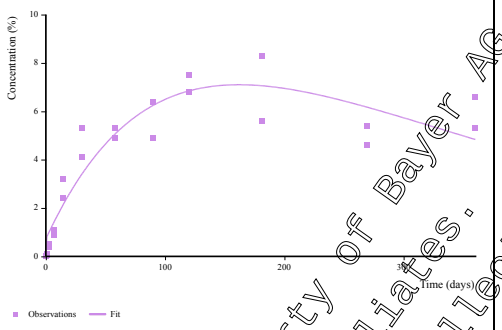
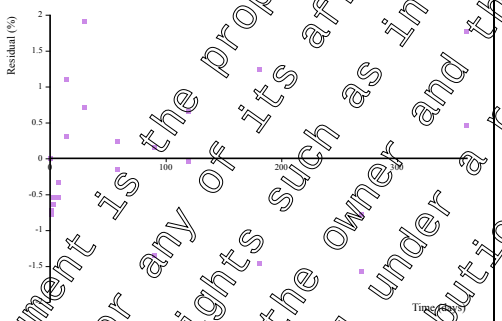
	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, no systematic errors	Good, no systematic errors
χ^2 error (%)	18.1	19.0
t-test	*k*: $p < 0.05$	*k*: $p < 0.05$
DT_{50} (days)	78.2	95.4
DT_{90} (days)	260[1]	317[1]
Modelling DT_{50} (days)	55.2[2]	67.4[2]
Formation fraction	0.182	0.113
Assessment	Fit acceptable Visual fit is good and rate parameter differs significantly from zero, however, χ^2 error is slightly high. Nevertheless, residuals show no large, systematic error	Fit acceptable Visual fit is good and rate parameter differs significantly from zero, however, χ^2 error is slightly high. Nevertheless, residuals show no large, systematic error
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints

[1] Interpret with care – extrapolated beyond experimental period
[2] Normalised to pF 2.0 soil moisture

Appendix 3.2.5.3. Metabolite kinetics (M03)

Step 1: Run parent and metabolites all SFO. SFO fit for metabolite acceptable?

SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO M03
Plot	
Residuals	
Visual fit	Good, no systematic errors
χ^2 error (%)	15.1
t-test	k_1: $p < 0.05$
DT_{50} (days)	152
DT_{90} (days)	507
Modelling DT_{50} (days)	107[2]
Formation fraction	0.171
Assessment	Fit acceptable Visual fit is good and rate parameter differs significantly from zero, however, χ^2 error is slightly high. Nevertheless, residuals show no large, systematic error
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints

[1] Interpret with care – extrapolated beyond experimental period
[2] Normalised to pF 2.0 soil moisture

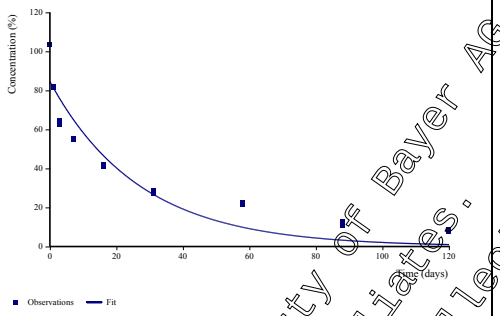
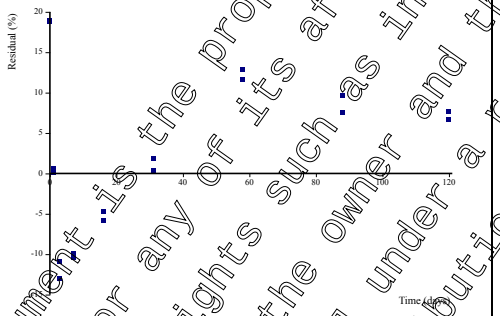
Summary:

For M01 use DFOP/SFO. $DT_{50\ mod}$ = 55.3 days, $f.f._{from\ parent}$ = 0.182.
For M02 use DFOP/SFO. $DT_{50\ mod}$ = 67.4 days, $f.f._{from\ parent}$ = 0.113.
M03 use DFOP/SFO. $DT_{50\ mod}$ = 107 days, $f.f._{from\ parent}$ = 0.171.

Appendix 3.2.6. Degradation of ^{14}C-dioxolane labelled spiroxamine, M01, M02 and M03 in Hoefchen am Hohenseh soil (KCA 7.1.1.1/05 (M-303803-01-1))

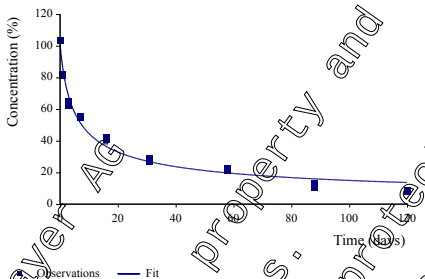
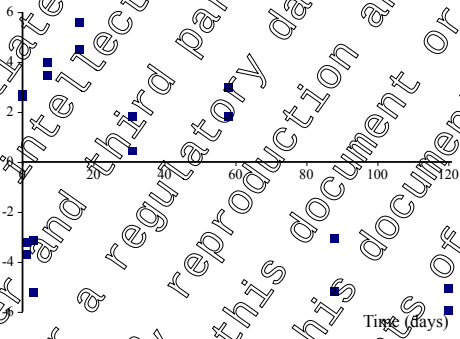
Appendix 3.2.6.1. Spiroxamine kinetics

Step 1: Run SFO. SFO statistically and visually acceptable?

	SFO
Plot	
Residuals	
Visual fit	Poor, residuals show systematic error and fit not conservative
χ^2 error (%)	17.4
t-test	ns. $p < 0.05$
DT_{50} (days)	18.7
DT_{90} (days)	62.2
Modelling DT_{50} (days)	18.2[1]
Assessment	Fit not acceptable Rate parameter differs significantly from zero, however, visual fit is poor (residuals show systematic error and fit not conservative) and χ^2 error is slightly high
Discussion	i) SFO statistically and visually acceptable? No ii) Run modified fitting. SFO statistically and visually acceptable? Deviation from SFO is not due to outliers or experimental artefacts Step 2: Correction procedure: iii) Bi-phasic pattern. Yes. iv) Aim: modelling fate of parent only? Yes. v) 10% initially measured concentration reached within experimental period? No Go to step 2 below:

[1] Normalised to pF 2.0 soil moisture

Step 2: Correction procedure (continued). 10% of initial parent mass reached? Yes, run FOMC.

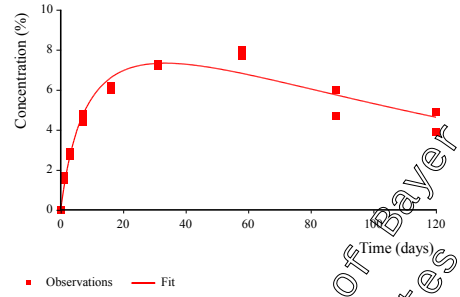
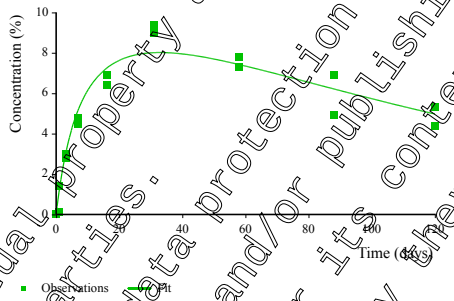
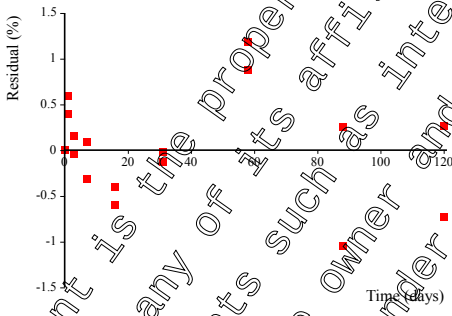
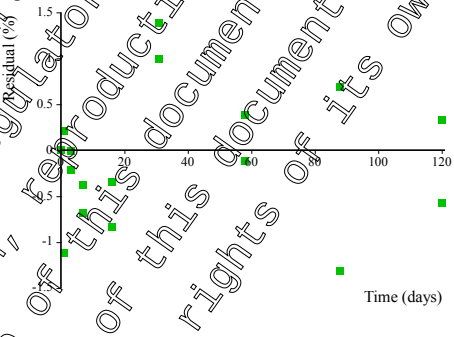
	FOMC
Plot	
Residuals	
Visual fit	Visual fit is excellent, with low χ^2 and no evidence of systematic errors in residuals. Fit versus final time points is conservative.
χ2 error (%)	6.97
t-test	N/A
DT_{50} (days)	7.36
DT_{90} (days)	217
Modelling DT_{50} (days)	65.4
Assessment	FOMC fit is acceptable. Low χ^2 and no evidence of systematic error in residuals.
Discussion	FOMC is acceptable. Use DT90/3.32 for modelling endpoint

Summary:

For spiroxamine use FOMC. $DT_{50\ mod}$ = 65.4 days (based on FOMC DT90/3.32) normalised to pF 2.0.

Appendix 3.2.6.2. Metabolite kinetics (M01 and M02)

Step 1: Run parent and metabolites all SFO. SFO fit for metabolite acceptable?

	Parent FOMC, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 error (%)	7.84	8.21
t-test	k: $p < 0.05$	k: $p < 0.05$
DT_{50} (days)	78	75.9
DT_{90} (days)	259	252
Modelling DT_{50} (days)	78	75.9
Formation fraction	0.1336	0.147
Assessment	Fit acceptable Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero	Fit acceptable Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints

[2] Normalised to pF 2.0 soil moisture

Appendix 3.2.6.3. Metabolite kinetics (M03)

Metabolite M03 was not observed for this soil.

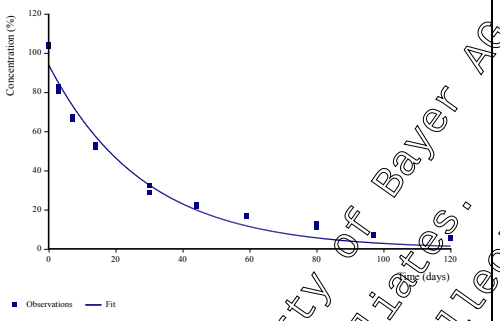
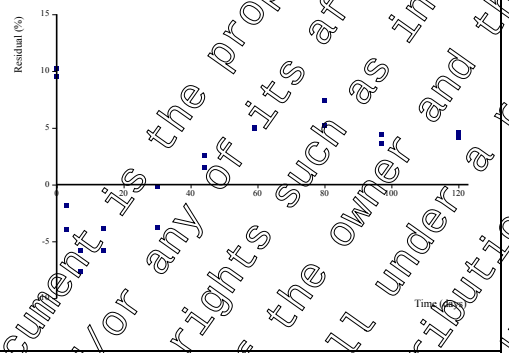
Summary:

For M01 use FOMC/SFO. $DT_{50\ mod}$ = 78.0 days, $f.f._{from\ parent}$ = 0.1336.
For M02 use FOMC/SFO. $DT_{50\ mod}$ = 75.9 days, $f.f._{from\ parent}$ = 0.147.
M03 was not observed for this soil.

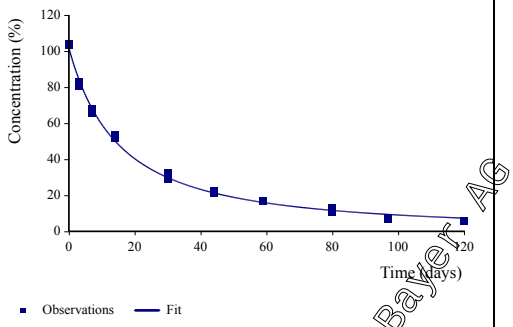
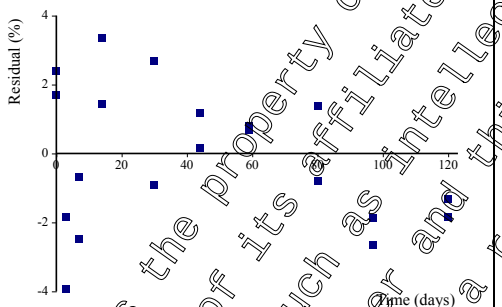
Appendix 3.2.7. Degradation of ^{14}C-cyclohexyl labelled spiroxamine, M01, M02, M03 and M06 in Longwoods soil (KCA 7.1.1.1/06 (M-762349-01-1))

Appendix 3.2.7.1. Spiroxamine kinetics

Step 1: Run SFO. SFO statistically and visually acceptable?

	SFO
Plot	
Residuals	
Visual fit	Intermediate, residuals show systematic error and fit is not conservative
χ^2 error (%)	10.8
t-test	ns. $p < 0.05$
DT_{50} (days)	19.6
DT_{90} (days)	65.2
Modelling DT_{50} (days)	19.6
Assessment	Fit not acceptable χ^2 error is low and rate parameter differs significantly from zero, however, visual fit is intermediate (residuals show systematic error and fit not conservative).
Discussion	i) SFO statistically and visually acceptable? No ii) Run modified fitting. SFO statistically and visually acceptable? Deviation from SFO is not due to outliers or experimental artefacts Step 2: Correction procedure: iii) Bi-phasic pattern. Yes. iv) Aim: modelling fate of parent only? Yes. v) 10% initially measured concentration reached within experimental period? No Go to step 2 below:

Step 2: Correction procedure (continued). 10% of initial parent mass reached? Yes, run FOMC.

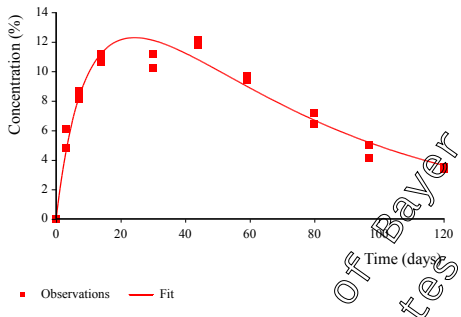
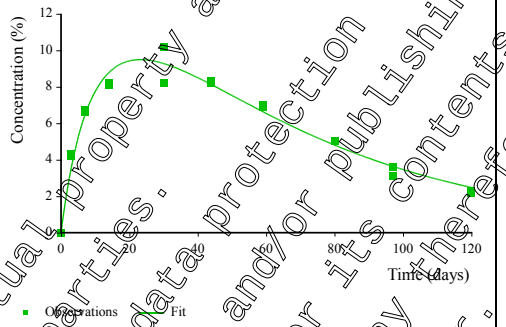
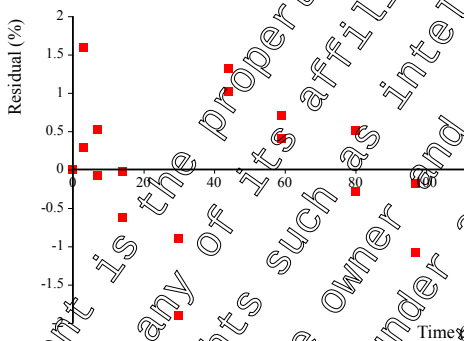
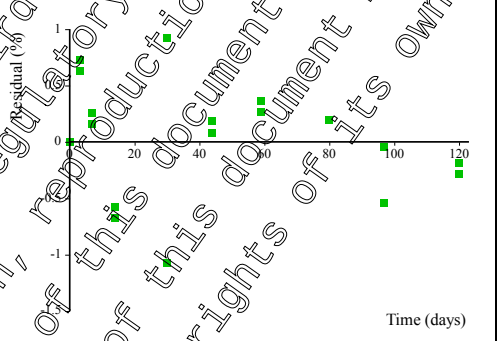
	FOMC
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 error (%)	3.68
t-test	N/A
DT_{50} (days)	13.7
DT_{90} (days)	90.2
Modelling DT_{50} (days)	27.1
Assessment	Fit acceptable Visual fit is excellent, χ^2 error is low.
Discussion	FOMC is visually acceptable with a low χ^2 and no evidence of systematic errors. Use DT90/3.32 for DT50 modelling endpoint.

Summary

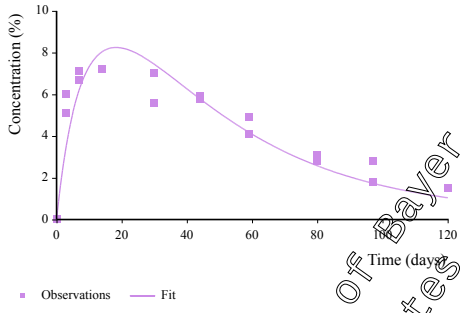
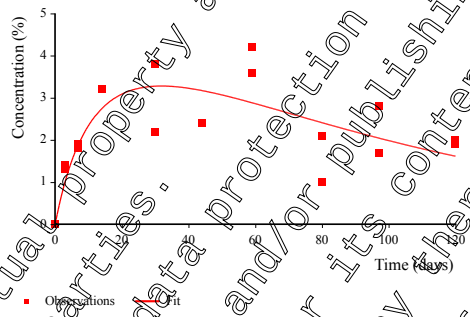
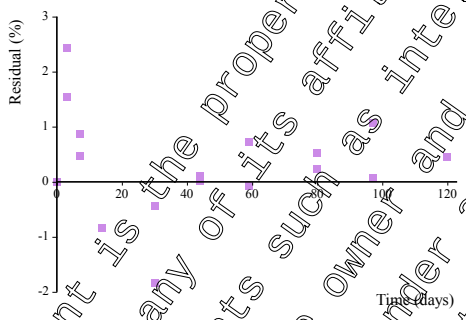
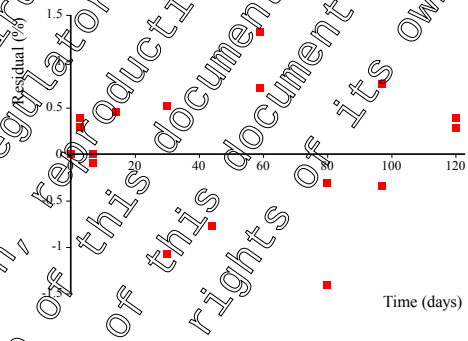
For spiroxamine use FOMC. $DT_{50\ mod}$ = 27.1 days (based on DT90/3.32).

Appendix 3.2.7.2. Metabolite kinetics (M01 and M02)

Step 1: Run parent and metabolites all SFO. SFO fit for metabolite acceptable?

	Parent FOMC, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 error (%)	7.85	4.85
t-test	*k*: p < 0.05	*k*: p < 0.05
DT_{50} (days)	34	31.2
DT_{90} (days)	113	103
Modelling DT_{50} (days)	34	31.2
Formation fraction	0.2464	0.1957
Assessment	Fit acceptable Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero	Fit acceptable Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints

Appendix 3.2.7.3. Metabolite kinetics (M03 and M06)

	Parent FOMC, metabolites SFO	
	M03	M06
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error	Intermediate, residuals show no systematic error
χ^2 error (%)	14.9	19.1
t-test	*k*: p < 0.05	*k*: p < 0.05
DT_{50} (days)	19.8	52.9
DT_{90} (days)	65.8	176
Modelling DT_{50} (days)	19.8	52.9
Formation fraction	0.199	0.05855
Assessment	Fit acceptable Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero	Fit acceptable Visual fit is good, χ^2 error is slightly high and rate parameter differs significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints

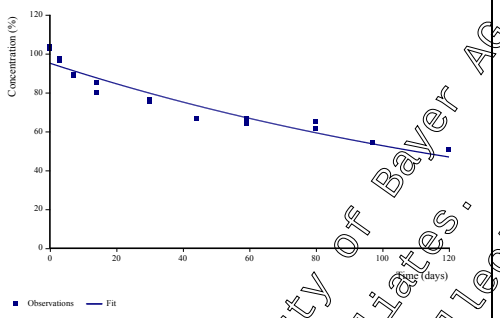
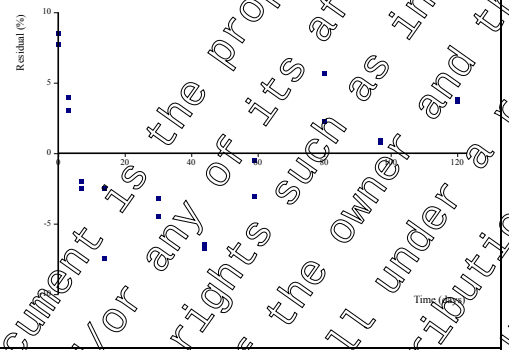
Summary:

For M01 use FOMC/SFO. $DT_{50\ mod}$ = 34.0 days, $f.f._{from\ parent}$ = 0.2464.
For M02 use FOMC/SFO. $DT_{50\ mod}$ = 31.2 days, $f.f._{from\ parent}$ = 0.1957.
For M03 use FOMC/SFO. $DT_{50\ mod}$ = 19.8 days, $f.f._{from\ parent}$ = 0.199.
For M06 use FOMC/SFO. $DT_{50\ mod}$ = 52.9 days, $f.f._{from\ parent}$ = 0.0586.

Appendix 3.2.8. Degradation of ^{14}C-cyclohexyl labelled spiroxamine, M01, M02, M03 and M06 in Refesol 02-A soil (KCA 7.1.1.1/06 (M-762349-01-1))

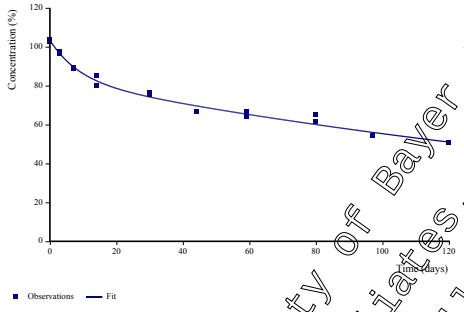
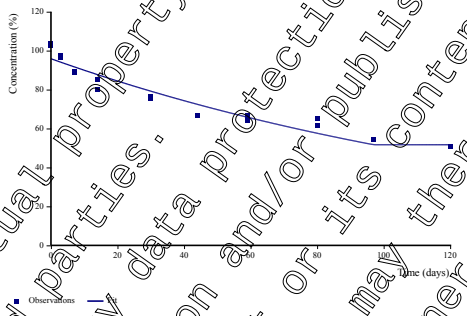
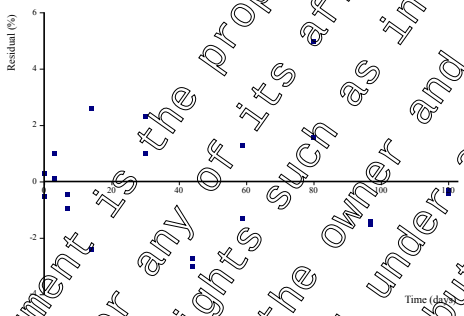
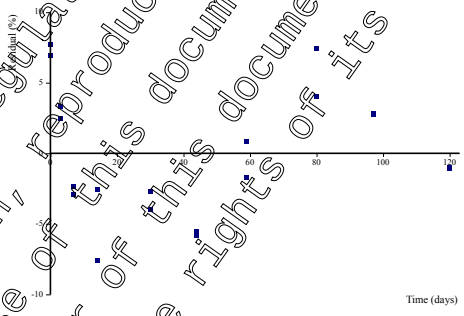
Appendix 3.2.8.1. Spiroxamine kinetics

Step 1: Run SFO. SFO statistically and visually acceptable?

	SFO
Plot	
Residuals	
Visual fit	Intermediate residuals show no systematic error
χ^2 error (%)	4.9
t-test	✗. $p < 0.05$
DT_{50} (days)	117
DT_{90} (days)	389[1]
Modelling DT_{50} (days)	117
Assessment	Fit potentially acceptable. Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero
Discussion	i) SFO statistically and visually acceptable? Potentially, but as fitting using SFO negatively impacts metabolite fits (total formation fraction >1, see appendix 2.8.3) bi-phasic models investigated below. ii) Run modified fitting. SFO statistically and visually acceptable? Deviation from SFO is not due to outliers or experimental artefacts Step 2. Correction procedure: iii) Bi-phasic pattern. Yes. iv) Aim: modelling fate of parent only? Yes. v) 10% initially measured concentration reached within experimental period? No Go to step 2 below:

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Correction procedure (continued). Run HS or DFOP. vi) HS or DFOP statistically and visually acceptable?

	DFOP	HS
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error	Good, residuals show no systematic error
χ^2 error (%)	1.9	4.9
t-test	k_1: p >0.05 >0.1 k_2: p < 0.05	k_1: p <0.05 k_2: p >0.1
DT_{50} (days)	116	>10,000[1]
DT_{90} (days)	510[1]	>10,000[1]
Modelling DT_{50} (days)	k_2 DT_{50} = 170[1]	k_2 DT_{50} = >10,000[1]
Assessment	Fit acceptable Visual fit is excellent, χ^2 error is low and rate parameters differ significantly from zero	Fit not acceptable Visual fit is good, χ^2 error is low, however k_2 rate parameter not significantly different to zero
Discussion	vi) HS or DFOP statistically and visually acceptable? DFOP acceptable. Use DT50 from slow phase of DFOP model for fate modelling	

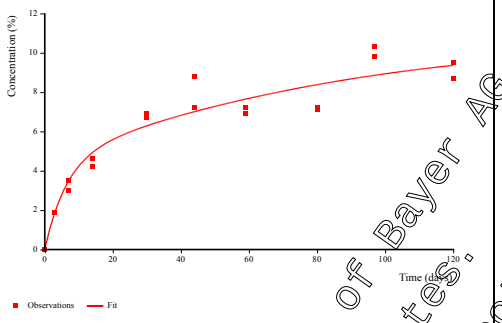
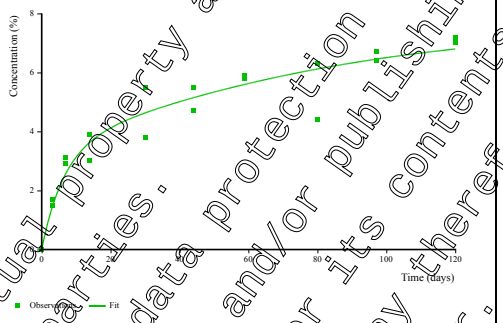
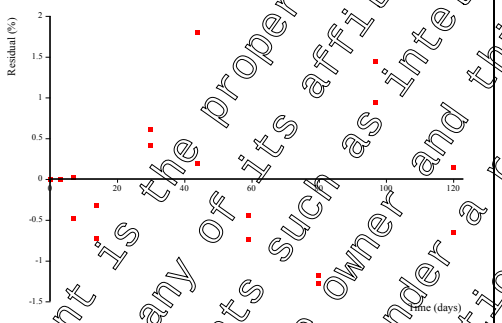
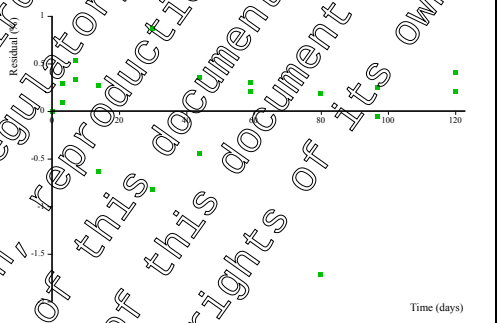
1 Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use DFOP. DT_{50mod} = 170 days (based on k_2 DFOP).

Appendix 3.2.8.2. Metabolite kinetics (M01 and M02)

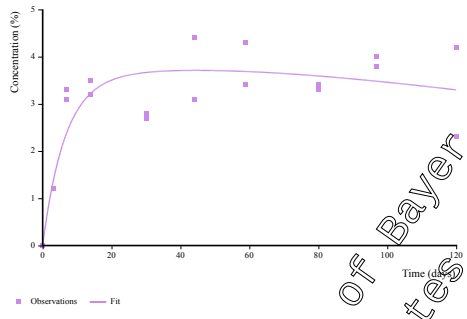
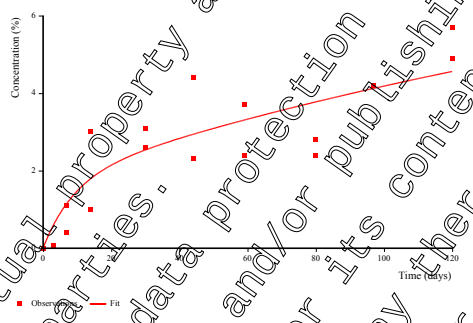
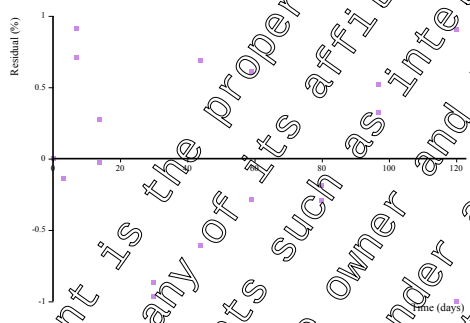
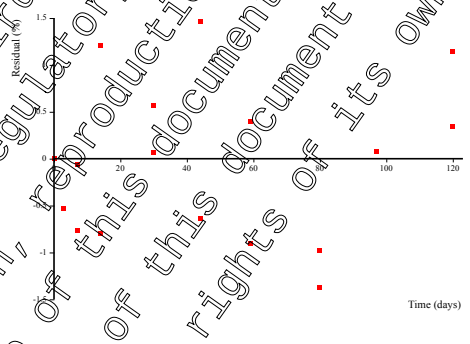
Step 1: Run parent and metabolites all SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Intermediate, residuals show no systematic error
χ^2 error (%)	9.3	5.6
t-test	*k*: p <0.05	*k*: p < 0.05
DT_{50} (days)	219[1]	204[1]
DT_{90} (days)	727[1]	678[1]
Modelling DT_{50} (days)	219[1]	204[1]
Formation fraction	0.225	0.166
Assessment	Fit acceptable Visual fit is good, χ^2 error is low, and rate parameter differs significantly from zero	Fit acceptable Visual fit is intermediate, χ^2 error is low and rate parameter differs significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints

[1] Interpret with care – extrapolated beyond experimental period

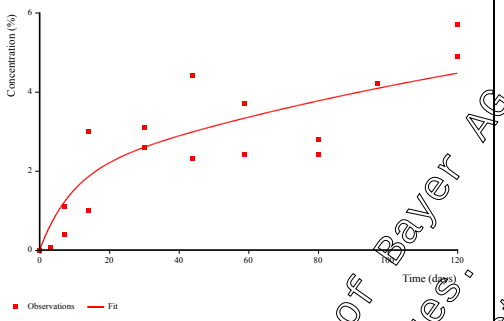
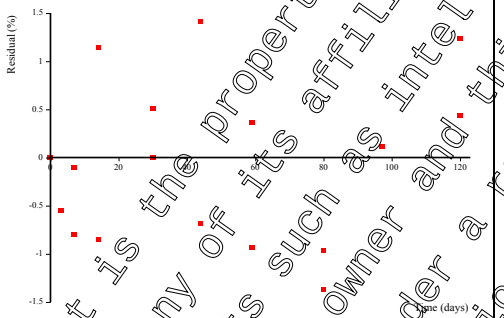
Appendix 3.2.8.3. Metabolite kinetics (M03 and M06)

Step 1: Run parent and metabolites all SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M03	M06
Plot		
Residuals		
Visual fit	Intermediate, residuals show no systematic error	Intermediate, residuals show no systematic error
χ^2 error (%)	10.2	16.6
t-test	*k*: p < 0.05	*k*: p > 0.1
DT_{50} (days)	53.1	>10,000[1]
DT_{90} (days)	176[1]	>10,000[1]
Modelling DT_{50} (days)	53.1	>10,000[1]
Formation fraction	0.160	0.087
Assessment	Fit acceptable Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero	Fit not acceptable Visual fit is intermediate and χ^2 error is only slightly high, however rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints	iii) SFO fit for metabolite acceptable? SFO is not considered acceptable. Therefore, DFOP or FOMC should be assessed. However, these models are not appropriate for metabolites which are formed gradually. Thereafter, decline after max should be assessed. However, this was not possible as there is no decline data to clearly establish the dissipation pattern. Therefore, use of default DT_{50} and formation fraction of 0.09 proposed as a worst case.

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Run parent best-fit and metabolite SFO, DT_{50} fixed to default value. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO M06
Plot	
Residuals	
Visual fit	Intermediate, residuals show no systematic error
χ^2 error (%)	16.1
t-test	NA
DT_{50} (days)	1000[1]
DT_{90} (days)	3,320
Modelling DT_{50} (days)	1000
Formation fraction	0.09
Assessment	Visual fit is intermediate and χ^2 error is only slightly high
Discussion	iii) SFO using a default DT_{50} is considered acceptable and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period

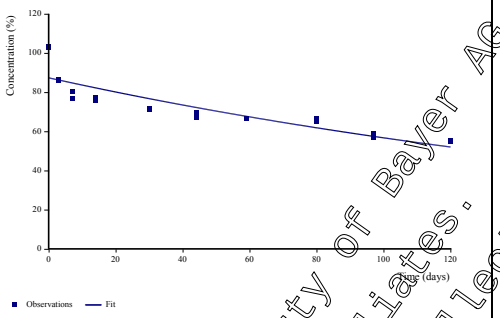
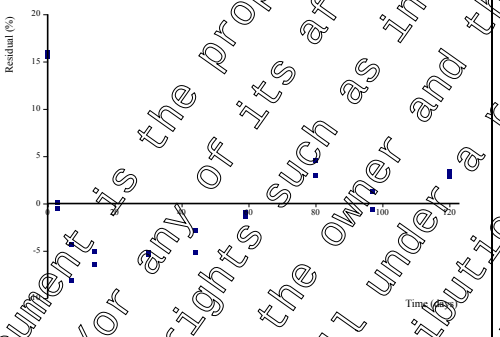
Summary:

For M01 use DFOP/SFO. $DT_{50\ mod}$ = 219 days, $f.f._{from\ parent}$ = 0.225.
For M02 use DFOP/SFO. $DT_{50\ mod}$ = 204 days, $f.f._{from\ parent}$ = 0.166.
For M03 use DFOP/SFO. $DT_{50\ mod}$ = 53.1 days, $f.f._{from\ parent}$ = 0.160.
For M06 M02 use DFOP/SFO (using conservative parameters). $DT_{50\ mod}$ = 1000 days, $f.f._{from\ parent}$ = 0.09.

Appendix 3.2.9. Degradation of ^{14}C-cyclohexyl labelled spiroxamine, M01, M02, M03 and M06 in Refesol 03-G soil (KCA 7.1.1.1/06 (M-762349-01-1))

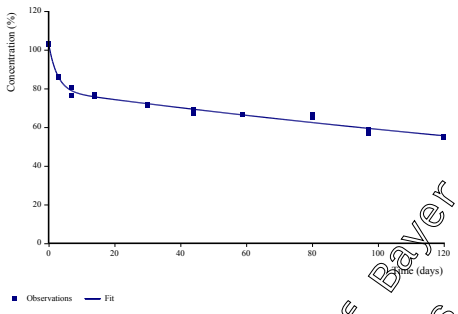
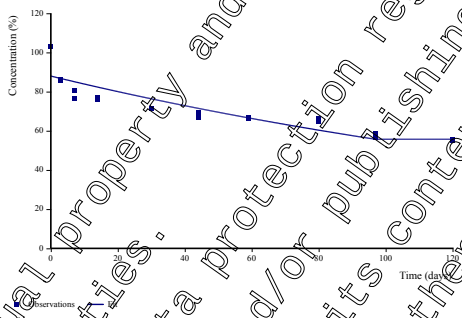
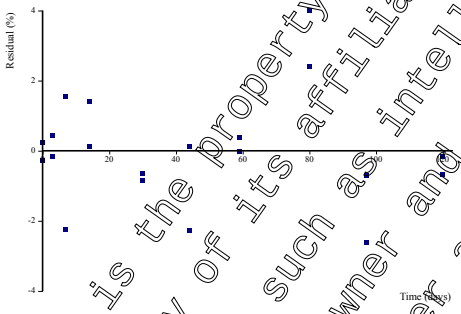
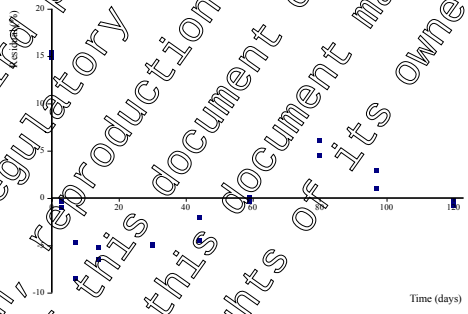
Appendix 3.2.9.1. Spiroxamine kinetics

Step 1: Run SFO. SFO statistically and visually acceptable?

	SFO
Plot	
Residuals	
Visual fit	Intermediate, residuals show systematic error and fit is not conservative
χ^2 error (%)	7.2
t-test	ns. $p < 0.05$
DT_{50} (days)	159[1]
DT_{90} (days)	527[1]
Modelling DT_{50} (days)	159[1]
Assessment	Fit not acceptable χ^2 error is low and rate parameter differs significantly from zero, however, visual fit is intermediate (residuals show systematic error)
Discussion	i) SFO statistically and visually acceptable? No. ii) Run modified fitting. SFO statistically and visually acceptable? Deviation from SFO is not due to outliers or experimental artefacts Step 2: Correction procedure: iii) Bi-phasic pattern. Yes. iv) Aim: modelling fate of parent only? Yes. v) 10% initially measured concentration reached within experimental period? No Go to step 2 below:

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Correction procedure (continued). Run HS or DFOP. vi) HS or DFOP statistically and visually acceptable?

	DFOP	HS
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error	Good, however residuals show systematic errors
χ^2 error (%)	1.5	7.1
t-test	k_1: $p < 0.05$ k_2: $p < 0.05$	k_1: $p < 0.05$ k_2: $p > 0.1$
DT_{50} (days)	142[1]	>10,000[1]
DT_{90} (days)	696[1]	>10,000[1]
Modelling DT_{50} (days)	k_2 DT_{50} = 239	k_2 DT_{50} = >10,000[1]
Assessment	Fit acceptable Visual fit is excellent, χ^2 error is low and rate parameters differ significantly from zero	Fit not acceptable Visual fit is good, χ^2 error is low, k_2 parameter does not differ significantly from zero
Discussion	vi) HS or DFOP statistically and visually acceptable? DFOP acceptable. Use DT_{50} from slow phase of DFOP model for fate modelling	

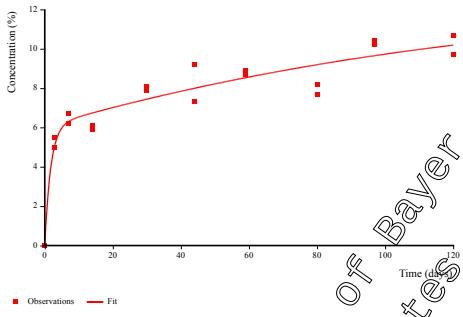
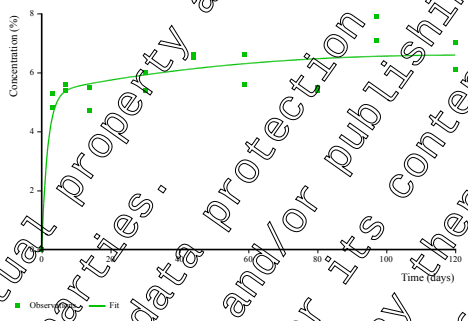
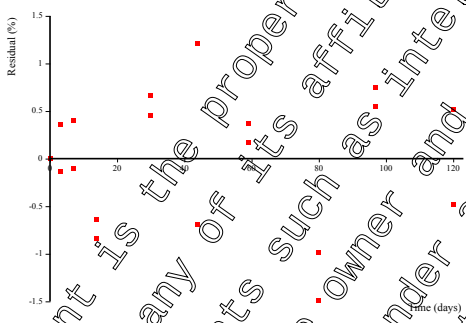
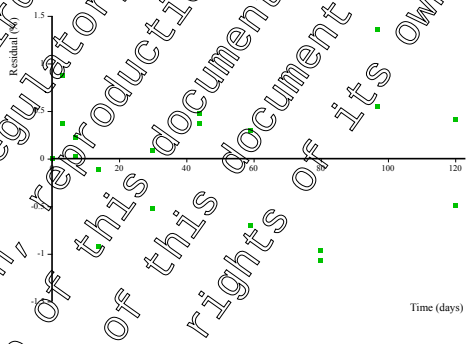
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use DFOP. $DT_{50\ mod}$ = 239 days (based on k_2 DFOP).

Appendix 3.2.9.2. Metabolite kinetics (M01 and M02)

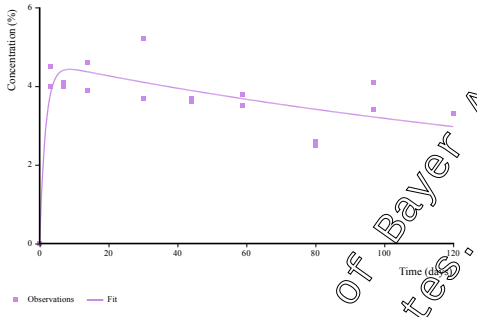
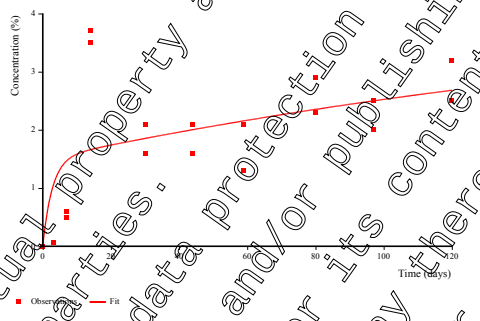
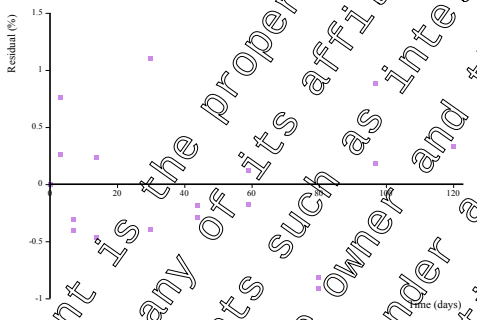
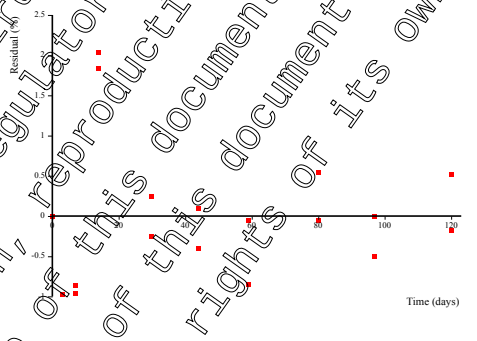
Step 1: Run parent and metabolites all SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 error (%)	5.8	7.6
t-test	*k*: $p < 0.05$	*k*: $p < 0.05$
DT_{50} (days)	304[1]	120
DT_{90} (days)	1,010[1]	399[1]
Modelling DT_{50} (days)	304[1]	120
Formation fraction	0.258	0.224
Assessment	Fit acceptable Visual fit is good and χ^2 error is low, however, rate parameter does not differ significantly from zero	Fit acceptable Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints

[1] Interpret with care – extrapolated beyond experimental period

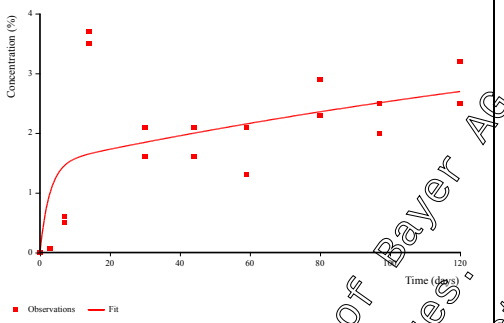
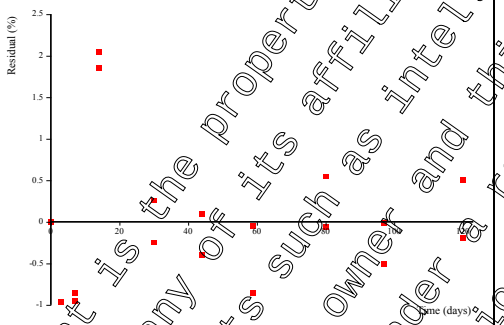
Appendix 3.2.9.3. Metabolite kinetics (M03 and M06)

Step 1: Run parent and metabolites all SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M03	M06
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Intermediate, residuals show no systematic error
χ^2 error (%)	9.3	33.9
t-test	*k*: $p < 0.05$	*k*: $p > 0.1$
DT_{50} (days)	48	1,380[1]
DT_{90} (days)	159[1]	4,570[1]
Modelling DT_{50} (days)	48	1,380[1]
Formation fraction	0.192	0.061
Assessment	Fit acceptable Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero	Fit not acceptable Visual fit is intermediate, χ^2 error is high and rate parameter does not differ significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints	iii) SFO fit for metabolite acceptable? SFO is not considered acceptable. Therefore, DFOP or FOMC should be assessed. However, these models are not appropriate for metabolites which are formed gradually. Thereafter, decline after max should be assessed. However, this was not possible as there is no decline data to clearly establish the dissipation pattern. Therefore, use of default DT_{50} and modelled formation fraction of 0.061 investigated as a realistic worst case.

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Run parent best-fit and metabolite SFO, DT_{50} fixed to default value. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO M06
Plot	
Residuals	
Visual fit	Intermediate, residuals show no systematic error
χ^2 error (%)	30.8
t-test	NA
DT_{50} (days)	1000[1]
DT_{90} (days)	3,320
Modelling DT_{50} (days)	1000
Formation fraction	0.061
Assessment	Visual fit is intermediate and χ^2 error is only slightly high
Discussion	iii) SFO using a default DT_{50} is considered acceptable and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period

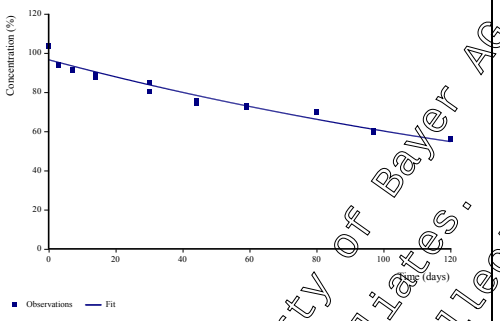
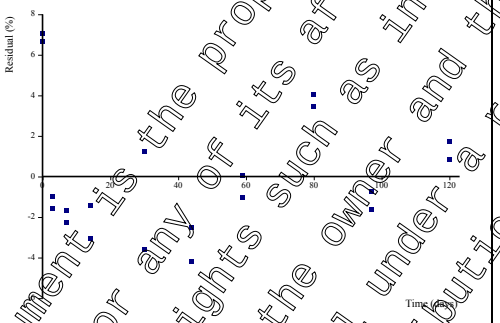
Summary:

For M01 use DFOP/SFO. $DT_{50\ mod}$ = 304 days, $f.f._{from\ parent}$ = 0.258.
For M02 use DFOP/SFO. $DT_{50\ mod}$ = 120 days, $f.f._{from\ parent}$ = 0.224.
For M03 use DFOP/SFO. $DT_{50\ mod}$ = 48 days, $f.f._{from\ parent}$ = 0.192.
For M06 use DFOP/SFO (using conservative parameters). $DT_{50\ mod}$ = 1000 days, $f.f._{from\ parent}$ = 0.061.

Appendix 3.2.10. Degradation of ^{14}C-cyclohexyl labelled spiroxamine, M01, M02, M03 and M06 in Speyer 6S soil (KCA 7.1.1.1/06 (M-762349-01-1))

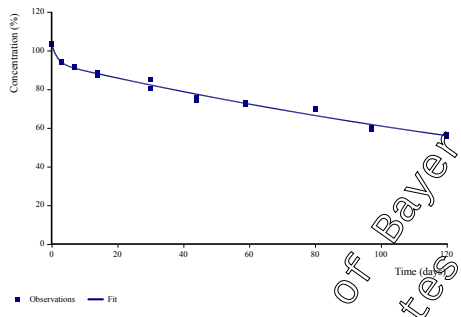
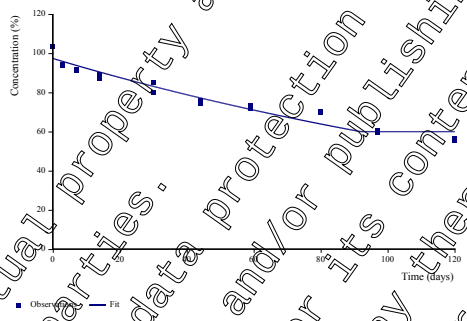
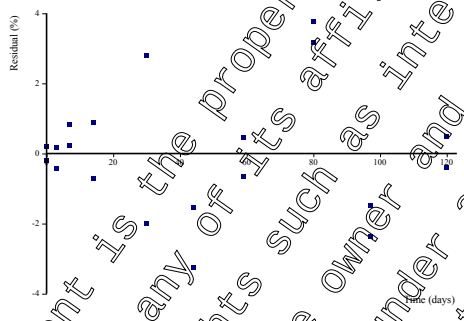
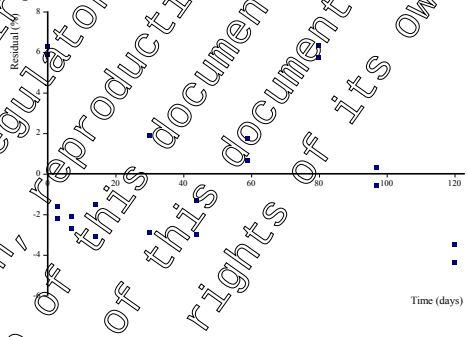
Appendix 3.2.10.1. Spiroxamine kinetics

Step 1: Run SFO. SFO statistically and visually acceptable?

	SFO
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error and fit is conservative
χ^2 error (%)	3.2
t-test	✓, $p < 0.05$
DT_{50} (days)	146[1]
DT_{90} (days)	484[1]
Modelling DT_{50} (days)	146
Assessment	Fit potentially acceptable. Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero
Discussion	i) SFO statistically and visually acceptable? Potentially, but as fitting using SFO negatively impacts metabolite fits (total formation fraction >1, see appendix) bi-phasic models investigated below. ii) Run modified fitting. SFO statistically and visually acceptable? Deviation from SFO is not due to outliers or experimental artefacts Step 2: Correction procedure: iii) Bi-phasic pattern. Yes. iv) Aim: modelling fate of parent only? Yes. v) 10% initially measured concentration reached within experimental period? No Go to step 2 below:

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Correction procedure (continued). Run HS or DFOP. vi) HS or DFOP statistically and visually acceptable?

	DFOP	HS
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error	Excellent, residuals show no systematic error
χ^2 error (%)	1.7	3.7
t-test	k_1: $p > 0.05$ k_2: $p < 0.05$	k_1: $p < 0.05$ k_2: $p > 0.1$
DT_{50} (days)	137[1]	>10,000[1]
DT_{90} (days)	514[1]	>10,000[1]
Modelling DT_{50} (days)	k_2 DT_{50} = 163[1]	k_2 DT_{50} = >10,000[1]
Assessment	Fit acceptable Visual fit is excellent, χ^2 error is low and rate parameters differ significantly from zero	Fit not acceptable Visual fit is good, χ^2 error is low, however k_2 rate parameter is not significantly different to zero
Discussion	vi) HS or DFOP statistically and visually acceptable? DFOP acceptable. DFOP should be used for modelling endpoints best acceptable fit) Use DT50 from slow phase of DFOP model for fate modelling	

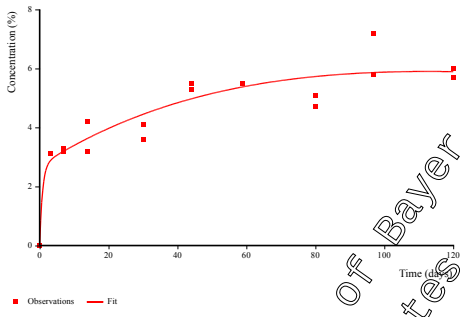
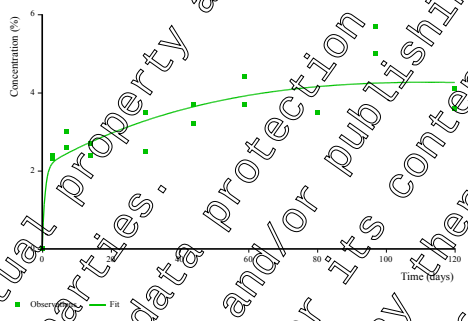
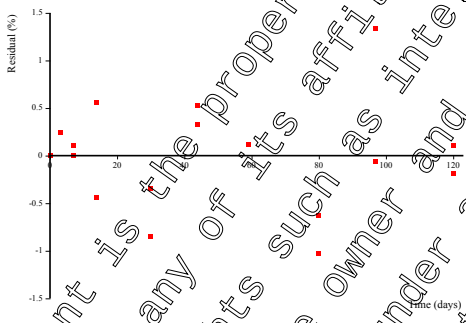
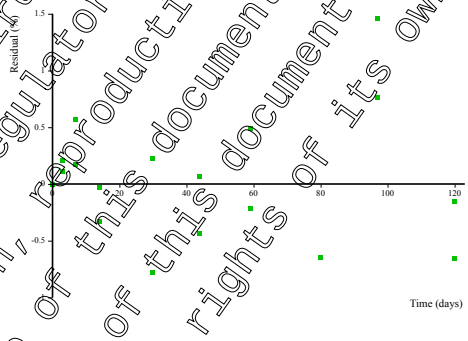
1 Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use DFOP. $DT_{50\ mod}$ = 163 days (based on k_2 DFOP).

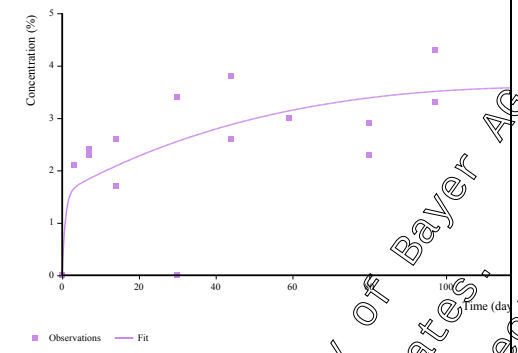
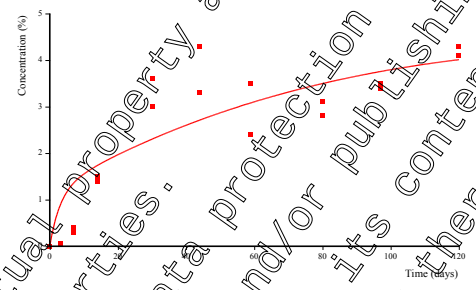
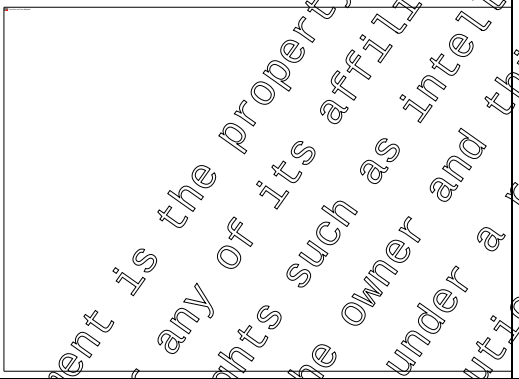
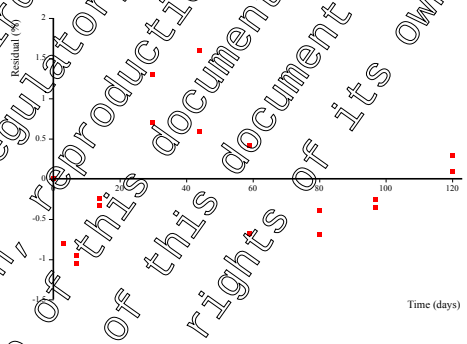
Appendix 3.2.10.2.Metabolite kinetics (M01 and M02)

Step 1: Run parent and metabolites all SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 error (%)	7.4	11.4
t-test	*k*: p <0.05	*k*: p <0.05
DT_{50} (days)	70.7	65.2
DT_{90} (days)	235[1]	217[1]
Modelling DT_{50} (days)	70.7	65.2
Formation fraction	0.242	0.183
Assessment	Fit acceptable Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero	Fit acceptable Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints

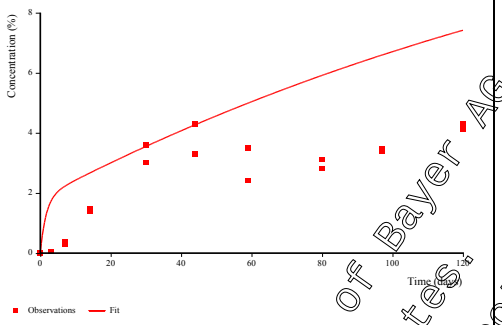
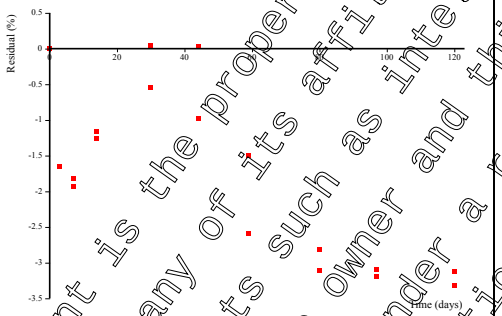
[1] Interpret with care – extrapolated beyond experimental period

Appendix 3.2.10.3.Metabolite kinetics (M03 and M06)

	Parent DFOP, metabolites SFO	
	M03	M06
Plot		
Residuals		
Visual fit	Good, fit is conservative, residuals show no systematic error	Intermediate, residuals show no systematic error
χ^2 error (%)	14.3	23.5
t-test	*k*: p < 0.05	*k*: p > 0.1
DT_{50} (days)	79	191[1]
DT_{90} (days)	262[1]	635[1]
Modelling DT_{50} (days)	79	191[1]
Formation fraction	0.137	0.112
Assessment	Fit acceptable Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero	Fit not acceptable Visual fit is intermediate and χ^2 error is slightly high, rate parameter does not differ significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints	iii) SFO fit for metabolite acceptable? SFO is not considered acceptable. Therefore, DFOP or FOMC should be assessed. However, these models will not improve the fit.. Thereafter, decline after max should be assessed. However, this was not possible as there is limited decline data to clearly establish the dissipation pattern. Therefore, use of default DT50 and formation fraction of 0.17 investigated as a realistic worst case.

Step 2: Case-by-case decision (alternative – but conservative – estimates).

SFO fit for metabolite acceptable?

	Parent DFOP, metabolites SFO M06
Plot	
Residuals	
Visual fit	Conservative
χ^2 error (%)	NA
t-test	NA
DT_{50} (days)	1000[1]
DT_{90} (days)	3,320[1]
Modelling DT_{50} (days)	1000[1]
Formation fraction	0.17
Assessment	Visual fit is conservative
Discussion	iii) SFO is considered conservative and should be used for modelling endpoints

[1] Interpret with care – extrapolated beyond experimental period

Summary:

For M01 use DFOP/SFO. $DT_{50\ mod}$ = 70.7 days, $f.f._{from\ parent}$ = 0.242.
For M02 use DFOP/SFO. $DT_{50\ mod}$ = 65.2 days, $f.f._{from\ parent}$ = 0.183.
For M03 use DFOP/SFO. $DT_{50\ mod}$ = 79.0 days, $f.f._{from\ parent}$ = 0.137.
For M06 use DFOP/SFO (using conservative parameters). $DT_{50\ mod}$ = 1,000 days, $f.f._{from\ parent}$ = 0.170.

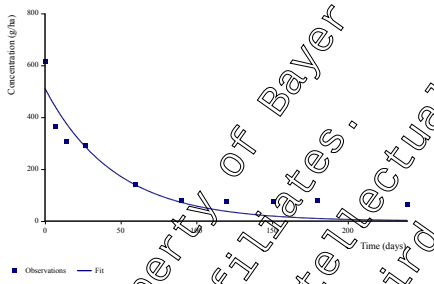
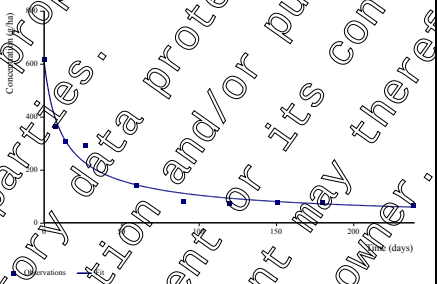
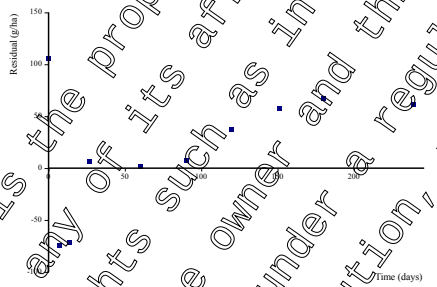
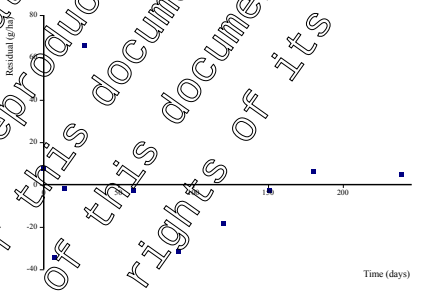
Appendix 4: Kinetic evaluation of field soil studies

Appendix 4.1: Kinetic evaluation for persistence/trigger endpoints

Appendix 4.1.1. Dissipation of spiroxamine in Höfchen soil trial no. 30122/1 (KCA 7.1.2.2.1.01 (M-006116-01-1))

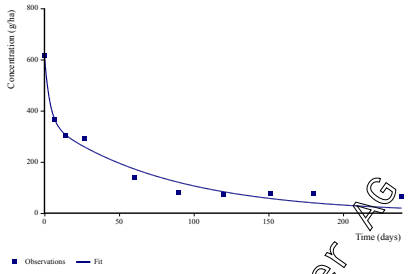
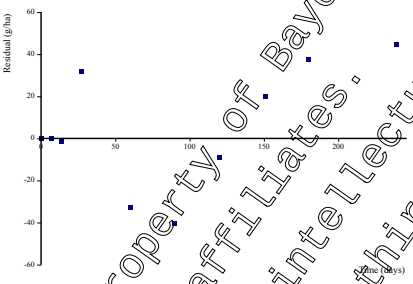
Appendix 4.1.1.1. Parent Fitting

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor; residuals show systematic error and fit not conservative.	Excellent; residuals show no potential systematic error.
χ^2 err %	22.7	10.7
t-test	*k*: $p < 0.05$	NA
$DissT_{50}$ (days)	31.9	14.3
$DissT_{90}$ (days)	106	230[1]
Assessment	Fit not acceptable. Rate parameter differs significantly from zero, however, visual fit is poor and χ^2 error is slightly high.	Fit acceptable. Visual fit is excellent and χ^2 error is acceptable.
Conclusion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed. iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No. Go to step 2 below.	

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Possible bi-phasic decline. Fit DFOP.

	DFOP
Plot	
Residuals	
Visual fit	Intermediate, residuals show potential systematic error.
χ^2 err %	11.8
t-test	k_1: $p > 0.1$; $k_2 < 0.05$
$DissT_{50}$ (days)	13.8
$DissT_{90}$ (days)	145[1]
Assessment	Fit acceptable. Visual fit is reasonable and χ^2 error is acceptable, however, k_1 rate parameters do not differ significantly from zero at the 5% level.
Conclusion	iv) Determine which of the models (FOMC, DFOP) is best. Both give acceptable fit. v) Does the best-fit model give an acceptable description of the data? Yes with both models returning similar end-points. Both datasets were run for metabolites fits and FOMC was found to give a very poor fit (see Appendix 6). DFOP was therefore selected as best fit for parent.

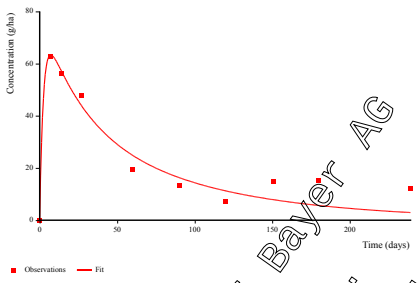
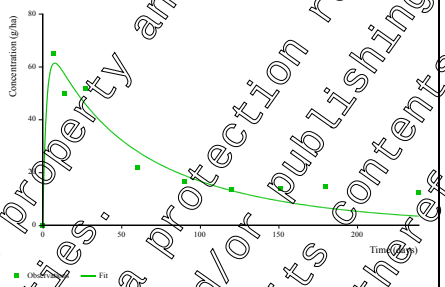
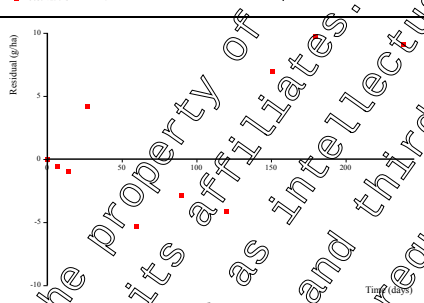
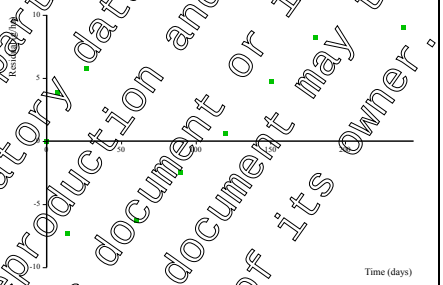
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use DFOP. $DissT_{50}$ = 13.8 days, $DissT_{90}$ = 145 days.

Appendix 4.1.1.2. Metabolite fitting (M01 and M02)

Step 4: Run parent best-fit and metabolite SFO. iii) SFO fit for metabolite acceptable?

	Parent DFOP, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Intermediate, final time points underestimated	Intermediate, final time points underestimated
χ^2 err %	16.6	16.5
t-test	*k*: p < 0.05	*k*: p < 0.05
$DissT_{50}$ (days)	18.1	21
$DissT_{90}$ (days)	60.2	69.6
Assessment	Fit acceptable. Rate parameter differs significantly from zero, χ^2 error is slightly high and visual fit is intermediate. Residuals at final three time points are considered to be due to data scatter rather than the kinetic fit being inappropriate.	Fit acceptable. Rate parameter differs significantly from zero, χ^2 error is slightly high and visual fit is intermediate. Residuals at final three time points are considered to be due to data scatter rather than the kinetic fit being inappropriate.
Discussion	iii) SFO fit for metabolite acceptable? is considered acceptable and should be used for persistence endpoints.	iii) SFO fit for metabolite acceptable? is considered acceptable and should be used for persistence endpoints.

Summary:

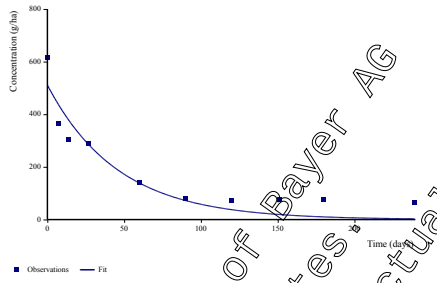
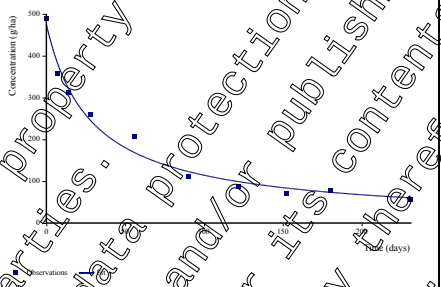
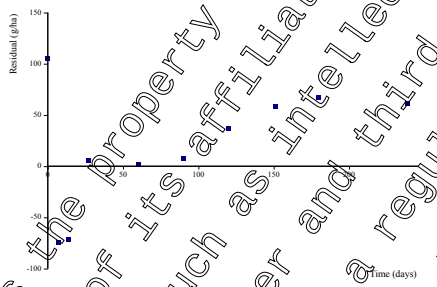
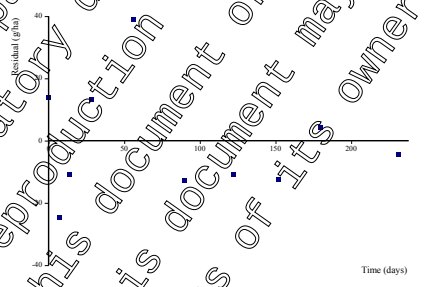
For metabolite M01 use DFOP/SFO $DissT_{50}$ = 18.1 days, $DissT_{90}$ = 60.2 days.

For metabolite M02 use DFOP/SFO $DissT_{50}$ = 21 days, $DissT_{90}$ = 69.6 days.

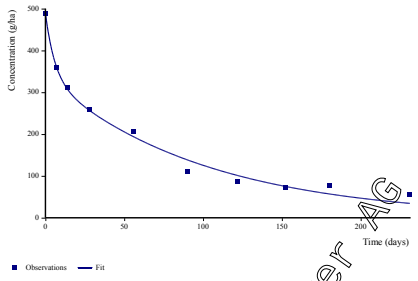
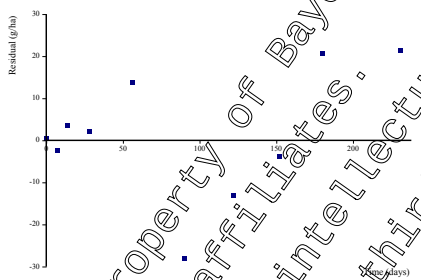
Appendix 4.1.2. Dissipation of spiroxamine in Laacher Hof soil trial no. 30124/8 (KCA 7.1.2.2.1/01 (M-006116-01-1))

Appendix 4.1.2.1. Parent Fitting

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor, residuals show systematic error and fit not conservative	Excellent, residuals show no systematic error
χ^2 err %	13.3	7.3
t-test	k_1 p < 0.05	NA
$DisST_{50}$ (days)	52.2	29.9
$DisST_{90}$ (days)	173[1]	308[1]
Assessment	Fit not acceptable. χ^2 error is acceptable and rate parameter differs significantly from zero, however, visual fit is poor (residuals show systematic error and fit not conservative).	Fit acceptable. Visual fit is excellent and χ^2 error is low.
Conclusion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed. iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No. Go to step 2 below.	

Step 2: Possible bi-phasic decline. Fit DFOP.

	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 err %	6.3
t-test	k_1: p < 0.05 k_2: p < 0.05
DissT50 (days)	32.6
$DissT_{90}$ (days)	196[1]
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	iv) Determine which of the models (FOMC, DFOP) is best. Both FOMC and DFOP give acceptable fits. DFOP selected (lower χ^2 error). v) Does the best-fit model give an acceptable description of the data? Yes. DFOP should be used for persistence endpoints.

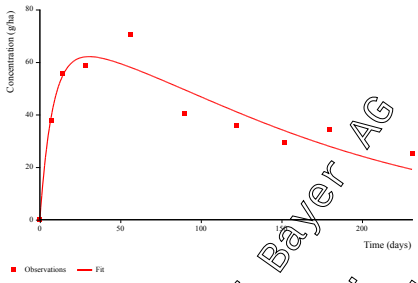
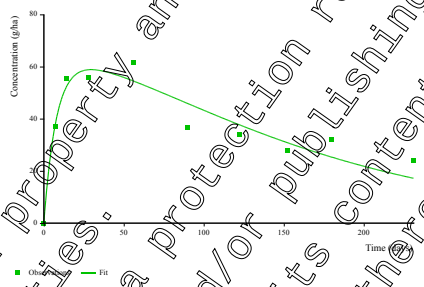
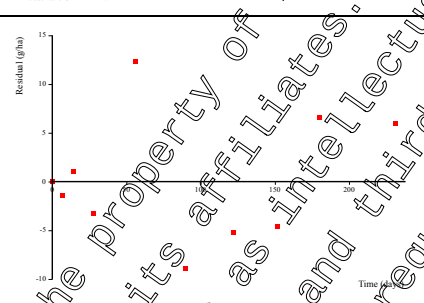
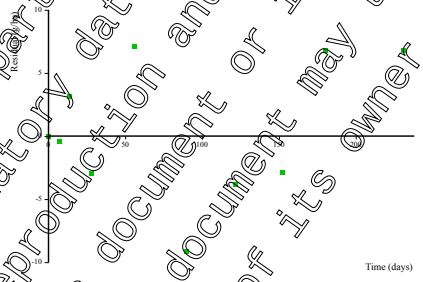
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use DFOP. DissT50 = 32.6 days, $DissT_{90}$ = 196 days.

Appendix 4.1.2.2. Metabolite fitting (M01 and M02)

Step 4: Run parent best-fit and metabolite SFO. iii) SFO fit for metabolite acceptable?

	Parent DFOP, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error	Excellent, residuals show no systematic error
χ^2 err %	11.9	10.8
t-test	*k*: p < 0.05	*k*: p < 0.05
DissT50 (days)	50.3	48.3
DT_{90} (days)	167	160
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is acceptable and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is excellent, χ^2 error is acceptable and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

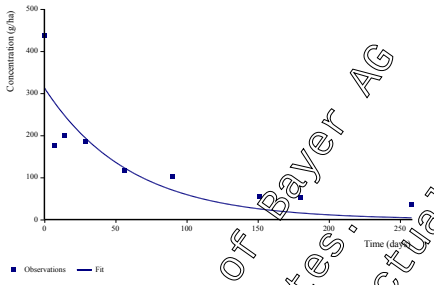
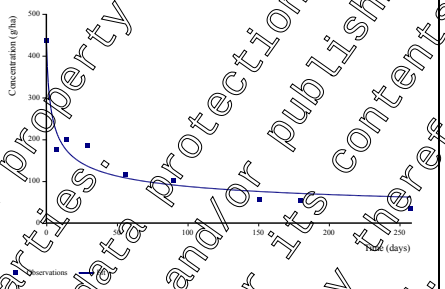
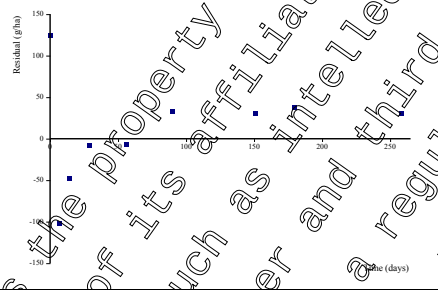
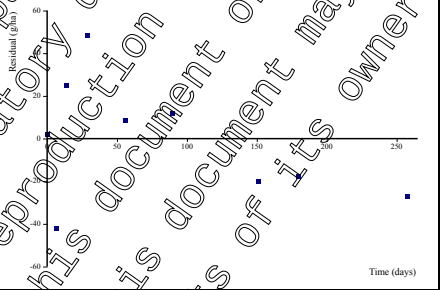
For metabolite M01 use DFOP/SFO DissT50 = 50.3 days, $DissT_{90}$ = 167 days.

For metabolite M02 use DFOP/SFO DissT50 = 48.3 days, $DissT_{90}$ = 160 days.

Appendix 4.1.3. Dissipation of spiroxamine in Elm Farm/Thurston soil trial no. 30262/7 (KCA 7.1.2.2.1/01 (M-006116-01-1))

Appendix 4.1.3.1. Parent Fitting

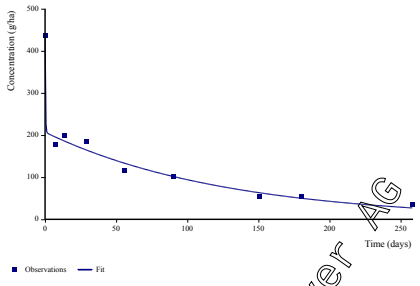
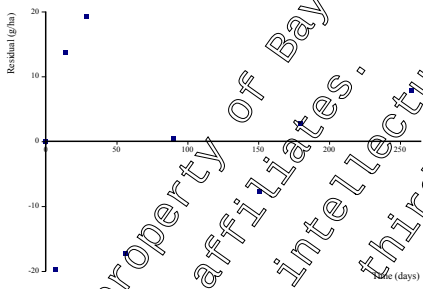
Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor, residuals show systematic error and fit not conservative.	Good, residuals show no systematic error.
χ^2 err %	31.8	14.9
t-test	k_1 p < 0.05	NA
$DisST_{50}$ (days)	41.7	7.13
$DisST_{90}$ (days)	138[1]	683[1]
Assessment	Fit not acceptable. Rate parameter differs significantly from zero, however, visual fit is poor and χ^2 error is high.	Fit potentially acceptable. Visual fit is excellent and χ^2 error is acceptable. However, significant extrapolation beyond experimental period to DT90[2].
Conclusion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed. iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No. Go to step 2 below.	

[1]Interpret with care – extrapolated beyond experimental period

[2] EFSA Scientific Report (2009) 338, 1-32: "Report on the PPR Stakeholder Workshop Improved Realism in Soil Risk Assessment (IRS) – How will pesticide risk assessment in soil be tackled tomorrow?"

Step 2: Possible bi-phasic decline. Fit DFOP.

	DFOP
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error
χ^2 err %	7.4
t-test	k_1: ns k_2: < 0.05
$DissT_{50}$ (days)	0.8
$DissT_{90}$ (days)	197[1]
Assessment	Fit potentially acceptable. Visual fit is excellent and χ^2 error is low, however, rate parameter (k_1 only) does not differs significantly from zero.
Conclusion	iv) Determine which of the models (FOMC, DFOP) is best. DFOP gives best fit (lowest χ^2 err). v) Does the best-fit model give an acceptable description of the data? Yes DFOP should be used for persistence endpoints.

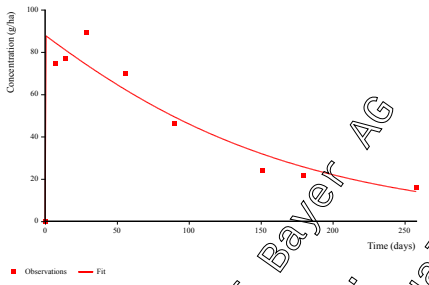
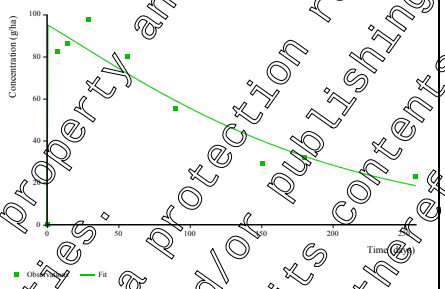
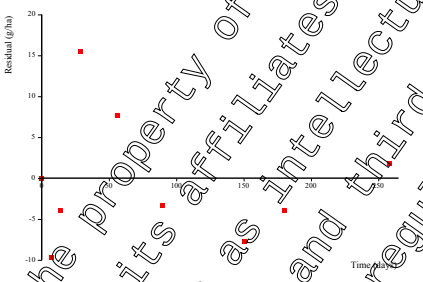
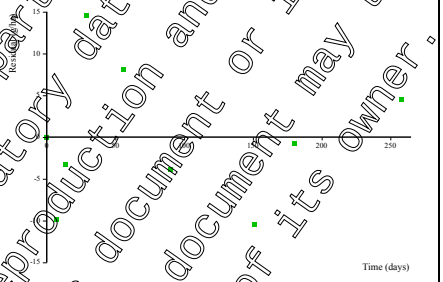
[1]Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use DFOP. $DissT_{50}$ = 0.8 days, $DissT_{90}$ = 197 days.

Appendix 4.1.3.2. Metabolite fitting (M01 and M02)

Step 4: Run parent best-fit and metabolite SFO. iii) SFO fit for metabolite acceptable?

	Parent DFOP, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error.	Good, residuals show no systematic error.
χ^2 err %	12.0	10.7
t-test	*k*: p < 0.05	*k*: p < 0.05
$DissT_{50}$ (days)	51.4	57.7
$DissT_{90}$ (days)	171	192
Assessment	Fit acceptable. Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints.

[1] Interpret with care – extrapolated beyond experimental period.

Summary:

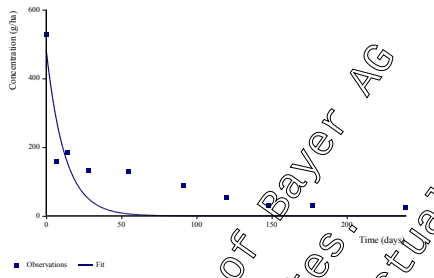
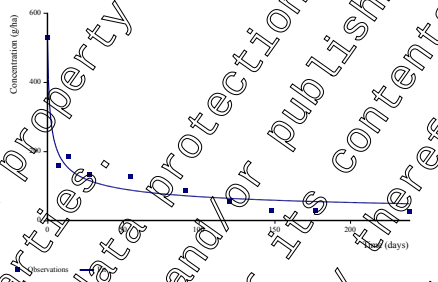
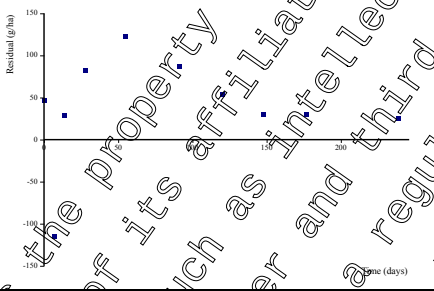
For metabolite M01 use FOMC/SFO $DissT_{50}$ = 51.4 days, $DissT_{90}$ = 171 days.

For metabolite M02 use FOMC/SFO $DissT_{50}$ = 57.7 days, $DissT_{90}$ = 192 days.

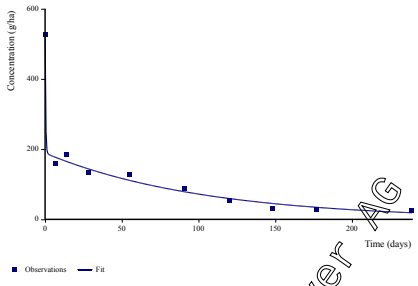
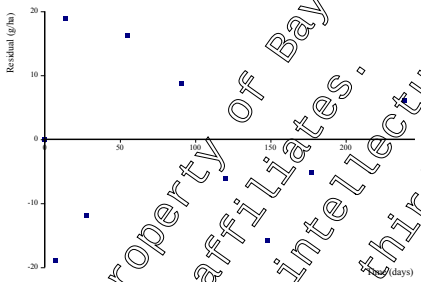
Appendix 4.1.4. Dissipation of spiroxamine in Pakenham soil trial no. 30263/5 (KCA 7.1.2.2.1/01 (M-006116-01-1))

Appendix 4.1.4.1. Parent Fitting

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor, residuals show systematic error and fit not conservative.	Good, residuals show no systematic error.
χ^2 err %	42.2	16.4
t-test	k_1 p< 0.05	NA
$DisST_{50}$ (days)	8.6	3.0
$DisST_{90}$ (days)	28.5	194
Assessment	Fit not acceptable. Rate parameter differs significantly from zero, however, visual fit is poor and χ^2 error is high.	Fit acceptable. Visual fit is good and χ^2 error is slightly high.
Conclusion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed. iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No. Go to step 2 below.	

Step 2: Possible bi-phasic decline. Fit DFOP.

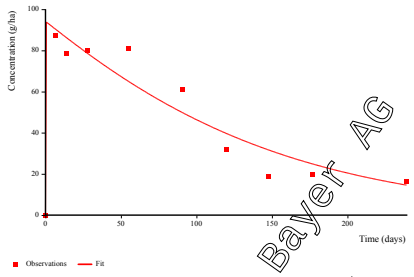
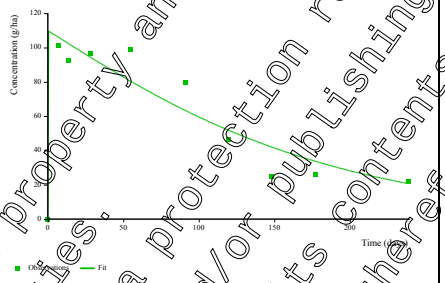
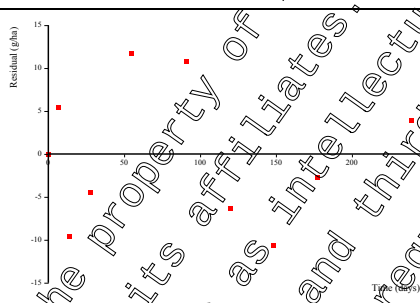
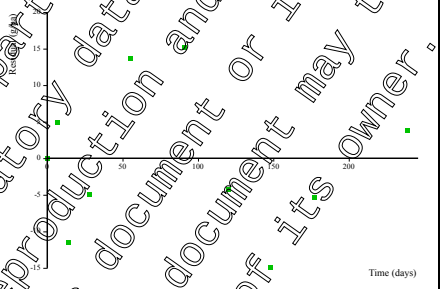
	DFOP
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error
χ^2 err %	8.2
t-test	k_1: p > 0.1 k_2: p< 0.05
$DissT_{50}$ (days)	0.5
$DissT_{90}$ (days)	132[1]
Assessment	Fit acceptable. Visual fit is excellent and χ^2 error is low, however, rate parameters do not differs significantly from zero.
Conclusion	iv) Determine which of the models (FOMC, DFOP) is best. DFOP gives best fit (lowest χ^2). v) Does the best-fit model give an acceptable description of the data? Yes DFOP should be used for persistence endpoints.

Summary

For spiroxamine use DFOP. $DissT_{50}$ = 0.5 days, $DissT_{90}$ = 132 days.

Appendix 4.1.4.2. Metabolite fitting (M01 and M02)

Step 4: Run parent best-fit and metabolite SFO. iii) SFO fit for metabolite acceptable?

	Parent FOMC, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 err %	13.5	13.7
t-test	*k*: p < 0.05	*k*: p < 0.05
$DissT_{50}$ (days)	57.4	63.8
$DissT_{90}$ (days)	191	212
Assessment	Fit acceptable. Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

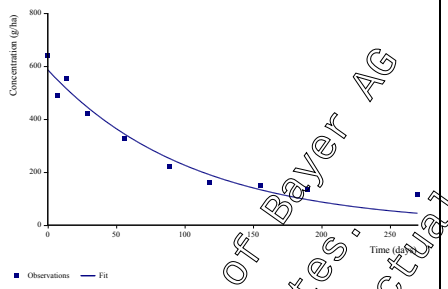
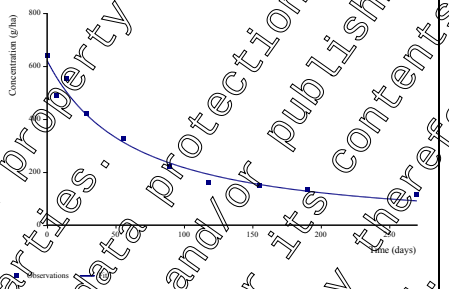
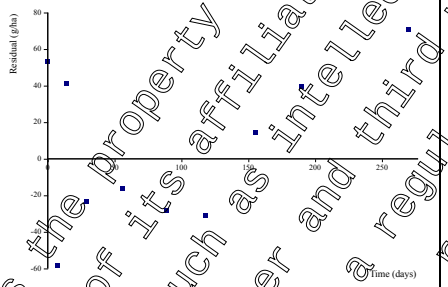
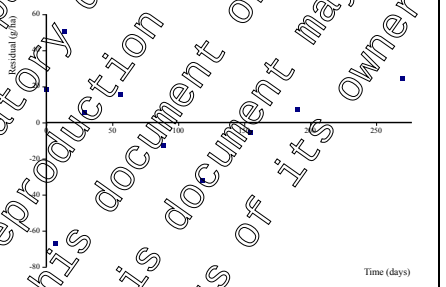
For metabolite M01 use FOMC/SFO $DissT_{50}$ = 57.4days, $DissT_{90}$ = 191 days.

For metabolite M02 use FOMC/SFO $DissT_{50}$ = 63.8 days, $DissT_{90}$ = 212 days.

Appendix 4.1.5. Dissipation of spiroxamine in Höfchen trial no. 40006/8 (KCA 7.1.2.2.1/02 (M-006126-01-1))

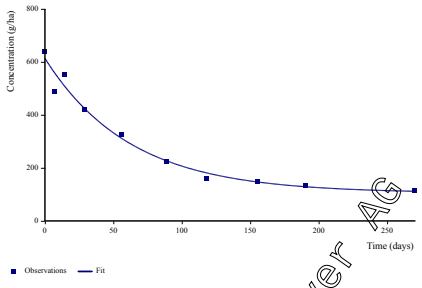
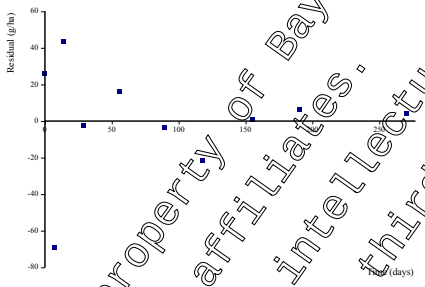
Appendix 4.1.5.1. Parent Fitting

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor, residuals show systematic error and fit not conservative.	Excellent, residuals show no systematic error.
χ^2 err %	10.4	8.1
t-test	k_1 p < 0.05	NA
$DisST_{50}$ (days)	72.8	56.6
$DisST_{90}$ (days)	242[1]	393[1]
Assessment	Fit not acceptable. χ^2 error is acceptable and rate parameter differs significantly from zero, however, visual fit is poor (residuals show systematic error and fit not conservative).	Fit acceptable. Visual fit is excellent and χ^2 error is low.
Conclusion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed. iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No. Go to step 2 below.	

1 Interpret with care – extrapolated beyond experimental period

Step 2: Possible bi-phasic decline. Fit DFOP.

	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 err %	7.0
t-test	k_1: p < 0.05 k_2: p > 0.1
$DissT_{50}$ (days)	57.5
$DissT_{90}$ (days)	>10,000[1]
Assessment	Fit potentially acceptable. Visual fit is excellent and χ^2 error is low, however, rate parameters do not differ significantly from zero at a 5% level.
Conclusion	iv) Determine which of the models (FOMC, DFOP) is best. FOMC gives best fit. v) Does the best-fit model give an acceptable description of the data? Yes Both biphasic models were evaluated for assessing metabolite kinetics. DFOP metabolites fits were poor, as such FOMC selected as best fit model.

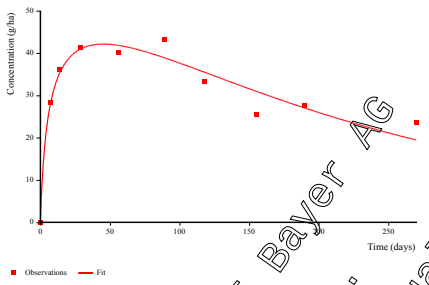
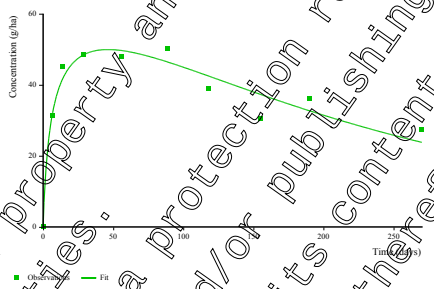
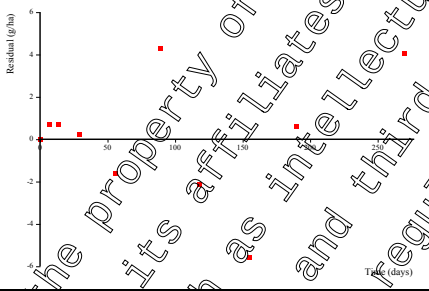
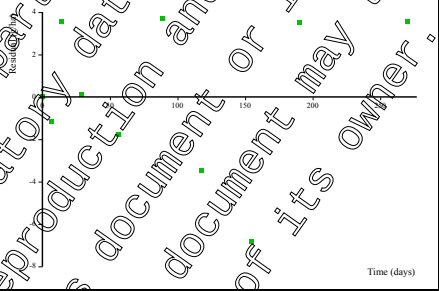
1 Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use FOMC. $DissT_{50}$ = 56.6 days, $DissT_{90}$ = 393 days.

Appendix 4.1.5.2. Metabolite fitting (M01 and M02)

Step 4: Run parent best-fit and metabolite SFO. iii) SFO fit for metabolite acceptable?

	Parent FOMC, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error	Excellent, residuals show no systematic error
χ^2 err %	6.93	7.19
t-test	*k*: p < 0.05	*k*: p < 0.05
$DisST_{50}$ (days)	130	135
$DisST_{90}$ (days)	432[1]	447[1]
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence end-points.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence end-points.

Summary:

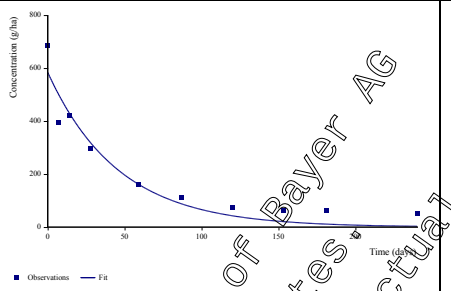
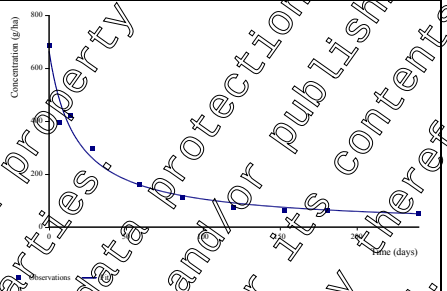
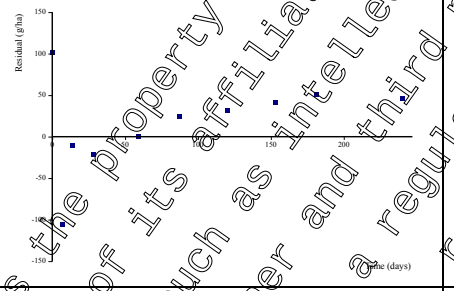
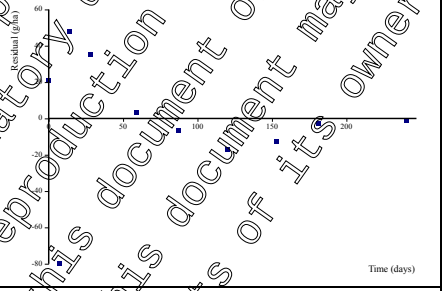
For metabolite M01 use FOMC/SFO $DisST_{50}$ = 130 days, $DisST_{90}$ = 432 days.

For metabolite M02 use FOMC/SFO $DisST_{50}$ = 135 days, $DisST_{90}$ = 447 days.

Appendix 4.1.6. Dissipation of spiroxamine in Laacher Hof soil trial no. 40007/6 (KCA 7.1.2.2.1/02 (M-006126-01-1))

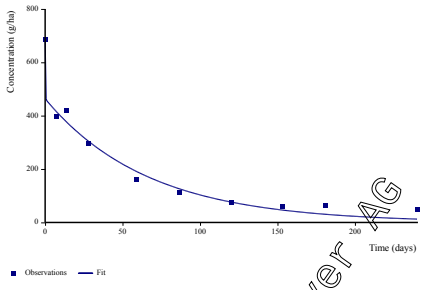
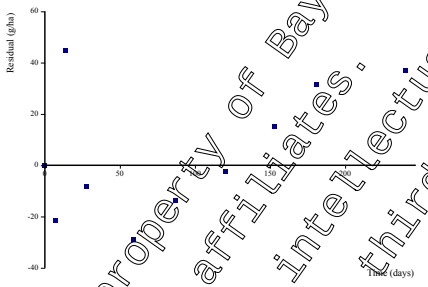
Appendix 4.1.6.1. Parent Fitting

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor, residuals show systematic error and fit not conservative	Excellent, residuals show no systematic error
χ^2 err %	18.9	12.0
t-test	k_1 p < 0.05	NA
DT_{50} (days)	31.8	17.9
DT_{90} (days)	106	175[1]
Assessment	Fit not acceptable. Rate parameter differs significantly from zero, however, visual fit is poor (residuals show systematic error and fit not conservative) and χ^2 error is slightly high.	Fit acceptable. Visual fit is excellent and χ^2 error is acceptable.
Conclusion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed. iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No. Go to step 2 below.	

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Possible bi-phasic decline. Fit DFOP.

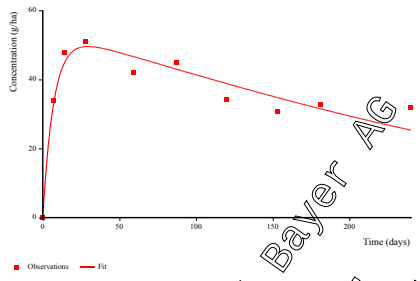
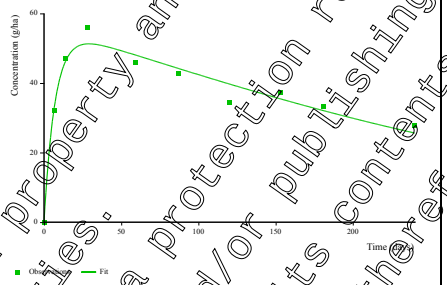
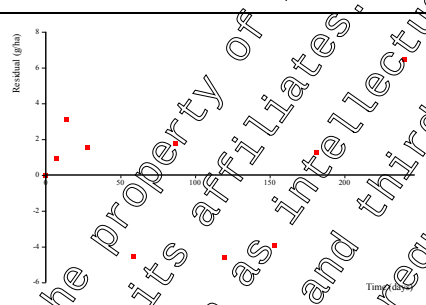
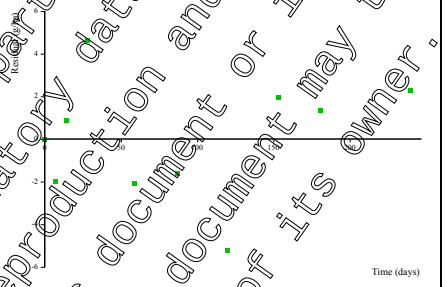
	DFOP
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error.
χ^2 err %	9.6
t-test	k_1: p > 0.1 k_2: p < 0.05
$DissT_{50}$ (days)	20.1
$DissT_{90}$ (days)	127
Assessment	Fit acceptable. Visual fit is good and χ^2 error is low, however, rate parameters do not differ significantly from zero.
Conclusion	iv) Determine which of the models (FOMC, DFOP) is best. DFOP gives best fit (lowest χ^2) v) Does the best-fit model give an acceptable description of the data? Yes DFOP should be used for persistence endpoints.

Summary

For spiroxamine use DFOP $DissT_{50}$ = 20.1 days, $DissT_{90}$ = 127 days.

Appendix 4.1.6.2. Metabolite fitting (M01 and M02)

Step 4: Run parent best-fit and metabolite SFO. iii) SFO fit for metabolite acceptable?

	Parent DFOP, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error.	Excellent, residuals show no systematic error.
χ^2 err %	7.42	5.67
t-test	*k*: p < 0.05	*k*: p < 0.05
$DissT_{50}$ (days)	125[1]	122
$DissT_{90}$ (days)	416[1]	406[1]
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence end-points.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence end-points.

[1] Interpret with care – extrapolated beyond experimental period

Summary

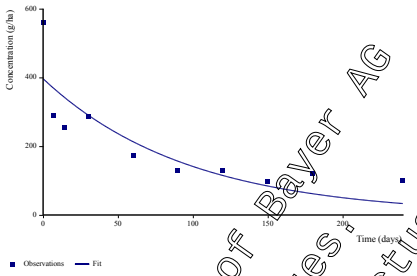
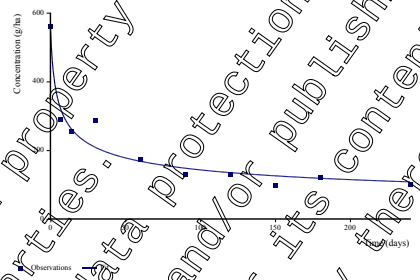
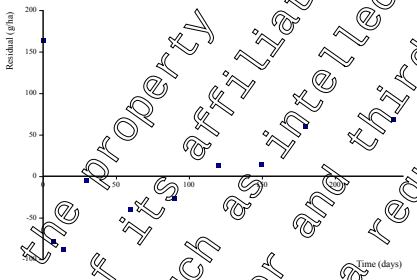
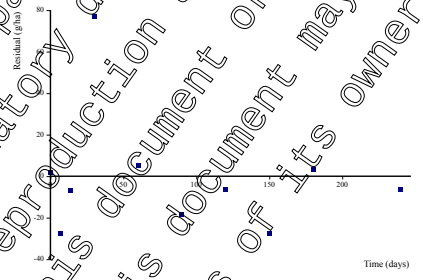
For metabolite M01 use FOMC/SFO $DissT_{50}$ = 125 days, $DissT_{90}$ = 416 days.

For metabolite M02 use FOMC/SFO $DissT_{50}$ = 122 days, $DissT_{90}$ = 406 days.

Appendix 4.1.7. Dissipation of spiroxamine in Maasen soil trial no. 40008/4 (KCA 7.1.2.2.1/02 (M-006126-01-1))

Appendix 4.1.7.1. Parent Fitting

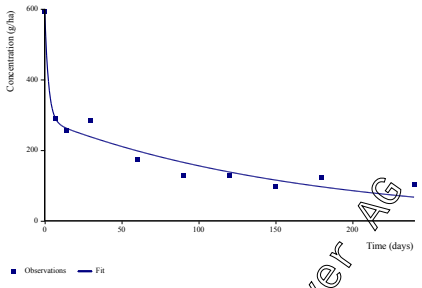
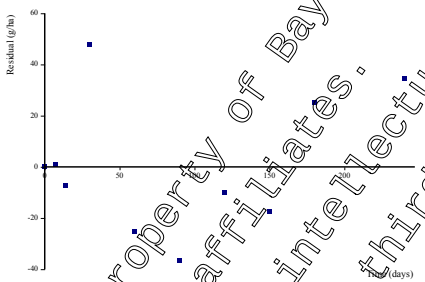
Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor, residuals show systematic error and fit not conservative.	Excellent, residuals show no systematic error.
χ^2 err %	26.9	11.1
t-test	*k*: $p < 0.05$	NA
$DisST_{50}$ (days)	67.1	11.3
$DisST_{90}$ (days)	223[1]	1,870[1]
Assessment	Fit not acceptable. Rate parameter differs significantly from zero, however, visual fit is poor and χ^2 error is high.	Fit potentially acceptable. Visual fit is excellent and χ^2 error is acceptable. However, significant extrapolation beyond experimental period to DT90[2].
Conclusion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not considered acceptable. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed. iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No. Go to step 2 below.	

1 Interpret with care – extrapolated beyond experimental period

2 EFSA Scientific Report (2009) 338, 1-32: "Report on the PPR Stakeholder Workshop Improved Realism in Soil Risk Assessment (IRIS) – How will pesticide risk assessment in soil be tackled tomorrow?"

Step 2: Possible bi-phasic decline. Fit DFOP.

	DFOP
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error.
χ^2 err %	10.6
t-test	k_1: p > 0.1 k_2: p < 0.05
$DisST_{50}$ (days)	8.4
$DisST_{90}$ (days)	271[1]
Assessment	Fit potentially acceptable. Visual fit is good and χ^2 error is acceptable, however, *k1* rate parameter does not differ significantly from zero.
Conclusion	iv) Determine which of the models (FOMC, DFOP) is best. DFOP gives best fit (lower χ^2). v) Does the best-fit model give an acceptable description of the data? Yes. DFOP should be used for persistence endpoints.

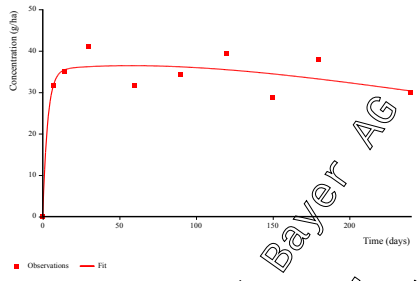
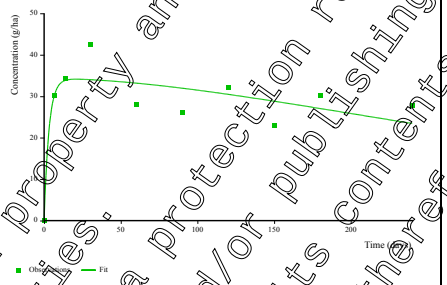
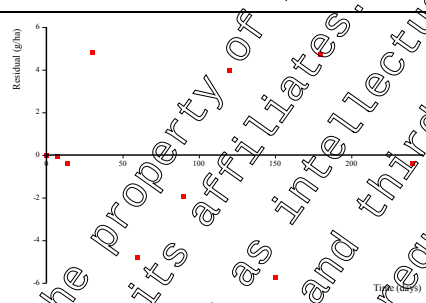
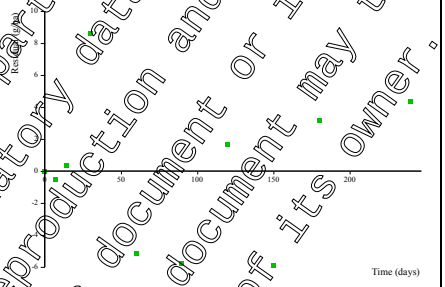
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use DFOP. $DisST_{50}$ = 8.4 days, $DisST_{90}$ = 271 days.

Appendix 4.1.7.2. Metabolite fitting (M01 and M02)

Step 4: Run parent best-fit and metabolite SFO. iii) SFO fit for metabolite acceptable?

	Parent DFOP, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error.	Excellent, residuals show no systematic error.
χ^2 err %	8.54	12.4
t-test	*k*: p < 0.05	*k*: p < 0.05
$DissT_{50}$ (days)	223	161
$DissT_{90}$ (days)	742[1]	533[1]
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence end-points.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence end-points.

1 Interpret with care – extrapolated beyond experimental period

Summary:

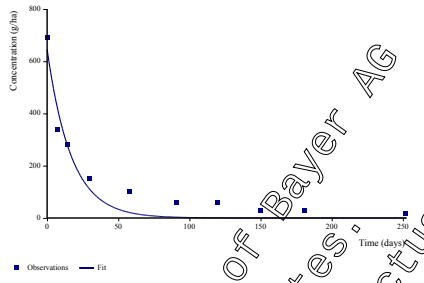
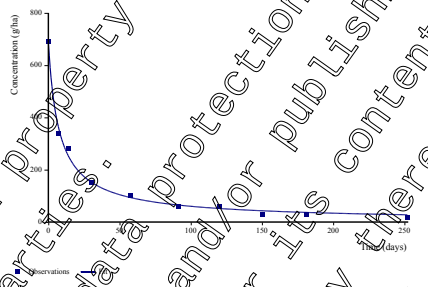
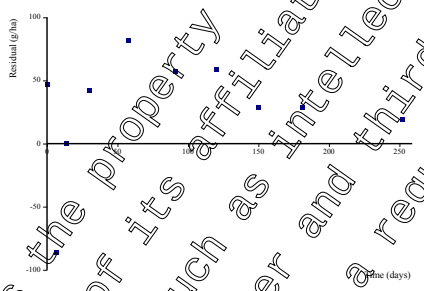
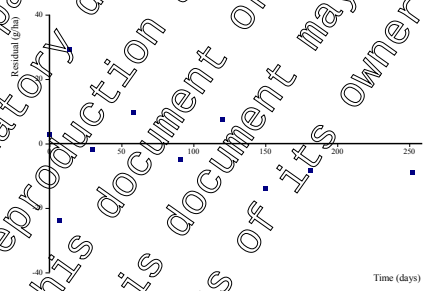
For metabolite M01 use FOMC/SFO $DissT_{50}$ = 223 days, $DissT_{90}$ = 742 days.

For metabolite M02 use FOMC/SFO $DissT_{50}$ = 161 days, $DissT_{90}$ = 533 days.

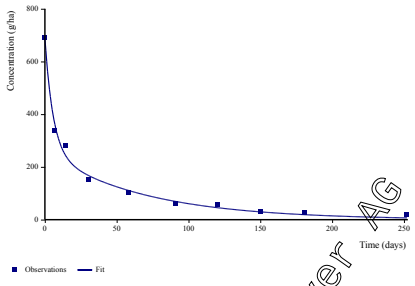
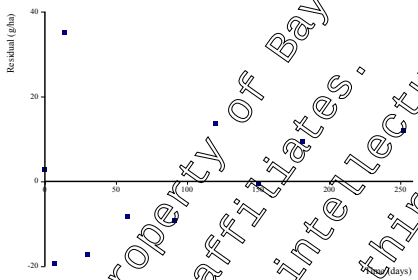
Appendix 4.1.8. Dissipation of spiroxamine in Swisttal-Hohn soil trial no. 40009/2 (KCA 7.1.2.2.1/02 (M-006126-01-1))

Appendix 4.1.8.1. Parent Fitting

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor, residuals show systematic error and fit not conservative.	Excellent, residuals show no systematic error.
χ^2 err %	23.6	6.7
t-test	k p < 0.05	NA
$DisST_{50}$ (days)	11.7	7.9
$DisST_{90}$ (days)	38.9	84.7
Assessment	Fit not acceptable. Rate parameter differs significantly from zero, however, visual fit is poor and χ^2 error is slightly high.	Fit acceptable. Visual fit is excellent and χ^2 error is low.
Conclusion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed. iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No. Go to step 2 below.	

Step 2: Possible bi-phasic decline. Fit DFOP.

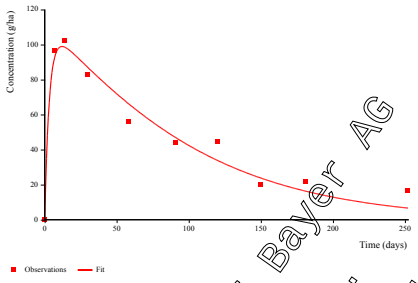
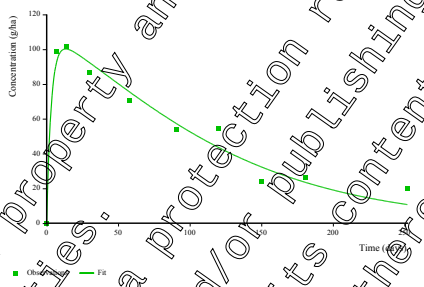
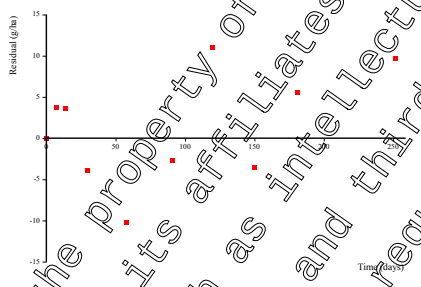
	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 err %	8.0
t-test	k_1: $p < 0.05$ k_2: $p < 0.05$
$DissT_{50}$ (days)	7.6
$DissT_{90}$ (days)	91.2
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	iv) Determine which of the models (FOMC, DFOP) is best. Both FOMC and DFOP give acceptable fits. FOMC selected for on basis of χ^2. v) Does the best-fit model give an acceptable description of the data? Yes, FOMC should be used for persistence endpoints.

Summary:

For spiroxamine use FOMC. $DissT_{50}$ = 7.9 days, $DissT_{90}$ = 84.7 days.

Appendix 4.1.8.2. Metabolite fitting (M01 and M02)

Step 4: Run parent best-fit and metabolite SFO. iii) SFO fit for metabolite acceptable?

	Parent FOMC, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error.	Excellent, residuals show no systematic error.
χ^2 err %	10.0	8.84
t-test	*k*: p < 0.05	*k*: p < 0.05
$DisST_{50}$ (days)	40	49.2
$DisST_{90}$ (days)	133	164
Assessment	Fit acceptable. Visual fit is good and rate parameter differs significantly from zero, however, χ^2 error is slightly high.	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence end-points.	ii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence end-points.

[1] Interpret with care – extrapolated beyond experimental period

Summary

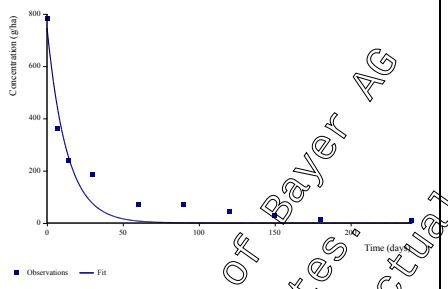
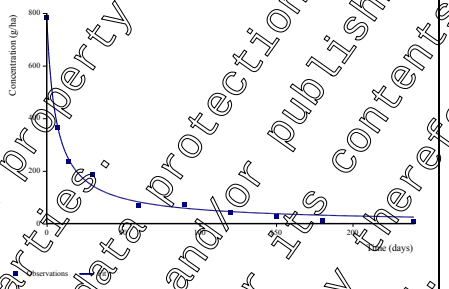
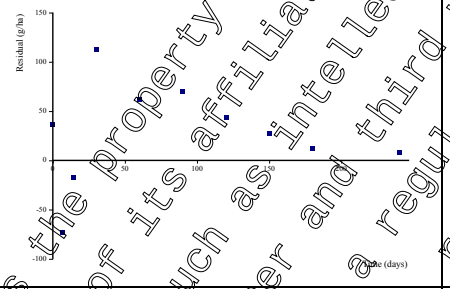
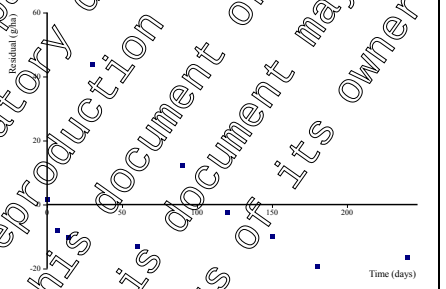
For metabolite M01 use FOMC/SFO $DisST_{50}$ = 40 days, $DisST_{90}$ = 133 days.

For metabolite M02 use FOMC/SFO $DisST_{50}$ = 49.2 days, $DisST_{90}$ = 164 days.

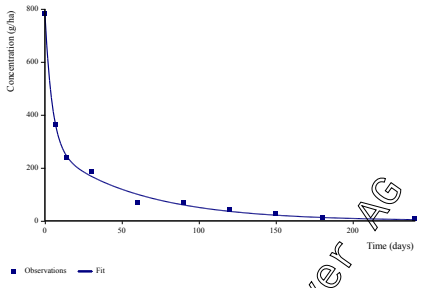
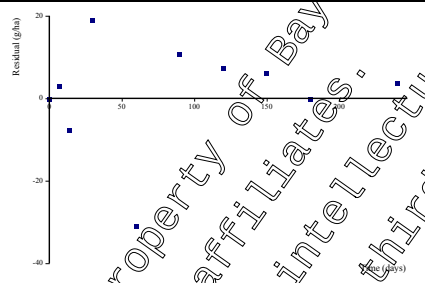
Appendix 4.1.9. Dissipation of spiroxamine in Albig soil trial no. 40010/6 (KCA 7.1.2.2.1/02 (M-006126-01-1))

Appendix 4.1.9.1. Parent Fitting

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor, residuals show systematic error and fit not conservative.	Excellent, residuals show no systematic error.
χ^2 err %	24.9	8.3
t-test	k p < 0.05	NA
$DisST_{50}$ (days)	9.0	6.3
$DisST_{90}$ (days)	30	64
Assessment	Fit not acceptable. Rate parameter differs significantly from zero, however, visual fit is poor and χ^2 error is high.	Fit acceptable. Visual fit is excellent and χ^2 error is low.
Conclusion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed. iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No. Go to step 2 below.	

Step 2: Possible bi-phasic decline. Fit DFOP.

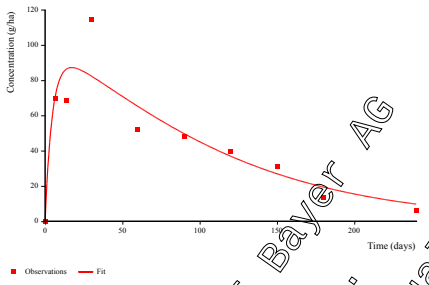
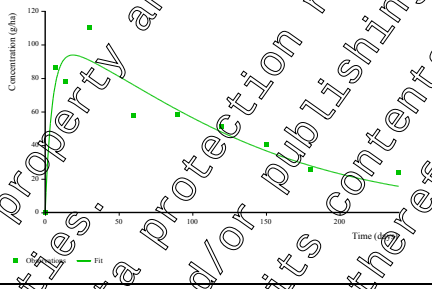
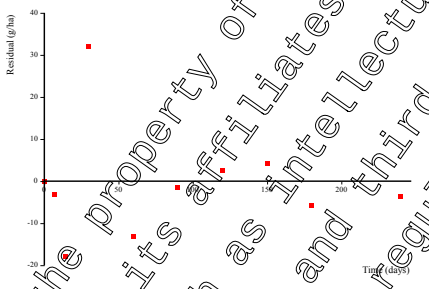
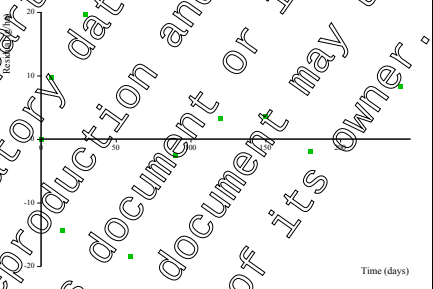
	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 err %	6.3
t-test	k_1: p< 0.05 k_2: p< 0.05
$DissT_{50}$ (days)	6.0
$DissT_{90}$ (days)	74.5
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	iv) Determine which of the models (FOMC, DFOP) is best. Both FOMC and DFOP give acceptable fits. DFOP selected (lower χ^2 error). v) Does the best-fit model give an acceptable description of the data? Yes. DFOP should be used for persistence endpoints.

Summary:

For parent spiroxamine use DFOP $DissT_{50}$ = 6.0 days, $DissT_{90}$ = 74.5 days.

Appendix 4.1.9.2. Metabolite fitting (M01 and M02)

Step 4: Run parent best-fit and metabolite SFO. iii) SFO fit for metabolite acceptable?

	Parent DFOP, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error.	Excellent, residuals show no systematic error.
χ^2 err %	21.7	18.7
t-test	*k*: p < 0.05	*k*: p < 0.05
$DisST_{50}$ (days)	52.3	63.3
$DisST_{90}$ (days)	174	210
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is only slightly high and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is excellent, χ^2 error is only slightly high and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence end-points.	ii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence end-points.

[1] Interpret with care – extrapolated beyond experimental period

Summary

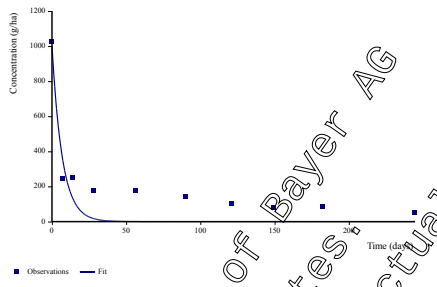
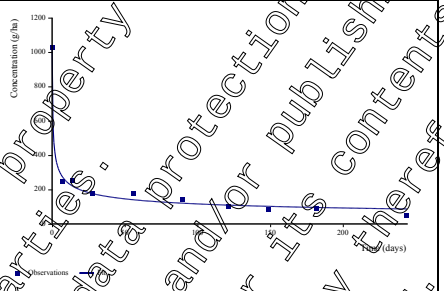
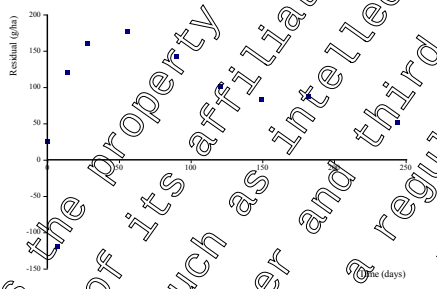
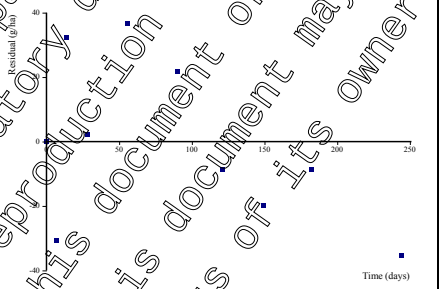
For metabolite M01 use DFOP/SFO $DisST_{50}$ = 52.3 days, $DisST_{90}$ = 174 days.

For metabolite M02 use DFOP/SFO $DisST_{50}$ = 63.3 days, $DisST_{90}$ = 210 days.

Appendix 4.1.10. Dissipation of spiroxamine in Elm Farm/Thurston soil trial no. 40097/1 (KCA 7.1.2.2.1/03 (M-006127-01-1))

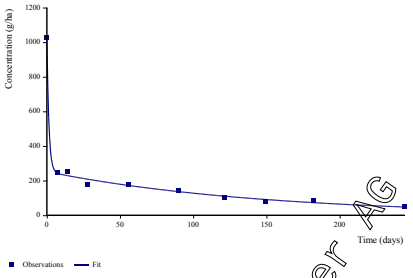
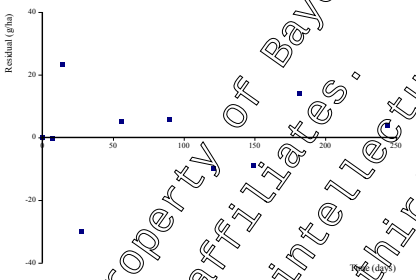
Appendix 4.1.10.1. Parent Fitting

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor, residuals show systematic error and fit not conservative.	Excellent, residuals show no systematic error.
χ^2 err %	39.7	8.5
t-test	$k_1 p < 0.05$	NA
$DisST_{50}$ (days)	4.8	0.9
$DisST_{90}$ (days)	15.9	147[1]
Assessment	Fit not acceptable. Rate parameter differs significantly from zero, however, visual fit is poor and χ^2 error is high.	Fit acceptable. Visual fit is excellent and χ^2 error is low.
Conclusion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed. iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No. Go to step 2 below.	

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Possible bi-phasic decline. Fit DFOP.

	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error
χ^2 err %	5.2
t-test	k_1: p > 0.1 k_2: p < 0.05
$DissT_{50}$ (days)	1.46
$DissT_{90}$ (days)	132[1]
Assessment	Fit acceptable. Visual fit is good and χ^2 error is low however, k_2 rate parameters does not differ significantly from zero
Conclusion	iv) Determine which of the models (FOMC, DFOP) is best. DFOP gives best fit (lowest χ^2). v) Does the best-fit model give an acceptable description of the data? Yes. DFOP should be used for persistence endpoints.

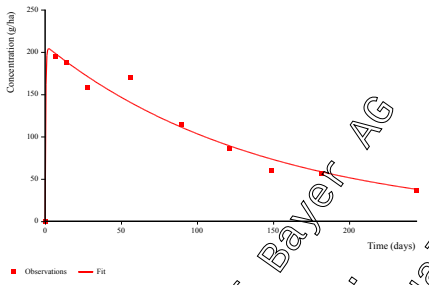
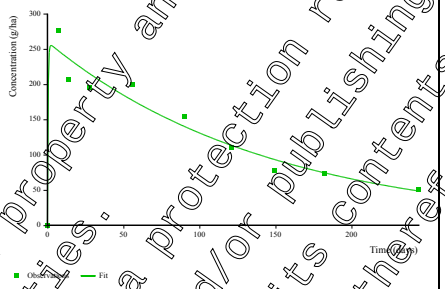
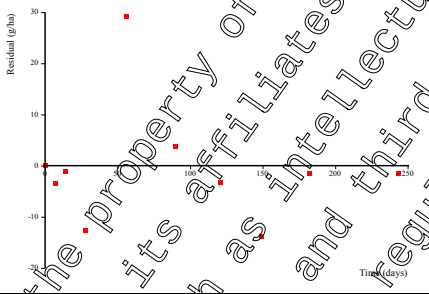
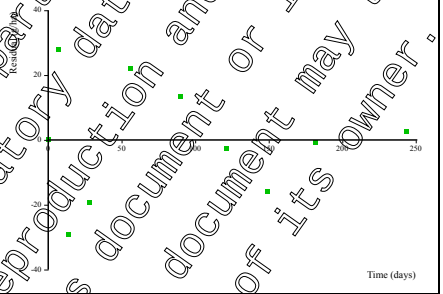
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use DFOP. $DissT_{50}$ = 1.46 days, $DissT_{90}$ = 132 days.

Appendix 4.1.10.2.Metabolite fitting (M01 and M02)

Step 4: Run parent best-fit and metabolite SFO. iii) SFO fit for metabolite acceptable?

	Parent DFOP, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error.	Excellent, residuals show no systematic error.
χ^2 err %	7.95	9.68
t-test	*k*: $p < 0.05$	*k*: $p < 0.05$
$DisST_{50}$ (days)	74.8	75.9
$DisST_{90}$ (days)	248	252
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is excellent, χ^2 error is acceptable and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary

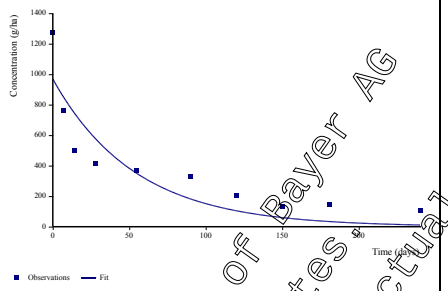
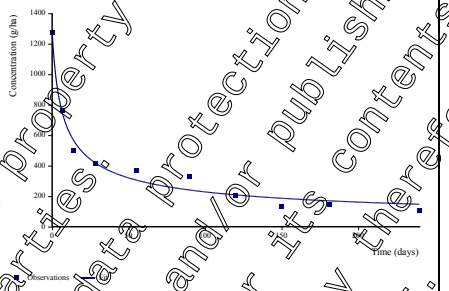
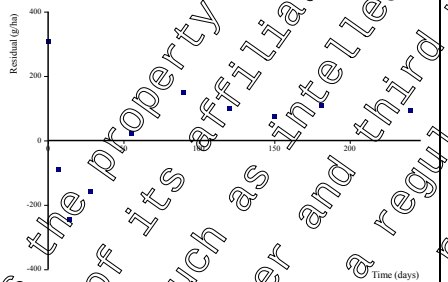
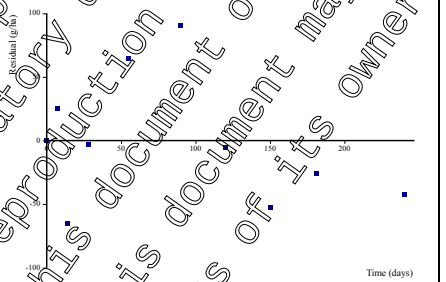
For metabolite M01 use DFOP/SFO $DisST_{50}$ = 74.8 days, $DisST_{90}$ = 248 days.

For metabolite M02 use DFOP/SFO $DisST_{50}$ = 75.9 days, $DisST_{90}$ = 252 days.

Appendix 4.1.11. Dissipation of spiroxamine in Pakenham soil trial no. 40099/8 (KCA 7.1.2.2.1/03 (M-006127-01-1))

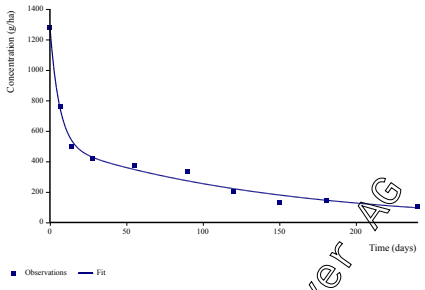
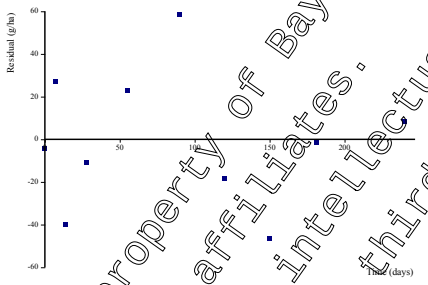
Appendix 4.1.11.1. Parent Fitting

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor, residuals show systematic error and fit not conservative.	Excellent, residuals show no systematic error.
χ^2 err %	29.8	9.5
t-test	t, $p < 0.05$	NA
$DisST_{50}$ (days)	37.4	10.3
$DisST_{90}$ (days)	124	319[1]
Assessment	Fit not acceptable. Rate parameter differs significantly from zero, however, visual fit is poor and χ^2 error is high.	Fit acceptable. Visual fit is excellent and χ^2 error is low.
Conclusion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed. iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No. Go to step 2 below.	

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Possible bi-phasic decline. Fit DFOP.

	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 err %	6.3
t-test	k_1: p <0.05 k_2: p <0.05
$DissT_{50}$ (days)	9.5
$DissT_{90}$ (days)	199[1]
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	iv) Determine which of the models (FOMC, DFOP) is best. Both FOMC and DFOP give acceptable fits. DFOP selected (lower χ^2 error). v) Does the best-fit model give an acceptable description of the data? Yes. DFOP should be used for persistence endpoints.

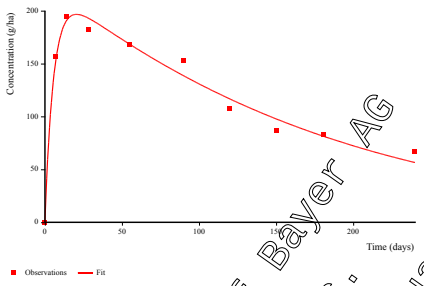
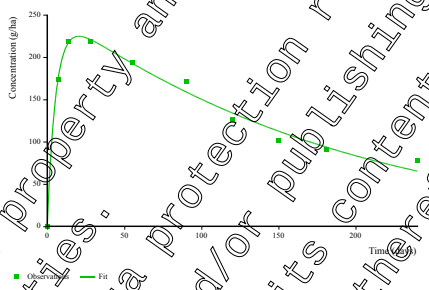
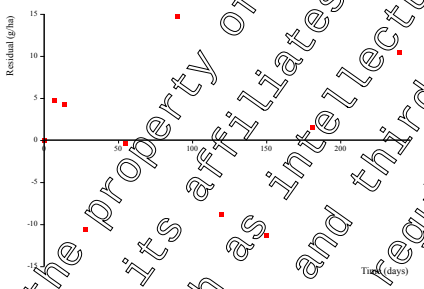
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use DFOP. $DissT_{50}$ = 9.5 days, $DissT_{90}$ = 199 days.

Appendix 4.1.11.2.Metabolite fitting (M01 and M02)

Step 4: Run parent best-fit and metabolite SFO. iii) SFO fit for metabolite acceptable?

	Parent DFOP, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error.	Excellent, residuals show no systematic error.
χ^2 err %	5.2	4.0
t-test	*k*: p <0.05	*k*: p <0.05
$DissT_{50}$ (days)	72.6	73.2
$DissT_{90}$ (days)	241	243
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence end-points.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence end-points.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

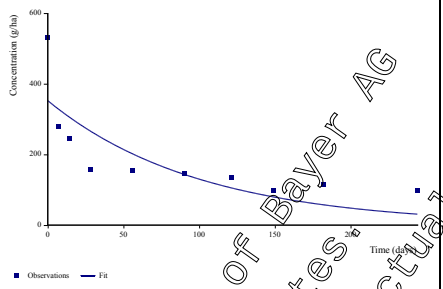
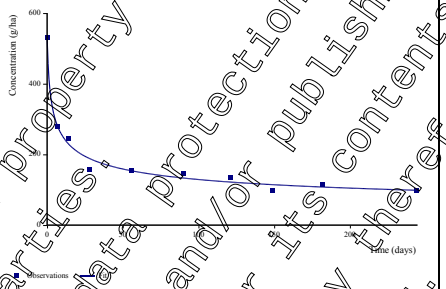
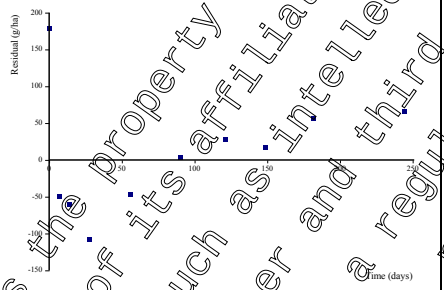
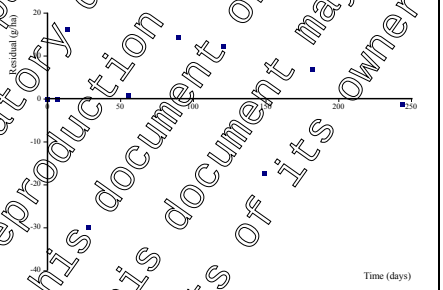
For metabolite M01 use DFOP/SFO $DissT_{50}$ = 72.6 days, $DissT_{90}$ = 241 days.

For metabolite M02 use DFOP/SFO $DissT_{50}$ = 73.2 days, $DissT_{90}$ = 243 days.

Appendix 4.1.12. Dissipation of spiroxamine in Elm Farm/Thurston soil trial no. 40100/5 (KCA 7.1.2.2.1/03 (M-006127-01-1))

Appendix 4.1.12.1. Parent Fitting

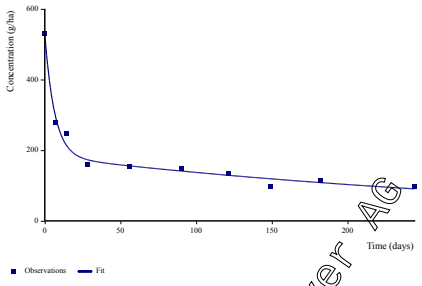
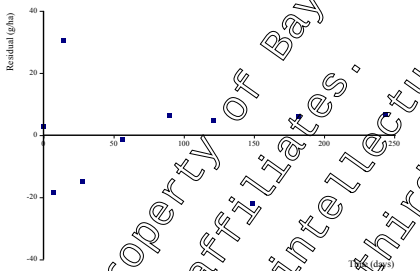
Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor, residuals show systematic error and fit not conservative.	Excellent, residuals show no systematic error.
χ^2 err %	31.9	5.9
t-test	k_1 p <0.05	NA
$DisST_{50}$ (days)	69.6	8.4
$DisST_{90}$ (days)	230	1,890[1]
Assessment	Fit not acceptable. Rate parameter differs significantly from zero, however, visual fit is poor and χ^2 error is high.	Fit acceptable. Visual fit is excellent and χ^2 error is low. However, significant extrapolation beyond experimental period to DT90[2].
Conclusion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed. iii) Deviation from SFO due to experimental artefact/decline in microbiall activity? No. Go to step 2 below.	

[1] Interpret with care – extrapolated beyond experimental period

[2] EFSA Scientific Report (2009) 338, 1-32. "Report on the PPR Stakeholder Workshop Improved Realism in Soil Risk Assessment (IRIS) – How will pesticide risk assessment in soil be tackled tomorrow?"

Step 2: Possible bi-phasic decline. Fit DFOP.

	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 err %	6.7
t-test	k_1: p <0.05 k_2: p <0.05
$DissT_{50}$ (days)	9.1
$DissT_{90}$ (days)	433[1]
Assessment	Fit acceptable. Visual fit is good and χ^2 error is low and rate parameters differ significantly from zero.
Conclusion	iv) Determine which of the models (FOMC, DFOP) is best. Both FOMC and DFOP give acceptable fits. DFOP selected (magnitude of residuals is smaller). v) Does the best-fit model give an acceptable description of the data? Yes. FOMC should be used for persistence endpoints.

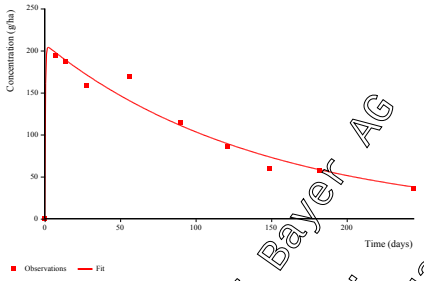
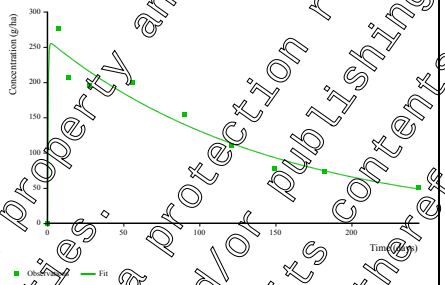
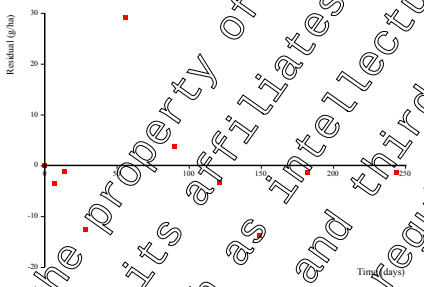
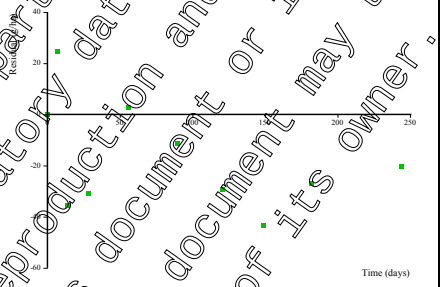
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use DFOP. $DissT_{50}$ = 9.1 days, $DissT_{90}$ = 433 days.

Appendix 4.1.12.2.Metabolite fitting (M01 and M02)

Step 4: Run parent best-fit and metabolite SFO. iii) SFO fit for metabolite acceptable?

	Parent DFOP, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error.	Excellent, residuals show no systematic error.
χ^2 err %	7.95	14.8
t-test	*k*: p <0.05	*k*: p <0.05
$DisST_{50}$ (days)	74.8	75.9
$DisST_{90}$ (days)	248	252
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is excellent, χ^2 error is acceptable and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints.	ii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary

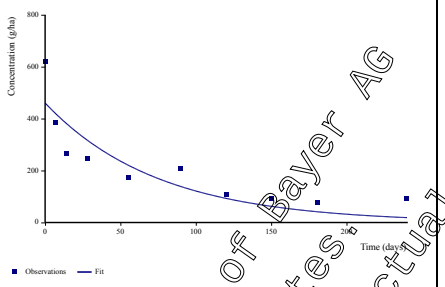
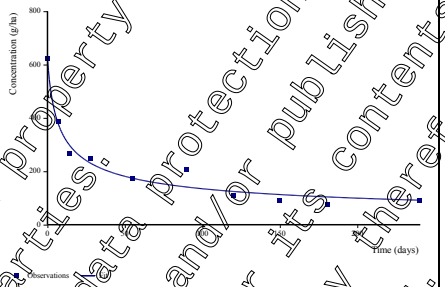
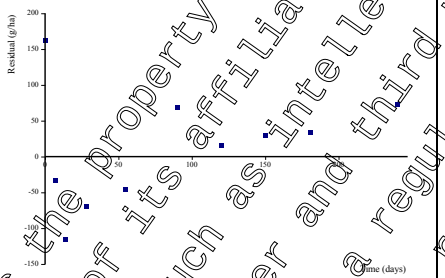
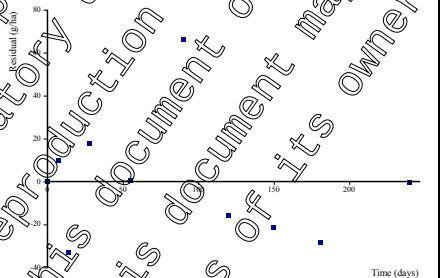
For metabolite M01 use DFOP/SFO $DisST_{50}$ = 74.8 days, $DisST_{90}$ = 248 days.

For metabolite M02 use DFOP/SFO $DisST_{50}$ = 75.9 days, $DisST_{90}$ = 252 days.

Appendix 4.1.13. Dissipation of spiroxamine in Pakenham soil trial no. 40101/3 (KCA 7.1.2.2.1/03 (M-006127-01-1))

Appendix 4.1.13.1. Parent Fitting

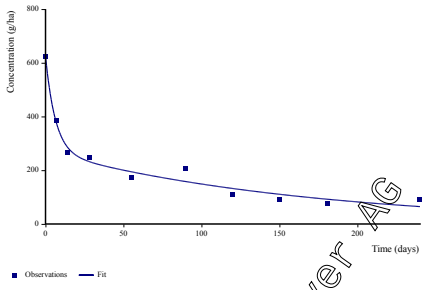
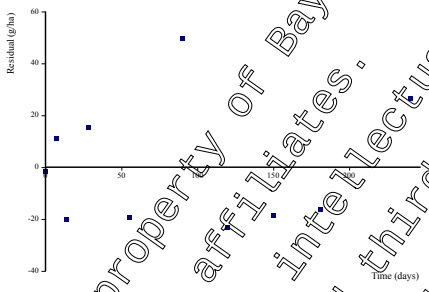
Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor, residuals show systematic error and fit not conservative.	Excellent, residuals show no systematic error.
χ^2 err %	27.3	10.1
t-test	t, $p < 0.05$	NA
$DisST_{50}$ (days)	52.0	12.6
$DisST_{90}$ (days)	173	578[1]
Assessment	Fit not acceptable. Rate parameter differs significantly from zero, however, visual fit is poor and χ^2 error is high.	Fit acceptable. Visual fit is excellent and χ^2 error is low. Significant extrapolation beyond experimental period to DT90[2].
Conclusion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed. iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No. Go to step 2 below.	

1 Interpret with care – extrapolated beyond experimental period

[2] EFSA Scientific Report (2009) 338, 1-32: "Report on the PPR Stakeholder Workshop Improved Realism in Soil Risk Assessment (IRIS) – How will pesticide risk assessment in soil be tackled tomorrow?"

Step 2: Possible bi-phasic decline. Fit DFOP.

	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error
χ^2 err %	9.2
t-test	*k1*: p < 0.05 *k2*: p < 0.05
$DisST_{50}$ (days)	11.2
$DisST_{90}$ (days)	247[1]
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	iv) Determine which of the models (FOMC, DFOP) is best. Both FOMC and DFOP give acceptable fits. DFOP selected (lower χ^2 error). v) Does the best-fit model give an acceptable description of the data? Yes. DFOP should be used for persistence endpoints.

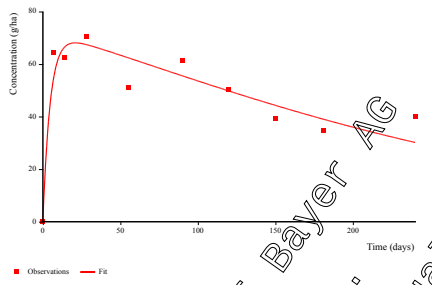
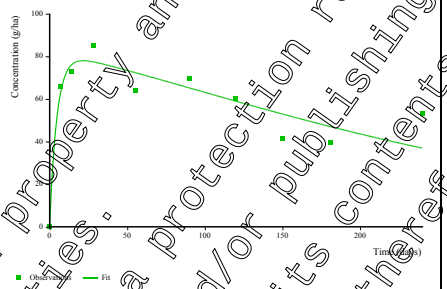
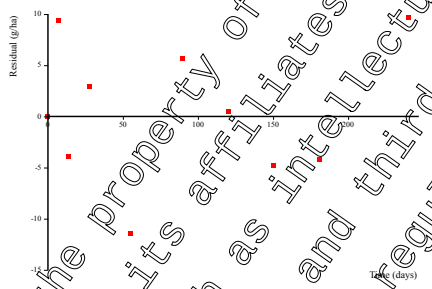
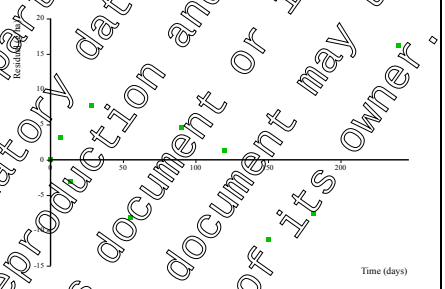
[1] Interpret with care – extrapolated beyond experimental period.

Summary:

For spiroxamine use DFOP $DisST_{50}$ = 11.2 days, $DisST_{90}$ = 247 days.

Appendix 4.1.13.2.Metabolite fitting (M01 and M02)

Step 4: Run parent best-fit and metabolite SFO. iii) SFO fit for metabolite acceptable?

	Parent DFOP, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error.	Good, residuals show no systematic error.
χ^2 err %	10.2	10.8
t-test	*k*: p < 0.05	*k*: p < 0.05
$DisST_{50}$ (days)	96.8	103
$DisST_{90}$ (days)	322[1]	342[1]
Assessment	Fit acceptable. Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence end-points.	ii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence end-points.

[1] Interpret with care – extrapolated beyond experimental period

Summary

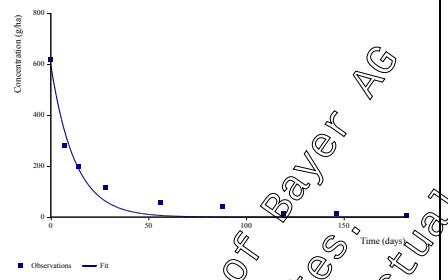
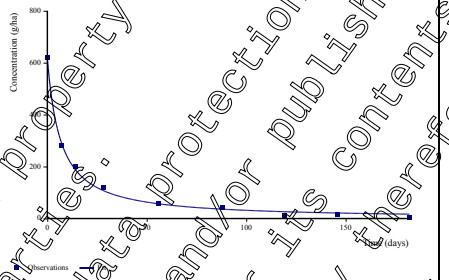
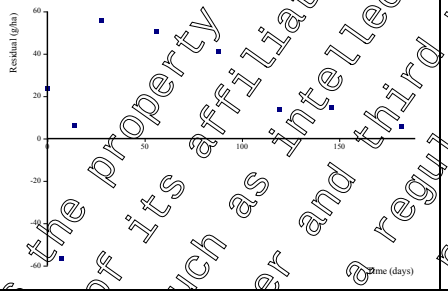
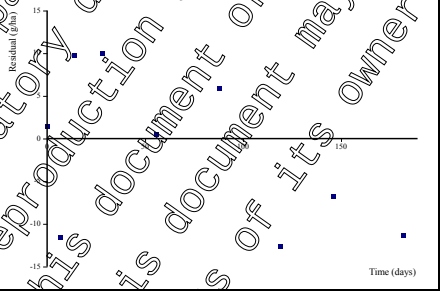
For metabolite M01 use DFOP/SFO $DisST_{50}$ = 96.8 days, $DisST_{90}$ = 322 days.

For metabolite M02 use DFOP/SFO $DisST_{50}$ = 103 days, $DisST_{90}$ = 342 days.

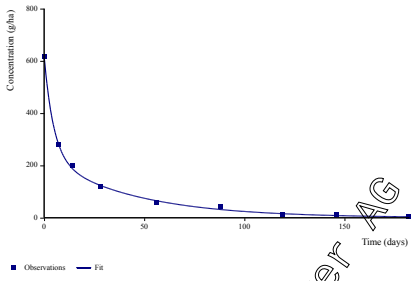
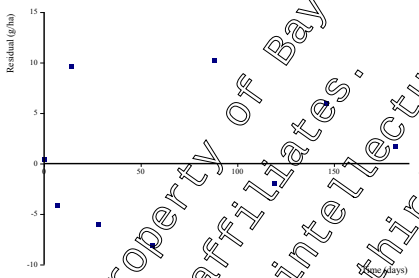
Appendix 4.1.14. Dissipation of spiroxamine in Touffreville soil trial no. 40193/5 (KCA 7.1.2.2.1/03 (M-006127-01-1))

Appendix 4.1.14.1. Parent Fitting

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor, residuals show systematic error.	Excellent, residuals show no systematic error.
χ^2 err %	19.1	5.0
t-test	k_1 p< 0.05	NA
$DisST_{50}$ (days)	8.6	6.4
$DisST_{90}$ (days)	28.6	51.2
Assessment	Fit not acceptable. Rate parameter differs significantly from zero, however, visual fit is poor and χ^2 error is slightly high.	Fit acceptable. Visual fit is excellent and χ^2 error is low.
Conclusion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed. iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No. Go to step 2 below.	

Step 2: Possible bi-phasic decline. Fit DFOP.

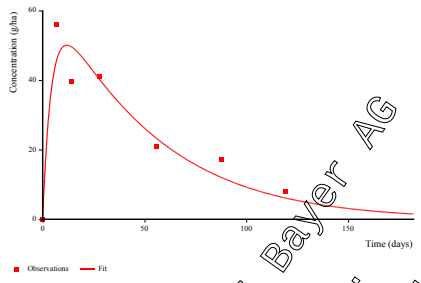
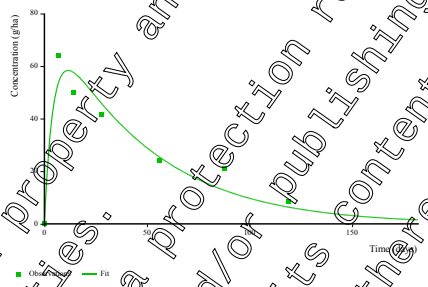
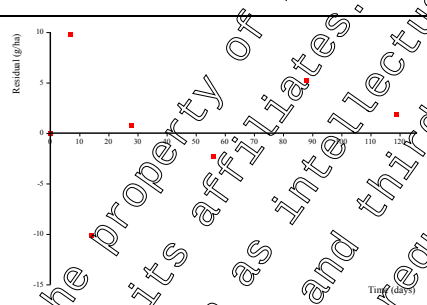
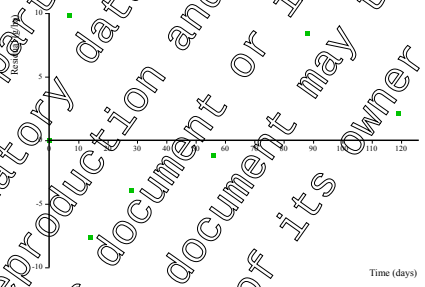
	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 err %	3.8
t-test	k_1: p< 0.05 k_2: p <0.05
$DissT_{50}$ (days)	6.1
$DissT_{90}$ (days)	58.4
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	iv) Determine which of the models (FOMC, DFOP) is best. Both FOMC and DFOP give acceptable fits. DFOP selected (lower χ^2 error). v) Does the best-fit model give an acceptable description of the data? Yes. DFOP should be used for persistence endpoints.

Summary:

For spiroxamine use DFOP. $DissT_{50}$ = 6.1 days. $DissT_{90}$ = 58.4 days.

Appendix 4.1.14.2.Metabolite fitting (M01 and M02)

Step 4: Run parent best-fit and metabolite SFO. iii) SFO fit for metabolite acceptable?

	Parent DFOP, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error.	Excellent, residuals show no systematic error.
χ^2 err %	16.4	14.6
t-test	*k*: p < 0.05	*k*: p < 0.05
$DissT_{50}$ (days)	22.6	21.3
$DissT_{90}$ (days)	75.2	70.7
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is acceptable and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is excellent, χ^2 error is acceptable and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints.

Summary:

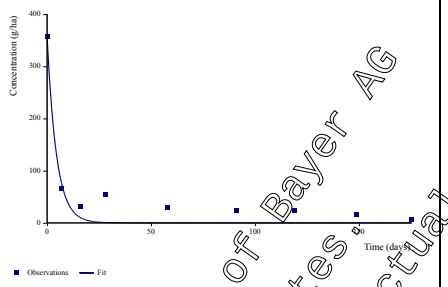
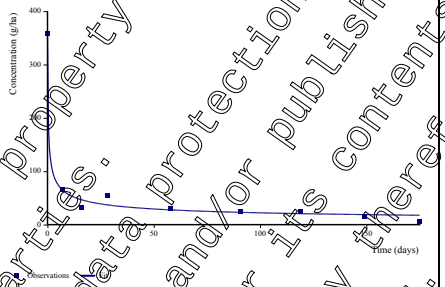
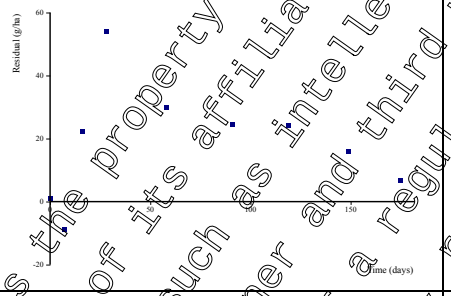
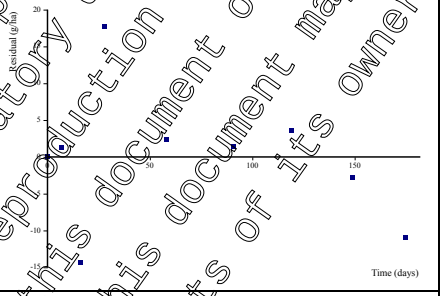
For metabolite M01 use DFOP/SFO $DissT_{50}$ = 22.6 days, $DissT_{90}$ = 75.2 days.

For metabolite M02 use DFOP/SFO $DissT_{50}$ = 21.3 days, $DissT_{90}$ = 70.7 days.

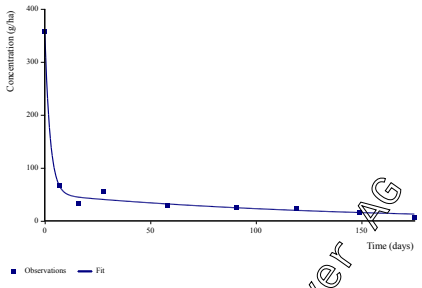
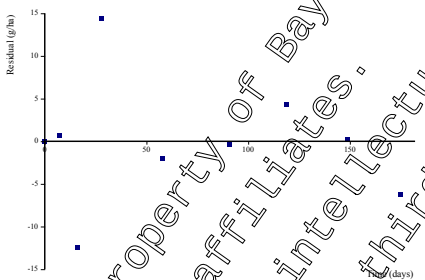
Appendix 4.1.15. Dissipation of spiroxamine in Laudun soil trial no. 40198/6 (KCA 7.1.2.2.1/04 (M-006128-01-1))

Appendix 4.1.15.1. Parent Fitting

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor, residuals show systematic error and fit not conservative.	Excellent, residuals show no systematic error.
χ^2 err %	30	10.7
t-test	k_1 $p < 0.05$	NA
$DisST_{50}$ (days)	3.1	0.5
$DisST_{90}$ (days)	10.4	30.7
Assessment	Fit not acceptable. Rate parameter differs significantly from zero, however, visual fit is poor and χ^2 error is high.	Fit acceptable. Visual fit is excellent and χ^2 error is low.
Conclusion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed. iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No. Go to step 2 below.	

Step 2: Possible bi-phasic decline. Fit DFOP.

	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 err %	9.1
t-test	k_1: p< 0.05 k_2: p <0.05
$DissT_{50}$ (days)	2.1
$DissT_{90}$ (days)	43.5
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	iv) Determine which of the models (FOMC, DFOP) is best. Both FOMC and DFOP give acceptable fits. DFOP selected (lower χ^2 error). v) Does the best-fit model give an acceptable description of the data? Yes, DFOP should be used for persistence endpoints.

Summary:

For spiroxamine use DFOP. $DissT_{50}$ = 2.1 days, $DissT_{90}$ = 43.5 days.

Appendix 4.1.15.2 Metabolite fitting (M01 and M02)

Insufficient data points to run metabolite (two data points per metabolite).

Summary:

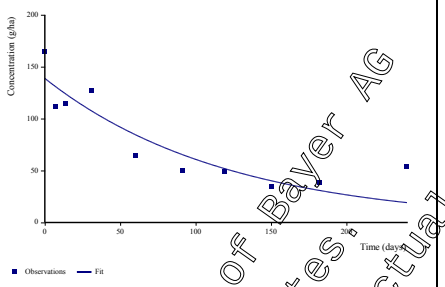
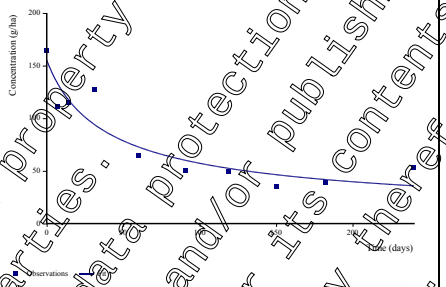
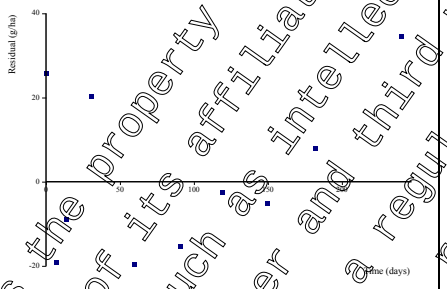
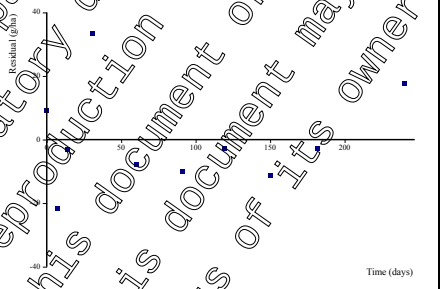
For metabolite M01 acceptable fit of decline cannot be obtained.

For metabolite M02 acceptable fit of decline cannot be obtained.

Appendix 4.1.16. Dissipation of spiroxamine in Filetto soil trial no. 40424/1 (KCA 7.1.2.2.1/04 (M-006128-01-1))

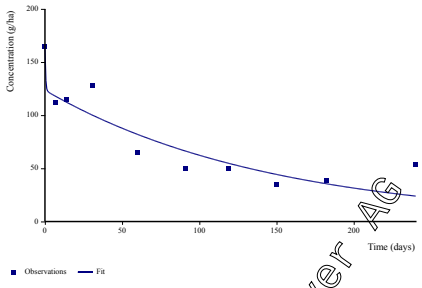
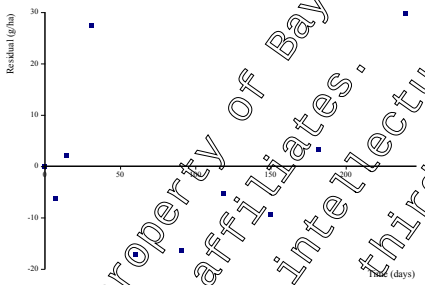
Appendix 4.1.16.1. Parent Fitting

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor, residuals show systematic error and fit not conservative.	Good, residuals show no systematic error.
χ^2 err %	18.3	15.8
t-test	k_1 p < 0.05	NA
$DisST_{50}$ (days)	84.0	51.7
$DisST_{90}$ (days)	279	994[1]
Assessment	Fit not acceptable. Rate parameter differs significantly from zero, however, visual fit is poor and χ^2 error is slightly high.	Fit potentially acceptable. Visual fit is intermediate and χ^2 error is slightly high. However, significant extrapolation beyond experimental period to DT90[2].
Conclusion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed. iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No. Go to step 2 below.	

[1] Interpret with care – extrapolated beyond experimental period
[2] EFSA Scientific Report (2008) 338, 1-32: "Report on the PPR Stakeholder Workshop Improved Realism in Soil Risk Assessment (IRIS) – How will pesticide risk assessment in soil be tackled tomorrow?"

Step 2: Possible bi-phasic decline. Fit DFOP.

	DFOP
Plot	
Residuals	
Visual fit	Intermediate, residuals show no systematic error.
χ^2 err %	16.9
t-test	k_1: p > 0.1 k_2: p < 0.05
$DissT_{50}$ (days)	59.6
$DissT_{90}$ (days)	295[1]
Assessment	Fit potentially acceptable. Visual fit is reasonable, however, χ^2 error is slightly high and rate parameters do not differ significantly from zero.
Conclusion	iv) Determine which of the models (FOMC, DFOP) is best. FOMC gives acceptable fit. v) Does the best-fit model give an acceptable description of the data? Yes. Although FOMC has the lowest χ^2, the DT90 was significantly extrapolated beyond the end of the study period. As such, DFOP selected as best fit model.

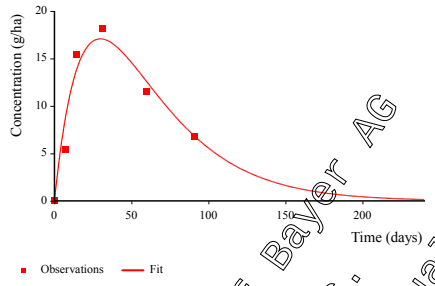
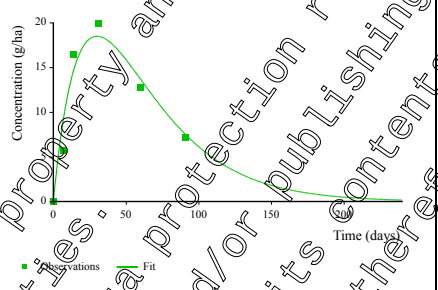
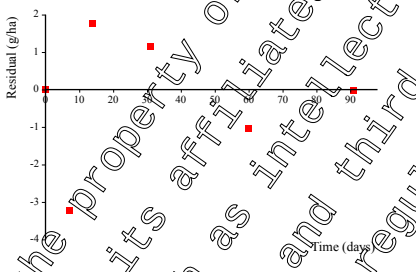
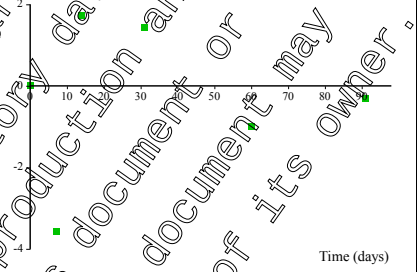
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use DFOP. $DissT_{50}$ = 59.6 days, $DissT_{90}$ = 295 days.

Appendix 4.1.16.2.Metabolite fitting (M01 and M02)

Step 4: Run parent best-fit and metabolite SFO. iii) SFO fit for metabolite acceptable?

	Parent DFOP, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error.	Excellent, residuals show no systematic error.
χ^2 err %	12.5	11.4
t-test	*k*: p < 0.05	*k*: p < 0.05
$DisST_{50}$ (days)	17.8	18.1
$DisST_{90}$ (days)	59	60.1
Assessment	Fit acceptable. Visual fit is excellent, however, χ^2 error is slightly high and rate parameter does not differ significantly from zero at a 5% level. Nevertheless, the p-value is < 0.10 and residuals show no large, systematic error.	Fit acceptable. Visual fit is excellent, however, χ^2 error is slightly high and rate parameter does not differ significantly from zero at a 5% level. Nevertheless, the p-value is < 0.10 and residuals show no large, systematic error.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence endpoints.

Summary:

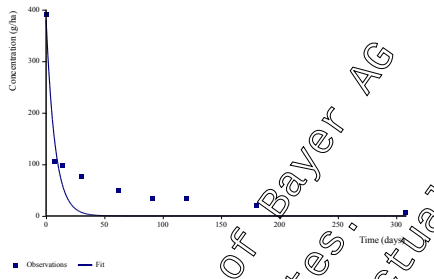
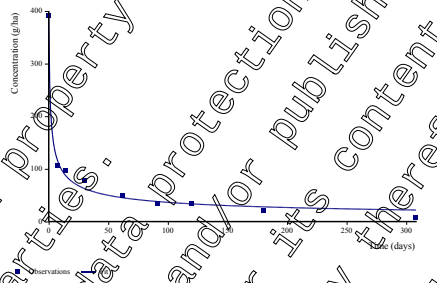
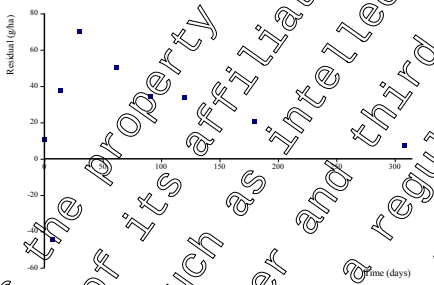
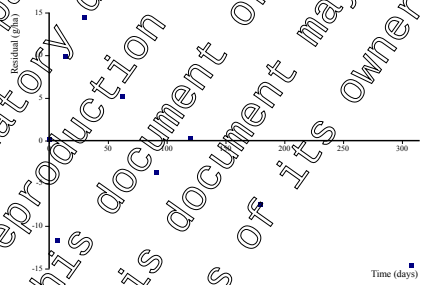
For metabolite M01 use FOMC/SFO $DisST_{50}$ = 17.8 days, $DisST_{90}$ = 59.0 days.

For metabolite M02 use FOMC/SFO $DisST_{50}$ = 18.1 days, $DisST_{90}$ = 60.1 days.

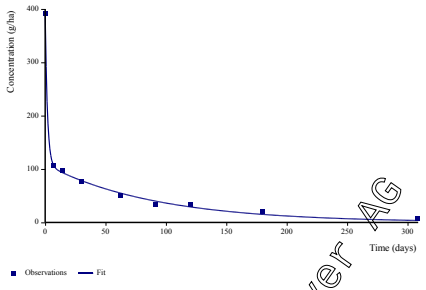
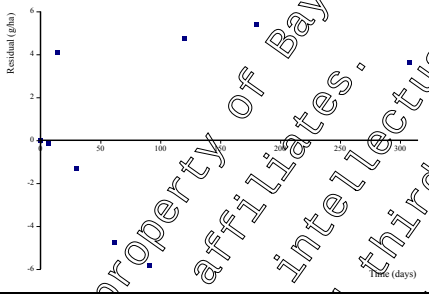
Appendix 4.1.17. Dissipation of spiroxamine in Laudun soil trial no. 50135/2 (KCA 7.1.2.2.1/05 (M-006129-01-1))

Appendix 4.1.17.1. Parent Fitting

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor, residuals show systematic error and fit not conservative	Excellent, residuals show no systematic error
χ^2 err %	34.3	8.5
t-test	k p < 0.05	NA
$DisST_{50}$ (days)	5.3	2.0
$DisST_{90}$ (days)	17.5	85.8
Assessment	Fit not acceptable. Rate parameter differs significantly from zero, however, visual fit is poor and χ^2 error is high.	Fit acceptable. Visual fit is excellent and χ^2 error is low.
Conclusion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed. iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No. Go to step 2 below.	

Step 2: Possible bi-phasic decline. Fit DFOP.

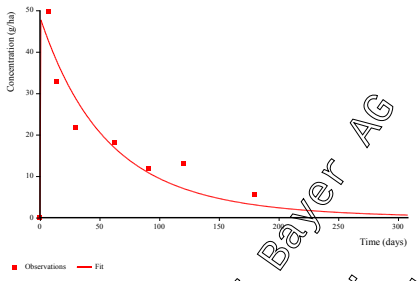
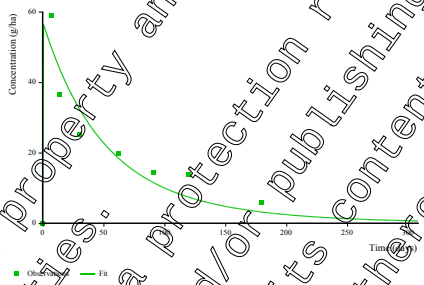
	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 err %	3.9
t-test	k_1: p< 0.05 k_2: p <0.05
$DissT_{50}$ (days)	2.1
$DissT_{90}$ (days)	93.3
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	iv) Determine which of the models (FOMC, DFOP) is best. Both FOMC and DFOP give acceptable fits. DFOP selected (lower χ^2 error). v) Does the best-fit model give an acceptable description of the data? Yes. DFOP should be used for persistence endpoints.

Summary:

For spiroxamine use DFOP. $DissT_{50}$ = 2.1 days, $DissT_{90}$ = 93.3 days.

Appendix 4.1.17.2.Metabolite fitting (M01 and M02)

Step 4: Run parent best-fit and metabolite SFO. iii) SFO fit for metabolite acceptable?

	Parent DFOP, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error.	Excellent, residuals show no systematic error.
χ^2 err %	17.7	19.1
t-test	*k*: p < 0.05	*k*: p < 0.05
$DisST_{50}$ (days)	30.9	28.9
$DisST_{90}$ (days)	103	96.1
Assessment	Fit acceptable. Visual fit is excellent and rate parameter differs significantly from zero, however, χ^2 error is slightly high.	Fit acceptable. Visual fit is excellent and rate parameter differs significantly from zero, however, χ^2 error is slightly high.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence end-points.	ii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence end-points.

Summary:

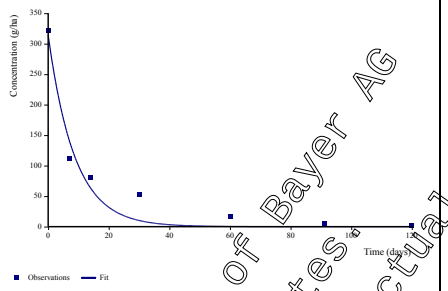
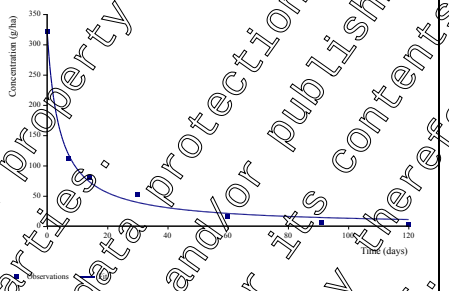
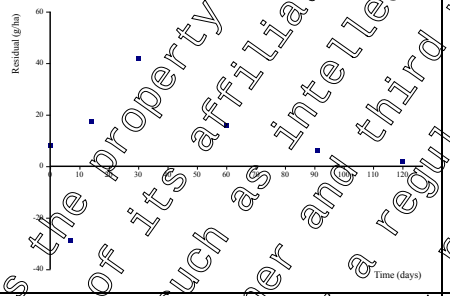
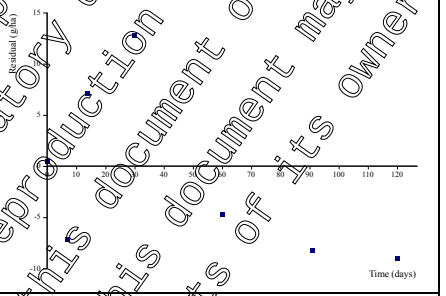
For metabolite M01 use DFOP/SFO $DisST_{50}$ = 30.9 days, $DisST_{90}$ = 103 days.

For metabolite M02 use DFOP/SFO $DisST_{50}$ = 28.9 days, $DisST_{90}$ = 96.1 days.

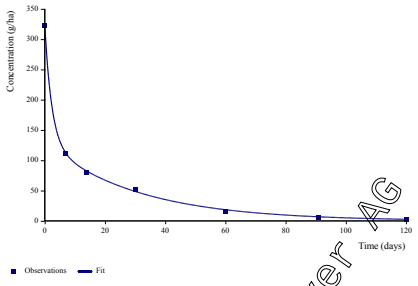
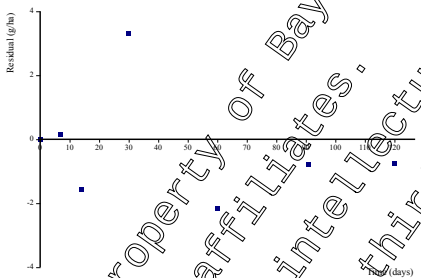
Appendix 4.1.18. Dissipation of spiroxamine in Nogarole Rocca soil trial no. 50136/0 (KCA 7.1.2.2.1/05 (M-006129-01-1))

Appendix 4.1.18.1. Parent Fitting

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
Visual fit	Poor, residuals show systematic error and fit not conservative.	Excellent, residuals show no systematic error.
χ^2 err %	20.1	8.1
t-test	t, $p < 0.05$	NA
$DisST_{50}$ (days)	6.3	4.3
$DisST_{90}$ (days)	20.9	38.9
Assessment	Fit not acceptable. Rate parameter differs significantly from zero, however, visual fit is poor and χ^2 error is slightly high.	Fit acceptable. Visual fit is excellent and χ^2 error is low.
Conclusion	i) SFO more appropriate than FOMC and gives acceptable fit? SFO is not more appropriate than FOMC. ii) Run modified fitting. SFO more appropriate than FOMC & fit acceptable (modified fitting)? Modified fitting not needed. iii) Deviation from SFO due to experimental artefact/decline in microbial activity? No. Go to step 2 below.	

Step 2: Possible bi-phasic decline. Fit DFOP.

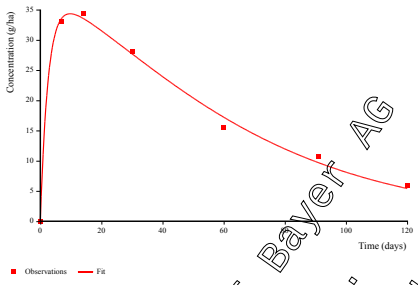
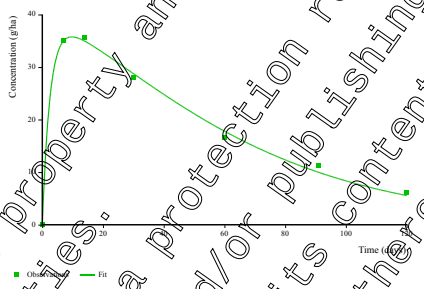
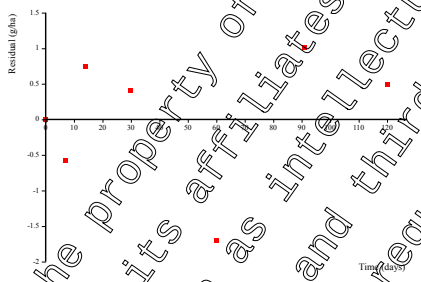
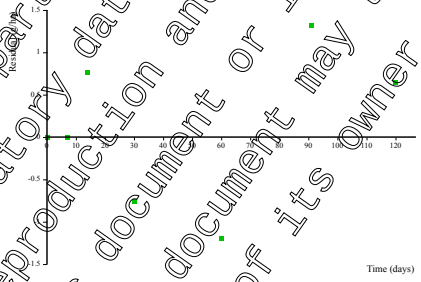
	DFOP
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 err %	1.9
t-test	k_1: p < 0.05 k_2: p <0.05
$DissT_{50}$ (days)	3.5
$DissT_{90}$ (days)	43.8
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	iv) Determine which of the models (FOMC, DFOP) is best. Both FOMC and DFOP give acceptable fits. DFOP selected (lower χ^2 error). v) Does the best-fit model give an acceptable description of the data? Yes, DFOP should be used for persistence endpoints.

Summary:

For spiroxamine use DFOP. $DissT_{50}$ = 3.5 days, $DissT_{90}$ = 43.8 days.

Appendix 4.1.18.2.Metabolite fitting (M01 and M02)

Step 4: Run parent best-fit and metabolite SFO. iii) SFO fit for metabolite acceptable?

	Parent DFOP, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error.	Excellent, residuals show no systematic error.
χ^2 err %	3.5	3.3
t-test	*k*: p < 0.05	*k*: p < 0.05
$DisST_{50}$ (days)	28.8	28.5
$DisST_{90}$ (days)	95.6	94.8
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence end-points.	ii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for persistence end-points.

Summary:

For metabolite M01 use DFOP/SFO $DisST_{50}$ = 28.8 days, $DisST_{90}$ = 95.6 days.

For metabolite M02 use DFOP/SFO $DisST_{50}$ = 28.5 days, $DisST_{90}$ = 94.8 days.

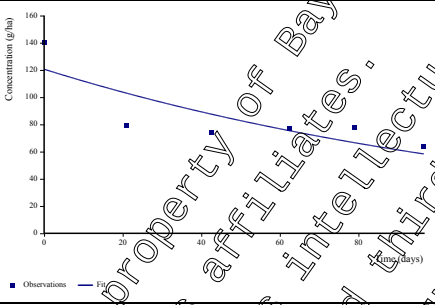
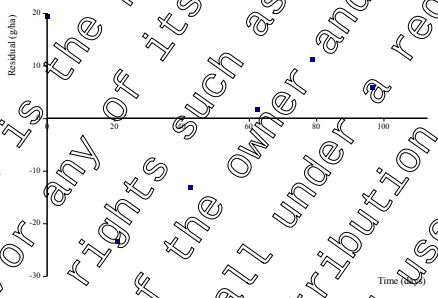
Appendix 4.2: Kinetic evaluation for modelling endpoints

Appendix 4.2.1. Degradation of spiroxamine in Höfchen soil trial no. 30122/1 (KCA 7.1.2.2.1/01 [M-006116-01-1])

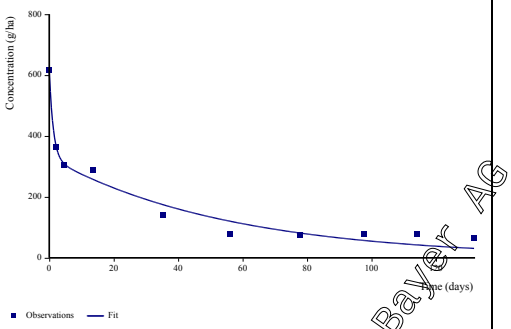
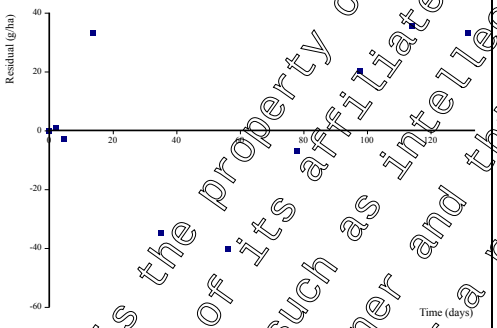
Appendix 4.2.1.1. Parent fitting

Step 1: Does decline in lab studies show a lag-phase or effects of long-term sorption kinetics? No

Step 3: Can field decline be described with SFO after eliminating data points before 10 mm rain ensuring no systematic deviations when examining residuals especially when residues at study end are above 10% of initially measured?

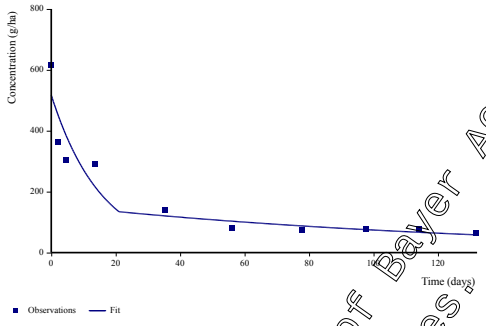
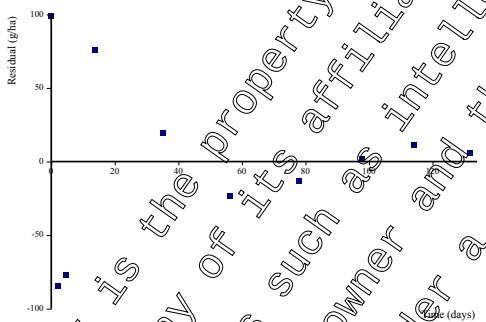
	SFO
Plot	
Residuals	
Visual fit	Poor, residuals show systematic error.
χ^2 err %	13.5
t-test	n.p < 0.05
DT_{50} (days)	92.2
DT_{90} (days)	306[1]
$DegT_{50\ matrix}$ (days)	92.2
Assessment	Fit not acceptable. χ^2 error is acceptable and rate parameter differs significantly from zero, however, visual fit is poor.
Conclusion	iii) SFO fit is acceptable. No. Go to step 5.

Step 5: Fit DFOP to complete dataset + estimate breakpoint?

	DFOP
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error.
χ^2 error (%)	11.1
t-test	k_1: p > 0.05-0.1 k_2: p < 0.05
DT_{50} (days)	44
DT_{90} (days)	93.4
Modelling DT_{50} (days)	k_2: DT_{50} = 38.7
g	0.47
k_1	0.819 (-0.296 - 1.934)
k_2	0.0179 (0.010 - 0.026)
Assessment	Fit not acceptable. Visual fit is good and χ^2 error is acceptable, however, rate parameter does not differ significantly from zero. Further, k_1 and k_2 overlap.
Discussion	vi) breakpoint estimate = *ca* 2.5 days. vi) g = 0.75? Yes. vii) k_{fast} and k_{slow} significantly different? No. Go to Hockey-stick flow chart.

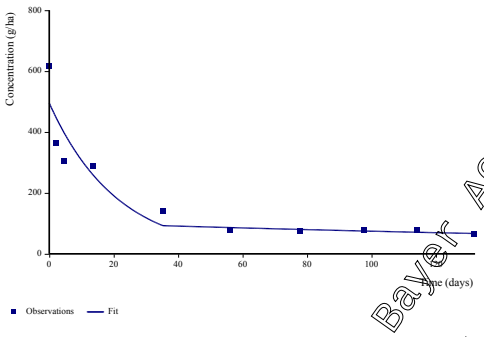
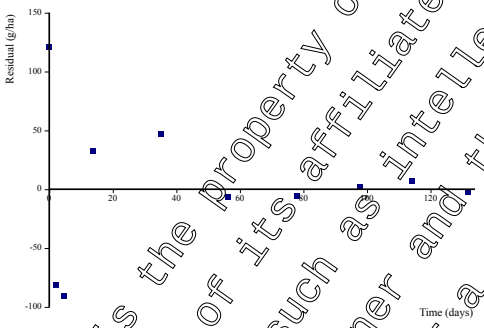
Hockey stick flow chart:

Step 1: Fit HS to complete dataset

	HS
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error.
χ^2 error (%)	23.3
t-test	k_1: p > 0.1; k_2 p < 0.05
DT_{50} (days)	10.8
DT_{90} (days)	148[1]
Modelling DT_{50} (days)	k_2 DT_{50} = 92.2
tb	21
>10 mm occurs after x days (normalised time)	35.2
k_1	0.0643 (-4.97E^{-5} - 0.129)
k_2	0.0975 (-0.0167 - 0.032)
Assessment	Fit not acceptable. Visual fit is good, however, χ^2 error is slightly high and rate parameter does not differ significantly from zero.
Discussion	ii) rain > 10 mm at breakpoint? No. Go to step 3 below.

[1] Interpret with care – extrapolated beyond experimental period

Step 3: Fix breakpoint to normalised time when rain > 10 mm and fit k_1 and k_2.

	HS
Plot	
Residuals	
Visual fit	Intermediate, residuals show no systematic error.
χ^2 error (%)	23.2
t-test	k_1: p < 0.05; k_2 p > 0.1
DT_{50} (days)	14.6
DT_{90} (days)	221[1]
Modelling DT_{50} (days)	k_3: DT_{50} = 206
k_2	0.0034 (-0.018 - 0.025)
Assessment	Fit acceptable Visual fit is reasonable, however χ^2 error is slightly high. k_2 fate parameter does not differ significantly from zero.
Discussion	Breakpoint fixed to 35.2 days. iv) fit acceptable? No Expert judgement. DFOP fit for this study was potentially acceptable with a very good visual fit, but DFOP was initially rejected because k_1 and k_2 were not significantly different. From [redacted] (2021), a lab investigation of Hofchen was found to reliably predict a DT_{50MOD} of 65.4 days, thereby providing confidence that the estimated DT_{50MOD} as k2 DFOP is reliable.

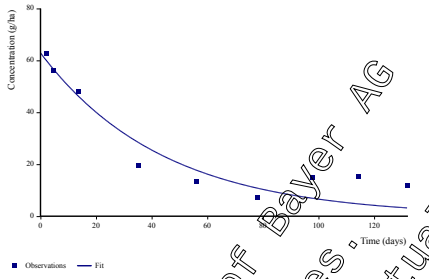
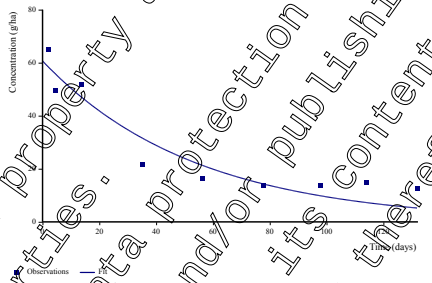
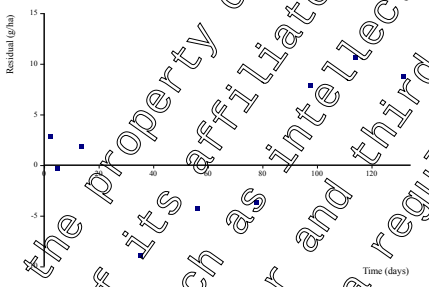
[1] Interpret with care – extrapolated beyond experimental period

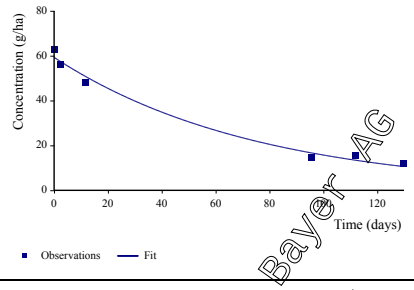
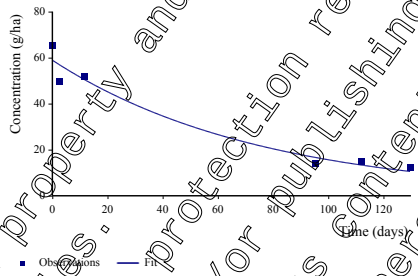
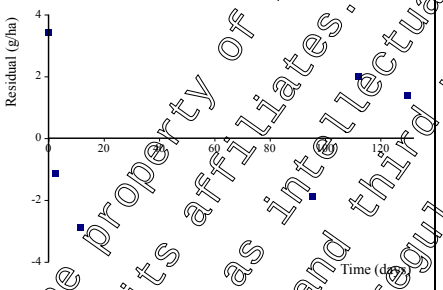
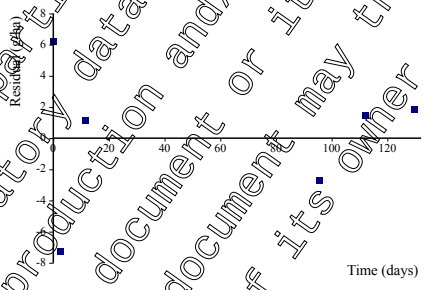
Summary:

For spiroxamine modelling, use DFOP $DegT_{50,matrix}$ based on k_2 parameter = 38.7 days.

Appendix 4.2.1.2. Metabolite fitting (M01 and M02)

Step 4: Run metabolite SFO from decline. iii) SFO fit for metabolite acceptable?

	M01	M02
Plot		
Residuals		
Visual fit	Intermediate, residuals show potential systematic error, and final three time points are underestimated	Intermediate, residuals show potential systematic error, and final three time points are underestimated
χ^2 err %	18.7	17.3
t-test	*k*: $p < 0.05$	*k*: $p < 0.05$
DT_{50} (days)	30.7	37.3
DT_{90} (days)	102	124
$DegT_{50matrix}$ (days)	30.7	37.3
Formation fraction	N/A	N/A
Assessment	Fit not acceptable. Visual fit is intermediate, and final three time points underestimated. χ^2 error is acceptable, and rate parameter differs significantly from zero. However, the final 3 timepoints are raised versus the other data raising a data quality issue. Try a modified SFO with removal of intermediate timepoints.	Fit not acceptable. Visual fit is intermediate, and final three time points underestimated. χ^2 error is acceptable, and rate parameter differs significantly from zero. However, the final 3 timepoints are raised versus the other data raising a data quality issue. Try a modified SFO with removal of intermediate timepoints.
Discussion	iii) SFO fit for metabolite acceptable? SFO is not considered acceptable but potentially impacted by data quality.	iii) SFO fit for metabolite acceptable? SFO is not considered acceptable.

	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error, and χ^2 very low.	Good, residuals show no systematic error, and χ^2 low.
χ^2 err %	5.17	9.64
t-test	*k*: p < 0.05	*k*: p < 0.05
DT_{50} (days)	52.3	52.3
DT_{90} (days)	174	174
$DegT_{50matrix}$ (days)	52.3	52.3
Formation fraction	N/A	N/A
Assessment	Fit acceptable. Visual fit is excellent with no evidence of residuals. χ^2 is low and the rate parameter is significantly different to zero. Assessment is conservative.	Fit acceptable. Visual fit is good with no evidence of residuals. χ^2 error is acceptable, and rate parameter differs significantly from zero. Assessment is conservative.
Discussion	iii) SFO fit for metabolite acceptable? SFO is acceptable.	iii) SFO fit for metabolite acceptable? SFO is acceptable.

Summary

For metabolite M01 use DT_{50} of SFO of decline phase, $DegT_{50, matrix}$ = 52.3 days

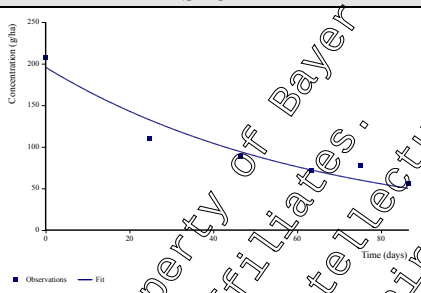
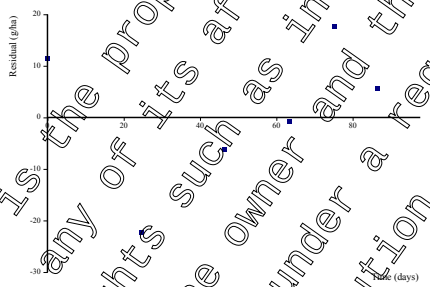
For metabolite M02 use DT_{50} of SFO of decline phase, $DegT_{50, matrix}$ = 52.3 days.

Appendix 4.2.2. Degradation of spiroxamine in Laacher Hof soil trial no. 30124/8 (KCA 7.1.2.2.1/01 (M-006116-01-1))

Appendix 4.2.2.1. Parent fitting

Step 1: Does decline in lab studies show a lag-phase or effects of long-term sorption kinetics? No

Step 3: Can field decline be described with SFO after eliminating data points before 10 mm rain ensuring no systematic deviations when examining residuals especially when residues at study end are above 10% of initially measured?

	SFO
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error
χ^2 err %	10.1
t-test	*k*: p < 0.05
DT_{50} (days)	44.1
DT_{90} (days)	146[1]
$DegT_{50matrix}$ (days)	44.1
Assessment	Fit acceptable Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero.
Conclusion	iii) SFO fit is acceptable. Yes. iv) Use this $DegT_{50matrix}$

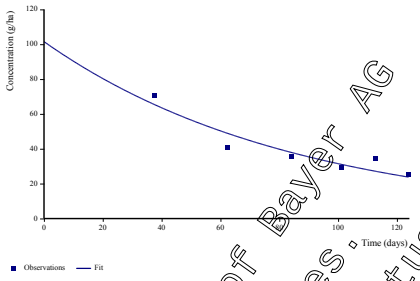
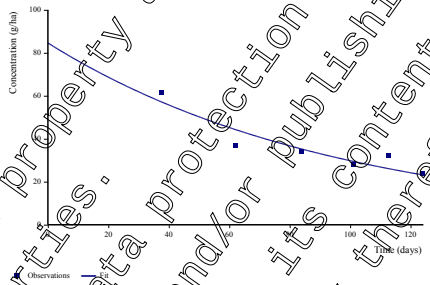
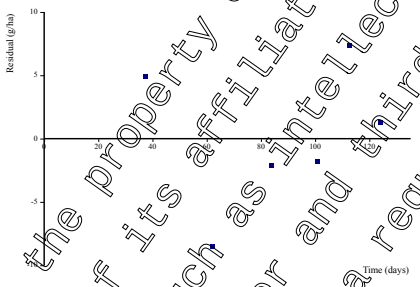
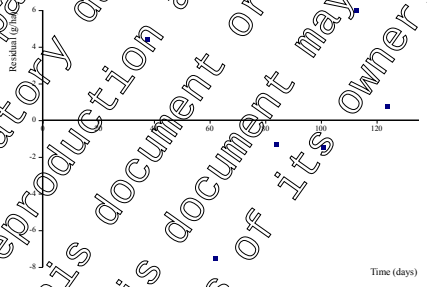
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use SFO $DegT_{50matrix}$ = 44.1 days.

Appendix 4.2.2.2. Metabolite fitting (M01 and M02)

Step 4: Run metabolite SFO from decline. iii) SFO fit for metabolite acceptable?

	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 err %	10.5	9.7
t-test	*k*: p < 0.05	*k*: p < 0.05
DT_{50} (days)	59.2	66.5
DT_{90} (days)	197	221[1]
$DegT_{50matrix}$ (days)	59.2	66.5
Formation fraction	N/A	N/A
Assessment	Fit acceptable. Visual fit is good, χ^2 error is acceptable, and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is good, χ^2 error is acceptable, and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

For metabolite M01 use SFO (from decline) $DegT_{50,\ matrix}$ = 59.2 days.

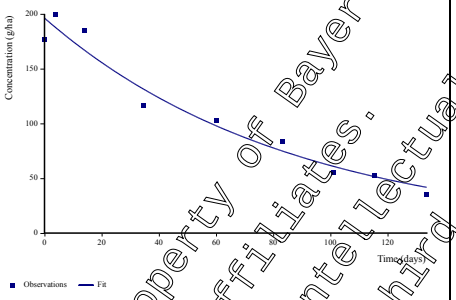
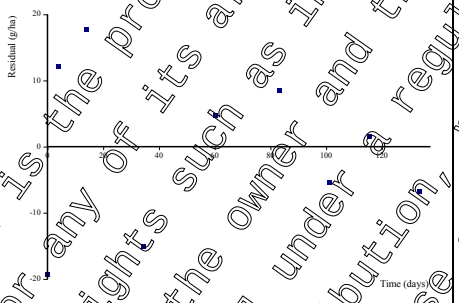
For metabolite M02 use SFO (from decline) $DegT_{50,\ matrix}$ = 66.5 days.

Appendix 4.2.3. Degradation of spiroxamine in Elm Farm/Thurston soil trial no. 30262/7 (KCA 7.1.2.2.1/01 (M-006116-01-1))

Appendix 4.2.3.1. Parent fitting

Step 1: Does decline in lab studies show a lag-phase or effects of long-term sorption kinetics? No

Step 3: Can field decline be described with SFO after eliminating data points before 10 mm rain ensuring no systematic deviations when examining residuals especially when residues at study end are above 10% of initially measured?

	SFO
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error.
χ^2 err %	8.4
t-test	*k*: p < 0.05
DT_{50} (days)	59.9
DT_{90} (days)	199[1]
$DegT_{50\ matrix}$ (days)	59.9
Assessment	Fit acceptable. Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	iii) SFO fit is acceptable. Yes. iv) Use this $DegT50_{matrix}$

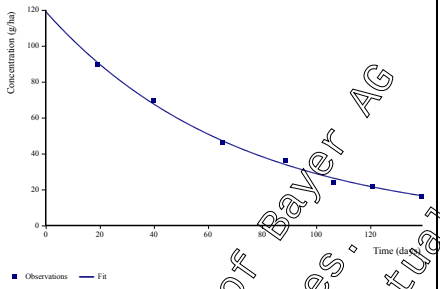
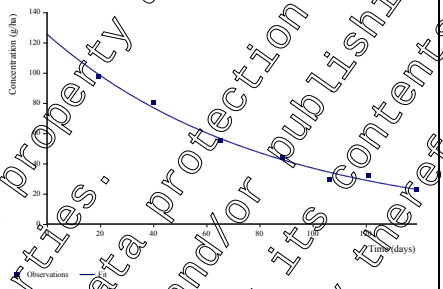
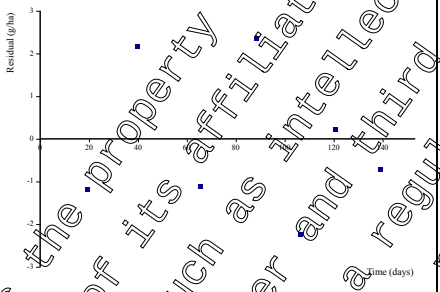
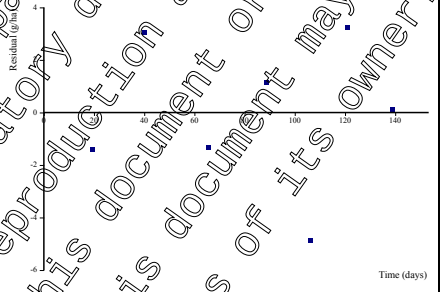
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use SFO $DegT_{50,\ matrix}$ = 59.9 days.

Appendix 4.2.3.2. Metabolite fitting (M01 and M02)

Step 4: Run metabolite SFO from decline. iii) SFO fit for metabolite acceptable?

	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 err %	2.98	4.07
t-test	*k*: $p < 0.05$	*k*: $p < 0.05$
DT_{50} (days)	48.9	56.7
DT_{90} (days)	162[1]	188[1]
$DegT_{50matrix}$ (days)	48.9	56.7
Formation fraction	N/A	N/A
Assessment	Fit acceptable. Visual fit is good, χ^2 error is low, and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is good, χ^2 error is low, and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

For metabolite M01 use SFO (from decline) $DegT_{50,\ matrix}$ = 48.9 days.

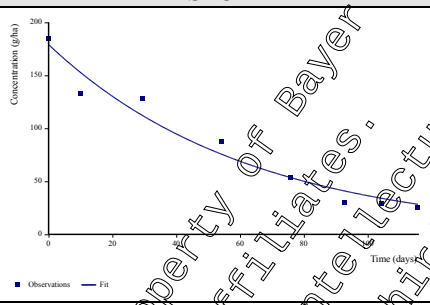
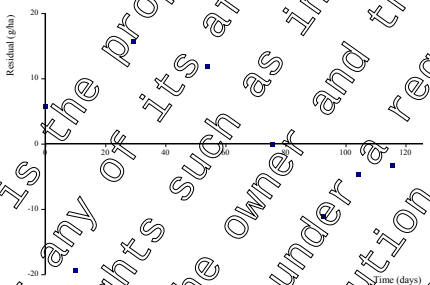
For metabolite M02 use SFO (from decline) $DegT_{50,\ matrix}$ = 56.7 days.

Appendix 4.2.4. Degradation of spiroxamine in Pakenham soil trial no. 30263/5 (KCA 7.1.2.2.1/01 (M-006116-01-1))

Appendix 4.2.4.1. Parent fitting

Step 1: Does decline in lab studies show a lag-phase or effects of long-term sorption kinetics? No

Step 3: Can field decline be described with SFO after eliminating data points before 10 mm rain ensuring no systematic deviations when examining residuals especially when residues at study end are above 10% of initially measured?

	SFO
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error
χ^2 err %	10.4
t-test	*k*: p < 0.05
DT_{50} (days)	43.6
DT_{90} (days)	145[1]
$DegT_{50\,matrix}$ (days)	43.6
Assessment	Fit acceptable. Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero.
Conclusion	iii) SFO fit is acceptable. Yes. iv) Use this $DegT50_{matrix}$

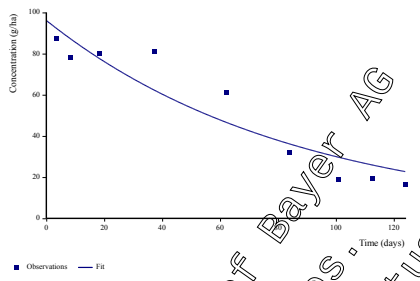
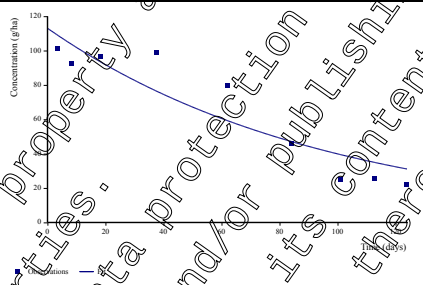
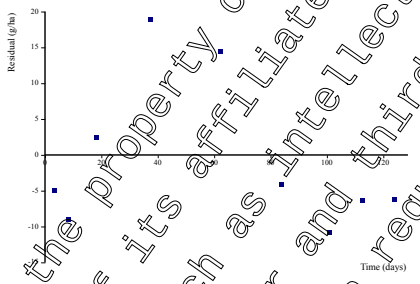
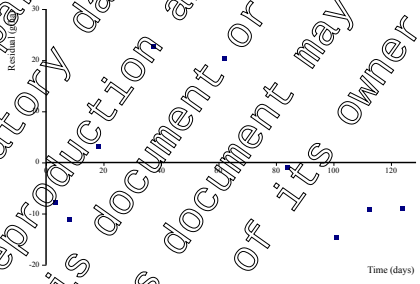
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use SFO $DegT_{50,matrix}$ = 43.6 days.

Appendix 4.2.4.2. Metabolite fitting (M01 and M02)

Step 4: Run metabolite SFO from decline. iii) SFO fit for metabolite acceptable?

	M01	M02
Plot		
Residuals		
Visual fit	Intermediate, residuals show no systematic error and fit is conservative	Intermediate, residuals show no systematic error and fit is conservative
χ^2 err %	15.1	15.8
t-test	*k*: p < 0.05	*k*: p < 0.05
DT_{50} (days)	59.5	66.6
DT_{90} (days)	198	221[1]
$DegT_{50matrix}$ (days)	59.5	66.6
Formation fraction	N/A	N/A
Assessment	Fit acceptable. Visual fit is accetpable, χ^2 error is only slightly high, and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is accetpable, χ^2 error is only slightly high, and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

For metabolite M01 use SFO (decline) $DegT_{50, matrix}$ = 59.5 days.

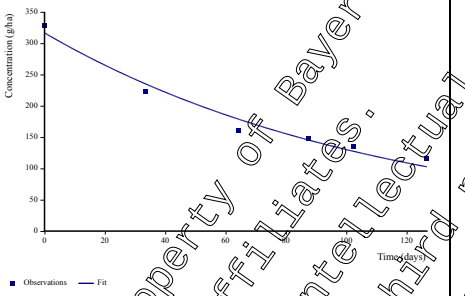
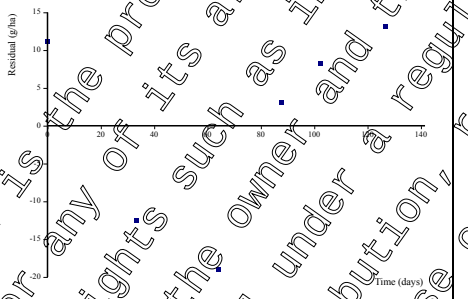
For metabolite M02 use SFO (decline) $DegT_{50, matrix}$ = 66.6 days.

Appendix 4.2.5. Degradation of spiroxamine in Höfchen soil trial no. 40006/8 (KCA 7.1.2.2.1/02 (M-006126-01-1))

Appendix 4.2.5.1. Parent fitting

Step 1: Does decline in lab studies show a lag-phase or effects of long-term sorption kinetics? No

Step 3: Can field decline be described with SFO after eliminating data points before 10 mm rain ensuring no systematic deviations when examining residuals especially when residues at study end are above 10% of initially measured?

	SFO
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error.
χ^2 err %	5.2
t-test	*k*: $p < 0.05$
DT_{50} (days)	78.0
DT_{90} (days)	259[1]
$DegT_{50\ matrix}$ (days)	78
Assessment	Fit acceptable. Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	iii) SFO fit is acceptable. Yes. iv) Use this $DegT50_{matrix}$

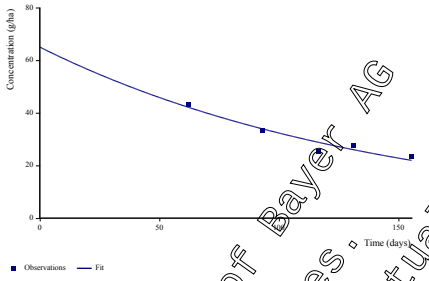
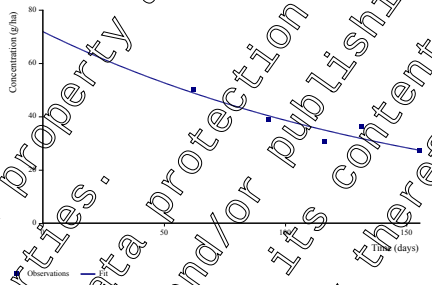
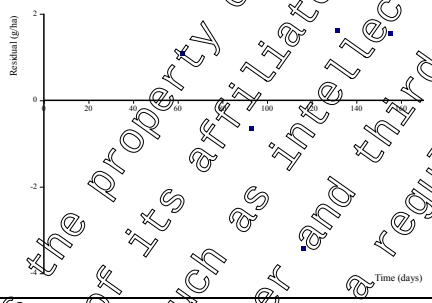
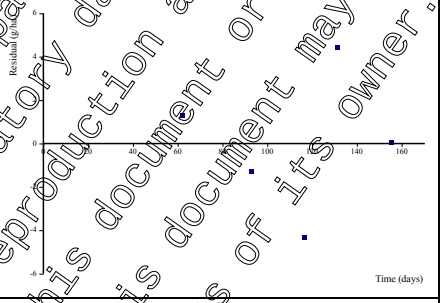
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use SFO $DegT_{50,\ matrix}$ = 78.0 days.

Appendix 4.2.5.2. Metabolite fitting (M01 and M02)

Step 4: Run metabolite SFO from decline. iii) SFO fit for metabolite acceptable?

	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error and fit is conservative	Good, residuals show no systematic error and fit is conservative
χ^2 err %	5.01	6.32
t-test	*k*: $p < 0.05$	*k*: $p < 0.05$
DT_{50} (days)	99.3	112
DT_{90} (days)	330	371[1]
$DegT_{50matrix}$ (days)	99.3	112
Formation fraction	N/A	N/A
Assessment	Fit acceptable. Visual fit is good, χ^2 error is low, and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is good, χ^2 error is low, and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

For metabolite M01 use SFO (decline) $DegT_{50, matrix}$ = 99.3 days.

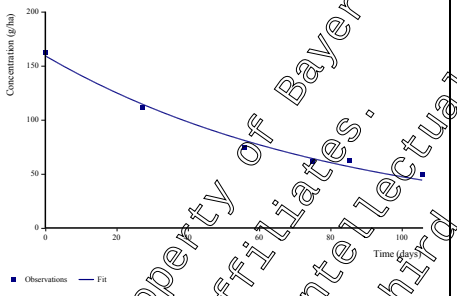
For metabolite M02 use SFO (decline) $DegT_{50, matrix}$ = 112 days.

Appendix 4.2.6. Degradation of spiroxamine in Laacher Hof soil trial no. 40007/6 (KCA 7.1.2.2.1/02 (M-006126-01-1))

Appendix 4.2.6.1. Parent fitting

Step 1: Does decline in lab studies show a lag-phase or effects of long-term sorption kinetics? No

Step 3: Can field decline be described with SFO after eliminating data points before 10 mm rain ensuring no systematic deviations when examining residuals especially when residues at study end are above 10% of initially measured?

	SFO
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error.
χ^2 err %	4.3
t-test	*k*: $p < 0.05$
DT_{50} (days)	57.2
DT_{90} (days)	190[1]
$DegT_{50\ matrix}$ (days)	57.2
Assessment	Fit acceptable. Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	iii) SFO fit is acceptable. Yes. iv) Use this $DegT50_{matrix}$

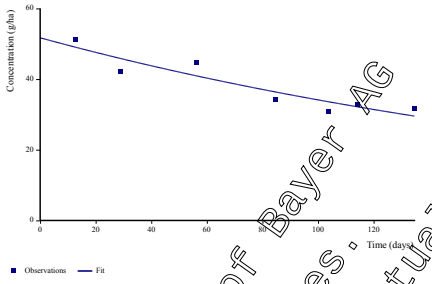
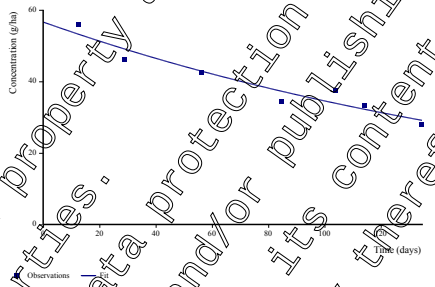
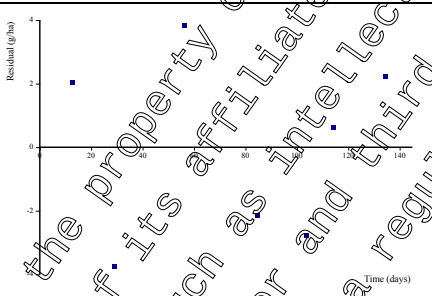
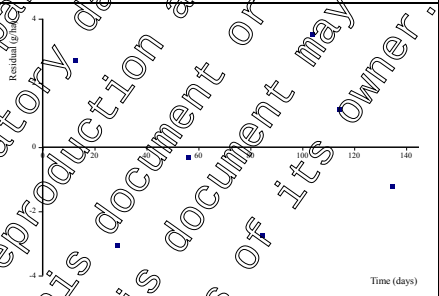
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use SFO $DegT_{50,\ matrix}$ = 57.2 days.

Appendix 4.2.6.2. Metabolite fitting (M01 and M02)

Step 4: Run metabolite SFO from decline. iii) SFO fit for metabolite acceptable?

	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 err %	5.6	4.77
t-test	*k*: p < 0.05	*k*: p < 0.05
DT_{50} (days)	167[1]	140[1]
DT_{90} (days)	554[1]	467[1]
$DegT_{50matrix}$ (days)	167[1]	140[1]
Formation fraction	N/A	N/A
Assessment	Fit acceptable. Visual fit is good, χ^2 error is low, and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is good, χ^2 error is low, and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

For metabolite M01 use SFO (decline) $DegT_{50, matrix}$ = 167 days.

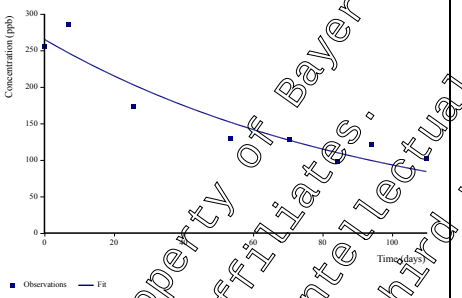
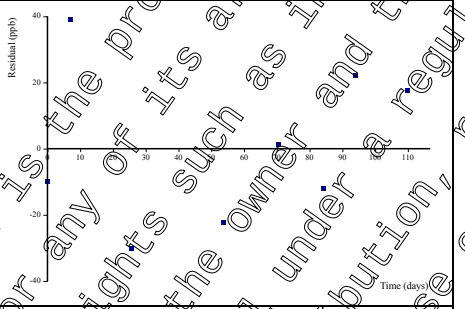
For metabolite M02 use SFO (decline) $DegT_{50, matrix}$ = 140 days

Appendix 4.2.7. Degradation of spiroxamine in Maasen soil trial no. 40008/4 (KCA 7.1.2.2.1/02 (M-006126-01-1))

Appendix 4.2.7.1. Parent fitting

Step 1: Does decline in lab studies show a lag-phase or effects of long-term sorption kinetics? No

Step 3: Can field decline be described with SFO after eliminating data points before 10 mm rain ensuring no systematic deviations when examining residuals especially when residues at study end are above 10% of initially measured?

	SFO
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error.
χ^2 err %	11.0
t-test	*k*: $p < 0.05$
DT_{50} (days)	66.4
DT_{90} (days)	221[1]
$DegT_{50\ matrix}$ (days)	66.4
Assessment	Fit acceptable. Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero.
Conclusion	iii) SFO fit is acceptable. Yes. iv) Use this $DegT50_{matrix}$.

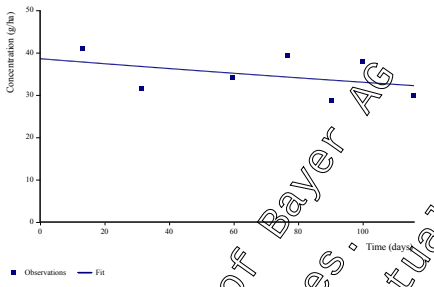
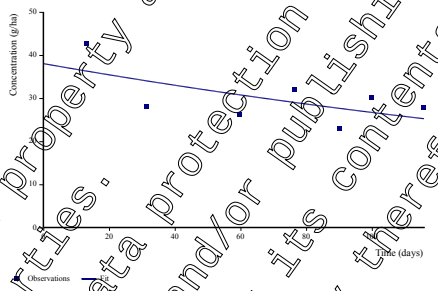
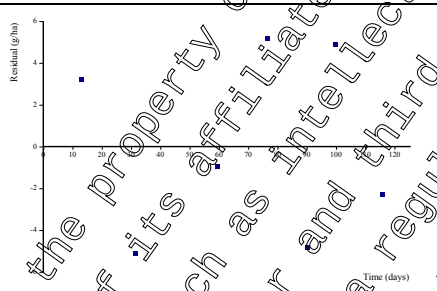
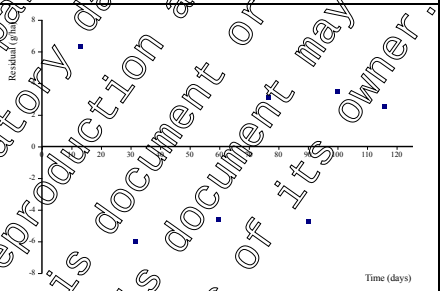
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use SFO $DegT_{50,\ matrix}$ = 66.4 days.

Appendix 4.2.7.2. Metabolite fitting (M01 and M02)

Step 4: Run metabolite SFO from decline. iii) SFO fit for metabolite acceptable?

	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 err %	9.35	12.2
t-test	*k*: p > 0.1	*k*: p < 0.1, >0.05
DT_{50} (days)	445[1]	196[1]
DT_{90} (days)	1,480[1]	651[1]
$DegT_{50matrix}$ (days)	445[1]	196[1]
Formation fraction	N/A	N/A
Assessment	Fit not acceptable. Visual fit is good and χ^2 error is acceptable, however rate parameter does not differ significantly from zero.	Fit acceptable. Visual fit is good, χ^2 error is acceptable, and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is not considered acceptable.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

For metabolite M01 acceptable fit of decline cannot be obtained using SFO. For M01 use conservative default $DegT_{50, matrix}$ = 1,000 days

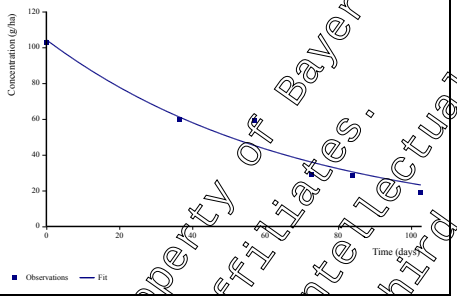
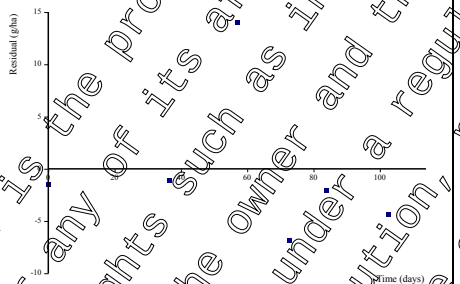
For metabolite M02 use SFO (decline) $DegT_{50, matrix}$ = 196 days

Appendix 4.2.8. Degradation of spiroxamine in Swisttal-Hohn soil trial no. 40009/2 (KCA 7.1.2.2.1/02 (M-006126-01-1))

Appendix 4.2.8.1. Parent fitting

Step 1: Does decline in lab studies show a lag-phase or effects of long-term sorption kinetics? No

Step 3: Can field decline be described with SFO after eliminating data points before 10 mm rain ensuring no systematic deviations when examining residuals especially when residues at study end are above 10% of initially measured?

	SFO
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error.
χ^2 err %	10.8
t-test	*k*: $p < 0.05$
DT_{50} (days)	47.3
DT_{90} (days)	157[1]
$DegT_{50\ matrix}$ (days)	47.3
Assessment	Fit acceptable. Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero.
Conclusion	iii) SFO fit is acceptable. Yes. iv) Use this $DegT50_{matrix}$.

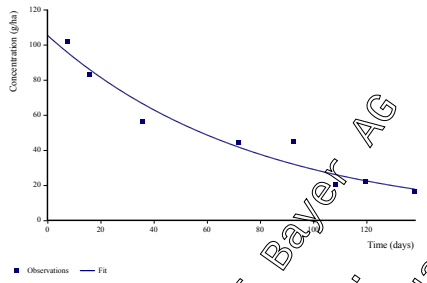
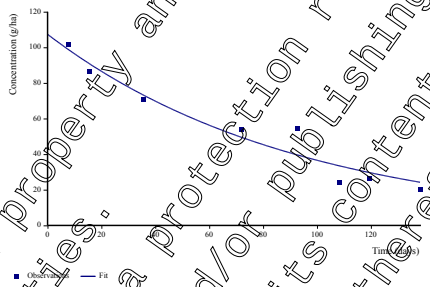
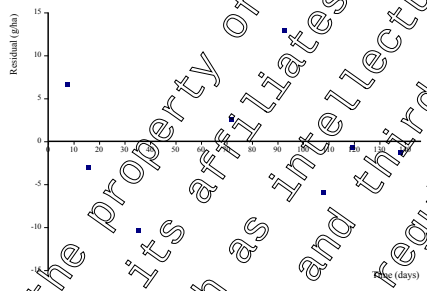
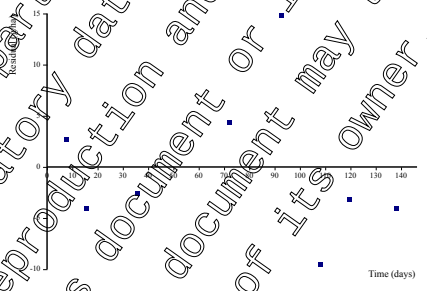
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use SFO $DegT_{50,matrix}$ = 47.3 days.

Appendix 4.2.8.2. Metabolite fitting (M01 and M02)

Step 4: Run metabolite SFO from decline. iii) SFO fit for metabolite acceptable?

	Parent SFO, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 err %	11.2	10.1
t-test	*k*: $p < 0.05$	*k*: $p < 0.05$
DT_{50} (days)	53.8	64.4
DT_{90} (days)	179[1]	214[1]
$DegT_{50matrix}$ (days)	53.8	64.4
Formation fraction	N/A	N/A
Assessment	Fit not acceptable. Visual fit is good, χ^2 error is acceptable, and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is good, χ^2 error is acceptable, and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

For metabolite M01 use SFO (decline) $DegT_{50, matrix}$ = 53.8 days.

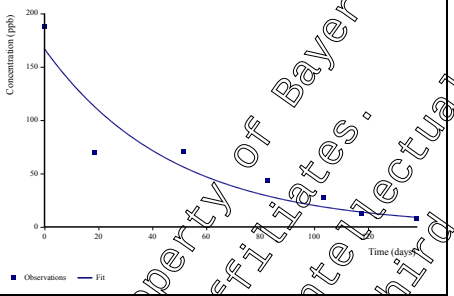
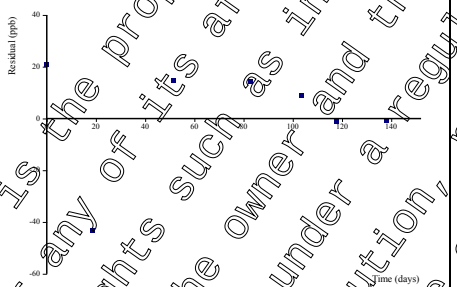
For metabolite M02 use SFO (decline) $DegT_{50, matrix}$ = 64.4 days

Appendix 4.2.9. Degradation of spiroxamine in Albig soil trial no. 40010/6 (KCA 7.1.2.2.1/02 (M-006126-01-1))

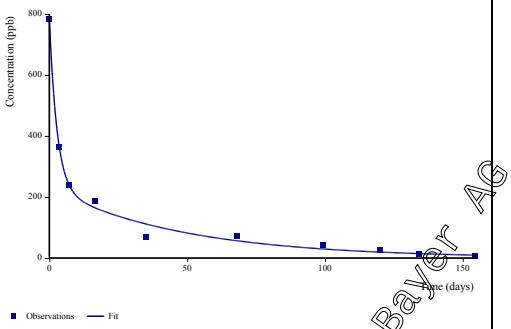
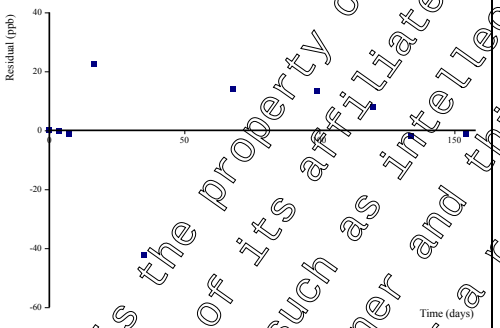
Appendix 4.2.9.1. Parent fitting

Step 1: Does decline in lab studies show a lag-phase or effects of long-term sorption kinetics? No

Step 3: Can field decline be described with SFO after eliminating data points before 10 mm rain ensuring no systematic deviations when examining residuals especially when residues at study end are above 10% of initially measured?

	SFO
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error
χ^2 err %	26.5
t-test	*k*: p < 0.05
DT_{50} (days)	32.8
DT_{90} (days)	109
$DegT_{50\ matrix}$ (days)	32.8
Assessment	Fit acceptable. Visual fit is good and rate parameter differs significantly from zero, however, χ^2 error is high.
Conclusion	iii) SFO fit is acceptable. No. Go to step 5.

Step 5: Fit DFOP to complete dataset + estimate breakpoint?

	DFOP
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error.
χ^2 error (%)	8.2
t-test	k_1: p < 0.05 k_2: p <0.05
DT_{50} (days)	33
DT_{90} (days)	52.2
Modelling DT_{50} (days)	k: DT_{50} = 33.7
g	0.71
k_1	0.362 (0.213 - 0.511)
k_2	0.021 (0.0096 - 0.032)
>10 mm occurs after x days (normalised time)	16.4
Assessment	Fit acceptable. Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero.
Discussion	v) breakpoint estimate = c.a 6 days. vi) g <0.75? Yes. vii) k_{fast} and k_{slow} significantly different? Yes. viii) rain > 10 mm at breakpoint? Yes.

Summary:

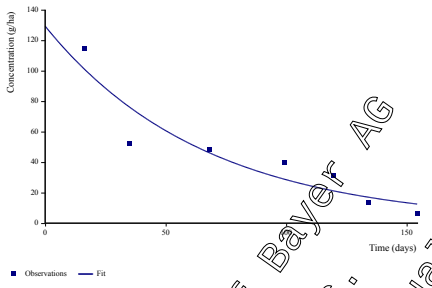
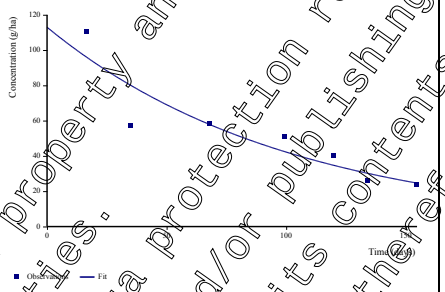
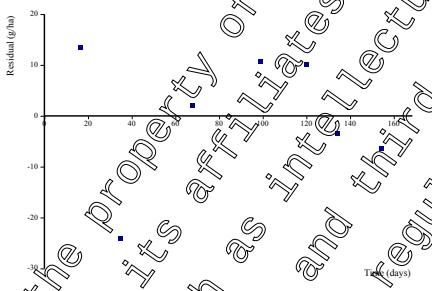
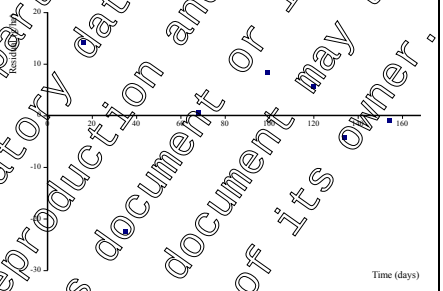
Following the decision flow in the guidance leads to the conclusion that for spiroxamine modelling endpoints are not derivable, however, this essentially results from the estimate of when > 10 mm of rainfall occurs compared to the estimated breakpoint. The recorded cumulative amount of rainfall on the day of the 3rd sampling occasion (i.e. 14 d non normalised time or 7.2 d normalised time) was 9 mm and the next recorded measurement, yielding 45 mm cumulative rainfall, at the next sampling occasion (i.e. 30 d non normalised time or 16.4 d normalised time) results in the exceedance of 10 mm. It is considered very likely that cumulative rainfall exceeded 10 mm shortly after 7.2 d normalised time, i.e. before the estimated breakpoint of 10 days, given measured rainfall for this site (see details in kinetic evaluation report **Error! Reference source not found.** (**Error! Reference source not found.**)).

Summary:

For spiroxamine use use DFOP k_{slow} $DegT_{50,\ matrix}$ = 33.7 days.

Appendix 4.2.9.2. Metabolite fitting (M01 and M02)

Step 4: Run metabolite SFO from decline. iii) SFO fit for metabolite acceptable?

	Parent SFO, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 err %	22.2	16.5
t-test	*k*: $p < 0.05$	*k*: $p < 0.05$
DT_{50} (days)	45.9	70.4
DT_{90} (days)	152	234[1]
$DegT_{50matrix}$ (days)	45.9	70.4
Formation fraction	N/A	N/A
Assessment	Fit acceptable. Visual fit is good, χ^2 error is slightly high, and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is good, χ^2 error is slightly high, and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

For metabolite M01 use SFO (decline) $DegT_{50, matrix}$ = 45.9 days.

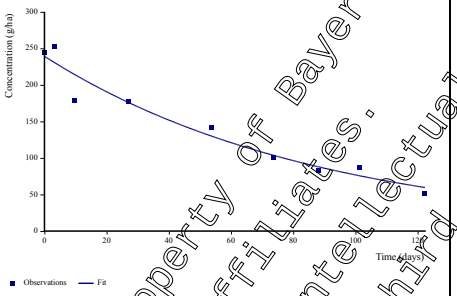
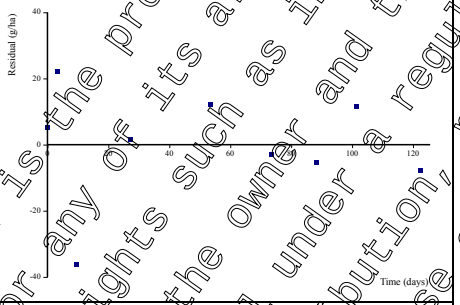
For metabolite M02 use SFO (decline) $DegT_{50, matrix}$ = 70.4 days.

Appendix 4.2.10. Degradation of spiroxamine in Elm Farm/Thurston soil trial no. 40097/1 (KCA 7.1.2.2.1/03 (M-006127-01-1))

Appendix 4.2.10.1. Parent fitting

Step 1: Does decline in lab studies show a lag-phase or effects of long-term sorption kinetics? No

Step 3: Can field decline be described with SFO after eliminating data points before 10 mm rain ensuring no systematic deviations when examining residuals especially when residues at study end are above 10% of initially measured?

	SFO
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error.
χ^2 err %	8.6
t-test	*k*: $p < 0.05$
DT_{50} (days)	61.0
DT_{90} (days)	203[1]
$DegT_{50\ matrix}$ (days)	61.0
Assessment	Fit acceptable. Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	iii) SFO fit is acceptable. Yes. iv) Use this $DegT50_{matrix}$

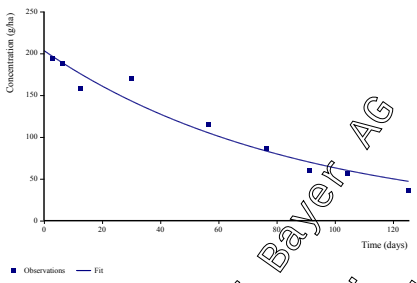
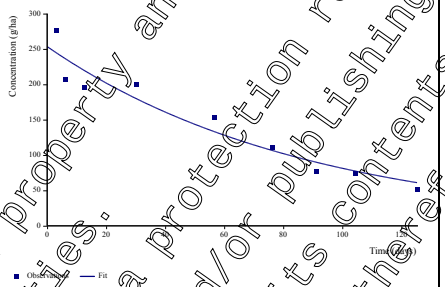
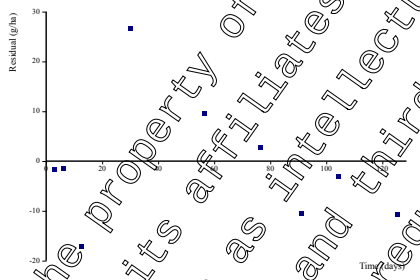
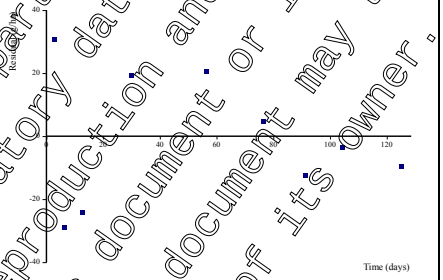
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use SFO $DegT_{50,\ matrix}$ = 61.0 days.

Appendix 4.2.10.2.Metabolite fitting (M01 and M02)

Step 4: Run metabolite SFO from decline. iii) SFO fit for metabolite acceptable?

	Parent SFO, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 err %	8.27	10.5
t-test	*k*: p < 0.05	*k*: p < 0.05
DT_{50} (days)	59.3	61
DT_{90} (days)	197[1]	203[1]
$DegT_{50matrix}$ (days)	59.3	61
Formation fraction	N/A	N/A
Assessment	Fit acceptable. Visual fit is good, χ^2 error is low, and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is good, χ^2 error is acceptable, and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

For metabolite M01 use SFO (decline) $DegT_{50, matrix}$ = 59.3 days.

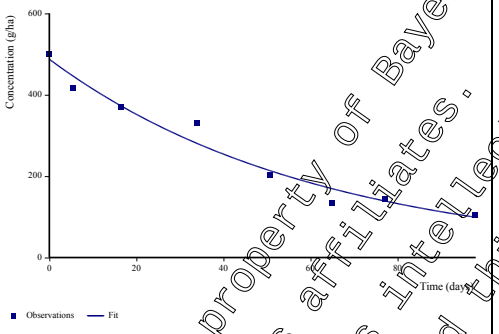
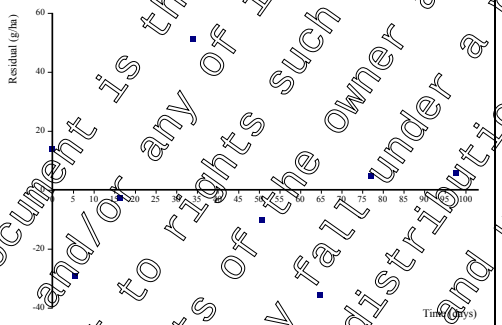
For metabolite M02 use SFO (decline) $DegT_{50, matrix}$ = 61 days.

Appendix 4.2.11. Degradation of spiroxamine in Pakenham soil trial no. 40099/8 (KCA 7.1.2.2.1/03 (M-006127-01-1))

Appendix 4.2.11.1. Parent fitting

Step 1: Does decline in lab studies show a lag-phase or effects of long-term sorption kinetics? No

Step 3: Can field decline be described with SFO after eliminating data points before 10 mm rain ensuring no systematic deviations when examining residuals especially when residues at study end are above 10% of initially measured?

	SFO
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error.
χ^2 error (%)	7.3
t-test	t, $p < 0.05$
DT_{50} (days)	42.6
DT_{90} (days)	142[1]
Modelling DT_{50} (days)	42.6
Assessment	Fit acceptable. Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero.
Discussion	iii) SFO fit is acceptable. Yes. iv) Use this $DegT50_{matrix}$.

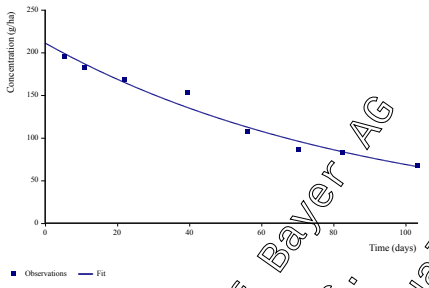
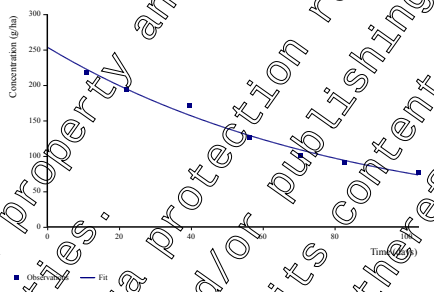
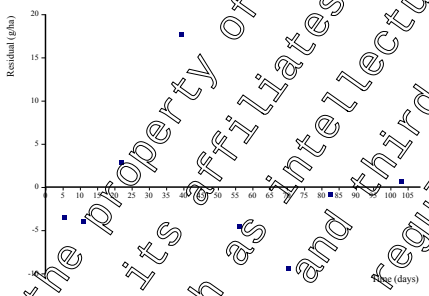
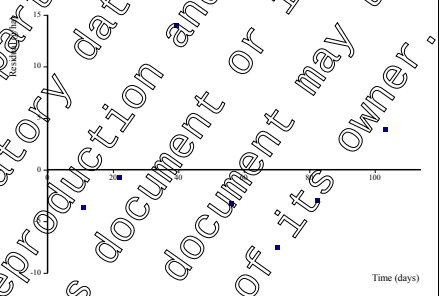
1 Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use SFO $DegT50_{matrix}$ = 42.6 days

Appendix 4.2.11.2.Metabolite fitting (M01 and M02)

Step 4: Run metabolite SFO from decline. iii) SFO fit for metabolite acceptable?

	Parent SFO, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 err %	4.64	3.72
t-test	*k*: $p < 0.05$	*k*: $p < 0.05$
DT_{50} (days)	61.8	57.7
DT_{90} (days)	205[1]	192[1]
$DegT_{50matrix}$ (days)	61.8	57.7
Formation fraction	N/A	N/A
Assessment	Fit acceptable. Visual fit is good, χ^2 error is low, and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is good, χ^2 error is low, and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

For metabolite M01 use SFO (decline) $DegT_{50, matrix}$ = 61.8 days.

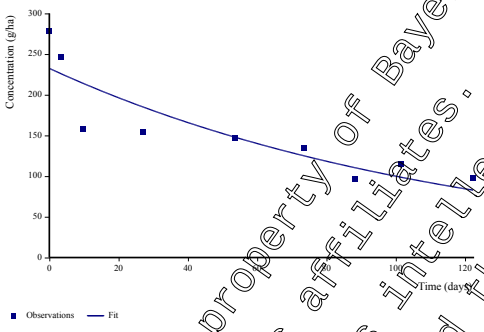
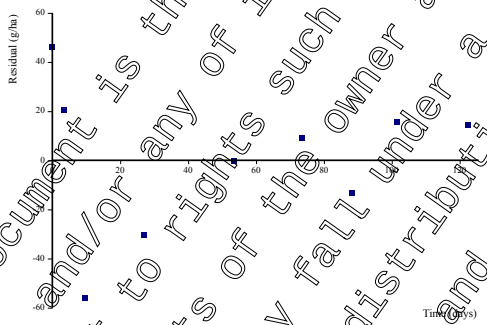
For metabolite M02 use SFO (decline) $DegT_{50, matrix}$ = 57.7 days.

Appendix 4.2.12. Degradation of spiroxamine in Elm Farm/Thurston soil trial no. 40100/5 (KCA 7.1.2.2.1/03 (M-006127-01-1))

Appendix 4.2.12.1. Parent fitting

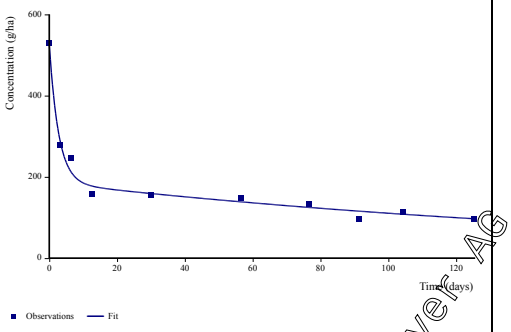
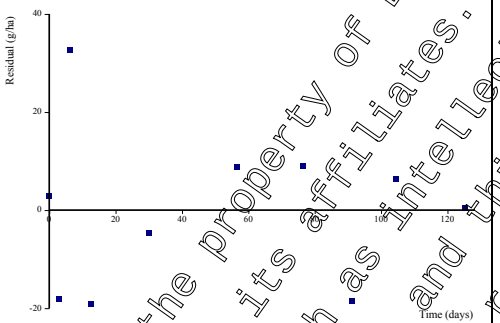
Step 1: Does decline in lab studies show a lag-phase or effects of long-term sorption kinetics? No

Step 3: Can field decline be described with SFO after eliminating data points before 10 mm rain ensuring no systematic deviations when examining residuals especially when residues at study end are above 10% of initially measured?

	SFO
Plot	
Residuals	
Visual fit	Good, however residuals show potential systematic error.
χ^2 error (%)	14.4
t-test	ns: $p < 0.05$
DT_{50} (days)	82.2
DT_{90} (days)	273[1]
Modelling DT_{50} (days)	82.2
Assessment	Fit not acceptable. Visual fit is poor, χ^2 error is high but rate parameter differs significantly from zero.
Discussion	iii) SFO fit is acceptable. No. Go to Step 5

[1] Interpret with care – extrapolated beyond experimental period

Step 5: Fit DFOP to complete dataset + estimate breakpoint?

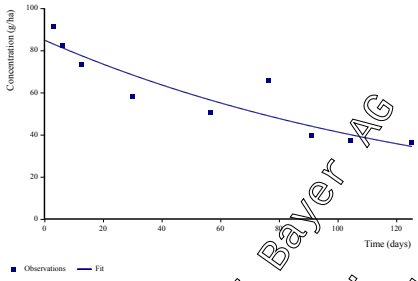
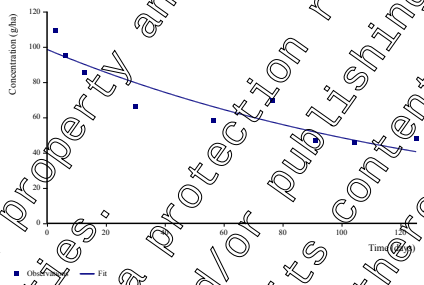
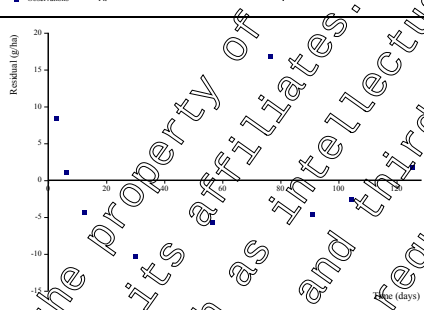
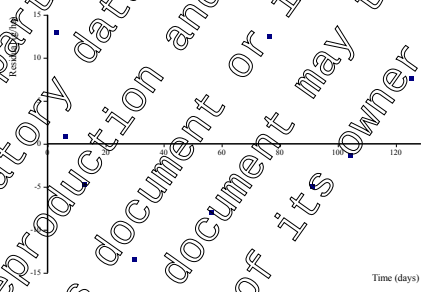
	DFOP
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error.
χ^2 error (%)	7.0
t-test	k_1: p< 0.05 k_2: p <0.05
DT_{50} (days)	3.9
DT_{90} (days)	243
Modelling DT_{50} (days)	k_2 DT_{50} = 133
g	0.65
k_1	0.367 (0.1914 - 0.542)
k_2	0.0052 (0.0014 - 0.009)
>10 mm occurs after x days (normalised time)	3.0
Assessment	Fit acceptable. Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero.
Discussion	v) breakpoint estimate ~*ca* 5.67 days. vi) g < 0.75? Yes. vii) k_{fast} and k_{slow} significantly different? Yes. viii) rain > 10 mm at breakpoint? Yes. Use DT50 based on k_{slow}.

Summary:

For spiroxamine use DFOP k_{slow} $DegT_{50,matrix}$ = 133 days.

Appendix 4.2.12.2.Metabolite fitting (M01 and M02)

Step 4: Run metabolite SFO from decline. iii) SFO fit for metabolite acceptable?

	Parent SFO, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show small potential systematic error	Good, residuals show no systematic error
χ^2 err %	10.5	9.96
t-test	*k*: $p < 0.05$	*k*: $p < 0.05$
DT_{50} (days)	96.2	98
DT_{90} (days)	320[1]	326[1]
$DegT_{50matrix}$ (days)	96.2	98
Formation fraction	N/A	N/A
Assessment	Fit acceptable. Visual fit is good, χ^2 error is acceptable, and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is good, χ^2 error is acceptable, and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

For metabolite M01 use SFO (decline) $DegT_{50, matrix}$ = 96.2 days.

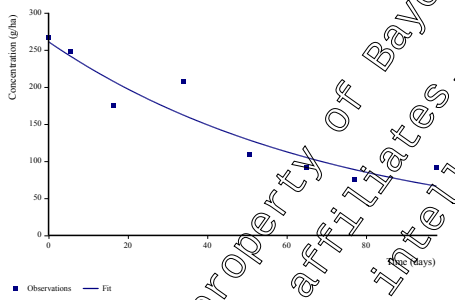
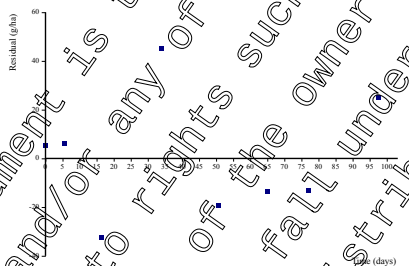
For metabolite M02 use SFO (decline) $DegT_{50, matrix}$ = 98 days.

Appendix 4.2.13. Degradation of spiroxamine in Pakenham soil trial no. 40101/3 (KCA 7.1.2.2.1/03 (M-006127-01-1))

Appendix 4.2.13.1. Parent fitting

Step 1: Does decline in lab studies show a lag-phase or effects of long-term sorption kinetics? No

Step 3: Can field decline be described with SFO after eliminating data points before 10 mm rain ensuring no systematic deviations when examining residuals especially when residues at study end are above 10% of initially measured?

	SFO
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error.
χ^2 error (%)	12.0
t-test	t test $p < 0.05$
DT_{50} (days)	49.5
DT_{90} (days)	165[1]
Modelling DT_{50} (days)	49.5
Assessment	Fit acceptable. Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero.
Discussion	iii) SFO fit is acceptable. Yes. iv) Use this $DegT50_{matrix}$.

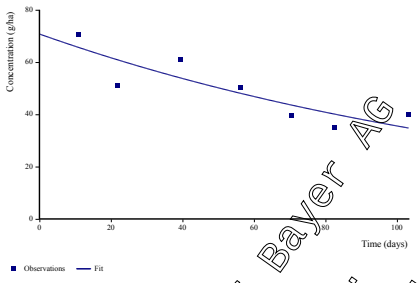
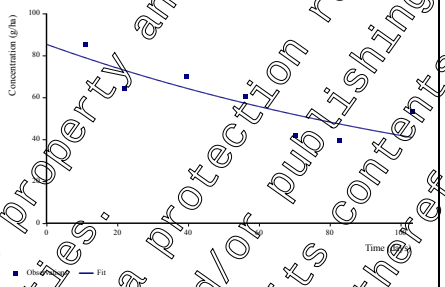
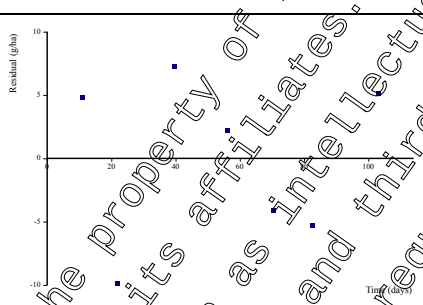
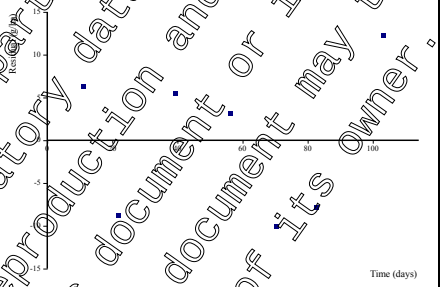
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use SFO $DegT50_{matrix}$ = 49.5 days

Appendix 4.2.13.2.Metabolite fitting (M01 and M02)

Step 4: Run metabolite SFO from decline. iii) SFO fit for metabolite acceptable?

	Parent SFO, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 err %	9.57	11.0
t-test	*k*: p < 0.05	*k*: p < 0.05
DT_{50} (days)	101	97.3
DT_{90} (days)	335[1]	323[1]
$DegT_{50matrix}$ (days)	101	97.3
Formation fraction	N/A	N/A
Assessment	Fit acceptable. Visual fit is good, χ^2 error is acceptable, and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is good, χ^2 error is acceptable, and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

For metabolite M01 use SFO (decline) $DegT_{50,matrix}$ = 101.0 days.

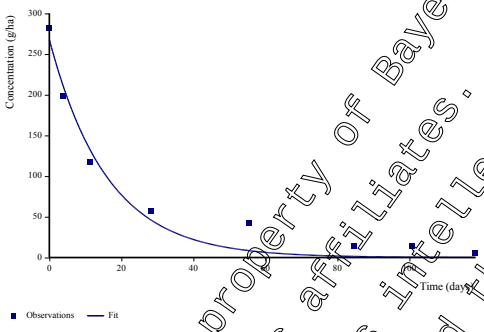
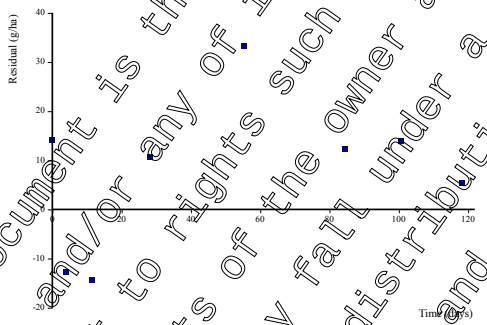
For metabolite M02 use SFO (decline) $DegT_{50,matrix}$ = 97.3 days.

Appendix 4.2.14. Degradation of spiroxamine in Touffreville soil trial no. 40193/5 (KCA 7.1.2.2.1/03 (M-006127-01-1))

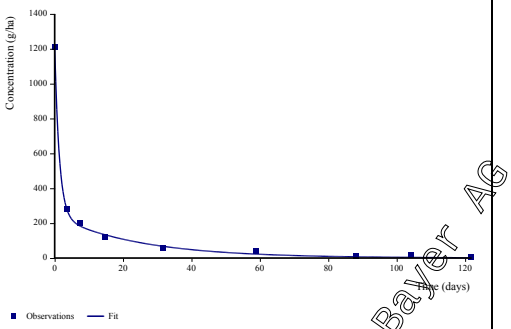
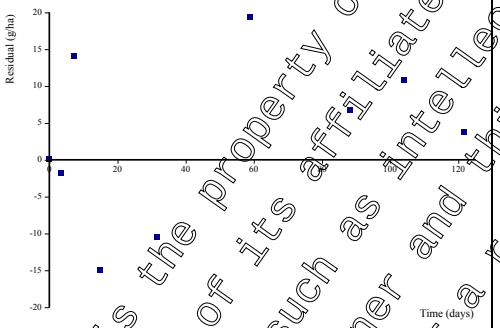
Appendix 4.2.14.1. Parent fitting

Step 1: Does decline in lab studies show a lag-phase or effects of long-term sorption kinetics? No

Step 3: Can field decline be described with SFO after eliminating data points before 10 mm rain ensuring no systematic deviations when examining residuals especially when residues at study end are above 10% of initially measured?

	SFO
Plot	
Residuals	
Visual fit	Poor, residuals show systematic error and fit not conservative.
χ^2 error (%)	14.4
t-test	ii: $p < 0.05$
DT_{50} (days)	111
DT_{90} (days)	37
Assessment	Fit not acceptable. χ^2 error is acceptable and rate parameter differs significantly from zero, however visual fit is poor.
Discussion	iii) SFO fit is acceptable. No. Go to step 5.

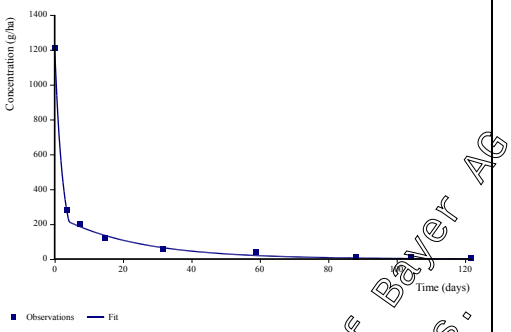
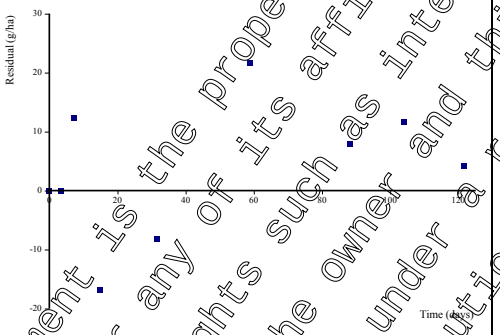
Step 5: Fit DFOP to complete dataset + estimate breakpoint?

	DFOP
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error
χ^2 error (%)	4.6
t-test	k_1: p < 0.05 k_2: p <0.05
DT_{50} (days)	13
DT_{90} (days)	17.2
Modelling DT_{50} (days)	k: DT_{50} = 17.2
g	0.80
k_1	0.7347 (0.481 - 0.989)
k_2	0.0404 (0.021 - 0.060)
Assessment	Fit acceptable. Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero.
Discussion	v) breakpoint estimate = 2.8 days v) g < 0.75? No Go to Hockey stick flow chart.

[1] Interpret with care – extrapolated beyond experimental period

Hockey stick flow chart:

Step 1: Fit HS to complete dataset

	HS
Plot	 Note: starting parameters amended to tb=10 to allow errors and t-test to be calculated (co-variance matrix error)
Residuals	
Visual fit	Excellent, residuals show no systematic error.
χ^2 error (%)	4.8
t-test	k_1: p < 0.05 k_2: p <0.05
DT_{50} (days)	1.7
DT_{90} (days)	17.4
Modelling DT_{50} (days)	k_2 DT_{50} = 16.1
tb	4.2
>10 mm occurs after x days (normalised time)	3.5
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.
Discussion	ii) rain >10 mm at breakpoint? Yes. iv) fit acceptable? Yes. v) k_2 significantly >0? Yes. Use k_2.

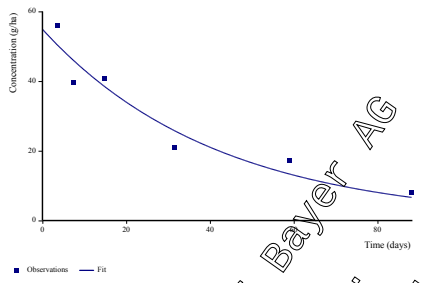
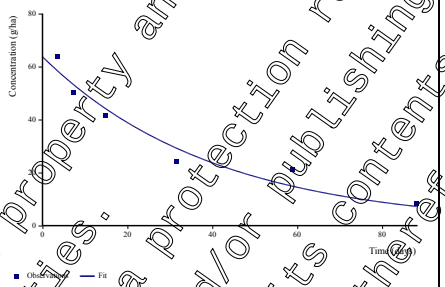
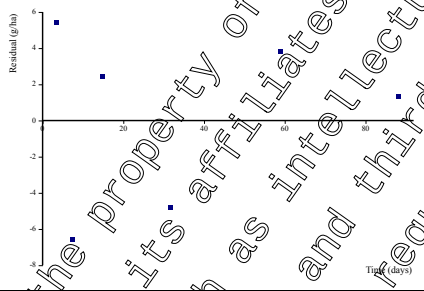
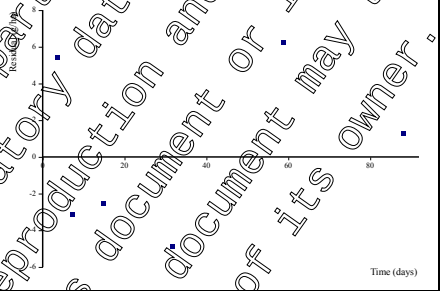
1 Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use HS k_{slow} $DegT_{50,\ matrix}$ = 16.1 days.

Appendix 4.2.14.2.Metabolite fitting (M01 and M02)

Step 4: Run metabolite SFO from decline. iii) SFO fit for metabolite acceptable?

	Parent SFO, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 err %	11.6	9.75
t-test	*k*: $p < 0.05$	*k*: $p < 0.05$
DT_{50} (days)	28.9	28.1
DT_{90} (days)	96.1[1]	93.2[1]
$DegT_{50matrix}$ (days)	28.9	28.1
Formation fraction	N/A	N/A
Assessment	Fit acceptable. Visual fit is good, χ^2 error is acceptable, and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is good, χ^2 error is acceptable, and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

For metabolite M01 use SFO (decline) $DegT_{50, matrix}$ = 28.9 days.

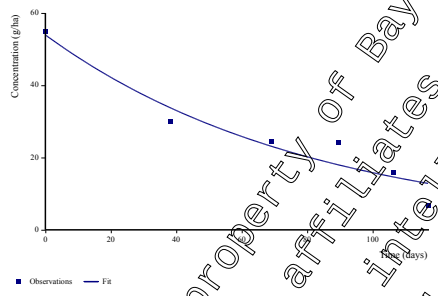
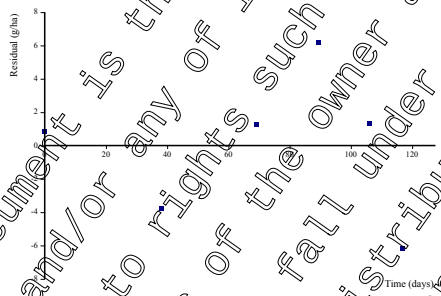
For metabolite M02 use SFO (decline) $DegT_{50, matrix}$ = 28.1 days.

Appendix 4.2.15. Degradation of spiroxamine in Laudun soil trial no. 40198/6 (KCA 7.1.2.2.1/04 (M-006128-01-1))

Appendix 4.2.15.1. Parent fitting

Step 1: Does decline in lab studies show a lag-phase or effects of long-term sorption kinetics? No

Step 3: Can field decline be described with SFO after eliminating data points before 10 mm rain ensuring no systematic deviations when examining residuals especially when residues at study end are above 10% of initially measured?

	SFO
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error.
χ^2 error (%)	12.2
t-test	ok, $p < 0.05$
DT_{50} (days)	56.5
DT_{90} (days)	188[1]
Modelling DT_{50} (days)	56.5
Assessment	Fit acceptable. Visual fit is good, rate parameter differs significantly from zero, and χ^2 error is acceptable.
Discussion	iii) SFO fit is acceptable. Yes. iv) Use this $DegT50_{matrix}$. iii) SFO fit is acceptable. Yes. Use SFO $DT_{50\ mod}$.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use SFO $DT_{50\ mod}$ = 56.5 days.

Appendix 4.2.15.2.Metabolite fitting (M01 and M02)

Insufficient data points to run metabolite. Only two data points for each metabolite.

Summary:

For metabolite M01 acceptable fit of decline cannot be obtained.

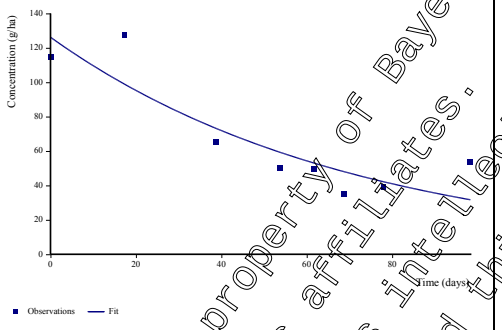
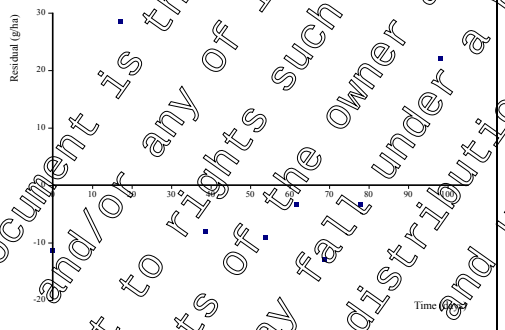
For metabolite M02 acceptable fit of decline cannot be obtained.

Appendix 4.2.16. Degradation of spiroxamine in Filetto soil trial no. 40424/1 (KCA 7.1.2.2.1/04 (M-006128-01-1))

Appendix 4.2.16.1. Parent fitting

Step 1: Does decline in lab studies show a lag-phase or effects of long-term sorption kinetics? No.

Step 3: Can field decline be described with SFO after eliminating data points before 10 mm rain ensuring no systematic deviations when examining residuals especially when residues at study end are above 10% of initially measured?

	SFO
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error.
χ^2 error (%)	17.8
t-test	t, p < 0.05
DT_{50} (days)	49.3
DT_{90} (days)	164[1]
Modelling DT_{50} (days)	49.3
Assessment	Fit potentially acceptable. Visual fit is good and rate parameter differs significantly from zero, however, χ^2 error is slightly high. Nevertheless, residuals show no large systematic error.
Discussion	iii) SFO fit is acceptable. Yes. iv) Use this $DegT50_{matrix}$.

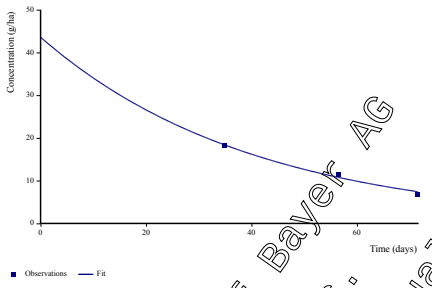
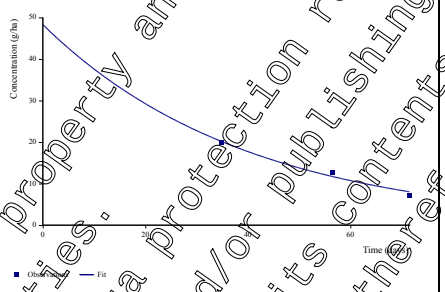
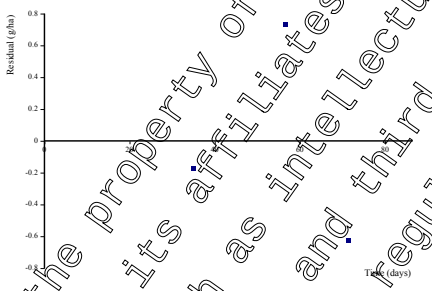
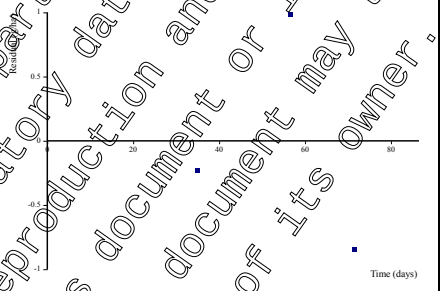
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use SFO $DegT_{50,\ matrix}$ = 49.3 days.

Appendix 4.2.16.2.Metabolite fitting (M01 and M02)

Step 4: Run metabolite SFO from decline. iii) SFO fit for metabolite acceptable?

	Parent SFO, metabolite SFO	
	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 err %	4.12	5.07
t-test	*k*: p < 0.05	*k*: p >0.1, >0.05
DT_{50} (days)	28	27.7
DT_{90} (days)	93[1]	91.9[1]
$DegT_{50matrix}$ (days)	28	27.7
Formation fraction	N/A	N/A
Assessment	Fit acceptable. Visual fit is good, χ^2 error is low, and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is good, χ^2 error is low, and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

For metabolite M01 use SFO (decline) $DegT_{50, matrix}$ = 28 days.

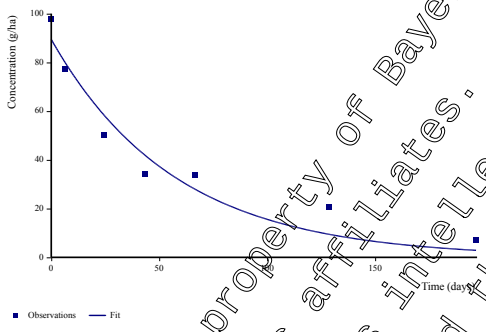
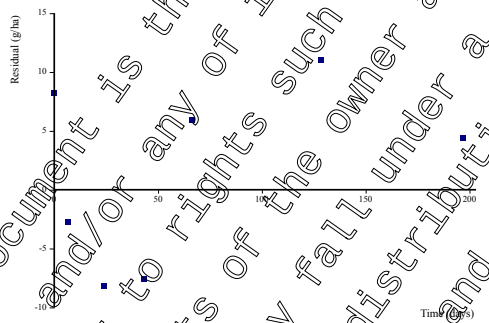
For metabolite M02 use SFO (decline) $DegT_{50, matrix}$ = 27.7 days.

Appendix 4.2.17. Degradation of spiroxamine in Laudun soil trial no. 50135/2 (KCA 7.1.2.2.1/05 (M-006129-01-1))

Appendix 4.2.17.1. Parent fitting

Step 1: Does decline in lab studies show a lag-phase or effects of long-term sorption kinetics? No

Step 3: Can field decline be described with SFO after eliminating data points before 10 mm rain ensuring no systematic deviations when examining residuals especially when residues at study end are above 10% of initially measured?

	SFO
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error.
χ^2 error (%)	12.7
t-test	k_1 p < 0.05
DT_{50} (days)	39.7
DT_{90} (days)	132[1]
Modelling DT_{50} (days)	39.7
Assessment	Fit acceptable. Visual fit is good, χ^2 error is acceptable and rate parameter differs significantly from zero.
Discussion	iii) SFO fit is acceptable. Yes. iv) Use this $DegT50_{matrix}$.

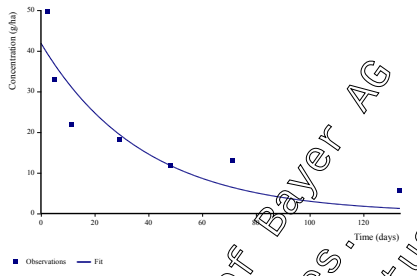
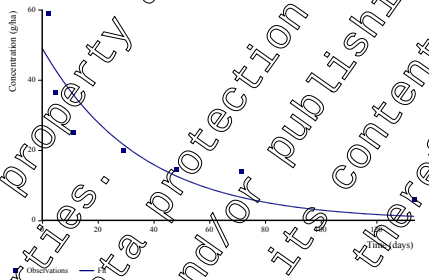
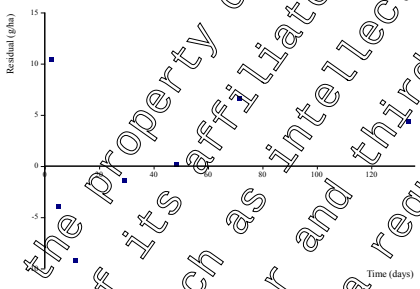
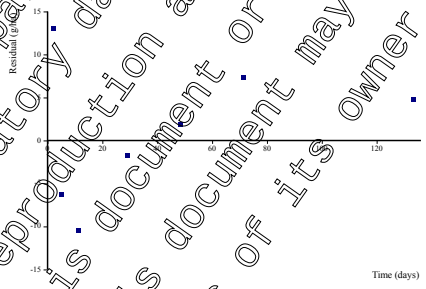
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use SFO $DegT_{50, matrix}$ = 39.7 days.

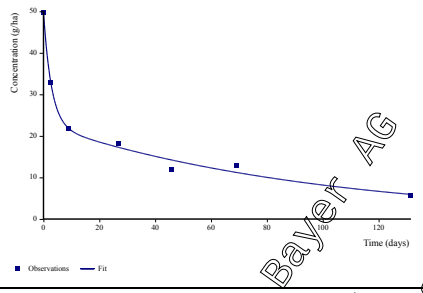
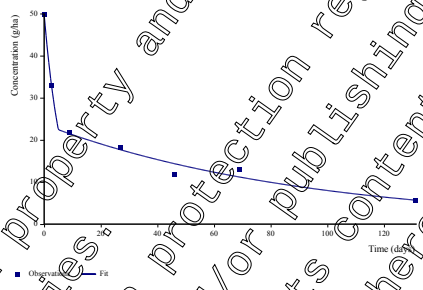
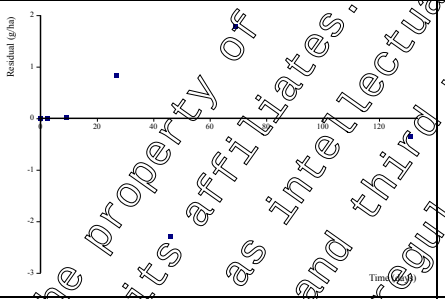
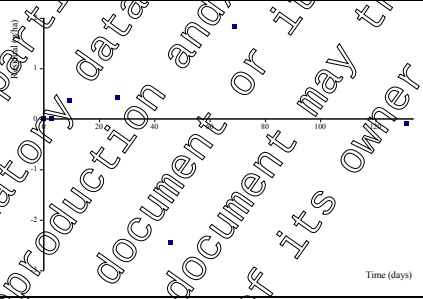
Appendix 4.2.17.2.Metabolite fitting (M01 and M02)

Step 4: Run metabolite SFO from decline. iii) SFO fit for metabolite acceptable?

	M01	M02
Plot		
Residuals		
Visual fit	Intermediate, residuals show potential systematic error and final two time points underestimated	Intermediate, residuals show potential systematic error and final three time points underestimated
χ^2 err %	22.7	24.2
t-test	*k*: p < 0.05	*k*: p < 0.05
DT_{50} (days)	26.4	24.6
DT_{90} (days)	87.6[1]	81.7[1]
$DegT_{50matrix}$ (days)	26.4	24.6
Formation fraction	N/A	N/A
Assessment	Fit not acceptable. Visual fit is intermediate, and final time points underestimated, with χ^2 error slightly high. However, rate parameter differs significantly from zero.	Fit not acceptable. Visual fit is intermediate, and final time points underestimated, with χ^2 error slightly high. However, rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.

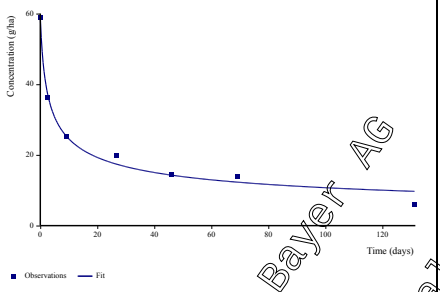
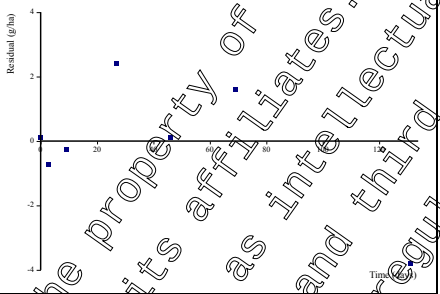
[1] Interpret with care – extrapolated beyond experimental period

Step 5: M01 - 10% reached in study period? No. Run metabolite DFOP and HS from decline. iii) DFOP or HS fit for metabolite acceptable?

	M01	
	DFOP	HS
Plot		
Residuals		
Visual fit	Excellent, residuals show no systematic error	Excellent, residuals show no systematic error
χ^2 err %	4.98	5.09
t-test	k_1: p <0.05 k_2: p <0.05	k_1: p < 0.05 k_2: p <0.05
DT_{50} (days)	5.79	4.35
DT_{90} (days)	149[1]	143[1]
$DegT_{50matrix}$ (days)	67.9[2]	63.8[2]
Formation fraction	N/A	N/A
Assessment	Fit acceptable. Visual fit is excellent and χ^2 error is low, however rate parameters differ significantly from zero.	Fit acceptable. Visual fit is excellent, χ^2 error is low, and rate parameters differ significantly from zero.
Discussion	iv) DFOP or HS fit for metabolite acceptable? Both are considered acceptable. DFOP chosen as conservative and should be used for modelling endpoints.	

[1] Interpret with care – extrapolated beyond experimental period
[2] Based on slow phase (k_2)

Step 5: M02 - 10% reached in study period? Yes. Run metabolite FOMC from decline. iii) FOMC fit for metabolite acceptable?

	M02
	FOMC
Plot	
Residuals	
Visual fit	Excellent, residuals show no systematic error
χ^2 err %	6.31
t-test	NA
DT_{50} (days)	5.75
DT_{90} (days)	302[1]
$DegT_{50matrix}$ (days)	151[2]
Formation fraction	N/A
Assessment	Fit acceptable. Visual fit is excellent and χ^2 error is low.
Discussion	iv) FOMC fit for metabolite acceptable? Yes. FOMC should be used for modelling endpoints.

[1] Interpret with care - extrapolated beyond experimental period
[2] Based on DT_{90} / 3.32

Summary

For metabolite M01 use slow phase (k_2) of DFOP of decline phase, $DegT_{50, matrix}$ = 67.9 days.

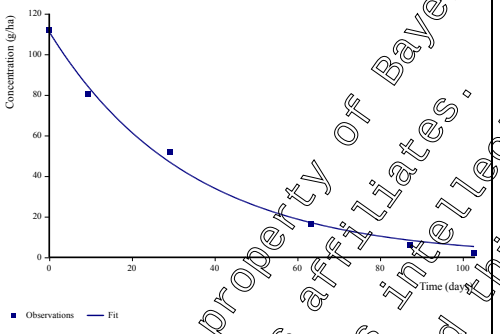
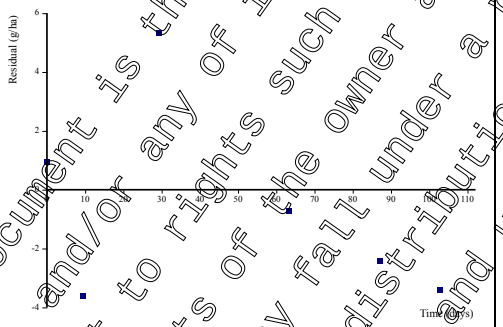
For metabolite M02 use DT_{90} / 3.32 of FOMC of decline phase, $DegT_{50, matrix}$ = 151 days.

Appendix 4.2.18. Degradation of spiroxamine in Nogarole Rocca soil trial no. 50136/0 (KCA 7.1.2.2.1/05 (M-006129-01-1))

Appendix 4.2.18.1. Parent fitting

Step 1: Does decline in lab studies show a lag-phase or effects of long-term sorption kinetics? No

Step 3: Can field decline be described with SFO after eliminating data points before 10 mm rain ensuring no systematic deviations when examining residuals especially when residues at study end are above 10% of initially measured?

	SFO
Plot	
Residuals	
Visual fit	Good, residuals show no systematic error.
χ^2 error (%)	5.6
t-test	k_2 p < 0.05
DT_{50} (days)	23.4
DT_{90} (days)	77.8
Modelling DT_{50} (days)	23.4
Assessment	Fit acceptable. Visual fit is excellent, χ^2 error is low and rate parameter differs significantly from zero.
Discussion	iii) SFO fit is acceptable. Yes. iv) Use this $DegT50_{matrix}$.

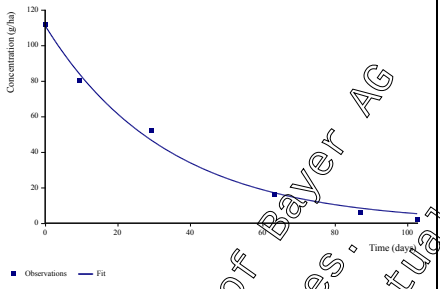
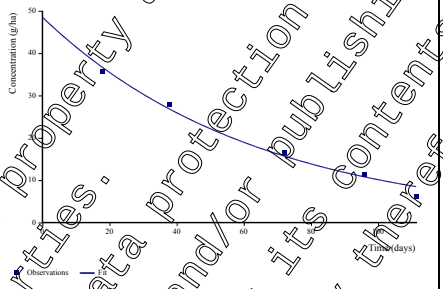
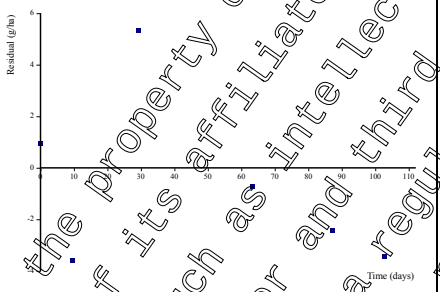
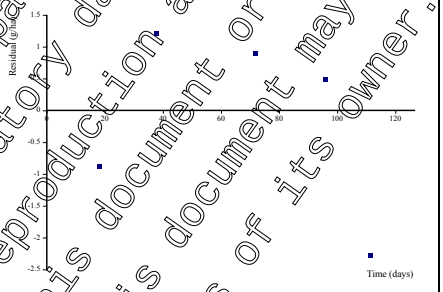
[1] Interpret with care – extrapolated beyond experimental period

Summary:

For spiroxamine use SFO $DegT50_{matrix}$ = 23.4 days.

Appendix 4.2.18.2.Metabolite fitting (M01 and M02)

Step 4: Run metabolite SFO from decline. iii) SFO fit for metabolite acceptable?

	M01	M02
Plot		
Residuals		
Visual fit	Good, residuals show no systematic error	Good, residuals show no systematic error
χ^2 err %	5.63	5.34
t-test	*k*: p < 0.05	*k*: p < 0.05
DT_{50} (days)	23.4	44.3
DT_{90} (days)	77.8	147[1]
$DegT_{50matrix}$ (days)	23.4	44.3
Formation fraction	N/A	N/A
Assessment	Fit acceptable. Visual fit is good, χ^2 error is low, and rate parameter differs significantly from zero.	Fit acceptable. Visual fit is good, χ^2 error is low, and rate parameter differs significantly from zero.
Discussion	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.	iii) SFO fit for metabolite acceptable? SFO is considered acceptable and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Summary:

For metabolite M01 use SFO (decline) $DegT_{50, matrix}$ = 23.4 days.

For metabolite M02 use SFO (decline) $DegT_{50, matrix}$ = 44.3 days.

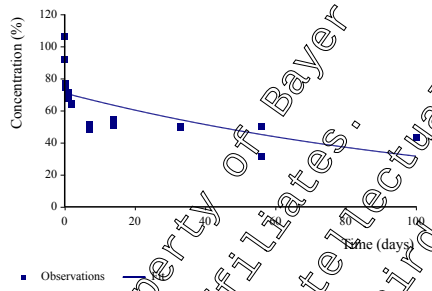
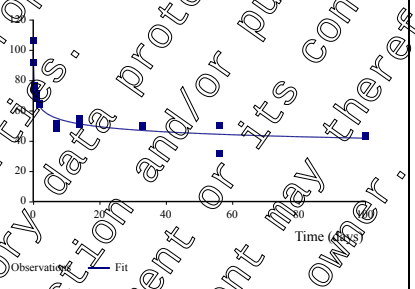
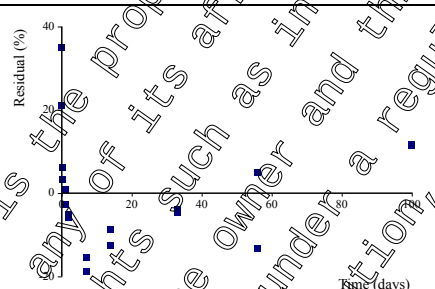
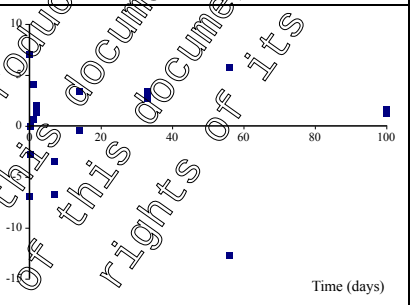
Appendix 5: Kinetic evaluation of water/sediment studies

Appendix 5.1: Kinetic evaluation for persistence/trigger endpoints

Appendix 5.1.1. KCA 7.2.2.3/01 (M-006015-01-1) Hoenniger Water

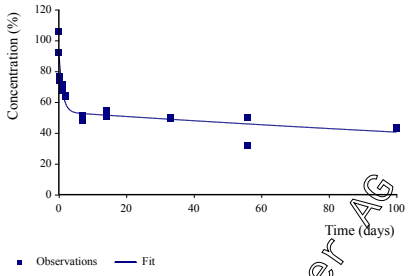
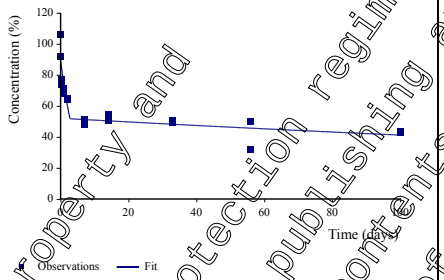
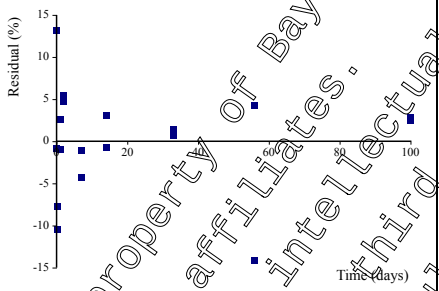
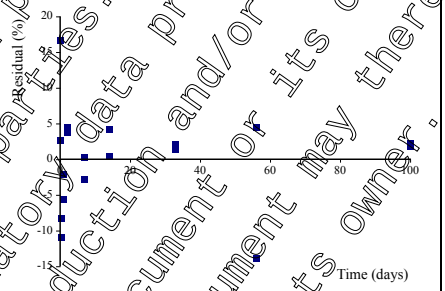
Appendix 5.1.1.1. Degradation of [cyclohexyl-1-^{14}C]-spiroxamine in Hoenniger total system (KCA 7.2.2.3/01 (M-006015-01-1))

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
χ^2 err %	16.6	3.67
Confidence measure	k: p > 0.10	Not applicable
Visual fit	Poor, residuals show clear trend.	Acceptable, though residuals show potential trend.
DT_{50} (days)	85.5	19.6
DT_{90} (days)	285[1]	>10,000[1]
Assessment	Not acceptable: Visual fit is poor, χ^2 err % is high but rate parameter differs significantly from zero.	Not acceptable: Visual fit is acceptable and χ^2 err % is good; however, significant extrapolation from the study period to the estimated DT90 is considered unreliable[2]
Conclusion	FOMC should better visually and statistically fit compared to SFO, however, DT_{90} values could not be determined using FOMC fit. Deviation from SFO is not considered to be due to outliers or experimental artefacts. Investigate DFOP and HS.	

[1] Interpret with care – extrapolated beyond experimental period
[2] EFSA (2009)

Step 2: FOMC better than SFO fit. Fit DFOP and HS

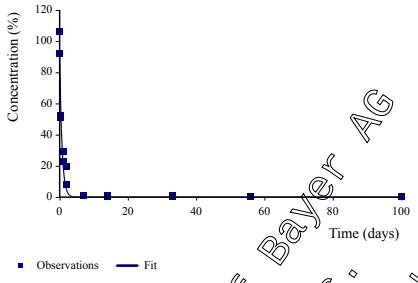
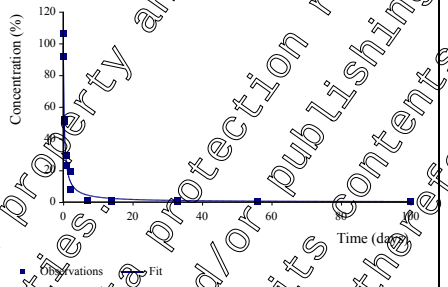
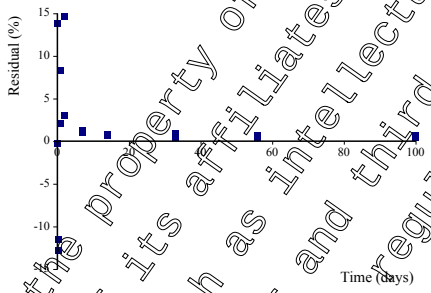
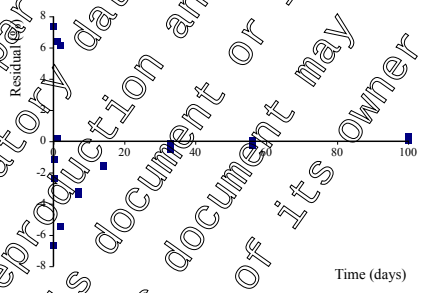
	DFOP	HS
Plot		
Residuals		
χ^2 err %	6.8	7.91
Confidence measure	k1: p <0.10 k2: p <0.10	k1: p <0.10 k2: p <0.10
Visual fit	Good, though residuals show potential trend.	Good, though residuals show potential trend.
DT_{50} (days)	51.4	65.2
DT_{90} (days)	628[1]	755[1]
Assessment	Acceptable: Visual fit is good, χ^2 error is low acceptable and rate parameters differ significantly from zero.	Acceptable: Visual fit is good, χ^2 error is low acceptable and rate parameters differ significantly from zero.
Conclusion	HS and DFOP fits are very similar. DFOP gives the best acceptable fit (lower χ^2) and should be used for persistence endpoints. FOMC not applicable due to the extensive extrapolation for DT90[2].	

[1] Interpret with care – extrapolated beyond experimental period.
[2] EFSA (2009)

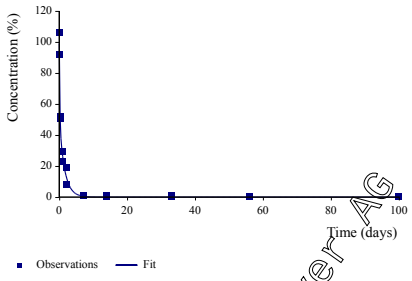
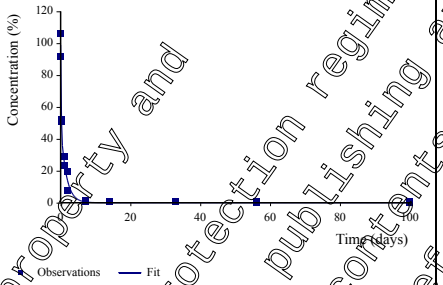
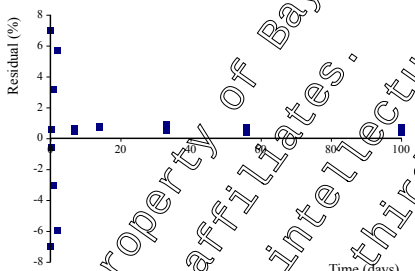
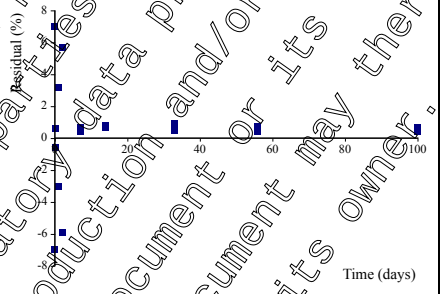
Summary:
For Spiroxamine use DFOP. DT_{50} = 51.4 days, DT_{90} = 628 days.

Appendix 5.1.1.2. Dissipation of [cyclohexyl-1-^{14}C]-spiroxamine in Hoenniger water phase (KCA 7.2.2.3/01 (M-006015-01-1))

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
χ^2 err %	21.5	6.87
Confidence measure	k_2p <0.10	N.A
Visual fit	Acceptable, residuals show no trend.	Acceptable, residuals show no trend.
DT_{50} (days)	0.468	0.294
DT_{90} (days)	1.55	2.83
Assessment	Acceptable: Visual fit is good, χ^2 error is high and rate parameter differ significantly from zero.	Acceptable: Visual fit is acceptable and χ^2 err % is good.
Conclusion	The FOMC fit is visually and statistically better than SFO. Deviation from SFO is not considered to be due to outliers or experimental artefacts. Investigate DFOP and HS.	

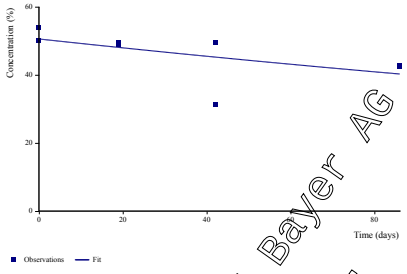
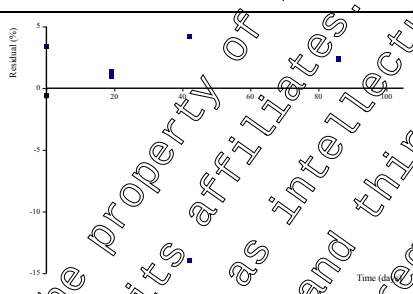
Step 2: FOMC gave the best fit. Investigate DFOP and HS fits.

	DFOP	HS
Plot		
Residuals		
χ^2 err %	1.96	1.96
Confidence measure	k1: p <0.05 k2: p <0.05	k1: p <0.05 k2: p <0.05
Visual fit	Acceptable, residuals show no trend.	Acceptable, residuals show no trend.
DT_{50} (days)	0.274	0.265
DT_{90} (days)	2.51	2.51
Assessment	Acceptable: Visual fit is good and χ^2 error is low.	Acceptable: Visual fit is good and χ^2 error is low..
Conclusion	HS and DFOP show an identical fit and are a significant improvement on FOMC. DFOP selected as a precautionary fit since DT50 is longer than for HS.	

Summary:
For Spiroxamine use DFOP. DT_{50} = 0.274 days, DT_{90} = 2.51.

Appendix 5.1.1.3. Dissipation of [cyclohexyl-1-^{14}C]-spiroxamine in Hoenniger sediment phase (KCA 7.2.2.3/01 (M-006015-01-1))

Step 1: SFO [top-down] acceptable fit?

	SFO [top-down]
Plot	
Residuals	
χ^2 err %	5.08
Confidence measure	k: p <0.10
Visual fit	Visual fit is intermediate, residuals show potential trend.
DT_{50} (days)	262[1]
DT_{90} (days)	871[1]
Assessment	Acceptable: Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	SFO using decline after max is considered acceptable and should be used for persistence endpoints. Biphasic kinetics could not be investigated as there was is insufficient decline data points.

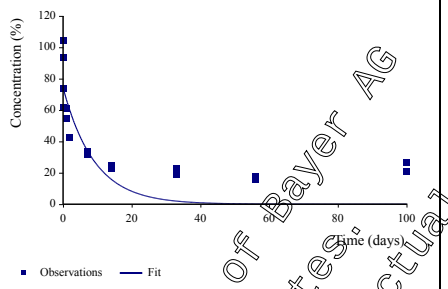
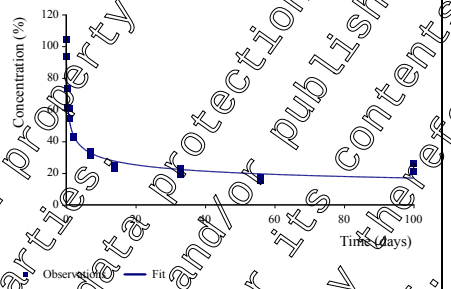
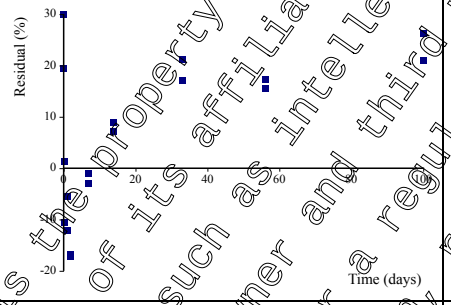
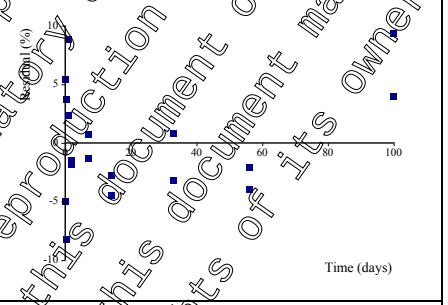
[1] Interpret with care – extrapolated beyond experimental period

Summary:
For Spiroxamine use [top-down] SFO: DT_{50} = 262 days, DT_{90} = 871 days.

Appendix 5.1.2. KCA 7.2.2.3/01 (M-006015-01-1) Stilwell

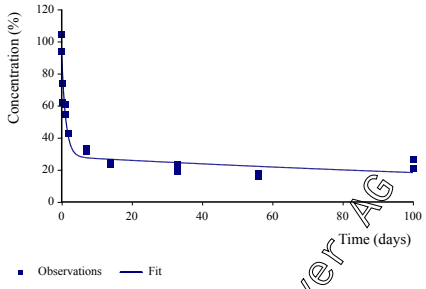
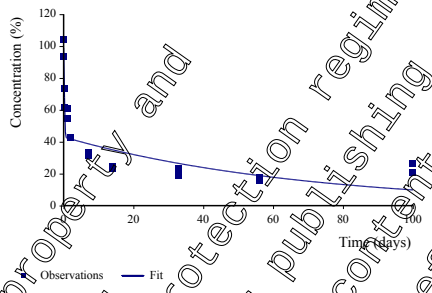
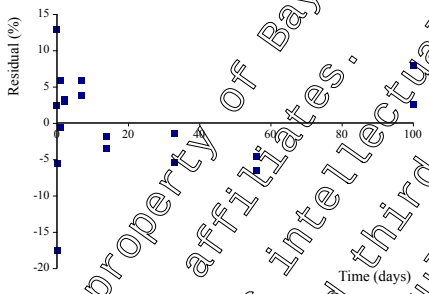
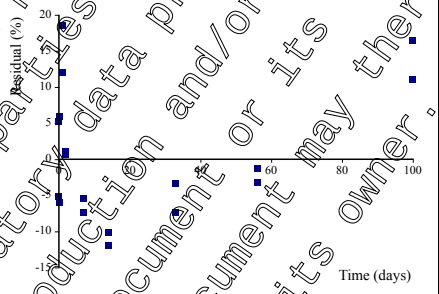
Appendix 5.1.2.1. Degradation of [cyclohexyl-1-^{14}C]-spiroxamine in Stilwell total system (KCA 7.2.2.3/01 (M-006015-01-1))

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?).

	SFO	FOMC
Plot		
Residuals		
χ^2 err %	29.6	6.83
Confidence measure	k: p <0.10	N.A.
Visual fit	Poor, residuals show clear trend.	Acceptable, residuals show no trend.
DT_{50} (days)	6.31	1.26
DT_{90} (days)	21.0	875[1]
Assessment	Not acceptable: Visual fit is poor, χ^2 err % is high but rate parameter significantly from zero.	Acceptable: Visual fit is acceptable and χ^2 err % is low. However, model is significantly extrapolated beyond the experimental study period[2].
Conclusion	The FOMC fit is visually and statistically better than SFO. Deviation from SFO is not considered to be due to outliers or experimental artefacts. Investigate DFOP and HS.	

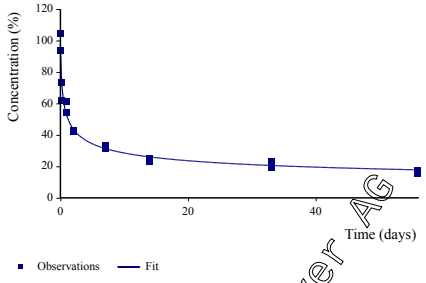
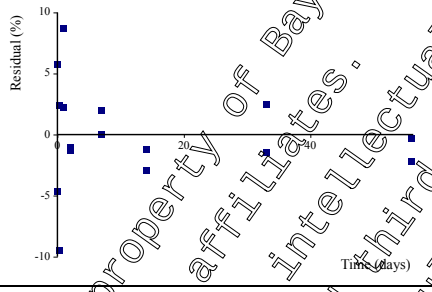
[1] Interpret with care – extrapolated beyond experimental period
[2] EFSA (2009)

Step 2: FOMC better than SFO fit. Fit DFOP and HS

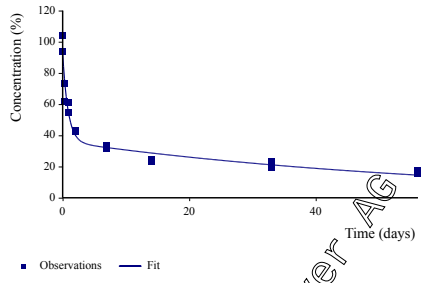
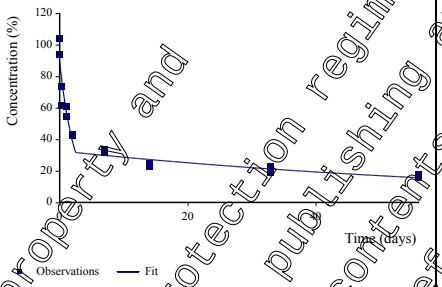
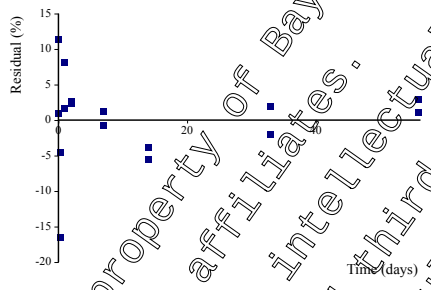
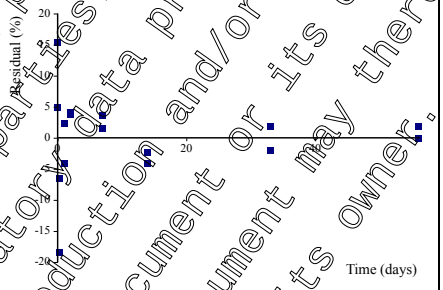
	DFOP	HS
Plot		
Residuals		
χ^2 err %	12.4	17.6
Confidence measure	k1: p <0.10 k2: p >0.10	k1: p <0.10 k2: p<0.10
Visual fit	Acceptable, residuals show no trend.	Acceptable, residuals show no trend.
DT_{50} (days)	1.37	0.455
DT_{90} (days)	83.7	99.6
Assessment	Acceptable: Visual fit is good, χ^2 error is acceptable, k2 rate parameter is not significantly different from zero	Acceptable: Visual fit is good, χ^2 error is acceptable, rate parameters both differ significantly from zero.
Conclusion	HS and DFOP fits are very similar but neither model presents rate parameters that are statistically acceptable. It was considered that the final measured time point was significantly impacting the modelled fit. Try a modified fitting routine (exclude final time point)	

[1] Interpret with care – extrapolated beyond experimental period

Step 3: Fit FOMC, DFOP and HS. Modified fitting routine (exclude final time point).

	FOMC
Plot	
Residuals	
χ2 err %	4.77
Confidence measure	N.A.
Visual fit	Acceptable, residuals show no trend.
DT50 (days)	127
DT90 (days)	487[1]
Assessment	Acceptable: Visual fit is acceptable and χ2 err % is low. However, model is significantly extrapolated beyond the experimental study period.
Conclusion	The FOMC fit is visually and statistically better than SFO. Deviation from SFO is not considered to be due to outliers or experimental artefacts. Investigate DFOP and HS.

[1] Interpret with care – extrapolated beyond experimental period
[2] EFSA (2009)

	DFOP	HS
Plot		
Residuals		
χ^2 err %	10.3	12.3
Confidence measure	k1: p <0.10 k2: p<0.10	k1: p <0.10 k2: p<0.10
Visual fit	Acceptable, residuals show no trend.	Acceptable, residuals show no trend.
DT_{50} (days)	1.37	1.67
DT_{90} (days)	83.7	99.2
Assessment	Acceptable: Visual fit is good, χ^2 error is acceptable, rate parameters both differ significantly from zero.	Acceptable: Visual fit is good, χ^2 error is acceptable, rate parameters both differ significantly from zero.
Conclusion	HS and DFOP fits are very similar but neither model presents rate parameters that are statistically acceptable. It was considered that the final measured time point was significantly impacting the modelled fit. Try a modified fitting routine (exclude final time point)	

Summary:
For Spiroxamine use DFOP DT_{50} = 1.37 days, DT_{90} = 83.7 days.

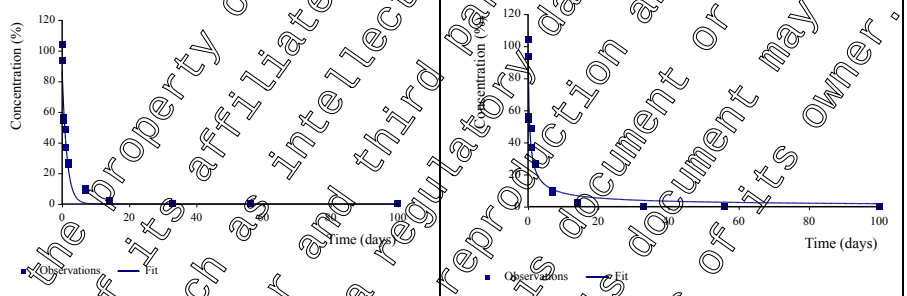
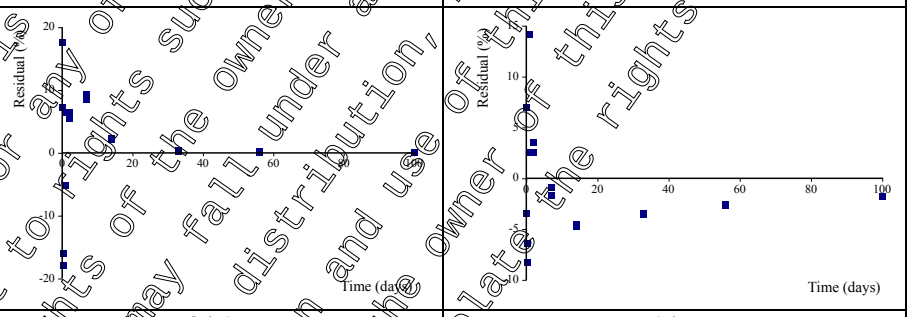
Appendix 5.1.2.2. Degradation of M06 in Stilwell total system (KCA 7.2.2.3/01 (M-006015-01-1))

As no decline phase was observed for M06 it is not possible to estimate degradation rates from the total system.

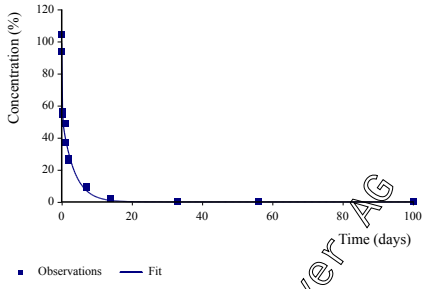
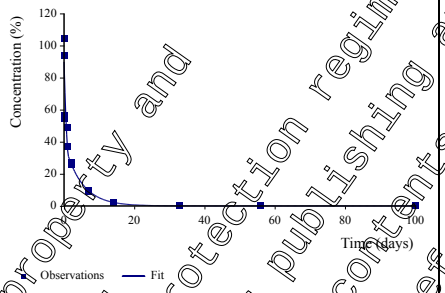
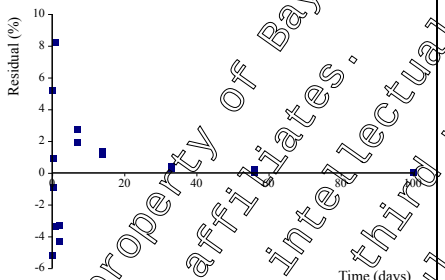
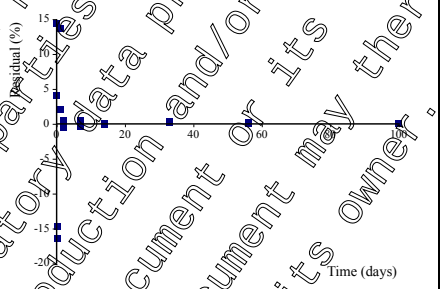
Summary:
M06 dissipation conservative default DT_{50} = 1,000 days

Appendix 5.1.2.3. Dissipation of [cyclohexyl-1-^{14}C]-spiroxamine in Stilwell water phase (KCA 7.2.2.3/01 (M-006015-01-1))

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
χ^2 err %	24.1	14.5
Confidence measure	k: p <0.10	N.A
Visual fit	Intermediate, residuals show clear trend.	Good, residuals show no trend.
DT_{50} (days)	0.965	0.491
DT_{90} (days)	3.21	8.21
Assessment	Not acceptable. Visual fit is poor; χ^2 err % is high. Rate parameter is significantly different to zero.	Acceptable: Visual fit is acceptable and χ^2 err % is good.
Conclusion	The FOMC fit is visually and statistically better than SFO. Deviation from SFO is not considered to be due to outliers or experimental artefacts. Investigate DFOP and HS.	

Step 2: FOMC gave the best fit. Investigate DFOP and HS fits.

	DFOP	HS
Plot		
Residuals		
χ^2 err %	6.03	22.6
Confidence measure	k1: p <0.10 k2: p <0.10	k1: p <0.10 k2: p <0.10
Visual fit	Good, residuals do not show a trend.	Intermediate, residuals show no trend.
DT_{50} (days)	0.401	0.736
DT_{90} (days)	5.89	7.27
Assessment	Acceptable: Visual fit is good with no evidence of systematic errors. χ^2 error is acceptable. Rate parameters all significantly different from zero.	Not acceptable: Rate parameters differs significantly from zero and visual fit is acceptable, however, χ^2 error is high.
Conclusion	DFOP showed more acceptable kinetic fit over HS and FOMC (lower χ^2). DFOP gives the best acceptable fit and should be used for persistence endpoints.	

Summary:
For Spiroxamine use DFOP. DT_{50} = 0.401 days, DT_{90} = 5.89 days.

Appendix 5.1.2.4. Dissipation of M06 in Stilwell water phase (KCA 7.2.2.3/01 (M-006015-01-1))

No observations of M06 > 5% in any time point for the water phase.

Appendix 5.1.2.5. Dissipation of [cyclohexyl-1-^{14}C]-spiroxamine in Stilwell sediment phase (KCA 7.2.2.3/01 (M-006015-01-1))

As no decline phase was observed for spiroxamine it is not possible to estimate degradation rates for the sediment.

For Spiroxamine use conservative default DT_{50} = 1,000 days

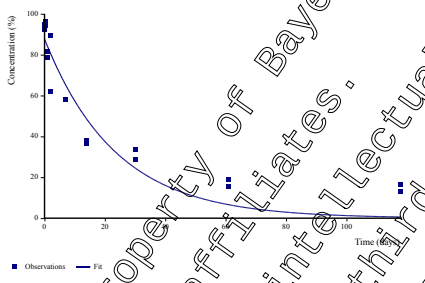
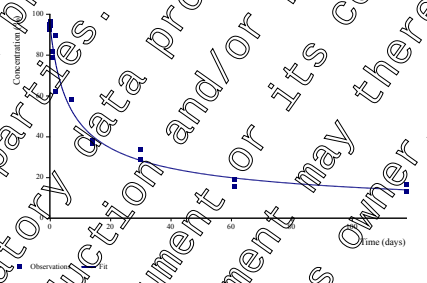
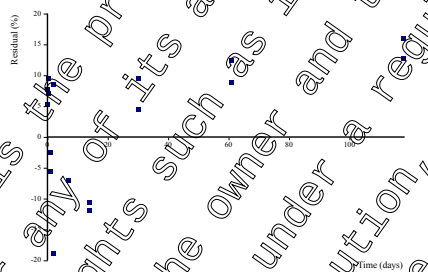
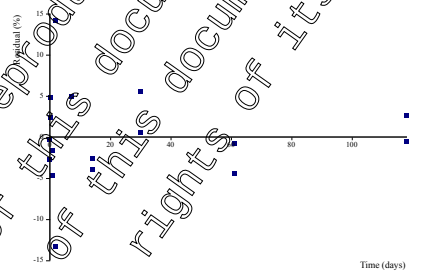
Appendix 5.1.2.6. Dissipation of M06 in Stilwell sediment phase (KCA 7.2.2.3/01 (M-006015-01-1))

No observations of M06 > 5% in any time point for the sediment phase.

Appendix 5.1.3. KCA 7.2.2.3/04 (M-303324-01-1) Anglerweiher System

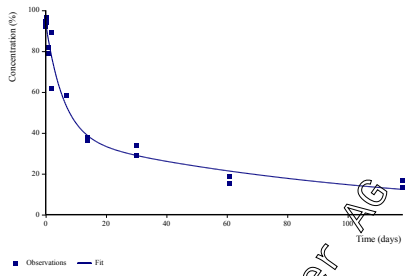
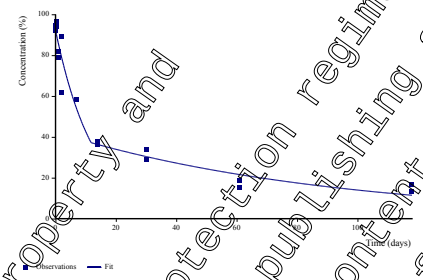
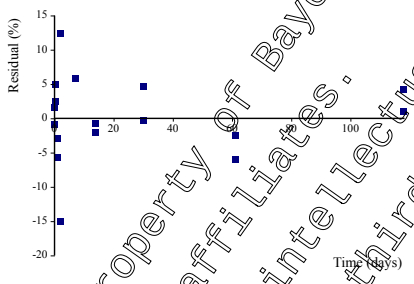
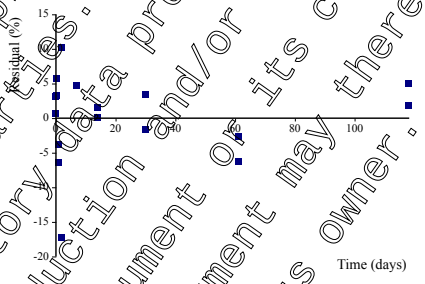
Appendix 5.1.3.1. Degradation of [1,3-dioxolane-4-^{14}C]-spiroxamine in Anglerweiher total system (KCA 7.2.2.3/04 (M-303324-01-1))

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
χ^2 err %	12.5	4.44
Confidence measure	k: p < 0.10	Not applicable
Visual fit	Poor, residuals show clear trend.	Good, residuals shows no clear trend.
DT_{50} (days)	16.3	9.47
DT_{90} (days)	54.2	237[1]
Assessment	Unacceptable: Visual fit is poor, χ^2 err % is ok and rate parameter differs significantly from zero	Acceptable: Visual fit is good and χ^2 error is low.
Conclusion	The FOMC fit shows a notable improvement over SFO. Deviation from SFO is not considered to be due to outliers or experimental artefacts. Investigate DFOP and HS kinetics.	

[1] Interpret with care – extrapolated beyond experimental period

Step 2: DFOP and HS fit investigated.

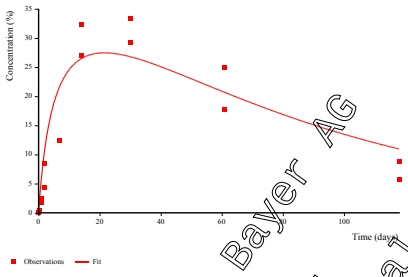
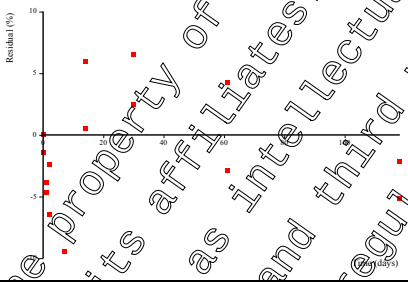
	DFOP	HS
Plot		
Residuals		
χ^2 err %	5.37	5.78
Confidence measure	k1: p <0.10 k2: p <0.10	k1: p <0.10 k2: p <0.10
Visual fit	Excellent, residuals shows no clear trend.	Excellent, residuals shows no clear trend.
DT_{50} (days)	9.11	8.99
DT_{90} (days)	147	138[1]
Assessment	Acceptable: Visual fit is excellent and χ^2 err % is low and rate parameters differ significantly from zero.	Acceptable: Visual fit is excellent, χ^2 err % is low and rate parameters differ significantly from zero.
Conclusion	FOMC showed more acceptable kinetic fit over DFOP and HS (lower χ^2). FOMC gives the best acceptable fit and should be used for persistence endpoints.	

[1] Interpret with care – extrapolated beyond experimental period

Summary:
For Spiroxamine use FOMC. DT_{50} = 9.47 days, DT_{90} = 237 days

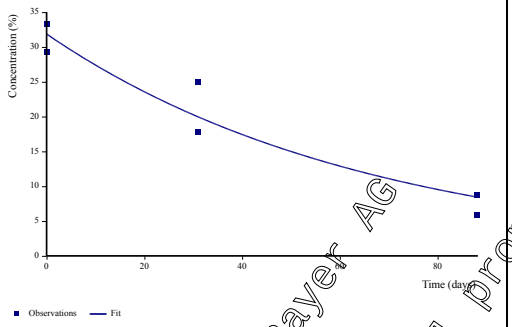
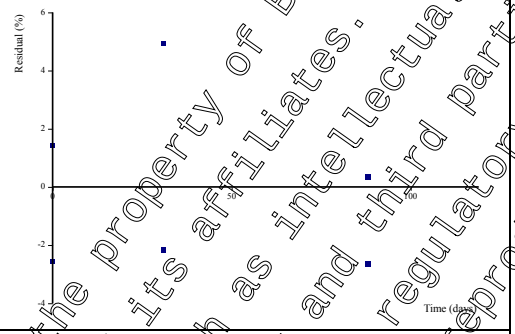
Appendix 5.1.3.2. Degradation of M06 in Anglerweiher total system (KCA 7.2.2.3/04 (M-303324-01-1))

Step 1: Run Spiroxamine best-fit FOMC. SFO fit for metabolite acceptable?

	Parent FOMC, Metabolite SFO
Plot	
Residuals	
χ^2 err %	26.7
Confidence measure	[1]: p <0.1
Visual fit	Visual fit is acceptable, residuals shows no clear trend
DT_{50} (days)	47
DT_{90} (days)	156
Assessment	Visual fit is acceptable, χ^2 err % is very high and rate parameter differs significantly from zero
Conclusion	SFO is considered acceptable despite high $\chi 2$. Fitting of decline phase was therefore attempted, using only three time points.

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Modified fitting routine [decline after max]. SFO fit for metabolite acceptable?

	SFO [top-down]
Plot	
Residuals	
χ^2 err %	4.82
Confidence measure	k: p < 0.1
Visual fit	Visual fit is acceptable, residuals shows no clear trend
DT_{50} (days)	46
DT_{90} (days)	153
Assessment	Visual fit is acceptable, χ^2 err % is low and rate parameter differs significantly from zero. Since the estimated DT50 is very similar to that previously estimated and as such the previous DT50 of 47 days accepted which was based on a larger number of data points.
Conclusion	Fitting decline confirms that parent-metabolite fit is representative yet conservative.

[1] Interpret with care – extrapolated beyond experimental

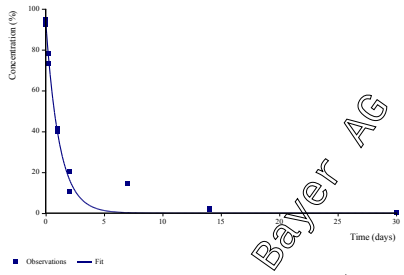
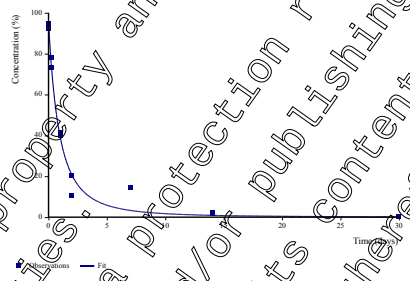
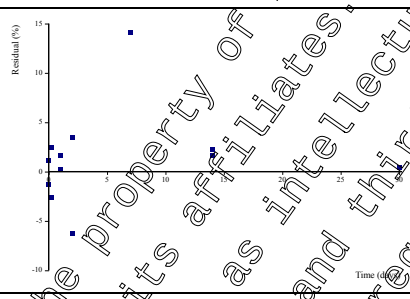
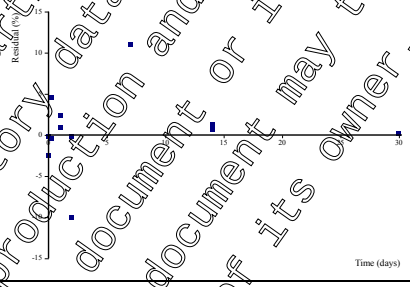
Summary

Summary

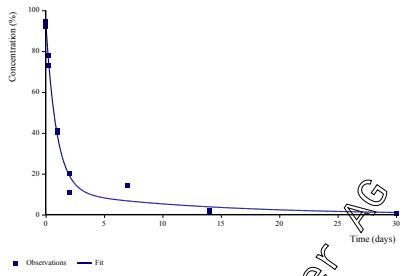
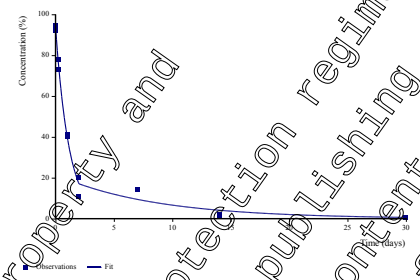
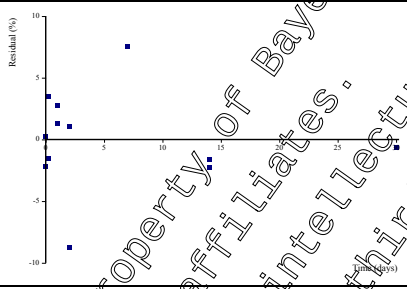
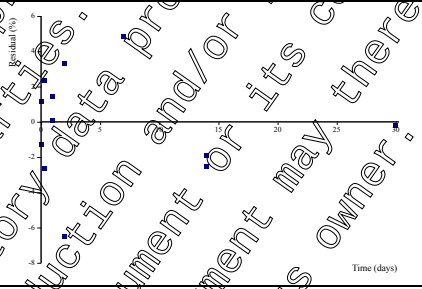
For M06 use DFOP-SFO DT_{50} = 47 days, DT_{90} = 156 days

Appendix 5.1.3.3. Dissipation of [1,3-dioxolane-4-^{14}C]-spiroxamine in Anglerweiher water phase (KCA 7.2.2.3/04 (M-303324-01-1))

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
χ^2 err %	12.4	11.8
Confidence measure	k: 0.10	Not applicable
Visual fit	Acceptable, residuals shows no clear trend.	Good, residuals shows no clear trend.
DT_{50} (days)	0.81	0.748
DT_{90} (days)	2.69	3.65
Assessment	Acceptable: Visual fit is intermediate and rate parameter differs significantly from zero; however, χ^2 err % is high.	Acceptable: Visual fit is good and χ^2 error is acceptable.
Conclusion	SFO and FOMC fits give similar visual fit. However, χ^2 err % is high in both. Deviation from SFO is not considered to be due to outliers or experimental artefacts.. Therefore DFOP and HS kinetics investigated.	

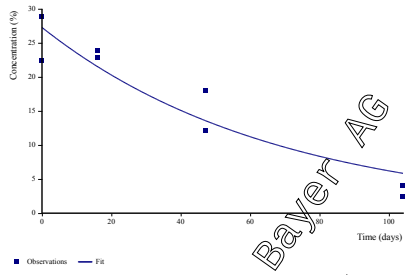
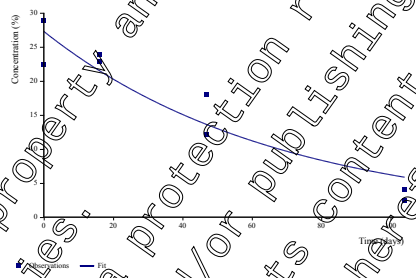
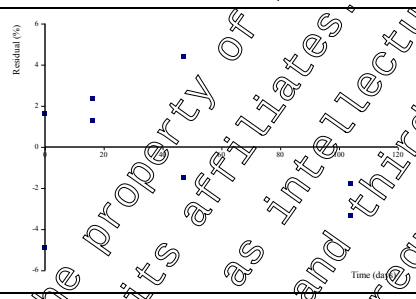
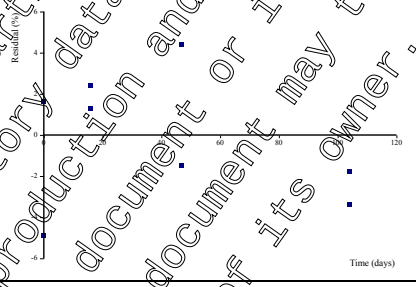
Step 2: DFOP and HS fit investigated.

	DFOP	HS
Plot		
Residuals		
χ^2 err %	9.39	5.84
Confidence measure	k1: p<0.10 k2: p >0.10	k1: p <0.10 k2: p <0.10
Visual fit	Excellent, residuals shows no clear trend.	Excellent, residuals shows no clear trend.
DT_{50} (days)	0.759	0.814
DT_{90} (days)	4.08	7.08
Assessment	Not acceptable: Visual fit is excellent and χ^2 err % is low and k_1 rate parameters differ significantly from zero, while k_2 rate parameter does not differ significantly from zero..	Acceptable: Visual fit is excellent, χ^2 err % is low and rate parameters differ significantly from zero.
Conclusion	The DFOP and HS are visually very similar; but k_2 rate parameter does not differ significantly from zero for DFOP. Use HS for persistence/trigger endpoints.	

Summary:
For Spiroxamine use HS, DT_{50} =0.814 days, DT_{90} =7.08 days

Appendix 5.1.3.4. Dissipation of M06 in Anglerweiher water phase (KCA 7.2.2.3/04 (M-303324-01-1))

Step 1: SFO [top-down] more appropriate than FOMC [top-down] and gives acceptable fit?)

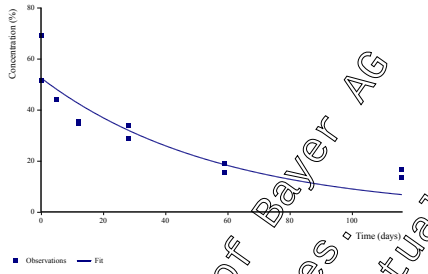
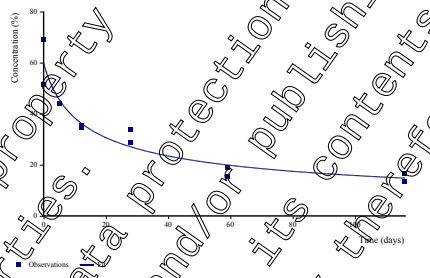
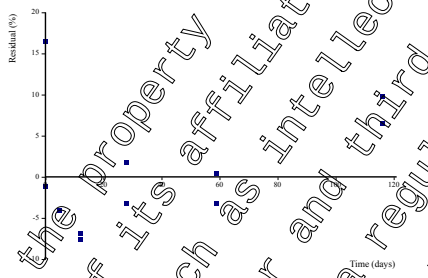
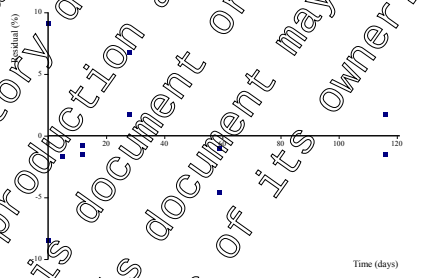
	SFO [top-down]	FOMC [top-down]
Plot		
Residuals		
χ^2 err %	9.26	11.6
Confidence measure	k_p <0.1	Not applicable
Visual fit	Visual fit is good, residuals show no clear trend.	Visual fit is good, residuals show no clear trend.
DT_{50} (days)	46.9	46.8
DT_{90} (days)	156[1]	156[1]
Assessment	Acceptable: Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero.	Acceptable: Visual fit is good, χ^2 error is low.
Conclusion	SFO using decline after max is considered acceptable and has a lower χ^2 err % compared to FOMC. Therefore, SFO should be used for persistence endpoints.	

[1] Interpret with care – extrapolated beyond experimental period

Summary:
For M06 use [top-down] SFO. DT_{50} = 46.9 days, DT_{90} = 156 days.

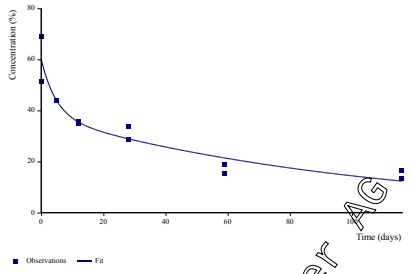
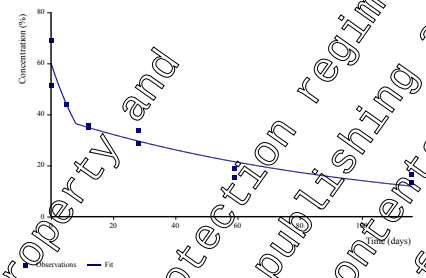
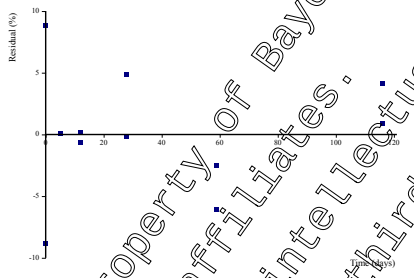
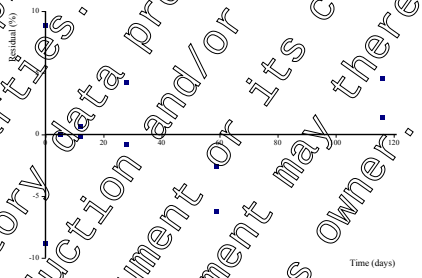
Appendix 5.1.3.5. Dissipation of [1,3-dioxolane-4-^{14}C]-spiroxamine in Anglerweiher sediment phase (KCA 7.2.2.3/04 (M-303324-01-1))

Step 1: Initial assessment (SFO [decline after max] more appropriate than FOMC [decline after max] and gives acceptable fit?)

	SFO [top down]	FOMC [top down]
Plot		
Residuals		
χ^2 err %	13.5	5.85
Confidence measure	k: p > 0.1	Not applicable
Visual fit	Visual fit is intermediate, residuals show potential for a trend	Visual fit is good, residuals show no clear trends
DT_{50} (days)	39.3	21.5
DT_{90} (days)	131[1]	819[1]
Assessment	Potentially acceptable: Visual fit is intermeidate, χ^2 error is acceptable and rate parameter differs significantly from zero.	Acceptable: Visual fit is good, χ^2 error is low. Significant extrapolation of data for DT90.
Conclusion	FOMC using decline after max is considered acceptable and should be used for persistence endpoints. Deviation from SFO is not considered to be due to outliers or experimental artefacts. HS and DFOP were investigated.	

[1] Interpret with care – extrapolated beyond experimental period.

Step 2: DFOP and HS [decline after max] fit investigated.

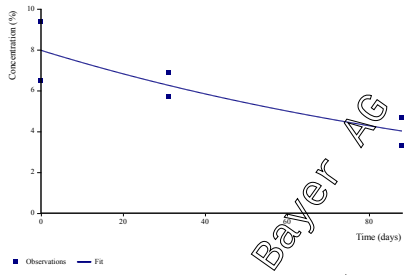
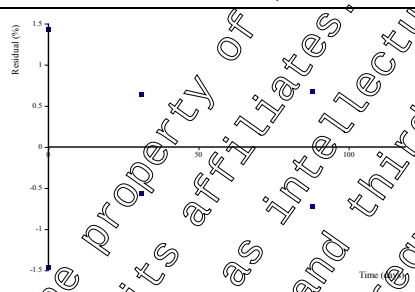
	DFOP [top down]	HS [top down]
Plot		
Residuals		
χ^2 err %	6.71	6.35
Confidence measure	k2: p <0.10 k2: p <0.10	k1: p <0.10 k2: p <0.10
Visual fit	Excellent, residuals shows no clear trend.	Excellent, residuals shows no clear trend.
DT_{50} (days)	24.3	20.6
DT_{90} (days)	191	183[1]
Assessment	Acceptable: Visual fit is excellent, χ^2 err % is low and rate parameters differ significantly from zero.	Acceptable: Visual fit is excellent, χ^2 err % is low and rate parameters differ significantly from zero.
Conclusion	The DFOP and HS are visually and statistically very similar. FOMC fit has significant extrapolation beyond the experimental period. DFOP selected as best persistent fit.	

[1] Interpret with care – extrapolated beyond experimental period.

Summary:
For Spiroxamine sediment phase use [top-down] DFOP, DT_{50} = 24.3 days, DT_{90} = 191 days.

Appendix 5.1.3.6. Dissipation of M06 in Anglerweiher sediment phase (KCA 7.2.2.3/04 (M-303324-01-1))

Step 1. SFO fit for M06 [decline after max] acceptable?

	SFO [top down]
Plot	
Residuals	
χ^2 err %	0.393
Confidence measure	k: p<0.1
Visual fit	Visual fit is good, residuals show no clear trend.
DT_{50} (days)	89.2
DT_{90} (days)	296[1]
Assessment	Acceptable: Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	SFO using decline after max is considered acceptable and should be used for persistence endpoints. Biphasic kinetics could not be investigated as there was is insufficient decline data points.

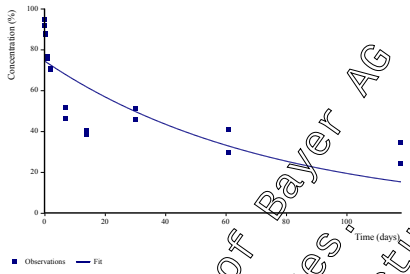
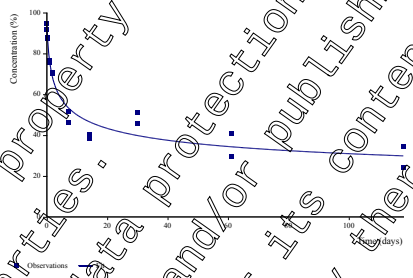
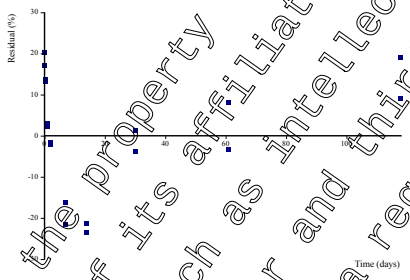
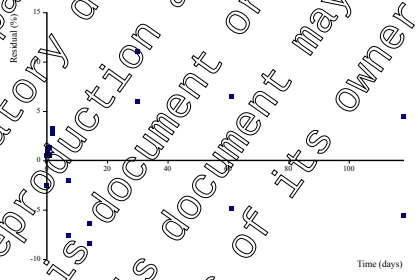
[1] Interpret with care – extrapolated beyond experimental period.

Summary:
For M06 use [top-down] SFO DT_{50} = 89.2 days, DT_{90} = 296 days.

Appendix 5.1.4. KCA 7.2.2.3/04 (M-303324-01-1) Hoenniger Water system

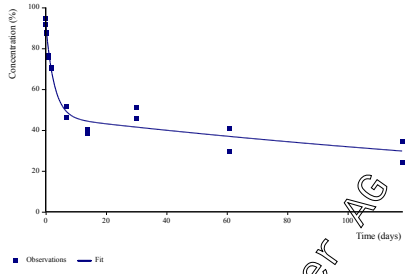
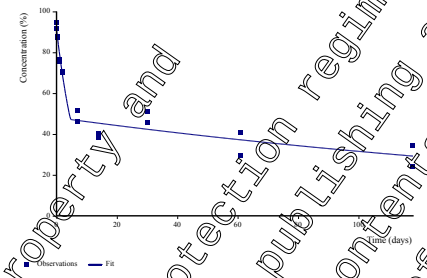
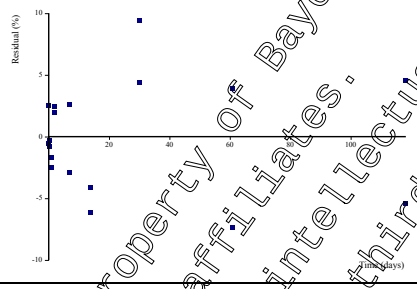
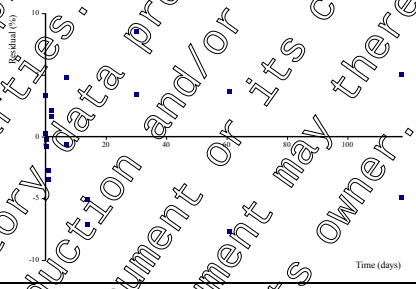
Appendix 5.1.4.1. Degradation of [1,3-dioxolane-4-^{14}C]-spiroxamine in Hoenniger total system (KCA 7.2.2.3/04 (M-303324-01-1))

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
χ^2 err %	18.2	6.14
Confidence measure	k: p <0.10	Not applicable
Visual fit	Poor, residuals shows a clear trend	Good, residuals show shows no clear.
DT_{50} (days)	81.1	13.4
DT_{90} (days)	171[1]	>10,000[1]
Assessment	Not acceptable: Visual fit is poor, χ^2 err % is high but rate parameter differs significantly from zero.	Not acceptable: Visual fit is good and χ^2 err % is low. However, significant extrapolation from tudy period to DT90 is not considered reliable[2].
Conclusion	The DOMC fit shows a notable improvement over SFO. SFO fit shows clear trend in residuals. Deviation from SFO is not considered to be due to outliers or experimental artefacts. FOMC fit is more acceptable than SFO. DFOP and HS investigated.	

[1] Interpret with care – extrapolated beyond experimental period
[2] EFSA (2009)

Step 2: DFOP and HS fit investigated.

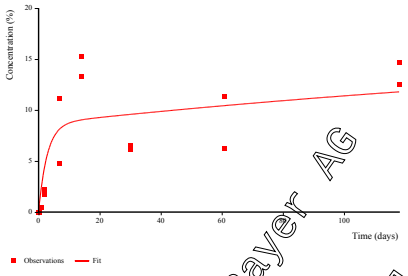
	DFOP	HS
Plot		
Residuals		
χ^2 err %	4.79	5.08
Confidence measure	k1: p <0.10 k2: p <0.10	k1: p <0.10 k2: p <0.10
Visual fit	Acceptable, residuals shows no clear trend.	Acceptable, residuals shows no clear trend.
DT_{50} (days)	9.82	12.3
DT_{90} (days)	429	395[1]
Assessment	Acceptable: Visual fit is acceptable and χ^2 err % is low and rate parameters differ significantly from zero.	Acceptable: Visual fit is acceptable, χ^2 err % is low and rate parameters differ significantly from zero.
Conclusion	The DFOP and HS are visually and statistically very similar; however, the DFOP χ^2 err % is slightly improved versus HS. Use DFOP for persistence/trigger endpoints.	

[1] Interpret with care – extrapolated beyond experimental period

Summary:
For Spiroxamine use DFOP. DT_{50} = 9.82 days, DT_{90} = 429 days

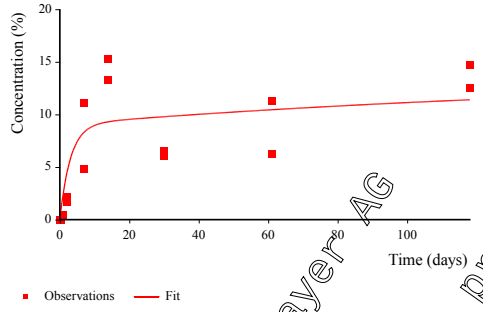
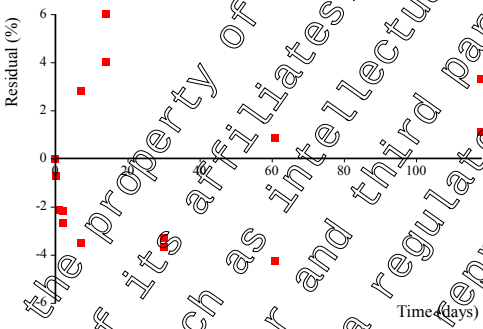
Appendix 5.1.4.2. Degradation of M06 in Hoenniger total system (KCA 7.2.2.3/04 (M-303324-01-1))

Step 1: Run Spiroxamine best-fit DFOP and metabolite SFO. SFO fit for metabolite acceptable?

	Parent DFOP, metabolite SFO
Plot	
Residuals	
χ^2 err %	3.09
Confidence measure	k-p = 0.5
Visual fit	Visual fit is intermediate, residuals show no trend
DT_{50} (days)	>10,000[1]
DT_{90} (days)	>10,000[1]
Assessment	Not acceptable: Visual fit is intermediate, χ^2 err % is very high and rate parameter does not differ significantly from zero.
Conclusion	SFO is not considered acceptable. Therefore, decline after max. should be assessed. However, this was not possible as there is insufficient decline data. Try a fit with default DT_{50} to demonstrate applicability of default 1,000 days as rate constant

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Run alternative, yet conservative fit.

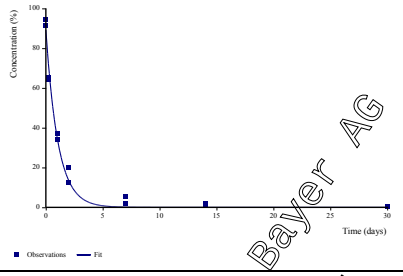
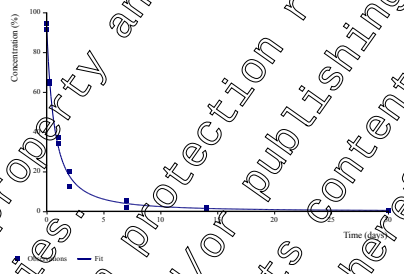
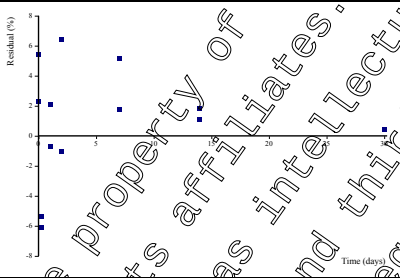
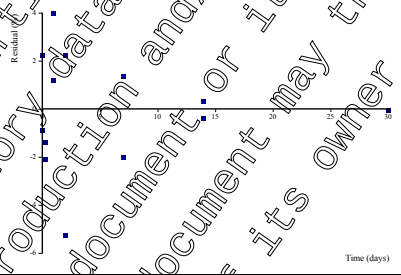
	Parent DFOP, metabolite SFO
Plot	
Residuals	
χ^2 err %	
Confidence measure	NA
Visual fit	Visual fit is intermediate, residuals show no trend
DT_{50} (days)	1,000[1]
DT_{90} (days)	1,000[1]
Assessment	Acceptable Visual fit is intermediate (as before) and residuals do not show a clear trend.
Conclusion	Use of the default 1,000 day DT50 is considered acceptable.

[1] Interpret with care – extrapolated beyond experimental period.

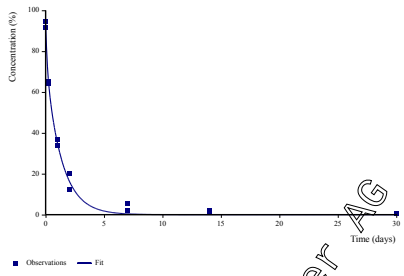
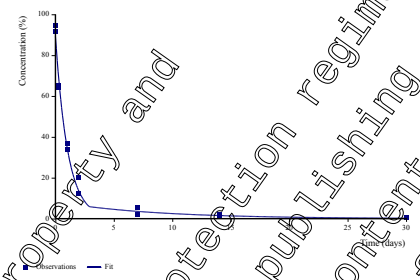
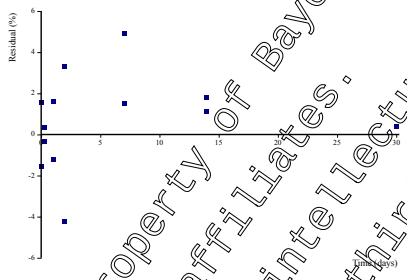
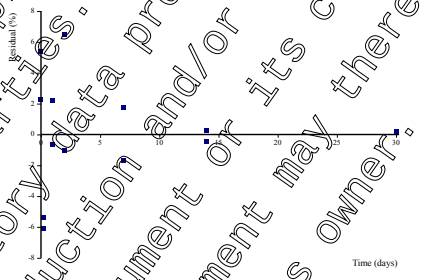
Summary:
For M06 use conservative default DT_{50} = 1,000 days.

Appendix 5.1.4.3. Dissipation of [1,3-dioxolane-4-^{14}C]-spiroxamine in Hoenninger surface water (KCA 7.2.2.3/04 (M-303324-01-1))

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
χ^2 err %	8.18	3.77
Confidence measure	k: p <0.10	Not applicable
Visual fit	Intermediate, residuals shows no clear trend.	Very good, residuals shows no clear trend.
DT_{50} (days)	0.728	0.596
DT_{90} (days)	2.44	3.6
Assessment	Acceptable. Visual fit is intermediate and residuals show a clear trend. Rate parameter differs significantly from zero.	Acceptable. Visual fit is very good, χ^2 err % is low.
Conclusion	FOMC gives a much better visual and statistical fit versus SFO. Deviation from SFO is not considered to be due to outliers or experimental artefacts. DFOP and HS fits investigated.	

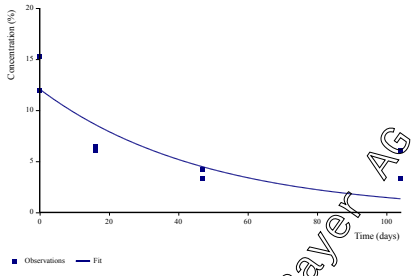
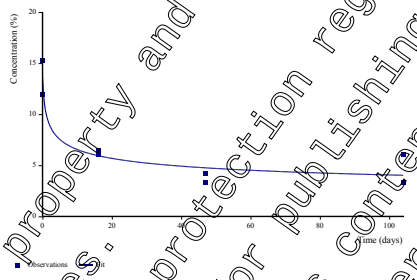
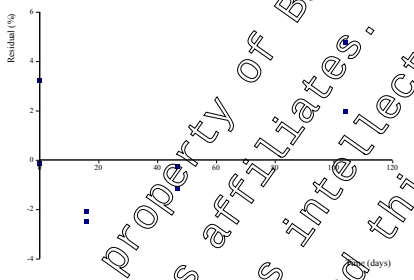
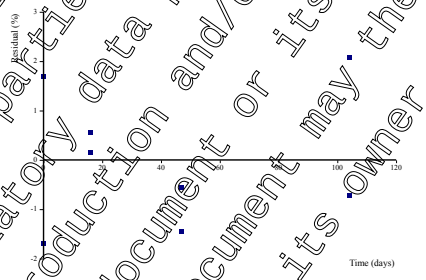
Step 2: DFOP and HS fit investigated.

	DFOP	HS
Plot		
Residuals		
χ^2 err %	4.16	8.68
Confidence measure	k2: p =0.23 k2: p <0.10	k1: p <0.10 k2: p =0.34
Visual fit	Good, some evidence of a trend in residuals.	Excellent, residuals shows no clear trend.
DT_{50} (days)	0.636	0.734
DT_{90} (days)	2.78	2.44
Assessment	Visual fit is intermediate, some evidence of a trend in residuals. χ^2 err % is low but k1 rate parameter is not significantly different to zero.	Visual fit is excellent, χ^2 err % is good but k2 rate parameter is not significantly different from zero.
Conclusion	The DFOP and HS fits are very similar but both fits return k values that do not differ significantly from zero. FOMC is the best fit model on the basis of χ^2 err % and residuals.	

Summary:
For Spiroxamine use FOMC. DT_{50} = 0.596 days, DT_{90} = 3.6 days.

Appendix 5.1.4.4. Dissipation of M06 in Hoenniger water phase (KCA 7.2.2.3/04 (M-303324-01-1))

Step 1: SFO [top-down] more appropriate than FOMC [top-down] and gives acceptable fit?)

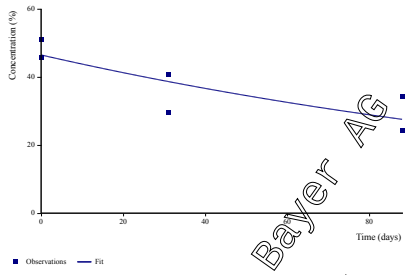
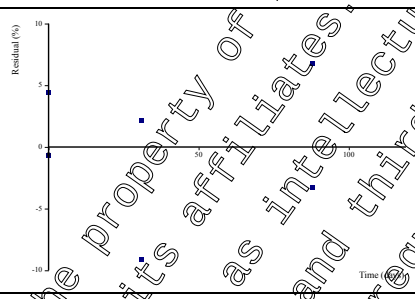
	Metabolite SFO [top down]	Metabolite FOMC [top down]
Plot		
Residuals		
χ^2 err %	25.4	9.07
Confidence measure	k: p <0.1	Not applicable
Visual fit	Visual fit is poor, some evidence of a trend in residuals	Very good, residuals shows no clear trend.
DT_{50} (days)	32.8	8.31
DT_{90} (days)	109	>10,000[1]
Formation fraction	Not applicable	Not applicable
Assessment	Visual fit is poor, χ^2 err % is very high and rate parameter does not differ significantly from zero.	Acceptable. Visual fit is very good, χ^2 err % is low.
Conclusion	SFO [decline only] is not considered acceptable. Deviation from SFO is not considered to be due to outliers or experimental artefacts. FOMC [decline only] showed acceptable fit, therefore DFOP [decline only] and HS [decline only] kinetics should be investigated, however, there are insufficient time points.	

[1] Interpret with care – extrapolated beyond experimental period.

Summary:
For M06 use FOMC [decline only]. DT_{50} = 8.31 days, DT_{90} = >10,000 days.

Appendix 5.1.4.5. Dissipation of [1,3-dioxolane-4-^{14}C]-spiroxamine in Hoenninger sediment phase (KCA 7.2.2.3/04 (M-303324-01-1))

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO [top down]
Plot	
Residuals	
χ^2 err %	5.9
Confidence measure	k: p < 0.10
Visual fit	Good, residuals show no evidence of a trend in residuals.
DT_{50} (days)	117
DT_{90} (days)	388[1]
Assessment	Acceptable: Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	SFO was acceptable and should be used for persistence endpoints. Biphasic fits could not be investigated as there was insufficient time points.

[1] Interpret with care – extrapolated beyond experimental period.

Summary:
For Spiroxamine use SFO [decline only]. DT_{50} = 117 days, DT_{90} = 388 days.

Appendix 5.1.4.6. Dissipation of M06 in Hoenniger Water sediment phase (KCA 7.2.2.3/04 (M-303324-01-1))

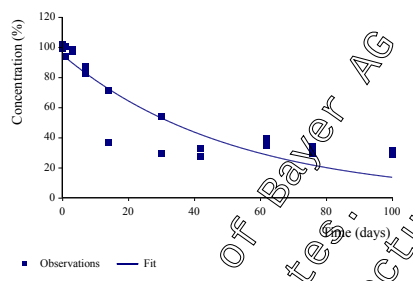
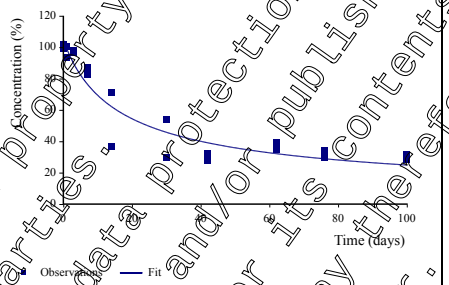
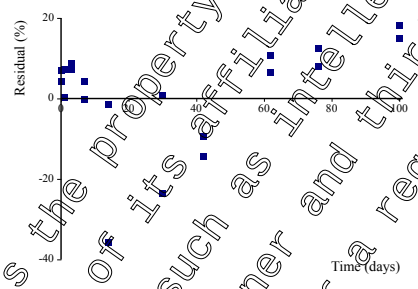
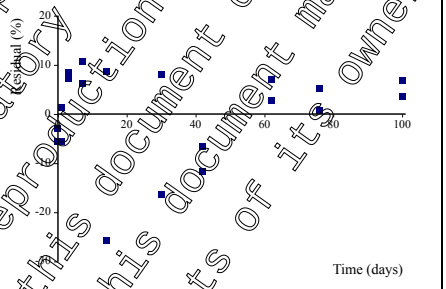
No decline phase was observed and as such endpoint cannot be derived

For M06 use conservative default. DT_{50} = 1,000 days.

Appendix 5.1.5. KCA 7.2.2.3/07 (M-763128-01-1) Calwich Abbey

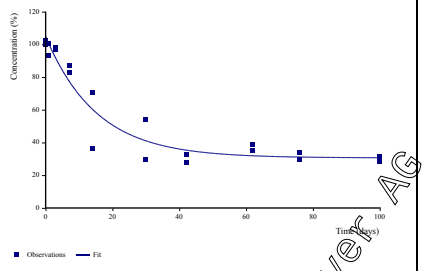
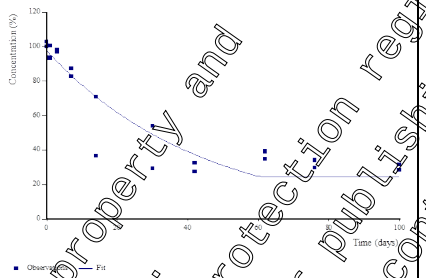
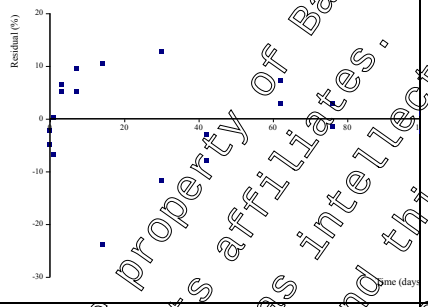
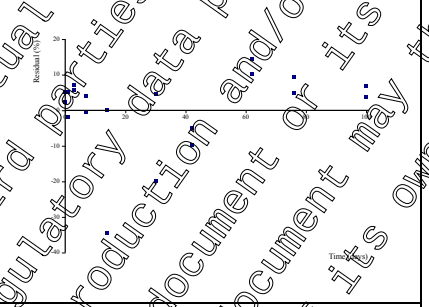
Appendix 5.1.5.1. Degradation of [cyclohexyl-1-^{14}C]-spiroxamine in Calwich Abbey total system (KCA 7.2.2.3/07 (M-763128-01-1))

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
χ^2 err %	14.4	8.7
Confidence measure	k: $p < 0.05$	NA
Visual fit	Visual fit is intermedaite but evidence of systematic errors	Visual fit is good although some evidence of systematic errors
DT_{50} (days)	35.7	22.0
DT_{90} (days)	118[1]	453[1]
Assessment	Not acceptable: Visual fit of data is poor giving an elevated χ^2 value. Rate parameter differs significantly from zero.	Acceptable: Visual fit of data is good with a low χ^2 value.Significant extrapolation beyond study duration.
Conclusion	SFO fit is more appropriate than FOMC. Deviation from SFO is not considered to be due to outliers or experimental artefacts. Investigate DFOP and HS.	

[1] Interpret with care – extrapolated beyond experimental period

Step 3: FOMC better than SFO fit. Fit DFOP and HS.

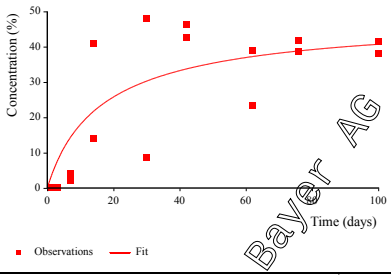
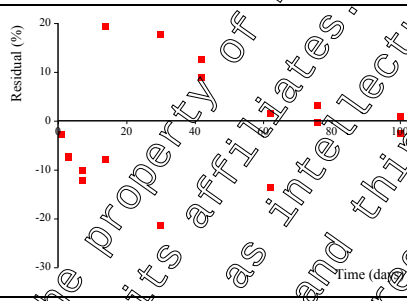
	DFOP	HS
Plot		
Residuals		
χ^2 err %	6.89	11.6
Confidence measure	k1: p <0.05 k2: p >0.5	k1: p <0.05 k2: p >0.5
Visual fit	Visual fit is excellent, no evidence of systematic error	Visual fit is good, some evidence of systematic error
DT_{50} (days)	18.9	30.4
DT_{90} (days)	>10,000[1]	>10,000[1]
Assessment	Visual fit is excellent with a low χ2 but rate constant does not differ significantly from zero. DT90 cannot be estimated.	Visual fit is good with a reasonable χ2 but rate constant does not differ significantly from zero. DT90 cannot be estimated.
Conclusion	Both DFOP and HS give similar visual and statistical fits. Visual fits are good with no evidence of systematic errors, but k_2 rate parameter does not different significantly from zero and DT90 estimates show significant extrapolation. Use FOMC fit.	

[1] Interpret with care – extrapolated beyond experimental period

Summary:
For Spiroxamine use FOMC. DT_{50} = 220 days, DT_{90} = 453 days

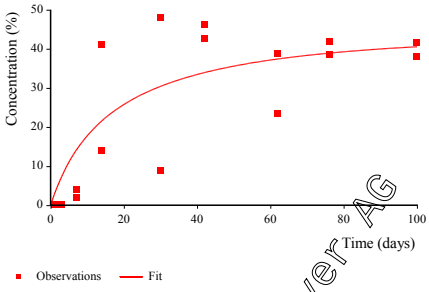
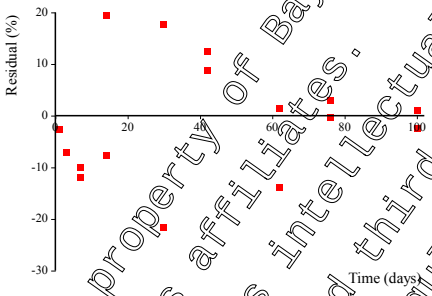
Appendix 5.1.5.2. Degradation of M06 in Calwich Abbey total system (KCA 7.2.2.3/07 (M-763128-01-1))

Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent FOMC, metabolite SFO
Plot	
Residuals	
χ^2 err %	21.6
Confidence measure	k: p > 0.1
Visual fit	Visual fit is good with no evidence of systematic error
DT_{50} (days)	>10,000[1]
DT_{90} (days)	>10,000[1]
Assessment	Visual fit is good with no evidence of systematic error but rate parameter does not differ significantly from zero
Conclusion	SFO is not considered acceptable. Therefore decline after max. should be assessed. However, this was not possible as there is insufficient decline data. Fix metabolite DT_{50} with the FOCUS default, does this give an acceptable fit?

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Run alternative, yet conservative fit.

	Parent FOMC, metabolite SFO
Plot	
Residuals	
χ^2 err %	20.4
Confidence measure	NA
Visual fit	Visual fit is good with no evidence of systematic error
DT_{50} (days)	1,000
DT_{90} (days)	3,320[1]
Assessment	Visual fit is good with no evidence of systematic error. Formation of metabolite is well described.
Conclusion	SFO is considered acceptable.

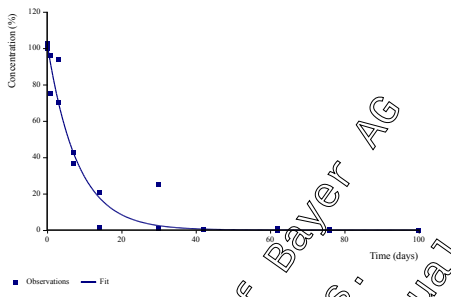
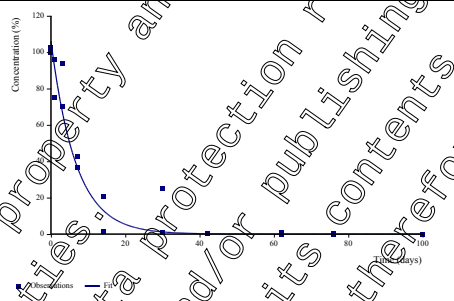
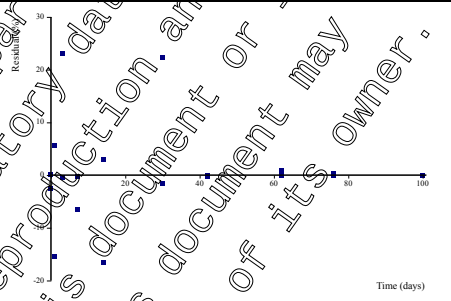
[1] Interpret with care – extrapolated beyond experimental period

Summary:
No models give an acceptable fit.

For M06 use conservative default. DT_{50} = 1,000 days.

Appendix 5.1.5.3. Dissipation of [cyclohexyl-1-^{14}C]-spiroxamine in Calwich Abbey water phase (KCA 7.2.2.3/07 (M-763128-01-1))

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
χ^2 err %	12.9	12.7
Confidence measure	t-p < 0.05	N.A
Visual fit	Visual fit is good with no evidence of systematic errors in residuals	Visual fit is good with no evidence of systematic errors in residuals
DT_{50} (days)	5.61	5.61
DT_{90} (days)	18.6	18.6
Assessment	Acceptable: Visual fit is good although χ2 is slightly high due to data scatter. Rate parameter differs significantly from zero.	Acceptable: Visual fit is good although χ2 is slightly high due to data scatter.
Conclusion	SFO and FOMC assessments are very similar giving the same estimated DT50 and DT90. SFO selected as χ2 was lower than for FOMC	

Summary:
For Spiroxamine use SFO. DT_{50} = 5.61 days, DT_{90} = 18.6 days.

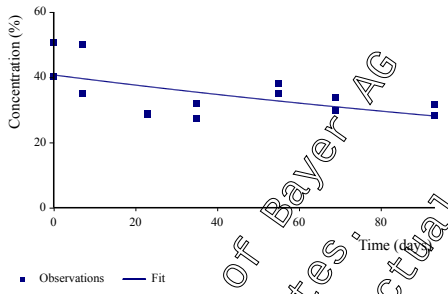
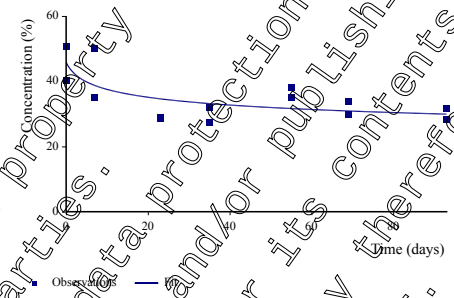
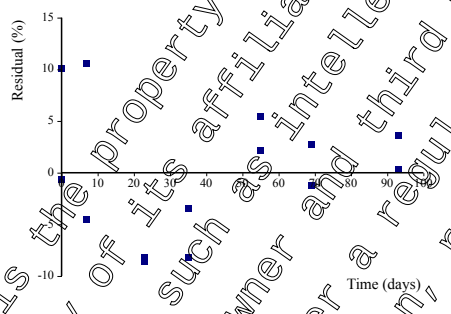
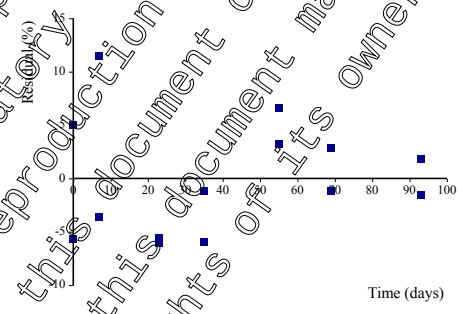
Appendix 5.1.5.4. Dissipation of M06 in Calwich Abbey water phase (KCA 7.2.2.3/07 (M-763128-01-1))

No decline phase was observed and as such endpoint cannot be derived

For M06 use conservative default. DT_{50} = 1,000 days.

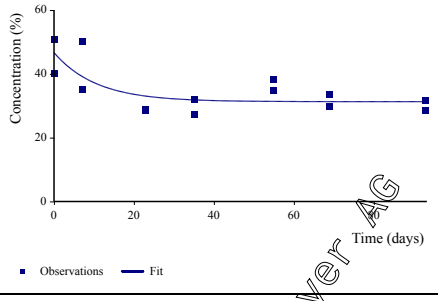
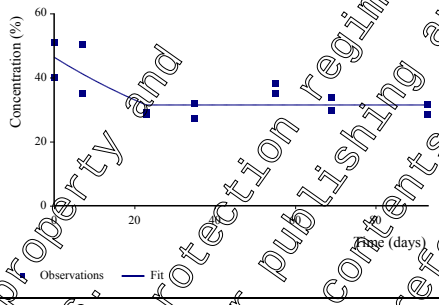
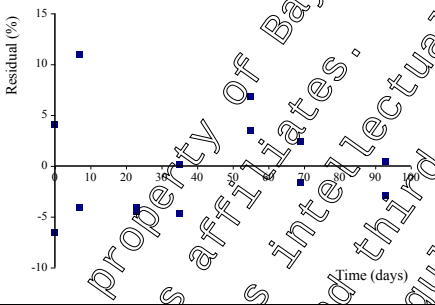
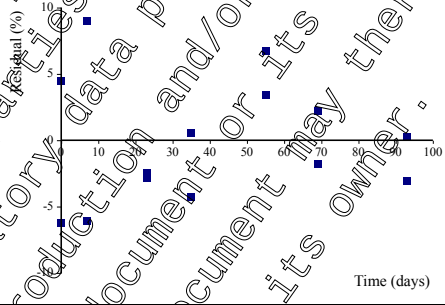
Appendix 5.1.5.5. Dissipation of [cyclohexyl-1-^{14}C]-spiroxamine in Calwich Abbey sediment phase (KCA 7.2.2.3/07 (M-763128-01-1))

Step 1: Initial assessment (SFO [decline after max] more appropriate than FOMC [decline after max] and gives acceptable fit?)

	SFO [top down]	FOMC [top down]
Plot		
Residuals		
χ^2 err %	10.7	8.66
Confidence measure	k; $p < 0.05$	N.A
Visual fit	Visual fit is good with no evidence of systematic errors. Rate parameter differs significantly from zero.	Visual fit is excellent with no evidence of systematic errors
DT_{50} (days)	175[1]	1,110[1]
DT_{90} (days)	580[1]	>10,000[1]
Assessment	Potentially acceptable: Visual fit is good with a low χ^2 and rate parameter differs from zero.	Not acceptable: Visual fit is excellent with no systematic errors. However, significant extrapolation from the study period to DT90 is not reliable[2].
Conclusion	SFO is not more appropriate than FOMC. Deviation from SFO is not considered to be due to outliers or experimental artefacts. Investigate DFOP and HS.	

[1] Interpret with care – extrapolated beyond experimental period.

Step 2: DFOP and HS [decline after max] fit investigated.

	DFOP [top down]	HS [top down]
Plot		
Residuals		
χ^2 err %	8.23%	6.59%
Confidence measure	k1: p = 0.1 k2: p = 0.5	k1: p < 0.05 k2: p = 0.5
Visual fit	Visual fit is excellent with a low χ2 and no evidence of systematic errors	Visual fit is excellent with a low χ2 and no evidence of systematic errors
DT_{50} (days)	>10,000[1]	>10,000[1]
DT_{90} (days)	>10,000[1]	>10,000[1]
Assessment	Not Acceptable: Visual fit is excellent with no evidence of systematic errors but rate parameters do not differ significantly from zero	Not Acceptable: Visual fit is excellent with no evidence of systematic errors but rate parameters do not differ significantly from zero.
Conclusion	DFOP and HS give very similar visual and kinetic fits. Visual fits are both very good but both k2 rate parameters are not statistically different from zero. No models provide adequate description of data, apply FOCUS default as a conservative worst case.	

[1] Interpret with care – extrapolated beyond experimental period.

Summary:
For Spiroxamine use conservative default DT_{50} = 1,000 days.

Appendix 5.1.5.6. Dissipation of M06 in Calwich Abbey sediment phase (KCA 7.2.2.3/07 (M-763128-01-1))

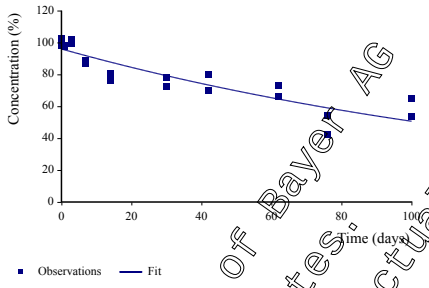
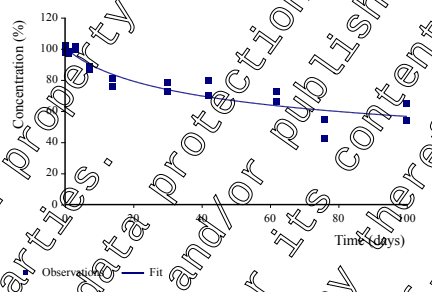
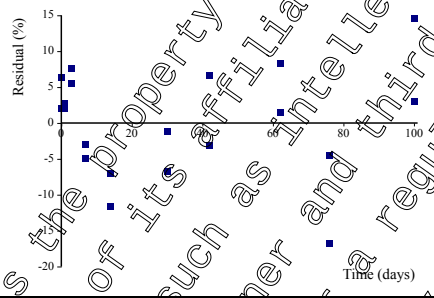
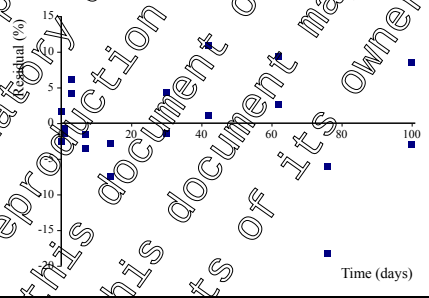
No decline phase was observed and as such endpoint cannot be derived.

For M06 use conservative default, DT_{50} = 1,000 days.

Appendix 5.1.6. KCA 7.2.2.3/07 (M-763128-01-1) Emperor Lake

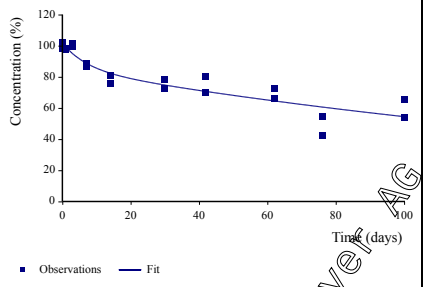
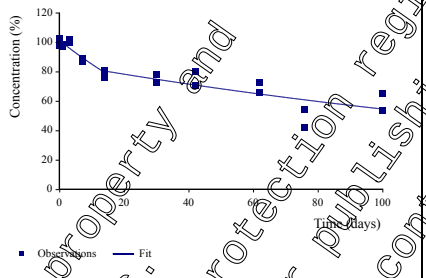
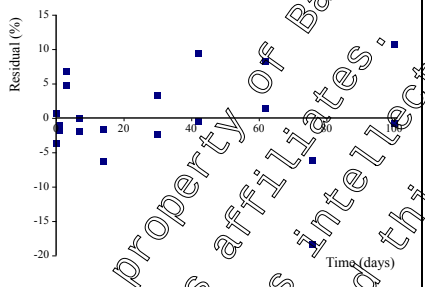
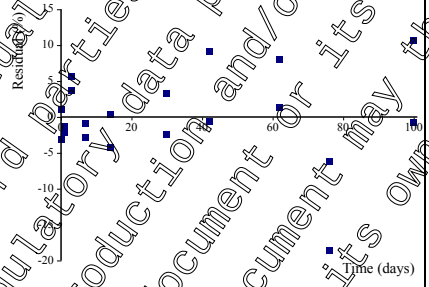
Appendix 5.1.6.1. Degradation of [cyclohexyl-1-^{14}C]-spiroxamine in Emperor Lake total system (KCA 7.2.2.3/07 (M-763128-01-1))

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
χ^2 err %	6.39	5.74
Confidence measure	k: p < 0.05	N.A
Visual fit	Visual fit is good with no evidence of systematic error.	Visual fit is good with no evidence of systematic error.
DT_{50} (days)	109	160[1]
DT_{90} (days)	360[1]	>10,000[1]
Assessment	Acceptable: Visual fit is good with low χ2 and no evidence of systematic errors. Rate parameter differs significantly from zero.	Not Acceptable: Visual fit is excellent with low χ2 and no evidence of systematic errors. However, significant extrapolation from the study period to estimated DT90 is not reliable[2].
Conclusion	SFO and FOMC give very similar fit but FOMC has lowest χ2. Deviation from SFO is not considered to be due to outliers or experimental artefacts. Assess DFOP and HS.	

[1] Interpret with care – extrapolated beyond experimental period

Step 3: FOMC better than SFO fit. Fit DFOP and HS.

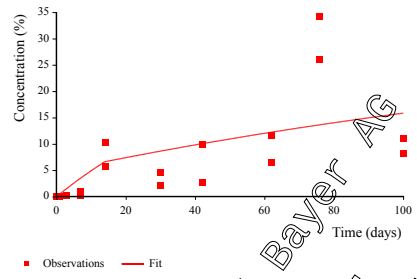
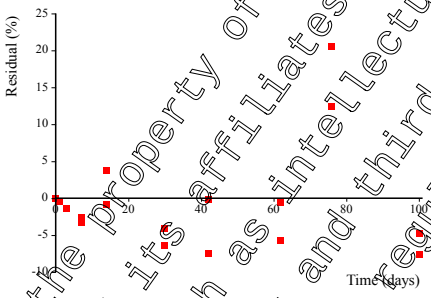
	DFOP	HS
Plot		
Residuals		
χ^2 err %	5.93	5.68
Confidence measure	k1: $p > 0.1$ k2: $p > 0.05$	k1: $p < 0.05$ k2: $p < 0.05$
Visual fit	Visual fit is excellent with no evidence of systematic errors.	Visual fit is excellent with no evidence of systematic errors.
DT_{50} (days)	117[1]	118[1]
DT_{90} (days)	476[1]	474[1]
Assessment	Not Acceptable: Visual fit is excellent with a low χ^2 and no systematic errors in the residuals. However, k_2 rate parameter does not differ significantly from zero.	Acceptable: Visual fit is excellent with a low χ^2 and no systematic errors in the residuals. Rate parameters differ significantly from zero.
Conclusion	HS is statistically and visually acceptable with the lowest χ^2. HS selected.	

[1] Interpret with care – extrapolated beyond experimental period

Summary:
For Spiroxamine use HS. DT_{50} = 118 days, DT_{90} = 474 days

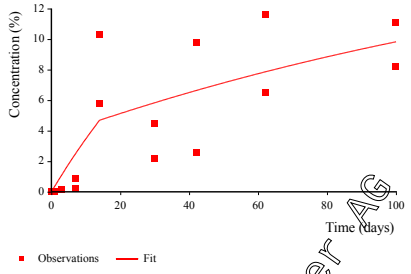
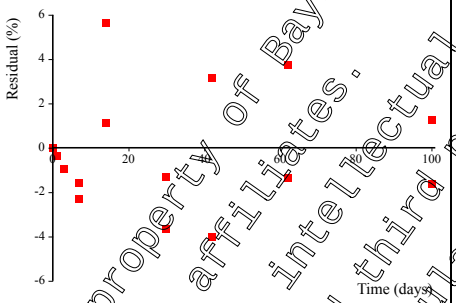
Appendix 5.1.6.2. Degradation of M06 in Emperor Lake total system (KCA 7.2.2.3/07 (M-763128-01-1))

Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	Parent HS, metabolite SFO
Plot	
Residuals	
χ^2 err %	69.1
Confidence measure	$t_{1/2}$ p = 0.5
Visual fit	Visual fit is poor with a high χ^2 and evidence of systematic error.
DT_{50} (days)	>10,000[1]
DT_{90} (days)	>10,000[1]
Assessment	Not Acceptable Visual fit is very poor with evidence of systematic errors. Rate parameter does not differ significantly from zero.
Conclusion	Fit is not acceptable. Try to improve fit by removing outlier.

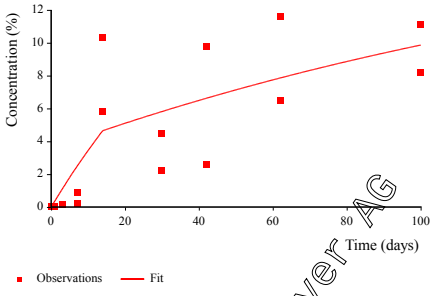
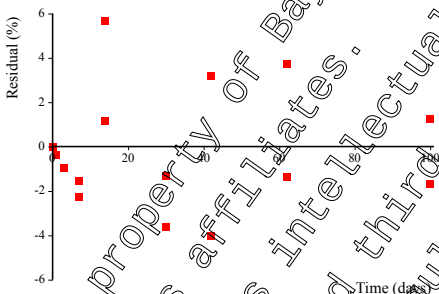
[1] Interpret with care – extrapolated beyond experimental period

Step 2: Run modified fitting routine (outlier removed). SFO fit for metabolite acceptable?

	Parent HS, metabolite SFO
Plot	
Residuals	
χ^2 err %	29.9
Confidence measure	k: p > 0.1
Visual fit	Visual fit is good with no evidence of systematic errors
DT_{50} (days)	773
DT_{90} (days)	2,570[1]
Assessment	Not Acceptable. Visual fit is good with no evidence of systematic error but rate parameter does not differ significantly from zero. Try to model with metabolite DT_{50} fixed to FOCUS default.
Conclusion	Dataset cannot be used to reliably estimate kinetic parameters

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Run alternative conservative fits

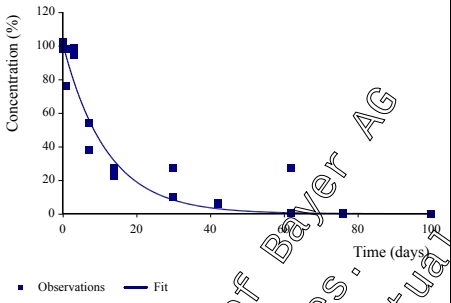
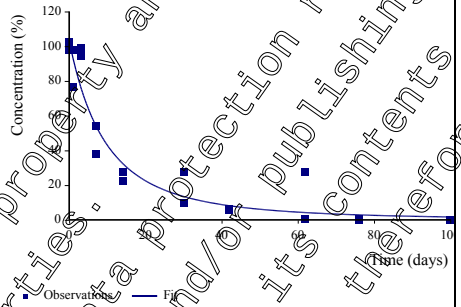
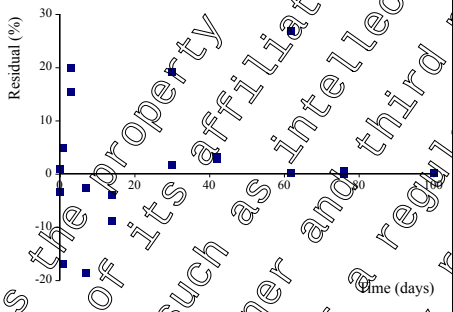
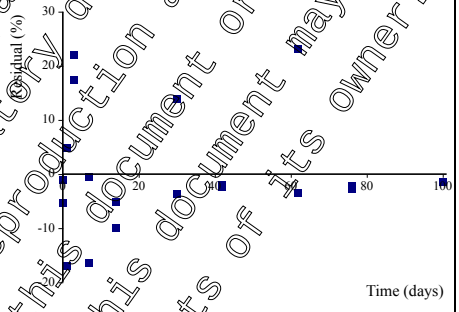
	Spiroxamine HS, metabolite SFO
Plot	
Residuals	
χ^2 err %	28.2
Confidence measure	NA
Visual fit	Visual fit is good with no evidence of systematic errors
DT_{50} (days)	1,000[1]
DT_{90} (days)	3,320[1]
Assessment	Acceptable. Use of default DT50 of 1,000 days gives an adequate description of the dataset.
Conclusion	Use of FOCUS default acceptable.

[1] Interpret with care – extrapolated beyond experimental period

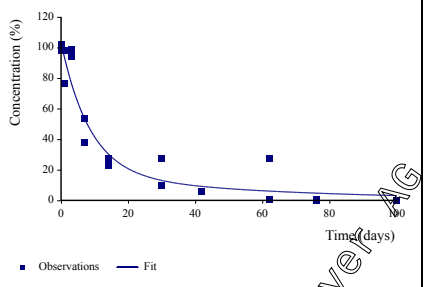
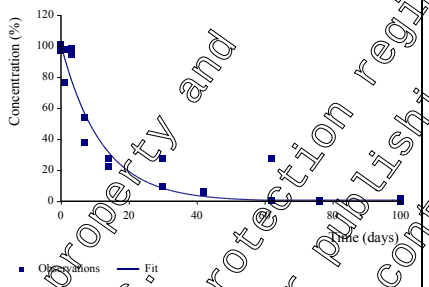
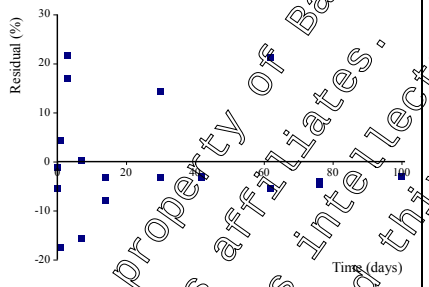
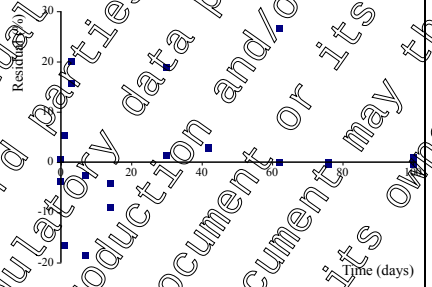
Summary:
For M06 use conservative default DT_{50} = 1,000 days

Appendix 5.1.6.3. Dissipation of [cyclohexyl-1-^{14}C]-spiroxamine in Emperor Lake water phase (KCA 7.2.2.3/07 (M-763128-01-1))

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO	FOMC
Plot		
Residuals		
χ^2 err %	18.3	18.0
Confidence measure	k: p > 0.05	N.A
Visual fit	Visual fit is good with some evidence of systematic errors in residuals.	Visual fit is excellent with no evidence of systematic error
DT_{50} (days)	8.35	7.69
DT_{90} (days)	27.7	36.4
Assessment	Acceptable: Visual fit is good but some evidence of systematic error. Rate parameter differs significantly from zero.	Acceptable: Visual fit is good but some evidence of systematic error.
Conclusion	FOMC is slightly improved on SFO. Deviation from SFO is not considered to be due to outliers or experimental artefacts. Investigate DFOP and HS	

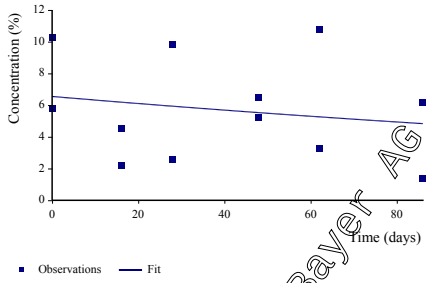
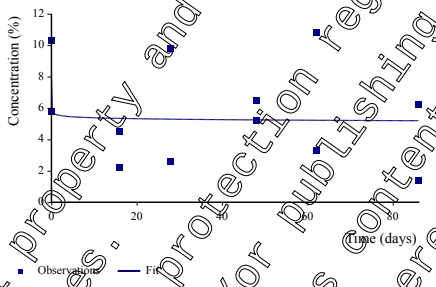
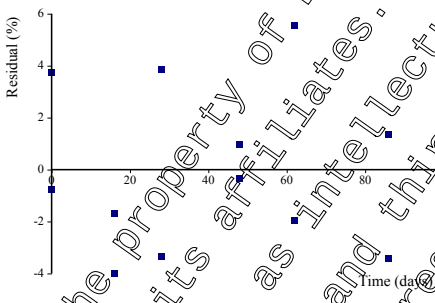
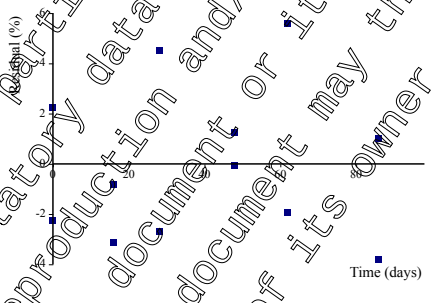
Step 3: FOMC better than SFO fit. Fit DFOP and HS.

	DFOP	HS
Plot		
Residuals		
χ^2 err %	18.3	19.2
Confidence measure	k1: p < 0.05 k2: p >0.1	k1: p < 0.05 k2: p = 0.5
Visual fit	Visual fit is good with limited evidence of systematic error	Visual fit is good with limited evidence of systematic error.
DT_{50} (days)	7.46	8.36
DT_{90} (days)	37.4	27.8
Assessment	Visual fit is good with limited evidence of systematic error but high χ^2. Rate parameter does not differ significantly from zero	Visual fit is good with limited evidence of systematic error but high χ^2. Rate parameter does not differ significantly from zero
Conclusion	Both DFOP and HS give similar visual; and statistical fits. However, k_2 rate parameter does not differ significantly from zero. FOMC gives best fit and selected.	

Summary
For Spiroxamine use FOMC. DT_{50} = 7.69 days, DT_{90} = 36.4 days

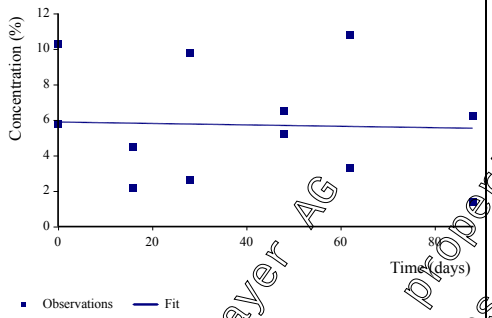
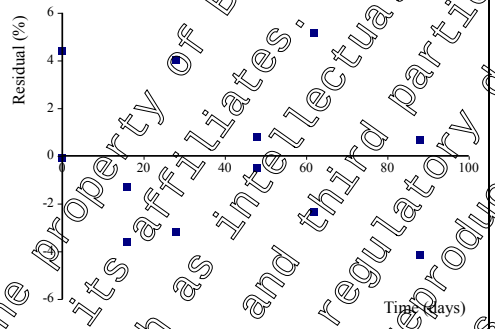
Appendix 5.1.6.4. Dissipation of M06 in water

Step 1: SFO [top-down] more appropriate than FOMC [top-down] and gives acceptable fit?)

	Metabolite SFO [top down]	Metabolite FOMC [top down]
Plot		
Residuals		
χ^2 err %	21.8	20.2
Confidence measure	k: p > 0.05	N/A
Visual fit	Visual fit is intermediate with some evidence of systematic errors	Visual fit is intermediate with some evidence of systematic errors
DT_{50} (days)	197[1]	>10,000[1]
DT_{90} (days)	653[1]	>10,000[1]
Assessment	Not Acceptable: Visual fit is intermediate with high χ^2 and some evidence of systematic error. Rate parameter does not differ significantly from zero.	Not Acceptable: Visual fit is intermediate with high χ^2 and some evidence of systematic error. Significant extrapolation from study period to DT90 is not considered reliable[2].
Conclusion	Both SFO and FOMC give very similar fits. It was considered that running DFOP and HS would not improve the fit. Insufficient decline phase to allow for an accurate prediction of endpoints. Try to fit default DT_{50} – does the fit give an acceptable description of the data?	

[1] Interpret with care – extrapolated beyond experimental period.
[2] EFSA (2009)

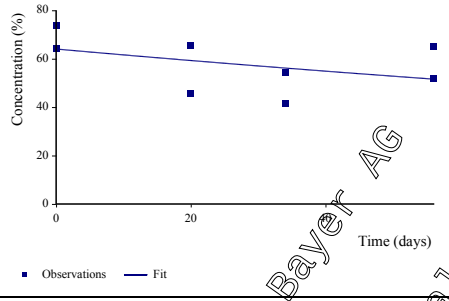
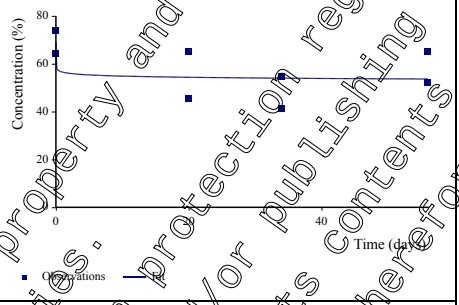
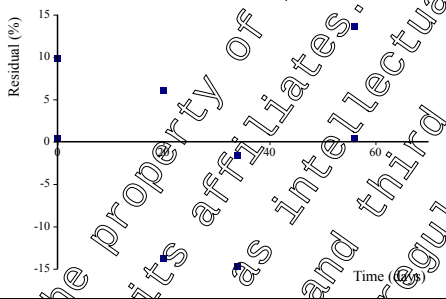
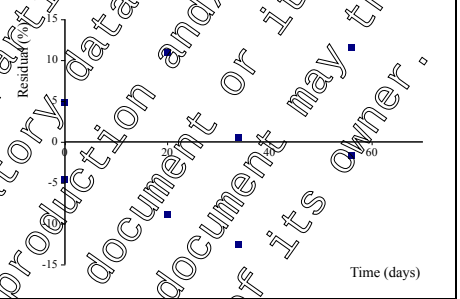
Step 2: Run alternative, yet conservative fit.

	Metabolite SFO [top down]
Plot	
Residuals	
χ^2 err %	21
Confidence measure	NA
Visual fit	Visual fit is intermediate with no evidence of systematic errors
DT_{50} (days)	1,000[1]
DT_{90} (days)	3,320[1]
Assessment	Acceptable: Visual fit is intermediate with high χ^2 and some evidence of systematic error. However, default DT_{50} adequately described data.

Summary:
For M06 dissipation from water use conservative default DT_{50} = 1,000 days.

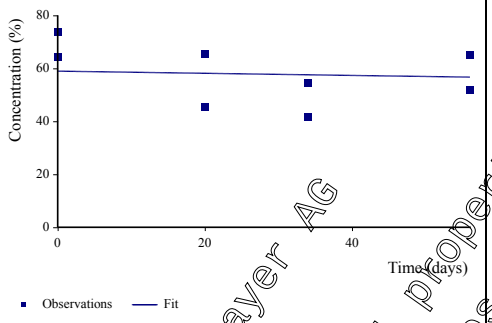
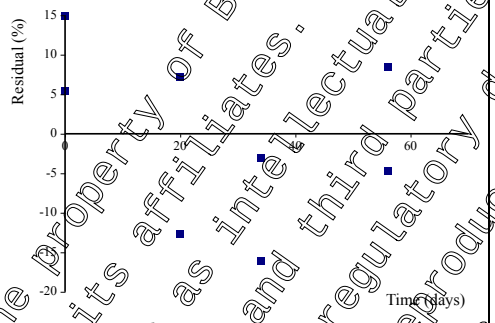
Appendix 5.1.6.5. Dissipation of [cyclohexyl-1-^{14}C]-spiroxamine in sediment

Step 1: Initial assessment (SFO more appropriate than FOMC and gives acceptable fit?)

	SFO [top-down]	FOMC [top-down]
Plot		
Residuals		
χ^2 err %	8.86	6.93
Confidence measure	k, p > 0.1	Insufficient data
Visual fit	Visual fit is good with no evidence of systematic error.	Visual fit is good with no evidence of systematic error.
DT_{50} (days)	180[1]	>10,000[1]
DT_{90} (days)	598[1]	>10,000[1]
Assessment	Not Acceptable: Visual fit is good with low χ^2 but rate parameter does not differ significantly from zero.	Not Acceptable: Insufficient data
Conclusion	SFO fit is not acceptable as rate parameter does not differ significantly from zero. Insufficient data for an assessment of biphasic models. Demonstrate that the default DT_{50} gives an acceptable fit?	

[1] Interpret with care – extrapolated beyond experimental period.

Step 2: Run alternative, yet conservative fit.

	SFO [top down]
Plot	
Residuals	
χ^2 err %	8.9
Confidence measure	NA
Visual fit	Visual fit is intermediate with some evidence of systematic errors
DT_{50} (days)	1,000[1]
DT_{90} (days)	3,320[1]
Assessment	Acceptable: Visual fit is intermediate with high χ^2 and some evidence of systematic error. However, default DT_{50} adequately described data

Summary:
For Spiroxamine use conservative default DT_{50} = 1,000 days.

Appendix 5.1.6.6. Dissipation of M06 in sediment

Insufficient data for a top-down assessment.

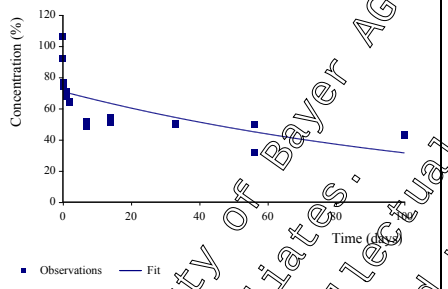
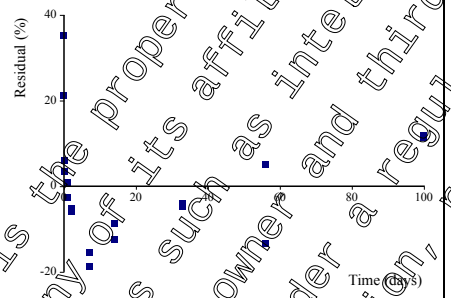
For M06 dissipation from water use conservative default DT_{50} = 1,000 days

Appendix 5.2: Kinetic evaluation for modelling endpoints

Appendix 5.2.1. KCA 7.2.2.3/01 (M-006015-01-1) Hoenniger Water

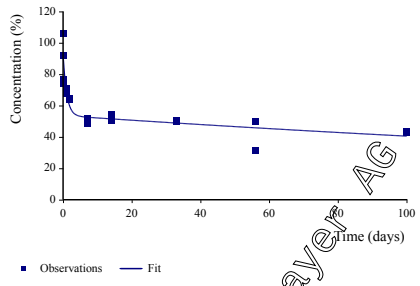
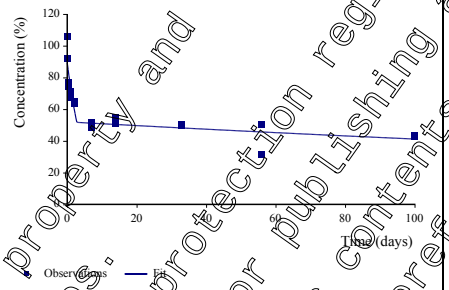
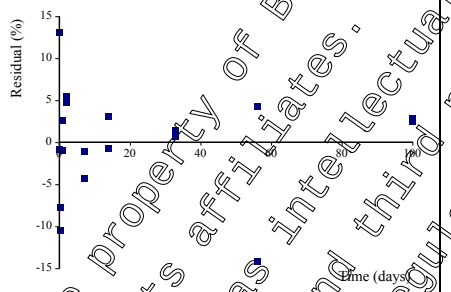
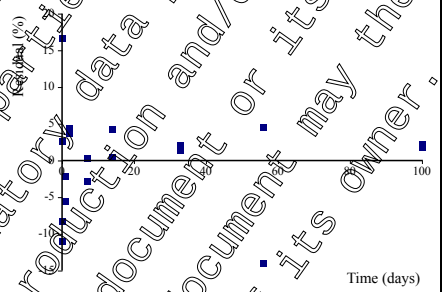
Appendix 5.2.1.1. Degradation of [cyclohexyl-1-^{14}C]-spiroxamine in Hoenniger total system (KCA 7.2.2.3/01 (M-006015-01-1))

Step 1: Initial assessment SFO.

	SFO
Plot	
Residuals	
χ^2 err %	16.6
Confidence measure	k: p < 0.10
Visual fit	Poor, residuals show clear trend.
DT_{50} (days)	85.8
DT_{90} (days)	285[1]
DT_{50MOD} (days)	85.8
Assessment	Not acceptable: Visual fit is poor, χ^2 err % is high and rate parameter differs significantly from zero.
Conclusion	SFO not acceptable as residues show clear trend. 10% initially measured concentration not met. Investigate DFOP and HS.

[1] Interpret with care – extrapolated beyond experimental period.

Step 2: 10% initially measured concentration not reached within experimental period.
Run HS and DFOP. HS/DFOP statistically and visually acceptable?

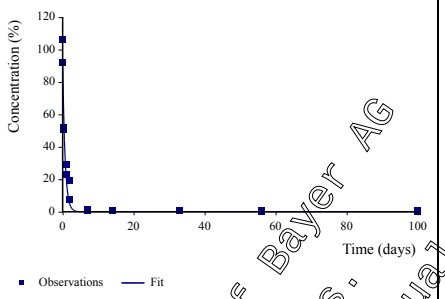
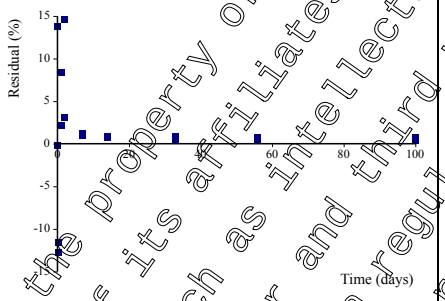
	DFOP	HS
Plot		
Residuals		
χ^2 err %	6.8	7.91
Confidence measure	k_1: p <0.05 k_2: p <0.05	k_1: p <0.05 k_2: p <0.10
Visual fit	Good, though residuals show potential trend.	Good, though residuals show potential trend.
DT_{50} (days)	51.4	65.2
DT_{50MOD} (days)	249[1,2]	297[1,2]
DT_{90} (days)	628[1]	755[1]
Assessment	Acceptable: Visual fit is good, χ^2 error is low and rate parameters differ significantly from zero.	Acceptable: Visual fit is good, χ^2 error is low acceptable and rate parameters differ significantly from zero.
Conclusion	HS and DFOP fits are very similar. DFOP gives the best acceptable fit (χ^2) and should be used for modelling endpoints.	

[1] Interpret with care – extrapolated beyond experimental period.
[2] k_2 DT_{50}

Summary:
For Spiroxamine use DFOP. DT_{50MOD} = 249 days (k_2 rate parameter).

Appendix 5.2.1.2. Dissipation of [cyclohexyl-1-^{14}C]-spiroxamine in Hoenniger water phase (KCA 7.2.2.3/01 (M-006015-01-1)

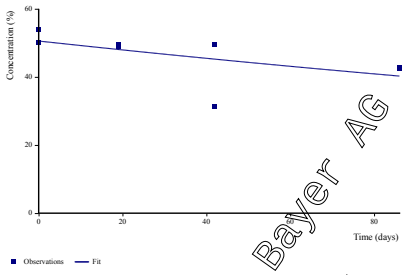
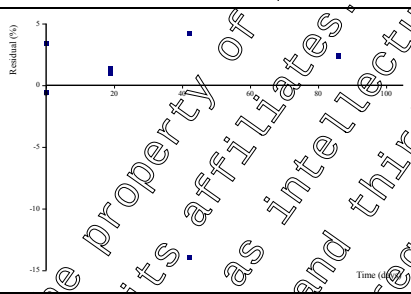
Step 1: Initial assessment SFO.

	SFO
Plot	
Residuals	
χ^2 err %	1.5
Confidence measure	k: p <0.10
Visual fit	Acceptable, residuals show no trend and are very small
DT_{50} (days)	0.468
DT_{90} (days)	1.55
DT_{50MOD} (days)	0.468
Assessment	Acceptable Visual fit is good, χ^2 error is high and rate parameters differ significantly from zero.
Conclusion	SFO statistically and visually acceptable

Summary:
For Spiroxamine use SFO. DT_{50MOD} =0.468 days.

Appendix 5.2.1.3. Dissipation of [cyclohexyl-1-^{14}C]-spiroxamine in Hoenniger sediment phase (KCA 7.2.2.3/01 (M-006015-01-1))

Step 1: SFO [top-down] acceptable fit?

	SFO [top-down]
Plot	
Residuals	
χ^2 err %	5.08
Confidence measure	k: p < 0.10
Visual fit	Intermediate, residuals show no evidence of a trend
DT_{50} (days)	262[1]
DT_{90} (days)	871[1]
DT_{50MOD} (days)	262[1]
Assessment	Acceptable: Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	SFO using decline after max is considered acceptable and should be used for modelling endpoints.

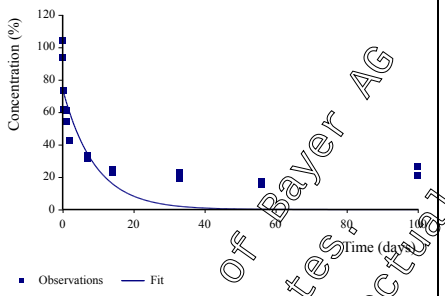
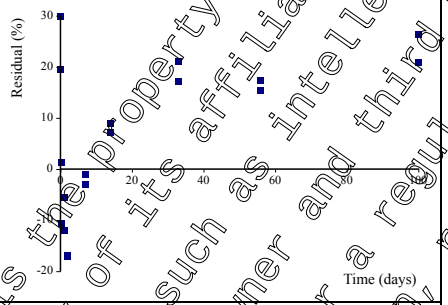
[1] Interpret with care – extrapolated beyond experimental period

Summary:
For Spiroxamine use [top-down] SFO DT_{50MOD} = 262 days

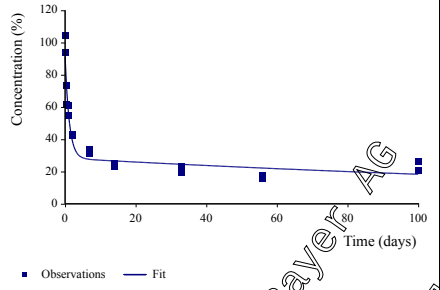
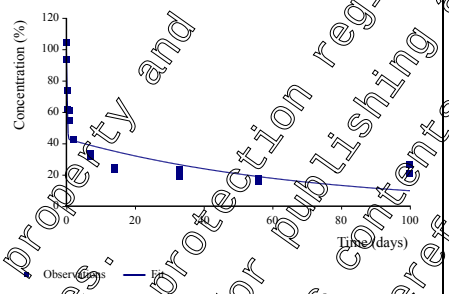
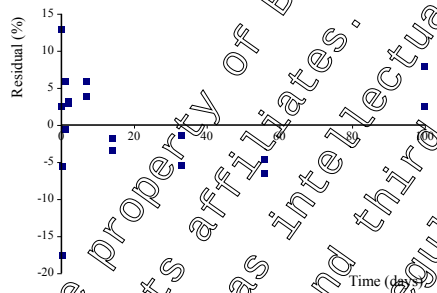
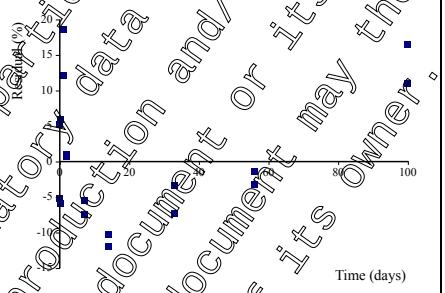
Appendix 5.2.2. KCA 7.2.2.3/01 (M-006015-01-1) Stilwell

Appendix 5.2.2.1. Degradation of [cyclohexyl-1-^{14}C]-spiroxamine in Stilwell total system (KCA 7.2.2.3/01 (M-006015-01-1))

Step 1: Initial assessment SFO.

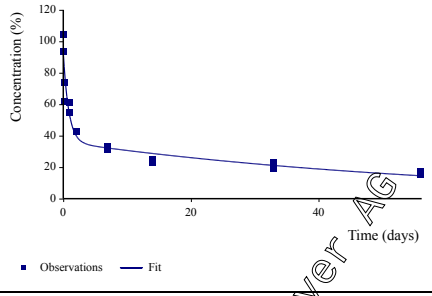
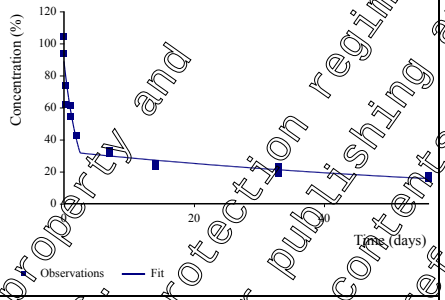
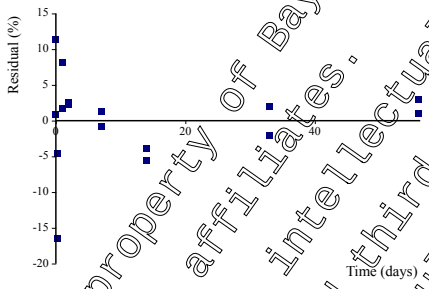
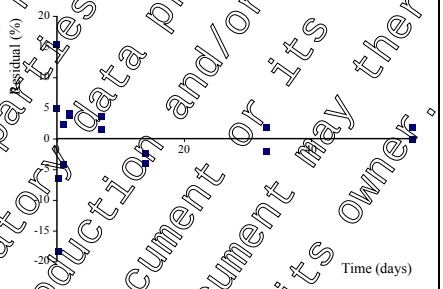
	SFO
Plot	
Residuals	
χ^2 err %	29.6
Confidence measure	k: p <0.10
Visual fit	Poor, residuals show clear trend.
DT_{50} (days)	6.34
DT_{50MOD} (days)	6.31
DT_{90} (days)	21.0
Assessment	Not acceptable: Visual fit is poor, χ^2 err % is high but rate parameter does differ significantly from zero.
Conclusion	SFO not acceptable as residues show clear trend. Deviation from SFO is not considered to be due to outliers or experimental artefacts. 10% of initial study mass not reached, investigate DFOP and HS.

Step 2: 10% initially measured concentration not reached within experimental period. Run HS and DFOP. HS/DFOP statistically and visually acceptable?

	DFOP	HS
Plot		
Residuals		
χ^2 err %	12.4	17.6
Confidence measure	k_1: p <0.10 k_2: p =0.11	k_1: p <0.10 k_2: p <0.10
Visual fit	Good, residuals show no trend.	Good, residuals show no trend.
DT_{50} (days)	1.5	0.455
DT_{50MOD} (days))	161[1,2]	47[2]
DT_{90} (days)	262[1]	99.6
Assessment	Not acceptable: Visual fit is good, χ^2 error is acceptable, however, the k_2 rate parameters does not differ significantly from zero. Nevertheless, residuals show no large, systematic error.	Acceptable: Visual fit is good, χ^2 error is acceptable, both rate parameters differ significantly from zero.
Conclusion	HS and DFOP fits are very similar. DFOP gives the best acceptable fit although k2 rate parameters does not differ significantly from zero. 100DAT timepoint considered to impacting modelling estimates	

[1] Interpret with care – extrapolated beyond experimental period
[2] k_2 DT_{50}

Step 2: Modified fit: exclusion of 100DAT

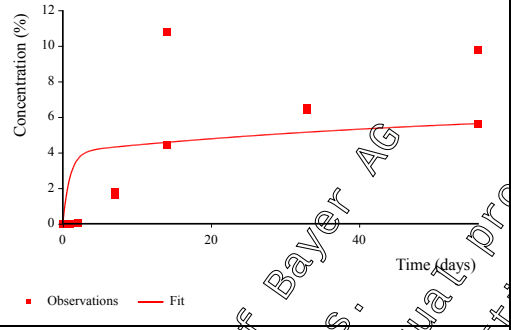
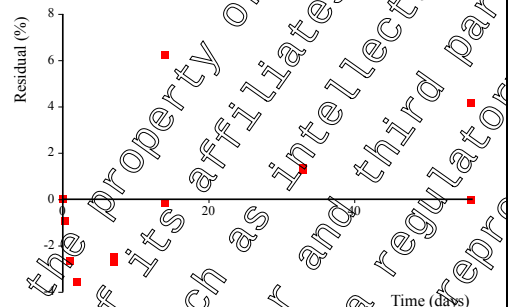
	DFOP	HS
Plot		
Residuals		
χ^2 err %	10.3	12.3
Confidence measure	k1: p <0.10 k2: p <0.10	k1: p <0.10 k2: p <0.10
Visual fit	Good, residuals show no trend.	Good, residuals show no trend.
DT_{50} (days)	1.37	1.67
Modelling DT_{50} (days)	42.8[1]	52.8[2]
DT_{90} (days)	83.7	99.2
Assessment	Acceptable: Visual fit is good, χ^2 error is acceptable, both rate parameters differ significantly from zero.	Acceptable: Visual fit is good, χ^2 error is acceptable, both rate parameters differ significantly from zero.
Conclusion	HS and DFOP fits are very similar. DFOP selected on the basis of most acceptable fit (χ^2)	

[1] Interpret with care – extrapolated beyond experimental period
[2] k_2 DT_{50}

Summary:
For Spiroxamine use DFOP (k_2 rate parameter), DT_{50modD} = 42.8 days

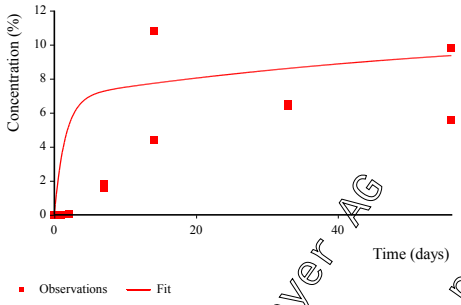
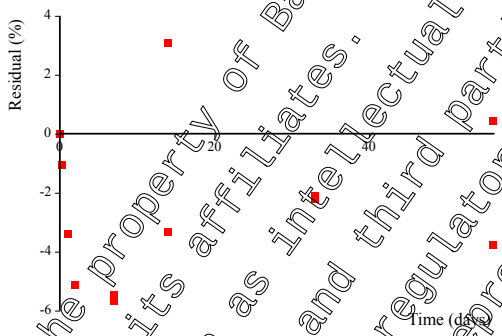
Appendix 5.2.2.2. M06 in Stilwell total system (KCA 7.2.2.3/01 (M-006015-01-1))

Step 1: Run parent best-fit DFOP. SFO fit for metabolite acceptable?

	Parent DFOP, Metabolite SFO
Plot	
Residuals	
χ^2 err %	58.5
Confidence measure	k: p = 0.5
Visual fit	Visual fit is poor and χ2 is very high. The rate parameter does not differ significantly from zero. Maximum metabolite formation not approached.
DT_{50} (days)	>10,000[1]
DT_{90} (days)	>10,000[1]
Formation fraction	0.073
Assessment	Not Acceptable: Visual fit is acceptable, but χ^2 err % is high and rate parameter does not differ significantly from zero
Conclusion	SFO is not considered acceptable and χ^2 err % is high. Estimated rate parameter does not differ from zero. Fit FOCUS default for rate parameter, increase formation fraction and evaluate fit to data.

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Alternative, yet conservative, fit.

	Parent DFOP, Metabolite SFO
Plot	
Residuals	
χ^2 err %	69.9
Confidence measure	NA
Visual fit	Visual fit is conservative.
DT_{50} (days)	1,000[1]
DT_{50MOD} (days)	1,000[1]
DT_{90} (days)	3,320[1]
Formation fraction	0.15
Assessment	Visual fit is sufficiently conservative
Conclusion	SFO using FOCUS default DT_{50} and ff of 0.15 is acceptable.

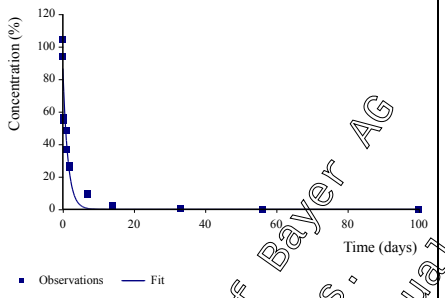
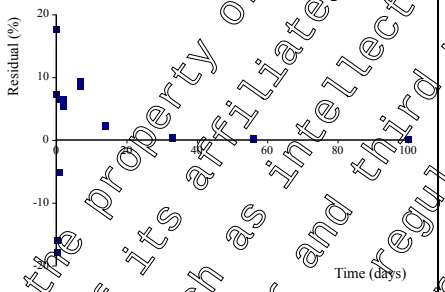
[1] Interpret with care – extrapolated beyond experimental period

Summary:
For M06 use FOCUS default as realistic worst case. DT_{50MOD} = 1,000 days.

Formation fraction from spiroxamine = 0.15

Appendix 5.2.2.3. Dissipation of [cyclohexyl-1-^{14}C]-spiroxamine in Stilwell water phase (KCA 7.2.2.3/01 (M-006015-01-1))

Step 1: Initial assessment SFO.

	SFO
Plot	
Residuals	
χ^2 err %	24.1
Confidence measure	k_2 p <0.10
Visual fit	Visual fit is acceptable; residuals show slight trend. Residuals are very small and occur after 90% of parent mass removed.
DT_{50} (days)	0.965
DT_{50MOD} (days)	0.965
DT_{90} (days)	3.21
Assessment	Acceptable: Visual fit is good, χ^2 error is high and rate parameters differ significantly from zero.
Conclusion	SFO is considered visually and statistically acceptable.

Summary:
For Spiroxamine use SFO. DT_{50MOD} =0.965 days.

Appendix 5.2.2.4. Dissipation of [cyclohexyl-1-^{14}C]-spiroxamine in Stilwell sediment phase (KCA 7.2.2.3/01 (M-006015-01-1))

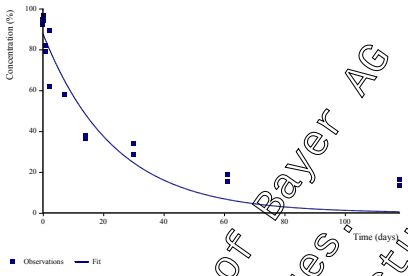
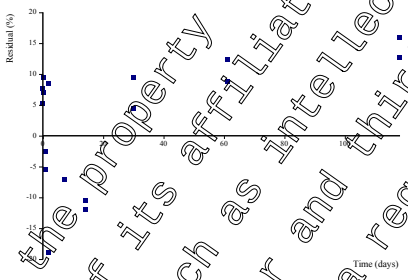
No decline phase observed so cannot be fitted.

Summary:
For Spiroxamine use FOCUS default as a conservative worst case. DT_{50MOD} =1,000 days.

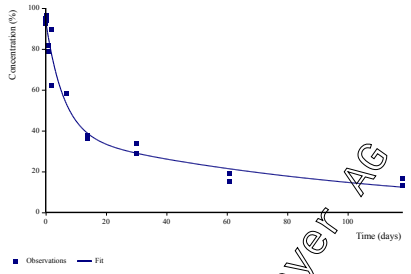
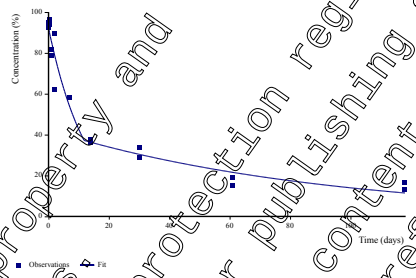
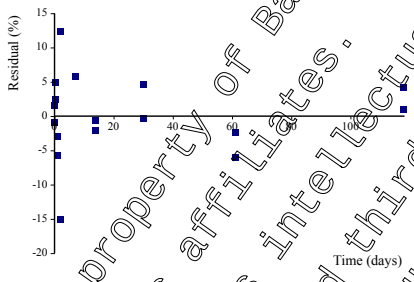
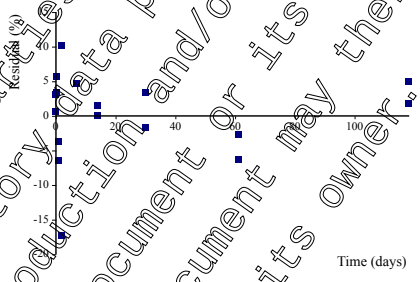
Appendix 5.2.3. KCA 7.2.2.3/04 (M-303324-01-1) Anglerweiher System

Appendix 5.2.3.1. Degradation of [1,3-dioxolane-4-^{14}C]-spiroxamine in Anglerweiher total system (KCA 7.2.2.3/04 (M-303324-01-1))

Step 1: Initial assessment (SFO acceptable?)

	SFO
Plot	
Residuals	
χ^2 err %	12.5
Confidence measure	k: p <0.10
Visual fit	Poor, residuals show clear trend.
DT_{50} (days)	16.3
DT_{90} (days)	54.2
DT_{50MOD} (days)	16.3
Assessment	Not Acceptable. Visual fit is poor, χ^2 err. % is ok and rate parameter differs significantly from zero.
Conclusion	SFO fit shows clear trend in residuals. 50% of initial measurable concentration not reached. Investigate DFOP and HS kinetics

Step 2: 10% initially measured concentration not reached within experimental period.
Run HS and DFOP. HS/DFOP statistically and visually acceptable?

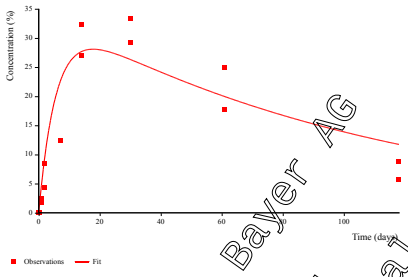
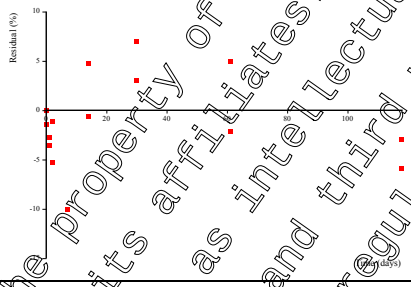
	DFOP	HS
Plot		
Residuals		
χ^2 err %	5.37	5.78
Confidence measure	k_1: p >0.10 k_2: p >0.10	k_1: p >0.10 k_2: p >0.10
Visual fit	Excellent, residuals shows no clear trend.	Excellent, residuals shows no clear trend.
DT_{50} (days)	9.11	8.99
DT_{50MOD} (days)	72.6	62.6
DT_{90} (days)	347[1]	138[1]
Assessment	Acceptable: Visual fit is excellent and χ^2 err % is low and rate parameters differ significantly from zero.	Acceptable: Visual fit is excellent, χ^2 err % is low and rate parameters differ significantly from zero.
Conclusion	DFOP and HS all give very good visual fits with no evidence of a trend in the residuals. DFOP selected on the basis of goodness of fit (χ^2)	

[1] Interpret with care – extrapolated beyond experimental period.

Summary:
For Spiroxamine use DFOP (k_2 rate parameter). DT_{50MOD} = 72.6 days.

Appendix 5.2.3.2. Degradation of M06 in Anglerweiher total system (KCA 7.2.2.3/04 (M-303324-01-1))

Step 1: Run parent best-fit DFOP. SFO fit for metabolite acceptable?

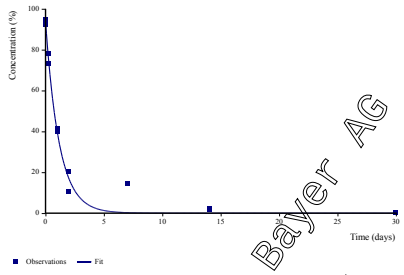
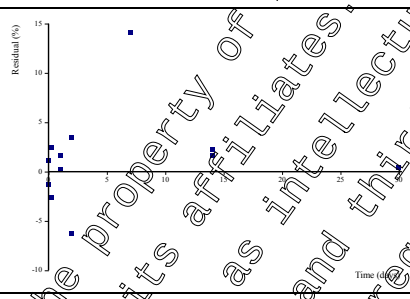
	Parent DFOP, Metabolite SFO
Plot	
Residuals	
χ^2 err %	26.2
Confidence measure	k: p <0.1
Visual fit	Visual fit is acceptable, residuals shows no clear trend
DT_{50} (days)	45.1
DT_{50MOD} (days)	45.1
DT_{90} (days)	150[1]
Formation fraction	0.7412
Assessment	Acceptable. Visual fit is acceptable, χ^2 err % is high and rate parameter differs significantly from zero.
Conclusion	SFO is considered Visually and statistically acceptable

[1] Interpret with care – extrapolated beyond experimental period

Summary:
For M06 use DFOP-SFO. DT_{50MOD} = 45.1 days
Formation fraction from spiroxamine = 0.7412

Appendix 5.2.3.3. Dissipation of [1,3-dioxolane-4-^{14}C]-spiroxamine in Anglerweiher water phase (KCA 7.2.2.3/04 (M-303324-01-1))

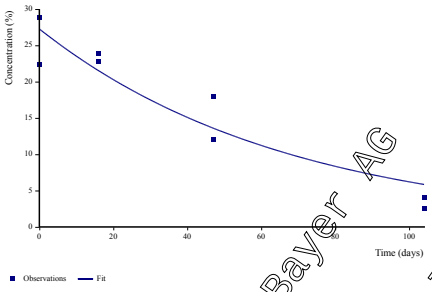
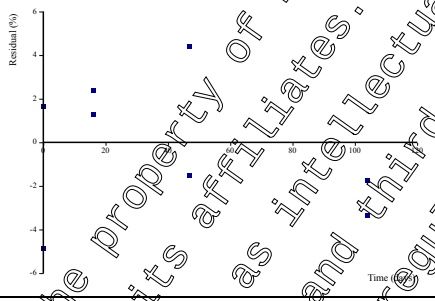
Step 1: Initial assessment SFO

	SFO
Plot	
Residuals	
χ^2 err %	12.4
Confidence measure	k: 0.10
Visual fit	Acceptable, residuals shows no clear trend and are generally very small.
DT_{50} (days)	0.81
DT_{50MOD} (days)	0.81
DT_{90} (days)	2.69
Assessment	Acceptable: Visual fit is intermediate and χ^2 err % is acceptable. Rate parameter differs significantly from zero.
Conclusion	SFO is considered visually and statistically acceptable.

Summary:
For Spiroxamine use SFO DT_{50MOD} = 0.81 days.

Appendix 5.2.3.4. Dissipation of M06 in water (KCA 7.2.2.3/04 (M-303324-01-1))

Step 2: SFO fit for M06 (decline after max) acceptable?

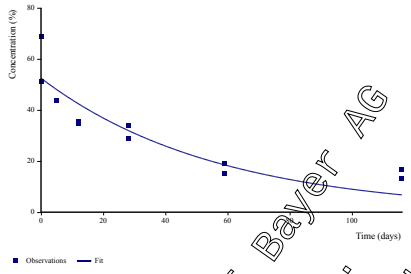
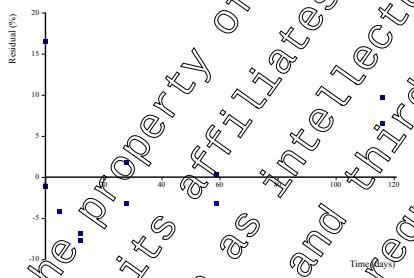
	SFO [top down]
Plot	
Residuals	
χ^2 err %	9.26
Confidence measure	k:1 $p < 0.05$
Visual fit	Visual fit is good with no evidence of systematic error
DT_{50} (days)	46.9
DT_{50MOD} (days)	46.9
DT_{90} (days)	156
Assessment	Acceptable: Visual fit is good with no evidence of systematic error. Rate parameter differs significantly from zero
Conclusion	SFO is considered visually and statistically acceptable.

[1] Interpret with care – extrapolated beyond experimental period

Summary:
For M06 use [top-down] SFO. DT_{50MOD} = 46.9 days

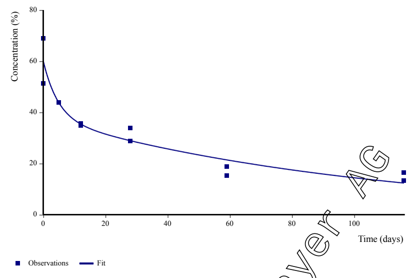
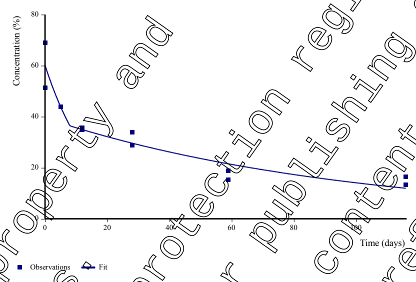
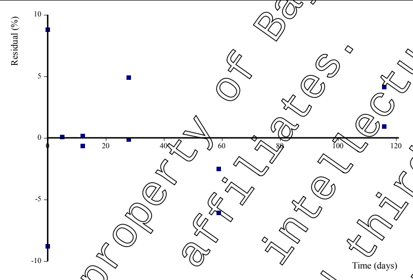
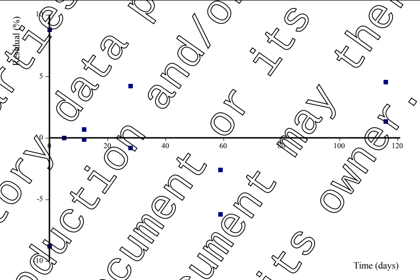
Appendix 5.2.3.5. Dissipation of [1,3-dioxolane-4-^{14}C]-spiroxamine in sediment (KCA 7.2.2.3/04 (M-303324-01-1))

Step 1: SFO fit for parent (decline after max) acceptable?

	SFO [top down]
Plot	
Residuals	
χ^2 err %	13.5
Confidence measure	ls: p <0.1
Visual fit	Visual fit is poor, residuals show a clear trend.
DT_{50} (days)	39.3
DT_{50MOD} (days)	39.3
DT_{90} (days)	131[1]
Assessment	Not Acceptable: Visual fit is poor, χ^2 error is acceptable and rate parameter differs significantly from zero.
Conclusion	Decline after max SFO is not considered acceptable. Therefore, biphasic kinetics were investigated.

[1] Interpret with care – extrapolated beyond experimental period

Step 2: 10% initially measured concentration not reached within experimental period. Run HS and DFOP. HS/DFOP statistically and visually acceptable?

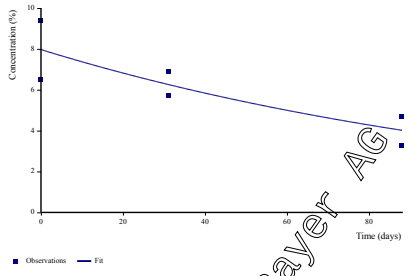
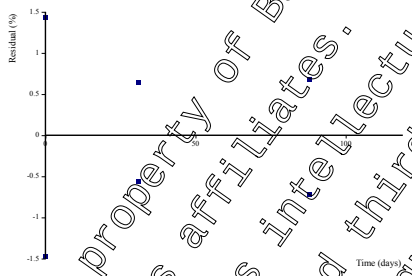
	DFOP [top down]	HS [top down]
Plot		
Residuals		
χ^2 err %	6.71	6.75
Confidence measure	k1: p <0.10 k2: p <0.10	k1: p <0.10 k2: p <0.10
Visual fit	Excellent, residuals shows no clear trend.	Excellent, residuals shows no clear trend.
DT_{50} (days)	24.3	26.6
Modelling DT_{50} (days)	72.0	67.3
DT_{90} (days)	191[1]	183[1]
Assessment	Acceptable: Visual fit is excellent, χ^2 err % is low and rate parameters differ significantly from zero.	Acceptable: Visual fit is excellent, χ^2 err % is low and rate parameters differ significantly from zero.
Conclusion	The DFOP and HS are visually and statistically very similar; however, DFOP selected on the basis of goodness of fit (χ^2).	

[1] Interpret with care – extrapolated beyond experimental period.

Summary:
For Spiroxamine use [top-down] DFOP (k_2 rate parameter). DT_{50MOD} = 72.0 days

Appendix 5.2.3.6. Dissipation of M06 in sediment (KCA 7.2.2.3/04 (M-303324-01-1))

Step 2: SFO fit for M06 (decline after max) acceptable?

	SFO [top down]
Plot	
Residuals	
χ^2 err %	0.399
Confidence measure	k: p<0.1
Visual fit	Visual fit is good, residuals show no clear trend.
DT_{50} (days)	89.2
DT_{50MOD} (days)	89.2
DT_{90} (days)	296[1]
Assessment	Acceptable: Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	SFO using decline after max is considered visually and statistically acceptable and should be used for modelling endpoints.

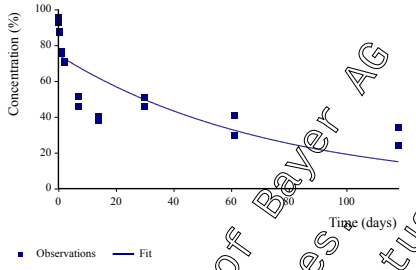
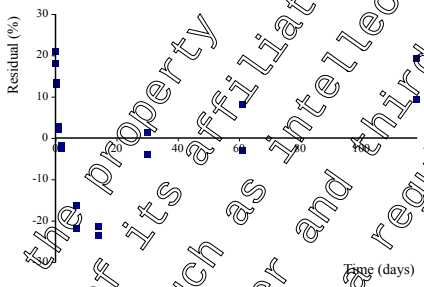
[1] Interpret with care – extrapolated beyond experimental period

Summary:
For M06 use [top-down] SFO. DT_{50MOD} = 89.2 days.

Appendix 5.2.4. KCA 7.2.2.3/04 (M-303324-01-1) Hoenniger Water

Appendix 5.2.4.1. Degradation of [1,3-dioxolane-4-^{14}C]-spiroxamine in Hoenniger total system (KCA 7.2.2.3/04 (M-303324-01-1))

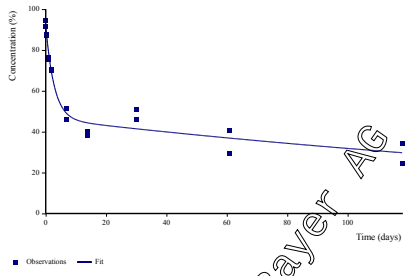
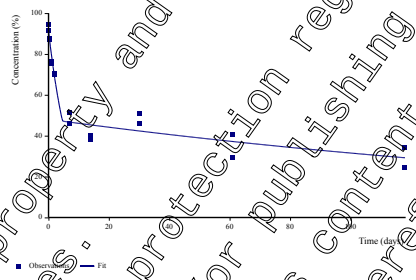
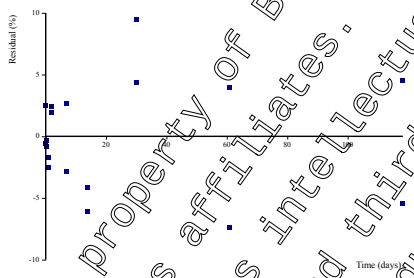
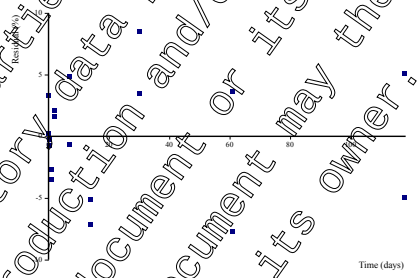
Step 1: Initial assessment SFO fit?

	SFO
Plot	
Residuals	
χ^2 err %	18.2
Confidence measure	k: p < 0.10
Visual fit	Poor, residuals shows a clear trend.
DT_{50} (days)	51.1
DT_{50MOD} (days)	51.1
DT_{90} (days)	171
Assessment	Not acceptable. Visual fit is poor, χ^2 err % is high but rate parameter differs significantly from zero.
Conclusion	SFO shows a high χ^2 err % and a clear trend in residuals. Deviation from SFO is not considered to be due to outliers or experimental artefacts. 10% of intial mass not reached in study period. DFOP and HS investigated.

[1] Interpret with care – extrapolated beyond experimental period

Step 2: 10% initially measured concentration not reached within experimental period.

Run HS and DFOP. HS/DFOP statistically and visually acceptable?

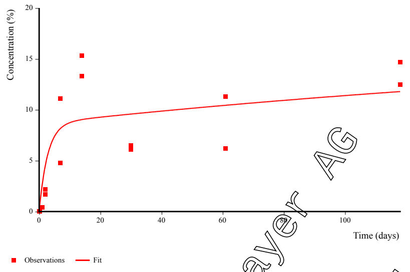
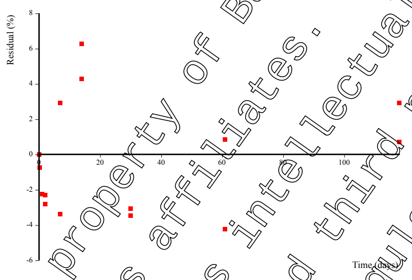
	DFOP	HS
Plot		
Residuals		
χ^2 err %	4.79	5.08
Confidence measure	k_1: p <0.10 k_2: p <0.10	k_1: p <0.10 k_2: p <0.10
Visual fit	Acceptable, residuals shows no clear trend.	Acceptable, residuals shows no clear trend.
DT_{50} (days)	9.82	12.3
Modelling DT_{50} (days)	184[1]	165[1]
DT_{90} (days)	429[1]	395[1]
Assessment	Acceptable: Visual fit is acceptable and χ^2 err % is low and rate parameters differ significantly from zero.	Acceptable: Visual fit is acceptable, χ^2 err % is low and rate parameters differ significantly from zero.
Conclusion	DFOP and HS all give very good visual fits with no evidence of a trend in the residuals. DFOP selected as best fit on basis of χ^2 err %.	

[1] Interpret with care – extrapolated beyond experimental period;

Summary:
For Spiroxamine use DFOP (k_2 rate parameter), DT_{50MOD} = 184 days.

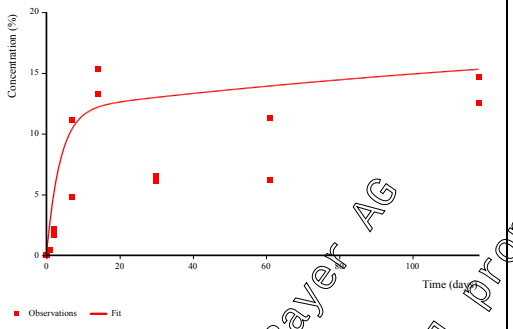
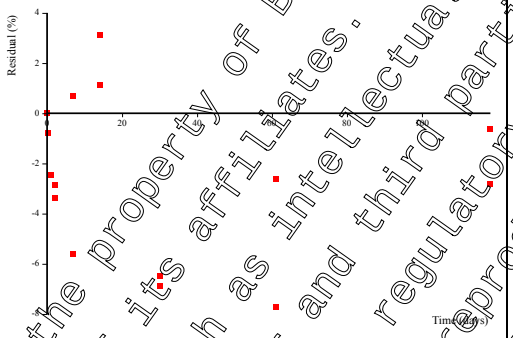
Appendix 5.2.4.2. Degradation of M06 in Hoenniger total system (KCA 7.2.2.3/04 (M-303324-01-1))

Step 1: Run parent best-fit and metabolite DFOP. SFO fit for metabolite acceptable?

	Parent DFOP, metabolite SFO
Plot	
Residuals	
χ^2 err %	31.9
Confidence measure	k: p = 0.50
Visual fit	Visual fit is intermediate, residuals show no trend
DT_{50} (days)	>10,000[1]
DT_{90} (days)	>10,000[1]
Formation fraction	0.189
Assessment	Not acceptable: Visual fit is poor, χ^2 err % is very high and rate parameter does not differ significantly from zero.
Conclusion	SFO is note acceptable. Therefore, decline after maximum should be assessed. However, this was not possible as there is insufficient decline data. Therefore, FOCUS default with an increased formation fraction wereassessed as potential modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period

Step 2: Alternative, yet conservative fit.

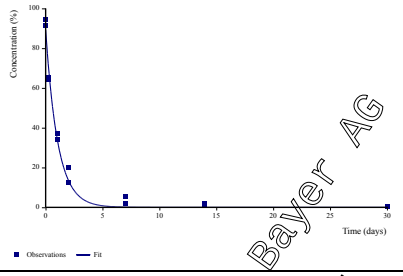
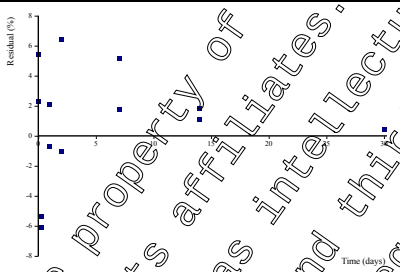
	Parent DFOP, metabolite SFO
Plot	
Residuals	
χ^2 err %	38.4
Confidence measure	N/A
Visual fit	Visual fit is conservative
DT_{50} (days)	1,000[1]
DT_{50MOD} (days)	1,000[1]
DT_{90} (days)	3,320[1]
Formation fraction	0.3
Assessment	Acceptable. Fit is sufficiently conservative
Conclusion	SFO using DT_{50} fixed to default and ff of 0.3 is considered acceptable for use in modelling

Summary:
For M06 use FOCUS default DT_{50MOD} = 1,000 days

Formation fraction from spiroxamine = 0.3.

Appendix 5.2.4.3. Dissipation of [1,3-dioxolane-4-^{14}C]-spiroxamine in Hoenninger surface water (KCA 7.2.2.3/04 (M-303324-01-1))

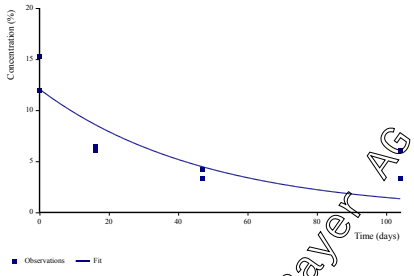
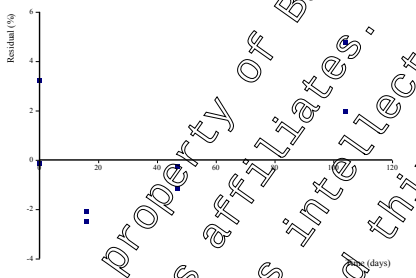
Step 1: Initial assessment SFO gives acceptable fit?

	SFO
Plot	
Residuals	
χ^2 err %	8.18
Confidence measure	k: p <0.10
Visual fit	Intermediate, residuals shows no clear trend
DT_{50} (days)	0.728
DT_{50MOD} (days)	0.728
DT_{90} (days)	2.42
Assessment	Acceptable: Visual fit is intermediate and residuals show a clear trend. Rate parameter differs significantly from zero.
Conclusion	SFO is considered visually and statsically acceptable and should be used for modelling endpoints.

Summary:
For Spiroxamine use SFO, DT_{50MOD} = 0.728 days.

Appendix 5.2.4.4. Dissipation of M06 in Hoenniger water phase (KCA 7.2.2.3/04 (M-303324-01-1))

Step 1: SFO fit for metabolite (decline after max) acceptable?

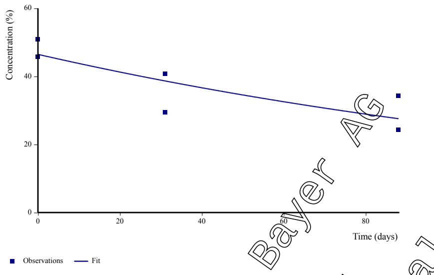
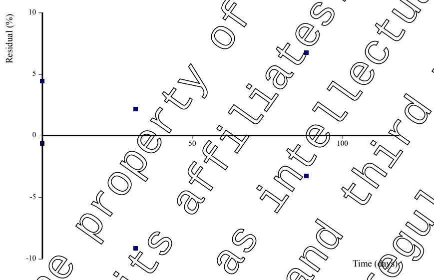
	Metabolite SFO [top down]
Plot	
Residuals	
χ^2 err %	25.4
Confidence measure	k: p <0.1
Visual fit	Visual fit is poor, some evidence of a trend in residuals
DT_{50} (days)	32.8
DT_{50MOD} (days)	32.8
DT_{90} (days)	109
Formation fraction	Not applicable
Assessment	Potentially Acceptable: Visual fit is poor, χ^2 err % is high but rate parameter differs significantly from zero.
Conclusion	Insufficient data for an assessment by FOCUS and HS. SFO [decline only] is considered acceptable despite high χ^2.

[1] Interpret with care – extrapolated beyond experimental period.

Summary:
For M06 use SFO. DT_{50MOD} = 32.8 days.

Appendix 5.2.4.5. Dissipation of [1,3-dioxolane-4-^{14}C]-spiroxamine in Hoenninger sediment phase (KCA 7.2.2.3/04 (M-303324-01-1))

Step 1: Initial assessment SFO gives acceptable fit?

	SFO [top down]
Plot	
Residuals	
χ^2 err %	5.9
Confidence measure	k: p <0.10
Visual fit	Good, residuals show no evidence of a trend in residuals.
DT_{50} (days)	117[1]
DT_{50MOD} (days)	117[1]
DT_{90} (days)	388[1]
Assessment	Acceptable: Visual fit is good, χ^2 error is low and rate parameter differs significantly from zero.
Conclusion	SFO was visually and statistically acceptable and should be used for modelling endpoints.

[1] Interpret with care – extrapolated beyond experimental period.

Summary:
For Spiroxamine use SFO [decline only]. DT_{50MOD} = 117 days.

Appendix 5.2.4.6. Dissipation of M06 in sediment (KCA 7.2.2.3/04 (M-303324-01-1))

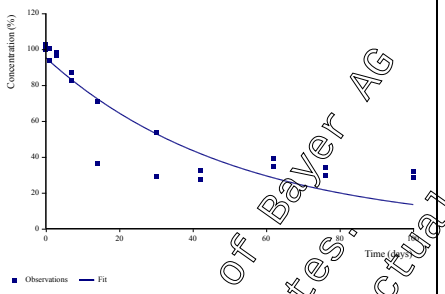
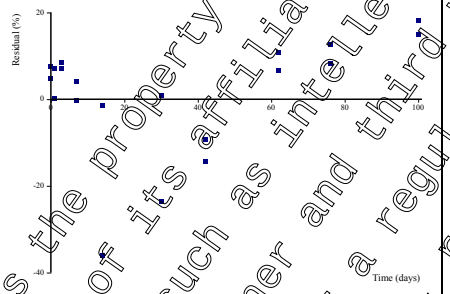
As no decline phase was observed for M06 it is not possible to estimate degradation rates from the sediment phase.

M06 sediment degradation use conservative default. DT_{50MOD} = 1,000 days

Appendix 5.2.5. KCA 7.2.2.3/07 (M-763128-01-1) Calwich Abbey

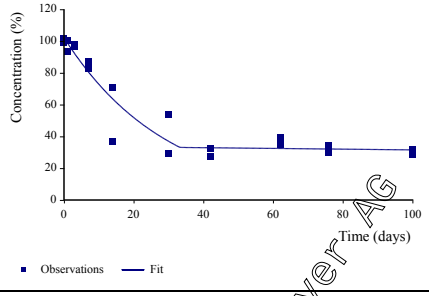
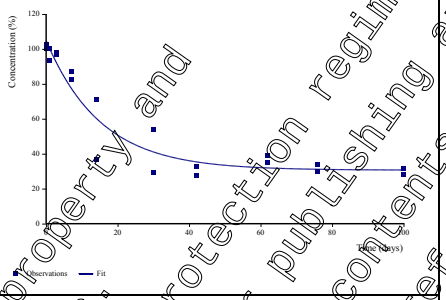
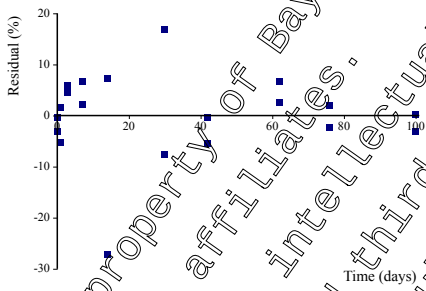
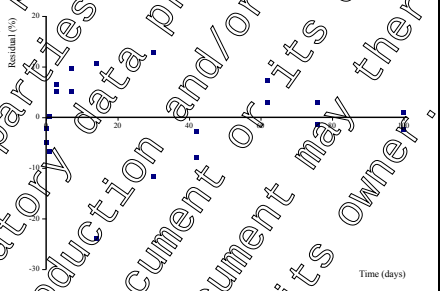
Appendix 5.2.5.1. Degradation of [cyclohexyl-1-^{14}C]-spiroxamine in Calwich Abbey total system (KCA 7.2.2.3/07 (M-763128-01-1))

Step 1: Initial assessment SFO.

	SFO
Plot	
Residuals	
χ^2 err %	14.4
Confidence measure	k: p < 0.05
Visual fit	Visual fit is intermediate with evidence of systematic error
DT_{50} (days)	35.7
DT_{50MOD} (days)	35.7
DT_{90} (days)	118[1]
Assessment	Not Acceptable: Visual fit is intermediate with evidence of systematic error. Rate parameter is significantly different from zero.
Conclusion	SFO not acceptable. Deviation from SFO is not considered to be due to outliers or experimental artefacts. 10% of initial measurable mass has not been reached. Investigate HS and DFOP.

[1] Interpret with care – extrapolated beyond experimental period.

Step 3: 10% of initially measured mass has not been reached, run HS and/or DFOP:

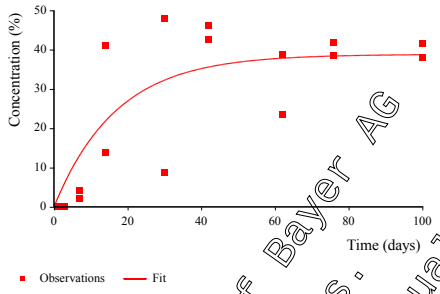
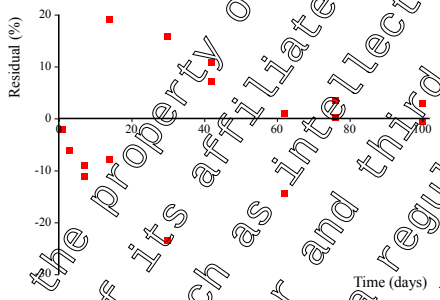
	HS	DFOP
Plot		
Residuals		
χ^2 err %	6.65	6.89
Confidence measure	k_1: p < 0.05 k_2: p > 0.1	k_1: p < 0.05 k_2: p = 0.5
Visual fit	Visual fit is good with no evidence of systematic errors	Visual fit is excellent with no evidence of systematic errors
DT_{50} (days)	20.5	18.9
DT_{50MOD} (days)	1,000*	1,000*
DT_{90} (days)	1,690[1]	>10,000[1]
Assessment	Not Acceptable: Visual fit is good with no evidence of systematic errors but k_2 rate constant does not differ significantly from zero	Not Acceptable: Visual fit is excellent with no evidence of systematic errors but k_2 rate constant does not differ significantly from zero
Conclusion	k_2 rate parameters do not differ significantly from zero and endpoint is not reliable. Use 1,000 day FOCUS default as modelling endpoint as a reasonable worst case.	

[1] Interpret with care – extrapolated beyond experimental period.
* FOCUS default

Summary:
For Spiroxamine use FOCUS default, DT_{50MOD} = 1,000 days.

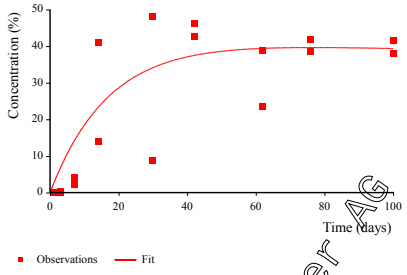
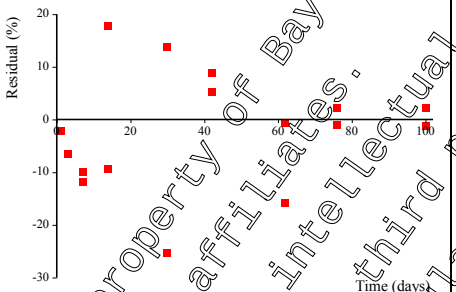
Appendix 5.2.5.2. Degradation of M06 in Calwich Abbey total system (KCA 7.2.2.3/07 (M-763128-01-1))

Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	parent DFOP, metabolite SFO
Plot	
Residuals	
χ^2 err %	20.1
Confidence measure	k: p = 0.5
Visual fit	Visual fit is intermediate with a high χ2 as a consequence of data scatter
DT_{50} (days)	>10,000[1]
DT_{90} (days)	>10,000[1]
Formation fraction	0.5163
Assessment	Potentially acceptable: Visual fit is intermediate with a high χ2 as a consequence of data scatter. Rate parameter is not statistically different to zero. Try to fit FOCUS default DT_{50} with conservative fit of 0.65
Conclusion	SFO is considered potentially acceptable

[1] Interpret with care – extrapolated beyond experimental period.
* FOCUS default

Step 2: Alternative, yet conservative fit.

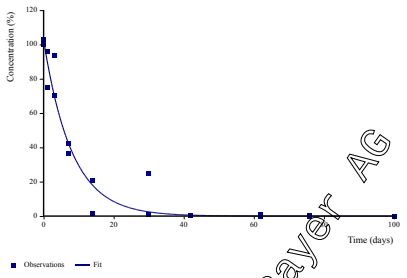
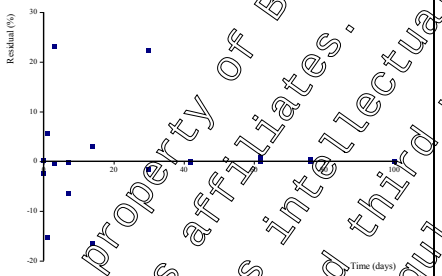
	parent DFOP, metabolite SFO
Plot	
Residuals	
χ^2 err %	20.1
Confidence measure	NA
Visual fit	Visual fit is conservative
DT_{50} (days)	1,000[1]
DT_{50MOD} (days)	1,000
DT_{90} (days)	3,320[1]
Formation fraction	0.65
Assessment	Fit is sufficiently conservative.
Conclusion	SFO using fixed DT_{50} of 1,000 and ff of 0.65 gives an acceptable representation of the data and can be considered a reasonable worst case for modelling estimates.

Summary:
For M06 use DFOP-SFO (FOCUS default). DT_{50MOD} = 1,000 days

Formation fraction from spiroxamine = 0.65

Appendix 5.2.5.3. Dissipation of [cyclohexyl-1-^{14}C]-spiroxamine in Calwich Abbey water phase

Step 1: Initial assessment SFO.

	SFO
Plot	
Residuals	
χ^2 err %	12.9
Confidence measure	*k*: p < 0.05
Visual fit	Visual fit is good with no evidence of systematic errors
DT_{50} (days)	5.61
$DT_{50\ MOD}$ (days)	5.61
DT_{90} (days)	18.6
Assessment	Acceptable: Visual fit is good with no evidence of systematic errors. Rate parameter differs significantly from zero.
Conclusion	SFO is visually and statistically acceptable and should be used for modelling endpoints

Summary:

For Spiroxamine use SFO, DT_{50MOD} = 5.61 days.

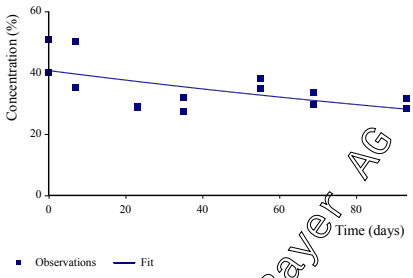
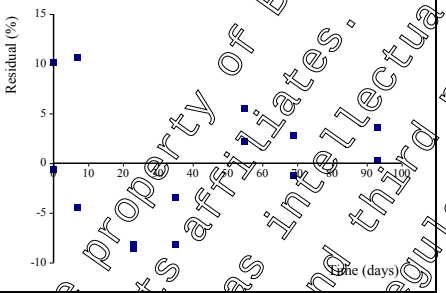
Appendix 5.2.5.4. Dissipation of M06 in Calwich Abbey water phase

No decline phase was observed and as such it is not possible to derive an endpoint for M06.

M06 degradation rate in water conservative default DT_{50MOD} = 1,000 days

Appendix 5.2.5.5. Dissipation of [cyclohexyl-1-^{14}C]-spiroxamine in Calwich Abbey sediment phase

Step 1: SFO [top-down] acceptable fit?

	SFO [top-down]
Plot	
Residuals	
χ^2 err %	10.7
Confidence measure	k: p < 0.05
Visual fit	Visual fit is good with no evidence of systematic error
DT_{50} (days)	175
$DT_{50\ MOD}$ (days)	175[1]
DT_{90} (days)	580[1]
Assessment	Acceptable: Visual fit is good with low χ2 and rate parameter differs significantly from zero
Conclusion	SFO is visually and statistically acceptable and should be used for modelling endpoints

[1] Interpret with care – extrapolated beyond experimental period.

Summary

For Spiroxamine use SFO. DT_{50MOD} = 175 days.

Appendix 5.2.5.6. Dissipation M06 from sediment phase

No decline phase was observed and as such it is not possible to derive an endpoint for M06.

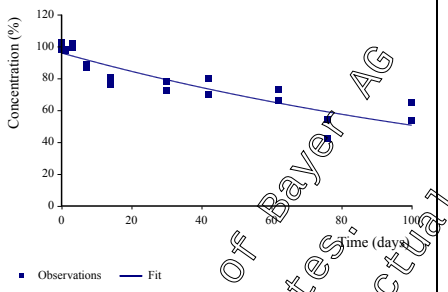
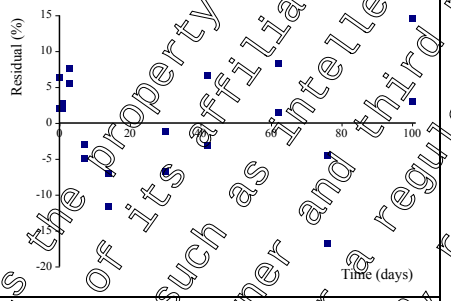
Summary

For M06 use conservative FOCUS default. DT_{50MOD} = 1,000 days.

Appendix 5.2.6. KCA 7.2.2.3/07 (M-763128-01-1) Emperor Lake

Appendix 5.2.6.1. Degradation of [cyclohexyl-1-^{14}C]-spiroxamine in Emperor lake total system (KCA 7.2.2.3/07 (M-763128-01-1))

Step 1: SFO statistically and visually acceptable?

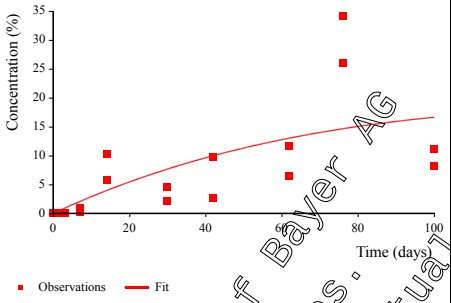
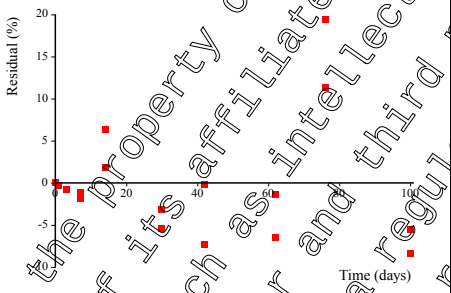
	SFO
Plot	
Residuals	
χ^2 err %	6.39
Confidence measure	k: p < 0.05
Visual fit	Visual fit is good with no evidence of systematic error.
DT_{50} (days)	109[1]
DT_{50MOD} (days)	109[1]
DT_{90} (days)	363[1]
Assessment	Visual fit is good with low χ^2 and no evidence of systematic errors. Rate parameter differs significantly from zero.
Conclusion	SFO is statistically and visually acceptable.

[1] Interpret with care – extrapolated beyond experimental period

Summary
For Spiroxamine use SFO. DT_{50MOD} = 109 days.

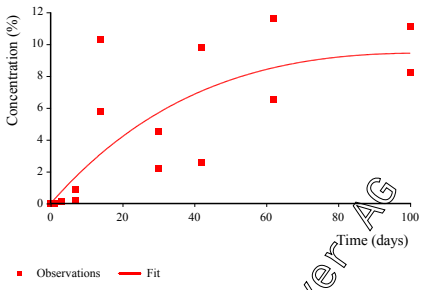
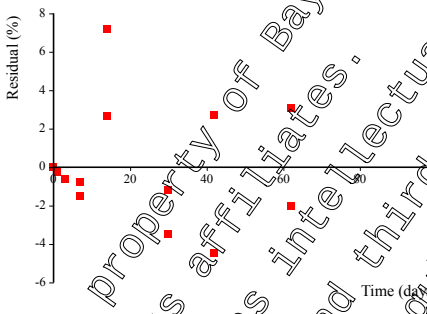
Appendix 5.2.6.2. Degradation of M06 in Emperor Lake total system (KCA 7.2.2.3/07 (M-763128-01-1))

Step 1: Run parent best-fit and metabolite SFO. SFO fit for metabolite acceptable?

	parent SFO, metabolite SFO
Plot	
Residuals	
χ^2 err %	67.4
Confidence measure	k: p< 0.1
Visual fit	Visual fit is intermediate with some evidence of systematic error.
DT_{50} (days)	120[1]
$DT_{50\ MOD}$ (days)	120[1]
DT_{90} (days)	399[1]
Formation Fraction	0.5024
Assessment	Not Acceptable: Visual fit is intermediate with some evidence of systematic error. Try to remove outlier data point
Conclusion	SFO is not appropriate. Try a modified fitting regime with removal of outlier data.

[1] Interpret with care – extrapolated beyond experimental period.

Step 2: Try modified fitting regime (outlier removal)

	parent SFO, metabolite SFO
Plot	
Residuals	
χ^2 err %	34.7
Confidence measure	k: p <0.1
Visual fit	Visual fit is good with no evidence of systematic errors.
DT_{50} (days)	48.4
DT_{90} (days)	161[1]
Formation Fraction	0.4251
$DT_{50\ MOD}$ (days)	48.4
Assessment	Acceptable: Visual fit is good with high $\chi 2$ as a consequence of dataset. Rate parameter differs significantly from zero.
Conclusion	SFO is visually and statistically acceptable and should be used for modelling endpoints.

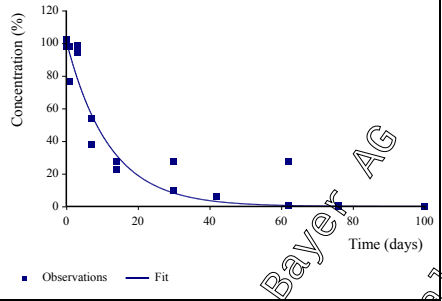
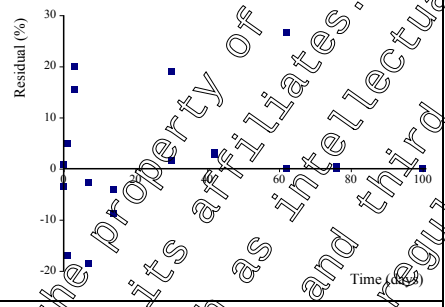
[1] Interpret with care – extrapolated beyond experimental period.

Summary:
For M06 use SFO-SFO: DT_{50MOD} = 48.4 days

Formation fraction from spiroxamine = 0.4251

Appendix 5.2.6.3. Dissipation of [cyclohexyl-1-^{14}C]-spiroxamine in Emperor Lake water phase

Step 1: Initial assessment SFO.

	SFO
Plot	
Residuals	
χ^2 err %	18.3
Confidence measure	k: p < 0.05
Visual fit	Visual fit is good although χ2 high. No evidence of systematic error.
DT_{50} (days)	8.35
$DT_{50\ MOD}$ (days)	8.35
DT_{90} (days)	27.7
Assessment	Acceptable. Visual fit is good although χ2 high. No evidence of systematic error. Rate parameter differs significantly from zero.
Conclusion	SFO is statistically and visually acceptable.

Summary:

For Spiroxamine use SFO. DT_{50MOD} = 8.35 days.

Appendix 5.2.6.4. Dissipation of M06 in Emperor Lake water phase

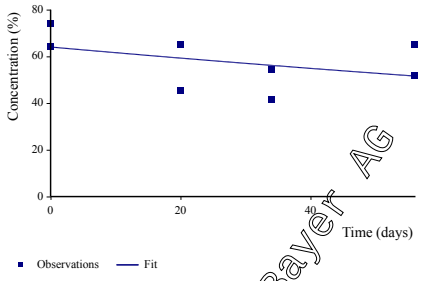
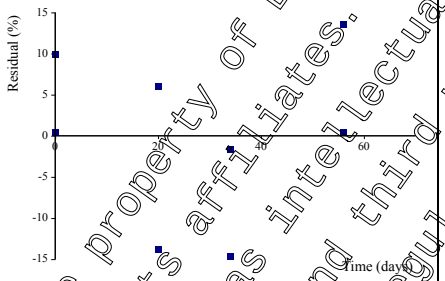
No decline phase was observed and as such it is not possible to derive an endpoint for M06.

Summary:

M06 modelling endpoint conservative default. DT_{50MOD} = 1,000 days

Appendix 5.2.6.5. Dissipation of [cyclohexyl-1-^{14}C]-spiroxamine in Emperor Lake sediment phase

Step 1: SFO [top-down] acceptable fit?

	SFO [top-down]
Plot	
Residuals	
χ^2 err %	8.86
Confidence measure	k: p > 0.1
Visual fit	Visual fit is good, no evidence of systematic errors
DT_{50} (days)	180
$DT_{50\ MOD}$ (days)	1000*
DT_{90} (days)	598[1]
Assessment	Not Acceptable. Visual fit is good with no evidence of systematic errors, but rate parameter does not significantly differ from zero.
Conclusion	SFO not statistically acceptable. Run HS and/or DFOP. Insufficient data points exist for these models. Rate parameter cannot be accurately estimated therefore use FOCUS default as a conservative worst case.

[1] Interpret with care – extrapolated beyond experimental period.
* FOCUS default

Summary
For Spiroxamine use SFO conservative default. DT_{50MOD} = 1,000 days

Appendix 5.2.6.6. Dissipation M06 from sediment

As no decline phase was observed for M06 it is not possible to estimate dissipation rates from the sediment phase.

Summary

For M06 use conservative default DT_{50MOD} = 1,000 days.