Document Title

Summary of the ecotoxicological studies
Spiroxamine EC 500 (500 g/L)

Data Requirement(s)

Regulation (EC) No 1107/2009 & Regulation (EC) No 284/2013

Document MCP

Section 10: Ecotoxicological studies

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

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ERM
On behalf of Bayer AG
Crop Science Division

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Version history

Date [yyyy-mm-dd]	Data points containing amendments or additions[1] and brief description	Document identifier and version number

[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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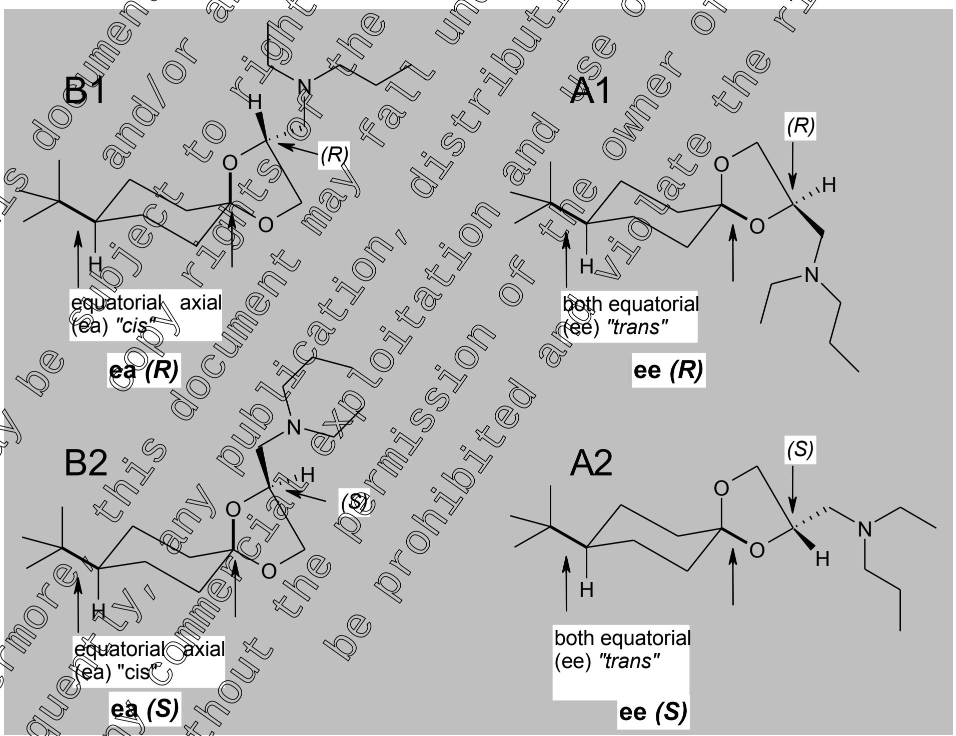
CP 10 ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

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 renewal of Spiroxamine under Council Directive 91/414/EEC and which were therefore not evaluated during the first 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 may 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.

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 A and B nomenclature presented may differ in some historical documentation 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.



CP 10.1 Effects on birds and other terrestrial vertebrates

CP 10.1.1 Effects on birds

The available avian toxicity data for spiroxamine and Spiroxamine EC 500 are summarized in the table below.

Table CP 10.1.1-1 Summary of avian toxicity studies with Spiroxamine and Spiroxamine EC 500

Organism		Test item	Test type	Endpoints		Reference
Bobwhite quail (*Colinus virginianus*)		Spiroxamine	Acute oral toxicity	LD_{50} 565 mg a.s./kg bw	EU	M-008095-02-1
Canary (*Serinus canarius*)		Spiroxamine	Acute oral toxicity	LD_{50} 250 - 500 mg a.s./kg bw	EU	M-008100-01-1
Bobwhite quail (*Colinus virginianus*)		Spiroxamine EC 500	Acute oral toxicity	LD_{50} 971 mg/kg bw (LD_{50} 477 mg a.s./kg bw)	EU	M-008102-01-1
Bobwhite quail (*Colinus virginianus*)		Spiroxamine	Short-term dietary toxicity	LC_{50} >5000 mg a.s./kg diet **(LDD_{50} >357 mg a.s./kg bw/day)**	EU	M-008081-02-1
Mallard duck (*Anas platyrhynchos*)		Spiroxamine	Short-term dietary toxicity	LC_{50} 5000 mg a.s./kg diet (LDD_{50} 871 mg a.s./kg bw/day)	EU	M-008047-02-1
Mallard duck (*Anas platyrhynchos*)		Spiroxamine	Short-term dietary toxicity	LC_{50} >312 mg a.s./kg diet (LDD_{50} >81.4 mg a.s./kg bw/day)	EU	M-008072-01-1
Bobwhite quail (*Colinus virginianus*)		Spiroxamine	Reproductive test	NOEC 29.3 mg a.s./kg diet NOEL 2.02 mg a.s./kg bw/day NOAEC 78.6 mg a.s./kg diet **NOAEL 5.40 mg a.s./kg bw/day**	EU	M-007470-03-1
Mallard duck (*Anas platyrhynchos*)		Spiroxamine	Reproductive test	NOEC 78.8 mg a.s./kg diet NOEL 10.6 mg a.s./kg bw/day	EU	M-008186-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
Values in **bold** have been used in the risk assessment

Toxicity endpoints for risk assessment

For the acute risk assessment the lowest reliable acute LD_{50} value for spiroxamine technical was determined to be 565 mg a.s./kg bw and for Spiroxamine EC 500 it was 477 mg a.s./kg bw. However, the short term dietary toxicity study with the bobwhite quail determined a lower LDD_{50} of >357 mg a.s./kg bw/day. Thus, the acute risk assessment has been conducted using the more conservative value of >357 mg a.s./kg bw/day.

An ecotoxicologically relevant NOAEL of 5.40 mg a.s./kg bw/day has been set and used in the risk assessment. Justification has been provided below.

The NOEC determined in the reproduction study with bobwhite quail (M-007470-03-1) was 29.3 mg a.s./kg diet (equivalent to 2.02 mg a.s./kg bw/day) and has been based on the statistically significant effects on 14-day survivor body weight at 78.6 mg a.s./kg diet. This NOEC is considered to be very conservative because there was only a 3.7% reduction in body weights relative to the control, at 78.6 mg a.s./kg diet. Whilst statistically significant, this reduction is not considered to be a true treatment related effect as the reduction is very minor and unlikely to cause an impact at the population level.

It may be a statistical anomaly or intrinsic variability instead of a substance related effect since over the weeks the body weights of 14 day survivors varied considerably. They were statistically reduced in three of the weeks but in two of the weeks they were reduced without statistical significance. In one of the weeks the body weights were equal to the control, but in three of the weeks they were higher than the control. In the last two weeks for example the mean body weights of 14 day old survivors were 34.3 g and 33.7 g, at 78.8 mg a.s./kg diet, while the control chicks weighed 32.3 g in that period. In contrast to these slight and partially contradictory effects, the results at the next highest test concentration (204 mg a.s./kg diet) were consistent and clear. The reduction of body weight of 14 days survivors compared to the control at this dose group amounted to 8.8 %. This also is not a dramatic decline but the average body weights were reduced over the whole exposure period (6 times statistically significant, 3 times without statistically significance). These findings indicate that at this test concentration (204 mg a.s./kg diet) the effects have to be considered treatment related. It is therefore considered that the true LOEC is 204 mg a.s./kg diet and the NOAEC is 78.6 mg a.s./kg diet (equivalent to 5.40 mg a.s./kg bw/day).

To confirm this conclusion, additional statistical analyses of the reproduction data were conducted and presented in report (M-279402-01-1). As part of the analyses, the data were re-evaluated using the mean body weight of all the 14-day old chicks which were produced by each single pair of adults. When the data were assessed in this way a NOEC of 78.6 mg a.s./kg food (5.40 mg a.s./kg bw/day) was determined. The analyses demonstrated how minor the body weight reductions at the 78.6 mg a.s./kg food dose group were in relation to the controls and that the NOEC could legitimately be increased from 29.3 mg a.s./kg diet (equivalent to 2.02 mg a.s./kg bw/day) to 78.6 mg a.s./kg food (5.40 mg a.s./kg bw/day).

Further supporting data has been provided in report M-304591-01-1 in the form of historical control data for 14-day old survivors to demonstrate that the mean body weight of 32.6 g achieved at the 78.6 mg a.s./kg diet dose group was well within the normal deviation of the historical control data from 59 regulatory studies and is not therefore a biologically relevant reduction.

In conclusion, the differences of the chick body weights between the 78.6 mg a.s./kg food dose group and the control were small (3.7%) and the statistical significance of the difference varies according to the various methods used to analyse the data. Taking all of the above information into account it is considered justified to set an ecotoxicologically relevant NOAEL of 5.40 mg a.s./kg bw/day.

According to the outcome of the pesticides peer review meeting on recurring issues in ecotoxicology (EFSA PPR meeting 133, September 2015), an ecotoxicologically relevant endpoint should be set in collaboration with mammalian toxicologists, where required, and should be used in all the steps of the risk assessment. The NOAEL of 5.40 mg a.s./kg bw/day has therefore been used in all tiers of the avian risk assessment for spiroxamine.

Metabolites

Numerous metabolites of spiroxamine are formed in plants following application to crops. In the Toxicology and Residues sections of the dossier the spiroxamine metabolites have been categorised into three distinct groups (Group A, B and C, respectively). Toxicology dara are available for several of these metabolites. Metabolite M13 has been used to represent all Group B metabolites therefore the toxicology data generated using this metabolite is also considered to cover the plant metabolites M35 and M36. Likewise, M28 has been used to represent all Group C metabolites therefore the toxicology data generated using this metabolite is also considered to cover the plant metabolites M29, M30 and M31. It is also noted that Group A metabolites are considered to be covered by parent spiroxamine.

The table below presents each plant metabolite along with the percentage TRR and actual residue value from the crop metabolism studies. Available toxicology data have also been presented as well as an indication of whether or not each plant metabolite was also found in the animal metabolism studies on laying hen, rat and goat. Finally an assessment is made regarding the relevance of each plant metabolite to the risk assessment. Only metabolites which were formed in plants at ≥10% TRR are considered to be potentially relevant to the bird and mammal risk assessment.

Note that only metabolites which were found in the crop metabolism studies have been presented below, however M13 and M13-acetate have been included in the table below because there are toxicology data which are relevant to other Group B plant metabolites.

Table CP 10.1.1-2 Assessment of potential exposure of birds to metabolites of spiroxamine formed in plants

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine - desethyl (M01) [GROUP A]	**Primary crops** Wheat Forage: 5.1% TRR; 1.11 mg/kg Straw: 2.0% TRR; 0.70 mg/kg Grain: 0.3% TRR; <0.001 mg/kg Grapes 2.1% TRR; 0.27 mg/kg Banana Pulp: 1.1% TRR; 0.005 mg/kg Peel: 2.7% TRR; 0.18 mg/kg **Rotational crops** Leafy vegetables 12.6% TRR; 0.026 mg/kg Cereals 20.0% TRR; 0.119 mg/kg Root & tuber vegetables 9.3% TRR; 0.083 mg/kg	Not found in goat or rat, found in laying hen (21.3% in liver, 9.3% in muscle, 8.4% in fat and 11.5% in eggs)	No data available.	Metabolite found in primary crops at <10% TRR therefore not considered relevant for risk assessment. Metabolite found >10% TRR in rotational crops but actual residue levels were very low. Metabolite found in hen metabolism study therefore toxicity data and associated assessment for parent considered to cover this metabolite.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine - despropyl (M02) [GROUP A]	**Primary crops** Wheat Forage: 4.6% TRR; 0.49 mg/kg Straw: 4.2% TRR; 3.48 mg/kg Grain: 3.0% TRR; 0.002 mg/kg Grapes 1.5% TRR; 0.20 mg/kg Banana Pulp: 0.5% TRR; 0.002 mg/kg Peel: 2.9% TRR; 0.19 mg/kg **Rotational crops** Leafy vegetables 51.2% TRR; 0.053 mg/kg Cereals 46.6% TRR; 0.190 mg/kg Root & tuber vegetables 21.1% TRR; 0.188 mg/kg	Not found in goat or rat. Found in laying hen (21.7% in liver, 11.3% in muscle, 3.4% in fat and 10.2% in eggs)	No data available.	Metabolite found in primary crops at <10% TRR therefore not considered relevant for risk assessment. Metabolite found <10% TRR in rotational crops but actual residue levels were very low. Metabolite found in hen metabolism study therefore toxicity data and associated assessment for parent considered to cover this metabolite.
Spiroxamine - N-oxide (M03) [GROUP A]	**Primary crops** Wheat Forage: 12.7% TRR; 3.06 mg/kg Straw: 22.0% TRR; 7.68 mg/kg Grain: 17.8% TRR; 0.012 mg/kg Grapes 4.7% TRR; 0.61 mg/kg Banana Pulp: 1.2% TRR; 0.007 mg/kg Peel: 4.9% TRR; 0.25 mg/kg **Rotational crops** Cereals 7.4% TRR; 0.235 mg/kg	Not found in goat or laying hen Found in rat (found in liver at low amounts of 0.14%)	Acute oral rat LD_{50} 707 mg/kg bw 28-day rat oral dietary NOAEL 12.9/13.2 mg/kg bw/day for males/females 90-day rat oral dietary NOAEL 8.8/9.7 mg/kg bw/day for males/females	Metabolite found in wheat at >10% TRR therefore considered relevant for risk assessment. Tox data are available and show that metabolite toxicity in the rat is comparable to that of spiroxamine. It is considered that this can also be extrapolated to birds therefore the avian reproductive risk assessment for spiroxamine covers the risk to this metabolite.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine - N-formyl-desethyl (M04) [GROUP A]	**Primary crops** Wheat Forage: 5.8% TRR; 1.40 mg/kg Straw: 9.7% TRR; 8.06 mg/kg Grain: 6.9% TRR; 0.005 mg/kg Grapes Not found Banana Not found **Rotational crops** Cereals 6.4% TRR; 0.204 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in primary crops and rotational crops at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine - hydroxyl (M05) [GROUP A]	**Primary crops** Wheat Forage: 7.1% TRR; 1.71 mg/kg Straw: 5.2% TRR; 4.32 mg/kg Grain: 1.6% TRR; 0.001 mg/kg Grapes 0.3% TRR; 0.04 mg/kg Banana Not found **Rotational crops** Leafy vegetables 17.2% TRR; 0.146 mg/kg Cereals 2.5% TRR; 0.49 mg/kg Root & tuber vegetables 3.6% TRR; 0.032 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops at >10% TRR in leafy vegetables but the actual residues level is very low therefore not considered relevant for risk assessment.
Spiroxamine - hydroxy-despropyl (M09) [GROUP A]	**Primary crops** Wheat Forage: not found Straw: 0.3% TRR; 0.21 mg/kg Grain: not found Grapes Not found Banana Not found	Not found in goat, rat or laying hen	No data available.	Metabolite found in primary crops and rotational crops at <10% TRR therefore not considered relevant for risk assessment.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine – cyclohexanol (M13) [Group B]	**Primary crops** Not found	Not found in goat, rat or laying hen	Acute oral rat LD_{50} 4200 mg/kg bw Acute dermal rabbit LD_{50} >5000 mg/kg bw 28-day rat oral (gavage) NOAEL 50 mg/kg bw/day It was concluded that M13 is less toxic than the parent, spiroxamine in the rat with a *ca.* 9-fold, 2-fold and 8-fold increase in sub-acute, maternal and developmental NOAELs respectively when compared to the spiroxamine equivalent studies	Metabolite not found in crop metabolism studies therefore not considered relevant for risk assessment. Tox data are available and confirm M13 to be less toxic than parent. M13 data used to represent the toxicity of all Group B metabolites.
Spiroxamine cyclohexanol acetate (M-13 acetate) [Group B]	**Primary crops** Not found	Not found in goat, rat or laying hen	Rat developmental NOAEL maternal toxicity 40 mg/kg bw/day Rat developmental NOAEL 160 mg/kg bw/day Group B metabolites considered to be covered by available data for M13 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite not found in crop metabolism studies therefore not considered relevant to the risk assessment. Tox data are available and suggest lower toxicity than parent spiroxamine.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine-diol (M14) [Group B]	**Primary crops** Banana pulp (12.8% TRR; 0.057 mg/kg- hydrolysis product) Grapes (13.0% TRR; 0.44 mg/kg- hydrolysis product) Spring wheat straw (0.2% TRR; 0.070 mg/kg- hydrolysis product) Spring wheat grain (2.6% TRR; 0.002 mg/kg- hydrolysis product) **Rotational crops** Swiss chard leaves (8.8-13.2% TRR; 0.01 mg/kg- hydrolysis product) Wheat straw (3.5-4% TRR; 0.05 mg/kg- hydrolysis product) Turnip tops (4.4-13.0% TRR; 0.02 mg/kg- hydrolysis product)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at >10% TRR but the actual residues level is very low therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine-ketone (M15) [Group B]	**Primary crops** Grapes (1.3% TRR- hydrolysis product) Spring wheat straw (5.5% TRR- hydrolysis product) Spring wheat grain (4.6% TRR- hydrolysis product)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine-hydroxy-ketone (M16) [Group B]	**Primary crops** 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; 0.04 mg/kg- hydrolysis product) Wheat straw (8.9-11.6% TRR; 0.15 mg/kg- hydrolysis product) Turnip tops (11.7-37.3% TRR; 0.17 mg/kg- hydrolysis product)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at >10% TRR but the actual residues level is very low therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine - hydroxy-N-oxide glucoside (M20) [Group A]	**Primary crops** Wheat Forage: 0.7% TRR; 0.08 mg/kg Straw: 2.0% TRR; 0.70 mg/kg Grain: not found Grapes Not found Banana Not found **Rotational crops** Swiss chard leaves (2.5% TRR; <0.01 mg/kg)) Wheat straw (2.1-2.6% TRR; 0.03 mg/kg) Turnip tops (8.4-10.4% TRR; 0.04 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in primary crops at <10% TRR with the exception of turnip tops but the actual residues level is very low. Therefore this metabolite is not considered relevant for risk assessment.
Spiroxamine - hydroxy-N-oxide malonyl glucoside (M21) [Group A]	**Primary crops** Wheat Forage: 2.0% TRR; 0.21 mg/kg Straw: 3.1% TRR; 2.57 mg/kg Grain: not found Grapes Not found Banana Not found **Rotational crops** Swiss chard leaves (1.6% TRR; <0.01 mg/kg) Wheat straw (4.4% TRR; 0.06 mg/kg) Turnip tops (1.7-3.7% TRR; <0.01 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at <10% TRR therefore not considered relevant for risk assessment.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine-diol-diglycoside (M24) [Group B]	**Primary crops** Grapes (14.8% TRR – main component of metabolite group 12; 0.50 mg/kg) **Rotational crops** Swiss chard leaves (3.0% TRR; <0.01 mg/kg) Wheat straw (1.9-2.2% TRR; 0.020 mg/kg- Turnip roots (7.8% TRR; <0.01 mg/kg) Turnip tops (2.0-4.3% TRR; <0.01 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in grapes at >10% TRR but the actual residues level is very low, therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine - aminodiol (M28) [GROUP C]	**Primary crops** Wheat Not found Grapes 37.5% TRR; 4.91 mg/kg Banana Pulp: 31.2% TRR; 0.173 mg/kg Peel: 37.2% TRR; 2.45 mg/kg **Rotational crops** Leafy vegetables 3.9% TRR; 0.014 mg/kg Cereals 0.6% TRR; 0.024 mg/kg Root & tuber vegetables 4.9% TRR; 0.005 mg/kg	Found in rat at 2.2 – 5.7% of dose	Acute oral rat LD_{50} >550 <2000 mg/kg bw 28-day rat oral dietary NOAEL 28.4/31.4 mg/kg bw/day for males/females Developmental rat oral (gavage) NOAEL maternal toxicity 150 mg/kg bw/day and developmental NOAEL 30 mg/kg bw/day It was concluded that M28 is less toxic than the parent, spiroxamine in the rat with a *ca.* 15-fold, 9-fold and 2-fold increase in sub-acute, maternal and developmental NOAELs, respectively when compared to the spiroxamine equivalent studies.	Metabolite found in grapes and banana at >10% TRR therefore relevant for the risk assessment. Tox data are available and confirm that toxicity is less than parent. It is considered that this can also be extrapolated to birds therefore the avian reproductive risk assessment for spiroxamine covers the risk to this metabolite. M28 data used to represent the toxicity of all Group C metabolites.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine - aminodiol-N-oxide (M29) [GROUP C]	**Primary crops** Wheat Not found Grapes 0.1% TRR; 0.01 mg/kg Banana Not found **Rotational crops** Leafy vegetables 5.2% TRR; 0.021 mg/kg Root & tuber vegetables 4.8% TRR; 0.005 mg/kg	Not found in goat, rat or laying hen	No data available. Group C metabolites considered to be covered by available data for M28 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in primary crops and rotational crops at <10% TRR therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M28 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine - desethyl-aminodiol (M30) [GROUP C]	**Primary crops** Wheat Not found Grapes 1.1% TRR; 0.14 mg/kg Banana Pulp: 0.6% TRR; 0.003 mg/kg Peel: 0.9% TRR; 0.06 mg/kg	Not found in goat, rat or laying hen	No data available. Group C metabolites considered to be covered by available data for M28 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in primary crops at <10% TRR therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M28 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine - despropyl-aminodiol (M31) [GROUP C]	**Primary crops** Wheat Not found Grapes 1.2% TRR; 0.16 mg/kg Banana Pulp: 0.6% TRR; 0.003 mg/kg Peel: 0.9% TRR; 0.06 mg/kg **Rotational crops** Cereals 1.7% TRR; 0.034 mg/kg Root & tuber vegetables 6.1% TRR; 0.006 mg/kg	Not found in goat, rat or laying hen	No data available. Group C metabolites considered to be covered by available data for M28 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in primary crops and rotational crops at <10% TRR therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M28 data confirm this group of metabolites to be less toxic than parent.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine-cyclohexanol-glucopyranosyl-pentose (M33) [GROUP B]	**Primary crops** Grapes (19.1% TRR; 0.650 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at >10% TRR but the actual residues level is very low therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine-cyclohexanol-glucopyranosyl-glucopyranosyl-pentose (M34) [GROUP B]	**Primary crops** Grapes (3.5% TRR; 0.130 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine docosanoic acid ester (M35) [GROUP B]	**Primary crops** <u>Wheat</u> Not found <u>Grapes</u> 13.0% TRR; 0.44 mg/kg <u>Banana</u> Not found	Not found in goat, rat or laying hen	No data on M35. Group B metabolites considered to be covered by available data for M13 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in grapes at >10% TRR. Available data with M13 used to cover this Group B metabolite and confirms that the toxicity is lower than that of the parent. Thus, the risk to M35 is considered to be covered by the assessment for parent.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine tetracosanoic acid ester (M36) [GROUP B]	**Primary crops** Wheat Not found Grapes 4.2% TRR; 0.14 mg/kg Banana Not found	Not found in goat, rat or laying hen	No data on M36. Group B metabolites considered to be covered by available data for M13 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in primary crops at <10% TRR therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine-cyclohexenol (M37) [GROUP B]	**Primary crops** Grapes (3.2% TRR; 0.11 mg/kg-hydrolysis product)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine – N-formyl-despropyl (M38) [GROUP A]	**Primary crops** Not found **Rotational crops** Cereals 7.6% TRR; 0.243 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops only and at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine – hydroxy-despropyl glycoside (M39) [GROUP A]	**Rotational crops** Leafy vegetables 2.8% TRR; 0.019 mg/kg Cereals 5.9% TRR; 0.232 mg/kg Root & tuber vegetables 21.3% TRR; 0.063 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops at >10% TRR in root and tuber vegetables but the actual residues level is very low therefore not considered relevant for risk assessment.
Spiroxamine – hydroxy glycoside (M40) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 4.7% TRR; 0.040 mg/kg Cereals 2.9% TRR; 0.088 mg/kg Root & tuber vegetables 7.6% TRR; 0.068 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops only and at <10% TRR therefore not considered relevant for risk assessment.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for avian risk assessment
Spiroxamine – hydroxy-desethyl glycoside (M42) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 1.6% TRR; 0.005 mg/kg Cereals 6.5% TRR; 0.129 mg/kg Root & tuber vegetables 14.6% TRR; 0.044 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops at >10% TRR in root and tuber vegetables but the actual residues level is very low therefore not considered relevant for risk assessment.
Spiroxamine – desethyl acid glycoside (M43) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 1.8% TRR; 0.015 mg/kg Cereals 3.4% TRR; 0.088 mg/kg Root & tuber vegetables 5.7% TRR; 0.051 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops only and at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine – acid glycoside (M44) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 4.6% TRR; 0.019 mg/kg Cereals 6.4% TRR; 0.126 mg/kg Root & tuber vegetables 11.6% TRR; 0.027 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops at >10% TRR in root and tuber vegetables but the actual residues level is very low therefore not considered relevant for risk assessment.
Spiroxamine – despropyl acid glycoside (M45) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 5.5% TRR; 0.019 mg/kg Cereals 3.7% TRR; 0.145 mg/kg Root & tuber vegetables 9.1% TRR; 0.002 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops only and at <10% TRR therefore not considered relevant for risk assessment.

M01 and M02 were found in the rotational crop studies at >10% TRR (although at low absolute amounts) but these metabolites were also found in the hen metabolism study therefore the risk assessment of parent spiroxamine is considered to cover the risk to these metabolites.

M03 was found in wheat at >10% TRR. Toxicology data for M03 demonstrate that this metabolite is of similar or lower toxicity than spiroxamine. It is considered reasonable to assume that this would also be the case for birds therefore, assuming extrapolation from mammals to birds, the risk assessment of parent spiroxamine is considered to cover the risk to this metabolite.

M28 was found in grapes and banana at >10% TRR. Toxicology data are available for M28 and confirm that this metabolite is 15-fold, 9-fold and 2-fold less toxic in sub-acute, maternal and developmental parameters than spiroxamine. It is considered reasonable to assume that this would also be the case for birds therefore, assuming extrapolation from mammals to birds, it is considered that this metabolite will be less toxic to birds than spiroxamine therefore the risk from exposure to this metabolite is considered to be covered by the assessment for parent.

M35 was also found in grapes at >10%TRR. The toxicology data generated for M28 are considered to also cover this metabolite and confirm that the metabolite is less toxic than parent. Thus, the risk from exposure to this plant metabolite is considered to be covered by the assessment for parent.

M04, M05, M09, M14, M15, M16, M20, M21, M24, M29, M30, M31, M33, M34, M36, M37, M38, M39, M40, M42, M43, M44 and M45 were found in the crop metabolism studies at either <10% TRR or very low absolute amounts and were therefore not considered to be relevant for risk assessment.

Specific dietary risk assessment for these plant metabolites of spiroxamine is therefore not considered to be necessary.

Dietary risk assessment for birds

Exposure

In order to present risk assessments which fully cover the range of options available in the GAP, the following six exposure regimes have been considered here with dietary risk assessments presented for each regime:

- 1 x 200 g a.s./ha BBCH 13 - 19
- 1 x 300 g a.s./ha BBCH 13 - 19
- 1 x 300 g a.s./ha BBCH 53 - 85
- 2 x 300 g a.s./ha BBCH 53 - 85 (10-day interval)
- 1 x 200 g a.s./ha BBCH 13 - 19 and 1 x 300 g a.s./ha BBCH 20 (53) - 85 (10-day interval)
- 1 x 300 g a.s./ha BBCH 13 - 19 and 1 x 300 g a.s./ha BBCH 20 (53) - 85 (10-day interval)

Isomers

The risk assessments for birds & mammals involves potential chronic exposure of these organisms to residues in plants therefore it may be necessary to apply an uncertainty factor (UF) to the chronic avian and mammalian risk assessments. The acute risk assessment need not have an UF applied as exposure in this scenario is immediate. However, chronic risk assessment considers exposure over a prolonged period therefore potential changes in isomeric ratio needs to be considered. For the bird and mammal risk assessments the same approach taken in the residues section for the consumer risk assessment, with respect to isomers, has been followed. Based on the current residues data set for spiroxamine, there are no indications of a significant change in isomer ratios therefore no additional factor need be applied to the risk assessments below (*i.e.* an UF of 1.0 has been used).

Risk assessment

The risk assessments have been conducted in accordance with the EFSA (2009) Bird & Mammal Guidance document. Each assessment starts with a screening step followed by a Tier I assessment if required. Finally, refined risk assessments have been presented where required.

The acute 'daily dietary dose' (DDD) is calculated by multiplying the shortcut value (SV) based on the 90th percentile residues by the application rate in kg a.s/ha.

DDD_A = application rate (kg a.s./ha) x SV_{90}

The long-term 'daily dietary dose' (DDD) is calculated by multiplying the SV based on the mean residues by the application rate in kg a.s./ha and (only for the long-term risk assessment) a time weighted average residue exposure (f_{twa}). The f_{twa} based upon a default DT_{50} of 10 days is 0.53, as given in EFSA guidance (2009).

DDD_{LT} = application rate (kg a.s./ha) x SV_m x f_{twa}

Acute risk is assessed by comparing the relevant daily dietary dose (DDD) values with the appropriate LD_{50} endpoint to give an acute Toxicity: Exposure Ratio (TER_A):

$$TER_A = \frac{LD_{50}\ (\text{mg/kg bw})}{\text{DDD}}$$

TER_A values which exceed a trigger value of 10 indicate an acceptable acute risk.

Derivation of the short-term toxicity exposure ratio is no longer a requirement according to the EFSA Guidance Document (2009) so a short-term risk assessment has not been presented. However, the endpoint from the short-term dietary study with the bobwhaite quail has been used in the acute risk assessment.

Long-term risk is assessed by comparing the long-term DDD values with the worst case NOAEL from the reproduction studies, expressed as daily dietary dose to give a long-term toxicity exposure ratio (TER_{LT}):

$$TER_{LT} = \frac{NOAEL\ (\text{mg/kg bw/day})}{\text{DDD}\ (\text{mg/kg bw/day})}$$

TER_{LT} values which exceed a trigger value of 5 indicate acceptable chronic risk.

1 x 200 g a.s./ha BBCH 13 - 19

The screening step assessments for the acute and reproductive risks are presented below.

Table CP 10.1.1-3 Screening step assessment for acute and long-term/reproductive risk to birds for the proposed use of Spiroxamine EC 500 in grapes (BBCH 13 - 19)

Intended use	Grapes (BBCH 13 - 19)				
Active substance/product	Spiroxamine / Spiroxamine EC 500				
Application rate (g a.s./ha)	1 x 200				
Acute toxicity (mg a.s./kg bw)	>357				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Vineyard	Small omnivorous bird	95.3	1.0	19.1	>18.7
Reprod. toxicity (mg a.s./kg bw/d)	5.40				
TER criterion	5				
Crop scenario	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard	Small omnivorous bird	38.9	1.0 x 0.53	4.12	**1.31**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger

For the proposed use of Spiroxamine EC 500 on grapes (1 x 200 g a.s./ha at BBCH 13 - 19) the acute risk to birds from dietary exposure to spiroxamine are considered to be acceptable (TER ≥10) but potential reproductive risks have been identified (TER <5). A Tier I reproductive risk assessment has therefore been conducted and presented below.

Table CP 10.1.1-4 Tier I assessment for reproductive risk to birds for the proposed use of Spiroxamine EC 500 in grapes (BBCH 13 - 19)

Intended use	Grapes (BBCH 13 - 19)
Active substance/product	Spiroxamine / Spiroxamine EC 500
Application rate (g a.s./ha)	1 × 200
Reprod. toxicity (mg a.s./kg bw/d)	5.40
TER criterion	5

Crop scenario Growth stage	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard BBCH 10-19	Small insectivorous species "redstart"	11.5	1.0 x 0.53	4.22	**4.43**
Vineyard BBCH 10-19	Small granivorous bird "finch"	6.9	1.0 x 0.53	0.731	7.38
Vineyard BBCH 10-19	Small omnivorous bird "lark"	6.5	1.0 x 0.53	0.689	7.84

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

For the proposed use of Spiroxamine EC 500 on grapes (1 x 200 g a.s./ha at BBCH 13 - 19) the reproductive risks to birds from dietary exposure to spiroxamine are considered to be acceptable (TER ≥5) for the small granivorous bird "finch" and small omnivorous bird "lark" scenarios but potential reproductive risks have been identified for the small insectivorous species "redstart" scenario. A refined risk assessment for this individual scenario has been presented below.

Refinement

Small insectivorous species "redstart" scenario

For the refined risk assessment for the small insectivorous species "redstart" scenario, the black redstart has been used as the focal species. This is the default species in EFSA (2009) but is supported by the available focal species study M-242340-01-1. In this study four vineyards in Germany were observed between early April and early July for the bird species that were present on the sites. The black redstart was found to be present in three of the four sites and was found to be breeding in two of the sites. In study M-291192-01-1 bird species in vineyards in France and Italy were observed in order to determine focal species. The black redstart was found to be present in the vineyards in France. Thus, the black redstart is considered to be a typical insectivorous bird that may be found in vineyards in both Central and Southern Europe.

The Tier I risk assessment has assumed that birds obtain all of their food from within the treated area *(i.e.* a PT of 1.0), but in reality birds may also obtain food from outside of this area. In study M-427241-01-1 the use of vineyards by the black redstart in Southern France was investigated using observation and radio-tracking. Mean PT values of 28.5% (0.285) were determined for the black redstart with a 90th percentile value of 75% (0.75). A refined 90th percentile PT of 0.75 for black redstarts in vineyards has therefore been applied to the refined risk assessment.

The default diet in EFSA (2009) for this scenario is 50% ground invertebrates and 50% foliar invertebrates. In study M-487359-01-1 the foraging behavior of the black redstart was investigated in German vineyards and foraging events were observed, distinguishing between foraging on the ground, in vine rows and in the air. A total of 96.45% of all documented foraging events were located on the ground in vineyards, 0.75% on the vine plant and 2.75% were in-flight catches. These observations have been taken to provide a diet that consists of 96.45% ground invertebrates and 3.55% foliar invertebrates.

This diet has therefore been used in the refined risk assessment as a more realistic diet for the focal species, black redstart.

The refined risk assessment for the small insectivorous bird scenario in vineyards at BBCH 10 - 19 following application of Spiroxamine EC 500 at 1 x 200 g a.s./ha at BBCH 13 - 19 is presented below using the refinement parameters outlined above.

Table CP 10.1.1-5 Refinement of the small insectivorous bird scenario (BBCH 10-19) for the proposed use of Spiroxamine EC 500 in grapes (BBCH 13 - 19) – black redstart

App rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT [d]	Dep. factor [e]	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
1 x 0.200 kg a.s./ha	Invertebrates (ground dwelling)	0.778	7.5	1.0	0.53	1.75	1.0	0.464	0.512	10.5
	Invertebrates (foliar dwelling)	0.0286	21.0	1.0	0.53		1.0	0.0477		

[a] Values calculated using focal species dietary data. The EFSA bodyweight of 10.5 g used in the calculations
[b] Default RUD values from EFSA (2009) Appendix F
[c] Default values from EFSA (2009)
[d] Refined 90th percentile PT value from focal species study
[e] Deposition from Appendix A (EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Using the refined parameters discussed above the reproductive risks to insectivorous birds following the proposed use of Spiroxamine EC 500 on grapes at 1 x 200 g a.s./ha at BBCH 13 - 19 are considered to be acceptable (TER ≥ 5).

1 x 300 g a.s./ha BBCH 13 - 19

The screening step assessments for the acute and reproductive risks are presented below.

Table CP 10.1.1-6 Screening step assessment for acute and long-term/reproductive risk to birds for the proposed use of Spiroxamine EC 500 in grapes (BBCH 13 - 19)

Intended use	Grapes (BBCH 13 - 19)				
Active substance/product	Spiroxamine / Spiroxamine EC 500				
Application rate (g a.s./ha)	1 × 300				
Acute toxicity (mg a.s./kg bw)	>357				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Vineyard	Small omnivorous bird	95.3	1.0	28.6	**3.12**
Reprod. toxicity (mg a.s./kg bw/d)	5.40				
TER criterion	5				
Crop scenario	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard	Small omnivorous bird	38.9	1.0 x 0.53	6.19	**0.873**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger

For the proposed use of Spiroxamine EC 500 on grapes (1 x 300 g a.s./ha at BBCH 13 - 19) the acute risk to birds from dietary exposure to spiroxamine are considered to be acceptable (TER ≥10) but potential reproductive risks have been identified (TER <5). A Tier I reproductive risk assessment has therefore been conducted and presented below.

Table CP 10.1.1-7 Tier I assessment for reproductive risk to birds for the proposed use of Spiroxamine EC 500 in grapes (BBCH 13 - 19)

Intended use	Grapes (BBCH 13 - 19)				
Active substance/product	Spiroxamine / Spiroxamine EC 500				
Application rate (g a.s./ha)	1 × 300				
Reprod. toxicity (mg a.s./kg bw/d)	5.40				
TER criterion	5				
Crop scenario Growth stage	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard BBCH 10-19	Small insectivorous species “redstart”	11.5	1.0 x 0.53	1.83	**2.95**
Vineyard BBCH 10-19	Small granivorous bird “finch”	6.9	1.0 x 0.53	1.10	**4.92**
Vineyard BBCH 10-19	Small omnivorous bird “lark”	6.5	1.0 x 0.53	1.03	5.22

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger

For the proposed use of Spiroxamine EC 500 on grapes (1 x 300 g a.s./ha at BBCH 13 - 19) the reproductive risk to birds from dietary exposure to spiroxamine are considered to be acceptable (TER

≥5) for the small omnivorous bird "lark" scenario but potential reproductive risks have been identified for the small insectivorous species "redstart" and the small granivorous bird "finch" scenarios. A refined risk assessment for these individual scenarios have been presented below.

Refinement

Small insectivorous species "redstart" scenario

For the refined risk assessment for the small insectivorous species "redstart" scenario, the black redstart has been used as the focal species. As discussed in the previous sub-section, a 90th percentile PT for the black redstart of 0.75 and a diet consisting of 96.45% ground invertebrates and 3.55% foliar invertebrates has been used in the refined risk assessment.

The refined risk assessment for the small insectivorous bird scenario in vineyards at BBCH 10 - 19 following application of Spiroxamine EC 500 at 1 x 300 g a.s./ha at BBCH 13 - 19 is presented below.

Table CP 10.1.1-8 Refinement of the small insectivorous bird scenario (BBCH 10-19) for the proposed use of Spiroxamine EC 500 in grapes (BBCH 13 - 19) – black redstart

App rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT [d]	Dep. factor [e]	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
1 x 0.300 kg a.s./ha	Invertebrates (ground dwelling)	0.778	7.5	1.0	0.53	0.75	1.0	0.686	0.768	7.03
	Invertebrates (foliar dwelling)	0.0286	21.0	1.0	0.53		1.0	0.0716		

[a] Values calculated using focal species dietary data. The EFSA bodyweight of 16.5 g used in the calculations
[b] Default RUD values from EFSA (2009) Appendix F
[c] Default values from EFSA (2009)
[d] Refined 90th percentile PT value from focal species study
[e] Deposition from Appendix A (EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Using the refined parameters discussed above the reproductive risks to insectivorous birds following the proposed use of Spiroxamine EC 500 on grapes at 1 x 300 g a.s./ha at BBCH 13 - 19 are considered to be acceptable (TER ≥5).

Small granivorous bird "finch" scenario

For the refined risk assessment for the small granivorous bird "finch" scenario, the linnet has been used as the focal species. In study M-291192-01-1 a field survey program was carried out to identify and quantify bird species found in vineyards in France and Italy during different grapevine growth stages. The linnet was found to be the most characteristic species present in vineyards in France and this species also featured at significant levels in vineyards in Southern Italy. As such the linnet was considered to be a suitable granivorous focal species for vineyards in Southern Europe.

This focal species is further supported by study M-291784-01-1 in which bird trapping, radio-tracking and visual observations of four bird species, including the linnet, took pace in a typical wine growing region in France. For linnets the mean PT value was determined to be 0.78 with a 90th percentile value of 0.97, thereby confirming the linnet to be a suitable focal species for vineyards. This study also analysed faeces and/or stomach contents in order to determine typical diets of these species. For the linnet the diet was overwhelmingly comprised of seeds, as expected for a granivorous species. Study M-516702-01-1 also supports this by demonstrating that the linnet obtains its diet predominantly from the ground vegetation. EFSA (2009) assumes a diet of 100% weed seeds for this scenario but for the refined risk assessment a diet of 97.3% weed seeds and 2.7% ground invertebrates has been considered. A 90th percentile PT of 0.97 has also been considered.

The refined risk assessment for the small granivorous bird scenario in vineyards at BBCH 10 - 19 following application of Spiroxamine EC 500 at 1 x 300 g a.s./ha at BBCH 13 - 19 is presented below.

Table CP 10.1.1-9 Refinement of the small granivorous bird scenario for the proposed use of Spiroxamine EC 500 in grapes (BBCH 13 - 19) - Linnet

Application rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT [d]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
1 x 0.300 kg a.s./ha	Weed seeds	0.282	40.2	1.0	0.53	0.97	0.67	1.05	1.06	5.09
	Invertebrates (ground dwelling)	0.00782	7.5	1.0	0.53		1.0	0.00905		

[a] Values calculated using focal species dietary data. The EFSA bodyweight of 15.3 g used in the calculations
[b] Default RUD values from EFSA (2009) Appendix I
[c] Default values from EFSA (2009)
[d] Refined 90th percentile PT value from focal species study
[e] Deposition value based on 40% crop interception (Appendix A; EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Using the refined parameters discussed above the reproductive risks to granivorous birds following the proposed use of Spiroxamine EC 500 on grapes at 1 x 300 g a.s./ha at BBCH 13 - 19 are considered to be acceptable (TER ≥5).

1 x 300 g a.s./ha BBCH 53 - 85

The screening step assessments for the acute and reproductive risks are presented below.

Table CP 10.1.1-10 Screening step assessment for acute and long-term/reproductive risk to birds for the proposed use of Spiroxamine EC 500 in grapes (BBCH 53 - 85)

Intended use	Grapes (BBCH 53 - 85)				
Active substance/product	Spiroxamine / Spiroxamine EC 500				
Application rate (g a.s./ha)	1 × 300				
Acute toxicity (mg a.s./kg bw)	>357				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Vineyard	Small omnivorous bird	95.3	1.0	28.6	>12.5
Reprod. toxicity (mg a.s./kg bw/d)	5.40				
TER criterion	5				
Crop scenario	Indicator/generic focal species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard	Small omnivorous bird	38.9	1.0 x 0.53	6.19	**0.873**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger

For the proposed use of Spiroxamine EC 500 on grapes (1 x 300 g a.s./ha at BBCH 53 - 85) the acute risk to birds from dietary exposure to spiroxamine are considered to be acceptable (TER ≥10) but

potential reproductive risks have been identified (TER <5). A Tier I reproductive risk assessment has therefore been conducted and presented below.

Table CP 10.1.1-11 Tier I assessment for reproductive risk to birds for the proposed use of Spiroxamine EC 500 in grapes (BBCH 53 - 85)

Intended use	Grapes (BBCH 53 - 85)				
Active substance/product	Spiroxamine / Spiroxamine EC 500				
Application rate (g a.s./ha)	1 × 300				
Reprod. toxicity (mg a.s./kg bw/d)	5.40				
TER criterion	5				
Crop scenario Growth stage	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard BBCH >20	Small insectivorous species “redstart”	9.9	1.0 x 0.53	1.57	**3.43**
Vineyard BBCH >40	Small granivorous bird “finch”	3.4	1.0 x 0.53	0.541	9.99
Vineyard BBCH >40	Small omnivorous bird “lark”	3.3	1.0 x 0.53	0.525	10.3
Vineyard Ripening	Frugivorous bird “Thrush/starling”	14.4	1.0 x 0.53	2.29	**2.36**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio; TER values shown in bold fall below the relevant trigger

For the proposed use of Spiroxamine EC 500 on grapes (1 x 300 g a.s./ha at BBCH 53 - 85) the reproductive risk to birds from dietary exposure to spiroxamine are considered to be acceptable (TER ≥5) for the small granivorous bird “finch” and small omnivorous bird “lark” scenarios but potential reproductive risks have been identified for the small insectivorous species “redstart” and the frugivorous bird “thrush/starling” scenarios. A refined risk assessment for these individual scenarios have been presented below.

Refinement

Small insectivorous species “redstart” scenario

For the refined risk assessment for the small insectivorous species “redstart” scenario, the black redstart has been used as the focal species. As discussed above, a 90th percentile PT for the black redstart of 0.75 and a diet consisting of 96.45% ground invertebrates and 3.55% foliar invertebrates has been used in the refined risk assessment.

The refined risk assessment for the small insectivorous bird scenario in vineyards at BBCH >20 following application of Spiroxamine EC 500 at 1 x 300 g a.s./ha at BBCH 53 - 85 is presented below.

Table CP 10.1.1-12 Refinement of the small insectivorous bird scenario (BBCH >20) for the proposed use of Spiroxamine EC 500 in grapes (BBCH 53 - 85) – black redstart

App rate	Food type	FIR/bw a)	RUD b)	MAF c)	f_{twa} c)	PT d)	Dep. factor e)	DDD [mg a.s./kg b.w./d]	Total DDD f)	TER g)
1 x 0.300 kg a.s./ha	Invertebrates (ground dwelling)	0.778	3.5	1.0	0.53	0.75	1.0	0.325	0.397	13.6

	Invertebrates (foliar dwelling)	0.0286	21.0	1.0	0.53		1.0	0.0716		

a) Values calculated using focal species dietary data. The EFSA bodyweight of 16.5 g used in the calculations
b) Default RUD values from EFSA (2009) Appendix F
c) Default values from EFSA (2009)
d) Refined 90th percentile PT value from focal species study
e) Deposition from Appendix A (EFSA, 2009)
f) Sum of DDD values for individual diet components
g) TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Using the refined parameters discussed above the reproductive risks to insectivorous birds following the proposed use of Spiroxamine EC 500 on grapes at 1 x 300 g a.s./ha at BBCH 53-85 are considered to be acceptable (TER ≥5).

Frugivorous bird scenario

For refinement of the frugivorous bird scenario reference is made to report M-414948-01-1. Data are available for a total of 54 studies in which initial residues of spiroxamine parent compound on grapes were either measured directly or were recalculated from total residue values determined immediately after the last application. The RUD derived for the total set of studies (n=54) is in line with the RUD derived only for those studies in which initial residues of spiroxamine parent itself on grapes were measured (n=24). Therefore, taking into account a dataset of 54 residue studies for spiroxamine in total, it is proposed to replace the generic RUD of 8.51 by a more realistic RUD of 1.63 that can be used in the refined risk assessment for Spiroxamine EC 500. Here the refined frugivorous bird scenario is applicable to the frugivorous bird "song thrush" (*Turdus philomelos)* which according to EFSA (2009) presents a FIR/bw value of 1.73.

Table CP 10.1.1-13 Refinement of the frugivorous bird scenario for the proposed use of Spiroxamine EC 500 in grapes (BBCH 53 - 85)

Application rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT [c]	Dep. factor [c]	DDD [mg a.s./kg b.w./d]	TER [d]
1 x 0.300 kg a.s./ha	Grapes	1.73	1.63	1.0	0.53	1.0	1.0	0.448	12.1

a) Value taken from Appendix A (EFSA, 2009)
b) Measured mean RUD value from 54 residues trials using spiroxamine on grapes
c) Default values from EFSA (2009)
d) TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Using the refined RUD value discussed above the reproductive risks to frugivorous birds following the proposed use of Spiroxamine EC 500 on grapes at 1 x 300 g a.s./ha at BBCH 53 - 85 are considered to be acceptable (TER ≥5).

2 x 300 g a.s./ha BBCH 53 - 85

The screening step assessments for the acute and reproductive risks are presented below.

Table CP 10.1.1-14 Screening step assessment for acute and long-term/reproductive risk to birds for the proposed use of Spiroxamine EC 500 in grapes (BBCH 53 - 85)

Intended use		Grapes (BBCH 53 - 85)			
Active substance/product		Spiroxamine / Spiroxamine EC 500			
Application rate (g a.s./ha)		2 × 300			
Acute toxicity (mg a.s./kg bw)		>357			
TER criterion		10			
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Vineyard	Small omnivorous bird	95.3	1.3	37.2	**>9.61**
Reprod. toxicity (mg a.s./kg bw/d)		5.40			
TER criterion		5			
Crop scenario	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard	Small omnivorous bird	38.9	1.5 x 0.53	9.28	**0.582**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger

For the proposed use of Spiroxamine EC 500 on grapes (2 x 300 g a.s./ha at BBCH 53 - 85) potential acute and reproductive risks have been identified. Tier I acute and reproductive risk assessments have therefore been conducted and presented below.

Table CP 10.1.1-15 Tier I assessment for acute risk to birds for the proposed use of Spiroxamine EC 500 in grapes (BBCH 53 - 85)

Intended use		Grapes (BBCH 53 - 85)			
Active substance/product		Spiroxamine / Spiroxamine EC 500			
Application rate (g a.s./ha)		2 × 300			
Acute toxicity (mg a.s./kg bw)		>357			
TER criterion		10			
Crop scenario Growth stage	Generic focal species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Vineyard BBCH >20	Small insectivorous species "redstart"	25.7	1.3	10.0	35.6
Vineyard BBCH >40	Small granivorous bird "finch"	7.4	1.3	2.89	124
Vineyard BBCH >40	Small omnivorous bird "lark"	7.2	1.3	2.81	127
Vineyard Ripening	Frugivorous bird "Thrush/starling"	28.9	1.3	11.3	31.7

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio

For the proposed use of Spiroxamine EC 500 on grapes (2 x 300 g a.s./ha at BBCH 53 - 85) the acute risks to birds from dietary exposure to spiroxamine are considered to be acceptable (TER ≥10) for all relevant scenarios. No further acute risk assessment is necessary for this use of Spiroxamine EC 500.

Table CP 10.1.1-16 Tier I assessment for reproductive risk to birds for the proposed use of Spiroxamine EC 500 in grapes (BBCH 53 - 85)

Intended use	Grapes (BBCH 53 - 85)
Active substance/product	Spiroxamine / Spiroxamine EC 500
Application rate (g a.s./ha)	2 × 300
Reprod. toxicity (mg a.s./kg bw/d)	5.40
TER criterion	5

Crop scenario Growth stage	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard BBCH >20	Small insectivorous species “redstart”	9.6	1.5 x 0.53	2.36	**2.29**
Vineyard BBCH >40	Small granivorous bird “finch”	3.4	1.5 x 0.53	0.811	6.66
Vineyard BBCH >40	Small omnivorous bird “lark”	3.3	1.5 x 0.53	0.787	6.86
Vineyard Ripening	Frugivorous bird “Thrush/starling”	14.4	1.5 x 0.53	3.43	**1.57**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio

For the proposed use of Spiroxamine EC 500 on grapes (2 x 300 g a.s./ha at BBCH 53 - 85) the reproductive risks to birds from dietary exposure to spiroxamine are considered to be acceptable (TER ≥5) for the small granivorous bird “finch” and small omnivorous bird “lark” scenarios but potential reproductive risks (TER <5) have been identified for the small insectivorous species “redstart” and the frugivorous bird “thrush/starling” scenarios. A refined risk assessment for these individual scenarios has been presented below.

Refinement

Small insectivorous species “redstart” scenario

For the refined risk assessment for the small insectivorous species “redstart” scenario, the black redstart has been used as the focal species. As discussed above, a 90th percentile PT for the black redstart of 0.75 and a diet consisting of 96.45% ground invertebrates and 3.55% foliar invertebrates has been used in the refined risk assessment.

The refined risk assessment for the small insectivorous bird scenario in vineyards at BBCH >20 following application of Spiroxamine EC 500 at 2 x 300 g a.s./ha at BBCH 53 - 85 is presented below.

Table CP 10.1.1-17 Refinement of the small insectivorous bird scenario (BBCH >20) for the proposed use of Spiroxamine EC 500 in grapes (BBCH 53 - 85) – black redstart

App rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT [d]	Dep. factor [e]	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
2 x 0.300 kg a.s./ha	Invertebrates (ground dwelling)	0.778	3.5	1.5	0.53	0.75	1.0	0.487	0.594	9.09
	Invertebrates (foliar dwelling)	0.0286	21.0	1.5	0.53		1.0	0.107		

[a] Values calculated using focal species dietary data. The EFSA bodyweight of 16.5 g used in the calculations

b) Default RUD values from EFSA (2009) Appendix F
c) Default values from EFSA (2009)
d) Refined 90th percentile PT value from focal species study
e) Deposition from Appendix A (EFSA, 2009)
f) Sum of DDD values for individual diet components
g) TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Using the refined parameters discussed above the reproductive risks to insectivorous birds following the proposed use of Spiroxamine EC 500 on grapes at 2 x 300 g a.s./ha at BBCH 53 - 85 are considered to be acceptable (TER ≥5).

Frugivorous bird scenario

For refinement of the frugivorous bird scenario reference is made to report M-414948-01-1 in which the generic RUD of 8.3 has been replaced by a more realistic RUD of 1.63 that can be used in the refined risk assessment for spiroxamine. It is noted that this measured RUD was determined following multiple applications of Spiroxamine therefore a MAF 1.0 has been used in the refined risk assessment as multiple applications have already been accounted for. Here the refined frugivorous bird scenario is applicable to the frugivorous bird "song thrush" (*Turdus philomelos)* which according to EFSA (2009) presents a FIR/bw value of 1.73.

The refined risk assessments for the frugivorous bird scenario in vineyards following application of Spiroxamine EC 500 at 2 x 300 g a.s./ha at BBCH 53 – 85 is presented below.

Table CP 10.1.1-18 Refinement of the frugivorous bird scenario for the proposed use of Spiroxamine EC 500 in grapes (BBCH 53 - 85)

Application rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	$f_{(a+d)}$ [d]	PT [d]	Dep. factor [d]	DDD [mg a.s./kg b.w./d]	TER [e]
2 x 0.300 kg a.s./ha	Grapes	1.73	1.63	1.0	0.53	1.0	1.0	0.448	12.1

a) Value taken from Appendix A (EFSA, 2009)
b) Measured mean RUD value from 54 residues trials using spiroxamine on grapes
c) MAF of 1.0 applied as multiple applications accounted for in the refined RUD
d) Default values from EFSA (2009)
e) TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Using the refined RUD value discussed above the reproductive risks to frugivorous birds following the proposed use of Spiroxamine EC 500 on grapes at 2 x 300 g a.s./ha at BBCH 53 - 85 are considered to be acceptable (TER ≥5).

1 x 200 g a.s./ha BBCH 13 - 19 and 1 x 300 g a.s./ha BBCH 20 (53) - 85

In order to cover the GAP use of this mixed application rate the risk assessment has taken a conservative approach and assumed that two applications of 200 g a.s./ha could be made at BBCH 13-19 or that two applications of 300 g a.s./ha could be made at BBCH 20-85. It is noted that BBCH growth stages between 19 and 53 do not exist. However, for completeness the EFSA scenarios at BBCH 20-39 have been included in the risk assessment.

The risk assessment starts at Tier I which is presented below.

Table CP 10.1.1-19 Tier I assessment for acute risk to birds for the proposed use of Spiroxamine EC 500 in grapes

Intended use	Grapes
Active substance/product	Spiroxamine / Spiroxamine EC 500
Application rate (g a.s./ha)	2 × 200 (BBCH 13-19) or; 2 × 300 (BBCH 53-85)
Acute toxicity (mg a.s./kg bw)	>357
TER criterion	10

Crop scenario Growth stage	Generic focal species	App. Rate (kg a.s./ha)	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Vineyard BBCH 10-19	Small insectivorous species “redstart”	0.2	27.4	1.3	7.12	50.1
Vineyard BBCH 10-19	Small granivorous bird “finch”	0.2	14.8	1.3	3.85	92.9
Vineyard BBCH 10-19	Small omnivorous bird “lark”	0.2	14.4	1.3	3.74	95.4
Vineyard BBCH >20	Small insectivorous species “redstart”	0.3	25.7	1.3	10.0	35.6
Vineyard BBCH 20-39	Small granivorous bird “finch”	0.3	12.4	1.3	4.84	73.8
Vineyard BBCH 20-39	Small omnivorous bird “lark”	0.3	12.0	1.3	4.68	76.3
Vineyard BBCH >40	Small granivorous bird “finch”	0.3	7.4	1.3	2.89	124
Vineyard BBCH >40	Small omnivorous bird “lark”	0.3	7.2	1.3	2.81	127
Vineyard Ripening	Frugivorous bird “Thrush/starling”	0.3	28.9	1.3	11.3	31.7

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.
Scenarios shaded may not be required as BBCH 20-39 does not exist for grapes

For the proposed use of Spiroxamine EC 500 on grapes (1 x 200 g a.s./ha at BBCH 13 – 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85) the acute risks to birds from dietary exposure to spiroxamine are considered to be acceptable (TER ≥ 10) for all relevant scenarios. No further acute risk assessment is necessary for this use of Spiroxamine EC 500.

Table CP 10.1.1-20 Tier I assessment for reproductive risk to birds for the proposed use of Spiroxamine EC 500 in grapes

Intended use	Grapes
Active substance/product	Spiroxamine / Spiroxamine EC 500
Application rate (g a.s./ha)	2 × 200 g a.s./ha (BBCH 13-19) or; 2 × 300 g a.s./ha (BBCH 53-85)
Reprod. toxicity (mg a.s./kg bw/d)	5.40
TER criterion	5

Crop scenario Growth stage	Generic focal species	App. Rate (kg a.s./ha)	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard BBCH 10-19	Small insectivorous species “redstart”	0.2	12.5	1.5 x 0.53	1.83	**2.95**
Vineyard BBCH 10-19	Small granivorous bird “finch”	0.2	6.9	1.5 x 0.53	1.00	**4.92**
Vineyard BBCH 10-19	Small omnivorous bird “lark”	0.2	6.5	1.5 x 0.53	1.03	5.22
Vineyard BBCH >20	Small insectivorous species “redstart”	0.3	9.9	1.5 x 0.53	2.36	**2.29**
Vineyard BBCH 20-39	Small granivorous bird “finch”	0.3	5.7	1.5 x 0.53	1.36	**3.97**
Vineyard BBCH 20-39	Small omnivorous bird “lark”	0.3	5.4	1.5 x 0.53	1.29	**4.19**
Vineyard BBCH >40	Small granivorous bird “finch”	0.3	3.4	1.5 x 0.53	0.811	6.66
Vineyard BBCH >40	Small omnivorous bird “lark”	0.3	3.3	1.5 x 0.53	0.787	6.86
Vineyard Ripening	Frugivorous bird “Thrush/starling”	0.3	14.4	1.5 x 0.53	3.43	**1.57**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio
TER values shown in bold fall below the relevant trigger
Scenarios shaded may not be required as BBCH 20-39 does not exist for vines

For the proposed use of Spiroxamine EC 500 on grapes (1 x 200 g a.s./ha at BBCH 13 – 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85) the reproductive risks to birds from dietary exposure to spiroxamine are considered to be acceptable (TER ≥5) for the small omnivorous bird “lark” at BBCH 10-19 and BBCH >40 and the small granivorous bird “finch” at BBCH >40 scenarios but potential reproductive risks (TER <5) have been identified for several of the other scenarios. Refined risk assessments for these individual scenarios have been presented below.

Refinement

Small insectivorous species “redstart” scenario

For the refined risk assessment for the small insectivorous species “redstart” scenarios, the black redstart has been used as the focal species. As discussed above, a 90th percentile PT for the black redstart of 0.75 and a diet consisting of 96.45% ground invertebrates and 3.55% foliar invertebrates has been used in the refined risk assessment.

The refined risk assessments for the small insectivorous bird scenarios in vineyards at BBCH 10 - 19 and BBCH >20 following application of Spiroxamine EC 500 at 1 x 200 g a.s./ha at BBCH 13 – 19 and 1 x 300 g a.s./ha at BBCH 20 (53) – 85 are presented below.

Table CP 10.1.1-21 Refinement of the small insectivorous bird scenario (BBCH 10 - 19) for the proposed use of Spiroxamine EC 500 in grapes – black redstart

App rate	Food type	FIR/bw [a)]	RUD [b)]	MAF [c)]	f_{twa} [c)]	PT [d)]	Dep. factor [e)]	DDD [mg a.s./kg b.w./d]	Total DDD [f)]	TER [g)]
2 x 0.200 kg a.s./ha	Invertebrates (ground dwelling)	0.778	7.5	1.5	0.53	0.75	1.0	0.696	0.768	7.03
	Invertebrates (foliar dwelling)	0.0286	21.0	1.5	0.53		1.0	0.0716		

a) Values calculated using focal species dietary data. The EFSA bodyweight of 16.5 g used in the calculations
b) Default RUD values from EFSA (2009) Appendix F
c) Default values from EFSA (2009)
d) Refined 90th percentile PT value from focal species study
e) Deposition from Appendix A (EFSA, 2009)
f) Sum of DDD values for individual diet components
g) TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Table CP 10.1.1-22 Refinement of the small insectivorous bird scenario (BBCH >20) for the proposed use of Spiroxamine EC 500 in grapes – black redstart

App rate	Food type	FIR/bw [a)]	RUD [b)]	MAF [c)]	f_{twa} [e)]	PT [d)]	Dep. factor [e)]	DDD [mg a.s./kg b.w./d]	Total DDD [f)]	TER [g)]
2 x 0.300 kg a.s./ha	Invertebrates (ground dwelling)	0.778	3.5	1.5	0.53	0.75	1.0	0.487	0.594	9.09
	Invertebrates (foliar dwelling)	0.0286	21.0	1.5	0.53		1.0	0.107		

a) Values calculated using focal species dietary data. The EFSA bodyweight of 16.5 g used in the calculations
b) Default RUD values from EFSA (2009) Appendix F
c) Default values from EFSA (2009)
d) Refined 90th percentile PT value from focal species study
e) Deposition from Appendix A (EFSA, 2009)
f) Sum of DDD values for individual diet components
g) TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Using the refined parameters discussed above the reproductive risks to insectivorous birds following the proposed use of Spiroxamine EC 500 on grapes at 1 x 200 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85 are considered to be acceptable (TER ≥5).

Small granivorous bird "finch" scenario

For the refined risk assessment for the small granivorous bird "finch" scenario, the linnet has been used as the focal species. As already discussed, the refined risk assessment using the linnet has been conducted assuming a diet of 97.3% weed seeds and 2.7% ground invertebrates and a 90th percentile PT of 0.97. By way of an additional refinement for this use, a DT_{50} of 4 days to represent spiroxamine decline on weedheads has been applied to the risk assessment. In study M-090880-01-1 residues of spiroxamine were measured in or on potential avian food items including grapes, grape leaves, invertebrates and weed heads associated with vineyards. A DT_{50} of *ca.* 4 days was determined for weedheads and has therefore been taken here as a suitable DT_{50} to represent weed seeds. Using a moving time window approach and based on two applications with a 10-day interval, a combined MAF x f_{twa}

value of 0.506 has been determined and replaces the default MAF and f_{twa} values of 1.5 and 0.53, respectively.

The refined risk assessments for the small granivorous bird scenarios in vineyards at BBCH 10 - 19 and BBCH 20 - 39 following application of Spiroxamine EC 500 at 1 x 200 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85 are presented below.

Table CP 10.1.1-23 Refinement of the small granivorous bird scenario for the proposed use of Spiroxamine EC 500 in grapes (BBCH 10 - 19) - Linnet

Application rate	Food type	FIR/bw [a)]	RUD [b)]	MAF	f_{twa}	PT [d)]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f)]	TER [g)]
2 x 0.200 kg a.s./ha	Weed seeds	0.282	40.2	0.506 [c)]		0.97	0.6 [e)]	0.668	0.677	7.98
	Invertebrates (ground dwelling)	0.00782	7.5	1.5	0.53		1.0	0.00905		

[a)] Values calculated using focal species dietary data. The EFSA bodyweight of 15.3 g used in the calculations
[b)] Default RUD values from EFSA (2009) Appendix F
[c)] A MAF×TWA value calculated using a moving time window and a DT_{50} value of 4 days
[d)] Refined 90th percentile PT value from focal species study
[e)] Deposition value based on 40% crop interception (Appendix A, EFSA, 2009)
[f)] Sum of DDD values for individual diet components
[g)] TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Table CP 10.1.1-24 Refinement of the small granivorous bird scenario for the proposed use of Spiroxamine EC 500 in grapes (BBCH 20 - 39) - Linnet

Application rate	Food type	FIR/bw [a)]	RUD [b)]	MAF	f_{twa}	PT [d)]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f)]	TER [g)]
2 x 0.300 kg a.s./ha	Weed seeds	0.282	40.2	0.506 [c)]		0.97	0.5 [e)]	0.835	0.841	6.42
	Invertebrates (ground dwelling)	0.00782	3.5	1.5	0.53		1.0	0.00633		

[a)] Values calculated using focal species dietary data. The EFSA bodyweight of 15.3 g used in the calculations
[b)] Default RUD values from EFSA (2009) Appendix F
[c)] A MAF×TWA value calculated using a moving time window and a DT_{50} value of 4 days
[d)] Refined 90th percentile PT value from focal species study
[e)] Deposition value based on 50% crop interception (Appendix A, EFSA, 2009)
[f)] Sum of DDD values for individual diet components
[g)] TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Using the refined parameters discussed above the reproductive risks to granivorous birds following the proposed use of Spiroxamine EC 500 on grapes at 1 x 200 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) – 85 are considered to be acceptable (TER ≥5).

Small omnivorous bird "lark" scenario

To refine the small omnivorous bird "lark" scenario at BBCH 20 – 39, the woodlark has been selected as the focal species for the refined risk assessment. In study M-291784-01-1 a radio-tracking program was carried out in a typical European wine growing region in France during the spring and summer to obtain measured data on PT and PD values for refined exposure assessment, including the woodlark. Bird trapping, radio-tracking, visual observations together with measurement of faecal and stomach content were methods used to characterise PT and PD in vineyards.

For woodlarks a mean PT value of 0.86 was determined with a 90th percentile value of 1.0. The diet of woodlarks consisted basically of invertebrates (PD of 0.921) with insect adults as the most important

food items. The remainder of the analysed diets consisted of seeds. The default EFSA diet for this scenario is 25% crop leaves, 25% weed seeds and 50% ground arthropods, however in the refined risk assessment this has been replaced with a diet of 92.1% ground arthropods and 7.9% weed seeds, as determined in study M-291784-01-1. A PT value of 1.0 continues to be used in the refined assessment, as confirmed by this study.

The refined risk assessment for the small omnivorous bird scenario in vineyards at BBCH 20 - 39 following application of Spiroxamine EC 500 at 1 x 200 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85 is presented below.

Table CP 10.1.1-25 Refinement of the small omnivorous bird scenario for the proposed use of Spiroxamine EC 500 in grapes (BBCH 20 - 39) - woodlark

Application rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT [c]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [e]	TER [f]
2 x 0.300 kg a.s./ha	Invertebrates (ground dwelling)	0.541	7.5	1.5	0.53	1.0	0.5 [d]	0.484	0.706	7.65
	Weed seeds	0.0464	40.2	1.5	0.53			0.222		

[a] Values calculated using focal species dietary data
[b] Default RUD values from EFSA (2009) Appendix F
[c] Default values from EFSA (2009)
[d] 50% crop interception used (Appendix A: EFSA (2009)
[e] Sum of DDD values for individual diet components
[f] TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Using the refined parameters discussed above the reproductive risks to omnivorous birds following the proposed use of Spiroxamine EC 500 on grapes at 1 x 200 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) – 85 are considered to be acceptable (TER ≥5).

Frugivorous bird scenario

For refinement of the frugivorous bird scenario reference is made to report M-414948-01-1 in which the generic RUD of 8.3 has been replaced by a more realistic RUD of 1.63 that can be used in the refined risk assessment for spiroxamine. It is noted that this measured RUD was determined following multiple applications of Spiroxamine therefore a MAF 1.0 has been used in the refined risk assessment as multiple applications have already been accounted for. Here the refined frugivorous bird scenario is applicable to the frugivorous bird "song thrush" (*Turdus philomelos)* which according to EFSA (2009) presents a FIR/bw value of 1.73.

The refined risk assessments for the frugivorous bird scenario in vineyards following application of Spiroxamine EC 500 at 1 x 200 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85 is presented below.

Table CP 10.1.1-26 Refinement of the frugivorous bird scenario for the proposed use of Spiroxamine EC 500 in grapes

Application rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [d]	PT [d]	Dep. factor [d]	DDD [mg a.s./kg b.w./d]	TER [e]
2 x 0.300 kg a.s./ha	Grapes	1.73	1.63	1.0	0.53	1.0	1.0	0.448	12.1

[a] Value taken from Appendix A (EFSA, 2009)
[b] Measured mean RUD value from 54 resiudes trials using spiroxamine on grapes
[c] MAF of 1.0 applied as multiple applications accounted for in the refined RUD
[d] Default values from EFSA (2009)
[e] TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Using the refined RUD value discussed above the reproductive risks to frugivorous birds following the proposed use of Spiroxamine EC 500 on grapes at 1 x 200 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85 are considered to be acceptable (TER ≥5).

1 x 300 g a.s./ha BBCH 13 - 19 and 1 x 300 g a.s./ha BBCH 20 (53) - 85

In order to cover the GAP use of this mixed application rate the risk assessment has taken a conservative approach and assumed that two applications of 300 g a.s./ha could be made at BBCH 13-19 or that two applications of 300 g a.s./ha could be made at BBCH 20-85. It is noted that BBCH growth stages between 19 and 53 do not exist. However, for completeness the EFSA scenarios at BBCH 20-39 have been included in the risk assessment.

The risk assessment starts at Tier I which is presented below.

Table CP 10.1.1-27 Tier I assessment for acute risk to birds for the proposed use of Spiroxamine EC 500 in grapes

Intended use	Grapes					
Active substance/product	Spiroxamine, Spiroxamine EC 500					
Application rate (g a.s./ha)	2 × 300 (BBCH 13-19) or; 2 × 300 (BBCH 53-85)					
Acute toxicity (mg a.s./kg bw)	>357					
TER criterion	10					
Crop scenario Growth stage	Generic focal species	App. Rate (kg a.s./ha)	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Vineyard BBCH 10-19	Small insectivorous species “redstart”	0.3	27.4	1.3	10.7	33.4
Vineyard BBCH 10-19	Small granivorous bird “finch”	0.3	14.8	1.3	5.77	61.9
Vineyard BBCH 10-19	Small omnivorous bird “lark”	0.3	14.4	1.3	5.62	63.6
Vineyard BBCH >20	Small insectivorous species “redstart”	0.3	25.7	1.3	10.0	35.6
Vineyard BBCH 20-39	Small granivorous bird “finch”	0.3	12.4	1.3	4.84	73.8
Vineyard BBCH 20-39	Small omnivorous bird “lark”	0.3	12.0	1.3	4.68	76.3
Vineyard BBCH >40	Small granivorous bird “finch”	0.3	7.4	1.3	2.89	124
Vineyard BBCH >40	Small omnivorous bird “lark”	0.3	7.2	1.3	2.81	127
Vineyard Ripening	Frugivorous bird “Thrush/starling”	0.3	28.9	1.3	11.3	31.7

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.
Scenarios shaded may not be required as BBCH 20-39 does not exist for grapes

For the proposed use of Spiroxamine EC 500 on grapes (1 x 200 g a.s./ha at BBCH 13 – 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85) the acute risks to birds from dietary exposure to spiroxamine are

considered to be acceptable (TER ≥10) for all relevant scenarios. No further acute risk assessment is necessary for this use of Spiroxamine EC 500.

Table CP 10.1.1-28 Tier I assessment for reproductive risk to birds for the proposed use of Spiroxamine EC 500 in grapes

Intended use	Grapes					
Active substance/product	Spiroxamine / Spiroxamine EC 500					
Application rate (g a.s./ha)	2 × 300 (BBCH 13-19) or; 2 × 300 (BBCH 53-85)					
Reprod. toxicity (mg a.s./kg bw/d)	5.40					
TER criterion	5					
Crop scenario Growth stage	Generic focal species	App. Rate (kg a.s./ha)	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard BBCH 10-19	Small insectivorous species “redstart”	0.3	11.5	1.5 x 0.53	3.74	**1.97**
Vineyard BBCH 10-19	Small granivorous bird “finch”	0.3	6.9	1.5 x 0.53	1.65	**3.28**
Vineyard BBCH 10-19	Small omnivorous bird “lark”	0.3	6.5	1.5 x 0.53	1.55	**3.48**
Vineyard BBCH >20	Small insectivorous species “redstart”	0.3	9.9	1.5 x 0.53	2.36	**2.29**
Vineyard BBCH 20-39	Small granivorous bird “finch”	0.3	5.7	1.5 x 0.53	1.36	**3.97**
Vineyard BBCH 20-39	Small omnivorous bird “lark”	0.3	5.4	1.5 x 0.53	1.29	**4.19**
Vineyard BBCH >40	Small granivorous bird “finch”	0.3	3.4	1.5 x 0.53	0.811	6.66
Vineyard BBCH >40	Small omnivorous bird “lark”	0.3	3.3	1.5 x 0.53	0.787	6.86
Vineyard Ripening	Frugivorous bird “Thrush/starling”	0.3	14.4	1.5 x 0.53	3.43	**1.57**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.
TER values shown in bold fall below the relevant trigger
Scenarios shaded may not be required as BBCH 20-39 does not exist for grapes

For the proposed use of Spiroxamine EC 500 on grapes (1 x 300 g a.s./ha at BBCH 13 – 19 and 1 x 300 g a.s./ha at BBCH 20 (53) – 85) the reproductive risks to birds from dietary exposure to spiroxamine are considered to be acceptable (TER ≥5) for the small omnivorous bird “lark” at BBCH >40 and the small granivorous bird “finch” at BBCH >40 scenarios but potential reproductive risks (TER <5) have been identified for the remaining scenarios. Refined risk assessments for these individual scenarios have been presented below.

Refinement

Small insectivorous species “redstart” scenario

For the refined risk assessment for the small insectivorous species "redstart" scenarios, the black redstart has been used as the focal species. As discussed above, a 90th percentile PT for the black redstart of 0.75 and a diet consisting of 96.45% ground invertebrates and 3.55% foliar invertebrates has been used in the refined risk assessment.

The refined risk assessments for the small insectivorous bird scenarios in vineyards at BBCH 10 – 19 and BBCH >20 following application of Spiroxamine EC 500 at 1 x 300 g a.s./ha at BBCH 13 – 19 and 1 x 300 g a.s./ha at BBCH 20 (53) – 85 are presented below.

Table CP 10.1.1-29 Refinement of the small insectivorous bird scenario (BBCH 10 - 19) for the proposed use of Spiroxamine EC 500 in grapes – black redstart

App rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT [d]	Dep. factor [e]	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
2 x 0.300 kg a.s./ha	Invertebrates (ground dwelling)	0.778	7.5	1.5	0.53	0.75	1.0	1.04	1.15	**4.70**
	Invertebrates (foliar dwelling)	0.0286	21.0	1.5	0.53		1.0	0.107		

[a] Values calculated using focal species dietary data. The EFSA bodyweight of 16.5 g used in the calculations
[b] Default RUD values from EFSA (2009) Appendix F
[c] Default values from EFSA (2009)
[d] Refined 90th percentile PT value from focal species study
[e] Deposition from Appendix A (EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day
Values in **bold** are below the trigger value of 5

Table CP 10.1.1-30 Refinement of the small insectivorous bird scenario (BBCH >20) for the proposed use of Spiroxamine EC 500 in grapes – black redstart

App rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT [d]	Dep. factor [e]	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
2 x 0.300 kg a.s./ha	Invertebrates (ground dwelling)	0.778	3.5	1.5	0.53	0.75	1.0	0.487	0.594	9.09
	Invertebrates (foliar dwelling)	0.0286	21.0	1.5	0.53		1.0	0.107		

[a] Values calculated using focal species dietary data. The EFSA bodyweight of 16.5 g used in the calculations
[b] Default RUD values from EFSA (2009) Appendix F
[c] Default values from EFSA (2009)
[d] Refined 90th percentile PT value from focal species study
[e] Deposition from Appendix A (EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Using the refined parameters discussed above the reproductive risks to insectivorous birds following the proposed use of Spiroxamine EC 500 at 1 x 300 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) – 85 are considered to be acceptable (TER ≥5) for the use in vineyards at BBCH >20. For the use at BBCH 10 – 19 the TER value was 4.70 which is slightly below the trigger value of 5. However, the risk assessment here has taken a conservative approach by assuming two applications at growth stages BBCH 10 – 19 [to account for the multiple exposure] but in reality the use is for only one application at this growth stage followed by a second application at higher growth stages. As the risk assessment for the two applications at the slightly higher growth stage of BBCH >20 is acceptable, it is therefore considered that the risk to insectivorous birds at BBCH 10 - 19 is in fact acceptable.

Small granivorous bird "finch" scenario

For the refined risk assessment for the small granivorous bird "finch" scenario, the linnet has been used as the focal species. As already discussed, the refined risk assessment using the linnet has been conducted assuming a diet of 97.3% weed seeds and 2.7% ground invertebrates and a 90th percentile PT of 0.97. By way of an additional refinement for this use, a DT_{50} of 4 days to represent spiroxamine decline on weedheads has been applied to the risk assessment. In study M-090880-01-1 residues of spiroxamine were measured in or on potential avian food items including grapes, grape leaves, invertebrates and weed heads associated with vineyards. A DT_{50} of ca. 4 days was determined for weedheads and has therefore been taken here as a suitable DT_{50} to represent weed seeds. Using a moving time window approach and based on two applications with a 10-day interval, a combined MAF x f_{twa} value of 0.506 has been determined and replaces the default MAF and f_{twa} values of 1.5 and 0.53, respectively.

The refined risk assessments for the small granivorous bird scenarios in vineyards at BBCH 10 - 19 and BBCH 20 - 39 following application of Spiroxamine EC 500 at 1 x 300 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85 are presented below.

Table CP 10.1.1-31 Refinement of the small granivorous bird scenario for the proposed use of Spiroxamine EC 500 in grapes (BBCH 10 - 19) - Linnet

Application rate	Food type	FIR/bw [a]	RUD [b]	MAF	f_{twa}	PT [d]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
2 x 0.300 kg a.s./ha	Weed seeds	0.282	40.2	0.506 [c]		0.97	0.5 [e]	1.00	1.01	5.35
	Invertebrates (ground dwelling)	0.00782	7.5	1.5	0.53		1.0	0.0136		

[a] Values calculated using focal species dietary data. The EFSA bodyweight of 15.3 g used in the calculations
[b] Default RUD values from EFSA (2009) Appendix E
[c] A MAF×TWA value calculated using a moving time window and a DT_{50} value of 4 days
[d] Refined 90th percentile PT value from focal species study
[e] Deposition value based on 40% crop interception (Appendix A: EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Table CP 10.1.1-32 Refinement of the small granivorous bird scenario for the proposed use of Spiroxamine EC 500 in grapes (BBCH 20 - 39) - Linnet

Application rate	Food type	FIR/bw [a]	RUD [b]	MAF	f_{twa}	PT [d]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
2 x 0.300 kg a.s./ha	Weed seeds	0.282	40.2	0.506 [c]		0.97	0.5 [e]	0.835	0.841	6.42
	Invertebrates (ground dwelling)	0.00782	3.5	1.5	0.53		1.0	0.00633		

[a] Values calculated using focal species dietary data. The EFSA bodyweight of 15.3 g used in the calculations
[b] Default RUD values from EFSA (2009) Appendix F
[c] A MAF×TWA value calculated using a moving time window and a DT_{50} value of 4 days
[d] Refined 90th percentile PT value from focal species study
[e] Deposition value based on 50% crop interception (Appendix A: EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Using the refined parameters discussed above the reproductive risks to granivorous birds following the proposed use of Spiroxamine EC 500 on grapes at 1 x 300 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) – 85 are considered to be acceptable (TER ≥5).

Small omnivorous bird "lark" scenario

To refine the small omnivorous bird "lark" scenarios at BBCH 10 - 19 and BBCH 20 - 39, the woodlark has been selected as the focal species for the refined risk assessment. As discussed in the previous section the refined risk assessment this has been conducted assuming a diet of 92.1% ground arthropods and 7.9% weed seeds, as determined in study M-291784-01-1. A PT value of 1.0 continues to be used in the refined assessment, as confirmed by this study.

The refined risk assessments for the small omnivorous bird scenario in vineyards at BBCH 10 - 19 and BBCH 20 - 39 following application of Spiroxamine EC 500 at 1 x 300 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85 are presented below.

Table CP 10.1.1-33 Refinement of the small omnivorous bird scenario for the proposed use of Spiroxamine EC 500 in grapes (BBCH 10 - 19) - woodlark

Application rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [e]	TER [f]
2 x 0.300 kg a.s./ha	Invertebrates (ground dwelling)	0.541	4.5	1.5	0.53	1.0	0.6 [d]	0.581	0.848	6.37
	Weed seeds	0.0464	40.2	1.5	0.53			0.267		

[a] Values calculated using focal species dietary data
[b] Default RUD values from EFSA (2009) Appendix F
[c] Default values from EFSA (2009)
[d] 40% crop interception used (Appendix A: EFSA, 2009)
[e] Sum of DDD values for individual diet components
[f] TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Table CP 10.1.1-34 Refinement of the small omnivorous bird scenario for the proposed use of Spiroxamine EC 500 in grapes (BBCH 20 - 39) - woodlark

Application rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [e]	TER [f]
2 x 0.300 kg a.s./ha	Invertebrates (ground dwelling)	0.541	7.5	1.5	0.53	1.0	0.5 [d]	0.484	0.706	7.65
	Weed seeds	0.0464	40.2	1.5	0.53			0.222		

[a] Values calculated using focal species dietary data
[b] Default RUD values from EFSA (2009) Appendix F
[c] Default values from EFSA (2009)
[d] 50% crop interception used (Appendix A: EFSA, 2009)
[e] Sum of DDD values for individual diet components
[f] TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Using the refined parameters discussed above the reproductive risks to omnivorous birds following the proposed use of Spiroxamine EC 500 on grapes at 1 x 300 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) – 85 are considered to be acceptable (TER ≥5).

Frugivorous bird scenario

For refinement of the frugivorous bird scenario reference is made to report M-414948-01-1 in which the generic RUD of 8.3 has been replaced by a more realistic RUD of 1.63 that can be used in the refined risk assessment for spiroxamine. It is noted that this measured RUD was determined following multiple applications of Spiroxamine therefore a MAF 1.0 has been used in the refined risk assessment as multiple applications have already been accounted for. Here the refined frugivorous bird scenario is

applicable to the frugivorous bird "song thrush" (*Turdus philomelos)* which according to EFSA (2009) presents a FIR/bw value of 1.73.

The refined risk assessments for the frugivorous bird scenario in vineyards following application of Spiroxamine EC 500 at 1 x 300 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85 is presented below.

Table CP 10.1.1-35 Refinement of the frugivorous bird scenario for the proposed use of Spiroxamine EC 500 in grapes

Application rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twg} [d]	PT [d]	Ded. factor [d]	DDD [mg a.s./kg b.w./d]	TER [e]
2 x 0.300 kg a.s./ha	Grapes	1.73	1.63	1.0	0.53	1.0	1.0	0.448	12.1

[a] Value taken from Appendix A (EFSA, 2009)
[b] Measured mean RUD value from 54 resiudes trials using spiroxamine on grapes
[c] MAF of 1.0 applied as multiple applications accounted for in the refined RUD
[d] Default values from EFSA (2009)
[e] TER calculated based on reproductive endpoint of 5.40 mg a.s./kg bw/day

Using the refined RUD value discussed above the reproductive risks to frugivorous birds following the proposed use of Spiroxamine EC 500 on grapes at 1 x 300 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85 are considered to be acceptable (TER ≥ 5).

Risks for birds through drinking water

In addition to dietary items, birds may also be exposed to residues occurring in drinking water. The daily dietary dose (DDD) values used in the standard dietary risk assessments do not encompass drinking water and therefore the potential risk from this exposure route has not been covered in the dietary risk assessment. Two scenarios are considered:

Leaf scenario

This scenario assumes pooling of spray solution in leaf whorls following application and is relevant only for certain crops and growth stages *e.g.* leafy vegetables forming heads or with a morphology that might facilitate collection of spray and from BBCH principle growth stage 4 until harvest. This scenario is not considered to be applicable to the proposed use on grapes.

Puddle scenario

This scenario considers puddles occuring on the soil surface following a rainfall event after application and is considered possible in all crop types.

In accordance with the EFSA Guidance Document (2009), based on the characteristics of the exposure scenario and standard assumptions for water uptake by animals no specific requirement for drinking water exposure calculation and TER determination based on the puddle scenario is required:

- for substances with a Koc <500 L/kg (less sorptive); if the ratio of application rate (g a.s./ha) to effective endpoint mg a.s./kg bw/d does not exceed 50;
- for substances with a Koc ≥ 500 L/kg (more sorptive); if the ratio of application rate (g a.s./ha) to effective endpoint mg a.s./kg bw/d does not exceed 3000.

The geomean Koc for spiroxamine is 4111 L/kg therefore spiroxamine belongs to the group of more sorptive substances. The ratio calculation is based on two applications of the highest rate at 300 g a.s./ha.

Table CP 10.1.1-36 Ratios of effective application rate (AR_{eff}) to acute and long-term endpoints for spiroxamine following the proposed use of Spiroxamine EC 500 on grapes - puddle scenario

Test substance	AR_{eff} (g/ha)[a]	Toxicological endpoint (mg a.s./kg bw/d)	Ratio (AR_{eff}/endpoint)	Trigger
Acute				
Spiroxamine	390	LD_{50} >357	1.09	3000
Long-term				
Spiroxamine	450	NOAEL 5.40	83.3	3000

[a] AR_{eff} = based on an application rate of 300 g a.s./ha with a MAF of 1.3 and 1.5 applied for acute and reproductive risk assessments, respectively

The ratios for both acute and reproductive risks are below the relevant trigger of 3000 for spiroxamine therefore a quantitative risk assessment is not necessary. Thus, there are no unacceptable risks to birds from exposure to spiroxamine *via* drinking water.

Secondary poisoning

The Log P_{ow} of spiroxamine is 2.79 and 2.98 at pH 7 for diastomers A and B respectively but at pH 9 these value are 4.88 and 5.08, respectively. Thus the trigger value of 3 for a secondary poisoning risk assessment is met.

Consideration of secondary poisoning risk due to metabolites

The Log P_{ow} of spiroxamine-desethyl (M01) is 2.41, 1.97 and >3.64 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-despropyl (M02) is 1.95, 1.41 and >3.44 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-N-oxide (M03) is 2.82, 2.59 and 2.63 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-carboxylic acid (M06) is 0.45, -0.25 and 0.10 at pH 4, 7 and 9, respectively. Thus, an assessment of the potential risk from bioconcentration of spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02) also needs to be addressed in the risk assessment.

Risk assessment for earthworm-eating birds *via* secondary poisoning

According to EFSA (2009), the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

To achieve a concise risk assessment, the risk envelope approach is applied whereby the maximum application rate of 2 x 300 g a.s./ha has been considered. For spiroxamine, M01 and M02, the $PEC_{soil\ accumulation}$ has been used in he risk assessment as these values are higher than the 21-day TWA PEC_{soil} values. There are no avian reproductive toxicity data available for M01 and M02 therefore the NOAEL of 5.40 mg/kg bw/day for spiroxamine has been used as a surrogate value.

The secondary poisoning risk assessments for earthworm-eating birds from exposure to spiroxamine, KWG 4168-desethyl (M01) and KWG 4168-despropyl (M02) are presented in the tables below.

Table CP 10.1.1-37 Assessment of the risk for earthworm-eating birds due to exposure to spiroxamine *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Spiroxamine	Comments
PEC_{soil} (mg a.s./kg soil)	0.555	Accumulation PEC_{soil} used as worst-case
Log P_{ow} / P_{ow}	4.0 / 10000	Mean value of 4.0 has been used based on the values for diastomers A and B at pH 7 and 9
K_{oc}	4111	Geoean

f_{oc}	0.02	Default
BCF_{worm}	1.47	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC_{worm}	0.816	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg a.s./kg bw/d)	0.856	$DDD = PEC_{worm} \times 1.05$
NOAEL (mg a.s./kg bw/d)	5.40	From study M-007470-03-1
TER_{LT}	6.30	Acceptable risks (TER>5)

Table CP 10.1.1-38 Assessment of the risk for earthworm-eating birds due to exposure to spiroxamine-desethyl (M01) *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Spiroxamine-desethyl	Comments
PEC_{soil} (mg/kg soil)	0.064	Accumulation PEC_{soil} used as worst-case
Log P_{ow} / P_{ow}	3.64 / 4365	-
K_{oc}	3271	Geomean
f_{oc}	0.02	Default
BCF_{worm}	0.814	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC_{worm}	0.0521	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0547	$DDD = PEC_{worm} \times 1.05$
NOAEL (mg/kg bw/d)	5.40	Value determined for spiroxamine used as a surrogate
TER_{LT}	98.8	Acceptable risks (TER>5)

Table CP 10.1.1-39 Assessment of the risk for earthworm-eating birds due to exposure to spiroxamine-despropyl (M02) *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Spiroxamine-despropyl	Comments
PEC_{soil} (mg/kg soil)	0.040	Accumulation PEC_{soil} used as worst-case
Log P_{ow} / P_{ow}	3.44 / 2754	-
K_{oc}	2695	Geomean
f_{oc}	0.02	Default
BCF_{worm}	0.629	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC_{worm}	0.0252	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0264	$DDD = PEC_{worm} \times 1.05$
NOAEL (mg/kg bw/d)	5.40	Value determined for spiroxamine used as a surrogate
TER_{LT}	204	Acceptable risks (TER>5)

For the secondary poisoning risk assessments for earthworm-eating birds from exposure to spiroxamine, M01 and M02 the TER values are >5 thereby demonstrating an acceptable risk to birds *via* this route of

exposure. The Tier I secondary poisoning risk assessment has used the default parameters from the EFSA Bird & Mammal Guidance Document in order to determine the BCF_{worm} value. However, earthworm bioaccumulation data are available for spiroxamine (study M-411910-01-1), which determined a BAF of 1.56. Although not required, an updated risk assessment for spiroxamine has been conducted and presented below using this BAF value in place of the Tier I BCF_{worm} value.

Table CP 10.1.1-40 Assessment of the risk for earthworm-eating birds due to exposure to spiroxamine *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Spiroxamine	Comments
PEC_{soil} (mg a.s./kg soil)		Accumulation PEC_{soil} used as worst-case
Log P_{ow} / P_{ow}	4.0 / 10000	Mean value of 4.0 has been used based on the values for diastomers A and B at pH 7 and 9
K_{oc}	4111	Mean
f_{oc}	0.02	Default
BCF_{worm}	1.56	From study M-411910-01-1
PEC_{worm}	0.866	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg a.s./kg bw/d)	0.909	$DDD = PEC_{worm} \times 1.28$
NOAEL (mg a.s./kg bw/d)	5.40	From study M-007470-03-1
TER_{LT}	5.94	Acceptable risks (TER >5)

When the experimentally determined BAF value is applied to the risk assessment, the risk to earthworm-eating birds from exposure to spiroxamine has also been demonstrated to be acceptable with a TER value >5.

Risk assessment for fish-eating birds *via* secondary poisoning

According to EFSA (2009), the risk for piscivorous birds is assessed for a bird of 1000 g body weight with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

To achieve a concise risk assessment, the risk envelope approach is applied whereby the maximum application rate of 2 x 300 g a.s./ha has been considered in the risk assessment. The highest Step 3 TWA PEC_{sw} of 2.627 µg a.s./L for spiroxamine has been used in the risk assessment. For M01 the highest Step 2 PEC_{sw} value of 1.084 µg/L has been used and for M02 the highest Step 2 PEC_{sw} value of 0.917 µg/L has been used. For M01 and M02 there are no BCF values available therefore the BCF value determined for spiroxamine (87 L/kg) has been used. Furthermore, there are no avian reproductive toxicity data available for M01 and M02 therefore the NOAEL of 5.40 mg/kg bw/day for spiroxamine has been used as a surrogate value.

The secondary poisoning risk assessments for fish-eating birds from exposure to spiroxamine, KWG 4168-desethyl (M01) and KWG 4168-despropyl (M02) are presented in the tables below.

Table CP 10.1.1-41 Assessment of the risk for fish-eating birds due to exposure to spiroxamine *via* bioaccumulation in fish (secondary poisoning)

Parameter	Spiroxamine	Comments
PEC_{sw} (mg a.s./L)	0.002627	FOCUS Step 3 TWA PEC_{sw} (calculated for vines: D6 ditch, 2 x 300 g a.s./ha, late application)

PEC_{water} (mg a.s./L)	0.002627	TWA PEC_{sw} value used
BCF_{fish}	87	From study M-006479-01-1
PEC_{fish}	0.229	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg a.s./kg bw/d)	0.0363	$DDD = PEC_{fish} \times 0.159$
NOAEL (mg a.s./kg bw/d)	5.40	From study M-007470-03-1
TER_{LT}	149	Acceptable risks (TER>5)

Table CP 10.1.1-42 Assessment of the risk for fish-eating birds due to exposure to spiroxamine-desethyl (M01) *via* bioaccumulation in fish (secondary poisoning)

Parameter	Spiroxamine-desethyl	Comments
PEC_{sw} (mg/L)	0.001084	FOCUS Step 2 maximum PEC_{sw} (calculated for vines; 2 x 300 g a.s./ha)
PEC_{water} (mg/L)	0.000575	PEC_{water} = max $PEC_{sw} \times f_{twa}$, where f_{twa} = 0.53; in line with approach in EFSA (2009)
BCF_{fish}	87	Value determined for spiroxamine used as a surrogate
PEC_{fish}	0.0500	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.00795	$DDD = PEC_{fish} \times 0.159$
NOAEL (mg/kg bw/d)	5.40	Value determined for spiroxamine used as a surrogate
TER_{LT}	679	Acceptable risks (TER>5)

Table CP 10.1.1-43 Assessment of the risk for fish-eating birds due to exposure to spiroxamine-despropyl (M02) *via* bioaccumulation in fish (secondary poisoning)

Parameter	Spiroxamine-despropyl	Comments
PEC_{sw} (mg/L)	0.000917	FOCUS Step 2 maximum PEC_{sw} (calculated for vines; 2 x 300 g a.s./ha)
PEC_{water} (mg/L)	0.000486	PEC_{water} = max $PEC_{sw} \times f_{twa}$, where f_{twa} = 0.53; in line with approach in EFSA (2009)
BCF_{fish}	87	Value determined for spiroxamine used as a surrogate
PEC_{fish}	0.0423	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.00672	$DDD = PEC_{fish} \times 0.159$
NOAEL (mg/kg bw/d)	5.40	Value determined for spiroxamine used as a surrogate
TER_{LT}	803	Acceptable risks (TER>5)

For the secondary poisoning risk assessments for fish-eating birds from exposure to spiroxamine, M01 and M02, the TER values are >5 thereby demonstrating an acceptable risk to birds *via* this route of exposure.

Biodiversity

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on birds. Therefore, it is considered that the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects *via* alteration of the food web, are covered by the risk assessment for birds in this section and in the ED hazard assessment.

CP 10.1.1.1 Acute oral toxicity

A summary of the available avian formulation data is presented below.

Data Point:	KCP 10.1.1.1/01
Report Author:	[redacted]
Report Year:	1995
Report Title:	KWG 4168 (EC 500): Acute oral toxicity to bobwhite quail
Report No:	VB-032
Document No:	M-008102-01-1
Guideline(s) followed in study:	U.S. EPA E 71-1 (1982)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1999), RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The acute oral toxicity of KWG 4168 EC 500 to 20-week old Bobwhite quail (*Colinus virginianus*) was assessed over 14 days. KWG 4168 EC 500 was administered to five groups of 10 birds (5 per sex) in a single oral dose by gelatine capsules at 152, 289, 551, 1050 and 2000 mg/kg bw.

Birds dosed with 551, 1050 and 2000 mg/kg bw exhibited partly severe symptoms of intoxication including severe apathy, laying on the side and convulsions. Slight reductions in body weight were observed in the male birds at the higher dose concentrations of 551 and 1050 mg/kg bw, however there were hardly perceivable differences in body weights in the female birds. A reduced feed intake was observed in the groups treated with 289, 551 and 1050 mg/kg bw. Towards the end of the test period, females started laying eggs.

Mortalities and symptoms of intoxication occurred only on the first three days after application.

The 14-day acute oral LD_{50} was 971 mg product/kg bw. The 14-day NOEL of KWG 4168 EC 500 to Bobwhite quail, based on symptoms of intoxication and, at the 551 mg/kg bw, one case of mortality was 289 mg/kg bw.

Materials and Methods

Materials

Test Material	KWG 4168 EC 500
Lot/Batch #:	089A
Purity:	491.4 g/L
Description:	Clear yellow liquid

Reanalysis/Expiry date:	16 December 1994
Treatments	
Test rates:	151, 289, 551, 1050 and 2000 mg/kg bw
Analysis of test concentrations:	No
Test organisms	
Species:	Bobwhite quail (*Colinus virginianus*), 20-weeks old
Source:	Morris Quail Farm Inc. Florida 33170-5399
Feeding:	Food and drinking water administered throughout the study with the exception of an 18-hour fast immediately prior to dosing
Test design	
Test vessel:	Stainless steel wire cages (18 x 23 x 13 cm)
Replication:	One group of 10 birds per treatment. Birds individually housed
No. animals/vessel:	Ten animals per dosage concentration (five of each sex)
Duration of test:	14 days
Environmental test conditions	
Temperature:	20 ± 2 °C
Relative humidity:	30 - 90%
Photoperiod:	16 hours light, 8 hours dark

Study Design

This study was conducted in order to assess the acute toxicity of KWG 4168 EC 500 on Bobwhite quail in an avian single dose LD_{50} study over 14 days. The test concentrations were selected based upon results from a previously conducted range-finding study.

Bobwhite quail were used for this study and were approximately 20 weeks old at test initiation. There was an equal number of female and males birds used in the study.

The birds were placed individually into stainless steel wire cages of 18 x 23 x 13 cm. Paper was used as cage bedding and changes three times per week throughout the duration of the study. Single oral dosages of the test substance were administered orally as gelatine capsules at test initiation.

The test vessels were maintained at a temperature of 20 ± 2ºC with a relative humidity of 30 to 90%. Daily light length corresponded with a 16-hour light, 8- hour dark photoperiod.

Five groups of ten birds (five per sex) were given a single oral dose of 152, 289, 551, 1050 or 2000 mg/kg bw. One additional group of ten birds (five per sex) were similarly dosed with an empty capsule and maintained as concomitant control.

Food and drinking water were available to the birds prior to and throughout the duration of the study with the exception of approximately 18 hours immediately prior to dosing, during which time the birds were fasted.

Body weights were obtained prior to test initiation, on study day 7 and at test termination. Feed consumptions for each group were recorded on study days 3, 7 and 14. Mortality and toxicity observations were made continuously for the first hour post-dosing, approximately hourly on the first

day and then once daily for 14 days (with the exception of weekends when no symptoms were noted the day prior). Necropsy examinations were conducted on all surviving birds in the treatment groups 551 and 1050 mg/kg bw as well as on all deceased birds during the in-life phase of the study.

The LD_{50} value with 95%-confidence intervals was calculated by using a computer program which estimated the LD_{50} using one of three statistical techniques: moving average, binominal probability or probit. The appropriate method was determined on the basis of the data characteristics.

Results and Discussion

Validity criteria were not assessed as part of the study report.

Mortalities and symptoms of intoxication occurred only on the first three days after application. There were 10%, 50% and 100% morality observed at 551, 1050 and 2000 mg/kg bw, respectively. There were symptoms of intoxication observed at 551, 1050 and 2000 mg/kg bw.

Table CP 10.1.1.1/01-1 Mortality and toxic observations at test termination

Dose level (mg/kg bw)	No. of birds dosed	No. of mortalities	Toxic symptoms
0	10	0	0
152	10	0	0
289	10	0	0
551	10	1	4 (AP)
1050	10	5	9 (AP, CO)
2000	10	10	10 (SA, LS, CO)

AP = apathy, SA = severe apathy, LS = laying on the side, CO = convulsions

Table CP 10.1.1.1/01-2 Mean body weights of birds during the study

Dose level (mg/kg bw)	Male body weights (g)			Female body weights (g)		
	Day -1	Day 7	Day 14	Day -1	Day 7	Day 14
0	185.4	186.6	187.6	192.4	198.0	208.0
152	193.4	192.0	197.4	209.2	224.0	235.4
289	181.8	178.4	182.0	196.2	199.4	211.8
551	181.2	170.8	180.8	215.6	213.0	232.2
1050	190.2	170.5	184.3	189.0	187.0	211.0
2000	192.4	-	-	200.8	-	-

Table CP 10.1.1.1/01-3 Mean food consumption per bird per day

Dose level (mg/kg bw)	Daily feed consumption per bird (g)		
	Days 0 - 3	Days 3 - 7	Days 7 - 14
0	13.5	12.7	14.5
152	14.7	18.1	16.9
289	10.3	15.5	15.4
551	3.6	16.8	17.9

Dose level (mg/kg bw)	Daily feed consumption per bird (g)		
	Days 0 - 3	Days 3 - 7	Days 7 - 14
1050	0.8	17.2	21.1
2000	2.2	0.0	0.0

Conclusion

The acute oral LD_{50} for bobwhite quail orally dosed with KWG 4168 EC 500 was calculated to be 971 mg/kg bw (equivalent to 477 mg a.s./kg bw). The NOEL was determined to be 289 mg/kg bw based on symptoms of intoxication and one case of mortality at the 551 mg/kg bw level. Mortalities and symptoms of intoxication occurred only on the first three days after application. No mortalities were observed either in the control, or in the groups dosed with 152 and 289 mg/kg bw.

Assessment and conclusion by applicant:

The study was conducted to the U.S. EPA E 71-1 (1982) test guideline but the test methodology used is considered to be consistent with the requirements of the current OECD 223 test guideline and is therefore considered acceptable.

The study was assessed against the current OECD test guideline "OECD 223: Avian acute oral toxicity test", adopted 29 July 2016.

Validity criteria according to OECD 223 were met:

- Control mortality to not exceed 10% at the end of the test (actual: 0.0%).

The study is therefore considered acceptable.

The acute oral LD_{50} for bobwhite quail orally dosed with KWG 4168 EC 500 was calculated to be 971 mg/kg bw (equivalent to 477 mg a.s./kg bw).

CP 10.1.1.2 Higher tier data on birds

Ecological data

The following ecological data are available and considered relevant to the proposed use of Spiroxamine EC 500 in grapes.

Data Point:	KCP 10.1.1.2/16
Report Author:	[redacted]
Report Year:	2003
Report Title:	Vogelcoenosen suedwestdeutscher Weinberge
Report No:	Lit. 8234
Document No:	M-242340-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Introduction

The species composition of birds in south-west German vineyards was investigated at four study sites from April to July 2002 using the territory mapping method. The selection of areas with different habitat elements allowed to investigate the influence of habitat elements on the bird species composition. Vineyards are used by a number of bird species as breeding or foraging habitat. Twenty-four of 44 species were confirmed breeding species and six were potentially breeding. The linnet proved to be the only characteristic breeding species for vineyards, while the other species were only associated with certain habitat elements. At the same time structural diversity encourages biological diversity. Compared with other habitat elements, the actual vineyards is only used to a limited amount by the different bird species.

I. Material and methods

Four vineyards in the southwest of Germany were observed: [redacted] and [redacted] in the vineyard region Baden, and two vineyards in the [redacted] region ([redacted]). Each study area was 15.8 ha and contained structural elements like vineyard area, forest, bushes, garden, meadow, field, houses, path and huts.

For the bird species recording the territory mapping method as recommended by Erz *et al*. (1968) was used, whereby the evaluation of edge-inhabiting breeding birds was done according to Scherner (1981).

From the early April to early July 2002 ten observations were performed; eight in the early mornings and two in the evening. All visual and acoustic observations were recorded

II. Results

Forty-four bird species were recorded in the four study sites.

Table CP 10.1.1.2/16-1 List of observed bird species in four vineyards in southwest Germany

	Katzenberg	Kuhberg	Fuchsen	Löhrer Berg
Common Buzzard	N	N	N	N
Common Kestrel	N	N	N	N
Grey Partridge				P
Common Pheasant			P	
Common Pigeon				N
Common Wood Pigeon	N	N	N	N

	Katzenberg	Kuhberg	Fuchsen	Löhrer Berg
European Turtle Dove			N	N
European Green Woodpecker	N	N		
Great Spotted Woodpecker	N			
Eurasian Skylark			B	B
Barn Swallow	N			N
Tree Pipit	B			
White Wagtail	B			
Eurasian Wren	B		P	
Dunnock		B		
European Robin	B	B	B	
Black Redstart	B	B		N
European Stonechat			B	
Song Thrush	B	P	N	
Fieldfare		P		
Common Blackbird	B	B	B	N
Garden Warbler	P	B	B	
Eurasian Blackcap	B	B	B	
Common Whitethroat	B	B	B	
Common Chiffchaff	B	B	B	
Common Firecrest	P			
Great Tit	B	B	B	
Eurasian Blue Tit	B	B		
Eurasian Nuthatch	B			
Red-backed Shrike		P	B	
Eurasian Magpie	N	N	N	
Eurasian Jay		N	N	
Western Jackdaw			N	N
Carrion Crow	N	N	N	N
Common Starling	P	N	N	N
Eurasian Golden Oriole			N	
House Sparrow	P			
Eurasian Tree Sparrow	B	B		
Common Chaffinch	B	B	B	
Common Linnet	B	B	B	B
European Goldfinch	P	P		
European Greenfinch	B	P	B	N

	Katzenberg	Kuhberg	Fuchsen	Löhrer Berg
European Serin	B	B	B	N
Yellowhammer		B	B	B

B: breeding bird, P: potential breeding bird, N: food guest

In the following, the results are only presented for the black redstart as it is the focus of the risk assessment.

Table CP 10.1.1.2/16-2 Territories, abundance and dominance of the black redstart in the four study sites

Katzenberg			Kuhberg			Fuchsen			Löhrer Berg		
R	A	D	R	A	D	R	A	D	R	A	D
4.0	2.5	11.3	1.5	0.9	4.8	0.0	0.0	0.0	0.0	0.0	0.0

R: territories, A: abundance [territories/10 ha]; D: Dominance [%]

III. Conclusions

Vineyards provide a breeding or feeding habitat for a number of bird species, a structural diversity promotes species richness. Only the linnet was found to be the characteristic bird species in vineyards. All other bird species are dependent on certain habitat structures. The actual vineyard area is only used to a very limited amount. The actual vineyard serves only for a few bird species as territory, like the linnet, serin and yellowhammer, as they breed in the vine. The skylark is especially dominant in more open locations. The occurrence of the black redstart and tree sparrow depends on suitable nesting sites, such as huts. In the presence of field shrubs, a number of other species are present, like the blackbird, robin and great tit. Branch warbler species also occur here, but do not use the vineyard itself. As a foraging habitat, vineyards are important for some bird species. In addition to breeding birds, 14 other bird species - mainly pigeons, ravens and birds of prey have been recorded - who visited the study sites as food guests.

Assessment and conclusion by applicant:

This ecological monitoring study was not conducted to GLP nor was it conducted according to a specific test guideline. However, this is typical of studies of this type therefore the study is still considered to be acceptable for regulatory purposes by supporting various aspects of the Bird & Mammal risk assessment.

The results of this study have been used as part of the refined risk assessment of the small insectivorous species “redstart” scenario, specifically to support the use of the black redstart as a suitable focal species in vineyards.

Data Point:	KCP 10.1.1.2/01
Report Author:	[redacted]
Report Year:	2006
Report Title:	Bird species in vineyards in France and Italy field data for the determination of focal species
Report No:	RA05-005
Document No:	M-291192-01-1
Guideline(s) followed in study:	EU Council Directive 91/414/EEC amended by the Commission Directive 96/68/EC; SANCO 4145/2000
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In this generic study, a field survey program was carried out to identify and quantify bird species found in vineyards in France and Italy during different grapevine growth stages. Investigated parameters included the qualitative composition of the bird community encountered in vineyards, the frequency of occurrence and dominance of species in vineyards, and the variation of these parameters in relation to seasonal changes. Finally, these species were assigned to foraging guilds, *i.e.* ground-foraging or foliage-foraging, diet guilds and size classes. The aim was to propose a list of candidate bird species in selected vineyards that can be addressed as focal bird species in a refined risk assessment for plant protection products. The objectives of this generic study were to determine the qualitative and quantitative composition of the bird community employing the parameters, frequency of occurrence (FO_{field} and FO_{survey}) and dominance both as overall and as grapevine growth stage specific descriptors. Another objective was then to allocate the selected species to defined foraging guilds, diet guilds and size classes.

The linnet and wood lark were the most characteristic and stable elements of the bird community in vineyards in France across all vine growth stages. The blackbird and chaffinch were the most characteristic and stable elements of the bird community in vineyards in Northern Italy ([redacted]) across all vine growth stages. The goldfinch was the most characteristic and stable element of the bird community in vineyards in Southern Italy ([redacted]) across all vine growth stages. All species listed can be considered as potential candidates for focal bird species in a refined risk assessment for plant protection products in vineyards in France and Italy.

Study area

The study was conducted in the [redacted] regions of France and the [redacted] and [redacted] regions of Italy, encompassing 30, 15 and 15 vineyards, respectively.

The total transect area across all fields was 87.1 ha, 39.7 ha and 49.4 ha, respectively.

I. Method and parameters

The Field Phase of this study was carried out during spring / summer (March to July) 2005.

In order to cover different vine growth stages, three line transect surveys were conducted in 2005 for each vineyard during dormancy and/or bud development (survey 1), leaf development or inflorescence emergence (survey 2) and inflorescence emergence and/or development of fruits (survey 3). A standard line transect consisted of an ‘in-crop transect band’ (a 50 m wide recording band of 25 m to each side

of the observer moving along a longitudinal in-crop field transect). For the assessment of the bird community, frequency of occurrence (FO_{field} and FO_{survey}) and dominance were determined.

Data recording and analysis

Data were analysed using the "Ecology Research Database System" (ERDS). The ranking of species within the list of focal species candidates was carried out in decreasing order of importance, *i.e.* FO_{field}>FO_{survey}>dominance. This list of candidates of focal species was then used to allocate the respective species to defined foraging guilds, diet guilds and size classes.

FO_{field}: denotes the number of vineyards in which a defined species was recorded, given as percentage of the total number of vineyards regardless of the number of individuals observed. This approach serves as a measure for the spatial frequency of occurrence. A FO_{field} of 100% for one species indicates that this species was observed in all vineyards (*e.g.* n=30 in France) during at least one survey.

FO_{survey}: denotes the number of surveys in which a defined species was recorded, given as percentage of the total number of surveys. This approach gives an approximation for the temporal evenness of occurrence throughout the complete study period. A FO_{survey} of 100% means the species was recorded in each survey (e.g. n=90 in France) in every vineyard with at least one individual.

Dominance is reported as the percentage number of individuals of the respective species compared to the total number of individuals throughout all species (calculated as arithmetic means over all vineyards).

II. Results and Discussion

The study was deemed to be acceptable based on the criteria set out in Bibby *et al.* 1992, in the absence of an OECD guideline.

A total of 722, 739 and 184 individual bird contacts, comprising 36, 33 and 18 different species, was recorded throughout all surveys within the 'in-crop transect bands' in the Champagne-Ardenne/Bourgogne region of France, and the Tyrol and Apulia regions of Italy, respectively. The following species listed in the tables below were recorded with a frequency of occurrence (FO_{field}) >20% and were thus determined as the main candidates for focal bird species in the respective country. In most cases the findings were supported by FO_{survey} and dominance data (see tables below).

Table CP 10.1.1.2/01-1 Frequency of occurrence, dominance and list of candidates of focal species in vineyards in France (Champagne-Ardenne/Bourgogne)

Species	FO_{field} n=30 (%)	FO_{survey} n=90 (%)	Dominance n=30 (%)
Linnet (*Carduelis cannabina*)	76.7	42.2	23.3
Wood lark (*Lullula arborea*)	66.7	40	11.2
Skylark (*Alauda arvensis*)	63.3	34.4	6
Carrion crow (*Corvus corone*)	60	22.2	5
Blackbird (*Turdus merula*)	56.7	33.3	7.6
Chaffinch (*Fringilla coelebs*)	40	18.9	3.6
Cirl bunting (*Emberiza cirlus*)	36.7	25.6	8.9
Great tit (*Parus major*)	36.7	17.8	3.3
Greenfinch (*Carduelis chloris*)	36.7	14.4	3.3
Black redstart (*Phoenicurus ochruros*)	30	12.2	2.5
Goldfinch (*Carduelis carduelis*)	20	6.7	1.4

FO_{field}: denotes the number of vineyards in which a defined species was recorded, given as percentage of the total number of vineyards, regardless of the number of individuals observed. This approach serves as a measure for the spatial frequency of occurrence

FO_{survey}: denotes the number of surveys in which a defined species was recorded, given as percentage of the total number of surveys. This approach gives an approximation for the temporal evenness of occurrence throughout the study period

Dominance: denotes the relative occurrence of bird species within the bird community. It is reported as the percentage of individuals of the respective species compared to the total number of individuals of all species (calculated as arithmetic means over all vineyards)

Table CP 10.1.1.2/01-2 Frequency of occurrence, dominance and list of candidates of focal species in vineyards in Northern Italy (Tyrol)

Species	FO_{field} n=30 (%)	FO_{survey} n=90 (%)	Dominance n=30 (%)
Blackbird (*Turdus merula*)	100.0	98.0	36.4
Chaffinch (*Fringilla coelebs*)	100.0	82.4	15.2
Greenfinch (*Carduelis chloris*)	76.5	39.2	5.3
Serin (*Serinus serinus*)	76.5	31.4	5.2
Tree sparrow (*Passer montanus*)	70.6	39.2	8.5
Spotted flycatcher (*Muscicapa striata*)	64.7	27.5	4.6
Song thrush (*Turdus philomelos*)	41.2	23.5	6.6
Fieldfare (*Turdus pilaris*)	41.2	17.6	3.1
Great tit (*Parus major*)	41.2	13.7	1.2
Wryneck (*Jynx torquilla*)	35.3	11.8	0.8
Goldfinch (*Carduelis carduelis*)	23.5	9.8	1.4
Wheatear (*Oenanthe oenanthe*)	23.5	7.8	1.1
Hoopoe (*Upupa epops*)	23.5	7.8	1.1

FO_{field}: denotes the number of vineyards in which a defined species was recorded, given as percentage of the total number of vineyards, regardless of the number of individuals observed. This approach serves as a measure for the spatial frequency of occurrence

FO_{survey}: denotes the number of surveys in which a defined species was recorded, given as percentage of the total number of surveys. This approach gives an approximation for the temporal evenness of occurrence throughout the study period

Dominance: denotes the relative occurrence of bird species within the bird community. It is reported as the percentage of individuals of the respective species compared to the total number of individuals of all species (calculated as arithmetic means over all vineyards)

Table CP 10.1.1.2/01-3 Frequency of occurrence, dominance and list of candidates of focal species in vineyards in Southern Italy (Apulia)

Species	FO_{field} n=30 (%)	FO_{survey} n=90 (%)	Dominance n=30 (%)
Serin (*Serinus serinus*)	93.3	33.3	12.5
Goldfinch (*Carduelis carduelis*)	73.3	40	14.4
Crested lark (*Galerida cristata*)	73.3	31.1	9.8
Tree sparrow (*Passer montanus*)	60	28.9	11.4

Species	FO_{field} n=30 (%)	FO_{survey} n=90 (%)	Dominance n=30 (%)
Linnet (Carduelis cannabina)	60	24.4	22.8
Magpie (*Pica pica*)	53.3	22.2	9.2
House sparrow (*Passer domesticus*)	40	15.6	7.6
Hoopoe (*Upupa epops*)	20	6.7	1.6

FO_{field}: denotes the number of vineyards in which a defined species was recorded, given as percentage of the total number of vineyards, regardless of the number of individuals observed. This approach serves as a measure for the spatial frequency of occurrence

FO_{survey}: denotes the number of surveys in which a defined species was recorded, given as percentage of the total number of surveys. This approach gives an approximation for the temporal evenness of occurrence throughout the study period

Dominance: denotes the relative occurrence of bird species within the bird community. It is reported as the percentage of individuals of the respective species compared to the total number of individuals of all species (calculated as arithmetic means over all vineyards)

Frequency of occurrence values during different vine stages of growth

When analysing bird frequencies of occurrence during different vine stages the focal species, some variation regarding to their occurrence was observed over time.

In France, the linnet showed high FO values throughout the season with a maximum of 50.0% during the first survey period; the skylark, blackbird and wood lark also showed high FO values during the third survey with peaks of 50.0%, 46.7% and 43.3%, respectively. FO values of the chaffinch, great tit, cirl bunting, carrion crow and greenfinch showed small maxima (23.3 - 36.7%) during the first survey period, though levels in some cases were lower during the second and third survey periods.

In Northern Italy, the blackbird showed moderate to high FO values throughout the season with maxima of 100% during the second and third survey periods; the chaffinch also showed high FO values with a peak of 88.2% during both the first and second surveys. FO values for the tree sparrow, serin, wryneck and great tit reached their maxima (23.5 to 52.9%) during the second survey period. The spotted flycatcher, greenfinch and song thrush showed increasing FO values with peaks of 35.3 to 52.9% during the third survey, whereas the fieldfare showed a high FO value (41.2%) only during the first survey.

In Southern Italy, the goldfinch and tree sparrow showed homogeneous FO values throughout the season with maxima of 46.7% and 33.3% during the first and second survey periods, respectively. The serin displayed high FO values during the first and third surveys with a peak of 53.3% during the first survey, whereas the linnet, crested lark, magpie and house sparrow showed decreasing FO values with peaks of 20.0 to 60.0% during the first survey. The recorded candidates of focal bird species were assigned to the following guilds in accordance with the SANCO guidance document (ranked by their respective frequency of occurrence and dominance):

France

Small insectivore: great tit (ground/foliage) >black redstart (ground)

Small granivore: linnet >goldfinch (all ground/foliage)

Small omnivore: wood lark (ground) >skylark (ground) >chaffinch (ground/foliage) >cirl bunting (ground) >greenfinch (ground/foliage)

Medium omnivore: blackbird (ground/foliage)

Large omnivore: carrion crow (ground)

Northern Italy

Small insectivore: spotted flycatcher (foliage) >great tit (ground/foliage) >wryneck

	(ground/foliage) >wheatear (ground)
Small granivore	serin >goldfinch (all ground/foliage)
Small omnivore	chaffinch >greenfinch >tree sparrow (all ground/foliage)
Medium insectivore	hoopoe (ground)
Medium omnivore	blackbird >song thrush >fieldfare (all ground/foliage)
Southern Italy	
Small granivore	serin >goldfinch >linnet (all ground/foliage)
Small omnivore	crested lark (ground) >tree sparrow >house sparrow (all ground/foliage)
Medium insectivore	hoopoe (ground)
Medium omnivore	magpie (ground/foliage)

III. Conclusion

The linnet and wood lark were the most characteristic and stable elements of the bird community in vineyards in France across all vine growth stages. Analogous results were detected for the blackbird, cirl bunting and carrion crow, but with lower FO_{field} values. Other species, showing peak FO values >20% for individual vine growth stages only were the skylark (for surveys 2 and 3), chaffinch, great tit and greenfinch (for survey 1). The blackbird and chaffinch were the most characteristic and stable elements of the bird community in vineyards in Northern Italy (Tyrol) across all vine growth stages. Similar results were obtained for the greenfinch and tree sparrow, but with lower FO_{field} values. Other species, showing peak FO values >20% for individual vine growth stages only were the serin and spotted flycatcher (for surveys 2 and 3), song thrush (for surveys 1 and 3), fieldfare (for survey 1), wryneck, great tit and wheatear (for survey 2).

The goldfinch was the most characteristic and stable element of the bird community in vineyards in Southern Italy (Apulia) across all vine growth stages. Analogous results were detected for the tree sparrow, but with lower FO_{field} values. Other species, showing peak FO values >20% for individual vine growth stages only were the serin (for surveys 1 and 3), crested lark (for surveys 1 and 2), linnet, magpie and house sparrow (for survey 1). All the species listed in the tables above can be considered as potential candidates for focal bird species in a refined risk assessment for plant protection products in vineyards in France and Italy, respectively.

Assessment and conclusion by applicant:

This ecological monitoring study was not conducted to GLP but this is typical of studies of this type. Recognised bird census methodology was adopted as part of this study. The study is therefore still considered to be acceptable for regulatory purposes by supporting various aspects of the Bird & Mammal risk assessment.

The results of this study have been used as part of the refined risk assessment of the small insectivorous species "redstart" scenario, specifically to support the use of the black redstart as a suitable focal species in vineyards.

The results of this study have also been used as part of the refined risk assessment of the small granivorous bird "finch" scenario, specifically to support the use of the linnet as a suitable granivorous focal species in vineyards.

Data Point:	KCP 10.1.1.2/17
Report Author:	[redacted]
Report Year:	2007
Report Title:	The use of vineyards by birds in Southern France: An ecological study to refine the risk assessment for insecticide use
Report No:	ER-07-KCB-277
Document No:	M-427241-01-1
Guideline(s) followed in study:	SANCO/4145/2000
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in the Addendum No. 2 to the DAR (2012)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	No

Executive Summary

In this large-scale field study, a combination of visual observations and radio-tracking was carried out to determine the species present in commercial vineyard habitats in Southern France. A specific aim of the study was to determine what proportion of time selected focal species spent "in-crop" and "off-crop" and to generate data to show the distribution of PT.

Radio-tracking data showed that the proportion of the total number of readings for Black Redstarts taken that were in-crop ranged from 0% to 74.8%. Of the 20 birds tracked in this study, 1 individual (5%) spent more than 90% of its foraging time in the vineyards. Two birds (10%) spent 70-80% of their time in the vineyards and one (5%) spent 40-50% of it's time in vineyards. Three birds (15%) spent 30-40% of their foraging time in vineyards, five birds (25%) spent 20-30% of their time and two birds (10%) spent 10-20% of their active time in vineyards. Four birds (20%) spent 1-10% of their time in vineyards and only two individuals spent none of their time in the vineyards. The mean PT for Black Redstarts derived from a sample size of 20 birds was 28.5% with a standard deviation of 25.6% with a 90th centile value of 75%.

Study area

The study was conducted in a block of eight mature commercial vineyards at [redacted], [redacted], [redacted], [redacted], France with an area of approximately 9 ha., with every second row kept short by mowing for tractor access. Each vineyard had grass between the rows and each row was 2.4 m apart. The off-crop areas of the study site were surveyed on 21 June 207 and the details of the 22 different areas recorded. A habitat map of the site was loaded into Field Track Map Measure software (Charles Collinson, Co-ordinated Computer Integration, [redacted], UK) to determine the proportion of the surface area of the site map made up of vineyards compared to non-crop habitat. The surface area taken up by the vineyards in an 800m × 800 m square (64 ha in area).

I. Methods

A preliminary survey of the field site was carried out from 11 to 12 May 2007. The field phase of the study was carried out between 18 May and 21 June 2007.

An electronic data-logging weather station was established at the site to record maximum and minimum air temperature (1m above the ground) and daily rainfall.

Crop growth stage and general health

The BBCH growth stage of the vines was recorded at the study site each week throughout the study. In order to maintain a healthy and commercially viable vine crop the grower applied fungicides to the crop

throughout the study period, particularly after periods of heavy or prolonged rainfall. Similar fungicide products were being applied to neighbouring vines, which were later used by birds being tracked in this study.

Black redstarts catching, colour ringing and radio-tagging

Preliminary observation of the study site recorded Black Redstart as the insectivorous bird species spending the most time within the vine crop. At least twenty Black Redstart were caught and radio-tracked to determine the proportion of time each bird spent in the-crop (33 birds were caught, with 21 being successfully tagged. One bird shed its tag before tracking could take place). Birds were caught with mist-nets established at the site or in nearby farmland, within the vine crop rows or across the ends of the rows. Black Redstars on nests were also caught and radio-tagged by placing the mist-nets close to the nest early morning or at dusk.

Radio tags were attached to the tail feathers using cyanoacrylate glue. Each bird was also ringed with a uniquely marked metal ring and colour ringed for visual identification. During ringing, each bird was aged, weighed, sexed and its wing length recorded. The transmitting life of the tags was approximately 12 days.

Tracking for each bird began at 06:00 and continued until 21:00 and its location was recorded every 5 minutes throughout the day. Bird behaviour, whether in-crop or off-crop and whether seen feeding was also recorded for each time point. If the bird was not detected at a given time point then that point was missed and the next 5 minute interval used. Time spent on the nest, or conducting activities which were not foraging, such as singing or preening, were excluded from daily total before apportioning PT.

Vantage point surveys

Vantage Point Surveys were conducted for whole days (06:00 – 21:00) at twenty vineyards in the region of the main study site to record the extent of vineyard bird activity over a wider area. Although the study site and vineyards used for the black-redstart work were mostly grassy, both within and between rows, there were many vineyards in the area of Vaison la Romaine which comprised bare soil only or had only limited vegetation between the rows. Vineyards adjacent to the tracks were classified as being bare soil with virtually no vegetation (1), limited vegetation between rows (2) or long grass between at least 50% of the vines.

Suitable fields were those deemed to be small enough to see birds entering the crop from the vantage point but not so small as to be unlikely to have birds visiting them. Suitable sites had a vantage point high enough to enable the whole field to be seen. Vineyards for the vantage point surveys were chosen using a ratio based on the results of the ground cover surveys giving ten fields of bare soil vineyards (1), five fields of limited vegetation (2) and five of long grass on 50% or more of the rows (3).

Surveys were carried out to observe whole vineyards and record the species and number of birds seen or heard entering or leaving the vineyard under observation. In addition, a list of species seen or heard at that location but not in the vineyard was generated. These were the birds that could have entered the vineyard, because they were known to be in the vicinity but did not do so.

II. Results and Discussion

The highest temperature during the study was 31.75°C and the lowest temperature recorded was 1.33°C during the study.

Crop growth stage

The BBCH growth stage of the vines ranged from 65 to 79 during the study.

Radio-tracking

Ten male and ten female Black Redstarts were caught, fitted with radio-tags and tracked for one day from 06:00 to 21:00. Nine of these birds were caught and tracked on or near to the core study site at Put du Maupas and the remaining eleven were on neighbouring farms.

Table CP 10.1.1.2/17-1 Body weight and wing length of radio-tagged birds

Bird No.	Age	Sex	Wing length (mm)	Body mass (g)	Nesting status
1	5	F	83	n.a..	Feeding fledgling
2	6	M	89	16.3	Feeding fledglings
3	6	M	92	16.6	Chicks in nest
4	5	F	84	15.7	Feeding fledglings
5	5	M	85	16.2	Chicks in nest
6	6	F	81	14.2	Feeding fledglings
7	6	M	87	15.5	Not nesting
8	5	F	81	15.4	Not nesting
9	5	M	85	14.1	Chicks in nest
10	6	M	88	16.9	Chicks in nest
11	6	M	89	16.5	Feeding fledglings
12	5	F	81	15.1	Feeding fledglings
13	5	F	84	15.3	Eggs in nest
14	6	M	86	15.6	Eggs in nest
15	5	M	86	16.4	Not nesting
16	5	F	84	17.3	Eggs in nest
17	5	M	84	14.4	Eggs in nest
18	5	F	81	16.3	Eggs in nest
19	5	F	83	15.6	Chicks in nest
20	5	F	81	13.3	Feeding fledglings
Mean			84.7	15.6	-

n.a. = not applicable

Table CP 10.1.1.2/17-2 Summary of radio-tracking data

Bird No.	Total number of recordings	Number of recordings In-crop	Number of recordings Off-crop	Number of recordings in "nest area"	Proportion of time spent In-Crop	Proportion of range that is vines (%)	Estimated PT (excluding "nest area")%
19	135	101	34	23	74.81	77.94	90.18[2]
15	148	37	111	100	43.15	43.15	77.08[1]
12	140	85	55	26	75.44	75.44	74.56[1]
4	142	39	103	53	50.32	50.32	43.82[2]
13	159	12	147	122	25.24	25.24	32.43[2]
3	147	33	114	40	46.61	46.61	30.84[2]
6	168	28	140	77	3.62	3.62	30.77[2]
9	146	35	111	27	62.20	62.20	29.41[1,2]

5	94	22	72	13	21.36	21.36	27.16[1]
11	135	27	108	32	40.92	40.92	26.28[1]
2	143	23	120	54	20.14	20.14	25.84[1]
20	140	23	117	42	31.91	31.91	23.47[2]
10	117	17	100	20	81.49	81.49	17.53[2]
14	154	10	144	74	0.00	0.00	12.5[2]
16	169	2	167	148	0.00	0.00	9.52[2]
17	174	10	164	25	5.47	5.47	6.71[2]
8	157	9	148	8	22.11	22.11	6.04[2]
7	123	5	118	24	16.52	16.52	5.05[2]
1	131	0	131	4	0.00	0.00	0[2]
18	181	0	181	157	0.00	0.00	0[2]

[1]Taken from visual observations that the bird is not feeding, not foraging and not provisioning

[2]Taken from number of locations in the areas nominated as "nest area". May include locations where visual observation was not possible

Vantage point survey data

Vantage point survey data from the additional twenty vineyards in the area showed that twenty-seven different bird species were recorded. Eight species were only seen on only one site and the number of birds seen on a single site ranged from one to thirteen. Whilst species diversity may be enhanced with long grass the relationship is not straightforward as three bare soil sites all had seven or more species seen during the period from 06:00 to 21:00 hours. Excluding the aerial feeders, which were deemed to have little exposure to residues, Black Redstart was the insectivorous species with the highest recorded time in vines (2h 40 minutes) over 83 visits) and was seen in 9 of the 20 vineyards. Woodlark (*Lullula arboreа*), although only seen in five of the 20 vineyards, had a total recorded time in-crop of 2h 11 minutes over 24 visits. Cirl Bunting (*Emberiza cirlus*) spent 1h 52 minutes in-crop over 16 visits.

III. Conclusion

Excluding aerial feeders, the insectivorous species observed spending the most time within vines were Black Redstart, Wood Lark, and Cirl Bunting. As the most frequently observed and abundant in-crop species over a landscape scale Black Redstart was considered to be a suitable focal species for small insectivorous birds in vines in Southern France.

When the crop represented approximately 50% of the land area the mean PT (proportion of diet taken from treated area) for Black Redstarts was 28.5 % with a standard deviation of 25.6% (after correction for time spent on non-foraging activity) with a 90th centile value of 75%.

Of the 20 birds tracked in this study, 1 individual (5%) spent more than 90% of its foraging time in the vineyards. Two birds (10%) spent 70-80% of their time in the vineyards and one (5%) spent 40-50% of it's time in vineyards. Three birds (15%) spent 30-40% of their foraging time in vineyards, five birds (25%) spent 20-30% of their time and two birds (10%) spent 10-20% of their active time in vineyards. Four birds (20%) spent 1-10% of their time in vineyards and only two individuals spent none of their time in the vineyards.

The Black Redstarts tracked in this study had territories ranging from 0.03 ha (for a female with eggs in the nest) to 1.74 ha for a female with chicks in the nest. Males and females of the same pair had clearly overlapping territories but did not always forage in the same places. The mean PT for Black Redstarts derived from a sample size of 20 birds was 28.5% with a standard deviation of 25.6% with a 90th centile value of 75%.

Vantage point survey data from the additional twenty vineyards in the area showed that twenty-seven different bird species were recorded. Eight species were only seen on only one site and the number of birds seen on a single site ranged from one to thirteen. Whilst species diversity may be enhanced with long grass the relationship is not straightforward as three bare soil sites all had seven or more species seen during the period from 06:00 to 21:00 hours.

Excluding the aerial feeders, which were deemed to have little exposure to residues, Black Redstart was the insectivorous species with the highest recorded time in vines (2h 40 minutes) over 83 visits and was seen in 9 of the 20 vineyards. Woodlark (*Lullula arborea*), although only seen in five of the 20 vineyards, had a total recorded time in-crop of 2h 11 minutes over 24 visits. Cirl Bunting (*Emberiza cirlus*) spent 1h 52 minutes in-crop over 16 visits.

Values of PT derived in this study were generated from the area before treatment with chlorpyrifos. After insecticide treatment birds would be expected to obtain less of their diet from the treated crop due to the absence of suitable prey.

Assessment and conclusion by applicant:

This ecological monitoring study was conducted to GLP and followed the principles set out in the previous Bird & Mammal Guidance Document (SANCO/4145/2000). Thus, the study is considered to be acceptable for regulatory purposes by supporting various aspects of the Bird & Mammal risk assessment.

The results of this study have been used as part of the refined risk assessment of the small insectivorous species "redstart" scenario, specifically to justify a refined 90th percentile PT value of 0.75 for the black redstart in vineyards.

Data Point:	KCP 10.1.1.2/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	An ecological study of the use of vineyards by birds in Southern France
Report No:	Lit. 8943
Document No:	M-304340-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Current Guideline, not applicable
Previous evaluation:	yes, evaluated and accepted in the DAR (2011)
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Introduction

A field study was conducted in [redacted], Southern France, during May and June 2007 to determine which bird species make use of commercial vineyard habitats and the proportion of their diet obtained within a treated crop (PT) for small insectivorous birds. The study site comprised 9.25 ha of vines, divided by a mostly dry water course with deciduous woodland over part of its length. 49.5% of the surface area comprised vineyard with the remainder being scrub, woodland, gardens and grassed areas.

I. Materials and Methods

Vantage Point Surveys were conducted (06.00 – 21.00) at twenty vineyards in the vicinity of the site. The proportion of the different ground cover types in 505 vineyards in the area of the study was surveyed to apportion the vantage point surveys to vineyards in a similar ratio. Surveys were carried out by pairs of ornithologists with binoculars and/or telescopes observing whole vineyards and recording the species and number of birds seen or heard entering or leaving the vineyard under observation.

Black Redstart (*Phoenicurus ochruros*) was the insectivorous bird spending the most time within the vine crop at the study site. Mist nets were established at the site or in nearby farmland, either within the vine crop rows or across the ends of rows to catch and radio-tag birds. Where condition and suitable feathers allowed a "Pip 3" tail-mounted radiotag in the frequency range 147-150 MHz (Biotrack, Wareham, Dorset, U.K.) was fitted and birds were colour ringed before being released. Black Redstarts were radio-tracked from 06:00 until 21:00 Where possible the location of the target bird was recorded every 5 minutes throughout the day. Bird behaviour, whether in-crop or off-crop and whether seen feeding or not was also recorded for each time point. As the study progressed the area used to catch birds was increased to include neighbouring farms to the North, South and East of the original site.

II. Results

Table CP 10.1.1.2/02-1 Frequency of observation in field during vantage point surveys

Site No.	3	4	8	10	11	12	14	15	18	1	2	13	19	20	5	6	7	9	16	17	Total
Vegetation type																					
Jay	●		●	●	●		●		●	●		●		●	●	●	●	●	●	●	15
Barn Swallow	●	●	●	●	●					●	●		●	●	●	●		●	●	●	11
Serin		●	●	●		●	●		●		●			●		●	●	●	●	●	13
Black Redstart	●				●				●	●					●	●		●	●	●	9
House Martin		●		●	●			●				●						●	●	●	6
Bee Eater	●			●	●				●					●		●		●			7
Swift			●	●	●							●				●			●	●	7
Woodlark		●							●			●	●	●					●		5
Goldfinch					●									●				●	●		4
Great Tit				●						●								●		●	4
Woodpigeon	●														●			●	●		4
Common Kestrel				●					●							●					3
House Sparrow	●															●			●		3
Magpie													●		●	●					3
Stone Chat				●														●		●	3
White Wagtail				●							●								●		3
Carrion Crow	●										●										2
Cirl Bunting																●		●			2
Starling				●														●			2
Black Kite																				●	1
Blue Tit																		●			1
Buzzard		●																			1
Domestic Dove					●																1
Grey Partridge										●											1
Robin															●						1
Short-Toed Eagle																			●		1
Tawny Pipit																			●		1
Total	7	5	4	11	8	1	2	1	6	5	4	4	3	6	6	10	2	13	12	9	

Vineyards with little or no grass beneath vines and between rows

Vineyards with some grass beneath vines and between rows

Vineyards classed as very grassy beneath vines and between rows

Table CP 10.1.1.2/02-2 Frequency of observation in field during vantage point surveys

Bird No.	Total Number of Recordings	Number of Recordings In-Crop	Number of Recordings Off-Crop	Number of recordings in 'Nest Area'	Proportion of time spent In-Crop (%)	Proportion of Range that is Vines (%)	Estimated PT (excluding 'Nest Area') %
19	135	101	34	23	74.81	77.94	90.18
15	148	37	111	100	25.00	43.15	77.08
12	140	85	55	26	60.71	75.44	74.56
4	142	39	103	53	27.46	50.32	43.82
13	159	12	147	122	7.55	25.24	32.43
3	147	33	114	40	22.45	46.61	30.84
6	168	28	140	77	16.67	3.62	30.77
9	146	35	111	27	23.97	62.20	29.41
5	94	22	72	13	23.40	1.36	27.16
11	135	27	108	32	20.00	10.92	26.21
2	143	23	120	54	16.08	20.14	25.84
20	140	23	117	42	16.43	34.91	23.47
10	117	17	100	20	14.53	81.49	17.5
14	154	10	144	74	6.49	0.00	12.5
16	169	2	167	148	1.18	0.00	9.52
17	174	10	164	47	5.75	5.67	6.71
8	157	9	148	8	5.73	32.11	6.06
7	123	5	118	24	3.97	16.52	3.05
1	131	0	131	4	0.00	0.00	0
18	181	0	181	137	0.00	0.00	0

Figure CP 10.1.1.2/02-1 Total time recorded in crop by species in vantage point surveys

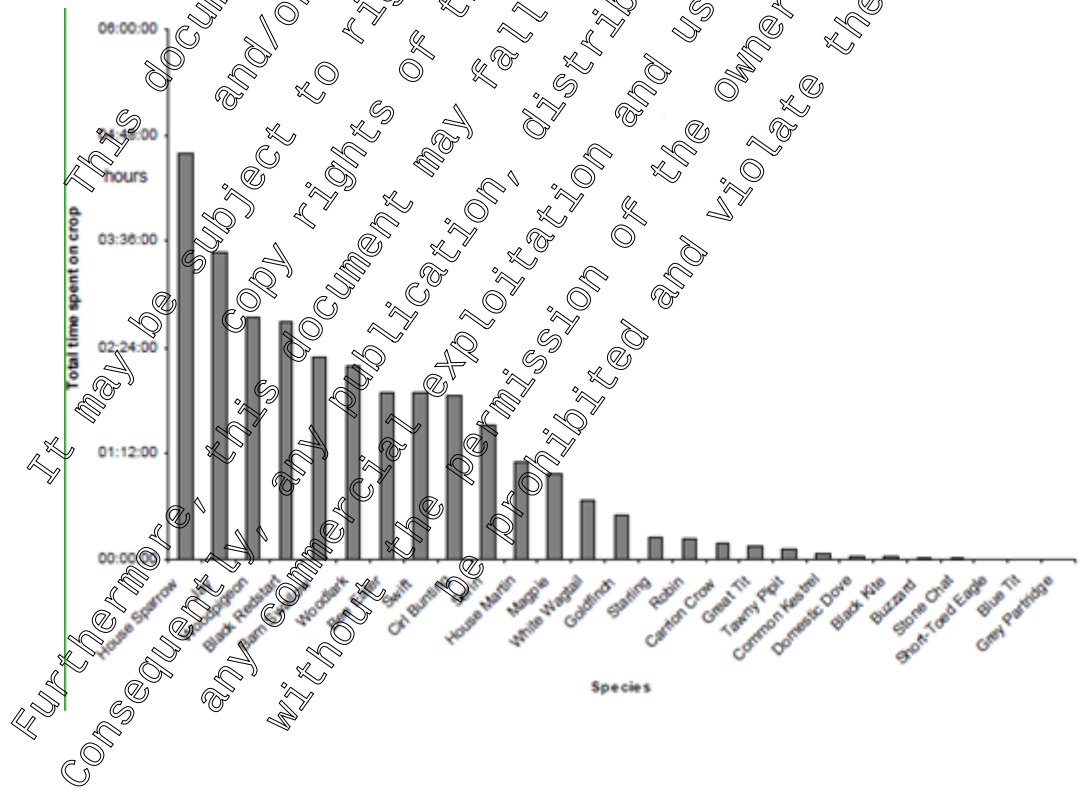


Figure CP 10.1.1.2/02-2 Locations (squares) and home ranges (polygons) of black redstarts at the core study site

III. Conclusions

Considering frequency of observation and time spent within vineyards, black redstart was selected as a focal species in vines. Sampling 20 birds for one day each generated a mean estimate of PT for black redstart of 28% with a 90th percentile of 75%.

Assessment and conclusion by applicant:

This document is a poster presentation of the GLP field study M-427241-01-1 which is summarised above. This has been included here for completeness.

The results of this study have been used as part of the refined risk assessment of the small insectivorous species "redstart" scenario, specifically to justify a refined 90th percentile PT value of 0.75 for the black redstart in vineyards.

Data Point:	KCP 10.1.1.2/18
Report Author:	[redacted]
Report Year:	2012
Report Title:	Foraging behaviour of the black redstart in vineyards in Germany
Report No:	423079
Document No:	M-487359-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted in the Addendum No. 4 to the DAR (rev 2017)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The foraging behaviour of the insectivorous black redstart (*Phoenicurus ochruros*) was investigated in German vineyards. As a consequence of their relatively small body size (average weight = 16 g), feeding on prey exposed to plant protection products (PPPs) could particularly affect this bird species, which is a relevant indicator species for the risk assessment within vineyards. Visual observations of foraging behaviour of 20 black redstarts was recorded. Using radio transmitters, 20 independent foraging events were recorded for each individual bird, distinguishing between foraging on the ground, in vine rows and in the air. Ground-foraging was further divided into foraging that took place either close to or distant from the vine rows. Other behaviours of each bird were also observed and classified into foraging-related and non-foraging-related behaviour. The height of the bird or the vegetation height if on the ground were also recorded.

Black redstarts foraged frequently within the winegrowing area. 96.45 % of all documented *foraging-events* were located on the ground in vineyards. There was no preference in whether the birds chose to forage close to or distant from the vine rows (close: 51.96%, distant: 48.04%), but birds preferred bare compared to overgrown ground when foraging (bare: 69.43%, overgrown: 30.57%). The structures within the vine rows, like the poles, wires and the wine plant itself were used as shelter, look-out and for general, *non-foraging-related* behaviour, *e.g.* preening and resting. The additional behavioural observations strongly support this result: the majority of *foraging-related* behaviour occurred on the ground, whereas the majority of *non-foraging-related* behaviour occurred above the ground.

In summary, the results support previous qualitative studies indicating that the black redstart forages mainly on the ground.

Study area

The study was conducted in vineyards in the Rhine-Hesse, part of the German province of Rhineland-Palantinate in southwest Germany.

I. Methods

The field phase of the study was carried out during summer (June to July) 2012.

Black redstarts trapping, marking and radio-tagging

Two trapping areas were established, both located in the North of the village within the vineyards and were about 150 m apart from each other. The study aimed at tracking at least 20 individuals of each species (25 were trapped but sufficient data was not obtained from 5 of the birds). Birds were trapped using common mist nets (polyester nets, 15 mm mesh wide, 7 m length, 2 m height), which were spanned between poles at a height of up to 3 m and placed into the flying routes of the birds. Captured birds were ringed with a foot ring displaying a unique identification number. Additional coloured rings allowed for

visual identification from a distance. Each black redstart was equipped with a radio transmitter, attached with medical skin adhesive to the back of the bird. The adhesive lasted for about 8 days, after which time the device dropped off. The transmitters were <5% of the bird's body weight (around 3.5% of body weight). Morphological measurements were taken from all captured birds.

Tracking began no less than 3 hours after release to ensure the bird was recovered from treatment. In total, the behaviour of 20 black redstarts within vineyards was observed. Data recording was stopped when there was no movement for longer than 45 minutes observed. Tracking continued in another tracking session once activity was resumed.

Foraging events (forging, pecking, diving, hovering and in-flight catches) were recorded, with distinctions between three different locations being made (ground, air, vine rows). Observations of foraging events were taken at intervals of at least 5 minutes to ensure that recorded events were independent of each other. Additionally, ground-foraging was recorded as either close (<30 cm) or distant (≥30 m) from the vine row.

To assess whether foraging behaviour changed during the tracking period, tracking days were grouped into four groups, each consisting of 4 – 5 successive tracking days, with the proportion of ground and non-ground foraging events.

Additional behaviour data

Behavioural observations were taken at 10 minute intervals. Types of locations were categorised in a similar manner to foraging data, with ground, air and vine row, in addition to other which incorporated all other locations (*e.g.* village, garden, wood pile).

Behavioural data was classified as either *foraging-related* behaviour or *non-foraging-related* behaviour (*foraging*: see *foraging-event*, *non-foraging*: singing, flying, sitting, preening). The sampling of the additional behavioural data was also complemented by recording the height of the observations in intervals of 0.5 m, and if birds were observed on the ground the ground vegetation height in intervals of 10 cm was estimated. It was tested whether the height observation of *foraging-related* behaviour differ significantly from the height of *non-foraging-related* behaviour using in R version 2.13.0.

Habitat mapping and visibility across the season

The grape-vine's BBCH growth stage was recorded, given that the study was conducted over much of the growing season of the grape-vines and as the plants grew, visibility of birds was reduced.

For each individual, it foraging-ground was mapped as minimum covex polygons (MCP) on a specific habitat map, with the habitat classified into six types;

1. Vineyards
2. Hedgerows and trees
3. Ruderal areas
4. Cereal fields
5. Area covered by the village
6. Buildings not connected to the village

II. Results and Discussion

Foraging data

In total 400 foraging-events were obtained (20 for each of the 20 tracked individuals). 96.5% of all observed foraging-events occurred on the ground, 0.75% on the vine plant and 2.75% were in-flight catches. The height of observed foraging-events not on the ground was on average 0.67 m. Of the ground foraging-events 69.43% were on bare ground, 30.57% on overgrown ground with a mean vegetation height of 19 cm. Of the ground foraging-events, 51.96% were close to the vine rows and 48.04% were distant. Number of ground foraging-events and non-ground-foraging-events did not differ significantly

between individuals or among tracking groups, indicating that foraging behaviour did not change during the course of the study.

Behavioural data

In total, 411 behavioural observations (mean: 20.5 per bird, range 15 – 31 per bird) were recorded. In 401 cases, the position of the bird within the habitat was determined (*n*non-vineyard = 93, *n*vinyard = 308). In 306 out of the 401 cases, behavioural information was obtained (*n*non-vineyard = 54, *n*vinyard = 252). Classification of the behavioural observations resulted in 103 (33.66 %) *foraging-related* (*n*non-vineyard = 93, *n*vinyard = 10) and 203 (66.34%) *non-foraging-related* data (*n*non-vineyard = 73, *n*vinyard = 130). A significant difference in the height of *foraging-related* and height of *non-foraging-related* behavioural observations (Wilcoxon two-sided signed-rank test: $p < 0.005$). 92.23 % (all data, 96.51% in vineyards) of *foraging-related* behaviour was observed on the ground whereas only 5.42% (all data, 5.42% in vineyards) of the *non-foraging-related* behaviour occurred on the ground.

Classification into the location-types of behavioural observations (*foraging- & non-foraging-related* behaviour) from within the vineyard resulted in 35.10% *ground*, 45.10% *vine rows* and 19.80% *air*. Notably, 95.35% of *foraging-related* behaviour within the vineyards occurred on the ground and 96.34% of *non-foraging-related* behaviour occurred above the ground.

III. Conclusion

Black redstarts foraged frequently within the wine-growing area. 96.45 % of all documented *foraging-events* were located on the ground in vineyards. Whereas no preference in whether the birds chose to forage close to or distant from the vine rows was detected (close: 51.96%, distant: 48.04%), birds preferred bare compared to overgrown ground when foraging (bare: 69.43%, overgrown: 30.57%). The structures within the vine-rows, like the poles, wires and the wine plant itself were used as shelter, look-out and for general, *non-foraging-related* behaviour, e.g. preening and resting. The additional behavioural observations strongly support this result: the majority of *foraging-related* behaviour occurred on the ground, whereas the majority of *non-foraging-related* behaviour occurred above the ground.

Foraging behaviour was consistent throughout the study period, indicating that the growth stage of vine plants and weather conditions did not influence the birds' foraging strategy.

In summary, the results support previous qualitative studies indicating that the black redstart forages mainly on the ground.

Assessment and conclusion by applicant:

This ecological monitoring study was not conducted to GLP nor was it conducted to a specific method but this is typical of studies of this type. The study is therefore still considered to be acceptable for regulatory purposes by supporting various aspects of the Bird & Mammal risk assessment.

The results of this study have been used as part of the refined risk assessment of the small insectivorous species "redstart" scenario, specifically to support the use of a refined diet of the black redstart of 96.45% ground invertebrates and 3.55% foliar invertebrates.

Data Point:	KCP 10.1.1.2/03
Report Author:	[redacted]
Report Year:	2007
Report Title:	Generic field monitoring of birds in vineyards in France
Report No:	RA05-223/2
Document No:	M-291784-01-1
Guideline(s) followed in study:	EU Council Directive 91/414/EEC amended by the Commission Directive 96/68/EC; SANCO/4145/2000
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In this generic study, a radio-tracking program was carried out in a typical European wine growing region in France during the spring and summer to obtain measured data on PT and PD values for refined exposure assessment. Four wild bird species were monitored in this study, the cirl bunting (*Emberiza cirlus)*, great tit (*Parus major*), linnet (*Cardueli cannabina*) and woodlark (*Lullula arborea*). In the present study bird trapping, radio-tracking, visual observations together with measurement of faecal and stomach content were methods used to characterise PT and PD in vineyards.

The study provided reliable refined parameters of PT and PD for use in higher tier risk assessments for birds foraging in vineyards.

Study area

The study was conducted in the Burgundy region around the municipalities of Santenay, Dezize-lès-Maranges and Cheilly-lès-Maranges (France), a typical wine growing district, and encompassed a study area of 1220 ha.

I. Methods

The study was carried out during spring and summer 2006.

Bird trapping, marking and radio-tagging

The study aimed at tracking at least 20 individuals of each species. A total of 21 cirl buntings, 23 great tits, 28 linnets and 21 woodlarks were actually trapped and tagged, to account for all birds not being successfully tracked afterwards. Birds were trapped using mist nets (polyester nets, 16 mm mesh wide, 9 m length, 2.5 m height, 5 shelves). For woodlarks, a whoosh net was used.

All captured birds were marked with a metal ring in order to enable recognition of individuals during subsequent visual contacts. Colour rings were used to mark birds selected to carry a telemetry transmitter. Transmitters were mounted on their backs and had a range of 0.8 to 3 km in open habitat with no obstacles and had an operational lifespan of two to three weeks.

Individual birds were tracked continuously over an entire activity period (from dawn till dusk). In the case of two cirl buntings, one linnet and three woodlarks, individuals were tracked for 2 daily activity periods. For analysis the respective sessions were pooled. The proportion of time foraging in vineyards (compared with the total potential foraging time) was estimated from the data obtained by radiotracking and visual observation. These values are regarded as equivalent to the proportion of diet obtained from the treated area (PT). To help interpret the PT values, the PT of each bird on the vineyard is compared with the proportion of vineyards in the home range. This comparison (calculated as the Jacobs' index

[D]) illustrates the preference or avoidance of the individual bird for vineyards as a feeding habitat during each tracking session.

To estimate the proportion of different food types in the diet (PD), faeces and/or stomach contents were sampled and analysed. Correction factors derived from the literature were applied to estimate the number of items actually ingested by the birds from the number of arthropods and seeds recognised within the food samples. Estimations of the prey length provided information on the food size selection of the birds. Length-weight regressions of invertebrates and seeds identified via the literature were used to calculate the proportion of dry weight of each food type in the diet actually ingested by the birds.

Individual PT was calculated as:

$$\frac{\text{Time potentially foraging in vineyards}}{\text{Time potentially foraging in all known habitats}}$$

Faeces sampling

To estimate the proportion of different food types in the diet (PD), sampling of faeces and stomach flushings were carried out.

Additional observations

The whole study area was mapped for habitat types and crops at least once during the study period.

The daily average temperature and daily precipitation data were obtained from the nearest weather recording station.

II. Results and Discussion

The study was deemed to be acceptable.

The mean temperature during the study period was 13.8°C. Total precipitation was 432.3 mm, with a daily average of 2.8 mm.

PT values

The combination of radio-tracking with visual appraisal and the trapping scheme as presented here, allowed an accurate and representative assessment of potential foraging times in given home ranges, thus making it possible to calculate reliable PT values. All birds were closely associated with vineyards and had the opportunity to use these fields as a foraging habitat. The results can thus be considered a worst case in terms of potential exposure. Based on the study results (Table, see below), the mean PT value derived for cirl buntings was 0.43 (90th percentile=0.77), for great tits the mean PT value was 0.05 (90th percentile=0.08), for linnets the mean PT was 0.78 (90th percentile=0.97) and for woodlarks 0.86 (90th percentile=1). It is important to emphasise that these values represent the total time spent potentially foraging in vineyards. These values include times when birds may not actually be foraging but information is not available to exclude this possibility. Therefore, the calculated PT values for all species can be regarded as a conservative assumption.

Table CP 10.1.1.2/03-1 Overview of PT values in vineyards

Overview of the PT				
Proportion of the diet obtained in vineyards determined by radio-tracking (PT) = 'potential foraging' time in vineyards as a proportion of the total 'potential foraging' time				
Percentiles	Cirl bunting[1]	Great tit[1]	Linnet[1]	Woodlark[1]
50% tile	0.39	0.02	0.85	0.96
90% tile	0.77	0.08	0.97	1.0
Mean	0.43	0.05	0.78	0.86

[1]Based on 20 tracked individuals

PD values

Cirl buntings ingested invertebrate items and plant seeds in similar proportions (0.499 and 0.501, respectively) related to the total dry weight of their diet. Insect adults and Poaceae seeds were the most important taxa with PD values of 0.297 and 0.334, respectively. Great tits fed mainly on invertebrates (PD value of 0.955), whereas the PD value for seeds was only 0.045. Insect adults and larvae were the most important food items, providing together a PD of 0.788. In contrast, linnets ingested mainly seeds, with a PD of 0.973. Most important were Brassicaceae seeds, providing a PD value of 0.508. The diet of woodlarks consisted basically of invertebrates (PD of 0.921) with insect adults as the most important food items (PD of 0.527).

Table CP 10.1.1.2/03-2 Diet composition values in vineyards

Overview of the PD					
Proportion of different food types in the diet (PD) = invertebrates and plant items actually eaten by individuals foraging in and around vineyards [proportion of dry weight]					
Food type		Cirl bunting[1]	Great tit[2]	Linnet[1]	Woodlark[3]
Invertebrate matter	Insecta* (adult)	0.297	0.405	0.01	0.527
	Insecta* (larvae)	0.151	0.383	-	0.209
	Araneida	0.003	0.165	0.017	0.014
	Gastropoda	0.048	0.002	-	0.172
	TOTAL	0.499	0.955	0.027	0.921
Plant matter (seeds)	Asteraceae	0.002	-	0.042	0.007
	Brassicaceae	-	-	0.508	-
	Caryophyllaceae	0.095	0.0004	0.146	0.0002
	Chenopodiaceae	0.011	-	0.023	-
	Geraniaceae	-	-	0.115	0.001
	Pinaceae	0.005	-	-	0.007
	Poaceae	0.334	0.016	0.084	0.03
	Polygonaceae	0.053	-	-	0.006
	Ranunculaceae	-	-	-	0.001
	Resedaceae	-	-	0.002	-
	Violaceae	0.0001	-	0.004	-
	Labiatae	-	-	-	0.026
	Other seeds	-	0.029	0.05	0.001
	TOTAL	0.501	0.045	0.973	0.079
Proportion of different item length in the diet (PD) = Length of food items actually eaten by individuals foraging in and around vineyards [proportions of dry weight]					
	Size class (mm)	Cirl bunting[1]	Great tit[2]	Linnet[1]	Woodlark[3]
Length of food item	>5	0.472	0.151	1	0.422
	>5-10	0.137	0.414		0.142
	>10-15	0.024	0.050		0.035
	>15-20	0.178	0.223		0.227

	>20	0.188	0.162		0.174

[1]Based on 17 faeces and 3 flushing samples

[2]Based on 16 faeces and 4 flushing samples

[3]Based on 20 faeces samples

*Summarised values for all insect taxa

III. Conclusion

Overall this study provides reliable refined parameters of PT and PD for use in higher tier risk assessments for birds foraging in vineyards.

Assessment and conclusion by applicant:

This ecological monitoring study was conducted to GLP. The study is therefore considered to be acceptable for regulatory purposes by supporting various aspects of the Bird & Mammal risk assessment.

The results of this study have been used as part of the refined risk assessment of the small granivorous bird "finch" scenario, specifically to support the use of a refined 90th percentile PT value of 0.97 for the linnet and to support the use of a diet for the linnet of 97.3% weed seeds and 2.7% ground invertebrates.

The results of this study have also been used as part of the refined risk assessment of the small omnivorous bird "lark" scenario, specifically to support the use of the woodlark as a focal species for vineyards with a 90th percentile PT value of 1.0 and to support the use of a diet for the woodlark of 92.1% ground arthropods and 7.9% weed seeds.

Data Point:	KCP 10.1.1.2/19
Report Author:	[redacted]
Report Year:	2015
Report Title:	Generic field study (GLP) to assess the foraging strata of linnets in vineyards in Germany
Report No:	724069
Document No:	M-516702-01-1
Guideline(s) followed in study:	not applicable
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 foraging strata (*i.e.* on the ground (in vegetation or on bare soil), in the foliage or on upstanding herbaceous perennials) of the linnet (*Carduelis cannabina*) was investigated in two wine-growing regions of South-western Germany. Linnets were observed during two different periods, from mid-April until the beginning of May and the beginning of June until mid-July. For evaluations regarding foraging behaviour, observations were divided into two study periods according to BBCH stages (BBCH 13-19 and BBCH ≥53). During each observation BBCH growth stage, ground vegetation, main plant species of ground vegetation and average ground vegetation height were also recorded.

The preferred foraging stratum of linnets in vineyards was the ground vegetation, followed by bare ground and the top of the ground vegetation. Linnets were never observed to use the vine canopy for foraging. Vineyard characteristics (*i.e.* management strategy and ground vegetation height) seemed to have an impact on the linnets' choice of foraging vineyards, with linnets preferring high ground vegetation followed by those with cut vegetation. The mean ground vegetation height preferred by linnets was 20 cm.

Study area

The study was conducted in vineyards in two wine-growing regions in South-western Germany (three study areas in [redacted] and five study areas in the [redacted]). A vineyard was defined as an area where grapevines grow plus the surrounding area of 1m. Hence, fallow land within the study area was excluded, even though fallow land is highly attractive for foraging linnets. In total, an area of about 275 ha was searched for linnets.

I. Methods

The field phase of the study was carried out in two different time periods, from mid-April until beginning of May 2014 and beginning of June until mid-July 2014.

Linnet observations

Foraging behaviour observations were divided into two study periods according to BBCH stages (Period 1: BBCH 13-19 and Period 2: BBCH >53). Observations of foraging linnets were done during periods when linnets are normally active and only during suitable weather conditions.

Most linnets were searched for by slowly walking through the study area and actively looking for individuals by using acoustic and visual signals to locate them in the area and then by following them to their foraging spot. Sometimes (when possible) linnets were searched for near known linnet nests. These methods of searching for linnets were independent of foraging strata, and it can therefore be assumed that the data collection (*i.e.* how linnets were searched for) had no influence on the probability of detecting and locating birds in specific strata. Once linnets were found they were observed using a pair of binoculars and/or eye sight.

During each study period, about 100 data points were collected in each study region. A data point was defined as a single observation of one foraging individual. Each single observation started when an individual was observed foraging or potentially foraging and took a maximum of about 5 minutes (depending on the time the bird could be followed). When linnets were found foraging in flocks or pairs, then, a second and a third and so on the individual of the flock was observed until either all individuals of the flock were observed once or until the observer could not distinguish between observed and non-observed birds any more. Then, the observer searched for a different flock/pair/individual in the same study area. However, because of the high mobility of linnets it cannot be excluded that individuals in the new flock had been previously observed.

For each data point the following parameters were recorded (partly directly and partly after the observation at the spot the linnet was observed): vineyard ID, time start and end of observation, GPS coordinates of the bird's foraging location, strata of the bird foraging and potentially foraging in and estimated time spent foraging and potentially foraging in each stratum (*i.e.* on bare ground, in ground vegetation, on top of the ground vegetation/upstanding herbaceous perennials, in the vine canopy), estimated percentage of ground cover (in 10% steps in the 1 m radius around the bird), height of ground vegetation (in 1 m radius around the bird), where possible the plant species linnets were feeding on, estimated percentage of seed bearing herbs, ground vegetation of the vineyard (*e.g.* high, cut, bare soil; plus a note if different between several rows), ground vegetation height in the vineyard, and BBCH growth stage.

Data was analysed using Microsoft® Excel 2010.

II. Results and Discussion

For the first study period the mean daily temperature ranged between 7.4°C and 18.8°C with a mean of 12.9°C. On 12 out of 20 days rainfall was registered with a total of 55.6mm. Over this period the precipitation ranged from 0.0 mm to 21.0 mm.

For the second study period, the mean daily temperature ranged between 13.2°C and 26.4°C with a mean of 19.3°C. On 16 out of 45 days rainfall was registered with a total of 95.7mm. Over this period the precipitation raged from 0.0 mm to 15.0 mm.

Foraging data

In total 399 foraging-events were observed. These included several events where foraging was recorded in more than one stratum. Overall, most foraging took place in the ground vegetation (59.74%), followed by foraging events on the bare ground (21.68%) and on top of the vegetation (18.58%). Foraging in the vine canopy was never observed. This pattern was similar in both study regions with ground vegetation being the preferred stratum.

Table CP 10.1.1.2/19-1 Number of foraging events per stratum

Region	On the ground		In the ground vegetation		On top of the ground vegetation	
	Number of foraging events	% foraging events	Number of foraging events	% foraging events	Number of foraging events	% foraging events
Bergstraße/Odenwald	86	25.83	153	45.95	94	28.23
Pfälzer Wald	33	15.28	175	81.02	8	3.70
Total	119	21.68	328	59.74	102	18.58

During 165 foraging events one or more food plants were identified. In total, 14 different genera or species could be distinguished. Among those dandelion (40% *Taraxacum officinale*) and amaranth (32%, *Amaranthus spec.*) were the most used food plants. Most likely due to differences in availability, amaranth was the preferred food plant in the [redacted] region, while in the [redacted] region dandelion was chosen most of the times.

Table CP 10.1.1.2/19-2 Food plants of foraging linnets

Common name	Species	Both study regions	[redacted]	[redacted]
Amaranth	*Amaranthus spec.*	31.98 (55)	50.47 (54)	1.54 (1)
Cockspur grass	*Echinochloa crus-galli*	2.33 (4)	3.74 (4)	0.00 (0)
Common fumitory	*Fumaria officinalis*	0.58 (1)	0.93 (1)	0.00 (0)
Corn salad	*Valerianella spec.*	5.23 (9)	8.41 (9)	0.00 (0)
Daisy	*Bellis perennis*	0.58 (1)	0.93 (1)	0.00 (0)
Dandelion	*Taraxacum officinale*	40.12 (69)	17.76 (19)	76.92 (50)
Dock	*Rumex spec.*	0.58 (1)	0.00 (0)	1.54 (1)
Foxtail or bristle grass	*Setaria spec.*	5.81 (10)	9.35 (10)	0.00 (0)
Grass spec.	*Poales spec.*	5.23 (9)	0.00 (0)	13.85 (9)

Common name	Species	Both study regions	[redacted]	[redacted]
Hairy bittercress	*Cardamine hirsuta*	1.16 (2)	1.87 (2)	0.00 (0)
Hoary alyssum	*Berteroa incana*	1.74 (3)	0.00 (0)	4.62 (3)
Sow thistle	*Sonchus spec.*	0.58 (1)	0.93 (1)	0.00 (0)
Vetch	*Vicia spec.*	1.74 (3)	1.87 (2)	1.54 (1)
Wild lettuce	Lactuca virosa	2.33 (4)	3.74 (4)	0.00 (0)

Vegetation characteristics at foraging spot

The vegetation at the foraging spot (*i.e.* the 1 m radius around the foraging bird) was characterised according to the percentage of ground cover, ground vegetation height and percentage of seed bearing herbs. The percentage of ground cover at foraging spots was very similar in both study regions and both study periods. Generally, it was around 69% with a range of 0 - 100%. Likewise, the ground vegetation height at foraging spots was very similar between study regions and periods with an average of 14 cm and a range of 0 – 50 cm. In contrast, the percentage of seed bearing plants was generally higher in the first study period, and even more in the [redacted] region. This could have been due to differences in management practices and vegetation composition between the regions. The overall percentage of seed bearing plants at foraging spots was 43% with a range of 0 – 100%.

Table CP 10.1.1.2/19-3 Vegetation characteristics at foraging spot (all foraging events)

		[redacted] region	[redacted] region	Total
Ground cover (%)	Mean (SEM)	68.76 (2.18)	68.28 (1.69)	68.52 (1.38)
	Range	10-100	0-100	0-100
Vegetation height (cm)	Mean (SEM)	16.00(0.67)	11.82 (0.54)	13.93 (0.44)
	Range	1-50	0-40	0-50
Seed plants (%)	Mean (SEM)	34.15 (1.46)	51.57 (2.25)	42.79 (1.40)
	Range	5-80	0-100	0-100

32 (8%) of these 399 foraging events were exclusively on bare ground. These foraging events were excluded to calculate the vegetation characteristics for foraging events in the ground vegetation. This resulted in slightly higher ground cover (mean: 73%; range: 10 – 100%), vegetation height (mean: 15 cm; range: 5 – 50 cm) and percentage of seed bearing plants (mean: 45%; range: 0 – 100%).

Table CP 10.1.1.2/19-4 Vegetation characteristics at foraging spot (excluding foraging events on bare ground)

		[redacted] region	[redacted] region	Total
Ground cover (%)	Mean (SEM)	72.93 (2.11)	72.40 (1.44)	72.67 (1.28)
	Range	10-100	10-100	10-100
Vegetation height (cm)	Mean (SEM)	16.74 (0.69)	12.27 (0.56)	14.51 (0.46)
	Range	5-50	5-40	5-50

		[redacted] region	[redacted] region	Total
Seed plants (%)	Mean (SEM)	35.35 (1.52)	53.77 (2.32)	44.54 (1.47)
	Range	5-80	0-100	0-100

Vineyard characteristics

Linnets preferred vineyards with high ground vegetation (50%) followed by those with cut vegetation (20%). In both study regions the percentage of vineyards with high ground vegetation decreased from BBCH 13-19 to BBCH ≥53 (Period 1 to 2). The mean ground vegetation height of vineyards preferred by linnets was quite uniform in both study regions and periods with an average of 20 cm.

The predominant management practices in neighbouring vineyards not chosen by foraging linnets were high or cut ground vegetation (36% or 33% respectively). In the [redacted] region high ground vegetation was more prominent in the first study period while in the second study period alternating stripes of bare ground and cut vegetation were more dominant. In the [redacted] region, in both study periods the majority of vineyards had cut ground vegetation. The mean ground vegetation height was 16 cm. It was similar in both study regions but generally higher in period 1 (BBCH 13-19).

Table CP 10.1.1.2/19-5 Foraging vineyards per management category (%)

Region	Bare ground	Bare ground and cut vegetation	Bare ground and high ground vegetation	Cut ground vegetation	Cut ground vegetation and high ground vegetation	High ground vegetation
[redacted] region	12.82	2.56	2.56	28.21	2.56	51.28
[redacted] region	4.76	4.76	28.57	4.76	9.52	47.62
Total	10.00	3.33	11.67	20.00	5.000	50.00

Table CP 10.1.1.2/19-6 Non-foraging vineyards per management category (%)

Region	Bare ground	Bare ground and cut vegetation	Bare ground and high ground vegetation	Cut ground vegetation	Cut ground vegetation and high ground vegetation	High ground vegetation
[redacted] region	13.51	10.81	2.70	48.65	0.00	24.32
[redacted] region	9.52	14.29	9.52	4.76	4.76	57.14
Total	12.07	12.07	5.17	32.76	1.72	36.21

Table CP 10.1.1.2/19-7 Vineyard characteristics – ground vegetation height (cm) in foraging and non-foraging vineyards

Region	Foraging vineyards		Non-foraging vineyards	
	Mean (SEM) (cm)	Range (cm)	Mean (SEM) (cm)	Range (cm)

[redacted] region	19.45 (1.31)	5.5-35.0	14.08 (1.71)	0.00-35.0
[redacted] region	19.88 (2.52)	2.5-45.0	19.05 (2.25)	2.5-42.5
Total	19.60 (1.21)	2.5-45.0	15.88 (1.38)	0.00-42.5

Foraging behaviour in relation to vineyard management strategies

Linnets preferred foraging in the ground vegetation independent of vineyard characteristics. Overall and in both study regions as well as study periods, the ground vegetation was the favoured stratum throughout the different management strategies. For vineyards known to be under bare ground management the most frequented foraging stratum was the bare ground.

III. Conclusion

The preferred foraging stratum of linnets in vineyards was the ground vegetation, followed by bare ground and the top of the ground vegetation. Linnets were never observed to use the vine canopy for foraging. The preference for foraging in the ground vegetation persisted throughout the different vineyard management strategies. Vineyard characteristics (*i.e.* management strategy and ground vegetation height) seemed to have an impact on the linnets' choice of foraging vineyards.

Assessment and conclusion by applicant:

This ecological monitoring study was conducted to GLP. The study is therefore considered to be acceptable for regulatory purposes by supporting various aspects of the Bird & Mammal risk assessment.

The results of this study are considered to support the findings of the previous study (M-291784-01-1) by demonstrating that the linnet obtains its diet predominantly from the ground vegetation.

Data Point:	KCP 10.1.1.2/20
Report Author:	[redacted]
Report Year:	2009
Report Title:	Generic field monitoring of birds in vineyards in Spain
Report No:	R09123-1
Document No:	M-401943-01-1
Guideline(s) followed in study:	No official test guideline(s) available at present
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

In this generic study, a radio-tracking program was carried out in a typical southern European vine growing region in Spain during spring and summer, to obtain data on PT and PD values for refined exposure assessment. Two wild bird species were monitored in this study, the serin (*Serinus serinus*) and the crested lark (*Galerida cristata*). These species were identified as critical focal species based on their frequency of occurrence and hence high potential for exposure to plant protection products in

vineyards. In this study, bird trapping, radio-tracking, visual observations and analysis of faecal content were conducted to characterise PT and PD/diet estimations in vineyards.

The study provided reliable refined parameters of PT for both focal species, PD values for crested larks and dietary estimates for serins for use in higher tier risk assessments for birds foraging in vineyards in southern Europe.

Study area

The study was conducted in vineyards in the vicinity of [redacted] a municipality in the [redacted] region in north-eastern Spain, a typical area for vine cultivation in southern Europe. The final extent of the study area, determined by the home ranges of all the tracked birds, was 942.8 ha.

I. Methods

The Field Phase of this study was carried out during spring / summer (March to July) 2009.

Bird trapping, marking and radio-tagging

The study aimed at tracking at least 20 individuals of each species. A total of 61 individual serins and 30 crested larks were trapped with mist nets (polyester nets, 16-20 mm mesh wide, 9 m length, 2.5 m height) inside of vineyards (26 serins and 22 crested larks were tagged to account for all birds not being successfully tracked afterwards. 20 individuals of each species were successfully tracked). All captured birds were marked with a metal ring in order to enable recognition of individuals during subsequent visual contacts. Colour rings were used to mark birds selected to carry a telemetry transmitter. Transmitters were mounted on their backs and did not exceed 5% of the bird's body weight. Radio-transmitters had a range of 0.8 to 3 km (for serins) and 2 to 6 km (for crested larks) in open habitat with no obstacles and had an operational lifespan of 9 days for serin tags and 35 days for crested lark tags.

Birds were not tracked for at least 24 hours following trapping and tagging. Individual birds were tracked continuously over an entire activity period (from dawn till dusk). The proportion of time potentially foraging in vineyards (compared to the total potential foraging time in all habitats) was estimated by means of data obtained by radio-tracking and visual observation. These values were regarded as conservative equivalents to the proportion of diet obtained from the treated area (PT).

Individual PT was calculated as:

$$\frac{\text{Time potentially foraging in vineyards}}{\text{Time potentially foraging in all known habitats}}$$

Faeces sampling

To estimate the proportion of different food types in the diet (PD), 18 faeces samples of crested larks and 20 faeces samples of serins were sampled and analysed. For the crested lark correction factors determined by Green (1978) for the related skylark (*Alauda arvensis*) were applied to take into account losses during the digestion process. For the serin no correction factors are available. Therefore, weight-length and weight-area relationships based on reference data for all food categories (*i.e.* invertebrates, seeds and green plant material), collected during the study, were used to calculate the proportion of dry weight of each food category found in the faeces of the serin.

Additional observations

The whole study area was mapped for habitat types and crops and the vegetation status of the vineyards in the study area was determined by changes of BBCH principle growth stages during the study period.

The temperature (daily minimum, maximum and average) and daily precipitation data were obtained from the nearest weather recording station.

II. Results and Discussion

The temperature during the study period ranged between 1.6 and 35.8°C. The mean daily temperature was 18.6°C. Precipitation ranged from 1.3 to 31.8 mm, with 30 rainy days in total.

PT values

The combination of radio-tracking with visual observations and the trapping scheme as presented here (*i.e.* trapping inside of vineyards), allowed an accurate and representative assessment of potential foraging times in given home ranges in order to calculate reliable PT values. All birds were closely associated with vineyards and had the opportunity to use these as foraging habitat. Therefore the results can be considered as conservative in terms of potential dietary exposure. PT values were calculated for individual birds and as overall PT values (*i.e.* 50%tile, 90%tile, and mean) for both bird species. Based on the study results, the mean PT values calculated for the serin and the crested lark were 0.42 (90%tile = 0.73) and 0.52 (90%tile = 0.84), respectively.

Table CP 10.1.1.2/20-1 Overview of PT in vineyards

Proportion of diet obtained in vineyards determined by radio-tracking (PT)		
'potential foraging' time in vineyards as a proportion of the total 'potential foraging' time equals the proportion of diet obtained		
Serin[1]	50% tile	0.37
	90% tile	0.73
	Mean	0.42
Crested lark[1]	50% tile	0.48
	90% tile	0.84
	Mean	0.52

[1]Based on 20 individuals in 20 tracking sessions

Diet estimate and PD values

The analysis of the serin faeces followed the most evolved methodology for the granivorous diet guild, which is biomass dry weight determination by means of comparison with a reference data base. The dry weight proportions for the different food categories (*e.g.* invertebrates, seeds, green plant material, dry wood / bark) in the faeces samples are presented below.

Table CP 10.1.1.2/20-2 Diet composition of the serin

Based on 20 faeces samples analysed for their dry weight proportions (% dry weight) of different food categories			
Food category	**50% tile**	**90% tile**	**mean**
Invertebrates	0.00	18.92	11.76
Seeds	99.03	100.00	87.91
Green plant material (stem)	0.00	0.00	0.10
Dry wood/bark	0.00	0.00	0.23

The crested lark faeces analysis followed the most evolved methodology for larks. The fragment area of the different food categories (*e.g.* invertebrates, seeds, green plant material) in the faeces was used to determine the composition of diet actually ingested. For this correction factors according to Green (1978) were applied to the total fragment area of categories in the samples in order to derive the proportion each category contributed to the actual ingested diet (PD). The PD values for the different food categories of the diet of crested larks are presented below.

Table CP 10.1.1.2/20-3 Diet composition for the crested lark

Based on 18 faeces samples analysed for their area proportions of different food categories and subsequent use of correction factors according to Green (1978)			
Food category	**50% tile**	**90% tile**	**mean**
Invertebrates	0.62	0.96	0.60
Seeds	0.30	0.53	0.29
Green plant material (dicots)	0.03	0.25	0.11
Green plant material (monocots)	0.00	0.00	0.01

III. Conclusion

Overall this study provides reliable refined parameters of PT for both focal species, PD values for crested larks and dietary estimates for serins for use in higher tier risk assessments for birds foraging in vineyards in southern Europe.

Assessment and conclusion by applicant:

This ecological monitoring study was conducted to GLP. The study is therefore considered to be acceptable for regulatory purposes by supporting various aspects of the Bird & Mammal risk assessment.

The results of this study are considered to be valid but have not specifically been relied upon for the refined avian risk assessments for the proposed uses of Spiroxamine EC 500 in grapes.

Data Point:	KCP 10.1.2.2.1
Report Author:	[redacted]
Report Year:	2003
Report Title:	Insectivorous mammals and birds in orchards and vineyards, a literature survey
Report No.:	Lit. 8206
Document No:	M-105688-01-1
Guideline(s) followed in study:	
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

A literature survey has been conducted on the diet and the foraging behaviour of relevant insectivorous mammals and birds inhabiting European vineyards and orchards.

Wood mouse and common shrew have been identified as relevant insectivorous mammal species in orchards and vineyards. Insectivorous bird species predominating in orchards were chaffinch and great tit, while blackbird and yellowhammer prevail in vineyards.

The proportion of animal matter part in the diet of the wood mouse depends on the availability of seeds in respective habitat. In habitats with sufficient seeds the amount of animal matter in the diet of the wood mouse ranges between 10% and 20%. The diet basically consisted of a endogeic prey such as lumbricids and epigeic prey such as various taxa of arthropods. The animal matter proportion in the diet of the wood

mouse varies according to season. In central and northern Europe animal matter prevails in spring and early summer while in southern Europe during winter. The mean body weight of wood mice is about 20 g. The daily dietary demand wood mice is estimated to 5 g animal matter. The foraging time of wood mice is largely nocturnal. The species predominantly forages on the ground. The daily distances covered can be up to more than 1.5 km.

The diet of common *Sorex araneaus* and Millet´s shrew *Sorex coronatus* predominantly consists of animal matter throughout the year. The proportion of endogeic prey amounts to about 30%. The mean body weight of common shrews ranges between 8.0 and 9.0 g. The daily dietary demand of common shrews is estimated to be about 90% to 100% of its body weight during summer, 150% when lactating and 80% of its body weight during winter. The foraging time of common shrews is both diurnal and nocturnal with a greater activity during darkness. The species predominantly forages above and below the ground surface and is able to locate prey up to a soil depth of 12 cm. The diameter of the home range varied between 25m and 60m.

The diet of adult great tit largely consists of arthropods from spring to summer. Irrespective of habitat the nestlings diet is predominantly composed of lepidopteran larvae and spiders. The body weight of adult great tits averages at 20 g. The daily energy consumption of adults during breeding season is about 95 kJ. The daily food intake of nestlings varied between 30% and 40% of the body weight. The species is diurnal. The foraging distance is mostly less than 45 m from the nest. Foraging in modern apple orchards is about 48% of total foraging. Ground foraging predominates from autumn to spring while it decreases in the summer months. From March to August on the average 31% of foraging takes place on the ground.

During the breeding season the blackbird feeds mostly on arthropods and earthworms. The proportion of animal matter in the diet of blackbirds has a peak in spring (60% to 90%) and decreases towards winter (7% - 40%). The diet of nestlings of blackbirds consists of endogeic invertebrates such as earthworms, isopods and myriapods and epigeic invertebrates. The proportion of endogeic prey varies between less than 10% to more than 80%, depending on availability. The average body weight of blackbirds in Central Europe is about 95 g. The daily food intake is estimated to be 35 g fresh weight. The blackbirds activity pattern is diurnal. The foraging territory ranges between 0.18 ha and 0.34 ha. Blackbirds forage almost exclusively on the ground.

The chaffinch is considered to be omnivorous with a shift of being more carnivorous during the breeding season and consuming more vegetational food outside the breeding season. Between March and August appr. half to two thirds of the species diet consists of animal matter. From March to July the species forages 53% (10% - 76%) in trees gleaning foliage while is forages 27% (0% - 100%) of its time on the ground in the same period of time. The mean foraging distance during the breeding season is 100 m or less. The body weight of adult chaffinches averages at about 20 g. Over the year the basal metabolic rate of caged chaffinches was 32.2- 41.6 kJ per day. The maximum daily intake was 7 g fresh weight (138kJ).

The yellowhammer is considered to be a characteristic bird species of vineyards. While the diet of the adults consists of seed and animal matter the diet of the nestlings is composed almost exclusively of epigeic invertebrates with dipteran and lepidopteran larvae prevailing. The average body weight of adult yellowhammers is 28 g. The yellowhammer forages almost exclusively on the ground. The average foraging distance is mostly below 250 m from the nest.

I. Materials and Methods

Study Design

After spray applications of crop protection products mammal and birds may be exposed to residues by ingestion of contaminated food. Hence a risk assessment of crop protection products on mammals and birds requires thorough knowledge of those species inhabiting a certain crop, their foraging behaviour and the composition of their diet. The diet of the majority of small birds and mammals is known to consist at least partially of animal matter, *i.e.* invertebrates.

The arthropod residues used in the Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC (Sanco 4145/2000, rev. 6, 25.09.02) are based on residues found on seeds or pods, which were proposed as surrogate values for arthropods by Kenaga in 1973.

Thus, especially the residue level for small insects represents a default value in order to cover the worst case. The Guidance Document sates that "the residue estimate for small insects appears unsatisfactory, and as soon as better information becomes available this surrogate should be replaced. Research is highly desirable to develop more robust data for residues in insects, also with regard to the temporal pattern." Currently a large part of the PPP's submitted for registration in the EU fail the existing risk assessment based on default values, requiring a depth refinement based on generic data and / or expensive and long-term compound specific studies.

In order to assess the risk of insectivorous mammals and birds feeding in orchards and vineyards generic monitoring studies have been conducted in Central and southern Europe ([redacted] 2002, [redacted] *et al*. 2003a, [redacted] *et al*. 2003b, [redacted] & [redacted] 2003, [redacted] & Wilkens 2003). From the results of these monitoring studies a list of relevant species has been emerged for birds and mammals in central European vineyards ([redacted] 2002, [redacted] *et al*. 2003a, [redacted] *et al*. 2003b). Orchard monitoring studies on birds revealed lists of relevant bird species in central ([redacted] & [redacted] 2003) and southern Europe ([redacted] & [redacted] 2003).

In order to assess the risk of crop protection products for insectivorous mammals and birds a literature survey has been conducted on the dietary composition and the foraging behaviour of relevant insectivorous mammal and bird species foraging in vineyards and orchards.

The literature survey was organized in several steps:

1. Literature search through electronic data bases cited below and collection of additional citations from the reference lists of scientific articles which were already in our archive.
2. Screening of the articles for relevant data
3. Compilation of report

A number of electronic data bases were used to conduct the literature search. These included: ZOOLOGICAL RECORDS®, AGRIS®, SCIRUS®, BIOSIS®, AGRICOLA®. Additional citations were collected from the reference lists of scientific articles which were already archived.

The articles were screened for relevant data on the diet and foraging behaviour on the species identified relevant for orchards and vineyards.

II. Results and Discussion

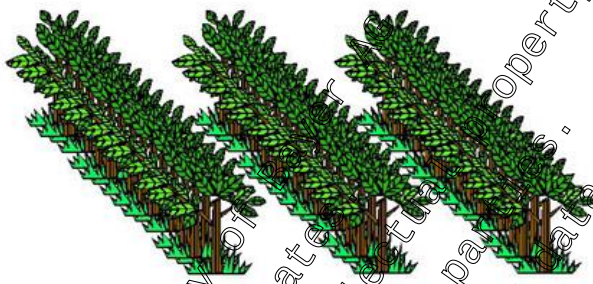
Insectivorous mammals of vineyards and orchards

A survey on the small mammal fauna in three vineyards in south-western Germany was conducted from April to August 2002 by live-trapping ([redacted] 2002, [redacted] *et al*. 2003a). Two species, wood mouse *Apodemus sylvaticus* and common vole *Microtus arvalis*, were observed in small numbers in vineyards characterized by a grassy understorey. No populations were recorded in vineyards devoid of a green ground cover. The existence of a green understorey proved to be the determining factor for the occurrence of small mammals in vineyards in south-western Germany. No exclusively insectivorous species such as shrews were caught. Based on the results of this study the wood mouse was the only non-volatile mammal species which occurs in vineyards and regularly feeds at least partially on animal matter.

Commonly two major types of orchards can be distinguished. Traditional orchards are characterized by large trees and a rich vegetation on the ground. From a vegetation structural point they resemble open woodland and hence characteristic woodland species such as the yellow-necked mouse *Apodemus flavicollis* are to be expected. The majority of orchards in contemporary agriculture are modern orchards which are characterized by dense rows of small trees approximately 2 m high. Normally there is no understorey at the base of the trees while grassy strips prevail between the tree rows. No mammalian

monitoring data has been available from modern orchards but their vegetation structure is expected to be more similar to vineyards than to traditional orchards. Hence the mammalian fauna is expected to more resemble the vineyard fauna than a woodland fauna. The wood mouse was chosen as characteristic species.

Figure CP 10.1.1.2/21-1 Schematical depiction of modern orchard or vineyards



Additionally the common shrew *Sorex araneus* and its western European sibling species Millet´s shrew *Sorex coronatus* were included to represent exclusively insectivorous small mammals. In the British Isles common shrews are found almost everywhere provided some vegetation cover is available ([redacted] 1991) and in Central Europe the common shrew is most abundant in thick grass, bushy scrub and deciduous woodland ([redacted] 2000). In an analysis on the habitat of small mammals in Baden-Württemberg, Germany, the common shrew proved to be the characteristic shrew species inhabiting arable land ([redacted] et al. 2003). *Sorex araneus* is one of the best studied European shrew species and a wealth of data has been collected from various parts of its range. In western Europe, *i.e.* France and Spain, the species is replaced by Millet´s shrew Sorex coronatus which was discovered as a sibling species of Sorex araneus in 1964. Sorex coronatus has about the same ecological niche as *Sorex araneus* (Castien & Gosalbez 1995). Sorex coronatus is apparently expanding its range to the detriment of the more coldadapted *Sorex araneus* in recent times. This seems to be linked to climatic change (Andera 1999).

Wood mouse (*Apodemus sylvaticus, Muridae, Rodentia*)

The wood mouse relies on concentrated food items such as animal matter or seeds beside less nutritious foliage matter. In seed rich habitats the bulk of the wood mouse diet is made up of seeds. In habitats characterized by a low seed availably the diet of the wood mouse is characterized by an increased amount of animal matter. The seed availability in modern orchards and vineyards is expected to be reduced compared to natural habitats such as deciduous forests or grasslands. An increased amount of animal matter of at least 50% of the diet can be assumed for wood mice inhabiting vineyards and orchards. The animal diet basically consisted of an endogeic prey such as lumbricids and epigeic prey such as various taxa of arthropods. The animal matter proportion in the diet of the wood mouse shows varies according to season. In central and northern Europe animal matter prevails in spring and early summer while in southern Europe during winter. The mean body weight of wood mice is about 20 g. The daily dietary demand wood mice is estimated to 5 g animal matter. The foraging time of wood mice is largely nocturnal. The species predominantly forages on the ground. The daily distances covered can be up to more than 1.5 km.

Common shrew *Sorex araneus* and Millet´s shrew *Sorex coronatus*

The diet of common and Millet´s shrew predominantly consists of animal matter throughout the year. The proportion of endogeic prey amounts to about 30%. The mean body weight of common shrews ranges between 8.0 and 9.0 g. The daily dietary demand of common shrews is estimated to be about 90% to 100% of its body weight during summer, 150% when lactating and 80% of its body weight during winter. The foraging time of common shrews is both diurnal and nocturnal with a greater activity

during darkness. The species predominantly forages above and below the ground surface and is able to locate prey up to a soil depth of 12 cm. The diameter of the home range varied between 25m and 60m.

Insectivorous birds of orchards and vineyards

Commonly two major types of orchards can be distinguished. Traditional orchards are characterized by large trees and a rich vegetation on the ground ([redacted]r & [redacted] 1985, [redacted] 1983). From a vegetation structural point they resemble open woodland or parkland and they are characterized by a great diversity of birds. The bird fauna of traditional orchards has been documented by various authors and concern has been expressed about the impoverishment of the bird fauna when traditional orchards are converted to modern orchards ([redacted] & [redacted] 1985, [redacted] 1975, [redacted] 1983).

A survey on the birds inhabiting vineyards in southwestern Germany has been conducted ([redacted] 2002, [redacted] *et al*. 2003). Among the birds typically foraging within vineyards the linnet, the yellowhammer and the blackbird were those species identified as characteristic, *i.e.* they are encountered regularly feeding in the vineyards. The linnets diet predominantly consists of seeds ([redacted] 1989), thus the yellowhammer was chosen as representative insectivorous birds species foraging in vineyards.

Chaffinch and great tit were the most common insectivorous birds inhabiting modern orchards in Switzerland ([redacted] 1983).

According to monitoring data on birds in modern spindle bush apple orchards in southern Germany the great tit proved to be the most common insectivorous species while blackbird and chaffinch were the most common species partly feeding on arthropods ([redacted]l & [redacted] in prep.). The same results were obtained in modern apple orchards of southern Europe ([redacted] & [redacted] in prep.).

Table CP 10.1.1.2/21-1 Steadiness of relevant bird species observed in orchards and vineyards

Species	Orchards	Vineyards
Blackbird	XXX	XXX
Chaffinch	XXX	X
Great tit	XX	-
Yellowhammer	X	XX

XXX: highest steadiness XX: high steadiness X: low steadiness -: not encountered

Great tit (*Parus major*, Paridae)

The diet of adult great tit largely consists of arthropods from spring to summer. Irrespective of habitat the nestlings diet is predominantly composed of lepidopteran larvae and spiders. The body weight of adult great tits averages at 20 g. The daily energy consumption of adults during breeding season is about 95 kJ. The daily food intake of nestlings varied between 30% and 40% of the body weight. The species is diurnal. The foraging distance is mostly less than 45 m from the nest. Foraging in modern apple orchards is about 48% of total foraging. Ground foraging predominates from autumn to spring while it decreases in the summer months. From March to August on the average 31% of foraging takes place on the ground.

Blackbird (*Turdus merula*, Turdidae)

During the breeding season the blackbird feeds mostly on arthropods and earthworms. The proportion of animal matter in the diet of blackbirds has a peak in spring (60% to 90%) and decreases towards winter (7% - 40%). The diet of nestlings of blackbirds consists of endogeic invertebrates such as earthworms, isopods and myriapods and epigeic invertebrates. The proportion of endogeic prey varies between less than 10% to more than 80%, depending on availability. The average body weight of blackbirds in Central Europe is about 95 g. The daily food intake is estimated to be 35 g fresh weight.

The blackbirds activity pattern is diurnal. The foraging territory ranges between 0.18 ha and 0.34 ha. Blackbirds forage almost exclusively on the ground.

Chaffinch (*Fringilla coelebs*, Fringillidae)

The chaffinch is considered to be omnivorous with a shift of being more carnivorous during the breeding season and consuming more vegetational food outside the breeding season. Between March and August appr. half to two thirds of the species diet consists of animal matter. From March to July the species forages 53% (10% - 76%) in trees gleaning foliage while is forages 27% (0% - 100%) of its time on the ground in the same period of time. The mean foraging distance during the breeding season is 100 m or less. The body weight of adult chaffinches averages at about 20 g. Over the year the basal metabolic rate of caged chaffinches was 32.2-41.6 kJ per day. The maximum daily intake was 7 g fresh weight (138kJ).

Yellowhammer (*Emberiza citrinella*, Emberizidae)

The yellowhammer is considered to be a characteristic bird species of vineyards. While the diet of the adults consists of seed and animal matter the diet of the nestlings is composed almost exclusively of epigeic invertebrates with dipteran and lepidopteran larvae prevailing. The average body weight of adult yellowhammers is 28 g. The yellowhammer forages almost exclusively on the ground. The average foraging distance is mostly below 250 m from the nest.

III. Conclusion

The proportion of animal matter parts in the diet of the wood mouse depends on the availability of seeds in respective habitat. In habitats with sufficient seeds the amount of animal matter in the diet of the wood mouse ranges between 10% and 20%. The diet basically consisted of a endogeic prey such as lumbricids and epigeic prey such as various taxa of arthropods. The animal matter proportion in the diet of the wood mouse shows varies according to season. In central and northern Europe animal matter prevails in spring and early summer while in southern Europe during winter. The mean body weight of wood mice is about 20 g. The daily dietary demand of wood mice is estimated to 5 g animal matter. The foraging time of wood mice is largely nocturnal. The species predominantly forages on the ground. The daily distances covered can be up to more than 1.5 km.

The diet of common *Sorex araneaus* and Millet's shrew *Sorex coronatus* predominantly consists of animal matter throughout the year. The proportion of endogeic prey amounts to about 30%. The mean body weight of common shrews ranges between 8.0 and 9.0 g. The daily dietary demand of common shrews is estimated to be about 90% to 100% of its body weight during summer, 150% when lactating and 80% of its body weight during winter. The foraging time of common shrews is both diurnal and nocturnal with a greater activity during darkness. The species predominantly forages above and below the ground surface and is able to locate prey up to a soil depth of 12 cm. The diameter of the home range varied between 25m and 60m.

The diet of adult great tit largely consists of arthropods from spring to summer. Irrespective of habitat the nestlings diet is predominantly composed of lepidopteran larvae and spiders. The body weight of adult great tits averages at 20 g. The daily energy consumption of adults during breeding season is about 95 kJ. The daily food intake of nestlings varied between 30% and 40% of the body weight. The species is diurnal. The foraging distance is mostly less than 45 m from the nest. Foraging in modern apple orchards is about 48% of total foraging. Ground foraging predominates from autumn to spring while it decreases in the summer months. From March to August on the average 31% of foraging takes place on the ground.

During the breeding season the blackbird feeds mostly on arthropods and earthworms. The proportion of animal matter in the diet of blackbirds has a peak in spring (60% to 90%) and decreases towards winter (7% - 40%). The diet of nestlings of blackbirds consists of endogeic invertebrates such as earthworms, isopods and myriapods and epigeic invertebrates. The proportion of endogeic prey varies between less than 10% to more than 80%, depending on availability. The average body weight of blackbirds in Central Europe is about 95 g. The daily food intake is estimated to be 35 g fresh weight.

The blackbirds activity pattern is diurnal. The foraging territory ranges between 0.18 ha and 0.34 ha. Blackbirds forage almost exclusively on the ground.

The chaffinch is considered to be omnivorous with a shift of being more carnivorous during the breeding season and consuming more vegetational food outside the breeding season. Between March and August appr. half to two thirds of the species diet consists of animal matter. From March to July the species forages 53% (10% - 76%) in trees gleaning foliage while is forages 27% (0% - 100%) of its time on the ground in the same period of time. The mean foraging distance during the breeding season is 100 m or less. The body weight of adult chaffinches averages at about 20 g. Over the year the basal metabolic rate of caged chaffinches was 32.2- 41.6 kJ per day. The maximum daily intake was 7 g fresh weight (138 kJ).

The yellowhammer is considered to be a characteristic bird species of vineyards. While the diet of the adults consists of seed and animal matter the diet of the nestlings is composed almost exclusively of epigeic invertebrates with dipteran and lepidopteran larvae prevailing. The average body weight of adult yellowhammers is 28 g. The yellowhammer forages almost exclusively on the ground. The average foraging distance is mostly below 250 m from the nest.

Assessment and conclusion by applicant:

This literature survey was conducted in order to provide supporting information for use in the Bird & Mammal risk assessment.

The results of this study are considered to be valid but have not specifically been relied upon for the refined avian risk assessments for the proposed uses of Spiroxamine EC 500 in grapes.

Residues studies

The following residues data are available and considered relevant to the proposed use of Spiroxamine EC 500 in grapes.

Data Point:	KCP 10.1.1.2/04
Report Author:	[redacted]
Report Year:	1998
Report Title:	KWG 4168: An evaluation of residues in environmental matrices after application to California vineyards
Report No:	108068
Document No:	M-090880-01-1
Guideline(s) followed in study:	US EPA Pesticide Assessment Guideline, Subdivision 71-5, US EPA Draft OPPTS 850.2500
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Residues of KWG 4168 were measured in or on potential avian food items including grapes, grape leaves, invertebrates and weed heads associated with vineyards in the cF4 s3x:7?˚ wät?/0 of California. The study focused on measuring residues of spiroxamine immediately following a single application of 400 g a.s./ha and up to 28 days later.

Mean residues detected in grape foliage decreased over the study period from 43.4 µg a.s./g spiroxamine equivalents following application to 9.43 µg a.s./g spiroxamine equivalents (SD=1.31) twenty-eight days later. The half-life was calculated at 14.21 days. Residues in weed heads collected from the vineyards were slightly more than half as high as levels detected in grape foliage (26.9 µg a.s./g spiroxamine equivalents following application, decreasing to 11.6 µg a.s./g spiroxamine equivalents four days later) Residues appeared to dissipate more rapidly in weed heads as compared to grape foliage.

Residues detected in grapes and invertebrates were low, with a mean of 0.857 µg a.s./g spiroxamine equivalents in grapes and 0.689 µg a.s./g spiroxamine equivalents in invertebrates following application. Mean levels decreased to 0.529 µg a.s./g spiroxamine equivalents in grapes and 0.484 µg a.s./g spiroxamine equivalents in invertebrates four days later. Levels detected in invertebrates should be handled with caution since the high coefficient of variation of 84 % indicates inconsistency among the samples.

I. Materials

Test Material	KWG 4168 300 CS
Lot/Batch #:	7030114
Purity:	32.4% spiroxamine
Description:	Liquid
Stability of test compound:	Stable under ambient conditions
Reanalysis/Expiry date:	April 1998
Density:	Not stated
Treatments	
Test rates:	Single application of 400 g a.s./ha
Solvent/vehicle:	Water was used as a carrier (200 gallons/acre)
Analysis of test concentrations:	Determined using gas chromatography with mass selective detection (GC-MSD)
Test design	
Test area:	Vineyard in Fresno County, California (3 plots approximately 0.1 acre each, with 4 stations per plot. Each plot contained 20 pitfall traps)
Replication:	Samples were composited for analysis from 4 stations on each plot on each sampling day
Duration of test:	28 days
Environmental test conditions	
Temperature:	During application: 29.9 to 31.7 Maximum: 27 to 39 Minimum: 13 to 23
Relative humidity:	During application: 47 to 51%
pH:	6.1 to 8.4 (soil pH)
Photoperiod:	Not stated

II. Study Design

The objective of this study was to measure residues of the test substance in or on potential avian food items including grapes, grape leaves, invertebrates and weed heads associated with vineyards in the San Joaquin Valley of California. The study focused on measuring residues of spiroxamine immediately following a single application and up to 28 days later. The applications were made to three plots of commercial grape vineyards approximately 0.1 acre in size. Single applications were made on June 17, 1997 to each test plot at the maximum use rate of approximately 400 g a.s./ha. The equipment calibration was confirmed and the application was monitored.

A total of 156 samples were collected for residue analysis including 84 grape foliage samples and 24 samples each of grapes, invertebrates and weed heads. Samples from each matrix were composited from the four stations on each plot for each sampling day. A total of 39 composited samples were analysed using GC-MSD.

Samples of grape foliage were collected prior to application, immediately following the application and on days 4, 7, 14, 21 and 28 following the application. Samples were composited from each of the plots on each sampling day resulting in a total of 21 grape foliage samples being analysed. Samples of grapes, invertebrates and weed heads (including the seed head, stem or stalk) were collected immediately following application and on day 4 after application. Samples were composited for analysis from four stations on each plot on each sampling day. Twenty pitfall traps at each residue station were used to capture ground dwelling invertebrates while sweep nets were used to capture insects that were in the vineyard vegetation or flying. Sweep netting was conducted for approximately 10 minutes at each residue station using mesh nets and sweeping over the vineyard vegetation.

Observations of wildlife within the study area were made. Sixty-one observations of birds of 20 species were documented during wildlife observations in test vineyards. A total of 126 birds were recorded in the vineyards and adjacent habitat during the course of the study.

Weather information was obtained from the National Oceanic and Atmospheric Administration station located in Fresno, California for the duration of the study period.

Analytical method

Samples of grapes, grape foliage, weed heads and invertebrates were analysed using the validated analytical method M-090880-01-1, report reference M-090880-01-1 **(see Doc MCP Section 5)**.

II. Results and Discussion

The study was deemed to be acceptable based on the criteria set out in the US EPA Pesticide Assessment Guideline, Subdivision 71-5 and US EPA Draft OPPTS 850.2500, in the absence of an OECD guideline.

The average application rate on the three plots was 398 g KWG 4168 equivalents/ha and ranged from 389.7 to 401.7 g KWG 4168 equivalents/ha.

The highest total residues were detected in grape foliage with a peak of 54.6 µg a.s./g spiroxamine equivalents on Plot 2 immediately following the application. Mean residues detected in grape foliage decreased over the study period from 43.4 µg a.s./g spiroxamine equivalents (SD=14.2) following application to 9.43 µg a.s./g spiroxamine equivalents (SD=1.31) twenty-eight days later. The half-life was calculated at 14.21 days. Residues in weed heads collected from the vineyards were slightly more than half as high as levels detected in grape foliage. A mean value of 26.9 µg a.s./g spiroxamine equivalents (SD=3.10) was detected following the application with residues decreasing to a mean of 11.6 µg a.s./g spiroxamine equivalents (SD=1.63) four days later. Residues appeared to dissipate more rapidly in weed heads as compared to grape foliage.

Residues detected in grapes and invertebrates were low, with a mean of 0.857 µg a.s./g spiroxamine equivalents (SD=0.415) in grapes and 0.689 µg a.s./g spiroxamine equivalents (SD=0.578) in invertebrates collected immediately following application. Mean levels decreased to 0.529 µg a.s./g

spiroxamine equivalents (SD=0.215) in grapes and 0.474 µg a.s./g spiroxamine equivalents (SD=0.113) in invertebrates four days later.

Table CP 10.1.1.2/04-1 Measured residues of KWG 4168 detected in grapes, weed heads and invertebrates

Compartment	Mean initial residues measured (µg a.s./g)	Mean residues measured (µg a.s./g)	Recalculated initial residues[1] (mg/kg)	DT_{50} (days)
Grape foliage	43.4	9.43 (day 28)	108.5	14.21
Weed heads	26.9	11.6 (day 4)	67.25	*ca.* 4
Grapes	0.857	0.529 (day 4)	2.1425	NA
Invertebrates	0.689	0.474 (day 4)	1.7225	NA

[1] recalculated for an application rate of 1.0 kg a.s./ha
NA = Not Applicable

III. Conclusion

It is important to note that the mean residue value of 0.689 µg a.s./g spiroxamine equivalents (SD=0.578) detected in invertebrates should be handled with caution since the high coefficient of variation of 84 % indicates inconsistency among the samples. A more conservative approach would be to use the maximum residue value of 1.34 µg/g spiroxamine equivalents detected in plot no. 2. Accordingly, the recalculated value for an application rate of 1.0 kg a.s./ha would be 3.35 mg/kg. However, this deviation does not change the outcome of the risk assessment and was therefore not considered in the risk assessment. Besides the small sample size (10 sweep net samples vs. 12 pitfall trap samples) another noteworthy shortcoming was that data on the taxonomy of collected invertebrates were completely missing. In contrast to invertebrates sampling, mean residue values detected in grapes and weed heads showed less inconsistency among the samples and are therefore considered acceptable.

Assessment and conclusion by applicant:

The study was conducted to the US EPA Pesticide Assessment Guideline, Subdivision 71-5 and US EPA Draft OPPTS 850.2500. In the absence of an OECD guideline, the study is considered valid according to the EPA guidelines followed.

The study is relatively old and therefore does not take in to account some of issues which are now expected as part of a residues decline trial. For example there were relatively few sampling timepoints shortly after application (Day 0, 4, 7, 14, 21 and 28). That being said, this regime does appear to be adequate for the derived DT_{50} on grape foliage with a value of 14 days. For weed heads an estimated DT_{50} of *ca.* 4 days has been reported and this has been estimated based on the residues value being roughly 50% of the starting value at Day 4 (and not determined *via* kinetic analysis). However, the value is still considered to be a reliable measurement and clearly demonstrates that the decline in spiroxamine residues on weed heads is approximately 50% after 4 days therefore a DT_{50} of 4 days is considered to be suitable for use in the risk assessment.

It is also noted that, although weather data are available, no information such as precipitation was collected at the study site itself. It is also noted that the study was conducted in the U.S. and not the EU but the chemical properties of spiroxamine are considered to be the same regardless of the continent in which the study was conducted.

The study is considered to be valid in its own right and the results of this study, particularly the DT_{50} of *ca.* 4 days on weed heads, has been used in the risk assessment of Spiroxamine EC 500 to refine the granivorous bird scenario.

Data Point:	KCP 10.1.1.2/05
Report Author:	[redacted]
Report Year:	2006
Report Title:	Determination of Spiroxamine residues in the carabid beetle Poecilus cupreus L. - extended laboratory study -
Report No:	31861007
Document No:	M-281566-01-1
Guideline(s) followed in study:	Auswirkungen von Pflanzenschutzmitteln auf Imagines von Poecilus cupreus L. als Vertreter der Familie Carabidae (= Laufkäfer) im Laboratorium, ([redacted] 1991); " A method for testing effects of plant protection products on the carabid beetle Poecilus cupreus (Coleoptera, Carabidae) under laboratory and semi-field conditions, [redacted] (2000). ; " SANCO/825/00 rev.7 guidance document on residue ana-lytical methods which gives guidance on requirements for residue methods suitable for post-registration monitoring).
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the residues of spiroxamine after certain ageing intervals in the carabid beetle *P. cupreus*. The test item was sprayed upon the beetles, the food for the first three days and the substrate (natural soil). After spray application of the test item, the beetles were maintained in the laboratory on natural soil substrate. After defined time intervals, beetles were removed from the test units, deep frozen and were used for residue analysis of spiroxamine in the beetles. Beetles maintained in separate test units were used as untreated blank controls.

The dry weight of 10 beetles (5 males and 5 females) was determined. The mean dry weight of one beetle *P. cupreus* was determined to be 45.6 mg/beetle.

During day 0 and day 4 the beetles consumed a mean of 0.55 test item contaminated fly pupae/beetle in the test item group compared to 0.51 untreated fly pupae/ beetle in the control group.

Following the application of 1509 g Spiroxamine EC 500 G/ha the total residues of spiroxamine in and on carabid beetles were determined after different ageing intervals. The residue analysis was done by Bayer CropScience after transfer of the deep frozen specimens.

Materials

Test Material Spiroxamine EC 500

Lot/Batch #: PF9008768

Content of a.s.: KWG 4168: 498.98 g/L, according to certificate of analysis

Description: Clear brown liquid

Stability of test compound: Sufficient based on the expiry date

Reanalysis/Expiry date: 11 January 2007

Density:	1.006 g/mL
Treatments	
Test rates:	1509 g Spiroxamine EC 500 g/ha, corresponding to 750 g a.s./ha
Solvent/vehicle:	The test item was applied in 300 L water/ha
Analysis of test concentrations:	Residue analysis of the test item in the beetles were conducted in a separate study at Bayer CropScience after transfer of the deep frozen specimen (beetles) from IBACON to Bayer CropScience.
Test design	
Test species:	Carabid beetle (*Poecilus cupreus* L.), age: about 3 - 4 weeks old; source: Bio-Test Labor GmbH, Sagerheide, Germany.
Test units:	Plastic boxes (18.3 x 13.6 x 6 cm), containing 250 g dry soil
Replication:	(2 males and two females per replicate)
Duration of test:	14 days
Environmental test conditions	
Temperature:	19 – 21°C
Relative humidity:	63 – 84%
pH:	6.1 to 8.4 (soil pH)
Photoperiod:	810 – 1590 lux

I. Study Design

The purpose of this study was to determine the residues of spiroxamine after certain ageing intervals in the carabid beetle *P. cupreus*. The test item was sprayed upon the beetles, the food for the first three days and the substrate (natural soil). After spray application of the test item, the beetles were maintained in the laboratory on natural soil substrate. After defined time intervals, beetles were removed from the test units, deep frozen and were used for residue analysis of spiroxamine in the beetles. Beetles maintained in separate test units, were used as untreated blank controls.

The test item was sprayed upon the beetles, the food for the first three days and the substrate (natural soil). After spray application of the test item, the beetles were maintained in the laboratory on natural soil substrate. After defined time intervals, beetles were removed from the test units, deep frozen and were used for residue analysis.

Two variants (determination of residues of spiroxamine and untreated control); 4 individuals (2 males and 2 females)/test unit. 8 individuals (2 test units = one sample) were used for each sampling date. Sampling intervals: application day (1 - 2 hours after application [day 0]) and on day 1, 2, 3, 4, 6, 8, 10, 12 and 14 following the application day.

250 g air-dried, natural soil was filled in plastic boxes (18.3 cm x 13.6 cm x 6 cm; length, width, height; substrate surface of about 175 cm2) containing a layer of about 1 cm natural soil. At the beginning the soil was moistened to about 55 % ± 5 % of its maximum water holding capacity (WHC = 37 %).

Natural soil collected from a field at a distance of *ca.* 100 m from the IBACON Building ("am Griinberg" Flur Nr. 42). The soil to be used as a substrate was collected on August 8, 2005 at a depth of 10 cm.

With punctured deep frozen fly pupae (Calliphora spec.} (Grebenstein GmbH, Kiisterstr. 4, D-31180 Giesen) at a rate of one pupa per beetle per feeding date. Feeding was conducted as follows: application day (before application), day 1, 2, 4 for the first week and thereafter on day 7, 9, 11 after application (3 x per week). First food was sprayed with the test item solution.

Analytical method

Samples of insects were analysed using the validated analytical method 00721/M001, report reference M-283574-01-1 **(see Doc MCP Section 5)**.

II. Results and Discussion

The dry weight of 10 organisms (5 untreated male and 5 untreated female beetles) was determined. The mean dry weight of the beetles was found to be 45.6 mg per beetle.

Table CP 10.1.1.2/05-1 Dry weight of carabid beetles

Sample	Wet weight [g]	Dry weight [g]	Water [g]	Water content [% beetle $_{wet}$][1]	Dry weight %
Female beetle	0.1158	0.0500	0.0658	56.82	43.18
Female beetle	0.1114	0.0455	0.0659	59.16	40.84
Female beetle	0.1099	0.0477	0.0622	56.60	43.40
Female beetle	0.1015	0.0487	0.0528	52.02	47.98
Female beetle	0.0963	0.0465	0.0498	51.71	48.29
Male beetle	0.1004	0.0516	0.0488	48.61	51.39
Male beetle	0.0975	0.0444	0.0531	54.46	45.54
Male beetle	0.0938	0.0400	0.0538	57.36	42.64
Male beetle	0.0914	0.0356	0.0558	61.05	38.95
Male beetle	0.1041	0.0458	0.0583	56.00	44.00
Mean	**0.1022**	**0.0456**	**0.0566**	**55.38**	**44.62**
SD	**0.0080**	**0.0047**	**0.0062**	**3.74**	**3.74**

The food consumption during the first 4 days was recorded in order to assess the food uptake of contaminated and uncontaminated fly pupae per beetle.

Mean Consumed Pupae over 4 days: Control: 0.51 pupa per beetle and test item: 0.55 pupa per beetle.

Table CP 10.1.1.2/05-2 Food consumption during the first 4 days

Time	Spiroxamine EC 500 G		Control	
	Pupae/ beetle #	Consumption %	Pupae/ beetle #	Consumption %
Days 0 – 1	0.55	100.0	0.55	100.0
Days 1 – 2	0.53	160.6	0.33	100.0
Days 2 – 4	0.56	86.2	0.65	100.0
Mean	**0.55**	**115.6**	**0.51**	**100.0**

III. Conclusion

The dry weight of 10 beetles (5 males and 5 females) was determined. The mean dry weight of one beetle *P. cupreus* was determined to be 45.6 mg/beetle.

During day 0 and day 4 the beetles consumed a mean of 0.55 test item contaminated fly pupae/beetle in the test item group compared to 0.51 untreated fly pupae/ beetle in the control group.

Following the application of 1509 g Spiroxamine EC 500 G/ha the total residues of spiroxamine in and on carabid beetles were determined after different ageing intervals. The residue analysis was done by Bayer CropScience after transfer of the deep frozen specimens.

Assessment and conclusion by applicant:

The study was conducted to an old BBA guideline but it is recognised that there are no specific test guidelines for residues decline studies, just recommendations provided in the Guidance Documents (for example EFSA, 2009).

This study investigated residues on beetles which were sprayed within the laboratory and then frozen for analysis at timepoints of Day 0, 1, 2, 3, 4, 6, 8, 10, 12 and 14 following the application.

The analytical results of the study have been conducted as part of a separate study, M-288174-01-2, which is summarised below.

The study was conducted within the laboratory and therefore the results achieved may not be representative of the 'field' situation. However, the study is considered to be valid and the analytical regime is considered to meet the current standards with a sufficient number of analytical timepoints to be able to derive reliable DT_{50} values.

The study is considered acceptable but the results have not been relied on in the risk assessment of Spiroxamine EC 500.

Data Point:	KCP 10.1.2/06
Report Author:	[redacted]
Report Year:	2007
Report Title:	Determination of the residues of spiroxamine (KWG 4168) in/on insects after application with spiroxamine 750 g a.s./ha
Report No:	MR-07/224
Document No:	M-288174-01-2
Guideline(s) followed in study:	EU 91/414/EEC amended by 96/68/EC; EU Annex II (part A, section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99; US EPA: OPPTS 860.1340
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of the study was to determine the residues of spiroxamine in/on insects after applications with Spiroxamine 750 g a.s./ha.

Residue values of spiroxamine in/on insects on day 0 after application with Spiroxamine EC 500 G were 8.7 mg/kg and decreased to 0.21 mg/kg on day 18, calculated to dry material. All residue values in control samples were below the LOQ (0.01 mg/kg).

Based on the residue analysis a half-life period for Spiroxamine in/on insects of 1.28 days was calculated.

I. Materials

Test Material	Spiroxamine (KWG 4168)
Lot/Batch #:	M28197
Purity.:	97.4%
Description:	Not reported
Stability of test compound:	Sufficient based on the expiry date
Reanalysis/Expiry date:	22-10-2007
Density:	Not reported

Study Design

The purpose of the study is to determine the residues of spiroxamine in/on insects after applications with Spiroxamine 750 g a.s./ha.

All samples were deep-frozen directly after sampling by the test facility IBACON and then stored at around -18°C or below until dispatch with dry ice to the analytical laboratory of R. Schöning (BCS-D-ROCS, D-40789 Monheim). All samples arrived in the laboratory in good condition.

The samples were stored in a deep-freezer at -18°C or below in dark conditions until analysis in the laboratory of R. Schöning.

Spiroxamine residues were extracted from beetles (approx. 1 g) with a mixture of acetone/water. After filtration, an aliquot of the extract was concentrated to the aqueous remainder. The residues were transferred into a 10 mL volumetric flask with acetonitrile and filled up with water/acetic acid (9/1, v/v). The residues were quantified by reversed phase HPLC with electrospray and MS/MS-detection using stable labelled standard solutions as internal standard.

II. Results and Discussion

The individual recovery values for spiroxamine with method 00721/M001 (report reference M-283574-01-1 (see Doc MCP Section 5) ranged from 106 to 122% with an overall recovery of 112% and with a relative standard deviation (RSD) of 5.0% (n = 6). All results of the method validation were in accordance with the general requirements for residue analytical methods, therefore the method was validated successfully.

The Limit of Quantitation (LOQ), defined as the lowest validated fortification level, was 0.01 mg/kg for Spiroxamine in/on insects. Residue values of Spiroxamine in/on insects on day 0 after application with Spiroxamine EC 500 C were 8.7 mg/kg and decreased to 0.21 mg/kg on day 18, calculated to dry material. All residue values in control samples were below the LOQ (0.01 mg/kg).

Table CP 10.1.1.2/06-1 Residue concentration of spiroxamine in the analysed samples

Results of Spiroxamine in/on beetles			
Days after application	**Control beetles**	**Treated beetles**	
		Spiroxamine in [mg/kg] wet*	**Spiroxamine in [mg/kg] dry**
0	<LOQ	3.8	8.7
1	<LOQ	1.5	3.3
2	<LOQ	1.4	3.2
3	<LOQ	1.0	2.2

Results of Spiroxamine in/on beetles			
Days after application	Control beetles	Treated beetles	
		Spiroxamine in [mg/kg] wet*	Spiroxamine in [mg/kg] dry
4	<LOQ	0.66	1.5
6	<LOQ	0.44	1.0
8	<LOQ	0.32	0.72
10	<LOQ	0.22	0.48
12	<LOQ	0.16	0.36
14	<LOQ	0.08	0.18
18	<LOQ	0.09	0.21

*= water content of the samples were 55.3%

Assessment and conclusion by applicant:

This study reports the analytical results achieved following analysis of the beetle samples taken in study M-281566-01-1. The study is considered to be valid and has followed the analytical guidance in place at the time of conduct including SANCO/3029/99. A validated analytical method was used.

The study is considered acceptable.

The residues data have been used to determine DT_{50} and an associated ftwa value for use in the Bird & Mammal risk assessment in report M-293626-01-1. This has been summarised below.

Data Point:	KCP 10.1.1.2/07
Report Author:	[redacted]
Report Year:	2007
Report Title:	Spiroxamine (KWG 4168): Summary of an additional study that has been conducted to determine dissipation of residues of Spiroxamine from insects plus an calculation of the fTWA values
Report No:	M-293626-01-1
Document No:	M-293626-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the residues of spiroxamine after certain ageing intervals on insects based on the carabid beetle *P. cupreus*.

Residue values of spiroxamine in/on insects on day 0 after application with Spiroxamine EC 500 were 8.7 mg/kg and decreased to 0.21 mg/kg on day 18, calculated to dry material. All residue values in control samples were below the LOQ (0.01 mg/kg). Based on the residue analysis a half-life period for

Spiroxamine in/on insects of 1.28 days can be calculated, by function of 1.5st order (3.38 days by an exponential function of 1st order).

An evaluation of the f_{TWA} value based on the area under the curve method resulted for a TWA interval of 14 days in an f_{TWA} value of 0.21 and for an TWA interval of 21 days in an f_{TWA} vaule of 0.15. Further f_{TWA} values for TWA periods of 2 to 21 days were also calculated.

I. Materials

Test Material	Spiroxamine EC 500 G
Lot/Batch #:	PF90087683
Content of a.s.:	498.98 g/L (according to C of A)
Description:	Not reported
Stability of test compound:	Not reported
Reanalysis/Expiry date:	Not reported
Density:	Not reported
Treatments	
Test rates:	1509 g Spiroxamine EC 500/ha, corresponding to 750 g a.i./ha.
Solvent/vehicle:	Water
Analysis of test concentrations:	Yes
Test organisms	
Species:	Carabid beetle (*Poecilus cupreus* L.)
Source:	Bio-Test Labor GmbH, Sagerheide, Germany
Acclimatisation period:	Not reported
Feeding:	Fly pupae (*Calliphora spec.*)
Treatment for disease:	Not reported
Test design	
Test units:	Plastic boxes (18.3 x 13.6 x 6 cm) containing 250 g dry soil
Replication:	Not reported
Duration of test:	14 days
Environmental test conditions	
Temperature:	19 – 21°C
Relative humidity:	63 – 84%
pH:	Not reported
Photoperiod:	16 hour light : 8 hour dark

Study Design

The purpose of this study was to determine the residues of Spiroxamine after certain ageing intervals on insects based on the carabid beetle *P. cupreus*. The test item Spiroxamine EC 500 (at a rate corresponding to 750 g a.s./ha) was sprayed upon the beetles, the food for the first three days and the substrate (natural soil). After defined time intervals (1 - 2 hours after application [day 0], and on day 1, 2, 3, 4, 6, 8, 10, 12 and 14 following the application day), beetles were deep frozen and then were used for residue analysis. Untreated beetles were used as a blank control.

Test units were plastic boxes (18.3 x 13.6 x 6 cm), containing 250 g dry soil.

1509 g Spiroxamine EC 500/ha, corresponding to 750 g a.s./ha. The test item was applied in 300 L water/ha. The test item was sprayed upon the substrate, the beetles and the first offered food via laboratory spray applicator (a sufficient additional amount of food was sprayed as well and given as treated food for the following 2 days). Untreated beetles were used as control beetles.

Following the application of 1509 g Spiroxamine EC 500/ha the total residues of Spiroxamine in and on carabid beetles were determined after different ageing intervals. The residue analysis was done by Bayer CropScience after transfer of the deep frozen specimen.

Residues of spiroxamine in/on insects were determined according to method 00721/M001. Spiroxamine residues were extracted from beetles (approx. 1 g wet weight) with a mixture of acetone/water. After filtration, an aliquot of the extract was concentrated to the aqueous remainder.

The residues were transferred into a 10 mL volumetric flask and filled up with acetonitrile. The residues were quantified by reversed phase HPLC with electrospray and MS/MS-detection using stable labelled standard solutions as internal standard.

II. Results and Discussion

The individual recovery values for Spiroxamine with method 00721/M001 ranged from 106 to 122% with an overall recovery of 112% and with a relative standard deviation (RSD) of 5.0% (n = 6). All results of the method validation were in accordance with the general requirements for residue analytical methods, therefore the method was validated successfully.

The Limit of Quantitation (LOQ), defined as the lowest validated fortification level, was 0.01 mg/kg for Spiroxamine in/on insects.

Residue values of spiroxamine in/on insects on day 0 after application with Spiroxamine EC 500 G were 8.7 mg/kg and decreased to 0.21 mg/kg on day 18, calculated to dry material. All residue values in control samples were below the LOQ (0.01 mg/kg).

Based on the residue analysis a half-life period for spiroxamine in/on insects of 1.28 days can be calculated, by function of 1.5st order or 3.38 days by an exponential function of 1st order.

To calculate the average concentration over a specific exposure period based on the maximum concentration the Time-Weighted-Average factor (f_{TWA}) values are used. Assuming an exponential 1st order dissipation the f_{TWA} value can be calculated based on the exponential 1st order DT_{50} value.

The initial dissipation between day 1 to day 18 is well described by an exponential 1st order function with and corresponding DT_{50} of 3.38 days, but this function does not reflect the fast dissipation during the first day of the exposure. Therefore, using tha DT_{50} value of 3.38 days as the basis to calculate an f_{TWA} value will overestimates the f_{TWA} value and in consequence overestimate the average exposure. As recommended by the EU Guidance Document on Risk Assessment for Birds and Mammals (SANCO/4145/2000, Sep. 2002), the mean concentration and correspondingly an f_{TWA} value can also be determined by the area under the curve method. In the case of spiroxamine this second approach will give an more realistic estimate of the f_{TWA} values.

To conduct such an evaluation the area under the curve has been calculated by multiplying the residue value measured on each day by the duration of the time period that is represented by this value (1 day).

For days for which no value was available the residue value from the last measurement before this day was used. Summing up these values for the appropriate period of time and dividing the cumulative area by the duration (days) of the period gives the average concentration during this time period. Dividing this average concentration by the initial concentration on day 0 gives the corresponding f_{TWA} value.

Table CP 10.1.1.2/07-1 Result of spiroxamine analysis in/on beetles

Days after application	Control beetles	Treated beetles	
		Spiroxamine in [mg/kg] wet	Spiroxamine in [mg/kg] dry
0	<LOQ	3.8	8.7
1	<LOQ	1.5	3.3
2	<LOQ	1.4	3.2
3	<LOQ	1.0	2.2
4	<LOQ	0.66	1.5
6	<LOQ	0.44	1.0
8	<LOQ	0.32	0.72
10	<LOQ	0.22	0.48
12	<LOQ	0.16	0.36
14	<LOQ	0.08	0.18
18	<LOQ	0.09	0.21

The f_{TWA} values are given in table 2 for f_{TWA} periods of 2 to 21 days. When reading this table it has to be considered that the analytical measurements refer to the first day as day 0. Therefore, the f_{TWA} factor for a TWA period of 14 days refers to the measured values between day 0 and day 13 and the f_{TWA} values is found in the line for day 13 (i.e. f_{TWA} = 21%).

Table CP 10.1.1.2/07-2 Calculation of f_{TWA} values (residue data in italics are extrapolated from the last measurement point before this day)

Day	Residue (mg/kg)	Cumulative area (mg/kg*day)	Average residue (mg/kg)	TWA period (counted in days from day 0)	f_{TWA} (% of intimal)
0	8.7		-	-	-
1	3.3	12.00	6.00	2	69
2	3.2	15.20	5.07	3	58
3	2.2	17.40	4.35	4	50
4	1.5	18.90	3.78	5	43
5	1.5	20.40	3.40	6	39
6	1.0	21.40	3.06	7	35
7	1.0	22.40	2.80	8	32
8	0.72	23.12	2.57	9	30
9	0.72	23.84	2.38	10	27
10	0.48	24.32	2.21	11	25

Day	Residue (mg/kg)	Cumulative area (mg/kg*day)	Average residue (mg/kg)	TWA period (counted in days from day 0)	f_{TWA} (% of intimal)
11	0.36	24.80	2.07	12	24
12	0.36	25.16	1.94	13	22
13	0.18	25.52	1.82	14	21
14	0.18	25.70	1.71	15	20
15	0.18	25.88	1.62	16	19
16	0.18	26.06	1.53	17	18
17	0.18	26.24	1.46	18	17
18	0.21	26.45	1.39	19	16
19	0.21	26.66	1.33	20	15
20	0.21	26.87	1.28	21	15

Assessment and conclusion by applicant:

This reports presents the outcome of an assessment of the DT_{50} values determined in the beetles residues decline study (biological report: M-281566-01-1, analytical report: M-288174-01-2) in order to determine f_{TWA} values for use in a refined risk assessment.

The results are considered acceptable but have not been used in the refined risk assessment for Spiroxamine EC 500.

Data Point:	KCP 10.1.1.2/22
Report Author:	[redacted]
Report Year:	2021
Report Title:	Refinement of mean RUD for spiroxamine in grapes
Report No:	M-414948-01-1
Document No:	M-414948-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

Refined reproductive risk assessment (TER_{LT}) may be required for frugivorous birds that could be exposed to residues of spiroxamine on grape berries after application in vineyards. Therefore, a more realistic evaluation of the mean RUD was performed, based on compound-specific residue measurements (RUD) on grapes. Data are available for a total of 54 studies in which initial residues of spiroxamine parent compound on grapes were either measured directly or were recalculated from total residue values determined immediately after the last application. The RUD derived for the total set of studies (n=54) is in line with the RUD derived only for those studies in which initial residues of spiroxamine parent itself on grapes were measured (n=24).

Therefore, taking into account a dataset of 54 residue studies for spiroxamine in total, it is proposed to replace the generic RUD of 8.3 by a more realistic RUD of 1.63 that can be used in the refined risk assessment for spiroxamine.

I. Methods

Refinement of RUD for spiroxamine in grapes

The Tier 1 risk assessment for frugivorous birds eating grape berries in vineyards is based on the RUDs provided by the EFSA GD (2009, Appendix F). Unlike the RUDs recommended for food categories like "grass & cereals" (n = 132 studies) or "nongrass weeds" (n = 230 studies), the database behind this recommended RUDs for grape berries (the destemmed grapes themselves) is poor (n = 8 studies according to EFSA GD 2009). Furthermore, the data were taken from a published review of North American residue studies (Baril et al 2005) comprising residue and thus of unclear relevance for the EU. With regard to using measured residue data for higher tier refinements, the EFSA GD (2009) requires justification "*why new measured residue data will override the existing residue values presented…, as several studies were used to generate these generic RUDs. Therefore it is unlikely that one study will be appropriate to replace the generic RUD value*" (2009, Appendix F). Taking into account this requirement, a large number of regulatory residue studies (n=54) in grapevines with late season applications of spiroxamine was evaluated in order to present compound-specific measured residue data that can be considered fully appropriate to replace the generic mean RUD = 8.3 for grape berries. Residue trials were conducted in southern and northern Europe. In some earlier trials (1994-96), only the total residue of spiroxamine (determined as aminodiol and expressed in spiroxamine equivalents) was determined. For trials such as these, parent-compound residue levels were derived *via* recalculation from total residue determinations. The reason for this "recalculation" is the change in the regulatory evaluation of spiroxamine in Europe during the original EU review process. The residue definition as proposed originally by Bayer for grapes was the total residue of spiroxamine, determined as aminodiol and expressed in spiroxamine equivalents. The EU rapporteur member state for spiroxamine (Germany) decided to change the residue definition to spiroxamine parent only, which is reflected in the EU MRLs published in 2000. At the time of publishing Commission Directive 2000/81/EC, all residue data submitted had presented results only for the total residue of spiroxamine. Using a factor derived from the metabolism studies, the parent-compound residue levels and, from them, MRLs were calculated by the rapporteur from the total residue results. Since that time, "new"studies have generally been conducted using both the old and new residue definitions. However, some "older" studies (reporting total residues only) are also included in this evaluation in order to further support the proposal for a refined RUD for spiroxamine after application in grape.

II. Results and Discussion

The table below represents a comparison between all data residue available for spiroxamine residues on grape berries to only those newer studies in which initial spiroxamine parent compound residues were measured. The average number of applications made in the new trials was 4.0. A DT_{50} was not determined.

Table CP 10.1.1.2/22-1 Summary of residues of spiroxamine on grapes for compound specific RUD derivation

n	Locations	Year	Last AR (kg a.s./ha)	Mean # of applications (± SD)	Mean RUD (± SD)
54[1]	N & S EU	1994-2007	0.16-0.64	3.48 (± 0.97)	1.44 (± 0.86)
24	N & S EU	1997-2007	0.16-0.64	4.0 (± 1.25)	1.63 (± 0.87)

[1] all: complete data set based on studies both with measured residues of spiroxamine itself and on recalculated residues from total residue determinations

[2] new: subset of data set comprising of only studies with measurement of initial residues of spiroxamine itself

n = number of studies; AR = application rate

The RUD derived for the totality of studies (RUD=1.44) is in line with the RUD derived for the subset of the database which only considers initial measured residues of spiroxamine (RUD=1.63). As a conservative approach, the residues measured directly after the last application can be used as refinement for C = AR × MAF. The average RUD was 1.63.

III. Conclusion

Taking into account a dataset of 54 residue studies for spiroxamine in total, the compound-specific RUD_m of 1.63 can replace the generic RUD presented in the EFSA GD (only 8 data sets) in a higher tier risk assessment for spiroxamine.

Assessment and conclusion by applicant:

This report is a review of available residues studies using spiroxamine (54 studies).

The dataset is considered to be sufficiently large to replace the default RUD values used in the Bird & Mammal Guidance Document (EFSA, 2009). It is also noted that these studies, unlike the EFSA values, are specific to spiroxamine and therefore considered to be more relevant.

The study is considered acceptable.

The determined RUD of 1.63 has been used in the risk assessment to refine the frugivorous bird and mammal scenarios by replacing the default RUD values specified in EFSA (2009).

For procedural reasons studies listed in the Table CP 10.1.1.2-1 below are included in the current dossier as available data or information previously submitted but not necessarily evaluated. However, these reports have been fully superseded by newer studies. Consequently, no summaries of the reports have been included in the dossier.

Table CP 10.1.1.2-1: Studies previously submitted and not relied upon for the risk assessment

Data Point	Document No.	Date	Title
KCP 10.1.1.2/08	M-285584-01-1	2006	Bekanntmachung Nr. 06-02/26 ueber die Umsetzung des EU-Guidance Document fuer Voegel und Saeuger
KCP 10.1.1.2/09	M-263242-01-1	2005	Review on initial residue levels of pesticides in arthropods sampled in field studies
KCP 10.1.1.2/10	M-305599-01-1	1965	Zur Kenntnis des Samenöffnens und der Struktur des hörnernen Gaumens bei körnerfressenden Oscines
KCP 10.1.1.2/11	M-108441-01-1	2002	AGROBIRD - Database 2002 - Turdus merula
KCP 10.1.1.2/12	M-291198-01-1	2006	Bird species in modern pome fruit orchards in Germany: field data for the determination of focal species
KCP 10.1.1.2/13	M-291194-01-1	2006	Bird species in pome fruit orchards in Poland and Italy: field data for the determination of focal species
KCP 10.1.1.2/14	M-291211-01-1	2007	Generic field monitoring of selected bird species in orchards in Southern Germany
KCP 10.1.1.2/15	M-266856-01-1	1988	Handbuch der Voegel Mitteleuropas - Turdus merula (Linnaeus 1758) - Amsel

CP 10.1.2 Effects on terrestrial vertebrates other than birds

The available mammalian toxicity data for spiroxamine and Spiroxamine EC 500 are summarised in the table below.

Table CP 10.1.2-1 Summary of mammalian toxicity studies with spiroxamine and Spiroxamine EC 500

Organism	Test item	Test type	Endpoints		Reference
Rat	Spiroxamine	Acute oral toxicity	LD_{50} 595 mg a.s./kg bw (male) LD_{50} >500<560 mg a.s./kg bw (female)	EU	M-007781-01-1
Mouse	Spiroxamine	Acute oral toxicity	LD_{50} **460 mg a.s./kg bw** (male) LD_{50} 561 mg a.s./kg bw (female)	EU	M-007804-01-1
Rat	Spiroxamine	Chronic, 2-generation	NOAEL (parental) ♂/♀ 5.5 / 6.7 mg a.s./kg bw/day NOAEL (reproductive) ♂/♀ **21.0** / 21.2 mg a.s./kg bw/day NOAEL (offspring) ♂/♀ 6.5 / 6.7 mg a.s./kg bw/day	EU	M-304231-01-1
Rat	Spiroxamine EC 500	Acute oral toxicity	LD_{50} ~1000 mg/kg bw (males) LD_{50} >200<1000 mg/kg bw (females) Equivalent to LD_{50} ~500 mg a.s./kg bw (males) LD_{50} >100<500 mg a.s./kg bw (females)	EU	M-016267-01-1
Rat	Spiroxamine EC 500	Acute oral toxicity	LD_{50} >500 mg/kg bw (males and females) Equivalent to LD_{50} >250 mg a.s./kg bw (males and females)	EU	M-016680-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
Values in **bold** have been used in the risk assessment

Toxicity endpoints for risk assessment

The acute risk assessment for spiroxamine has used the lowest available LD_{50} value which is 460 mg a.s./kg bw which was determined in male mice.

For the reproductive risk assessment of spiroxamine, an ecotoxicologically relevant endpoint has been determined. According to the outcome of the pesticides peer review meeting on recurring issues in ecotoxicology (EFSA PPR meeting 133, September 2015), an ecotoxicologically relevant endpoint should be set in collaboration with mammalian toxicologists and should be used in all the steps of the risk assessment.

Report M-762441-01-1 presents an assessment of the available mammalian toxicology data with spiroxamine along with a summary of the specific effects seen for various parameters for each study. A NOAEL of 21.0 mg a.s./kg bw/day has been determined from the rat two generation study and considered suitable for ecotoxicological risk assessment of wild mammals. The refinement is based on the assumption that the effects reported at the 300 ppm top dose level do not influence the population success and the total reproductive outcome of mammals in treated areas. At this dose parental animals showed slight decreases of body weight (up to 8.3 %) or body weight gain (up to 14.2 %) as well as irritation induced hyperkeratosis of the oesophagus epithelium. There were delays to developmental milestones of reaching puberty, *i.e.* preputial separation (PPS) in males and vaginal opening (VO) in females, in the F_1 offspring only which were apparently treatment related, but these were relatively small and are not considered to have an adverse effect at the population level. It is noted that in the same study the reproductive parameters (mating, fertility, oestrous cycling, sperm motility, sperm count, sperm morphology, pregnancy, natural delivery, litter observations, mean ovarian follicles, *corpora lutea*) were unaffected at the highest dose therefore it has been demonstrated that these small delays in PPS and VO do not have an adverse effect on the parameters that are considered to be relevant at the population level.

Thus, the lowest ecotoxicologically relevant NOAEL, suitable for use in the mammalian reproductive risk assessment, was considered to be 21.0 mg a.s./kg bw/day. Details of the assessment can be found in report M-762441-01-1 which has been summarized at the end of this section.

Literature paper M-669216-01-1 presents the results of population modelling conducted in order to assess the impact of body weight effects on the population development of the common vole. The study revealed that there was no detectable influence of common vole body weight on the reproductive success and survival during most times of the year and that reproductive success was mainly influenced by the date of birth. This study supports the position that the relatively small reductions in body weight recorded in the rat two-generation study are unlikely to have an adverse effect at the population level and are therefore not ecotoxicologically relevant.

The NOAEL of 21.0 mg a.s./kg bw/day has been used in all tiers of the reproductive risk assessment for spiroxamine.

Metabolites

Numerous metabolites of spiroxamine are formed in plants following application to crops. In the Toxicology and Residues sections of the dossier the spiroxamine metabolites have been categorised into three distinct groups (Group A, B and C, respectively). Toxicology dara are available for several of these metabolites. Metabolite M13 has been used to represent all Group B metabolites therefore the toxicology data generated using this metabolite is also considered to cover the plant metabolites M35 and M36. Likewise, M28 has been used to represent all Group C metabolites therefore the toxicology data generated using this metabolite is also considered to cover the plant metabolites M29, M30 and M31. It is also noted that Group A metabolites are considered to be covered by parent spiroxamine.

The table below presents each plant metabolite along with the percentage TRR and actual residue value from the crop metabolism studies. Available toxicology data have also been presented as well as an indication of whether or not each plant metabolite was also found in the animal metabolism studies on laying hen, rat and goat. Finally, an assessment is made regarding the relevance of each plant metabolite

to the risk assessment. Only metabolites which were formed in plants at ≥10% TRR are considered to be potentially relevant to the bird and mammal risk assessment.

Note that only metabolites which were found in the crop metabolism studies have been presented below however M13 and M13-acetate have been included in the table below because there are toxicology data which are relevant to other Group B plant metabolites.

Table CP 10.1.2-2 Assessment of potential exposure of mammals to metabolites of spiroxamine formed in plants

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine - desethyl (M01) [GROUP A]	**Primary crops** Wheat Forage: 5.1% TRR; 1.11 mg/kg Straw: 2.0% TRR; 0.70 mg/kg Grain: 0.5% TRR; <0.001 mg/kg Grapes 2.1 % TRR; 0.27 mg/kg Banana Pulp: 1.1% TRR; 0.005 mg/kg Peel: 2.7% TRR; 0.18 mg/kg **Rotational crops** Leafy vegetables 12.6% TRR; 0.026 mg/kg Cereals 20.0% TRR; 0.119 mg/kg Root & tuber vegetables 9.9% TRR; 0.083 mg/kg	Not found in goat or rat. Found in laying hen (21.3% in liver, 9.3% in muscle, 8.4% in fat and 11.5% in eggs)	No data available.	Metabolite found in primary crops at <10% TRR therefore not considered relevant for risk assessment. Metabolite found >10% TRR in rotational crops but actual residue levels were very low therefore not considered relevant for risk assessment.
Spiroxamine - despropyl (M02) [GROUP A]	**Primary crops** Wheat Forage: 4.6% TRR; 0.49 mg/kg Straw: 4.2% TRR; 3.48 mg/kg Grain: 3.0% TRR; 0.002 mg/kg Grapes 1.5% TRR; 0.20 mg/kg Banana Pulp: 0.5% TRR; 0.002 mg/kg Peel: 2.9% TRR; 0.19 mg/kg **Rotational crops** Leafy vegetables 51.2% TRR; 0.053 mg/kg Cereals 46.6% TRR; 0.190 mg/kg Root & tuber vegetables 21.1% TRR; 0.188 mg/kg	Not found in goat or rat. Found in laying hen (21.7% in liver, 11.3% in muscle, 3.4% in fat and 10.2% in eggs)	No data available.	Metabolite found in primary crops at <10% TRR therefore not considered relevant for risk assessment. Metabolite found >10% TRR in rotational crops but actual residue levels were very low therefore not considered relevant for risk assessment.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine - N-oxide (M03) [GROUP A]	**Primary crops** Wheat Forage: 12.7% TRR; 3.06 mg/kg Straw: 22.0% TRR; 7.68 mg/kg Grain: 17.8% TRR; 0.012 mg/kg Grapes 4.7% TRR; 0.61 mg/kg Banana Pulp: 1.2% TRR; 0.007 mg/kg Peel: 4.9% TRR; 0.23 mg/kg **Rotational crops** Cereals 7.4% TRR; 0.235 mg/kg	Not found in goat or laying hen Found in rat (found in liver at low amounts of 0.11%)	Acute oral rat LD_{50} 707 mg/kg bw 28-day rat oral dietary NOAEL 12.9/13.2 mg/kg bw/day for males/females 90-day rat oral dietary NOAEL 8.8/9.7 mg/kg bw/day for males/females	Metabolite found in wheat at >10% TRR therefore considered relevant for risk assessment. Tox data are available and show that metabolite toxicity is comparable to that of spiroxamine. Metabolite found in the rat metabolism study therefore toxicity data and associated assessment for parent considered to cover this metabolite.
Spiroxamine - N-formyl-desethyl (M04) [GROUP A]	**Primary crops** Wheat Forage: 5.8% TRR; 1.40 mg/kg Straw: 9.7% TRR; 8.06 mg/kg Grain: 6.9% TRR; 0.005 mg/kg Grapes Not found Banana Not found **Rotational crops** Cereals 6.4% TRR; 0.204 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in primary crops and rotational crops at <10% TRR therefore not considered relevant for risk assessment.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine - hydroxyl (M05) [GROUP A]	**Primary crops** Wheat Forage: 7.1% TRR; 1.71 mg/kg Straw: 5.2% TRR; 4.32 mg/kg Grain: 1.6% TRR; 0.001 mg/kg Grapes 0.3% TRR; 0.04 mg/kg Banana Not found **Rotational crops** Leafy vegetables 17.2% TRR; 0.146 mg/kg Cereals 2.5% TRR; 0.49 mg/kg Root & tuber vegetables 3.6% TRR; 0.032 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops at >10% TRR in leafy vegetables but the actual residues level is very low therefore not considered relevant for risk assessment.
Spiroxamine - hydroxy-despropyl (M09) [GROUP A]	**Primary crops** Wheat Forage: not found Straw: 0.3% TRR; 0.01 mg/kg Grain: not found Grapes Not found Banana Not found	Not found in goat, rat or laying hen	No data available.	Metabolite found in primary crops and rotational crops at <10% TRR therefore not considered relevant for risk assessment.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine – cyclohexanol (M13) [Group B]	**Primary crops** Not found	Not found in goat, rat or laying hen	Acute oral rat LD_{50} 4200 mg/kg bw Acute dermal rabbit LD_{50} >5000 mg/kg bw 28-day rat oral (gavage) NOAEL 50 mg/kg bw/day It was concluded that M13 is less toxic than the parent, spiroxamine in the rat with a *ca.* 9-fold, 2-fold and 8-fold increase in sub-acute, maternal and developmental NOAELs respectively when compared to the spiroxamine equivalent studies	Metabolite not found in crop metabolism studies therefore not considered relevant for risk assessment. Tox data are available and confirm M13 to be less toxic than parent. M13 data used to represent the toxicity of all Group B metabolites.
Spiroxamine cyclohexanol acetate (M-13 acetate) [Group B]	**Primary crops** Not found	Not found in goat, rat or laying hen	Rat developmental NOAEL maternal toxicity 40 mg/kg bw/day Rat developmental NOAEL 160 mg/kg bw/day Group B metabolites considered to be covered by available data for M13 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite not found in crop metabolism studies therefore not considered relevant to the risk assessment. Tox data are available and suggest lower toxicity than parent spiroxamine.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine-diol (M14) [Group B]	**Primary crops** Banana pulp (12.8% TRR; 0.057 mg/kg- hydrolysis product) Grapes (13.0% TRR; 0.44 mg/kg- hydrolysis product) Spring wheat straw (0.2% TRR; 0.070 mg/kg- hydrolysis product) Spring wheat grain (2.6% TRR; 0.002 mg/kg- hydrolysis product) **Rotational crops** Swiss chard leaves (8.8-13.2% TRR; 0.01 mg/kg- hydrolysis product) Wheat straw (3.5-4% TRR; 0.05 mg/kg- hydrolysis product) Turnip tops (4.4-13.0% TRR; 0.02 mg/kg- hydrolysis product)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at >10% TRR but the actual residues level is very low therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine-ketone (M15) [Group B]	**Primary crops** Grapes (1.3% TRR- hydrolysis product) Spring wheat straw (5.5% TRR- hydrolysis product) Spring wheat grain (4.6% TRR- hydrolysis product)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine-hydroxy-ketone (M16) [Group B]	**Primary crops** 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; 0.04 mg/kg- hydrolysis product) Wheat straw (8.9-11.6% TRR; 0.15 mg/kg- hydrolysis product) Turnip tops (11.7-37.3% TRR; 0.17 mg/kg- hydrolysis product)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at >10% TRR but the actual residues level is very low therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine - hydroxy-N-oxide glucoside (M20) [Group A]	**Primary crops** Wheat Forage: 0.7% TRR; 0.08 mg/kg Straw: 2.0% TRR; 0.70 mg/kg Grain: not found Grapes Not found Banana Not found **Rotational crops** Swiss chard leaves (2.5% TRR; <0.01 mg/kg)) Wheat straw (2.1-2.6% TRR; 0.03 mg/kg) Turnip tops (8.4-10.4% TRR; 0.04 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in primary crops at <10% TRR with the exception of turnip tops but the actual residues level is very low. Therefore this metabolite is not considered relevant for risk assessment.
Spiroxamine - hydroxy-N-oxide malonyl glucoside (M21) [Group A]	**Primary crops** Wheat Forage: 2.0% TRR; 0.21 mg/kg Straw: 3.1% TRR; 2.57 mg/kg Grain: not found Grapes Not found Banana Not found **Rotational crops** Swiss chard leaves (1.6% TRR; <0.01 mg/kg) Wheat straw (4.4% TRR; 0.06 mg/kg) Turnip tops (1.7-3.7% TRR; <0.01 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at <10% TRR therefore not considered relevant for risk assessment.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine-diol-diglycoside (M24) [Group B]	**Primary crops** Grapes (14.8% TRR – main component of metabolite group 12; 0.50 mg/kg) **Rotational crops** Swiss chard leaves (3.0% TRR; <0.01 mg/kg) Wheat straw (1.9-2.2% TRR; 0.020 mg/kg- Turnip roots (7.8% TRR; <0.01 mg/kg) Turnip tops (2.0-4.3% TRR; <0.01 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in grapes at >10% TRR but the actual residues level is very low, therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine - aminodiol (M28) [GROUP C]	**Primary crops** Wheat Not found Grapes: 37.5% TRR; 4.91 mg/kg Banana Pulp: 31.2% TRR; 0.173 mg/kg Peel: 37.2% TRR; 2.45 mg/kg **Rotational crops** Leafy vegetables 3.9% TRR; 0.014 mg/kg Cereals 0.6% TRR; 0.024 mg/kg Root & tuber vegetables 4.9% TRR; 0.005 mg/kg	Found in rat at 2.2 – 5.7% of dose	Acute oral rat LD_{50} >550 <2000 mg/kg bw 28-day rat oral dietary NOAEL 28.4/31.4 mg/kg bw/day for males/females Developmental rat oral (gavage) NOAEL maternal toxicity 150 mg/kg bw/day and developmental NOAEL 30 mg/kg bw/day It was concluded that M28 is less toxic than the parent, spiroxamine in the rat with a *ca.* 15-fold, 9-fold and 2-fold increase in sub-acute, maternal and developmental NOAELs, respectively when compared to the spiroxamine equivalent studies.	Metabolite found in grapes and banana at >10% TRR therefore relevant for the risk assessment. Tox data are available and confirm that toxicity is less than parent. Furthermore, M28 was found in the rat metabolism study therefore the toxicity and the associated risk assessment is considered to be covered by the assessment of parent spiroxamine. M28 data used to represent the toxicity of all Group C metabolites.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine - aminodiol-N-oxide (M29) [GROUP C]	**Primary crops** Wheat Not found Grapes 0.1% TRR; 0.01 mg/kg Banana Not found **Rotational crops** Leafy vegetables 5.2% TRR; 0.021 mg/kg Root & tuber vegetables 4.8% TRR; 0.005 mg/kg	Not found in goat, rat or laying hen	No data available. Group C metabolites considered to be covered by available data for M28 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in primary crops and rotational crops at <10% TRR therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M28 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine - desethyl-aminodiol (M30) [GROUP C]	**Primary crops** Wheat Not found Grapes 1.1% TRR; 0.14 mg/kg Banana Pulp: 0.6% TRR; 0.003 mg/kg Peel: 0.9% TRR; 0.06 mg/kg	Not found in goat, rat or laying hen	No data available. Group C metabolites considered to be covered by available data for M28 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in primary crops at <10% TRR therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M28 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine - despropyl-aminodiol (M31) [GROUP C]	**Primary crops** Wheat Not found Grapes 1.2% TRR; 0.16 mg/kg Banana Pulp: 0.6% TRR; 0.003 mg/kg Peel: 0.9% TRR; 0.06 mg/kg **Rotational crops** Cereals 1.7% TRR; 0.034 mg/kg Root & tuber vegetables 6.1% TRR; 0.006 mg/kg	Not found in goat, rat or laying hen	No data available. Group C metabolites considered to be covered by available data for M28 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in primary crops and rotational crops at <10% TRR therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M28 data confirm this group of metabolites to be less toxic than parent.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine-cyclohexanol-glucopyranosyl-pentose (M33) [GROUP B]	**Primary crops** Grapes (19.1% TRR; 0.650 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at >10% TRR but the actual residues level is very low therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine-cyclohexanol-glucopyranosyl-glucopyranosyl-pentose (M34) [GROUP B]	**Primary crops** Grapes (3.5% TRR; 0.130 mg/kg)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine docosanoic acid ester (M35) [GROUP B]	**Primary crops** <u>Wheat</u> Not found <u>Grapes</u> 13.0% TRR; 0.44 mg/kg <u>Banana</u> Not found	Not found in goat, rat or laying hen	No data on M35. Group B metabolites considered to be covered by available data for M13 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in grapes at >10% TRR. Available data with M13 used to cover this Group B metabolite and confirms that the toxicity is lower than that of the parent. Thus, the risk to M35 is considered to be covered by the assessment for parent.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine tetracosanoic acid ester (M36) [GROUP B]	**Primary crops** Wheat Not found Grapes 4.2% TRR; 0.14 mg/kg Banana Not found	Not found in goat, rat or laying hen	No data on M36. Group B metabolites considered to be covered by available data for M13 which confirm that this metabolite is less toxic than spiroxamine.	Metabolite found in primary crops at <10% TRR therefore not considered relevant for risk assessment. Toxicity would be covered by parent assessment as M13 data confirm this group of metabolites to be less toxic than parent.
Spiroxamine-cyclohexenol (M37) [GROUP B]	**Primary crops** Grapes (3.2% TRR; 0.11 mg/kg-hydrolysis product)	Not found in goat, rat or laying hen	No data available.	Metabolite found in plants at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine – N-formyl-despropyl (M38) [GROUP A]	**Primary crops** Not found **Rotational crops** Cereals 7.6% TRR; 0.243 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops only and at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine – hydroxy-despropyl glycoside (M39) [GROUP A]	**Rotational crops** Leafy vegetables 2.8% TRR; 0.019 mg/kg Cereals 5.9% TRR; 0.232 mg/kg Root & tuber vegetables 21.3% TRR; 0.063 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops at >10% TRR in root and tuber vegetables but the actual residues level is very low therefore not considered relevant for risk assessment.
Spiroxamine – hydroxy glycoside (M40) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 4.7% TRR; 0.040 mg/kg Cereals 2.9% TRR; 0.088 mg/kg Root & tuber vegetables 7.6% TRR; 0.068 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops only and at <10% TRR therefore not considered relevant for risk assessment.

Plant Metabolite	Maximum levels of residue in plants	Metabolite found in animal studies?	Mammalian toxicity data available?	Conclusion on relevance for mammalian risk assessment
Spiroxamine – hydroxy-desethyl glycoside (M42) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 1.6% TRR; 0.005 mg/kg Cereals 6.5% TRR; 0.129 mg/kg Root & tuber vegetables 14.6% TRR; 0.044 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops at >10% TRR in root and tuber vegetables but the actual residues level is very low therefore not considered relevant for risk assessment.
Spiroxamine – desethyl acid glycoside (M43) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 1.8% TRR; 0.015 mg/kg Cereals 3.4% TRR; 0.088 mg/kg Root & tuber vegetables 5.7% TRR; 0.051 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops only and at <10% TRR therefore not considered relevant for risk assessment.
Spiroxamine – acid glycoside (M44) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 4.6% TRR; 0.019 mg/kg Cereals 6.4% TRR; 0.126 mg/kg Root & tuber vegetables 11.6% TRR; 0.027 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops at >10% TRR in root and tuber vegetables but the actual residues level is very low therefore not considered relevant for risk assessment.
Spiroxamine – despropyl acid glycoside (M45) [GROUP A]	**Primary crops** Not found **Rotational crops** Leafy vegetables 5.5% TRR; 0.019 mg/kg Cereals 3.7% TRR; 0.145 mg/kg Root & tuber vegetables 9.1% TRR; 0.002 mg/kg	Not found in goat, rat or laying hen	No data available.	Metabolite found in rotational crops only and at <10% TRR therefore not considered relevant for risk assessment.

M01 and M02 were found in the rotational crop studies at >10% TRR however the absolute residue values were very low therefore these metabolites are not considered to be relevant to the mammalian risk assessment.

M03 was found in wheat at >10% TRR. Toxicology data for M03 demonstrate that this metabolite is of similar or lower toxicity than spiroxamine. Furthermore, this metabolite was found in the rat metabolism study. It is therefore considered that the risk assessment of parent spiroxamine covers the risk from exposure to this metabolite.

M28 was found in grapes and banana at >10% TRR. Toxicology data are available for M28 and confirm that this metabolite is 15-fold, 9-fold and 2-fold less toxic in sub-acute, maternal and developmental parameters than spiroxamine. Furthermore, this metabolite was found in the rat metabolism study. Therefore the risk from exposure to this metabolite is considered to be covered by the assessment for parent.

M35 was also found in grapes at >10%TRR. The toxicology data generated for M28 are considered to also cover this metabolite and confirm that the metabolite is less toxic than parent. Thus, the risk from exposure to this plant metabolite is considered to be covered by the assessment for parent.

M04, M05, M09, M14, M15, M16, M20, M21, M24, M29, M30, M31, M33, M34, M36, M37, M38, M39, M40, M42, M43, M44 and M45 were found in the crop metabolism studies at either <10% TRR or very low absolute amounts and were therefore not considered to be relevant for risk assessment.

Specific dietary risk assessment for these plant metabolites of spiroxamine is therefore not considered to be necessary.

Dietary risk assessment for mammals

Exposure

In order to present risk assessments which fully cover the range of options available in the GAP, the following six exposure regimes have been considered here with dietary risk assessments presented for each regime:

- 1 x 200 g a.s./ha BBCH 13 - 19
- 1 x 300 g a.s./ha BBCH 13 - 19
- 1 x 300 g a.s./ha BBCH 53 - 85
- 2 x 300 g a.s./ha BBCH 53 - 85 (10-day interval)
- 1 x 200 g a.s./ha BBCH 13 - 19 and 1 x 300 g a.s./ha BBCH 20 (53) - 85 (10-day interval)
- 1 x 300 g a.s./ha BBCH 13 - 19 and 1 x 300 g a.s./ha BBCH 20 (53) - 85 (10-day interval)

Isomers

The risk assessments for birds & mammals involves potential chronic exposure of these organisms to residues in plants therefore it may be necessary to apply an uncertainty factor (UF) to the chronic avian and mammalian risk assessments. The acute risk assessment need not have an UF applied as exposure in this scenario is immediate. However, the chronic risk assessment considers exposure over a prolonged period therefore potential changes in isomeric ratio needs to be considered. For the bird and mammal risk assessments the same approach taken in the residues section for the consumer risk assessment, with respect to isomers, has been followed. Based on the current residues data set for spiroxamine, there are no indications of a significant change in isomer ratios therefore no additional factor need be applied to the risk assessments below (*i.e.* an UF of 1.0 has been used).

Risk assessment

The risk assessments have been conducted in accordance with the EFSA (2009) Bird & Mammal Guidance document. Each assessment starts with a screening step followed by a Tier I assessment if required. Finally, refined risk assessments have been presented where required.

The acute 'daily dietary dose' (DDD) is calculated by multiplying the shortcut value (SV) based on the 90th percentile residues by the application rate in kg a.s./ha.

DDD = application rate (kg a.s/ha) x SV_{90}

The long-term 'daily dietary dose' (DDD) is calculated by multiplying the SV based on the mean residues by the application rate in kg a.s./ha and (only for the long-term risk assessment) a time weighted average residue exposure (f_{twa}). The f_{twa} based upon a default DT_{50} of 10 days is 0.53, as given in EFSA guidance (2009).

DDD_{LT} = application rate (kg a.s./ha) x SV_m x f_{twa}

Acute risk is assessed by comparing the relevant daily dietary dose (DDD) values with the appropriate LD_{50} endpoint to give an acute Toxicity: Exposure Ratio (TER_A):

$$TER_A = \frac{LD_{50}\ (\text{mg/kg bw})}{\text{DDD}}$$

TER_A values which exceed a trigger value of 10 indicate an acceptable acute risk.

Derivation of the short-term toxicity exposure ratio is no longer a requirement according to the EFSA Guidance Document (2009) so a short-term risk assessment has not been presented.

Long-term risk is assessed by comparing the long-term DDD values with the worst case NOAEL from the reproduction studies, expressed as daily dietary dose, to give a long-term toxicity exposure ratio (TER_{LT}):

$$TER_{LT} = \frac{NOAEL\ (\text{mg/kg bw/day})}{\text{DDD}\ (\text{mg/kg bw/day})}$$

TER_{LT} values which exceed a trigger value of 5 indicate acceptable chronic risk.

1 x 200 g a.s./ha BBCH 13 - 19

The screening step assessments for the acute and reproductive risks are presented below.

Table CP 10.1.2-3 Screening step assessment for acute and long-term/reproductive risk to mammals for the proposed use of Spiroxamine EC 500 in grapes (BBCH 13 - 19)

Intended use	Grapes (BBCH 13 - 19)				
Active substance/product	Spiroxamine / Spiroxamine EC 500				
Application rate (g a.s./ha)	1 × 200				
Acute toxicity (mg a.s./kg bw)	460				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Vineyard	Small herbivorous mammal	136.4	1.0	27.3	16.9
Reprod. toxicity (mg a.s./kg bw/d)	21.0				
TER criterion	5				
Crop scenario	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard	Small herbivorous mammal	72.3	1.0 x 0.53	7.66	**2.74**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger

For the proposed use of Spiroxamine EC 500 on grapes (1 x 200 g a.s./ha at BBCH 13 - 19) the acute risks to mammals from dietary exposure to spiroxamine are considered to be acceptable (TER ≥10) but potential reproductive risks have been identified (TER <5). A Tier I reproductive risk assessment has therefore been conducted and presented below.

Table CP 10.1.2-4 Tier I assessment for reproductive risk to mammals for the proposed use of Spiroxamine EC 500 in grapes (BBCH 13 - 19)

Intended use	Grapes (BBCH 13 - 19)
Active substance/product	Spiroxamine / Spiroxamine EC 500
Application rate (g a.s./ha)	1 × 200
Reprod. toxicity (mg a.s./kg bw/d)	21.0
TER criterion	5

Crop scenario Growth stage	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard BBCH 10-19	Large herbivorous mammal “lagomorph”	6.7	1.0 x 0.53	0.710	29.6
Vineyard BBCH 10-19	Small insectivorous mammal “shrew”	4.2	1.0 x 0.53	0.445	47.2
Vineyard BBCH 10-19	Small herbivorous mammal “vole”	43.4	1.0 x 0.53	4.60	**4.57**
Vineyard BBCH 10-19	Small omnivorous mammal “mouse”	4.7	1.0 x 0.53	0.498	42.2

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger

For the proposed use of Spiroxamine EC 500 on grapes (1 x 200 g a.s./ha at BBCH 13 - 19) the reproductive risks to mammals from dietary exposure to spiroxamine are considered to be acceptable (TER ≥5) for all of the relevant scenarios with the exception of the small herbivorous mammal “vole” scenario. A refined risk assessment for this individual scenario has been presented below.

Refinement of the small herbivorous mammal “vole” scenario

The relevance of the vole in agricultural areas has frequently been discussed and its use in risk assessment questioned. In the literature paper M-476622-01-1 it is stated that the preferred primary habitat of common voles, is steppe, which comprises grassland, pasture and meadow with mixed grassland, herbs and weeds that provide appropriate cover to avoid predation. It is also stated that although the common vole is indicated as the representative generic focal species in screening and tier 1 risk assessments under EFSA (2009), population dynamics and habitat preferences indicate that in the period between population outbreaks the likelihood of significant numbers of common voles being found in secondary habitats is low. Thus, it is considered that the common vole is not necessarily a good choice of focal species to represent small mammals in vineyards.

It is therefore proposed to use the wood mouse as a more representantive focal species to represent small mammals in vineyards. Study M-291785-01-1 was a generic monitoring study of mammals in vineyards in which a radio-tracking program was carried out in a typical wine growing region of France during Spring and Summer. Trapping, radio-tracking and visual observations together with analyses of stomach content were carried out for the wood mouse. The study showed that the wood mouse was far more prevalent in vineyards and the surrounding areas when compared to other small mammals such as the vole or shrew. It is therefore considered to be a highly relevant small mammal focal species, far more so than the vole which was seen in very low numbers. The study also found that, whilst the wood mouse did spend time within the vineyards, they found the surrounding areas more attractive. A 90th percentile PT value for the wood mouse of 0.41 was determined in this study and has been applied to the refined risk assessment. The study also analysed stomach contents which confirmed that the diet was almost exclusively plant matter with seeds being the primary component. The study is considered to support

the default EFSA (2009) diet for omnivorous mammals of 25% weeds, 50% weed seeds and 25% ground arthropods therefore this EFSA (2009) diet has been considered in the refined risk assessment below.

Study M-405593-01-1 is also a generic field monitoring study of mammals in vineyards but this time in Spain. The study can be used as supporting evidence that the wood mouse is a suitable focal species to represet small mammals in vineyards. A 90th percentile PT value of 0.17 was determined in this study but the refined risk assessment below has used the more conservative PT value of 0.41. Study M-237095 provides further supporting data for the presence of the wood mouse in vineyards in Germany.

Table CP 10.1.2-5 Refinement of the small herbivorous mammal vole scenario (BBCH 10-19) for the proposed use of Spiroxamine EC 500 in grapes (BBCH 13 - 19) – Wood mouse

App rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT [d]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
1 x 0.200 kg a.s./ha	Weeds	0.0667	28.7	1.0	0.53	0.41	0.6 [e]	0.0499	0.202	104
	Weed seeds	0.133	40.2	1.0	0.53			0.139		
	Invertebrates (ground dwelling)	0.0667	43	1.0	0.53			0.0130		

[a] Values calculated using default dietary data from EFSA (2009)
[b] Default RUD values from EFSA (2009) Appendix F
[c] Default EFSA values
[d] Refined 90th percentile PT value from focal species study
[e] Deposition value based on 40% crop interception (Appendix A: EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Using the refined parameters discussed above, the reproductive risks to small mammals following application of Spiroxamine EC 500 to grapes at 1 x 200 g a.s./ha at BBCH 10 - 19 are considered to be acceptable (TER ≥ 5).

Alternative refinement of the small herbivorous mammal "vole" scenario

As an alternative refinement the vole has been used as the focal species. This is the generic focal species from EFSA (2009) and although it is not considered to be as prevalent in vineyards as the wood mouse, its presence in vineyards has been confirmed by monitoring study M-291785-01-1, as discussed above. A vole diet of 76.6% grass & cereals, 11.9% non-grass herbs and 11.5% weed seeds has been used in the refined risk assessment below. This diet has been taken from study M-439777-01-1 which reports the outcome of an analysis of vole diets from different habitats. The evidence suggests that voles do not feed exclusively on grasses and that other plant material such as dicots and seeds also made up part of the diet.

Several residues decline studies are available in which the residues of spiroxamine have been determined at frequent intervals following application of spiroxamine containing formulations to cereals. Report M-759383-01-1 summarises the kinetic analyses of all 24 available trials which have been conducted as part of five separate studies (M-301585-01-1, M-574326-01-1, M-578235-01-1, M-628347-02-1 and M-684671-01-1) covering wheat and barley plants in both Northern and Southern Europe. The individual validity of each study has been discussed in the evaluation section after each study summary but all of the trials used in this assessment were considered to be valid and suitable for use in the derivation of an overall crop dissipation half-life (DT_{50}) value. It was found that spiroxamine residues dissipated relatively quickly on cereals with an overall geomean DT_{50} of 3.03 days determined. A geomean DT_{50} value of 2.74 days was determined for Northern EU and a DT_{50} of 3.83 days for Southern EU. The overall geomean DT_{50} of 3.03 days has been applied to the refined risk assessment below and is considered to be suitably representative of the decline of spiroxamine residues throughout Europe. Note that the refined DT_{50} has only been applied to the grass/cereals component of the diet as this is the matrix upon which the residues were determined. The DT_{50} of 3.03 days has been used in place of the default value of 10 days to refine the f_{twa} value from 0.53 to 0.206.

Table CP 10.1.2-6 Refinement of the small herbivorous mammal vole scenario (BBCH 10-19) for the proposed use of Spiroxamine EC 500 in grapes (BBCH 13 - 19) – Vole

App rate	Food type	FIR/bw [a]	RUD [b]	MAF	f_{twa}	PT [d]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
1 x 0.200 kg a.s./ha	Grass/ cereals	0.558	54.2	1.0	0.206 [c]	1.0	0.6 [e]	0.748	1.12	18.8
	Non-grass herbs	0.0866	28.7	1.0	0.53			0.158		
	Weed seeds	0.0837	40.2	1.0	0.53			0.214		

[a] Values calculated using focal species dietary data
[b] Default RUD values from EFSA (2009) Appendix F
[c] A f_{twa} value calculated using a DT_{50} value of 3.03 days
[d] Default PT of 1.0 used
[e] Deposition value based on 40% crop interception (Appendix A EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Using the refined parameters discussed above, the reproductive risks to small herbivorous mammals, following application of Spiroxamine EC 500 to grapes at 1 x 200 g a.s./ha at BBCH 13 - 19, are considered to be acceptable (TER ≥5).

1 x 300 g a.s./ha BBCH 13 - 19

The screening step assessments for the acute and reproductive risks are presented below.

Table CP 10.1.2-7 Screening step assessment for acute and long-term/reproductive risk to mammals for the proposed use of Spiroxamine EC 500 in grapes (BBCH 13 - 19)

Intended use	Grapes (BBCH 13 - 19)				
Active substance/product	Spiroxamine / Spiroxamine EC 500				
Application rate (g a.s./ha)	1 × 300				
Acute toxicity (mg a.s./kg bw)	460				
TER criterion	10				
Crop scenario	Indicator species	SV_m	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Vineyard	Small herbivorous mammal	136.4	1.0	40.9	11.2
Reprod. toxicity (mg a.s./kg bw/d)	21.0				
TER criterion	5				
Crop scenario Growth stage	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{LT}
Vineyard	Small herbivorous mammal	72.3	1.0 x 0.53	11.5	**1.83**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger

For the proposed use of Spiroxamine EC 500 on grapes (1 x 300 g a.s./ha at BBCH 13 - 19) the acute risks to mammals from dietary exposure to spiroxamine are considered to be acceptable (TER ≥10) but potential reproductive risks have been identified (TER <5). A Tier I reproductive risk assessment has therefore been conducted and presented below.

Table CP 10.1.2-8 Tier I assessment for reproductive risk to mammals for the proposed use of Spiroxamine EC 500 in grapes (BBCH 13 - 19)

Intended use	Grapes (BBCH 13 - 19)
Active substance/product	Spiroxamine / Spiroxamine EC 500
Application rate (g a.s./ha)	1 × 300
Reprod. toxicity (mg a.s./kg bw/d)	21.0
TER criterion	5

Crop scenario Growth stage	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard BBCH 10-19	Large herbivorous mammal “lagomorph”	6.7	1.0 x 0.53	1.07	19.7
Vineyard BBCH 10-19	Small insectivorous mammal “shrew”	4.2	1.0 x 0.53	0.668	31.5
Vineyard BBCH 10-19	Small herbivorous mammal “vole”	43.4	1.0 x 0.53	6.90	**3.04**
Vineyard BBCH 10-19	Small omnivorous mammal “mouse”	4.7	1.0 x 0.53	0.747	28.1

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger

For the proposed use of Spiroxamine EC 500 on grapes (1 x 300 g a.s./ha at BBCH 13 - 19) the reproductive risks to mammals from dietary exposure to spiroxamine are considered to be acceptable (TER ≥5) for all of the relevant scenarios with the exception of the small herbivorous mammal “vole” scenario. A refined risk assessment for this individual scenario has been presented below.

Refinement of the small herbivorous mammal “vole” scenario

For the first refined risk assessment the wood mouse has been used as a focal species. As discussed in the previous section, this species is considered to be a suitable representative of small mammals in and around vineyards. As presented above, a refined PT value of 0.41 has been applied to the risk assessment below.

Table CP 10.1.2-9 Refinement of the small herbivorous mammal vole scenario (BBCH 10-19) for the proposed use of Spiroxamine EC 500 in grapes (BBCH 13 - 19) – Wood mouse

App rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT [d]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
1 x 0.300 kg a.s./ha	Weeds	0.0667	28.7	1.0	0.53	0.41	0.6 [e]	0.0749	0.304	69.1
	Weed seeds	0.133	40.2	1.0	0.53			0.209		
	Invertebrates (ground dwelling)	0.0667	7.3	1.0	0.53			0.0196		

[a] Values calculated using default dietary data from EFSA (2009)
[b] Default RUD values from EFSA (2009) Appendix F
[c] Default EFSA values
[d] Refined 90th percentile PT value from focal species study
[e] Deposition value based on 40% crop interception (Appendix A: EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Using the refined parameters discussed above, the reproductive risks to small mammals following application of Spiroxamine EC 500 to grapes at 1 x 300 g a.s./ha at BBCH 10 - 19 are considered to be acceptable (TER ≥5).

Alternative refinement of the small herbivorous mammal "vole" scenario

As an alternative refinement the vole has been used as the focal species. This is the generic focal species from EFSA (2009) and, although it is not considered to be as prevalent in vineyards as the wood mouse, its presence in vineyards has been confirmed by a monitoring study, as already discussed. A vole diet of 76.6% grass & cereals, 11.9% non-grass herbs and 11.5% weed seeds has been used in the refined risk assessment below. As presented before, a geomean DT_{50} of 3.03 days, from a suite of residues decline trials on cereals, has been applied to refine the f_{twa} value from 0.53 to 0.206 on the grass/cereals part of the diet in the refined risk assessment below.

Table CP 10.1.2-10 Refinement of the small herbivorous mammal vole scenario (BBCH 10-19) for the proposed use of Spiroxamine EC 500 in grapes (BBCH 13 - 19) – Vole

App rate	Food type	FIR/bw [a]	RUD [b]	MAF	f_{twa}	PT [d]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
1 x 0.300 kg a.s./ha	Grass/ cereals	0.558	54.2	1.0	0.206 [c]	1.0	0.6 [e]	7.12	1.68	12.5
	Non-grass herbs	0.0866	28.7	1.0	0.53			0.237		
	Weed seeds	0.0837	40.2	1.0	0.53			0.321		

[a] Values calculated using focal species dietary data
[b] Default RUD values from EFSA (2009) Appendix F
[c] A f_{twa} value calculated using a DT_{50} value of 3.03 days
[d] Default PT of 1.0 used
[e] Deposition value based on 40% crop interception (Appendix A, EFSA 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Using the refined parameters discussed above, the reproductive risks to small herbivorous mammals, following application of Spiroxamine EC 500 to grapes at 1 x 300 g a.s./ha at BBCH 10 – 19, are considered to be acceptable (TER ≥5).

1 x 300 g a.s./ha BBCH 53 - 85

The screening step assessments for the acute and reproductive risks are presented below.

Table CP 10.1.2-11 Screening step assessment for acute and long-term/reproductive risk to mammals for the proposed use of Spiroxamine EC 500 in grapes (BBCH 53 - 85)

Intended use	Grapes (BBCH 53 - 85)				
Active substance/product	Spiroxamine / Spiroxamine EC 500				
Application rate (g a.s./ha)	1 × 300				
Acute toxicity (mg a.s./kg bw)	460				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Vineyard	Small herbivorous mammal	136.4	1.0	40.9	11.2
Reprod. toxicity (mg a.s./kg bw/d)	21.0				
TER criterion	5				
Crop scenario	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard	Small herbivorous mammal	72.3	1.0 x 0.53	11.5	**1.83**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger.

For the proposed use of Spiroxamine EC 500 on grapes (1 x 300 g a.s./ha at BBCH 53 - 85) the acute risks to mammals from dietary exposure to spiroxamine are considered to be acceptable (TER ≥10) but potential reproductive risks have been identified (TER <5). A Tier I reproductive risk assessment has therefore been conducted and presented below.

Table CP 10.1.2-12 Tier I assessment for reproductive risk to mammals for the proposed use of Spiroxamine EC 500 in grapes (BBCH 53 - 85)

Intended use	Grapes (BBCH 53 - 85)				
Active substance/product	Spiroxamine / Spiroxamine EC 500				
Application rate (g a.s./ha)	1 × 300				
Reprod. toxicity (mg a.s./kg bw/d)	21.0				
TER criterion	5				
Crop scenario Growth stage	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard BBCH >20	Small insectivorous mammal "shrew"	1.9	1.0 x 0.53	0.302	69.5
Vineyard BBCH >40	Large herbivorous mammal "lagomorph"	3.3	1.0 x 0.53	0.525	40.0
Vineyard BBCH >40	Small herbivorous mammal "vole"	21.7	1.0 x 0.53	3.45	6.09
Vineyard BBCH >40	Small omnivorous mammal "mouse"	2.3	1.0 x 0.53	0.366	57.4

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio

For the proposed use of Spiroxamine EC 500 on grapes (1 x 300 g a.s./ha at BBCH 53 - 85) the reproductive risks to mammals from dietary exposure to spiroxamine are considered to be acceptable (TER ≥5) for all of the relevant scenarios. No further risk assessment is necessary for this proposed use of Spiroxamine EC 500.

2 x 300 g a.s./ha BBCH 53 - 85

The screening step assessments for the acute and reproductive risks are presented below.

Table CP 10.1.2-13 Screening step assessment for acute and long-term/reproductive risk to mammals for the proposed use of Spiroxamine EC 500 in grapes (BBCH 53 - 85)

Intended use	Grapes (BBCH 53 - 85)				
Active substance/product	Spiroxamine / Spiroxamine EC 500				
Application rate (g a.s./ha)	2 × 300				
Acute toxicity (mg a.s./kg bw)	460				
TER criterion	10				
Crop scenario	Indicator species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Vineyard	Small herbivorous mammal	156.4	1.3	53.2	**8.65**
Reprod. toxicity (mg a.s./kg bw/d)	21.0				
TER criterion	5				
Crop scenario	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard	Small herbivorous mammal	72.3	1.5 x 0.53	17.2	**1.22**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity exposure ratio. TER values shown in bold fall below the relevant trigger

For the proposed use of Spiroxamine EC 500 on grapes (2 x 300 g a.s./ha at BBCH 53 - 85) potential acute and reproductive risks to mammals from dietary exposure to spiroxamine have been identified (TER <5). A Tier I acute and reproductive risk assessment has therefore been conducted and presented below.

Table CP 10.1.2-14 Tier I assessment for acute risk to mammals for the proposed use of Spiroxamine EC 500 in grapes (BBCH 53 - 85)

Intended use	Grapes (BBCH 53 - 85)				
Active substance/product	Spiroxamine / Spiroxamine EC 500				
Application rate (g a.s./ha)	2 × 300				
Acute toxicity (mg a.s./kg bw)	460				
TER criterion	10				
Crop scenario Growth stage	Generic focal species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Vineyard BBCH >20	Small insectivorous mammal "shrew"	5.4	1.3	2.11	218
Vineyard BBCH >40	Large herbivorous mammal "lagomorph"	8.1	1.3	3.16	146

Intended use	Grapes (BBCH 53 - 85)				
Active substance/product	Spiroxamine / Spiroxamine EC 500				
Application rate (g a.s./ha)	2 × 300				
Acute toxicity (mg a.s./kg bw)	460				
TER criterion	10				
Crop scenario Growth stage	Generic focal species	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Vineyard BBCH >40	Small herbivorous mammal “vole”	40.9	1.3	16.0	28.8
Vineyard BBCH >40	Small omnivorous mammal “mouse”	5.2	1.3	2.03	227

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio

For the proposed use of Spiroxamine EC 500 on grapes (2 x 300 g a.s./ha at BBCH 53 - 85) the acute risks to mammals from dietary exposure to spiroxamine are considered to be acceptable (TER ≥10) for all of the relevant scenarios.

Table CP 10.1.2-15 Tier I assessment for reproductive risk to mammals for the proposed use of Spiroxamine EC 500 in grapes (BBCH 53 - 85)

Intended use	Grapes (BBCH 53 - 85)				
Active substance/product	Spiroxamine / Spiroxamine EC 500				
Application rate (g a.s./ha)	2 × 300				
Reprod. toxicity (mg a.s./kg bw/d)	21.0				
TER criterion	5				
Crop scenario Growth stage	Generic focal species	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard BBCH >20	Small insectivorous mammal “shrew”	1.9	1.5 x 0.53	0.453	46.3
Vineyard BBCH >40	Large herbivorous mammal “lagomorph”	3.3	1.5 x 0.53	0.787	26.7
Vineyard BBCH >40	Small herbivorous mammal “vole”	21.7	1.5 x 0.53	5.18	**4.06**
Vineyard BBCH >40	Small omnivorous mammal “mouse”	2.3	1.5 x 0.53	0.549	38.3

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio; TER values shown in bold fall below the relevant trigger

For the proposed use of Spiroxamine EC 500 on grapes (2 x 300 g a.s./ha at BBCH 53 - 85) the reproductive risks to mammals from dietary exposure to spiroxamine are considered to be acceptable (TER ≥5) for all of the relevant scenarios with the exception of the small herbivorous mammal “vole” scenario. A refined risk assessment for this individual scenario has been presented below.

Refinement of the small herbivorous mammal “vole” scenario

For the first refined risk assessment the wood mouse has been used as a focal species. As discussed in the previous section, this species is considered to be a suitable representative of small mammals in and

around vineyards. As presented above, a refined PT value of 0.41 has been applied to the risk assessment below.

Table CP 10.1.2-16 Refinement of the small herbivorous mammal vole scenario (BBCH >40) for the proposed use of Spiroxamine EC 500 in grapes (BBCH 53 - 85) – Wood mouse

App rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT [d]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
2 x 0.300 kg a.s./ha	Weeds	0.0667	28.7	1.5	0.53	0.41	0.3 [e]	0.0562	0.228	92.1
	Weed seeds	0.133	40.2	1.5	0.53			0.157		
	Invertebrates (ground dwelling)	0.0667	7.5	1.5	0.53			0.0147		

[a] Values calculated using default dietary data from EFSA (2009)
[b] Default RUD values from EFSA (2009) Appendix F
[c] Default EFSA values
[d] Refined 90th percentile PT value from focal species study
[e] Deposition value based on 70% crop interception (Appendix A: EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Using the refined parameters discussed above, the reproductive risks to small mammals following application of Spiroxamine EC 500 to grapes at 2 x 300 g a.s./ha at BBCH 53 - 85 are considered to be acceptable (TER ≥5).

Alternative refinement of the small herbivorous mammal "vole" scenario

As an alternative refinement the vole has been used as the focal species. This is the generic focal species from EFSA (2009) and, although it is not considered to be as prevalent in vineyards as the wood mouse, its presence in vineyards has been confirmed by a monitoring study, as already discussed. A vole diet of 76.6% grass & cereals, 11.9% non-grass herbs and 11.5% weed seeds has been used in the refined risk assessment below. A geomean DT_{50} of 3.03 days, from a suite of residues decline trials on cereals, has been applied to refine the f_{twa} value from 0.53 to 0.402 on the grass/cereals part of the diet in the refined risk assessment below. As multiple applications have been considered, a combined MAF and f_{twa} value, using a moving time window approach, has been used to give a MAF x TWA of 0.402.

Table CP 10.1.2-17 Refinement of the small herbivorous mammal vole scenario (BBCH >40) for the proposed use of Spiroxamine EC 500 in grapes (BBCH 53 – 85) – Vole

App rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa}	PT [d]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
2 x 0.300 kg a.s./ha	Grass/ cereals	0.558	54.2	0.402 [c]		1.0	0.3 [e]	1.09	1.51	13.9
	Non-grass herbs	0.0866	28.7	1.5	0.53			0.178		
	Weed seeds	0.0837	40.2	1.5	0.53			0.241		

[a] Values calculated using focal species dietary data
[b] Default RUD values from EFSA (2009) Appendix F
[c] A MAF/TWA value calculated using a moving time window and a DT_{50} value of 3.03 days
[d] Default PT of 1.0 used
[e] Deposition value based on 70% crop interception (Appendix A: EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Using the refined parameters discussed above, the reproductive risks to small herbivorous mammals, following application of Spiroxamine EC 500 to grapes at 2 x 300 g a.s./ha at BBCH 53 - 85, are considered to be acceptable (TER ≥5).

1 x 200 g a.s./ha BBCH 13 - 19 and 1 x 300 g a.s./ha BBCH 20 (53) - 85

In order to cover the GAP use of this mixed application rate the risk assessment has taken a conservative approach and assumed that two applications of 200 g a.s./ha could be made at BBCH 13-19 or that two applications of 300 g a.s./ha could be made at BBCH 20-85. It is noted that BBCH growth stages between 19 and 53 do not exist. However, for completeness the EFSA scenarios at BBCH 20-39 have been included in the risk assessment.

The risk assessment starts at Tier I which is presented below.

Table CP 10.1.2-18 Tier I assessment for acute risk to mammals for the proposed use of Spiroxamine EC 500 in grapes

Intended use	Grapes					
Active substance/product	Spiroxamine / Spiroxamine EC 500					
Application rate (g a.s./ha)	2 × 200 (BBCH 13-19) or 2 × 300 (BBCH 53-85)					
Acute toxicity (mg a.s./kg bw)	460					
TER criterion	10					
Crop scenario Growth stage	Generic focal species	App. Rate (kg a.s./ha)	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Vineyard BBCH 10-19	Large herbivorous mammal “lagomorph”	0.2	16.3	1.3	4.24	109
Vineyard BBCH 10-19	Small insectivorous mammal “shrew”	0.2	7.6	1.3	1.98	233
Vineyard BBCH 10-19	Small herbivorous mammal “vole”	0.2	81.9	1.3	21.3	21.6
Vineyard BBCH 10-19	Small omnivorous mammal “mouse”	0.2	10.3	1.3	2.68	172
Vineyard BBCH 20-39	Large herbivorous mammal “lagomorph”	0.3	13.6	1.3	5.30	86.7
Vineyard BBCH 20-39	Small herbivorous mammal “vole”	0.3	68.2	1.3	26.6	17.3
Vineyard BBCH 20-39	Small omnivorous mammal “mouse”	0.3	8.6	1.3	3.35	137
Vineyard BBCH >20	Small insectivorous mammal “shrew”	0.3	5.4	1.3	2.11	218
Vineyard BBCH >40	Large herbivorous mammal “lagomorph”	0.3	8.1	1.3	3.16	146
Vineyard BBCH >40	Small herbivorous mammal “vole”	0.3	40.9	1.3	16.0	28.8
Vineyard BBCH >40	Small omnivorous mammal “mouse”	0.3	5.2	1.3	2.03	227

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.
Scenarios shaded may not be required as BBCH 20-39 does not exist for grapes

For the proposed use of Spiroxamine EC 500 on grapes (1 x 200 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85) the acute risks to mammals from dietary exposure to spiroxamine are considered to be acceptable (TER ≥10) for all of the relevant scenarios.

Table CP 10.1.2-19 Tier I assessment for reproductive risk to mammals for the proposed use of Spiroxamine EC 500 in grapes

Intended use	Grapes					
Active substance/product	Spiroxamine / Spiroxamine EC 500					
Application rate (g a.s./ha)	2 × 200 (BBCH 13-19) or; 2 × 300 (BBCH 53-85)					
Reprod. toxicity (mg a.s./kg bw/d)	21.0					
TER criterion	5					
Crop scenario Growth stage	Generic focal species	App. Rate (kg a.s./ha)	SV_m	MAF_m TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard BBCH 10-19	Large herbivorous mammal “lagomorph”	0.2	6.7	1.5 x 0.53	1.07	19.7
Vineyard BBCH 10-19	Small insectivorous mammal “shrew”	0.2	4.2	1.5 x 0.53	0.668	31.5
Vineyard BBCH 10-19	Small herbivorous mammal “vole”	0.2	43.4	1.5 x 0.53	6.91	**3.04**
Vineyard BBCH 10-19	Small omnivorous mammal “mouse”	0.2	4.7	1.5 x 0.53	0.747	28.1
Vineyard BBCH 20-39	Large herbivorous mammal “lagomorph”	0.3	5.5	1.5 x 0.53	1.31	16.0
Vineyard BBCH 20-39	Small herbivorous mammal “vole”	0.3	36.1	1.5 x 0.53	8.61	**2.44**
Vineyard BBCH 20-39	Small omnivorous mammal “mouse”	0.3	3.9	1.5 x 0.53	0.930	22.6
Vineyard BBCH >20	Small insectivorous mammal “shrew”	0.3	1.9	1.5 x 0.53	0.453	46.3
Vineyard BBCH >40	Large herbivorous mammal “lagomorph”	0.3	3.3	1.5 x 0.53	0.787	26.7
Vineyard BBCH >40	Small herbivorous mammal “vole”	0.3	21.7	1.5 x 0.53	5.17	**4.06**
Vineyard BBCH >40	Small omnivorous mammal “mouse”	0.3	2.3	1.5 x 0.53	0.549	38.3

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger
Scenarios shaded may not be required as BBCH 20-39 does not exist for vines

For the proposed use of Spiroxamine EC 500 on grapes (1 x 200 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85) the reproductive risks to mammals from dietary exposure to spiroxamine are considered to be acceptable (TER ≥5) for all of the relevant scenarios with the exception of the small herbivorous mammal “vole” scenarios. Refined risk assessments for these scenarios have been presented below.

Refinement of the small herbivorous mammal "vole" scenario

For the first refined risk assessment the wood mouse has been used as a focal species. As discussed in the previous section, this species is considered to be a suitable representative of small mammals in and around vineyards. As presented above, a refined PT value of 0.41 has been applied to the risk assessment below. Three refined assessments have been presented which relate to the growth stages of the scenarios that required refinement; BBCH 10 - 19, BBCH 20 - 39 and BBCH >40.

Table CP 10.1.2-20 Refinement of the small herbivorous mammal vole scenario (BBCH 10 - 19) for the proposed use of Spiroxamine EC 500 in grapes – Wood mouse

App rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT [d]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
2 x 0.200 kg a.s./ha	Weeds	0.0667	28.7	1.5	0.53	0.41	0.6 [e]	0.0749	0.304	69.1
	Weed seeds	0.133	40.2	1.5	0.53			0.209		
	Invertebrates (ground dwelling)	0.0667	7.5	1.5	0.53			0.0196		

[a] Values calculated using default dietary data from EFSA (2009)
[b] Default RUD values from EFSA (2009) Appendix F
[c] Default EFSA values
[d] Refined 90th percentile PT value from focal species study
[e] Deposition value based on 40% crop interception (Appendix A: EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Table CP 10.1.2-21 Refinement of the small herbivorous mammal vole scenario (BBCH 20 - 39) for the proposed use of Spiroxamine EC 500 in grapes – Wood mouse

App rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT [d]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
2 x 0.300 kg a.s./ha	Weeds	0.0667	28.7	1.5	0.53	0.41	0.5 [e]	0.0936	0.379	55.4
	Weed seeds	0.133	40.2	1.5	0.53			0.261		
	Invertebrates (ground dwelling)	0.0667	7.5	1.5	0.53			0.0245		

[a] Values calculated using default dietary data from EFSA (2009)
[b] Default RUD values from EFSA (2009) Appendix F
[c] Default EFSA values
[d] Refined 90th percentile PT value from focal species study
[e] Deposition value based on 50% crop interception (Appendix A: EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Table CP 10.1.2-22 Refinement of the small herbivorous mammal vole scenario (BBCH >40) for the proposed use of Spiroxamine EC 500 in grapes – Wood mouse

App rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT [d]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
2 x 0.300 kg a.s./ha	Weeds	0.0667	28.7	1.5	0.53	0.41	0.3 [e]	0.0562	0.228	92.1
	Weed seeds	0.133	40.2	1.5	0.53			0.157		
	Invertebrates (ground dwelling)	0.0667	7.5	1.5	0.53			0.0147		

[a] Values calculated using default dietary data from EFSA (2009)

b) Default RUD values from EFSA (2009) Appendix F
c) Default EFSA values
d) Refined 90th percentile PT value from focal species study
e) Deposition value based on 70% crop interception (Appendix A: EFSA, 2009)
f) Sum of DDD values for individual diet components
g) TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Using the refined parameters discussed above, the reproductive risks to small mammals following application of Spiroxamine EC 500 to grapes at 1 x 200 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85 are considered to be acceptable (TER ≥5).

Alternative refinement of the small herbivorous mammal "vole" scenario

As an alternative refinement the vole has been used as the focal species. This is the generic focal species from EFSA (2009) and, although it is not considered to be as prevalent in vineyards as the wood mouse, its presence in vineyards has been confirmed by a monitoring study as already discussed. A vole diet of 76.6% grass & cereals, 11.9% non-grass herbs and 11.5% weed seeds has been used in the refined risk assessment below. As presented before, a geomean DT_{50} of 3.03 days, from a suite of residues decline trials on cereals, has been applied to refine the f_{twa} value from 0.53 to 0.402 on the grass/cereals part of the diet in the refined risk assessment below. As multiple applications have been considered, a combined MAF and f_{twa} value, using a moving time window approach, has been used to give a MAF x TWA of 0.402. Three refined assessments have been presented which relate to the growth stages of the scenarios that required refinement, BBCH 10 - 19, BBCH 20 - 39 and BBCH > 40.

Table CP 10.1.2-23 Refinement of the small herbivorous mammal vole scenario (BBCH 10 - 19) for the proposed use of Spiroxamine EC 500 in grapes – Vole

App rate	Food type	FIR/bw [a)]	RUD [b)]	MAF	f_{twa}	PT [d)]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f)]	TER [g)]
2 x 0.200 kg a.s./ha	Grass/ cereals	0.558	54.2	0.402 [c)]		1.0	0.6 [e)]	1.46	2.02	10.4
	Non-grass herbs	0.0866	28.7	1.5	0.53			0.237		
	Weed seeds	0.0837	40.2	1.5	0.53			0.321		

a) Values calculated using focal species dietary data
b) Default RUD values from EFSA (2009) Appendix F
c) A MAF×TWA value calculated using a moving time window and a DT_{50} value of 3.03 days
d) Default PT of 1.0 used
e) Deposition value based on 40% crop interception (Appendix A: EFSA, 2009)
f) Sum of DDD values for individual diet components
g) TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Table CP 10.1.2-24 Refinement of the small herbivorous mammal vole scenario (BBCH 20 - 39) for the proposed use of Spiroxamine EC 500 in grapes – Vole

App rate	Food type	FIR/bw [a)]	RUD [b)]	MAF	f_{twa}	PT [d)]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f)]	TER [g)]
2 x 0.300 kg a.s./ha	Grass/ cereals	0.558	54.2	0.402 [c)]		1.0	0.5 [e)]	1.82	2.52	8.33
	Non-grass herbs	0.0866	28.7	1.5	0.53			0.296		
	Weed seeds	0.0837	40.2	1.5	0.53			0.401		

a) Values calculated using focal species dietary data
b) Default RUD values from EFSA (2009) Appendix F
c) A MAF×TWA value calculated using a moving time window and a DT_{50} value of 3.03 days
d) Default PT of 1.0 used
e) Deposition value based on 50% crop interception (Appendix A: EFSA, 2009)
f) Sum of DDD values for individual diet components

[g] TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Table CP 10.1.2-25 Refinement of the small herbivorous mammal vole scenario (BBCH >40) for the proposed use of Spiroxamine EC 500 in grapes – Vole

App rate	Food type	FIR/bw [a]	RUD [b]	MAF	f_{twa}	PT [d]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD	TER [g]
2 x 0.300 kg a.s./ha	Grass/ cereals	0.558	54.2	0.402 [c]		1.0	0.3 [e]	1.09	1.51	13.9
	Non-grass herbs	0.0866	28.7	1.5	0.53			0.178		
	Weed seeds	0.0837	40.2	1.5	0.53			0.241		

[a] Values calculated using focal species dietary data
[b] Default RUD values from EFSA (2009) Appendix F
[c] A MAF×TWA value calculated using a moving time window and a DT$_{50}$ value of 3.03 days
[d] Default PT of 1.0 used
[e] Deposition value based on 70% crop interception (Appendix A; EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Using the refined parameters discussed above, the reproductive risks to small mammals following application of Spiroxamine EC 500 to grapes at 1 x 200 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85 are considered to be acceptable (TER ≥ 5).

1 x 300 g a.s./ha BBCH 13 - 19 and 1 x 300 g a.s./ha BBCH 20 (53) - 85

In order to cover the GAP use of this mixed application rate the risk assessment has taken a conservative approach and assumed that two applications of 300 g a.s./ha could be made at BBCH 13-19 or that two applications of 300 g a.s./ha could be made at BBCH 20-85. It is noted that BBCH growth stages between 19 and 53 do not exist. However, for completeness the EFSA scenarios at BBCH 20-39 have been included in the risk assessment.

The risk assessment starts at Tier I which is presented below.

Table CP 10.1.2-26 Tier I assessment for acute risk to mammals for the proposed use of Spiroxamine EC 500 in grapes

Intended use	Grapes					
Active substance/product	Spiroxamine / Spiroxamine EC 500					
Application rate (g a.s./ha)	2 × 300 (BBCH 13-19) or; 2 × 300 (BBCH 53-85)					
Acute toxicity (mg a.s./kg bw)	460					
TER criterion	10					
Crop scenario Growth stage	Generic focal species	App. Rate (kg a.s./ha)	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Vineyard BBCH 10-19	Large herbivorous mammal "lagomorph"	0.3	16.3	1.3	6.36	72.4
Vineyard BBCH 10-19	Small insectivorous mammal "shrew"	0.3	7.6	1.3	2.96	155
Vineyard BBCH 10-19	Small herbivorous mammal "vole"	0.3	81.9	1.3	31.9	14.4

Intended use	Grapes
Active substance/product	Spiroxamine / Spiroxamine EC 500
Application rate (g a.s./ha)	2 × 300 (BBCH 13-19) or; 2 × 300 (BBCH 53-85)
Acute toxicity (mg a.s./kg bw)	460
TER criterion	10

Crop scenario Growth stage	Generic focal species	App. Rate (kg a.s./ha)	SV_{90}	MAF_{90}	DDD_{90} (mg a.s./kg bw/d)	TER_A
Vineyard BBCH 10-19	Small omnivorous mammal “mouse”	0.3	10.3	1.3	4.02	115
Vineyard BBCH 20-39	Large herbivorous mammal “lagomorph”	0.3	13.6	1.3	5.30	86.7
Vineyard BBCH 20-39	Small herbivorous mammal “vole”	0.3	68.2	1.3	26.6	17.3
Vineyard BBCH 20-39	Small omnivorous mammal “mouse”	0.3	8.6	1.3	3.35	137
Vineyard BBCH >20	Small insectivorous mammal “shrew”	0.3	5.4	1.3	2.11	218
Vineyard BBCH >40	Large herbivorous mammal “lagomorph”	0.3	8.1	1.3	3.16	146
Vineyard BBCH >40	Small herbivorous mammal “vole”	0.3	40.9	1.3	16.0	28.8
Vineyard BBCH >40	Small omnivorous mammal “mouse”	0.3	5.2	1.3	2.03	227

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.
Scenarios shaded may not be required as BBCH 20-39 does not exist for grapes

For the proposed use of Spiroxamine EC 500 on grapes (1 x 300 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85) the acute risks to mammals from dietary exposure to spiroxamine are considered to be acceptable (TER ≥10) for all of the relevant scenarios.

Table CP 10.1.2-27 Tier I assessment for reproductive risk to mammals for the proposed use of Spiroxamine EC 500 in grapes

Intended use	Grapes					
Active substance/product	Spiroxamine / Spiroxamine EC 500					
Application rate (g a.s./ha)	2 × 300 (BBCH 13-19) or; 2 × 300 (BBCH 53-85)					
Reprod. toxicity (mg a.s./kg bw/d)	21.0					
TER criterion	5					
Crop scenario Growth stage	Generic focal species	App. Rate (kg a.s./ha)	SV_m	MAF_m × TWA	DDD_m (mg a.s./kg bw/d)	TER_{LT}
Vineyard BBCH 10-19	Large herbivorous mammal “lagomorph”	0.3	62	1.5 x 0.53	1.60	13.1
Vineyard BBCH 10-19	Small insectivorous mammal “shrew”	0.3	4.2	1.5 x 0.53	1.00	21.0
Vineyard BBCH 10-19	Small herbivorous mammal “vole”	0.3	43.4	1.5 x 0.53	10.4	**2.03**
Vineyard BBCH 10-19	Small omnivorous mammal “mouse”	0.3	4.7	1.5 x 0.53	1.12	18.7
Vineyard BBCH 20-39	Large herbivorous mammal “lagomorph”	0.3	5.5	1.5 x 0.53	1.31	16.0
Vineyard BBCH 20-39	Small herbivorous mammal “vole”	0.3	36.1	1.5 x 0.53	8.61	**2.44**
Vineyard BBCH 20-39	Small omnivorous mammal “mouse”	0.3	3.9	1.5 x 0.53	0.930	22.6
Vineyard BBCH >20	Small insectivorous mammal “shrew”	0.3	1.9	1.5 x 0.53	0.453	46.3
Vineyard BBCH >40	Large herbivorous mammal “lagomorph”	0.3	3.3	1.5 x 0.53	0.787	26.7
Vineyard BBCH >40	Small herbivorous mammal “vole”	0.3	21.7	1.5 x 0.53	5.17	**4.06**
Vineyard BBCH >40	Small omnivorous mammal “mouse”	0.3	2.3	1.5 x 0.53	0.549	38.3

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger
Scenarios shaded may not be required as BBCH 20-39 does not exist for grapes

For the proposed use of Spiroxamine EC 500 on grapes (1 x 300 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85) the reproductive risks to mammals from dietary exposure to spiroxamine are considered to be acceptable (TER ≥5) for all of the relevant scenarios with the exception of the small herbivorous mammal “vole” scenarios. Refined risk assessments for these scenarios have been presented below.

Refinement of the small herbivorous mammal “vole” scenario

For the first refined risk assessment the wood mouse has been used as a focal species. As discussed in the previous section, this species is considered to be a suitable representative of small mammals in and around vineyards. As presented above, a refined PT value of 0.41 has been applied to the risk assessment

below. Three refined assessments have been presented which relate to the growth stages of the scenarios that required refinement; BBCH 10 - 19, BBCH 20 - 39 and BBCH >40.

Table CP 10.1.2-28 Refinement of the small herbivorous mammal vole scenario (BBCH 10 - 19) for the proposed use of Spiroxamine EC 500 in grapes – Wood mouse

App rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT [d]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
2 x 0.300 kg a.s./ha	Weeds	0.0667	28.7	1.5	0.53	0.41	0.6 [e]	0.112	0.455	46.2
	Weed seeds	0.133	40.2	1.5	0.53			0.314		
	Invertebrates (ground dwelling)	0.0667	7.5	1.5	0.53			0.0294		

[a] Values calculated using default dietary data from EFSA (2009)
[b] Default RUD values from EFSA (2009) Appendix F
[c] Default EFSA values
[d] Refined 90th percentile PT value from focal species study
[e] Deposition value based on 40% crop interception (Appendix A: EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Table CP 10.1.2-29 Refinement of the small herbivorous mammal vole scenario (BBCH 20 - 39) for the proposed use of Spiroxamine EC 500 in grapes – Wood mouse

App rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT [d]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
2 x 0.300 kg a.s./ha	Weeds	0.0667	28.7	1.5	0.53	0.41	0.5 [e]	0.0936	0.379	55.4
	Weed seeds	0.133	40.2	1.5	0.53			0.261		
	Invertebrates (ground dwelling)	0.0667	7.5	1.5	0.53			0.0245		

[a] Values calculated using default dietary data from EFSA (2009)
[b] Default RUD values from EFSA (2009) Appendix F
[c] Default EFSA values
[d] Refined 90th percentile PT value from focal species study
[e] Deposition value based on 50% crop interception (Appendix A: EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Table CP 10.1.2-30 Refinement of the small herbivorous mammal vole scenario (BBCH >40) for the proposed use of Spiroxamine EC 500 in grapes – Wood mouse

App rate	Food type	FIR/bw [a]	RUD [b]	MAF [c]	f_{twa} [c]	PT [d]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
2 x 0.300 kg a.s./ha	Weeds	0.0667	28.7	1.5	0.53	0.41	0.3 [e]	0.0562	0.228	92.1
	Weed seeds	0.133	40.2	1.5	0.53			0.157		
	Invertebrates (ground dwelling)	0.0667	7.5	1.5	0.53			0.0147		

[a] Values calculated using default dietary data from EFSA (2009)
[b] Default RUD values from EFSA (2009) Appendix F
[c] Default EFSA values
[d] Refined 90th percentile PT value from focal species study
[e] Deposition value based on 70% crop interception (Appendix A: EFSA, 2009)
[f] Sum of DDD values for individual diet components

g) TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Using the refined parameters discussed above, the reproductive risks to small mammals following application of Spiroxamine EC 500 to grapes at 1 x 300 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85 are considered to be acceptable (TER ≥5).

Alternative refinement of the small herbivorous mammal "vole" scenario

As an alternative refinement the vole has been used as the focal species. This is the generic focal species from EFSA (2009) and, although it is not considered to be as prevalent in vineyards as the wood mouse, its presence in vineyards has been confirmed by a monitoring study, as already discussed. A vole diet of 76.6% grass & cereals, 11.9% non-grass herbs and 11.5% weed seeds has been used in the refined risk assessment below. As presented before, a geomean DT_{50} of 3.03 days, from a suite of residues decline trials on cereals, has been applied to refine the f_{twa} value from 0.53 to 0.402 on the grass/cereals part of the diet in the refined risk assessment below. As multiple applications have been considered, a combined MAF and f_{twa} value, using a moving time window approach, has been used to give a MAF x TWA of 0.402. Three refined assessments have been presented which relate to the growth stages of the scenarios that required refinement; BBCH 10 - 19, BBCH 20 - 39 and BBCH > 40.

Table CP 10.1.2-31 Refinement of the small herbivorous mammal vole scenario (BBCH 10 - 19) for the proposed use of Spiroxamine EC 500 in grapes – Vole

App rate	Food type	FIR/bw [a]	RUD [b]	MAF	f_{twa}	PT [d]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
2 x 0.300 kg a.s./ha	Grass/ cereals	0.558	54.2	0.402 [c]		1.0	0.6 [e]	2.19	3.03	6.93
	Non-grass herbs	0.0866	28.7	1.5	0.53			0.356		
	Weed seeds	0.0837	40.2	1.5	0.53			0.481		

[a] Values calculated using focal species dietary data
[b] Default RUD values from EFSA (2009) Appendix F
[c] A MAF×TWA value calculated using a moving time window and a DT_{50} value of 3.03 days
[d] Default PT of 1.0 used
[e] Deposition value based on 40% crop interception (Appendix A: EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Table CP 10.1.2-32 Refinement of the small herbivorous mammal vole scenario (BBCH 20 - 39) for the proposed use of Spiroxamine EC 500 in grapes – Vole

App rate	Food type	FIR/bw [a]	RUD [b]	MAF	f_{twa}	PT [d]	Dep. factor	DDD [mg a.s./kg b.w./d]	Total DDD [f]	TER [g]
2 x 0.300 kg a.s./ha	Grass/ cereals	0.558	54.2	0.402 [c]		1.0	0.5 [e]	1.82	2.52	8.33
	Non-grass herbs	0.0866	28.7	1.5	0.53			0.296		
	Weed seeds	0.0837	40.2	1.5	0.53			0.401		

[a] Values calculated using focal species dietary data
[b] Default RUD values from EFSA (2009) Appendix F
[c] A MAF×TWA value calculated using a moving time window and a DT_{50} value of 3.03 days
[d] Default PT of 1.0 used
[e] Deposition value based on 50% crop interception (Appendix A: EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Table CP 10.1.2-33 Refinement of the small herbivorous mammal vole scenario (BBCH >40) for the proposed use of Spiroxamine EC 500 in grapes – Vole

App rate	Food type	FIR/bw [a]	RUD [b]	MAF	f_{twa}	PT [d]	Dep. factor	DDD [mg a.s./kg bw/d]	Total DDD [f]	TER [g]
2 x 0.300 kg a.s./ha	Grass/ cereals	0.558	54.2	0.402 [c]		1.0	0.3 [e]	1.09	1.51	13.9
	Non-grass herbs	0.0866	28.7	1.5	0.53			0.178		
	Weed seeds	0.0837	40.2	1.5	0.53			0.241		

[a] Values calculated using focal species dietary data
[b] Default RUD values from EFSA (2009) Appendix F
[c] A MAF×TWA value calculated using a moving time window and a DT_{50} value of 7.03 days
[d] Default PT of 1.0 used
[e] Deposition value based on 70% crop interception (Appendix A, EFSA, 2009)
[f] Sum of DDD values for individual diet components
[g] TER calculated based on reproductive endpoint of 21.0 mg a.s./kg bw/day

Using the refined parameters discussed above, the reproductive risks to small mammals following application of Spiroxamine EC 500 to grapes at 1 x 300 g a.s./ha at BBCH 13 - 19 and 1 x 300 g a.s./ha at BBCH 20 (53) - 85 are considered to be acceptable (TER ≥5).

Risks for mammals through drinking water

In addition to dietary items, mammals may also be exposed to residues occurring in drinking water. The daily dietary dose (DDD) values used in the standard dietary risk assessments do not encompass drinking water and therefore the potential risk from this exposure route has not been covered in the dietary risk assessment.

The puddle scenario is relevant for mammals and considers puddles occurring on the soil surface following a rainfall event after application and is considered possible in all crop types.

In accordance with the EFSA Guidance Document (2009), based on the characteristics of the exposure scenario and standard assumptions for water uptake by animals no specific requirement for drinking water exposure calculation and TER determination based on the puddle scenario is required:

- for substances with a Koc <500 L/kg (less sorptive); if the ratio of application rate (g a.s./ha) to effective endpoint mg a.s./kg bw/d does not exceed 50;
- for substances with a Koc ≥ 500 L/kg (more sorptive); if the ratio of application rate (g a.s./ha) to effective endpoint mg a.s./kg bw/d does not exceed 3000.

The geomean Koc for spiroxamine is 4011 L/kg therefore spiroxamine belongs to the group of more sorptive substances. The ratio calculation is based on two applications of the highest rate at 300 g a.s./ha.

Table CP 10.1.2-34 Ratios of effective application rate (AR_{eff}) to acute and long-term endpoints for spiroxamine following the proposed use of Spiroxamine EC 500 - puddle scenario

Test substance	AR_{eff} (g/ha)[a]	Toxicological endpoint (mg a.s./kg bw/d)	Ratio (AR_{eff}/endpoint)	Trigger
Acute				
Spiroxamine	390	LD_{50} 460	0.848	3000
Long-term				
Spiroxamine	450	NOAEL 21.0	21.4	3000

[a] AR_{eff} = based on an application rate of 300 g a.s./ha with a MAF of 1.3 and 1.5 applied for acute and reproductive risk assessments, respectively

The ratios for both acute and reproductive risks are below the relevant trigger of 3000 for spiroxamine therefore a quantitative risk assessment is not necessary. Thus, there are no unacceptable risks to mammals from exposure to spiroxamine *via* drinking water.

Secondary poisoning

The Log P_{ow} of spiroxamine is 2.79 and 2.98 at pH 7 for diastomers A and B, respectively but at pH 9 these value are 4.88 and 5.08, respectively. Thus the trigger value of 3 for a secondary poisoning risk assessment is met.

Consideration of secondary poisoning risk due to metabolites

The Log P_{ow} of spiroxamine-desethyl (M01) is 2.41, 1.97 and >3.64 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-despropyl (M02) is 1.95, 1.41 and >3.44 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-N-oxide (M03) is 2.82, 2.59 and 2.63 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-carboxylic acid (M06) is 0.45, -0.25 and 0.10 at pH 4, 7 and 9, respectively. Thus, an assessment of the potential risk from bioconcentration of spiroxamine-desethyl (M01) and spiroxamine-despropyl (M02) also needs to be addressed in the risk assessment.

Risk assessment for earthworm-eating mammals *via* secondary poisoning

According to EFSA (2009), the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

To achieve a concise risk assessment, the risk envelope approach is applied whereby the maximum application rate of 2 x 300 g a.s./ha has been considered. For spiroxamine, M01 and M02, the $PEC_{soil\ accumulation}$ has been used in he risk assessment as these values are higher than the 21-day TWA PEC_{soil} values. There are no mammalian reproductive toxicity data available for M01 and M02 therefore the NOAEL of 21.0 mg/kg bw/day for spiroxamine has been used as a surrogate value.

The secondary poisoning risk assessments for earthworm-eating mammals from exposure to spiroxamine, KWG 4168-desethyl (M01) and KWG 4168-despropyl (M02) are presented in the tables below.

Table CP 10.1.2-35 Assessment of the risk for earthworm-eating mammals due to exposure to spiroxamine *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Spiroxamine	Comments
PEC_{soil} (mg a.s./kg soil)	0.555	Accumulation PEC_{soil} used as worst-case
Log P_{ow} / P_{ow}	4.0 / 10000	Mean value of 4.0 has been used based on the values for diastomers A and B at pH 7 and 9
K_{oc}	4111	Geomean
f_{oc}	0.02	Default
BCF_{worm}	1.47	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC_{worm}	0.816	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg a.s./kg bw/d)	1.04	$DDD = PEC_{worm} \times 1.28$
NOEL (mg a.s./kg bw/d)	21.0	M-762441-01-1
TER_{LT}	20.1	Acceptable risks (TER>5)

Table CP 10.1.2-36 Assessment of the risk for earthworm-eating mammals due to exposure to spiroxamine-desethyl (M01) *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Spiroxamine-desethyl	Comments
PEC_{soil} (mg/kg soil)	0.064	Accumulation PEC_{soil} used as worst-case
Log P_{ow} / P_{ow}	3.64 / 4365	-
K_{oc}	3271	Geomean
f_{oc}	0.02	Default
BCF_{worm}	0.814	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC_{worm}	0.0521	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0666	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	21.0	Value determined for spiroxamine used as a surrogate
TER_{LT}	315	Acceptable risks (TER>5)

Table CP 10.1.2-37 Assessment of the risk for earthworm-eating mammals due to exposure to spiroxamine-despropyl (M02) *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Spiroxamine-despropyl	Comments
PEC_{soil} (mg/kg soil)	0.040	Accumulation PEC_{soil} used as worst-case
Log P_{ow} / P_{ow}	3.44 / 2754	-
K_{oc}	2695	Geomean
f_{oc}	0.02	Default
BCF_{worm}	0.629	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC_{worm}	0.0252	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0322	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	21.0	Value determined for spiroxamine used as a surrogate
TER_{LT}	652	Acceptable risks (TER>5)

For the secondary poisoning risk assessments for earthworm-eating mammals from exposure to spiroxamine, M01 and M02 the TER values are >5 thereby demonstrating an acceptable risk to mammals *via* this route of exposure.

Risk assessment for fish-eating mammals via secondary poisoning

According to EFSA (2009), the risk for piscivorous mammals is assessed for a mammal of 3000 g body weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

To achieve a concise risk assessment, the risk envelope approach is applied whereby the maximum application rate of 2 x 300 g a.s./ha has been considered. The highest Step 3 TWA PEC_{sw} of 2.627 µg

a.s./L for spiroxamine has been used in the risk assessment. For M01 the highest Step 2 PEC_{sw} value of 1.084 µg/L has been used and for M02 the highest Step 2 PEC_{sw} value of 0.917 µg/L has been used. For M01 and M02 there are no BCF values available therefore the BCF value determined for spiroxamine (87 L/kg) has been used. Furthermore, there are no mammalian reproductive toxicity data available for M01 and M02 therefore the NOAEL of 21.0 mg/kg bw/day for spiroxamine has been used as a surrogate value.

The secondary poisoning risk assessments for fish-eating mammals from exposure to spiroxamine, KWG 4168-desethyl (M01) and KWG 4168-despropyl (M02) are presented in the tables below.

Table CP 10.1.2-38 Assessment of the risk for fish-eating mammals due to exposure to spiroxamine *via* bioaccumulation in fish (secondary poisoning)

Parameter	Spiroxamine	Comments
PEC_{sw} (mg a.s./L)	0.002627	FOCUS Step 3 TWA PEC_{sw} (calculated for vines: D6 ditch, 2 x 300 g a.s./ha, late application)
PEC_{water}	0.002627	TWA PEC_{sw} value used
BCF_{fish}	87	From study M-606479-01-1
PEC_{fish}	0.229	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg a.s./kg bw/d)	0.0325	$DDD = PEC_{fish} \times 0.142$
NOEL (mg a.s./kg bw/d)	21.0	M-702441-01-1
TER_{LT}	647	Acceptable risks (TER>5)

Table CP 10.1.2-39 Assessment of the risk for fish-eating mammals due to exposure to spiroxamine-desethyl (M01) *via* bioaccumulation in fish (secondary poisoning)

Parameter	Spiroxamine-desethyl	Comments
PEC_{sw} (mg/L)	0.001084	FOCUS Step 2 maximum PEC_{sw} (calculated for vines, 2 x 300 g a.s./ha)
PEC_{water}	0.000575	PEC_{water} = max $PEC_{sw} \times f_{twa}$, where f_{twa} = 0.53; in line with approach in EFSA (2009)
BCF_{fish}	87	Value determined for spiroxamine used as a surrogate
PEC_{fish}	0.0500	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.00710	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	21.0	Value determined for spiroxamine used as a surrogate
TER_{LT}	2959	Acceptable risks (TER>5)

Table CP 10.1.2-40 Assessment of the risk for fish-eating mammals due to exposure to spiroxamine-despropyl (M02) *via* bioaccumulation in fish (secondary poisoning)

Parameter	Spiroxamine-despropyl	Comments
PEC_{sw} (mg/L)	0.000917	FOCUS Step 2 maximum PEC_{sw} (calculated for

		vines; 2 x 300 g a.s./ha)
PEC_{water}	0.000486	PEC_{water} = max $PEC_{sw} \times f_{twa}$, where f_{twa} = 0.33, in line with approach in EFSA (2009)
BCF_{fish}	87	Value determined for spiroxamine used as a surrogate
PEC_{fish}	0.0423	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.00600	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	21.0	Value determined for spiroxamine used as a surrogate
TER_{LT}	3498	Acceptable risks (TER>5)

For the secondary poisoning risk assessments for fish-eating mammals from exposure to spiroxamine, M01 and M02, the TER values are >5 thereby demonstrating an acceptable risk to mammals *via* this route of exposure.

Biodiversity

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on mammals. Therefore, it is considered that the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects *via* alteration of the food web, are covered by the risk assessment for mammals in this section and in the ED hazard assessment.

CP 10.1.2.1 Acute oral toxicity to mammals

Please refer to Document M-CP Section 7 Toxicology for a summary of the acute oral rat studies using Spiroxamine EC 500 (M-016267-01-1 and M-016680-01-1).

CP 10.1.2.2 Higher tier data on mammals

The following summary relates to a report which has been submitted in order to justify the selection of the ecotoxicologically relevant NOAEL used in the chronic mammalian risk assessment.

Data Point:	KCP 10.1.2.2/08
Report Author:	[redacted]
Report Year:	2021
Report Title:	Spiroxamine: Consideration of long term mammalian toxicology endpoints for the bird and mammal risk assessment
Report No:	0471836-TOX3
Document No:	M-762441-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The available long-term Toxicology data has been reviewed in order to determine an ecotoxicologically relevant endpoint for the mammalian risk assessment. A NOAEL of 21.0 mg a.s./kg bw/day has been selected and considered to be appropriate.

I. Methods

The EFSA 2009 Bird & Mammal Guidance Document[1] sets out a different approach to determining ecotoxicologically relevant toxicity end points for wild birds and mammal risk assessments. The philosophy of these guidelines is to set a toxicity endpoint which would protect the dynamics of the population, rather than protecting any individual against an effect, perhaps inconsequential, to their health, survival or reproduction. According to the outcome of the pesticides peer review meeting on recurring issues in ecotoxicology (EFSA PPR meeting 1-3 September 2015[2]), an ecotoxicologically relevant endpoint should be set in collaboration with mammalian toxicologists and should be used in all the steps of the risk assessment. The available long-term data were assessed and considered in the table below.

II. Results and Discussion

The table below presents the results achieved in the long-term Toxicology studies.

Table CP 10.1.2.2/08-1 Consideration of Toxicology data for derivation of an ecotoxicologically relevant endpoint for risk assessment

Endpoint
Body weight change, behavioural effects and systemic toxicity : • NOAEL 125 ppm (equivalent ♂/♀: 1.9/2.7 mg/kg bw/day) based on systemic toxicity (hyperkeratosis of oesophagus and forestomach) and clinical chemistry paratmeters (↓ total cholesterol) [90 day oral (dietary) toxicity study in the rat], with the LOAEL of 625 ppm (♂/♀: 9.3/13.2 mg/kg bw/day). • NOAEL 80 ppm (equivalent to ♂/♀: 5.5/6.7 mg/kg bw/day) based on ↓ parental body weight gain in the 2008 two-generation study with evidence of systemic toxicity (hyperkeratosis of oesophagus).

[1] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. doi:10.2903/j.efsa.2009.1438

[2] EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924. 62 pp

Endpoint
Indices of gestation, litter size, pup and litter weight • NOAEL 80 ppm (F_1/F_2: 6.5/6.7 mg/kg bw/day) based on ↓ pup body weights in the 2008 two-generation study.
Indices of viability, pre- and post-implantation loss: • NOAEL of 300 ppm (♂/♀: 21.0/21.2 mg/kg bw/day), the highest dose tested in the 2008 two-generation study, with no effects on viability index or reproduction endpoints • In the rat developmental dose range finder toxicity study implantations, *corpora lutea* were all decreased, pre- and post implantation loss were increased with litter viability decreased with LOAEL of 150 mg/kg bw/day, with an NOAEL of 100 mg/kg bw/day. • In a further rat developmental toxicity study confirmed no effect on implantations, *corpora lutea*, pre- or post implantation loss or litter viability, with a NOAEL 100 mg/kg bw/day. • Pre- and post implantation loss was increased in the rabbit developmental dose range finder study NOAEL of 75 mg/kg bw/day (LOAEL 100 mg/kg bw/day), without any effect on litter viability. These effects occured in the presence of maternal toxicity (reduced body weight, body weight, food consumption, gastric ulceration, reduced faecal output). • In the rabbit developmental main toxicity study no effect on implantations, *corpora lutea*, pre- or post implantation loss or litter viability were observed with a NOAEL 80 mg/kg bw/day.
Embryo/fetal toxicity including teratological effects: • NOAEL (Embryo/foetal toxicity) of 80 mg/kg bw/day based on no embryo/fetal toxicity in the rabbit main study with a NOAEL 80 mg/kg bw/day. In the rat an NOAEL of 100 mg/kg bw/day was obtained in the presence of reduced fetal body weight. • NOAEL (teratological effects) of 30 mg/kg bw/day based increased incidence of palatoschisis (cleft palate) in the rat in the presence of maternal toxicity (↓ body weight, and body weight, food consumption, clinical signs). • In the rabbit, a NOAEL of 20 mg/kg bw/day was observed based on increased incidence of spontaneous malformations (conjoined sternebrae, caudal displacement of ears) in the presence of maternal toxicity. These malformations observed at 80 mg/kg bw/day (a dose level deemed to exceed the maximum tolerated dose) were deemed both incidental and occurred in the presence of overt maternal toxicity, rather than evidence of direct teratogenic effect of the compound. Unlike the rat, palatoschisis (cleft palate) was not observed.
Number aborting and number delivering early: • No evidence of abortions up to doses of 100 mg/kg bw/day in the rat (where examined at the highest dose level) or 80 mg/kg bw/day in the rabbit.
Systemic toxicity and effects on adult body weight: • NOAEL 125 ppm (equivalent ♂/♀: 1.9/2.7 mg/kg bw/day) based on systemic toxicity (hyperkeratosis of oesophagus and forestomach) and clinical chemistry paratmeters (↓ total cholesterol) [90 day oral (dietary) toxicity study in the rat (1)], with the LOAEL of 625 ppm (♂/♀: 9.3/13.2 mg/kg bw/day). • NOAEL 80 ppm (equivalent to ♂/♀: 5.5/6.7 mg/kg bw/day) based on ↓ parental body weight gain in the 2008 two-generation study with evidence of systemic toxicity (hyperkeratosis of oesophagus).
Indices of post-natal growth, indices of lactation and data on physical landmarks: • Evidence of effects on developmental landmarks were observed in the 2008 two-generation study. Both prepuptial separation and vaginal opening were delayed in F_1 offspring, with no effect on anogenital distance in F_2. Developmental landmarks were independent of offspring weight reductions, not endocrine driven but occured in the presence or maternal toxicity (↓ body weight gain, hyperkeratosis of the oesophagus observed at necropsy due to irritation of the digestive tract causing chronic maternal stress). A NOAEL of 80 ppm (F_1/F_2: 6.5/6.7 mg/kg bw/day) based on ↓ pup bodyweights in the 2008 two-generation study.
Survival and general toxicity up to sexual maturity: • NOAEL of 80 ppm (F_1/F_2: 6.5/6.7 mg/kg bw/day) based on ↓ pup bodyweights in the 2008 two-generation study, with no on survival up to the top dose of 300 ppm (F_1/F_2: 22.2/27.7 mg/kg bw/day) 2008 two-generation study.

Endpoint
• NOAEL 125 ppm (equivalent ♂/♀: 1.9/2.7 mg/kg bw/day) based on systemic toxicity (hyperkeratosis of oesophagus and forestomach) and clinical chemistry paratmeters (↓ total cholesterol) [90 day oral (dietary) toxicity study in the rat], with the LOAEL of 625 ppm (♂/♀: 9.3/13.2 mg/kg bw/day).

With regard to the selection of endpoints for the long-term assessment, it is considered that effects on populations will not occur if the survival rate, reproduction rate and development of individuals are not affected. Therefore, in principle, only endpoints in toxicity tests which are related to these key factors of population dynamics are ecotoxicologically relevant.

The refinement for the two-generation study (M-304231-01-1) is based on the assumption that the effects reported at highest dose level, 300 ppm do not influence the population success and the total reproductive outcome of mammals in treated areas. At this dose parental animals showed slight decreases of body weight (up to ↓8.3 %) or body weight gain (up to ↓14.2 %) as well as irritation induced hyperkeratosis of the oesophagus epithelium. However there were delays to developmental milestones of reaching puberty, i.e. preputial separation (PPS) in males and vaginal opening (VO) in females, in the F1 offspring only which were apparently treatment related. These effects occurred only in the presence of maternal toxicity (reduced body weight gain and hyperkeratosis of the oesophagus observed at necropsy due to irritation of the digestive tract causing chronic maternal stress).

The mean time of attainment of PPS was 42.0, 42.3, 42.8 and 44.6 days in 0, 20, 80 and 300 ppm groups, respectively, the delay at 300 ppm being statistically significant ($p < 0.01$). The mean time of attainment of VO was 34.3, 34.6, 35.2 and 38.4 days in 0, 20, 80 and 300 ppm groups, respectively, the delay at 300 ppm being statistically significant ($p < 0.01$). To understand if these delays in attainment of VO and PPS at 300 ppm were attributed to the marginal decreases in body weight effects, an analysis of covariance for the day of attainment of PPS and VO versus male and female pup body weight on post-natal day (PND 21), respectively, was undertaken.

Analysis of covariance for the day of attainment of PPS versus male pup body weight on PND 21 confirmed that the delay in PPS could not account for differences in PND 21 pup body weight. A similar conclusion was also reached for the day of attainment of VO versus female pup body weight on PND 21. Therefore, it cannot be concluded that differences in PND 21 pup body weight accounts for differences in PPS or VO.

III. Conclusion

In conclusion, the most plausible explanation of the effects observed in the two-generation study is that they are all directly caused by or are secondary to, the systemic toxicity of spiroxamine in parental females. As the effects are relatively small compared to the control, these are considered to have no effect at the population level and are therefore not considered to be ecologically relevant.

The resultant long-term effects and chronic endpoint is addressed with the NOAEL for reproductive toxicity assessment of 21.0 mg/kg bw/day.

Assessment and conclusion by applicant:

This report presents an assessment of the available mammalian toxicology data with spiroxamine along with a summary of the specific effects seen for various parameters for each study. A NOAEL of 21.0 mg a.s./kg bw/day has been determined from the rat two generation study and considered suitable for ecotoxicological risk assessment of wild mammals. The refinement is based on the assumption that the effects reported at the 300 ppm top dose level do not influence the population success and the total reproductive outcome of mammals in treated areas. At this dose parental animals showed slight decreases of body weight (up to 8.3 %) or body weight gain (up to 14.2 %) as well as irritation induced hyperkeratosis of the oesophagus epithelium. There were delays to developmental milestones of reaching puberty, *i.e.* preputial separation (PPS) in males and vaginal opening (VO) in females, in the F_1 offspring only which were apparently treatment related, but these were relatively small and are not considered to have an adverse effect at the population level. It is noted that in the

same study the reproductive parameters (mating, fertility, oestrous cycling, sperm motility, sperm count, sperm morphology, pregnancy, natural delivery, litter observations, mean ovarian follicles, *corpora lutea*) were unaffected at the highest dose therefore it has been demonstrated that these small delays in PPS and VO do not have an adverse effect on the parameters that are considered to be relevant at the population level.

Thus, the lowest ecotoxicologically relevant NOAEL, suitable for use in the mammalian reproductive risk assessment, was considered to be 21.0 mg a.s./kg bw/day.

The report is considered to be acceptable.

Ecological data

Data Point:	KCP 10.1.2.2/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	Generic field monitoring of mammals in vineyards in France
Report No:	RA05-223/1
Document No:	M-291785-01-1
Guideline(s) followed in study:	EU Council Directive 91/414/EEC by the Commission Directive 96/68/EC; SANCO/4145/2000
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In this generic study, a radio-tracking program was carried out in a typical wine growing region of France during spring and summer to obtain data on PT values for refined exposure assessment. Special emphasis was placed on the wood mouse (*Apodemus sylvaticus*), the focal species in vineyards. In the present study trapping, radio-tracking, visual observations together with analyses of stomach content were methods used to characterise PT in vineyards.

The wood mouse was the dominant focal species in the vineyard, however only a minor part of their time potentially foraging within the vineyards, with its preferred habitat was the surrounding areas. Stomach samples indicated wood mice consumed mainly plant matter, with seeds as the dominant food (80%).

Study area

The study was conducted in and around four different vineyards in the Burgundy region around the municipality of [redacted] (France), a typical wine growing district, and encompassed a study area of 487 ha.

I. Methods

The study was carried out during spring and summer 2006.

Mammal trapping, marking and radio-tagging

Small mammals were trapped in four vineyards and the adjacent surroundings to identify the species present and to define the focal species for further study. Regular trapping was performed on each study

plot on two consecutive trap nights every week. Each captured animal >10g body weight was individually marked with a passive integrated transponder, injected subcutaneously.

Adult individuals of the dominant species (wood mouse) were equipped with radio-collars. The weight of the collar did not exceed 10% of the animal's body mass.

In order to collect data on their time budget, habitat selection and home range sizes, 20 individuals (7 females, 13 males) of the focal species, the wood mouse (*Apodemus sylvaticus*), were radio-tracked over their whole activity period (from dusk till dawn). From the radio-tracking data, the proportion of time potentially foraging was compared for vineyards and all habitats in the individuals home range. These values were used for deriving reliable values of the proportion of diet obtained from the treated area (PT).

Individual PT was calculated as:

$$\frac{\text{Time potentially foraging in vineyards}}{\text{Time potentially foraging in all known habitats}}$$

All trapped and radio-tracked wood mice were closely associated with vineyards and had the opportunity to use them as a foraging habitat. The results can therefore be considered to provide a worst case estimate of dietary exposure.

The spatial portion of vineyards within the home range of the individual wood mice was calculated for each tracking session and compared to the corresponding PT value. This comparison (calculated as the Jacobs' index [D]) illustrates the preference or avoidance of the individual wood mouse for vineyards as a feeding habitat during each tracking session.

To estimate the proportion of different food types in the diet, stomach contents of wood mice were sampled and analysed. For each sample the proportions of different food items contributing to the volume of the diet samples were estimated.

Stomach sampling

Snap traps were set within the vineyard at night and checked early in the morning throughout the study period. Stomach samples were evaluated by microscopic observations.

Additional observations

The whole study area was mapped for habitat types and crops at least once during the study period.

The temperature (daily minimum, maximum and average) and daily precipitation data were obtained from the nearest weather recording station.

II. Results and Discussion

The study was deemed to be acceptable.

The temperature during the study period ranged between -1.9 and 26.5°C. The mean daily temperature was 13.8°C. Total precipitation was 432.9 mm, with a daily average of 2.8 mm.

The results of trapping indicate that the wood mouse (*Apodemus sylvaticus*) is the only species observed to regularly use vineyards and as such can be considered to be the focal species. Besides a few exceptions, yellow-necked mice (*Apodemus flavicollis*) and bank voles (*Clethrionomys glareolus)* were confined to hedgerows and woodland only. There were no shrews and only four captures of common vole (*Microtus arvalis*) in the vineyard out of 7680 trap nights. Live trapping revealed that the surrounding habitats were rather more attractive to wood mice than vineyards (captures/100 trapnights: surroundings 20.55, vineyard 3.75).

PT values

Based on the study results, the mean PT value derived for wood mice living within or in close vicinity to vineyards in spring/summer was 0.14 (90th percentile = 0.41). It is important to emphasise that these values represent the estimated proportion of time spent 'potentially foraging' in vineyards. All values

of 'time potentially foraging' include times when foraging was not evident but could not be precluded. The calculated PT value can therefore be regarded as a conservative estimate. The calculated mean Jacob's Index was -0.83, implies that vineyards were selected to a lesser extent for foraging than would be expected at random from the proportion of this habitat available within their home range.

Diet sample contents

The stomach samples of wood mice were mainly composed of plant material. Five out of nine samples were exclusively made up of plant matter. Among the plant matter found in the diet samples, seeds were most numerous in the diet of wood mice.

Table CP 10.1.2.2/01-1 Overview of small mammal abundance, PT and diet analysis

Relevant species in the vineyard (based on live-trapping)		
Species	**Mean trapping rate (captures/100 trappings)**	
	Vineyard[1]	**Surrounding[2]**
Apodemus sylvaticus	3.75	20.55
Apodemus flavicollis	0.16	6.99
Microtus arvalis	0.05	0.00
Clethrionomys glareolus	0.00	8.09
Shrews (*Crocidura sp., Sorex sp.*)	0.00	0.00
Proportion of diet obtained in vineyards determined by radio-tracking (PT)		
'potential foraging' time in vineyards as a proportion of the total 'potential foraging' time equals the proportion of diet obtained	Based on 20 radio-tracking sessions of 20 wood mouse individuals	
50% tile	0.09	
90% tile	0.41	
Mean	0.14	
Proportion of different food items in the diet		
Arthropod and plant items actually eaten by 20 individuals foraging in and around vineyards [mean portion]	Based on stomach samples of 20 wood mouse individuals	
Animal matter	3%	
Plant matter	97% (fruits 14.0%, weed seed 80.1%, others 2.9%)	

[1]Based on 7680 trappings

[2]Based on 2560 trappings

III. Conclusion

Of all small mammals, only the wood mouse (*Apodemus sylvaticus*) was found to be in significant numbers in vineyards. Other species were essentially confined to the surrounding, largely woodland habitat.

The wood mouse is the dominant species in vineyards. Live trapping revealed that the surrounding habitats were more attractive for this species than vineyards.

Radio-tracked wood mice spent only a small proportion of time foraging in vineyards (time potentially foraging). From a distribution generated from 20 individuals, the PT estimates for 50th and 90th percentiles were 0.15 and 0.41, respectively.

Wood mice avoid vineyard habitats, preferring surrounding habitats and vineyards are of marginal importance as feeding habitat for wood mice in spring and summer.

The main components of all sampled stomachs were seeds (80.1%) and fruits (14.0%).

Assessment and conclusion by applicant:

This ecological monitoring study was conducted to GLP but not to any specific methodology. However, this is typical of studies of this type therefore the study is still considered to be acceptable for regulatory purposes by supporting various aspects of the Bird & Mammal risk assessment.

The study showed that the wood mouse was far more prevalent in vineyards and the surrounding areas when compared to other small mammals such as the vole or shrew. It is therefore considered to be a highly relevant small mammal focal species, far more so than the vole which was seen in very low numbers. The study also found that, whilst the wood mouse did spend time within the vineyards, they found the surrounding areas more attractive. A 90th percentile PT value for the wood mouse of 0.41 was determined in this study and has been applied to the refined risk assessment. The study also analysed stomach contents which confirmed that the diet was almost exclusively plant matter with seeds being the primary component.

The results of this study have been used as part of the refined risk assessment of the small herbivorous mammal "vole" scenario, specifically to support the use of the wood mouse as a suitable focal species in vineyards, along with a 90th percentile PT value of 0.41. The study is also considered to support the use of the default omnivorous species diet from EFSA (2009) of 25% weeds, 50% weed seeds and 25% ground arthropods.

Data Point:	KCP 10.1.2.2/09
Report Author:	[redacted]
Report Year:	2010
Report Title:	Generic field monitoring of mammals in vineyards in Spain
Report No:	R09-123-2
Document No:	M-405593-01-1
Guideline(s) followed in study:	No official test guideline(s) available at present
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

In this generic study a live trapping and radio-tracking program was carried out in a typical southern European vine growing region in Spain during spring and summer, to obtain data on PT and diet estimation values for refined exposure assessment. Two small mammal species were monitored by radio-tracking the wood mouse (*Apodemus sylvaticus*) and the Algerian mouse (*Mus spretus*). In this study, live trapping, radio-tracking, visual observations and analysis of stomach contents were conducted to characterise PT and diet estimations in vineyards.

The study provided reliable refined parameters of PT for both focal species and dietary estimates for wood mice and Algerian mice for use in higher tier risk assessments for small mammals foraging in vineyards in southern Europe.

Study area

The study was conducted in vineyards in the vicinity of [redacted] a municipality in the [redacted] region in north-eastern Spain, a typical area for vine cultivation in southern Europe. Four vineyards were selected to represent a common size and structure of vineyards in the region, for suitable adjacent off-crop habitats and for the suitability of the intended study methods.

I. Methods

The field phase of the study was carried out during spring and summer (March to July) 2009.

Mammal trapping, marking and radio-tagging

Small mammals were trapped in four vineyards and the adjacent surroundings to identify the species present and to define the focal species for further study. Live trapping was conducted on all study vineyards with one trapping session (2 to 4 consecutive trap night) per week throughout the field phase. In the beginning of the study live trapping was carried out in an intensive manner to select suitable species for a radio-tracking program. Due to the results of the intensive trapping period the wood mouse (*Apodemus sylvaticus*) and the Algerian mouse (*Mus spretus*) were chosen as focal species for radio-telemetry.

Each captured animal (except individuals <10 g of body weight and shrews) was injected subcutaneously with a Passive Integrated Transponder (PIT), each with a unique ID number. Shrews were marked with a fur cut. Small mammal species captured inside the crop or in its close vicinity were equipped with radio collars. Individuals were regarded as suitable for tagging if the weight of the tag did not exceed 5% of the animal's body weight. Individuals were tracked at least 24 hours after tagging, to exclude any bias.

By radio-tracking of individual small mammals, their home range, habitat use and the portion of time they spent potentially foraging in vineyards was determined. A total of 20 individuals of each species were successfully radio-tracked. Individual Algerian mice were radio-tracked continuously over a period of 24 hours. Wood mice, known to be nocturnal, were radiotracked from dusk till dawn. The proportion of time potentially foraging in vineyards (compared to the total potential foraging time in all habitats) was estimated from the radiotracking data, supported by visual observations. These values were regarded as conservative equivalents to the proportion of diet obtained from the treated area (PT).

Individual PT was calculated as:

$$\frac{\text{Time potentially foraging in vineyards}}{\text{Time potentially foraging in all known habitats}}$$

To estimate the proportion of different food types in the diet (PD), stomach contents were analysed (20 stomachs of wood mice and Algerian mice, respectively). Since there are no correction factors available for small mammals, weight-length, weight-number and/or weight-area relationships based on reference data for all food categories (*e.g.* invertebrates, seeds and green plant material), collected during the study, were used to calculate the proportion of dry weight of each food category found in the analysed stomachs.

Stomach sampling

Snap traps were set within additional vineyards in proximity of the study vineyards over the whole study period. A minimum of 20 stomachs were sampled per species. Stomach samples were evaluated by microscopic observations.

Additional observations

The whole study area was mapped for habitat types and crops. The vegetation status of the vineyards was determined by changes in BBCH principal growth stages during the study period.

The temperature (daily minimum, maximum and average) and daily precipitation data were obtained from the nearest weather recording station.

II. Results and Discussion

The temperature during the study period ranged between 1.6 and 35.8°C. The mean daily temperature was 18.7°C. Precipitation was recorded on 30 days over this period with a total of 183.2 mm.

PT values

The combination of radio-tracking with visual observations and the trapping scheme as presented here (*i.e.* trapping inside of vineyards or close vicinity), allowed an accurate and representative assessment of potential foraging times in given home ranges in order to calculate reliable PT values. All small mammals were closely associated with vineyards and had the opportunity to use these as foraging habitat. Therefore the results can be considered as conservative in terms of potential dietary exposure. PT values were calculated for individual small mammals and as overall PT values (*i.e.* 50%tile, 90%tile and mean) for both species. Based on the study results, the mean PT values calculated for the wood mouse and the Algerian mouse were 0.05 (90%tile = 0.17) and 0.13 (90%tile = 0.43), respectively.

Table CP 10.1.2.2/09-1 Overview of PT in vineyards

Proportion of diet obtained in vineyards determined by radio-tracking (PT)		
'potential foraging' time in vineyards as a proportion of the total 'potential foraging' time		
Woodmouse[1]	50% tile	0.00
	90% tile	0.17
	Mean	0.05
Algerian mouse[1]	50% tile	0.00
	90% tile	0.43
	Mean	0.13

[1]Based on 20 individuals in 20 tracking sessions

Diet estimate and PD values

The analysis of the wood mouse and Algerian mouse stomach samples followed the most evolved methodology for the omnivorous diet guild, which is biomass dry weight determination by means of comparison with a reference data base. The dry weight proportions for the different food categories (*e.g.* invertebrates, seeds, green plant material, wood / bark) in the stomach samples are presented below.

Table CP 10.1.2.2/09-2 Diet composition of the wood mouse

Based on 20 faeces samples analysed for their dry weight proportions (% dry weight) of different food categories			
Food category	**50% tile**	**90% tile**	**mean**
Invertebrates	0.79	14.98	5.95
Seeds	98.96	99.95	92.78
Green plant material	0.01	0.25	1.11
Wood/bark	0.00	0.05	0.16

Table CP 10.1.2.2/09-3 Diet composition of the Algerian mouse

Based on 20 faeces samples analysed for their dry weight proportions (% dry weight) of different food categories			
Food category	**50% tile**	**90% tile**	**mean**
Invertebrates	0.07	46.47	12.22
Seeds	95.85	99.94	82.16
Green plant material	0.05	20.40	5.24
Wood/bark	0.00	1.11	0.37

III. Conclusion

Overall this study provides reliable refined parameters of PT for both local species and dietary estimates for wood mice and Algerian mice for use in higher tier risk assessments for small mammals foraging in vineyards in southern Europe.

Assessment and conclusion by applicant:

This ecological monitoring study was conducted to GLP but not to any specific methodology. However, this is typical of studies of this type therefore the study is still considered to be acceptable for regulatory purposes by supporting various aspects of the Bird & Mammal risk assessment.

The results of this study have been used as part of the refined risk assessment of the small herbivorous mammal "vole" scenario specifically to support the use of the wood mouse as a suitable focal species in vineyards. This study provided a 90th percentile PT value of 0.17 for the wood mouse. However, for the refined risk assessment for Spiroxamine EC 500, the more conservative PT value of 0.41 from study M-291785-01-1 has been used.

Data Point:	KCP 10.1.2.2/10
Report Author:	[redacted]
Report Year:	2012
Report Title:	Luna Experience - BCS response to the evaluation by the zonal rapporteur member state Greece - Refined risk assessment for small herbivorous mammals and omnivorous birds
Report No:	M-439777-01-4
Document No:	M-439777-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

I. Introduction

In its evaluation of Luna Experience as Rapporteur Member State for the Southern Zone of the EU, Greece concluded that the risk to the Common Vole is not acceptable for certain uses and that "Member States should carefully consider the vole scenarios are relevant and whether they should merit attention or not".

Such a conclusion would burden other Member States of the Southern Zone with additional work to resolve this question, if it could not be resolved by the Zonal RMS by providing a risk assessment that shows an acceptable risk for these scenarios. This document is intended to demonstrate that voles, even if the scenarios might be unrealistic in Southern Zone Member States, are not at risk by the intended uses of Luna Experience.

Refinement of ecological parameters of the common vole

Tier 1 Risk assessment for the vole as a small herbivorous mammal assumes that the animal only feeds on grasses. This may be true for a short period of time applicable for an acute risk assessment. However, it is not conceivable that voles would eat only grasses over a prolonged period relevant for a long-term risk assessment.

Lüthi *et al*. (2010) investigated diets of voles for various plants in natural habitats (n=98) and in recently sown wild flower fields (n=99) by analyzing stomach contents and faeces samples. The natural habitats were characterized by a prevalence of monocotyledonous plants whereas dicotyledonous plants prevailed in the wild flower fields.

The authors found a preference for monocots in both scenarios, supporting to notion that grasses might be the predominant feed for voles. They also found, however, that despite this preference for monocots, other feed items contributed to a significant part to the total diet. In the setting with dicots dominating the habitat, monocots represented on average 44.3% of the diet, dicots 19.6% and seeds made up for 19.4% (wet weight). The rest were unidentified items. In the natural habitat (monocots predominating), monocots made up for 71% of the diet, dicots represented 11% and seeds 10.7%. Again, the rest were unidentified items.

These findings demonstrate that, even in situations where they could easily satisfy their energy demands by feeding on monocots alone, voles will feed on dicots and other items like seeds and roots too. Therefore the assumption for the long-term risk assessment that voles will feed exclusively on grasses is unrealistically worst-case.

Because of these clear findings and the fact that the study was done within the typical range of distribution of this species the study and its results are deemed highly relevant for introducing more realistic elements into the risk assessment for this species.

For this reason a refinement is presented below using the dietary information from Lüthi *et al*. (2010).

Starting from the worst case situation (monocot dominated natural habitat) the diet composition was recalculated to account for the unidentified items portion (7.3%). Rather than assuming these were e.g. roots etc. this portion was proportionally distributed to the other three matrices, monocots (grasses), dicots (non-grass herbs), and seeds (weed seeds), resulting in percentages (PD's) for these items of 76.6, 11.9, and 11.5, respectively.

II. Conclusions

These findings demonstrate that, even in situations where they could easily satisfy their energy demands by feeding on monocots alone, voles will feed on dicots and other items like seeds and roots too. Therefore the assumption for the long-term risk assessment that voles will feed exclusively on grasses is unrealistically worst-case.

Assessment and conclusion by applicant:

The report presents the results of a review of the diet of the vole from different habitats. The evidence suggests that voles do not feed exclusively on grasses and that other plant material such as dicots and seeds also made up part of the diet. A vole diet of 76.6% grass & cereals, 11.9% non-grass herbs and 11.5% weed seeds has been established.

The information is considered suitable for use in a refined mammalian risk assessment for the vole.

Data Point:	KCP 10.1.2.2/11
Report Author:	[redacted]
Report Year:	2003
Report Title:	Small mammals in vineyards in south-west Germany
Report No:	M-237095-01-2
Document No:	M-237095-01-2
Guideline(s) followed in study:	not specified
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

A survey of small mammals was carried out between April and August 2002 in three vineyards in wine growing areas of south-west Germany. This was done by live trapping and individually marking the animals with cuts in their coat and $KMnO_4$ dye. Three different study areas with varying habitat features were selected in order to obtain information on the species composition according to the different habitat features. Two species - the common vole and the wood mouse – were identified in small numbers in vineyards with partial and full ground vegetation. There was no evidence of any small mammals in vineyards with no ground vegetation. The presence of ground vegetation in the vineyard was the determining factor for the occurrence of the common vole and the wood mouse in vineyards.

It can thus be stated in conclusion that the population densities of both species in vineyards are well below the figures determined for other agricultural areas. Vineyards represent a suboptimal habitat for both species. The amount of ground vegetation in the vineyard has a direct effect on the population density of the small mammals occurring there. A population of neither species should be expected in areas with no ground vegetation. Areas which have partial or full ground cover have low levels of colonisation, with the presence of the wood mouse restricted to the summer months.

I. Materials and Methods

Study areas in the wine-growing areas of [redacted]) and [redacted] districts near [redacted]) were selected. All three areas were at coliine level (150 - 400 m above sea level) and were characterised by a warm, mild climate.

The [redacted] study area ([redacted]) was in a wine-growing area of approximately 18 ha, which - apart from a few meadows and gardens – was covered almost exclusively by vineyards. There was a

wood to the north of the area. The study area itself was about 35 m from the edge of the wood and - like all the vineyards in this area - a layer of ground vegetation had formed in it.

The [redacted] area ([redacted]), which had ground vegetation In every other row, was surrounded by thickets on three sides at a distance of about 5 - 10 m. In the area surrounding the study area, large areas under vines alternated with thickets, meadows and fields during the study period.

The [redacted] study area ([redacted]), which did not have any ground vegetation, was in an area with few structural features where, apart from the actual vineyard, there were only meadows and fields: there were no thickets or woodland.

The Giessen standard method, as recommended by BOY'E & MENIG (1996), was used to record the data on small mammals. This gave an area of 0.25 ha for all study areas. A total of 64 wooden box traps made by [redacted] baited with sardines in oil, rolled oats and peanut butter were used ([redacted] 2001). Between April and August 2002, there was one trapping period per month in each of the study areas. The weight (PESOLA®, spring balance), species, sex and reproductive status of each animal caught was recorded.

Each animal was then individually marked by a cut in its coat and additionally fay $KmnO_4$ dye. The animals were then released at the same place immediately after the data had been recorded and they had been marked. The trapping success was calculated for each species as the total number of individuals caught during the study period in a study area in relation to 100 trap units. One trap unit was taken to be the period for which a trap was set during a monitoring round ([redacted] 1999).

The size of the species population was calculated using the minimum number alive method (MIMA) which, unlike other methods of estimation, determines the minimum population. With this method, the animals are evaluated as members of the population, even if they are not recorded in each sample. The MNA includes the number of individuals caught in an inspection plus the number of individuals not caught which were recorded in an earlier and later sample, but not in the current one. The number of individuals determined in this way was then multiplied by four in order to obtain the population densities of the 0.25 ha study areas which could be related to a hectare.

II. Results and Discussion

During the study period, 51 individuals were recorded in 15 trapping series with 9600 trap units and 63 small mammals trapped. Two species were identified in the study areas, each from different families:

Arvicolidae - common vole: *Microtus arvalis* (PALLAS) - field mouse and Muridae - true mice: *Apodemus sylvaticus* (L.) - wood mouse.

Common voles were found in only two out of the three study areas. In the [redacted] study area, the calculated catch rate was 1,06 individuals/100 trap units and in the Fuchsen study area it was 0.03 individuals/100 trap units. The catch rate for the wood mouse, which was identified in each of the study areas was 0.25 for [redacted] and 0.13 individuals/100 trap units for each of the other study areas ([redacted] and [redacted]).

The tables below show the number of individuals recorded, the number of re-catches, the population sizes in individuals/ha calculated by MNA, and the averages for each of the two species.

Table CP 10.1.2.2/11-1 Number of common vole catches

Trapping period	Individuals	Re-catches	Population size
Location: Kratzenberg			
1	6	0	24

Trapping period	Individuals	Re-catches	Population size
Location: Kratzenberg			
2	6	4	24
3	11	1	44
4	10	3*	40
5	3	0	12
6	7.2	1.6	28.8
Location: Fuchsen			
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	1	0	4
6	0.2	0	0.8
Location: Löhrer Berg			
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	0	0	0

*Includes two re-catches from previous trapping period

Of the total of 34 common vole individuals in the [redacted] study area, 17 were female, including one young animal. Of the 17 male individuals, eight were young animals. Only in the last trapping period was one female individual caught in the Fuchsen study area.

Seven of the eight wood mouse individuals in the [redacted] study area were males, two of them, like the female, young animals. Of the four individuals caught in the [redacted] study area, two were young male animals and one was a young female. An adult female was also caught. Only males were caught in the [redacted] study area, only one of which was a young animal.

Table CP 10.1.2.2/11-2 Number of wood mouse catches

Trapping period	Individuals	Re-catches	Population size
Location: Kratzenberg			
1	0	0	0
2	0	0	0

Trapping period	Individuals	Re-catches	Population size
Location: Kratzenberg			
3	3	0	12
4	2	0	8
5	3	2	12
6	1.6	0.5	6.4
Location: Fuchsen			
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	4	0	16
6	0.8	0	3.2
Location: Löhrer Berg			
1	1	0	4
2	0	0	0
3	2	0	8
4	0	0	0
5	1	0	4
6	0.8	0	3.2

III. Conclusion

It can thus be stated in conclusion that the population densities of both species in vineyards are well below the figures determined for other agricultural areas. Vineyards represent a suboptimal habitat for both species. The amount of ground vegetation in the vineyard has a direct effect on the population density of the small mammals occurring there. A population of neither species should be expected in areas with no ground vegetation. Areas which have partial or full ground cover have low levels of colonisation, with the presence of the wood mouse restricted to the summer months.

Assessment and conclusion by applicant:

This ecological monitoring study was not conducted to GLP and not to any specific methodology. However, this is typical of studies of this type therefore the study is still considered to be acceptable for regulatory purposes by supporting various aspects of the Bird & Mammal risk assessment.

The results of this study have been used to support the refined mammalian risk assessment, specifically the small herbivorous mammal scenario. The study demonstrates that both the wood mouse and the common vole are present in vineyards but at relatively low numbers compared to other agricultural areas. Furthermore, their presence is dependent on there being ground vegetation in the vineyard.

Data Point:	KCP 10.1.2.2/12
Report Author:	[redacted]; [redacted]; [redacted]
Report Year:	2019
Report Title:	Relevance of body weight effects for the population development of common voles and its significance in regulatory risk assessment of pesticides in the European Union
Report No:	M-669216-01-1
Document No:	M-669216-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

I. Background

The common vole (*Microtus arvalis*) is typically the wild mammal species driving regulatory pesticide risk assessment (RA) in Europe. The risk assessment endpoint for wild mammals is taken from the studies conducted mainly with rodents for the toxicological part of the dossier. Body weight effects in these studies are often driving the selection of the No Observed Adverse Effect Level (NOAEL) used for wildlife risk assessment. Thus, assessing body weight effects in voles very frequently constitutes a key scenario in the RA. Although many studies on ecology, reproductive biology, population genetics, and other aspects of common voles are available, the relevance of body weight for their survival and reproduction has not yet been specifically analysed. There is also little guidance on how to quantitatively deal with body weight effects in the regulatory risk assessment of pesticides.

II. Results

Population relevance of body weight effects on voles by analysis of a dataset from multi-annual study with repeated life-trapping and genotyping was evaluated and body weight with reproductive success was correlated, taking account of the seasonality of body weight. Body weight and growth were similar between reproducing and non-reproducing females. The number of confirmed offspring indicated no correlation with parental body weight. Reproductive success of the voles was mainly influenced by the date of birth, *i.e.*, animals born in Spring have a higher chance to reproduce. Body weight did not correlate with life span during most of the year, except for autumn. Animals weighing <15 g in October did not survive winter.

III. Discussion

In toxicology studies such as the rat reproduction study, in which animals are exposed to treated diet for several months, effects on body weight are frequently observed (most often in form of retarded growth, rather than actual body weight loss). Since the results from these studies are used for the wild mammal risk assessment of pesticides, the question arises to what extent effects on body weight may affect populations of free ranging animals exposed to pesticides under field conditions.

Already EFSA noted, that there were “no quantitative experimental data to define the level of body weight change that is associated with impaired mating performance or parental care”. However, no more guidance was then given in the relevant EFSA guidance on how to quantitatively interpret body weight effects observed in the laboratory or how to translate these to field conditions for risk assessment.

The generic focal species scenario “small herbivorous mammals”, *i.e.*, Common voles, often drives the initial steps of pesticide RA in the EU. To date, the relevance of body weight on free ranging common

voles has never been studied with regard to pesticides, although body weight is typically measured during capture–mark–recapture field effect studies.

The present evaluation is based on a unique dataset from a live trapping study conducted over 3 years and from genotyping of more than a thousand individual voles. Since animals were kept in outdoor enclosures from which they could not move away, the likelihood of trapping them was high. Therefore, information on their life span is considered robust. Since not all animals could be genotyped due to practical reasons, it is possible that some offspring were not detected. However, since a relatively large number of 1255 individuals were genotyped, the data can be considered adequate to address the objective.

A first remarkable result of the present analysis was that about 80% of all females and about 90% of all males had no genetically confirmed offspring. Hence, a considerable proportion of the population did not reproduce or did not reproduce successfully, while relatively few animals produced most offspring (females and males produced up to 13 and 32 pups, respectively).

This means that even under the optimal conditions of this study (grassland habitat, ground predators excluded, population density was in a normal range), the availability of home ranges was a limiting factor for the population. In turn, 80% and 90% of non-reproducing females and males, respectively, provided a considerable 'reproductive reserve', which could start to reproduce when becoming resident, or when reproducing animals disappeared (e.g., by emigration, predation or agricultural practice). This population resilience is not only relevant for pesticide risk assessment but also explains why common vole populations recover very quickly after rodenticide application. Although voles have short lifespans (during the breeding season most animals live only about a month), they exhibit a high reproductive output and often disperse from their natal areas.

Body weight effects, as observed in toxicological studies, could potential impact reproductive success of voles, for example during the "breeding phases" defined by EFSA: "Establish breeding site", "pairing" and "mating". For example, smaller female voles may potentially have a lower reproductive success due to competition over breeding territories.

However, females of small mammal species in agricultural fields are not very selective regarding mates, and males, which have larger home ranges encompassing a number of female home ranges, are only loosely connected with females and thus mate with a large number of females (*i.e.*, polygynous mating system with multiple paternity). Multiple paternity or polyandry is seen as a common female strategy to increase genetic diversity of offspring or to avoid infanticide. Multiple paternities within a litter have been described in common voles, several other small mammals and animals in general. Thus, the actual mating system of common voles is in fact very resilient to effects on individuals and this may also explain that differences in adult body weight of factor 2 or more were not associated in our study with measurable differences in reproductive success, a key element of the regulatory protection goal.

The focus of the present evaluation was to determine to what extent body weight had an effect on reproduction and survival, to inform the risk assessment or management of small mammals.

However, since common vole body weight showed a typical seasonal trend as previously reported, seasonality needed to be taken into account. The main factor influencing reproductive success (measured as the number of genetically confirmed offspring) was the month of birth. Females born early in the season had more offspring than females born later in the season. Body weights were generally higher early in the season (before population density reaches its peak) than in late summer or later. Hence, one might suspect that body weight is related to a higher number of offspring. However, this is not the case, because when seasonality was accounted for by comparing the number of confirmed offspring and female bodyweight month by month, it was found that larger body weight did not relate to more offspring. That is, for each month, there was no correlation between body weight and the number of confirmed offspring. Overall, this means that seasonally changing body weight does not seem to affect reproductive success, but that the single most influential factor affecting it is the time of birth (or in other words, the time animals have for reproduction). Also, when comparing the body weights of

successfully reproducing females (i.e., those with confirmed offspring) and unsuccessfully reproducing females (*i.e.*, those without confirmed offspring), no influence of body weight was found.

In contrast, regarding life span, an effect of body weight was found in young animals born late in the year: when comparing life span and body weight month by month (again to take account of seasonality), it was found that of all animals caught the first time in autumn (October), only those with a body weight of at least 15 g survived until the next year. However, survival of animals caught the first time in October was generally low (only 13% survived until the next year). But a low winter survival of animals born late in the season probably does not affect populations much, since only about 15% of all first captures were caught in October or later.

Before October, body weight and survival did not correlate. In this context, it is interesting to see that body weight is generally highest in late spring and summer when survival is typically lowest. These results are in line with findings in bank voles by Koskela who studied the impact of litter size manipulation in outdoor enclosures in Finland. An artificial increase of litter size related to a lower body weight at weaning and a reduction of litter size resulted in larger weaning weights. While litter size manipulation had no effect on winter survival, survival of pups during lactation was reduced for enlarged litters. However, a higher female weaning weight related to a slightly higher winter survival, independent of litter size manipulation (survival of male offspring was not analysed). Adult female weight did not, however, explain the probability of surviving over winter.

IV. Conclusions

These results demonstrate no detectable influence of common vole body weight on reproductive success and survival during most times of the year. The results of this study suggest that, additional to the hazard information from toxicity studies, ecological information on voles as a typical species of concern should be considered in the regulatory risk assessment of pesticides.

Assessment and conclusion by applicant:

This summary relates to a literature paper which has been written to highlight that the body weight of the common vole would not appear to affect the reproductive success of this species. The study revealed that there was no detectable influence of common vole body weight on the reproductive success and survival during most time of the year and that reproductive success was mainly influenced by the date of birth.

The paper has been referred to in the mammalian risk assessment to support the notion that the relatively small reductions in body weight recorded in the rat two-generation study will not have an adverse effect at the population level and are therefore not ecotoxicologically relevant.

Data Point:	KCP 10.1.2.2/13
Report Author:	[redacted]
Report Year:	2013
Report Title:	Common vole (Microtus arvalis) ecology and management: implications for risk assessment of plant protection products
Report No:	M-476622-01-1
Document No:	M-476622-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Abstract

Common voles (*Microtus arvalis*) are common small mammals in some European landscapes. They can be a major rodent pest in European agriculture and they are also representative generic focal small herbivorous mammal species used in risk assessment for plant protection products. In this paper, common vole population dynamics, habitat and food preferences, pest potential and use of the common vole as a model small wild mammal species in the risk assessment process were reviewed. Common voles are a component of agroecosystems in many parts in many parts of Europe, inhabiting agricultural areas (secondary habitats) when the carrying capacity of primary grassland habitats is exceeded. Colonisation of secondary habitats occurs during multiannual outbreaks, when population sizes can exceed 1000 individuals ha^{-1}. In such cases, in crop common vole population control management has been practised to avoid significant crop damage. The species' status as a crop pest, high fecundity, resilience to disturbance and intermittent colonisation of crop habitats are important characteristics that should be reflected in risk assessment. Based on the information provided in the scientific literature, it seems justified to modify elements of the current risk assessment scheme for plant protection products, including the use of realistic food intake rates, reduced assessment factors of the use of alternative focal rodent species in particular European regions. Some of these adjustments are already being applied in some EU member states. Therefore, it seems reasonably consistently to apply such pragmatic and realistic approaches in risk assessment for plant protection products across the EU.

I. Introduction

Agriculture provides food for more than six billion people globally, with agricultural production greatly increased owing to intensification of farming practices such as increased fertiliser applications, improved plant breeding techniques, irrigation, mechanisation and an increased used of plant protection products.

Plant protection products minimise pre- and post-harvest losses in many crops, including grains, vegetables and corn, but also in horticulture and forestry, by regulating plant disease and reducing the impact of invertebrates, weeds and occasionally vertebrates.

The use of plant protection products and their active ingredients is regulated at EU level and nationally at member state level to ensure that products are effective in managing crop pests and safe for humans and the environment. In the regulatory process, pesticide risk is assessed for formulated products and their active ingredients on the basis of scientific studies performed using recognised test procedures with resulting endpoints applied in the risk assessment models. Only active ingredients and formulated

products satisfying the requirements of the risk assessment to protect non-target organisms from effects associated with the application of the plant protection products can be registered for use.

Risk assessment approaches for wild mammals aim to evaluate the potential impact of a pesticide application on a model "representative" species that is likely to be present in the crop at the time of application. Typically, a model species will have high food intake rate (FIR), consume mostly a relevant type of food (*i.e.* a food type potentially carrying residues) and have a low body weight, all of which maximises the potential exposure to and risk from the pesticide. Under the current scheme of the mammalian risk assessment, the common vole is such a model species representing herbivorous mammals. In agroecosystems, the common vole is an important component of the food web, providing ecosystem can provide shelter for many other species.

However, the common vole is also important vertebrate pest species in many crop types across the European agricultural landscapes. It consumes plant material (*e.g.* leaves, stems, seeds, roots, bark) from several agricultural, horticultural and forestry plants, which can result in significant crop damage. Outbreaks of common voles occur every 2 – 5 years. During outbreaks, farmers typically manage the associated damage by applying rodenticides directly to tunnel entrances. Where possible, farmers can use indirect control methods to manage common voles, such as decreasing vegetation height and cover, which removes food and also reduces shelter from predation.

Within the framework of commission regulation 1107/2009 for placing of plant protection products on the market within the EU, mammalian environmental risk assessments are performed according to guidance presented in an EFSA (2009) guidance document. Within this guidance, the common vole is the representative generic focal small herbivorous mammal species used in the acute and chronic risk assessments, considered relevant for almost all crop types. With a low body weight and a high food intake rate, the common vole has a high potential exposure in crops following product application.

The uncertainty as to how to deal with common voles in risk assessment has remained a constant feature of small herbivorous mammal risk assessment under EFSA (2009) guidance.

This article presents a review of common vole population dynamics, biology and behaviour, including habitat preferences and crop damage potential relevant to risk assessment. In the review, refined approaches to the use of common voles in the risk assessment of plant protection products within the EU regulatory framework on the basis of realistic and scientifically based information are discussed.

II. Discussion

When considering voles in risk assessment, a realistic position on the importance of voles to the agroecosystem should be taken. Published information highlights opportunities to balance risk assessment with characteristics of common vole biology and ecology and pest status.

Habitat preferences

Common voles are essential food web components ensuring energy flow through the trophic levels as a significant primary consumer. They are an important food source for predators within the food chain; for example, raptor species adapt their abundance to coincide with outbreaks of small mammals. This has obvious ecological benefits, but can also result in conservation issues during times of common vole population decline when large predator populations search for alternative food. Common voles are an important pest species in multiple crops, causing significant levels of damage. Their distribution and damage potential is widespread across agricultural landscapes within Europe. Common vole populations peak usually seasonally during autumn. Multiannually, there is a long-term pattern of vole population growth and decline that results in outbreaks occurring in about 2–5 year periods, which means that naturally occurring seasonal and multiannual fluctuations are the rule for common vole populations. The preferred primary habitat of common voles is steppe, which comprises grassland, pasture and meadow with mixed grassland, herbs and weeds that provide appropriate cover to avoid predation. For common voles, many cropped areas are considered to be secondary habitats, and significant invasion into them occurs when there is a population outbreak. In contrast to primary habitats, these secondary habitats cannot maintain common vole populations sustainably for long periods owing to the seasonal nature of

farming, where populations are regularly disrupted by harvest and tilling. Although the common vole is indicated as the representative generic focal species in screening and tier 1 risk assessments under EFSA (2009), population dynamics and habitat preferences indicate that in the period between population outbreaks the likelihood of significant numbers of common voles being found in secondary habitats such as grain crops, vegetables and sugar beet is low. During vole population outbreaks, the density of voles in primary habitats is high, which is likely to provide a considerable buffer for potential adverse effects of plant protection products on common vole populations in secondary habitats such as cropped areas. Inclusion of different levels of comparative risk in primary and secondary habitats for a pest such as the common vole is considered to be appropriate to ensure a sufficient population density is maintained in the primary habitat. This contributes to maintaining the protection goal to avoid long-term detrimental effects on common vole populations.

Managing common vole populations

In Europe, few rodenticidal compounds are used regularly for direct control of common vole populations in crop habitats. The use of rodenticides and alternative methods can reduce crop damage. However, even with such extensive direct action during outbreaks, *Microtus* populations are seen to recover relatively quickly following rodenticide application, although no data are available for common voles. These findings, along with the exceptional reproductive potential of common voles, indicate that common voles are anticipated to overcome potential adverse effects of in-crop application of plant protection products at the landscape level.

Use of the common vole in risk assessment

Based on pest status, population dynamics, habitat preference, resilience and the reproductive potential of the common vole, its relevance to environmental risk assessments must be practically established to ensure that, at tier I of the risk assessment process, the risk to voles across multiple crops is realistically assessed. Risk assessment parameters for default generic focal species as defined in Appendix A of the EFSA (2009) bird and mammal guidance document do not always appear to concur with results of scientific observations in field and laboratory studies. For example, EFSA (2009) use of energy balance models indicates that a 25 g vole must consume 1.33 times its own body weight [the default food intake rate/body weight (FIR/bw)] to satisfy the theoretical daily energy expenditure (DEE). However, in laboratory studies, common voles have been found only to consume about a third of their body weight per day, and values as low as 10% based on the uptake of dry matter have been reported. As shown in laboratory studies, even at low temperatures, when food uptake is highest, an amount of food equivalent to about 50% of the body weight is eaten, although this was not verified under field conditions. Re-evaluating certain generic focal species food intake rates that are not in agreement with literature values is an area of future research that, coupled with additional research, could provide realistic food consumption data for use in risk assessment. The common vole is a model species that exists in cropped areas, and, given body weight and food intake rates, represents a worst-case exposure model. It therefore seems reasonable to consider an adjustment to Annex VI (trigger value) to account for the reduced uncertainty associated with the evaluation of derived TER values from acute and reproduction dietary risk assessments. This reduction could follow the model used in Germany, where lower TER trigger values (≥5 in the acute and ≥2 in the chronic risk assessment) are applied for common voles and wood mice. German regulators consider these species to be the worst case exposure models and not simply representatives of the worst-case exposure model. They also stress that mammalian toxicity endpoints are usually derived from studies with laboratory Norway rats (*Rattus norvegicus*) or house mice (*Mus musculus*) which have a close phylogenetic relationship to field rodent species, thereby reducing the interspecies uncertainty associated with extrapolating laboratory endpoints to wild mammals. Thus, adjusting the acute and chronic TER trigger points (as is the case in Germany) would be a realistic and pragmatic approach appropriate across all EU member states. The use of alternative focal species within the same feeding guild (*e.g.* field vole) is a pragmatic approach to risk assessment proposed for the Northern zone where common voles are not widely distributed. However, this position, although pragmatic, cannot be consistently applied across member states. More information is necessary for better assessment of resilience and recovery in common vole populations and for further development and

validation of modelling approaches that can be valuable in assisting decision-making in risk assessment. This information could be obtained from rodent control programmes and field monitoring data evaluating impacts on populations at the agroecosystem level. This information could be used to establish more accurate exposure estimates and to gain a better understanding of the differences in the dynamics of common vole populations when associated with different crop types.

III. Conclusion

Common voles are widely distributed in agroecosystems. The risk of side effects of plant protection products for common voles is limited to individuals present in crops during product application, while populations in off-crop primary habitat refuges remain unaffected. For many crops, the occurrence of common voles is restricted to population outbreaks and is associated with voles becoming significant agricultural pests. Their pest status, highly fluctuating population dynamics, habitat preferences, resilience and high reproductive potential should reduce potential pesticide impact upon common vole populations, but this is not fully reflected in the current risk assessment scheme.

Overall, based on the compelling evidence provided in this document, it is proposed that it would be justified to modify elements of the current risk assessment, for example by refining consumption estimates on the basis of expanded field-collected data on common voles, applying refined TER trigger values universally across all member states and/or advocating alternative focal species where this is considered to be a geographically appropriate. This will ensure that a more realistic and pragmatic approach to wild mammal risk assessment is taken in the assessment of plant protection products.

Assessment and conclusion by applicant:

This literature paper presents arguments for refining several of the assumptions used at Tier I of the EFSA Bird & Mammal risk assessment for the common vole.

The paper has been referred to as part of the refined risk assessment to justify the use of an alternative small mammal, the woodmouse, as a focal species.

Data Point:	KCP 10.1.2.2.14
Report Author:	[redacted]
Report Year:	2014
Report Title:	Population modeling - Use of scenarios to avoid different levels of protection
Report No:	M-489393-01-1
Document No:	M-489393-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Abstract

The calculation of TER values provides a simple method to obtain an idea of how likely it is to observe effects, given an estimated exposure and toxicity. While ecological and behavioural aspects can be considered in higher tier risk assessments, species specific reproduction or population ecology are not taken into account in TER. We exemplarily show that using the TER results in a different level of protection for different species. However, the use of conservative scenarios for population modelling,

developed here for the wood mouse and the common vole, provides a tool to apply the same level of protection in different species.

I. Introduction

TER only takes into account exposure and toxicity. Focussing on the protection goals defined in EFSA (2009) other factors additionally affect the risk on the population level, such as reproduction and population dynamics. This can be demonstrated when comparing recovery of modelled populations of wood mice and common voles in which litter sizes were reduced by 20% in May. While for wood mice a small reduction of population density is visible, no effect is visible in voles.

II. Landscape scenarios

Simulations were conducted in landscapes with varying size, in order to identify a minimum landscape size, which can sustain a "local populations" (a population in classical sense, *e.g.* MacArthur & Wilson, 1967; Wilson, 1971, or population genetic sense, Hardy, 1908, would be much larger). For wood mice landscapes of >25 ha size were needed, while for common voles 5 ha were sufficient.

To obtain conservative landscape scenarios for population modelling a GIS analysis was conducted calculating a landscape quality measure for all landscapes squares of 50 ha (wood mouse) and 5 ha (common vole) and ranking the resulting millions of landscapes for habitat quality (details in [redacted] and [redacted], 2013). Simulations were finally conducted with landscapes corresponding to the 10th, 20th, 50th, 80th and 90th percentiles and it was found that wood mice only consistently survived over 20 years in landscapes corresponding to the 10th percentile. This means that wood mice need a relatively large landscape with a considerable fraction of "good" habitat to survive on the long term, and voles can survive in almost any small landscapes, if there is at least a small fraction of useable habitat available.

Comparison of TER and population level risk

For comparison of TER calculations and population simulations, the following first tier risk assessment was considered as a basis:

Table CP 10.1.2.2/14-1 First tier risk assessment considered

Crop/stage[1]	Focal species	NOEL[2] [mg/kg bw]	DDD total [mg/kg bw]	TER_{LT}	Trigger value
Cereals, BBCH ≥ 40	Wood mouse (25% weeds, 50% weed seeds, 25% ground arthropods)	5	0.1563	32.0	5
Cereals, BBCH ≥ 40	Common vole (100% grass)	5	2.8734	1.7	5

[1]AR: 250 g a.s./ha [2]Effect: Reduced litter size

To calculate effects in population simulations it was assumed that the NOEL corresponds to the EC_{10} of a standard dose response curve. Simulations were conducted additionally with higher and lower doses for both species, which result in TER values between 0 and 10 in standard risk assessment.

TER results in a different level of protection for species

Simulations showed that doses which would result in the same TER value in a first tier risk assessment had different effects on the population level in each species. While in voles population level effects were visible only for TER values <1, in wood mice effects were visible for TER values <2-5. This demonstrates that TER results in a different level of protection for the common vole and the wood mouse.

III. Conclusion

The use of TER results in a different level of protection for different species. Conservative, species-specific landscape scenarios developed for population modelling provide a tool to reach the same level of protection in different species.

Assessment and conclusion by applicant:

M-489393-01-1 is a poster presentation summarising some population modelling work which suggests that the TER results in a different level of protection for different species with a focus here on the wood mouse and the common vole.

Residues studies

The following residues decline data are relevant for the residues of spiroxamine on cereal shoots (part of the vole diet used in the risk assessment of Spiroxamine EC 500).

Data Point:	KCP 10.1.22/15
Report Author:	[redacted]
Report Year:	2020
Report Title:	Spiroxamine: Kinetic assessment of residue decline study
Report No:	0471836-KIN1
Document No:	M-759383-01-1
Guideline(s) followed in study:	FOCUS (2014) and EFSA (2019)
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

The decline of spiroxamine residues in wheat and barley plants has been investigated in the field in five studies conducted at trial locations in northern and southern Europe. The objective of this project was to describe the calculation of kinetic endpoints from these studies according to the guidance of FOCUS (2014). Modelling DT_{50} values were calculated for use in deriving a crop dissipation half-life endpoint. Calculation of persistence endpoints was not considered necessary as there are no relevant study triggers for comparison.

The spiroxamine modelling DT_{50} values ranged from 1.14 to 6.93 days. The overall geometric mean was 3.03 days, with geomean for northern EU of 2.74 days and southern EU of 3.83 days.

I. Materials and Methods

Study Design

The decline of spiroxamine residues in wheat and barley plants has been investigated in the field in five studies conducted at trial locations in northern and southern Europe (M-301585-01-1, M-574326-01-1, M-578235-01-1, M-628347-02-1 and M-684671-01-1). The objective of this project was to describe the calculation of kinetic endpoints from these studies according to the guidance of FOCUS (2014). Modelling DT_{50} values were calculated for use in deriving a crop dissipation half-life endpoint.

Calculation of persistence endpoints was not considered necessary as there are no relevant study triggers for comparison.

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 (version 3.4) software package.

The FOCUS (2014) flowchart for calculating modelling endpoints has been followed. The residue decline behaviour of spiroxamine has been investigated in the field in twenty European trial sites. The results of this study have been used to determine the half-life in the crop canopy for spiroxamine under field conditions.

Modelling endpoints representing the decline rates of spiroxamine in wheat and barley plants have been calculated in accordance with the guidance of FOCUS (2014) and EFSA (2019), and are summarised in the tables below:

II. Results and Discussion

Table CP 10.1.2.2/15-1 Overall summary of modelling endpoints for spiroxamine

Study	Trial	DT_{50} (days)	χ^2 err %	Kinetic model
M-301585-01-1 [1]	UK R2007 0671/5 first application	3.02	1.09	SFO
	UK R2007 0671/5 second application	2.5	9.08	FOMC
	Geomean UK R2007 0671/5 value	2.75	-	-
	Sweden R2007 0698/7*	3.28	6.69	FOMC
	Southern France R 2007 0699/5 first application*	1.7	3.06	SFO
	Southern France R 2007 0699/5 second application*	1.76	3.73	FOMC
	Geomean Southern France R 2007 0699/5 value*	1.73	-	-
	Italy R 2007/2 first application	2.57	0.725	SFO
	Italy R 2007/2 second application	2.56	10.2	SFO
	Geomean Italy R 2007/2 value	2.56	-	-
M-574326-01-1	France 16-2958-01*	1.14	5.67	FOMC
	Germany 16-2958-02	2.91	6.53	SFO
	The Netherlands 16-2958-03*	3.34	10.4	FOMC
	Germany 16-2958-04*	6.3	2.03	DFOP
M-578235-01-1	France 16-2952-01*	3.3	3.47	FOMC

Study	Trial	DT_{50} (days)	$\chi 2$ err %	Kinetic model
	Spain 16-2952-02	4.93	4.27	DFOP
	Italy 16-2952-04	6.93	3.91	DFOP
	Portugal 16-2952-04*	6.34	6.8	HS
M-628347-02-1	Germany 17-2950-01*	6.23	4.57	DFOP
	Northern France 17-2950-02*	1.25	6.49	FOMC
	The Netherlands 17-2950-03	3.81	2.01	HS
	Belgium 17-2950-04	1.73	8.92	SFO
M-684671-01-1	Germany E19RP088-01	4.3	9.32	SFO
	Germany E19RP088-02	3	12.5	SFO
	Belgium E19RP088-03	2.38	7.04	SFO
	The Netherlands E19RP088-04	1.32	11.6	FOMC
Average (all data)		3.48		
Geometric mean (all data)		3.03		
Average (excluding trials with >1mm rainfall within 24 hours of application)		3.33		
Geometric mean (excluding trials with >1mm rainfall within 24 hours of application		3.01		

[1] For these trials, two applications were applied
*Trials with rainfall within 24 hours of application

Table CP 10.1.2.2/15-2 Overall summary of modelling endpoints for spiroxamine on Northern EU sites

Trial	DT_{50} (days)	$\chi 2$ err %	Kinetic model
UK R2007 0671/5 first application[1]	3.02	1.09	SFO
UK R2007 0671/5 second application[1)]	2.5	9.08	FOMC
Geomean UK R2007 0671/5 value	2.75	-	-
Sweden R2007 0698/7	3.28	6.69	FOMC
France 16-2958-01	1.14	5.67	FOMC
Germany 16-2958-02	2.91	6.53	SFO

Trial	DT_{50} (days)	χ2 err %	Kinetic model
The Netherlands 16-2958-03	3.34	10.4	FOMC
Germany 16-2958-04	6.3	2.03	DFOP
Germany 17-2950-01	6.23	4.57	DFOP
Northern France 17-2950-02	1.27	6.49	FOMC
The Netherlands 17-2950-03	3.81	2.01	HS
Belgium 17-2950-04	1.73	8.92	SFO
Germany E19RP088-01	4.3	9.32	SFO
Germany E19RP088-02	3	12.5	SFO
Belgium E19RP088-03	2.38	7.04	SFO
The Netherlands E19RP088-04	1.32	11.6	FOMC
Average	3.13		
Geomean	2.74		

[1] For these trials, two applications were applied

Table CP 10.1.2.2/15-3 Overall summary of modelling endpoints for spiroxamine on Southern EU sites

Trial	DT_{50} (days)	χ2 err %	Kinetic model
Southern France R 2007 0699/5 first application[1]	1.7	3.06	SFO
Southern France R 2007 0699/5 second application[1]	1.76	3.73	FOMC
Geomean Southern France R 2007 0699/5 value	1.73	-	-
Italy R 2007/2 first application[1]	2.57	0.725	FOMC
Italy R 2007/2 second application[1]	2.56	10.2	SFO
Geomean Italy R 2007/2 value	2.56	-	-
France 16-2952-01	3.3	3.47	FOMC
Spain 16-2952-02	4.93	4.27	DFOP
Italy 16-2952-04	6.93	3.91	DFOP
Portugal 16-2952-04	6.34	6.8	HS
Average	4.30		

Geomean	3.83	

[1] For these trials, two applications were applied

III. Conclusion

The residue decline behaviour of spiroxamine in wheat and barley plants has been investigated in the field in twenty European trial sites.

The spiroxamine modelling DT_{50} values ranged from 1.14 to 6.93 days. The overall geometric mean was 3.03 days, with geomean for northern EU of 2.74 days and southern EU of 3.83 days.

Assessment and conclusion by applicant:

This report has been generated in order to compile the decline data of spiroxamine on cereals from a total of 24 residues decline trials conducted as part of five studies. Residues of spiroxamine have been determined at frequent intervals following application of spiroxamine containing formulations to cereals. Studies M-301585-01-1, M-574326-01-1, M-578235-01-1, M-628347-02-1 and M-684671-01-1, covering wheat and barley plants in both Northern and Southern Europe, have been summarised and assessed separately below. The individual validity of each study has been discussed in the evaluation section after each study summary but all of the trials used in this assessment were considered to be valid and suitable for use in the derivation of an overall crop dissipation half-life (DT_{50}) value. It was found that spiroxamine residues dissipated relatively quickly on cereals with an overall geomean DT_{50} of 3.03 days determined. A geomean DT_{50} value of 2.74 days was determined for Northern EU and a DT_{50} of 3.83 days for Southern EU. These determined DT_{50} values have been used as part of the refined risk assessments to replace the default DT_{50} of 10 days with a more realistic experimentally determined value. It is noted that only the parts of the focal species diet that relates to cereals has used these refined values with the other parts of the diets still using the default value.

The report has followed the guidance set out in the FOCUS (2014) *Generic guidance for estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration, Version 1.1*. The analysis has also used up to date modelling software (CAKE). Thus, the results are considered to be valid and reliable and therefore suitable for use in risk assessment in situations where its use has been sufficiently justified.

Data Point:	KCP 10.1.2.2/02
Report Author:	[redacted]; [redacted]
Report Year:	2008
Report Title:	Determination of the residues of KWG 4168 in/on spring barley after spraying of KWG 4168 (500 EC) in the field in United Kingdom, Sweden, Southern France, and Italy
Report No:	RA-2648/07
Document No:	M-301585-01-1
Guideline(s) followed in study:	91/414/EEC of July 15, 1991, 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	No deviations (from study plan) occurred which had a negative influence on the study results
Previous evaluation:	yes, evaluated and accepted RAR (2010) Submitted and evaluated as part of the report M-301999-01-1
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of the presented study was to determine the magnitude of residues of KWG 4168 in/on spring barley green material sampled at various intervals after two spray applications with KWG 4168 (500 EC) at approx. 0.75 L/ha each, early in the growing season. The first application was made approx. at growth stage BBCH 25 (5 tillers visible), and the second approx. 14 days later. Two trials each were conducted in northern and southern Europe, with the individual trials located in the United Kingdom, Sweden, southern France, and Italy.

This study comprises four supervised field residue trials with spring barley in the United Kingdom, Sweden, Southern France, and Italy. A treated and an untreated plot were used for each trial.

The study was conducted according to the relevant guidelines. No residues were found in the control samples. The results for the recovery samples were in the range of 70 - 100%, in conformance with guidelines.

I. Materials

Test Material Spiroxamine EC 500E G

Lot/Batch #: PF90087683

Purity: 500 g/L (nominal), 501 g/L (analysed)

Description: Not stated

Stability of test compound: Not stated

Reanalysis/Expiry date: 31 January 2010

Density: Not stated

Treatments

Test rates: Two applications of 0.75 L/ha

Solvent/vehicle: Water was used as a carrier (200 L/ha)

Analysis of test concentrations:	Determination of spiroxamine was conducted using high performance liquid chromatography with mass spectrophotometric detection (HPLC-MS/MS).

Test design

Test area:	Four residue trials in the UK (sandy loam), Sweden, Southern France (clay silt) and Italy (silty sand). Each trial consisted of a treated and untreated plot. Plots ranged from 100 to 360 m^2
Sampling:	The sample material to be analysed was green material. Samples were collected on -14, -9, -4, -0, 0, 1, 2, 3, 5, 7, 10 and 14 after last treatment (DALT) from the UK and Italy study sites, on Day -14, -9, -4, -0, 0, 1, 2, 3, 8, 7, 10 and 14 DALT from the Sweden study site and -14, -9, -4, -0, 0, 2, 2, 3, 5, 7, 10 and 14 DALT from the Southern France site.
Duration of test:	14 days

Study design

The purpose of the presented study was to determine the magnitude of residues of KWG 4168 in/on spring barley green material sampled at various intervals after two spray applications with KWG 4168 (500 EC) at approx. 0.75 L/ha each, early in the growing season. The first application was made approx. at growth stage BBCH 25 (5 tillers visible), and the second approx. 14 days later. Two trials each were conducted in northern and southern Europe, with the individual trials located in the United Kingdom, Sweden, southern France, and Italy.

The test site for the field phase R 2007 0671/5 was Bayer CropScience Ltd., 230 Cambridge Science Park, Milton Road, Cambridge, CB4 0 WB. The test site for the field phase R 2007 0698/7 was Bayer Sverige AB S-20241 Malmö. The test site for the field phase R 2007 0699/5 was Bayer CropScience France 16 rue Jean Marie Leclerc F-69337 Lyon cedex 09, CP 310. The test site for the field part R 2007 0700/2 was Bayer CropScience Italy I-20156 Milano.

Samples were taken, prepared in the field where necessary, transported and stored according to EC guidance 7029/VI/95 rev. 5 (1997-07-22). The field sub-samples from all trials were stored deep-frozen within 24 hours after sampling and until dispatch to the Laboratory for Sampling, Preparation Technique and Sample Logistics (PVTL), Bayer CropScience AG in D-40789 Monheim am Rhein. All field sub-samples were shipped by deep-freeze lorry and arrived at PVTL in good condition. The field sub-samples were stored in a freezer at -18°C or below until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field sub-samples were shredded with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes separately for analysis (UP samples) and archiving (UR samples) and stored at -18°C or below until analysis.

The analytical method was developed for the determination of residues of BYF00587, Prothioconazole, and the metabolites BYF00587-desmethyl and TAU6476-desthio (SXX0665) in/on plant materials. 5 g of sample was extracted with a mixture of acetonitrile/water (4/1; v/v, containing cysteine hydrochloride) using a blender. After filtration of the extract, the stable isotopically labelled analytes were added. The solution was made up to volume, diluted and subjected to reversed phase HPLC-MS/MS without a further clean-up step. In modification M001 to method 01013, spiroxamine (KWG 4168) was extracted from barley (grain, green material, straw) as described above and detected in ESI positive mode. Residues were quantified using internal stable labelled standards. The LOQ for all compounds, defined as the lowest validated fortification level, was 0.01 mg/kg for all sample materials.

Analytical method

Samples of green material were analysed using the validated analytical method M-301585-01-1, report reference M-301585-01-1 (**see Doc MCP Section 5)**.

II. Results and Discussion

Temperature for Trial R 2007 0671/5 (UK) ranged between 9 – 14 °C and daily rainfall ranged between 0 – 26 mm. Temperature for Trial R 2007 0698/7 (Sweden) ranged between 10 – 21 °C and daily rainfall ranged between 0 – 4 mm. Temperature for Trial R 2007 0699/5 (France) ranged between 12 – 19 °C and daily rainfall ranged between 0 – 15 mm. Temperature for Trial R 2007 0700/2 (Italy) ranged between 15 – 23 °C and daily rainfall ranged between 0 – 6 mm.

The study was conducted according to the relevant guidelines. No residues were found in the control samples. The results for the recovery samples were in the range of 70-100%, in conformance with guidelines.

The analytical method 01013 was developed for the determination of residues of BYF00587, Prothioconazole, and the metabolites BYF00587-desmethyl and JAU6476-desthio (SXX0665) in/on plant materials. 5 g of sample was extracted with a mixture of acetonitrile/water (4/1; v/v containing cysteine hydrochloride) using a blender. After filtration of the extract, the stable isotopically labeled analytes were added. The solution was made up to volume, diluted and subjected to reversed phase HPLC-MS/MS without a further clean-up step.

In modification M001 to method 01013, spiroxamine (KWG 4168) was extracted from barley (grain, green material, straw) as described above and detected in ESI positive mode. Residues were quantified using internal stable labelled standards.

The LOQ for all compounds, defined as the lowest validated fortification level, was 0.01 mg/kg for all sample materials.

Table CP 10.1.2.2/02-1 Analytical results of control samples for KWG 4168, test system: spring barley

Trial No.	Growth stage (BBCH)	DALT	Sample material	KWG 4168 (mg/kg)
United Kingdom R 2007 0671/5	25	-14	Green material	<0.01
	31	-0	Green material	<0.01
	43	14	Green material	<0.01
Sweden R 2007 0698/7	24	-14	Green material	<0.01
	37	-0	Green material	<0.01
	61	14	Green material	<0.01
Southern France R 2007 0699/5	25	-14	Green material	<0.01
	31	-0	Green material	<0.01
	51	14	Green material	<0.01
Italy R 2007 0700/2	25	-14	Green material	<0.01
	32	-0	Green material	<0.01
	59	14	Green material	<0.01

DALT = Days after last application

Table CP 10.1.2.2/02-2 Analytical results of treated samples for KWG 4168, test system: spring barley

Trial No.	Growth stage (BBCH)	DALT	Sample material	KWG 4168 (mg/kg)
United Kingdom R 2007 0671/5	25	-14	Green material	18
	30	-9	Green material	5.6
	30	-4	Green material	1.9
	31	-0	Green material	0.85
	31	0	Green material	13
	31	1	Green material	4.3
	31	2	Green material	3.5
	32	3	Green material	2.7
	32	5	Green material	2.4
	32	7	Green material	1.4
	33	10	Green material	0.76
	43	14	Green material	0.34
Sweden R 2007 0698/7	24	-14	Green material	14
	24	-9	Green material	2.4
	37	-4	Green material	0.41
	37	0	Green material	14
	37	1	Green material	7.3
	37	2	Green material	6.0
	37	3	Green material	4.7
	39	5	Green material	2.9
	43	8	Green material	1.8
	49	10	Green material	1.3
	61	14	Green material	0.68
Southern France R 2007 0699/5	25	-14	Green material	12
	29	-9	Green material	1.5
	30	-4	Green material	0.43
	31	-0	Green material	0.16
	31	0	Green material	8.8
	31	2	Green material	2.2
	31	2	Green material	2.0
	31	3	Green material	1.4
	31	5	Green material	1.1
	31	7	Green material	0.85
	32	10	Green material	0.59

Trial No.	Growth stage (BBCH)	DALT	Sample material	KWG 4168 (mg/kg)
	51	15	Green material	0.25
Italy R 2007 0700/2	25	-14	Green material	19
	25	-9	Green material	5.0
	30	-4	Green material	1.2
	32	-0	Green material	0.39
	32	0	Green material	11
	32	1	Green material	6.9
	32	2	Green material	5.3
	33	3	Green material	4.7
	37	5	Green material	3.0
	39	7	Green material	1.9
	54	10	Green material	0.98
	59	14	Green material	0.68

DALT = days after last treatment

III. Conclusion

The study was conducted according to the relevant guidelines. No residues were found in the control samples. The results for the recovery samples were in the range of 70 - 100%, in conformance with guidelines.

Assessment and conclusion by applicant:

There is no formal test guideline for the conduct of residues decline trials but some guidance is provided in the EFSA Guidance document on Risk Assessment for Birds and Mammals (2009) as well as the more recent EFSA Technical Report on general recurring issues in ecotoxicology (EFSA supporting publication 2019:EN-1673). Up to date regulatory intelligence on the expectations of the test design of such trials has also been taken in to consideration.

The study comprised four trials over two countries in NEU (Sweden and the UK) and two countries in SEU (Sothern France and Italy). The crop used was spring barley which is highly relevant to the proposed representative GAP for cereals. The application rate was high enough in order to achieve sufficiently high residues of spiroxamine at the start of the trials, thereby allowing for a good decline curve to derive DT_{50} values from.

The analytical sampling regime adopted in the trials is considered to be suitable to derive reliable DT_{50} values, with sampling timepoints typically conducted before application and on Days 0, 1, 2, 3, 5, 7, 10 and 14. This regime is considered to meet the expectations for a robust residues decline trial, even with the relatively short expected DT_{50} for spiroxamine.

Weather data were adequately recorded from the nearest weather station.

Overall, the data are considered reliable and suitable for inclusion in the derivation of refined DT_{50} values in cereals for use in Bird & Mammal risk assessment.

Report M-759383-01-1 presents the results of the kinetic modeling for the spiroxamine data measured for these trials as well as the other available decline studies on cereals. An overall DT_{50} value for spiroxamine has been determined using all of these data. As part of the analysis, trials that measured

>1 mm rainfall in the first 24 hours were both included and excluded in order to assess the impact that this rainfall may have had on the overall DT_{50} value. It was concluded that there was very little variation in the mean DT_{50} value when these trials were either included or excluded. Thus, it is considered that any rainfall recorded in these trials has not adversely affected the results achieved therefore these trials are considered to be valid and can be included in the determination of the residue decline for spiroxamine.

Data Point:	KCP 10.1.2.2/16
Report Author:	[redacted]; [redacted]
Report Year:	2016
Report Title:	Determination of the residues of prothioconazole, tebuconazole and spiroxamine in/on winter wheat after spray application of PTZ & SPX & TBZ EC 425 in northern France, Germany and the Netherlands
Report No:	16-2958
Document No:	M-574326-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009), US EPA OCSPP 860.1500, Crop Field Trial
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

Residues of prothioconazole, spriroxamine and tebuconazole were determined in/on winter wheat (green material) after one spray application with PTZ & SPX & TBZ EC 425, an emulsifiable concentrate formulation containing 53 g/L prothioconazole, 224 g/L spiroxamine and 148 g/L tebuconazole. The study included four supervised residue trials conducted in the field in Northern Europe (France, the Netherlands and two sites in Germany) during the 2016 season.

Average recoveries were within the range of 70-110%. No residues above the LOQ were found in control samples except for tebuconazole with a value of 0.11 mg/kg.

I. Materials

Test Material PTZ & SPX & TBZ EC 425

Lot/Batch #: ECE2102270

Purity: 53 g/L Prothioconazole (nominal); 50.54 g/L (analysed)
224 g/L Spiroxamine (nominal); 221.3 g/L (analysed)
148 g/L Tebuconazole (nominal); 149.7 g/L (analysed)

Description: Not stated

Stability of test compound: Not stated

Reanalysis/Expiry date:	10 February 2017
Density:	Not stated

Treatments

Test rates:	Single application consisting of 0.053 kg a.s./ha prothioconazole, 0.224 kg a.s./ha spiroxamine and 0.148 kg a.s./ha tebuconazole with a test item rate of 1.0 L/ha
Solvent/vehicle:	Water was used as a carrier (250-400 L/ha)
Analysis of test concentrations:	Determination of each of the actives and their associated metabolites was conducted using high performance liquid chromatography with mass spectrophotometric detection (HPLC-MS/MS)

Test design

Test area:	Four residue trials in northern France (clayey silt), Germany (sandy loam) and the Netherlands (clay). Each trial consisted of a treated and untreated plot. Plots ranged from 108 to 125 m^2
Sampling:	The sample material to be analysed was green material. Samples were collected on Day 0, 1, 2, 3, 6, 7 and 10 after last treatment (DALT) from the French study site, on Day 0, 1, 2, 3, 5, 7 and 10 from both German sites, and Day 0, 1, 2, 3, 5, 7 and 10 from the Netherlands site
Duration of test:	10 days

Environmental test conditions

Temperature:	During application – 5.0 to 25.0°C
Relative humidity:	During application – 54.2 to 73 %
pH:	Soil pH in water - 6.6 in Germany, 8.4 in France Soil pH in $CaCl_2$ – 5.4 in Germany Soil pH in KCl – 7.5 in the Netherlands

Study Design

The objective of this study was to determine the magnitude of the residues of prothioconazole (comprising prothioconazole and its metabolite JAU 6476-desthio), spiroxamine and tebuconazole in/on winter wheat (BBCH 29-31, trial dependant) after one spray application with PTZ & SPX & TBZ EC 425. The study measured residues immediately following a single application (consisting of 0.053 kg a.s./ha prothioconazole, 0.224 kg a.s./ha spiroxamine and 0.148 kg a.s./ha tebuconazole with a test item rate of 1.0 L/ha), and up to 10 days later. The study included four supervised residue trials conducted in the field in Northern Europe (France, two sites in Germany and the Netherlands), with plots ranging from 108 to 125 m^2. At each trial site there was one untreated plot in addition to the treated plot(s). The treated and untreated plots were cultivated in the same manner

Sprayers were calibrated before each application and water was used as a carrier at a rate of between 250 and 400 L/ha, trial dependant.

The sample material to be analysed as green material. Analysis was conducted using HPLC-MS/MS.

Climatic and irrigation data were recorded (not according to GLP) during the conduct of the field trials.

Analytical method

Samples of wheat/green material were analysed using the validated analytical method 01089, report reference M-304677-01-1 (**see Doc MCP Section 5**).

II. Results and Discussion

The study was deemed to be acceptable based on the criteria set out in the US EPA OCSPP 860.1500, Crop Field Trial (1996) and OECD guideline 509 for The testing of Chemicals on Crop Field Trial (2009).

Mean temperatures ranged from 3 to 8°C in the French trial (16-2958-01), 6 to 13°C in the German trial (16-2958-02), 8 to 12°C in the Netherlands trial (16-2958-03) and 6 to 14°C in the other German trial (16-2958-04). Rainfall ranged from 0 to 10 mm in France, 0 to 4 mm in Germany, 0 to 4 mm in the Netherlands and 0 to 6 mm in the second German trial. No irrigation was applied in any of the four trials.

The application rate of prothioconazole, spiroxamine and tebuconazole for each trial was 0.053, 0.224 and 0.148 kg a.s./ha, respectively.

The residue levels determined in the treated samples are summarised in the tables below;

Table CP 10.1.2.2/16-1 Measured residues of Prothioconazole, JAU 6476-desthio, tebuconazole and spiroxamine in/on winter wheat

Country	BBCH growth stage	DALT	Residues (mg/kg)			
			prothioconazole		tebuconazole	spiroxamine
			Prothioconazole	JAU 6476-desthio		
France (16-2958-01)	29	0	0.70	2.5	16	16
	29	1	0.059	0.26	3.7	2.8
	29	2	0.043	0.19	3.3	2.2
	29	3	0.040	0.18	3.6	2.3
	30	6	0.024	0.11	2.9	1.3
	30	7	0.018	0.089	2.6	1.1
	30	10	0.012	0.063	2.1	0.66
Germany (16-2958-02)	29	0	0.44	1.4	10	8.8
	29	1	0.10	1.6	9.2	6.5
	29	2	0.049	1.2	8.2	5.4
	30	3	0.033	0.82	7.9	4.9
	30	5	0.019	0.25	3.8	2.1
	30	7	0.01	0.15	3.4	1.6
	30	10	<0.01	0.066	2.7	1.0
The Netherlands (16-2958-03)	29	0	0.48	1.4	9.9	9.4
	9	1	0.050	0.39	4.3	3.6
	30	2	0.055	0.31	4.5	3.3
	30	3	0.049	0.22	3.8	2.7
	30	5	0.026	0.12	3.3	1.5
	30	7	0.019	0.085	2.6	1.0

Country	BBCH growth stage	DALT	Residues (mg/kg)			
			prothioconazole		tebuconazole	spiroxamine
			Prothioconazole	JAU 6476-desthio		
	31	10	<0.01	0.043	1.6	0.53
Germany (16-2958-04)	29	0	0.47	1.0	7.4	7.5
	29	1	0.043	0.29	2.5	3.6
	30	2	0.025	0.18	2.3	2.9
	30	3	0.021	0.11	2.2	2.6
	30	5	0.014	0.061	1.8	2.0
	31	7	0.012	0.047	1.6	1.7
	31	10	0.01	0.027	1.2	1.1

DALT = Days after last application

Table CP 10.1.2.2/16-2 Summmary of measured residues in/on winter wheat after application of PTZ & SPX & TBZ EC 425

Analyte	BBCH growth stage	DALT	Residues (mg/kg)
prothioconazole	29	0	0.44-0.70
		1	0.043-0.10
	29-30	2	0.025-0.055
		3	0.021-0.049
	30	5	0.010-0.026
		6	0.024
	30-31	7	<0.01-0.019
		10	<0.01-0.012
JAU 6476-desthio	29	0	1.0-2.5
		1	0.26-1.6
	29-30	2	0.18-1.2
		3	0.11-0.82
	30	5	0.061-0.25
		6	0.11
	30-31	7	0.047-0.15
		10	0.027-0.066
spiroxamine	29	0	7.3-16
		1	2.8-6.5
	29-30	2	2.2-5.4
		3	2.3-4.9
	30	5	1.5-2.1

Analyte	BBCH growth stage	DALT	Residues (mg/kg)
		6	1.3
	30-31	7	1.0-1.7
		10	0.53-1.1
tebuconazole	29	0	7.4-16
		1	7.5-9.2
	29-30	2	2.3-8.2
		3	2.3-7.9
	30	5	1.8-3.8
		6	2.9
	30-31	7	1.6-3.4
		10	1.2-2.7

DALT = Days after last application

III. Conclusion

Average recoveries were within the range of 70-110%. No residues above the LOQ were found in control samples, except for tebuconazole with a value of 9.11 mg/kg.

Assessment and conclusion by applicant:

There is no formal test guideline for the conduct of residues decline trials but some guidance is provided in the EFSA Guidance document on Risk Assessment for Birds and Mammals (2009) as well as the more recent EFSA Technical Report on general recurring issues in ecotoxicology (EFSA supporting publication 2019:EN-1673). Up to date regulatory intelligence on the expectations of the test design of such trials has also been taken in to consideration.

The study comprised four trials over three countries in NEU (Germany, the Netherlands and Northern France). The crop used was winter wheat which is highly relevant to the proposed representative GAP for cereals. The application rate was high enough in order to achieve sufficiently high residues of spiroxamine at the start of the trials, thereby allowing for a good decline curve to derive DT_{50} values from.

The analytical sampling regime adopted in the trials is considered to be suitable to derive reliable DT_{50} values, with sampling timepoints typically conducted on Days 0, 1, 2, 3, 5, 7 and 10. This regime is considered to meet the expectations for a robust residues decline trial, even with the relatively short expected DT_{50} for spiroxamine.

Weather data were adequately recorded from the nearest weather station.

Overall, the data are considered reliable and suitable for inclusion in the derivation of refined DT_{50} values in cereals for use in Bird & Mammal risk assessment.

Report M-759383-01-1 presents the results of the kinetic modeling for the spiroxamine data measured for these trials as well as the other available decline studies on cereals. An overall DT_{50} value for spiroxamine has been determined using all of these data. As part of the analysis, trials that measured >1 mm rainfall in the first 24 hours were both included and excluded in order to assess the impact that this rainfall may have had on the overall DT_{50} value. It was concluded that there was very little variation in the mean DT_{50} value when these trials were either included or excluded. Thus, it is considered that any rainfall recorded in these trials has not adversely affected the results achieved

therefore these trials are considered to be valid and can be included in the determination of the residue decline for spiroxamine.

Data Point:	KCP 10.1.2.2/17
Report Author:	[redacted]
Report Year:	2017
Report Title:	Determination of the residues of prothioconazole, tebuconazole and spiroxamine in/on winter wheat after spray application of PTZ & SPX & TBZ EC 425 in southern France, Spain, Italy and Portugal
Report No:	16-2952
Document No:	M-578235-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500 Crop Field Trial
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

Residues of prothioconazole, spiroxamine and tebuconazole were determined in/on wheat (green material) after one spray application with PTZ & SPX & TBZ EC 425, an emulsifiable concentrate formulation containing 53 g/L prothioconazole, 224 g/L spiroxamine and 148 g/L tebuconazole. The study included four supervised residue trials conducted in the field in Southern Europe (France, Spain, Italy and Portugal) during the 2016 season.

Average recoveries were within the range of 70-110%. No residues above the LOQ were found in control samples.

I. Materials

Test Material PTZ & SPX & TBZ EC 425

Lot/Batch #: RCE2102770

Purity: 53 g/L Prothioconazole (nominal); 50.54 g/L (analysed)
224 g/L Spiroxamine (nominal); 221.3 g/L (analysed)
148 g/L Tebuconazole (nominal); 149.7 g/L (analysed)

Description: Not stated

Stability of test compound: Not stated

Reanalysis/Expiry date: 10 February 2017

Density: Not stated

Treatments

Test rates:	Single application consisting of 0.053 kg a.s./ha prothioconazole, 0.224 kg a.s./ha spiroxamine and 0.148 kg a.s./ha tebuconazole with a test item rate of 1.0 L/ha
Solvent/vehicle:	Water was used as a carrier (300-400 L/ha)
Analysis of test concentrations:	Determination of each of the actives and their associated metabolites was conducted using high performance liquid chromatography with mass spectrophotometric detection (HPLC-MS/MS).

Test design

Test area:	Four residue trials in Southern France (silty clay), Spain (clay), Italy (sandy loam) and Portugal (clay). Each trial consisted of a treated and untreated plot. Plots ranged from 45 to 87.5 m^2.
Sampling:	The sample material to be analysed was green material. Samples were collected on Day 0, 1, 2, 3, 5, 7 and 9 after last treatment (DALT) from the French study site, on Day 0, 1, 2, 4, 5, 7 and 10 from the Spanish site, Day 0, 1, 2, 3, 4, 8 and 10 from the Italian site and Day 0, 1, 2, 3, 5, 7 and 10 from the Portuguese site.
Duration of test:	10 days (9 for France)

Environmental test conditions

Temperature:	During application – 8.0 to 15.0°C
Relative humidity:	During application – 67.5 to 81 %
pH:	6.9 to 8.3 (soil pH in water)

Study Design

The objective of this study was to determine the magnitude of the residues of prothioconazole (comprising prothioconazole and its metabolite JAU 6476-desthio), spiroxamine and tebuconazole in/on winter wheat (BBCH 23-30, trial dependant) after one spray application with PTZ & SPX & TBZ EC 425. The study measured residues immediately following a single application (consisting of 0.053 kg a.s./ha prothioconazole, 0.224 kg a.s./ha spiroxamine and 0.148 kg a.s./ha tebuconazole with a test item rate of 1.0 L/ha) and up to 10 days later (with the exception of France where the last sampling was 9 DALT). The study included four supervised residue trials conducted in the field in Southern Europe (France, Spain, Italy and Portugal), with plots ranging from 45 to 87.5 m^2. Sprayers were calibrated before each application and water was used as a carrier at a rate of between 200 and 400 L/ha, trial dependant.

The sample material to be analysed as green material. Analysis was conducted using HPLC-MS/MS.

Climatic and irrigation data were recorded (not according to GLP) during the conduct of the field trials.

Analytical method

Samples of wheat green material were analysed using the validated analytical method 01089, report reference M-304677-01-1 (**see Doc MCP Section 5**).

II: Results and Discussion

The study was deemed to be acceptable based on the criteria set out in the US EPA OCSPP 860.1500, Crop Field Trial (1996) and OECD guideline 509 for The testing of Chemicals on Crop Field Trial (2009).

Mean temperatures ranged from 6 to 12°C in the French trial (16-2952-01), 6 to 13°C in the Spanish trial (16-2952-02), 9 to 15°C in the Italian trial (16-2952-03) and between 11 and 12°C in the Portuguese

trial (16-2952-04). Rainfall ranged from 0 to 15 mm in France, 0 mm in Spain, 0 mm in Italy and 0 to 15 mm in Portugal. No irrigation was applied in any of the four trials.

The application rate of prothioconazole, spiroxamine and tebuconazole for each trial was 0.053, 0.224 and 0.148 kg a.s./ha, respectively.

The residue levels determined in the treated samples are summarised in the tables below;

Table CP 10.1.2.2/17-1 Measured residues of Prothioconazole, JAU 6476-desthio, tebuconazole and spiroxamine in/on winter wheat

Country	BBCH growth stage	DALT	Residues (mg/kg)			
			prothioconazole		tebuconazole	spiroxamine
			prothioconazole	JAU 6476-desthio		
France (16-2952-01)	29	0	0.46	2.3	13	16
	29	1	0.10	0.78	8.6	7.1
	29	2	0.065	0.36	5.6	4.9
	29	3	0.050	0.27	5.5	4.3
	29	5	0.030	0.13	3.8	2.9
	30	7	0.018	0.066	3.1	2.1
	30	9	0.014	0.031	1.7	1.5
Spain (16-2952-02)	23	0	0.44	1.6	9.6	12
	23	1	0.24	1.2	11	6.1
	25	2	0.17	1.1	11	4.9
	25	4	0.046	0.54	5.1	3.4
	25	5	0.035	0.41	4.3	2.5
	29	7	0.032	0.42	4.7	2.4
	30	10	0.020	0.28	3.9	1.6
Italy (16-2952-03)	30	0	0.37	0.97	7.5	7.7
	30	1	0.11	0.43	4.5	4.3
	31	2	0.078	0.46	4.6	4.3
	31	3	0.042	0.39	3.6	3.8
	31	4	0.027	0.31	3.7	3.4
	32	8	0.011	0.11	3.4	2.4
	32	10	<0.01	0.046	2.3	1.6
Portugal (16-2952-04)	29	0	0.47	1.5	7.9	9.8
	29	1	0.039	0.35	3.8	4.6
	29	2	0.037	0.34	3.5	4.4
	29	3	0.035	0.33	3.0	4.6
	29	5	0.015	0.084	2.0	2.8
	29	7	0.011	0.057	2.0	2.4

Country	BBCH growth stage	DALT	Residues (mg/kg)			
			prothioconazole		tebuconazole	spiroxamine
			prothioconazole	JAU 6476-desthio		
	30	10	<0.01	0.033	1.6	1.9

DALT = Days after last application

Table CP 10.1.2.2/17-2 Summmary of measured residues in/on winter wheat after application of PTZ & SPX & TBZ EC 425

Analyte	BBCH growth stage	DALT	Residues (mg/kg)
prothioconazole	23-30	0	0.37-0.47
		1	0.039-0.24
	25-31	2	0.037-0.17
	25-31	3-4	0.035-0.050
	25-31	4-5	0.015-0.035
	29-32	7-8	0.0041-0.032
	30-32	9-10	<0.01-0.020
JAU 6476-desthio	23-30	0	0.97-2.3
		1	0.35-1.2
	25-31	2	0.34-1.1
	25-31	3-4	0.27-0.54
	25-31	4-5	0.084-0.41
	29-32	7-8	0.057-0.42
	30-32	9-10	0.031-0.28
tebuconazole	23-30	0	7.5-13
		1	3.8-11
	25-31	2	3.5-11
	25-31	3-4	3.0-5.5
	25-31	4-5	2.0-4.3
	29-32	7-8	2.0-4.7
	30-32	9-10	1.6-3.9
spiroxamine	23-30	0	7.7-16
		1	4.3-7.1
	25-31	2	4.3-4.9
	25-31	3-4	3.4-4.6
	25-31	4-5	2.5-3.4
	29-32	7-8	2.1-2.4
	30-32	9-10	1.5-1.9

DALT = Days after last application

III. Conclusion

Average recoveries were within the range of 70-110%. No residues above the LOQ were found in control samples.

Assessment and conclusion by applicant:

There is no formal test guideline for the conduct of residues decline trials but some guidance is provided in the EFSA Guidance document on Risk Assessment for Birds and Mammals (2009) as well as the more recent EFSA Technical Report on general recurring issues in ecotoxicology (EFSA supporting publication 2019:EN-1673). Up to date regulatory intelligence on the expectations of the test design of such trials has also been taken in to consideration.

The study comprised four trials over four countries in SEU (France, Spain, Italy and Portugal). The crop used was winter wheat which is highly relevant to the proposed representative GAP for cereals. The application rate was high enough in order to achieve sufficiently high residues of spiroxamine at the start of the trials, thereby allowing for a good decline curve to derive DT_{50} values from.

The analytical sampling regime adopted in the trials is considered to be suitable to derive reliable DT_{50} values, with sampling timepoints typically conducted on Days 0, 1, 2, 3, 5, 7 and 10. This regime is considered to meet the expectations for a robust residues decline trial, even with the relatively short expected DT_{50} for spiroxamine.

Weather data were adequately recorded from the nearest weather station.

Overall, the data are considered reliable and suitable for inclusion in the derivation of refined DT_{50} values in cereals for use in Bird & Mammal risk assessment.

Report M-759383-01-1 presents the results of the kinetic modeling for the spiroxamine data measured for these trials as well as the other available decline studies on cereals. An overall DT_{50} value for spiroxamine has been determined using all of these data. As part of the analysis, trials that measured >1 mm rainfall in the first 24 hours were both included and excluded in order to assess the impact that this rainfall may have had on the overall DT_{50} value. It was concluded that there was very little variation in the mean DT_{50} value when these trials were either included or excluded. Thus, it is considered that any rainfall recorded in these trials has not adversely affected the results achieved therefore these trials are considered to be valid and can be included in the determination of the residue decline for spiroxamine. It is acknowledged that there was no rainfall recorded during the trials in Spain and Italy.

Data Point:	KCP 10.1.2.2/18
Report Author:	[redacted]; [redacted]
Report Year:	2018
Report Title:	Determination of the residues of prothioconazole, spiroxamine and trifloxystrobin in/on wheat after spray application of PTZ & SPX & TFS EC 280.3 in Germany, northern France, the Netherlands and Belgium
Report No:	17-2950
Document No:	M-628347-02-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
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

Residues of prothioconazole, spiroxamine and trifloxystrobin were determined in/on wheat (green material) after one spray application with PTZ & SPX & TFS EC 280.3, an emulsifiable concentrate formulation containing 93.3 g/L prothioconazole, 107 g/L spiroxamine and 80 g/L trifloxystrobin. The study included four supervised residue trials conducted in the field in Northern Europe (Germany, Northern France, the Netherlands and Belgium).

Residues of prothioconazole comprised of prothioconazole and its metabolite JAU 6476-desthio. Residues of spiroxamine comprised of the spiroxamine enantiomers A1, A2, B1 and B2, the total residue of the parent spiroxamine as the sum of the four enantiomers. Residues of trifloxystrobin comprising trifloxystrobin and its isomers/metabolites CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466.

Average recoveries were within the range of 70-110%. No residues above the LOQ were found in control samples.

I. Materials

Test Material PTZ & SPX & TFS EC 280.3

Lot/Batch #: 201-001477

Purity: 93.3 g/L Prothioconazole (nominal); 92.40 g/L (analysed)
107 g/L Spiroxamine (nominal); 107.8 g/L (analysed)
80 g/L Trifloxystrobin (nominal); 79.55 g/L (analysed)

Description: Not stated

Stability of test compound: Not stated

Reanalysis/Expiry date: 21 March 2017

Density: Not stated

Treatments

Test rates:	Single application consisting of 0.140 kg a.s./ha prothioconazole, 0.161 kg a.s./ha spiroxamine and 0.120 kg a.s./ha trifloxystrobin with a test item rate of 1.5 L/ha
Solvent/vehicle:	Water was used as a carrier (100-350 L/ha)
Analysis of test concentrations:	Determination of each of the actives and their associated metabolites was conducted using high performance liquid chromatography with mass spectrophotometric detection (HPLC-MS/MS).

Test design

Test area:	Four residue trials in Germany (sandy loam), Northern France (clayey silt), the Netherlands (clay) and Belgium (silt loam). Each trial consisted of a treated and untreated plot. Plots ranged from 100 to 156 m^2
Sampling:	The sample material to be analysed was green material. Samples were collected on Day -0, 0, 1, 2, 3, 5, 7 and 10 after last treatment (DALT) from each of the four study sites.
Duration of test:	10 days (11 for Belgium)

Environmental test conditions

Temperature:	During application – 12.0 to 19.0°C
Relative humidity:	During application – 40 to 73%
pH:	6.7 to 8.1 (soil pH)

Study Design

The objective of this study was to determine the magnitude of the residues of prothioconazole (comprising prothioconazole and its metabolite JAU 6476-desthio), the residues of spiroxamine (comprising the spiroxamine enantiomers A1, A2, B1 and B2, the total residue of parent spiroxamine as the sum of the four enantiomers) and trifloxystrobin (comprising trifloxystrobin and its isomers/metabolites CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on wheat (BBCH 30) after one spray application with PTZ & SPX & TFS EC 280.3. The study measured residues immediately following a single application (consisting of 0.140 kg a.s./ha prothioconazole, 0.161 kg a.s./ha spiroxamine and 0.120 kg a.s./ha trifloxystrobin 400 g a.s./ha with a test item rate of 1.5 L/ha) and up to 10 days later (with the exception of Belgium where the last sampling was 11 DALT). The study included four supervised residue trials conducted in the field in Northern Europe (Germany, Northern France, the Netherlands and Belgium), with plots ranging from 100 to 156 m^2. Sprayers were calibrated before each application and water was used as a carrier at a rate of between 100 and 350 L/ha, trial dependant.

The sample material to be analysed was green material. Analysis was conducted using HPLC-MS/MS. For the control sample taken at -0 DALT, the total green material sample amount of 25 plants was weighed and recorded in order to obtain an approximate single plant weight. The weight of 25 plants in Germany, Northern France, The Netherlands and Belgium was 53 g, 63.5 g, 165 g and 792 g, respectively.

Climatic and irrigation data were recorded (without GLP) during the conduct of the field trials.

Analytical method

Samples of wheat/green material were analysed using the validated analytical method 01480, report reference M-628347-02-1 (**see Doc MCP Section 5**).

II. Results and Discussion

The study was deemed to be acceptable based on the criteria set out in the US EPA OCSPP 860.1500, Crop Field Trial (1996) and OECD guideline 509 for The testing of Chemicals on Crop Field Trial (2009).

Mean temperatures ranged from 8 to 16°C in the German trial (17-2950-01), 6 to 15°C in the Northern France trial (17-2950-02), 14 to 19°C in the Netherland trial (17-2950-03) and between 12 and 23°C in the Belgium trial (17-2950-04). Rainfall ranged from 0 to 3 mm in the German trial, 0 to 12 mm in Northern France, 0 to 21 mm in the Netherlands and 0 to 7 mm in Belgium. No irrigation was applied in any of the four trials.

The application rate of prothioconazole, spiroxamine and trifloxystrobin for each trial was 0.140, 0.161 and 0.120 kg a.s./ha, respectively.

The residue levels determined in the treated samples are summarised in the tables below;

Table CP 10.1.2.2/18-1 Measured residues of prothioconazole and JAU 6476-desthio in on wheat

Country	BBCH growth stage	DALT	Residues (mg/kg) a.s. prothioconazole	
			Prothioconazole	JAU 6476-desthio
Germany (17-2950-01)	30	0	2.3	4.2
	30	1	0.26	3.4
	30	2	0.11	2.2
	30	3	0.10	2.0
	30	5	0.085	1.2
	30	7	0.067	0.75
	30	10	0.077	0.37
Northern France (17-2950-02)	30	0	2.3	4.3
	30	1	0.13	0.62
	30	2	0.10	0.58
	30	3	0.042	0.28
	30	5	0.043	0.20
	31	7	0.028	0.084
	31	10	0.020	0.036
The Netherlands (17-2950-03)	30	0	1.6	2.9
	30	1	0.15	2.8
	31	2	0.062	2.4
	31	3	0.046	2.1
	32	5	0.024	1.4
	32	7	0.015	0.73
	39	10	0.011	0.31
Belgium	30	0	3.1	7.4

Country	BBCH growth stage	DALT	Residues (mg/kg)	
			a.s. prothioconazole	
			Prothioconazole	JAU 6476-desthio
(17-2950-04)	30	1	0.63	5.4
	30	2	0.12	4.8
	31	3	0.023	2.6
	31	5	0.015	1.6
	32	7	0.011	0.89
	37	11	<0.01	0.24

DALT = Days after last application

Table CP 10.1.2.2/18-2 Measured residues of spiroxamine and its enantiomers in/on wheat

Country	BBCH growth stage	DALT	Residues (mg/kg)				
			a.s. spiroxamine				
			KWG 4168-A1 enantiomer	KWG 4168-A2 enantiomer	KWG 4168-B1 enantiomer	KWG 4168-B2 enantiomer	Total residue of 4 enantiomers
Germany (17-2950-01)	30	0	1.7	1.7	1.4	1.3	6.1
	30	1	0.94	0.92	0.75	0.70	3.3
	30	2	0.57	0.53	0.45	0.44	2.0
	30	3	0.53	0.53	0.42	0.40	1.9
	30	5	0.43	0.42	0.32	0.33	1.5
	30	7	0.28	0.28	0.23	0.22	1.0
	30	10	0.22	0.23	0.17	0.17	0.78
Northern France (17-2950-02)	30	0	1.9	1.8	1.5	1.4	6.5
	30	1	0.31	0.30	0.26	0.25	1.1
	30	2	0.30	0.29	0.25	0.26	1.1
	30	3	0.20	0.20	0.19	0.18	0.77
	30	5	0.18	0.19	0.17	0.17	0.71
	31	7	0.12	0.12	0.11	0.11	0.45
	31	10	0.073	0.076	0.067	0.064	0.28
The Netherlands (17-2950-03)	30	0	1.0	1.0	0.82	0.79	3.6
	30	1	0.54	0.52	0.41	0.40	1.9
	31	2	0.47	0.45	0.36	0.36	1.6
	31	3	0.40	0.40	0.31	0.31	1.4
	32	5	0.25	0.24	0.19	0.20	0.88
	32	7	0.18	0.18	0.15	0.14	0.65
	39	10	0.10	0.10	0.086	0.082	0.37

Country	BBCH growth stage	DALT	Residues (mg/kg)				
			a.s. spiroxamine				
			KWG 4168-A1 enantiomer	KWG 4168-A2 enantiomer	KWG 4168-B1 enantiomer	KWG 4168-B2 enantiomer	Total residue of 4 enantiomers
Belgium (17-2950-04)	30	0	1.7	1.7	1.3	1.3	6.0
	30	1	0.98	0.97	0.80	0.77	3.5
	30	2	0.77	0.75	0.61	0.60	2.7
	31	3	0.44	0.42	0.34	0.35	1.6
	31	5	0.25	0.25	0.20	0.21	0.91
	32	7	0.20	0.20	0.18	0.17	0.75
	37	11	0.083	0.082	0.070	0.070	0.30

DALT = Days after last application

Table CP 10.1.2.2/18-3 Measured residues of trifloxystrobin and metabolites/isomers in on wheat

Country	BBCH growth stage	DALT	Residues (mg/kg)					
			a.s. trifloxystrobin					
			trifloxystrobin	CGA 331409	CGA 357262	CGA 357261	CGA 321113	CGA 373466
Germany (17-2950-01)	30	0	8.3	0.077	<0.01	0.17	0.11	<0.01
	30	1	5.5	0.17	0.027	0.38	0.35	0.014
	30	2	2.1	0.20	0.10	0.53	0.16	0.015
	30	3	1.6	0.23	0.17	0.57	0.13	0.031
	30	5	1.2	0.25	0.23	0.61	0.058	0.014
	30	7	0.56	0.17	0.19	0.34	0.032	<0.01
	30	10	0.39	0.11	0.13	0.22	0.022	<0.01
Northern France (17-2950-02)	30	0	8.2	0.035	<0.01	0.063	0.46	<0.01
	30	1	1.6	0.089	0.015	0.11	0.14	<0.01
	30	2	1.5	0.13	0.041	0.22	0.12	0.010
	30	3	0.36	0.054	0.019	0.060	0.057	<0.01
	30	5	0.32	0.060	0.031	0.092	0.023	<0.01
	31	7	0.15	0.042	0.035	0.068	<0.01	<0.01
	31	10	0.075	0.023	0.022	0.033	<0.01	<0.01
The Netherlands (17-2950-03)	30	0	5.6	0.055	<0.01	0.10	0.092	<0.01
	30	1	2.8	0.17	0.056	0.38	0.17	0.021
	30	2	3.0	0.26	0.17	0.65	0.16	0.029
	31	3	1.9	0.23	0.15	0.49	0.095	0.027
	32	5	0.94	0.18	0.15	0.28	0.043	0.013
	32	7	0.64	0.12	0.11	0.15	0.015	<0.01

Country	BBCH growth stage	DALT	Residues (mg/kg)					
			a.s. trifloxystrobin					
			trifloxystrobin	CGA 331409	CGA 357262	CGA 357261	CGA 321113	CGA 373466
	39	10	0.076	0.049	0.047	0.013	<0.01	<0.01
Belgium (17-2950-04)	30	0	10	0.31	0.023	0.58	0.34	0.026
	30	1	6.0	0.51	0.14	0.95	0.23	0.041
	30	2	2.9	0.57	0.31	1.0	0.30	0.096
	31	3	0.32	0.25	0.17	0.12	0.073	0.015
	31	5	0.091	0.15	0.11	0.035	0.026	<0.01
	32	7	0.047	0.080	0.069	0.027	0.010	<0.01
	37	11	<0.01	0.018	0.022	<0.01	<0.01	<0.01

DALT = Days after last application

III. Conclusion

Residues of prothioconozole comprised of prothioconazole and its metabolite JAU 6476-desthio. Residues of sprioxamine comprised of the spiroxamine enantiomers A1, A2, B1 and B2, the total residue of the parent spiroxamine as the sum of the four enantiomers. Residues of trifloxystrobin comprising trifloxystrobin and its isomers/metabolites CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466.

Average recoveries were within the range of 70-110%. No residues above the LOQ were found in control samples.

Assessment and conclusion by applicant:

There is no formal test guideline for the conduct of residues decline trials but some guidance is provided in the EFSA Guidance document on Risk Assessment for Birds and Mammals (2009) as well as the more recent EFSA Technical Report on general recurring issues in ecotoxicology (EFSA supporting publication 2019:EN-1673). Up to date regulatory intelligence on the expectations of the test design of such trials has also been taken into consideration.

The study comprised four trials over four countries in NEU (Germany, Northern France, the Netherlands and Belgium). The crop used was wheat which is highly relevant to the proposed representative GAP for cereals. The application rate was high enough in order to achieve sufficiently high residues of spiroxamine at the start of the trials, thereby allowing for a good decline curve to derive DT_{50} values from.

The analytical sampling regime adopted in the trials is considered to be suitable to derive reliable DT_{50} values, with sampling timepoints typically conducted on Days 0, 1, 2, 3, 5, 7 and 10. This regime is considered to meet the expectations for a robust residues decline trial, even with the relatively short expected DT_{50} for spiroxamine.

Weather data were adequately recorded from the nearest weather station.

Overall, the data are considered reliable and suitable for inclusion in the derivation of refined DT_{50} values in cereals for use in Bird & Mammal risk assessment.

Report M-759383-01-1 presents the results of the kinetic modeling for the spiroxamine data measured for these trials as well as the other available decline studies on cereals. An overall DT_{50} value for spiroxamine has been determined using all of these data. As part of the analysis, trials that measured >1 mm rainfall in the first 24 hours were both included and excluded in order to assess the impact

that this rainfall may have had on the overall DT_{50} value. It was concluded that there was very little variation in the mean DT_{50} value when these trials were either included or excluded. Thus, it is considered that any rainfall recorded in these trials has not adversely affected the results achieved therefore these trials are considered to be valid and can be included in the determination of the residue decline for spiroxamine.

Data Point:	KCP 10.1.2.2/19
Report Author:	[redacted]; [redacted]
Report Year:	2020
Report Title:	Determination of the residues of trifloxystrobin, prothioconazole and spiroxamine in/on wheat after spray application of PTZ & SPX & TFS EC 280.3 in the field in Germany, Belgium and the Netherlands - Final report
Report No:	E19RP088
Document No:	M-684671-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
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 purpose of the study E19RP088 was to determine the magnitude of the residues of prothioconazole (comprising prothioconazole and its degradation product JAU 6476-desthio), spiroxamine (comprising KWG 4168-A1 enantiomer, KWG 4168-A2 enantiomer, KWG 4168-B1 enantiomer, KWG 4168-B2 enantiomer, the total residue of spiroxamine parent as the sum of the four enantiomers, and the total residue of spiroxamine (*via* 4-tbutylcyclohexanone), the residues of trifloxystrobin (comprising trifloxystrobin, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on wheat (green material) after one spray application with PTZ & SPX & TFS EC 280.3, an emulsifiable concentrate (EC) formulation containing 93.3 g/L prothioconazole, 107 g/L spiroxamine and 80 g/L trifloxystrobin.

I. Materials

Test Material PTZ & SPX & TFS EC 280.3

Lot/Batch #: 201-001477

Purity: 93.3 g/L Prothioconazole (nominal); 90.42 g/L (analysed)
107 g/L Spiroxamine (nominal); 107.4 g/L (analysed)
80 g/L Trifloxystrobin (nominal); 73.83 g/L (analysed)

Description: Not stated

Stability of test compound:	Not stated
Reanalysis/Expiry date:	22 March 2022
Density:	Not stated

Treatments

Test rates:	E19RP088-01, Germany: Single application consisting of 0.142 kg a.s./ha prothioconazole, 0.163 kg a.s./ha spiroxamine and 0.122 kg a.s./ha trifloxystrobin with a test item rate of 1.52 L/ha E19RP088-02, Germany: Single application consisting of 0.150 kg a.s./ha prothioconazole, 0.172 kg a.s./ha spiroxamine and 0.129 kg a.s./ha trifloxystrobin with a test item rate of 1.61 L/ha E19RP088-03, Belgium: Single application consisting of 0.140 kg a.s./ha prothioconazole, 0.161 kg a.s./ha spiroxamine and 0.120 kg a.s./ha trifloxystrobin with a test item rate of 1.61 L/ha E19RP088-04, Netherlands: Single application consisting of 0.139 kg a.s./ha prothioconazole, 0.160 kg a.s./ha spiroxamine and 0.120 kg a.s./ha trifloxystrobin with a test item rate of 1.49 L/ha
Solvent/vehicle:	Water was used as a carrier (E19RP088-01, Germany: 306 L/ha, E19RP088-02, Germany: 322 L/ha, E19RP088-03, Belgium: 301 L/ha, E19RP088-04, Netherlands: 299 L/ha
Analysis of test concentrations:	Determination of each of the actives and their associated metabolites was conducted using high performance liquid chromatography with mass spectrophotometric detection (HPLC-MS/MS).

Environmental test conditions

Temperature:	E19RP088-01 = 7.0 to 14.0°C, E19RP088-02 = 12 to 20°C, E19RP088-03 = 9.0 to 12.0°C and E19RP088-04 = 8.0 to 17.0°C
Relative humidity:	Not stated
pH:	Not stated

Study design

The purpose of the study E19RP088 was to determine the magnitude of the residues of prothioconazole (comprising prothioconazole and its degradation product JAU 6476-desthio), spiroxamine (comprising KWG 4168-A1 enantiomer, KWG 4168-A2 enantiomer, KWG 4168-B1 enantiomer, KWG 4168-B2 enantiomer, the total residue of spiroxamine parent as the sum of the four enantiomers, and the total residue of spiroxamine (*via* 4-tbutylcyclohexanone)), the residues of trifloxystrobin (comprising trifloxystrobin, CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466) in/on wheat (green material) after one spray application with PTZ & SPX & TFS EC 280.3, an emulsifiable concentrate (EC) formulation containing 93.3 g/L prothioconazole, 107 g/L spiroxamine and 80 g/L trifloxystrobin.

Test site for the field phase E19RP088-01, E19RP088-02 was Bayer Crop Science BCSD, Elisabeth-Selbert-Strasse 4a, 40764 Langenfeld, Germany. The test site for the field phase E19RP088-03 was Bayer Crop Science SA-NV, J.E. Mommaertslaan 14, 1831 Diegem (Machelen), Belgium. The test site for the field phase E19RP088-04 was Bayer Crop Science SA-NV Netherlands, Energieweg 1, 3641 RT Mijdrecht, Netherlands.

The study measured residues immediately following a single application at the four trial locations (consisting of 0.140 kg a.s./ha prothioconazole, 0.161 kg a.s./ha spiroxamine and 0.120 kg a.s./ha trifloxystrobin).

The sample material to be analyzed was green material. The analysed substances were prothioconazole, JAU 6476-desthio, total residue of spiroxamine (*via* 4-t-butylcyclohexanone), KWG 4168-A1 enantiomer, KWG 4168-A2 enantiomer, KWG 4168-B1 enantiomer, KWG 4168-B2 enantiomer, total residue of 4 spiroxamine enantiomers, trifloxystrobin, CGA 321113, CGA 331409, CGA 357262, CGA 357261 and CGA 373466.

The application rates of the active substance(s) were calculated based on the nominal contents. No additional adjuvants, surfactants or mixing partners were used for the application.

Analytical method

Samples of wheat/green material were analysed using the validated analytical method 01480; report reference M-628347-02-1 (**see Doc MCP Section 5**).

II. Results and Discussion

Mean temperatures ranged from 7 to 14°C in the German trial (E19RP088-01), mean temperatures ranged from 12 to 20°C in the German trial (E19RP088-02), mean temperatures ranged from 9 to 12°C in the Belgium trial (E19RP088-03) and mean temperatures ranged from 8 to 17°C in the Netherlands trial (E19RP088-04).

Rainfall ranged from 0 to 8 mm in the German trial (E19RP088-01), 0 to 1 mm in the German trial (E19RP088-02), 0 to 6 mm in the Belgium trial (E19RP088-03) and 0 to 10 mm in the Netherlands trial (E19RP088-04).

The application rate of prothioconazole, spiroxamine and trifloxystrobin for each trial was 0.140, 0.161 and 0.120 kg a.s./ha, respectively.

Average recoveries were within the range of 70-110%. No residues above the LOQ were found in control samples with only two exceptions.

The residue levels determined in the treated samples are summarised in the tables below;

Table CP 10.1.2.2/19-1 Measured residues of prothioconazole and JAU 6476-desthio in/on wheat

Trial No. Country	BBCH growth stage	DALT	Residues (mg/kg)	
			Prothioconazole	JAU 6476-desthio
E19RP088-01 Germany	26	0	1.1	2.8
	26	1	0.16	2.9
	26	2	0.049	2.1
	30	3	0.037	1.9
	30	5	0.022	1.3
	30	7	0.014	0.84
	31	9	<0.01	0.42
E19RP088-02 Germany	22	0	2.0	3.9
	22	1	0.12	3.8
	30	2	0.039	3.0
	30	3	0.041	2.7

Trial No. Country	BBCH growth stage	DALT	Residues (mg/kg)	
			Prothioconazole	JAU 6476-desthio
	30	5	0.019	1.8
	31	7	0.019	1.0
	32	10	<0.01	0.41
E19RP088-03 Belgium	23	0	2.3	5.4
	23	1	0.30	3.0
	23	2	0.10	2.3
	23	3	0.042	1.6
	30	6	0.024	0.62
	30	7	0.019	0.47
	30	10	<0.01	0.16
E19RP088-04 The Netherlands	29	0	3.1	2.1
	29	1	0.33	2.9
	30	2	0.048	1.8
	30	3	0.037	1.3
	31	5	0.024	1.1
	32	7	0.018	0.81
	32	10	0.014	0.52

DALT = Days after last application

Table CP 10.1.2.2/19-2 Measured residues of KWG 4168-A1 enantiomer, KWG 4168-A2 enantiomer, KWG 4168-B1 enantiomer, KWG 4168-B2 enantiomer and total residue of 4 spiroxamine enantiomer in/on wheat

Trial No. Country	BBCH growth stage	DALT	Residues (mg/kg)				
			KWG 4168-A1 enantiomer	KWG 4168-A2 enantiomer	KWG 4168-B1 enantiomer	KWG 4168-B2 enantiomer	total residue of 4 spiroxamine enantiomer
E19RP088-01 Germany	26	0	1.1	1.1	0.92	0.94	4.0
	26	1	0.71	0.71	0.61	0.62	2.6
	26	2	0.75	0.76	0.64	0.66	2.8
	30	3	0.59	0.60	0.51	0.53	2.2
	30	5	0.44	0.45	0.38	0.37	1.7
	30	7	0.37	0.37	0.32	0.31	1.4
	31	9	0.21	0.21	0.17	0.17	0.75
E19RP088-02	22	0	1.5	1.4	1.2	1.2	5.3
	22	1	0.91	0.91	0.74	0.75	3.3

Trial No. Country	BBCH growth stage	DALT	Residues (mg/kg)				
			KWG 4168-A1 enantiomer	KWG 4168-A2 enantiomer	KWG 4168-B1 enantiomer	KWG 4168-B2 enantiomer	total residue of 4 spiroxamine enantiomer
Germany	30	2	0.67	0.67	0.55	0.57	2.5
	30	3	0.63	0.63	0.51	0.53	2.3
	30	5	0.45	0.45	0.38	0.40	1.7
	31	7	0.34	0.34	0.29	0.29	1.2
	32	10	0.24	0.24	0.20	0.20	0.88
E19RP088-03 Belgium	23	0	1.8	1.8	1.5	1.5	6.6
	23	1	1.1	1.1	0.98	1.0	4.2
	23	2	0.92	0.90	0.78	0.80	3.4
	23	3	0.75	0.75	0.65	0.63	2.8
	30	6	0.31	0.31	0.28	0.27	1.2
	30	7	0.27	0.27	0.22	0.23	0.98
	30	10	0.10	0.10	0.085	0.088	0.38
E19RP088-04 The Netherlands	29	0	1.7	1.7	1.4	1.4	6.1
	29	1	0.94	0.91	0.77	0.78	3.4
	30	2	0.37	0.36	0.30	0.30	1.3
	30	3	0.24	0.25	0.19	0.20	0.88
	31	5	0.18	0.18	0.14	0.14	0.65
	32	7	0.13	0.13	0.09	0.099	0.46
	32	10	0.10	0.096	0.073	0.074	0.34

DALT = Days after last application

Table CP 10.1.2.2/19-3 Measured residues of spiroxamine 1 (*via* 4-t-butylyclohexanone) in/on wheat

Trial No. Country	BBCH growth stage	DALT	Total residue of spiroxamine 1 (via 4-t-butylyclohexanone (mg/kg)
E19RP088-01 Germany	26	0	7.1
	26	1	4.7
	26	2	5.4
	30	3	4.3
	30	5	3.2
	30	7	2.4
	31	9	1.4
E19RP088-02	22	0	8.5

Trial No. Country	BBCH growth stage	DALT	Total residue of spiroxamine 1 (via 4-t-butylyclohexanone (mg/kg)
Germany	22	1	6.5
	30	2	4.5
	30	3	5.1
	30	5	3.0
	31	7	2.3
	32	10	1.7
E19RP088-03 Belgium	23	0	15
	23	1	6.7
	23	2	4.9
	23	3	3.8
	30	6	1.7
	30	7	1.5
	30	10	0.60
E19RP088-04 The Netherlands	29	0	8.3
	29	1	6.3
	30	2	2.4
	30	3	2.0
	31	5	1.4
	32	7	1.0
	32	10	0.83

DALT = Days after last application

Table CP 10.1.2.2/19-4 Measured residues of trifloxystrobin, CGA 321113, CGA 331409, CGA 357262, CGA 357261, CGA 373466 in/on wheat

Trial No. Country	BBCH growth stage	DALT	Residues (mg/kg)					
			Trifloxystrobin	CGA 321113	CGA 331409	CGA 357262	CGA 357261	CGA 373466
E19RP088-01 Germany	26	0	5.2	0.42	0.056	<0.01	0.12	0.013
	26	1	2.9	0.40	0.25	0.14	0.71	0.10
	26	2	1.9	0.18	0.30	0.31	0.98	0.11
	30	3	1.1	0.099	0.26	0.31	0.77	0.076
	30	5	0.55	0.057	0.18	0.23	0.45	0.050
	30	7	0.26	0.046	0.11	0.17	0.24	0.039
	31	9	0.067	0.013	0.049	0.064	0.047	<0.01
	22	0	6.8	0.30	0.12	0.016	0.22	0.016

Trial No. Country	BBCH growth stage	DALT	Residues (mg/kg)					
			Trifloxy-strobin	CGA 321113	CGA 331409	CGA 357262	CGA 357261	CGA 373466
E19RP088-02 Germany	22	1	4.3	0.41	0.39	0.16	0.79	0.072
	30	2	2.9	0.28	0.47	0.27	0.93	0.068
	30	3	2.3*	0.18*	0.44*	0.28*	0.85*	0.054*
	30	5	0.96	0.11	0.33	0.22	0.34	0.029
	31	7	0.51	0.023	0.20	0.18	0.29	0.012
	32	10	0.25	0.016	0.15	0.13	0.15	<0.01
E19RP088-03 Belgium	23	0	9.1*	0.45*	0.73*	0.28*	1.8*	0.10*
	23	1	3.5	0.32	0.48	0.19	0.59	0.076
	23	2	1.1	0.077	0.29	0.13	0.28	0.013
	23	3	0.48*	0.037*	0.20*	0.12*	0.20*	<0.01*
	30	6	0.10	<0.01	0.069	0.055	0.060	<0.01
	30	7	0.080	<0.01	0.053	0.047	0.053	<0.01
	30	10	0.034	<0.01	0.022	0.021	0.023	<0.01
E19RP088-04 The Netherlands	29	0	10	0.16	0.12	0.065	0.25	<0.01
	29	1	6.8	0.23	0.36	0.18	0.88	0.048
	30	2	2.8	0.13	0.31	0.16	0.34	0.017
	30	3	2.0	0.068	0.25	0.17	0.30	0.011
	31	5	1.2	0.036	0.21	0.16	0.24	<0.01
	32	7	0.95	0.025	0.16	0.14	0.18	<0.01
	32	10	0.94	0.022	0.12	0.11	0.16	<0.01

DALT = Days after last application

*mean value, sample was extracted and analysed multiple times

No deviations occurred during the conduct of this study which had any negative impact on the quality of this study.

III. Conclusion

Residues of prothioconozole comprised of prothioconazole and its metabolite JAU 6476-desthio. Residues of sprioxamine comprised of the spiroxamine enantiomers A1, A2, B1 and B2, the total residue of spiroxamine parent as the sum of the four enantiomers, and the total residue of spiroxamine (*via* 4-tbutylcyclohexanone. Residues of trifloxystrobin comprising trifloxystrobin and its isomers/metabolites CGA 331409, CGA 357262, CGA 357261, CGA 321113 and CGA 373466.

Average recoveries were within the range of 70-110%. No residues above the LOQ were found in control samples with only two exceptions.

Assessment and conclusion by applicant:

There is no formal test guideline for the conduct of residues decline trials but some guidance is provided in the EFSA Guidance document on Risk Assessment for Birds and Mammals (2009) as well as the more recent EFSA Technical Report on general recurring issues in ecotoxicology (EFSA

supporting publication 2019:EN-1673). Up to date regulatory intelligence on the expectations of the test design of such trials has also been taken in to consideration.

The study comprised four trials over three countries in NEU (Germany, the Netherlands and Belgium). The crop used was wheat which is highly relevant to the proposed representative GAP for cereals. The application rate was high enough in order to achieve sufficiently high residues of spiroxamine at the start of the trials, thereby allowing for a good decline curve to derive DT_{50} values from.

The analytical sampling regime adopted in the trials is considered to be suitable to derive reliable DT_{50} values, with sampling timepoints typically conducted on Days 0, 1, 2, 3, 5, 7 and 10. This regime is considered to meet the expectations for a robust residues decline trial, even with the relatively short expected DT_{50} for spiroxamine.

Weather data were adequately recorded from the nearest weather station. Rainfall was measured at the trial sites themselves in order to provide accurate precipitation measurements.

Overall, the data are considered reliable and suitable for inclusion in the derivation of refined DT_{50} values in cereals for use in Bird & Mammal risk assessment.

Report M-759383-01-1 presents the results of the kinetic modeling for the spiroxamine data measured for these trials as well as the other available decline studies on cereals. An overall DT_{50} value for spiroxamine has been determined using all of these data. As part of the analysis, trials that measured >1 mm rainfall in the first 24 hours were both included and excluded in order to assess the impact that this rainfall may have had on the overall DT_{50} value. It was concluded that there was very little variation in the mean DT_{50} value when these trials were either included or excluded. Thus, it is considered that any rainfall recorded in these trials has not adversely affected the results achieved therefore these trials are considered to be valid and can be included in the determination of the residue decline for spiroxamine.

For procedural reasons studies listed in the Table CP 10.1.2.2-1 below are included in the current dossier as available data or information previously submitted but not necessarily evaluated. However, these reports have been fully superseded by newer studies. Consequently, no summaries of the reports have been included in the dossier.

Table CP 10.1.2.2-1: Studies previously submitted and not relied upon for the risk assessment

Data Point	Document No.	Date	Title
KCP 10.1.2.2/03	M-236289-01-1	2000	Voles - Small graminivores with polyphasic patterns Microtine rodents, a special case of diel activity patterns
KCP 10.1.2.2/04	M-236295-01-1	1978	Short-term rhythms in foraging behaviour of the common vole, Microtus arvalis
KCP 10.1.2.2/05	M-075573-01-1	2003	Kleinsaeugercoenosen suedwestdeutscher Weinberge
KCP 10.1.2.2/06	M-065143-01-1	2001	AGROMAM - Database 2001 - Microtus agrestis, Microtus arvalis, Apodemus sylvaticus
KCP 10.1.2.2/07	M-108725-01-1	2001	AGROMAM - Database 2001 - Sorex araneus, Sorex coronatus

CP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

No data are available with Spiroxamine EC 500 for terrestrial vertebrates other than mammals and birds. No additional studies on other terrestrial vertebrates are required in accordance with Commission Regulation (EU) No 283/2013 or 284/2013 and there are currently no risk assessment schemes for reptiles or amphibians.

In the supporting publication by EFSA (2017)[3] to review the biological relevance of the magnitude of effects observed in studies with amphibians and reptiles, it is noted that fish-generated toxicity data seem to be appropriate to cover aquatic amphibians. For terrestrial organisms typically birds and mammals are shown to be more sensitive than amphibians and reptiles to a higher number of substances. Currently data do not allow for extrapolating between groups, however the frequency of cases in which amphibians or reptiles are more sensitive than birds or mammals is around 30%. It can therefore be reasonably assumed therefore that the risk assessment for fish, birds and mammals is likely to be protective of the risk to amphibians and reptiles.

CP 10.2 Effects on aquatic organisms

Toxicity data for spiroxamine, Spiroxamine EC 500 and the metabolites of spiroxamine are summarized in the table below. The data include studies previously reviewed and included in the DAR and EFSA conclusion for spiroxamine as well as any previously un-submitted or new studies which have been conducted.

Table CP 10.2-1 Summary of endpoints for toxicity of spiroxamine, Spiroxamine EC 500 and metabolites to aquatic organisms

Organism	Test item	Test type	Endpoints		Reference
Fish					
Oncorhynchus mykiss (Rainbow trout)	Spiroxamine	Acute toxicity 96 h (static)	96-hour LC_{50} 18,500 µg a.s./L (mm)	EU	M-006243-01-1
Lepomis macrochirus (Bluegill sunfish)	Spiroxamine	Acute toxicity 96 h (static)	96-hour LC_{50} 7,130 µg a.s./L (mm)	EU	M-006229-01-1
Danio rerio (Zebra fish)	Spiroxamine	Acute toxicity 96 h (static)	**96-hour LC_{50} 2,410 µg a.s./L (mm)**	EU	M-303809-02-1
Oncorhynchus mykiss (Rainbow trout)	Spiroxamine EC 500	Acute toxicity 96 h (static)	**96-hour LC_{50} 11,500 µg/L (mm)** (5,700 µg a.s./L)	EU	M-006610-01-1
Oncorhynchus mykiss (Rainbow trout)	Spiroxamine	Chronic toxicity (ELS) 93 d (flow through)	NOEC <62.5 µg a.s./L (nom) (EC_0) 14 µg a.s./L (nom)	EU	M-006232-01-1
		Statistical Re-analysis	EC_{10} >62.5 µg a.s./L (nom)	NEW	M-760407-01-1

[3] EFSA supporting publication 2017; EN-1251. Biological relevance of the magnitude of effects (considering mortality, sub-lethal and reproductive effects) observed in studies with amphibians and reptiles in view of population level impacts on amphibians and reptiles.

Organism	Test item	Test type	Endpoints		Reference
Oncorhynchus mykiss (Rainbow trout)	Spiroxamine	Chronic toxicity (ELS; radiolabelled) 96 d (flow through)	NOEC 14.2 µg a.s./L (mm)	EU	M-006449-01-1
		Statistical Re-analysis	EC_{10} 91.5 µg a.s./L (mm) EC_{20} 195 µg a.s./L (mm)	NEW	M-760405-01-1
Oncorhynchus mykiss (Rainbow trout)	Spiroxamine	Chronic toxicity (ELS; sediment system; pulsed exposure) 56 d	NOEC 3 x 60 µg a.s./L (mm)	EU	M-304369-01-1
Danio rerio (Zebra fish)	Spiroxamine	Chronic toxicity (FFLC) 230 d (flow through)	NOEC 2.6 µg a.s./L (nom)	EU	M-304458-02-1
		Statistical Re-analysis	**EC_{10} 1.88 µg a.s./L (nom)** EC_{20} 4.46 µg a.s./L (nom)	NEW	M-760413-01-1
Danio rerio (Zebra fish)	Spiroxamine	Chronic toxicity (FFLC sediment system; pulsed exposure) 56 d	EC_{10} (survival) 23.3 µg a.s./L (im) NOEC (biomarker VTG) **15.8 µg a.s./L (im)**	EU	M-467979-03-1
		Statistical Re-analysis	EC_{10} not determinable	NEW	M-760412-01-1
Pimephales promelas (Fathead minnow)	Spiroxamine	Fish screening assay	Growth and fertility not affected at up to and including 58.8 µg a.s./L (mm) No effects on endocrine specific biomarker endpoints at up to and including 18.9 µg a.s./L (mm)	EU	M-304833-01-1
Lepomis macrochirus (Bluegill sunfish)	Spiroxamine	BCF	**$BCF_{(whole\ fish)}$ 87** CT_{50} 13 - 19 hours	EU	M-006479-01-1
Aquatic invertebrates					
Daphnia magna	Spiroxamine	Acute toxicity 48 h (static)	48-hour EC_{50} 6,100 µg a.s./L (im)	EU	M-006245-01-1

Organism	Test item	Test type	Endpoints		Reference
Daphnia magna	Spiroxamine	Acute toxicity 48 h (radiolabelled; static)	48-hour EC_{50} 6,800 µg a.s./L (mm)	EU	M-006476-01-1
Daphnia magna	Spiroxamine	Acute toxicity 48 h (radiolabelled; flow-through)	**48-hour EC_{50} 3,000 µg a.s./L (mm)**	EU	M-006523-01-1
Daphnia magna	KWG 4168-N-oxide (M03)	Acute toxicity 48 h (static)	**48-hour EC_{50} >100,000 µg/L (nom)**	EU	M-006702-01-1
Daphnia magna	Spiroxamine EC 500	Acute toxicity 48 h (static)	**48-hour EC_{50} 10,300 µg/L (nom)** (5,070 µg a.s./L)	EU	M-006630-01-1
Daphnia magna	Spiroxamine	Chronic toxicity 21 d (static-renewal)	NOEC 100 µg a.s./L (nom)	EU	M-006401-01-1
		Statistical Re-analysis	EC_{10} 120 µg a.s./L (nom) EC_{20} 200 µg a.s./L (nom)	NEW	M-761546-01-1
Daphnia magna	Spiroxamine	Chronic toxicity 21 d (radiolabeled; flow-through)	**NOEC 34 µg a.s./L (mm)**	EU	M-006555-01-1
		Statistical Re-analysis	EC_{10} 32 µg a.s./L (mm)* EC_{20} 68 µg a.s./L (mm)*	NEW	M-760409-01-1
Daphnia magna	Spiroxamine	Chronic toxicity 21 d (radiolabeled; static-renewal)	NOEC 47 µg a.s./L (mm)	EU	M-006466-01-1
		Statistical Re-analysis	EC_{10} 39 µg a.s./L (mm) EC_{20} 65 µg a.s./L (mm)	NEW	M-761544-01-1
				EU	M-304557-01-1

Organism	Test item	Test type	Endpoints		Reference
Aquatic algae and invertebrates	Spiroxamine EC 500	Outdoor mesocosm	Class 1 effects: 1.0 µg a.s./L Class 2 effects: 2.1 µg a.s./L Class 3A effects: 9.3 µg a.s./L **ETO-RAC 0.5 µg a.s./L** ERO-RAC 3.1 µg a.s./L	NEW	M-690576-01-1
Sediment-dwelling organisms					
Chironomus riparius	Spiroxamine	Chronic toxicity 28 d (static, radiolabelled)	EC_{10} (development time) ~ 5,600 µg a.s./L (nom) **NOEC (emergence) 5,600 µg a.s./L (nom)**	EU	M-006549-01-1
		Statistical Re-analysis	EC_{10}/EC_{20} not determinable	NEW	M-760403-01-1
Chironomus riparius	Spiroxamine EC 500	Chronic toxicity 28 d (static)	NOEC ≥5.0 µg product/L (≥2.5 µg a.s./L)	EU	M-006626-01-1
		Statistical Re-analysis	EC_{10}/EC_{20} not determinable	NEW	M-760408-01-1
Lumbriculus variegatus	Spiroxamine	Chronic toxicity 28 d (static)	**EC_{10} 7,120 µg a.s./kg sediment (mm)** NOEC 16,700 µg a.s./kg sediment (mm)	NEW	M-688127-01-1
Amphibia					

Organism	Test item	Test type	Endpoints		Reference
Xenopus laevis	Spiroxamine	XETA	No indication of endocrine activity on the thyroid axis concluded. A statistically significant increase in fluorescence was observed at the 1.6 mg/L treatment but this concentration was above the MTC	NEW	M-762327-01-1
Algae					
Scenedesmus subspicatus	Spiroxamine	72 h (static)	**E_rC_{50} 12 µg a.s./L (mm)** E_bC_{50} 5.2 µg a.s./L (mm)	EU	M-006228-01-1
		Statistical Re-analysis	E_rC_{10} 1.56 µg a.s./L (mm) E_rC_{20} 3.51 µg a.s./L (mm) E_rC_{50} 11.9 µg a.s./L (mm) E_yC_{10} 0.84 µg a.s./L (mm) E_yC_{20} 1.44 µg a.s./L (mm) E_yC_{50} 3.28 µg a.s./L (mm)	NEW	M-761401-01-1
Pseudokirchneriella subcapitata	Spiroxamine	120 h (static)	E_rC_{50} 19.43 µg a.s./L (nom) E_bC_{50} 5.42 µg a.s./L (nom)	EU	M-006518-01-1
		Statistical Re-analysis	E_rC_{10} 9.20 µg a.s./L (nom) E_rC_{20} 10.9 µg a.s./L (nom) E_rC_{50} 15.2 µg a.s./L (nom) E_yC_{10} 3.60 µg a.s./L (nom) E_yC_{20} 4.73 µg a.s./L (nom) E_yC_{50} 7.99 µg a.s./L (nom)	NEW	M-761402-01-1
Pseudokirchneriella subcapitata	Spiroxamine	96 h (static)	E_rC_{50} >8.14 µg a.s./L (im) E_bC_{50} 5.5 µg a.s./L (im) EC_{50} (cell density) 5.7 µg a.s./L (im)	EU	M-006533-01-1

Organism	Test item	Test type	Endpoints		Reference
		Statistical Re-analysis	E_rC_{10} 4.93 µg a.s./L (im) E_rC_{20} 10.5 µg a.s./L (im) E_rC_{50} >8.14 µg a.s./L (im) E_yC_{10} 1.29 µg a.s./L (im) E_yC_{20} 2.78 µg a.s./L (im) E_yC_{50} 5.90 µg a.s./L (im)	NEW	M-761427-01-1
Desmodesmus subspicatus	Spiroxamine	72 h (static)	E_rC_{10} 0.53 µg a.s./L (nom) E_rC_{20} 11.4 µg a.s./L (nom) E_rC_{50} 175 µg a.s./L (nom)	EU	M-273962-01-1
		Statistical Re-analysis	E_yC_{10} not determinable E_yC_{20} not determinable E_yC_{50} 10.5 µg a.s./L (nom)	NEW	M-761457-01-1
Skeletonema costatum	Spiroxamine	96 h (static)	**E_rC_{50} 6.3 µg a.s./L (im)**	EU	M-006512-01-1
		Statistical Re-analysis	E_rC_{10} not determinable E_rC_{20} not determinable E_rC_{50} 6.33 µg a.s./L (im) E_yC_{10} not determinable E_yC_{20} not determinable E_yC_{50} 1.29 µg a.s./L (im)	NEW	M-761414-01-1
Anabaena flos-aquae	Spiroxamine	96 h (static)	EC_{50} (cell density) >990 µg a.s./L (mm)	EU	M-006537-01-1
Navicula pelliculosa	Spiroxamine	96 h (static)	E_rC_{50} 11.85 µg a.s./L (mm)	EU	M-006542-01-1
		Statistical Re-analysis	E_rC_{10} 8.36 µg a.s./L (mm) E_rC_{20} 9.44 µg a.s./L (mm) E_rC_{50} 11.9 µg a.s./L (mm)	EU	M-280532-01-1

Organism	Test item	Test type	Endpoints		Reference
		Statistical Re-analysis	E_yC_{10} 6.83 µg a.s./L (mm) E_yC_{20} 7.60 µg a.s./L (mm) E_yC_{50} 9.32 µg a.s./L (mm)	NEW	M-761438-01-1
Desmodesmus subspicatus	KWG 4168-desethyl (M01)	72 h (static)	E_rC_{10} not determinable E_rC_{20} 42.9 µg/L (nom) **E_rC_{50} 737 µg/L (nom)**	EU	M-288232-01-1
		Statistical Re-analysis	E_yC_{10} not determinable E_yC_{20} not determinable E_yC_{50} 30.6 µg/L (nom)	NEW	M-761465-01-1
Pseudokirchneriella subcapitata	KWG 4168-despropyl (M02)	72 h (static)	E_rC_{10} 20.3 µg/L (im) E_rC_{20} 55.7 µg/L (im) **E_rC_{50} 383 µg/L (im)** E_yC_{10} n.d. E_yC_{20} 14.8 µg/L (im) E_yC_{50} 42.5 µg/L (im)	NEW	M-680695-01-1
Desmodesmus subspicatus	KWG 4168-N-oxide (M03)	72 h (static)	E_rC_{10} 658 µg/L (nom) E_rC_{20} 2,500 µg/L (nom) **E_rC_{50} 31,700 µg/L (nom)**	EU	M-288235-01-1
		Statistical Re-analysis	E_yC_{10} 218 µg/L (nom) E_yC_{20} 526 µg/L (nom) E_yC_{50} 2835 µg/L (nom)	NEW	M-761467-01-1
Desmodesmus subspicatus	KWG 4168-acid (M06)	72 h (static)	E_rC_{10} >3,200 µg/L (nom) E_rC_{20} >3,200 µg/L (nom) **E_rC_{50} >3,200 µg/L (nom)**	EU	M-309818-01-1
		Statistical Re-analysis	Not determinable. E_yC_{50} considered to be >3,200 µg/L (nom)	NEW	M-761469-01-1

Organism	Test item	Test type	Endpoints		Reference
Scenedesmus subspicatus	Spiroxamine EC 500	72 h (static)	**E_rC_{50} 29 µg/L (nom)** (14.3 µg a.s./L)	EU	M-006617-01-1
		Statistical Re-analysis	E_rC_{10} 4.90 µg a.s./L (nom) E_rC_{20} 7.09 µg a.s./L (nom) E_rC_{50} 14.4 µg a.s./L (nom) E_yC_{10} 3.20 µg a.s./L (nom) E_yC_{20} 4.02 µg a.s./L (nom) E_yC_{50} 8.22 µg a.s./L (nom)	NEW	M-761431-01-1
Aquatic plants					
Lemna gibba	Spiroxamine	14 d (static)	**14-day EC_{50} (frond counts) 1,910 µg a.s./L (mm)** 14-day E_rC_{50} 2,650 µg a.s./L (mm)	EU	M-006497-01-1
		Statistical Re-analysis	frond number 7-day E_rC_{10} 2,060 µg a.s./L (mm) 7-day E_rC_{20} 3,110 µg a.s./L (mm) 7-day E_rC_{50} 6,780 µg a.s./L (mm)	EU	M-303421-01-1

Organism	Test item	Test type	Endpoints		Reference
			frond number 14-day E_rC_{10} 1,260 µg a.s./L (mm) 14-day E_rC_{20} 1,820 µg a.s./L (mm) 14-day E_rC_{50} 3,170 µg a.s./L (mm)		
		Statistical Re-analysis	7-day E_yC_{10} 220 µg a.s./L (mm) 7-day E_yC_{20} 620 µg a.s./L (mm) 7-day E_yC_{50} 3,020 µg a.s./L (mm) 14-day E_yC_{10} 560 µg a.s./L (mm) 14-day E_yC_{20} 930 µg a.s./L (mm) 14-day E_yC_{50} 1,990 µg a.s./L (mm)	NEW	M-760417-01-1
		14 d (static)	14-day EC_{50} (frond number) 2,760 µg a.s./L (mm) 14-day EC_{50} (biomass) 9,380 µg a.s./L (mm)	EU	M-006540-01-1
Lemna gibba	Spiroxamine	Statistical Re-analysis	frond number 7-day E_rC_{10} 3,510 µg a.s./L (mm) 7-day E_rC_{20} 4,130 µg a.s./L (mm) 7-day E_rC_{50} 5,600 µg a.s./L (mm) dry weight 14-day E_rC_{10} 4,760 µg a.s./L (mm) 14-day E_rC_{20} 7,960 µg a.s./L (mm) 14-day E_rC_{50} 21,200 µg a.s./L (mm)	EU	M-303443-01-1

Organism	Test item	Test type	Endpoints		Reference
		Statistical Re-analysis	frond number 14-day E_rC_{10} 2,530 µg a.s./L (mm) 14-day E_rC_{20} 2,790 µg a.s./L (mm) 14-day E_rC_{50} 3,670 µg a.s./L (mm) 7-day E_yC_{10} 2,340 µg a.s./L (mm) 7-day E_yC_{20} 2,860 µg a.s./L (mm) 7-day E_yC_{50} 4,230 µg a.s./L (mm) 14-day E_rC_{10} 1,290 µg a.s./L (mm) 14-day E_rC_{20} 1,770 µg a.s./L (mm) 14-day E_yC_{50} 2,860 µg a.s./L (mm)	NEW	M-760416-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
NEW: new study or data generated since the previous EU review or previously not submitted
Values in **bold** have been used in the risk assessment
mm = Results based on mean measured test concentrations
nom = Results based on nominal test concentrations
im = Results based on initial measured test concentrations
* EC_{10} considered unreliable therefore not used in risk assessment

Toxicity endpoints

For the long-term studies where EC_{10} and EC_{20} values were not already available, these values have been calculated. For each relevant study, a summary of the statistical re-evaluation work immediately follows the summary of the main study. In cases where a valid EC_{10} could be determined, the risk assessment has used the lower value out of the NOEC and the EC_{10}. Furthermore, for the algal and *Lemna* studies where yield had not been determined in the study report, the E_yC_{10}, E_yC_{20} and E_yC_{50} values have been determined, where possible. However, it is noted that the risk assessment has used the growth rate E_rC_{50} values, in accordance with the recommendations of the Aquatic Guidance Document[4].

Acute fish data are available using spiroxamine technical for three fish species. The most sensitive species was *Danio rerio* with a 96-hour LC_{50} of 2,410 µg a.s./L. Thus, this endpoint has been used in the acute risk assessment for fish. For chronic fish toxicity there are three fish early life stage studies as well as one standard fish full life cycle study using spiroxamine technical. The lowest endpoint comes

[4] Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp.

from the fish full life cycle study (M-304458-02-1) which gave a NOEC of 2.6 µg a.s./L. In the previous Renewal of Approval for spiroxamine an EC_{10} of 2 µg a.s./L was derived for this study and used in the assessment. EC_x re-evaluation has therefore been conducted for this study and an EC_{10} of 1.88 µg a.s./L has been derived. Thus, the EC_{10} of 1.88 µg a.s./L has been used at Tier I in the chronic fish risk assessment. It is noted that this EC_{10} is below the lowest concentration tested in the study and could therefore be considered unreliable, however, as a precedent for using an EC_{10} for this study has been set in the previous evaluation of spiroxamine the EC_{10} has also been used here. A refined fish full life cycle study using pulsed-exposure and conducted in the presence of sediment (M-467979-03-1) is also available and has been considered as part of a refined risk assessment. This refinement study has been discussed later on in the section.

Three acute *Daphnia* studies are available for spiroxamine technical from which the lowest EC_{50} of 3,000 µg a.s./L was derived. This endpoint has therefore been used in the acute aquatic invertebrate risk assessment. For the chronic aquatic invertebrate risk assessment there are also three *Daphnia* reproduction studies available, the lowest reliable NOEC/EC_{10} being 34 µg a.s./L. A NOEC of 34 µg a.s./L has therefore been used in the chronic aquatic invertebrate risk assessment.

For sediment-dwelling organisms two studies using *Chironomus riparius* are available. No effects were seen at any concentration tested in the formulation study (*i.e.* NOEC ≥2.5 µg a.s./L) therefore the higher NOEC from the technical material study of 5,600 µg a.s./L has been used in the risk assessment for sediment-dwelling organisms. Both studies showed a lack of significant effects therefore it is considered justified to take the highest of the two available NOEC values.

Spiroxamine is a fungicide and therefore, according to the Aquatic Guidance Document, the recommended test species for sediment-dwelling organisms is *Lumbriculus*. A *Lumbriculus* study is available using spiroxamine technical and provides a sediment-based endpoint for use in the risk assessment. The lowest endpoint from this study was an EC_{10} of 7,120 µg a.s./kg sediment. Thus, both the NOEC of 5,600 µg a.s./L from the *Chironomus* study and the EC_{10} of 7,120 µg a.s./kg sediment from the *Lumbriculus* study have been used in the risk assessment for surface water and sediment compartments, respectively.

For the algal risk assessment the lowest bound E_rC_{50} value from the studies with green algal species has been used in the risk assessment (E_rC_{50} of 12 µg a.s./L from study M-006228-01-1). A potentially lower endpoint of >8.14 µg a.s./L is available from study M-006533-01-1 but as this value is not bound (*i.e.* a 'greater than' value) it is considered more appropriate to use the derived E_rC_{50} of 12 µg a.s./L to represent green algae. Spiroxamine is a fungicide therefore additional studies with algal species from a different taxonomic group are not a data requirement, however, several studies are available using algal species which are not green algae, including *Skeletonema costatum, Navicula pelliculosa* and *Anabaena flos-aquae*. The lowest E_rC_{50} from these additional species is for the marine species *Skeletonema costatum* with an E_rC_{50} of 6.3 µg a.s./L. As this value is lower than the E_rC_{50} for the green algal species it is considered necessary to include this in the risk assessment. Thus, both the E_rC_{50} of 12 µg a.s./L and the E_rC_{50} of 6.3 µg a.s./L have been used in the risk assessment to represent freshwater and marine species, respectively.

Spiroxamine is a fungicide therefore data for aquatic macrophytes are not a core data requirement. However, two studies using *Lemna* are available therefore aquatic macrophytes have also been included in the risk assessment. The lowest EC_{50} value was determined to be 1,910 µg a.s./L and has therefore been used in the risk assessment.

A mesocosm study is available using Spiroxamine EC 500 which included zooplankton and algae. The study has been re-assessed against current requirements including MDD analysis and meets the minimum requirements. The NOEC based on Class 1 effects was 1.0 µg a.s./L which gives an ETO-RAC of 0.5 µg a.s./L when an assessment factor (AF) of 2 is applied, in accordance with the recommendations of the Aquatic Guidance Document. An ERO-RAC of 3.1 µg a.s./L was also derived based on Class 3A effects at 9.3 µg a.s./L with an AF of 3. For the risk assessment the more conservative ETO-RAC of 0.5 µg a.s./L has been used. The study has not been used as a refinement study in the risk

assessment but as the ETO-RAC of 0.5 µg a.s./L is lower than the lowest Tier I algal RAC of 0.63 µg a.s./L, the mesocosm endpoint has been included in the Tier I risk assessment alongside the algal and invertebrate risk assessments.

Formulation data

Studies have been conducted using the representative formulation, Spiroxamine EC 500, specifically an acute fish, acute *Daphnia* and an algal study. These three studies provided a fish LC_{50} of 11,500 µg/L (5,700 µg a.s./L), a *Daphnia* EC_{50} of 10,300 µg/L (5,070 µg a.s./L) and an algal E_rC_{50} of 29 µg/L (14.3 µg a.s./L). In terms of the active substance content the formulation toxicity is considered to be driven by spiroxamine as the endpoints are very similar to the respective technical material studies but it is noted that the technical material data provide the overall lowest endpoints. A separate formulation specific risk assessment has been conducted and presented below.

Metabolites

For the metabolites there are experimental data available for M01, M02, M03 and M06 with algae. A non-GLP acute *Daphnia* study is available using M03 which has been used in the risk assessment but there are no acute aquatic invertebrate data available for M01, M02 and M06. Furthermore, there are no acute fish data available for any of the metabolites. For the risk assessment it has therefore been necessary to estimate the metabolite toxicity for fish and aquatic invertebrates using the available data with the parent material. It is clear from the algal data that the metabolites are at least one order of magnitude less toxic than spiroxamine (spiroxamine E_rC_{50}: 12 µg a.s./L; M01 E_rC_{50}: 737 µg/L; M02 E_rC_{50}: 383 µg/L; M03 E_rC_{50}: 31,700 µg/L; M06 E_rC_{50}: >3,200 µg/L). The non-GLP acute *Daphnia* study with M03 gave an EC_{50} of >100,000 µg/L which is also much greater than the EC_{50} for spiroxamine of 3,000 µg a.s./L. It is therefore considered justified to use equivalent parent toxicity to represent the metabolites in cases where there are no experimentally determined values. This approach is still considered to be conservative given that the available data with the most sensitive organism group, algae, confirms that the metabolites are at least ten times less toxic than spiroxamine. Thus, the acute fish LC_{50} for M01, M02, M03 and M06 has been taken to be 2,410 µg/L and the the acute aquatic invertebrate EC_{50} for M01, M02 and M06 has been taken to be 3000 µg/L.

Exposure

FOCUS PEC_{SW} values have been determined for the proposed uses of Spiroxamine EC 500 on vines at rates of both 200 g a.s./ha and 300 g a.s./ha, considering both one and two applications as well as applications to early and to late growth stages. Full details of the calculation of PEC_{SW} values, including FOCUS Step 4 values, have been presented in M-CP Section 9, Environmental fate.

Isomers

In accordance with the isomer Guidance Document[5] it is necessary to consider the impact of selective degradation over time for isomeric substances, such as spiroxamine. In the absence of specific toxicity data on the individual isomers, an additional Uncertainty Factor (UF) is applied to the risk assessment if selective degradation could occur.

For parent spiroxamine further investigative environmental fate work is currently ongoing in order to clarify whether or not there is significant selective degradation of isomers in surface water and sediment over time. Until this work has been completed and submitted a conclusion on the issue of selective degradation of spiroxamine in surface water cannot be made. Thus, for the risk assessment below an

[5] 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

additional Uncertainty Factor (UF) has not been applied to the risk assessment for the parent materials spiroxamine and Spiroxamine EC 500 (*i.e.* an UF of 1.0 has been used).

For the metabolites of spiroxamine there are no chiral data available to be able to make an assessment over whether or not selective degradation occurs therefore there is a possibility that selective degradation of isomers could occur in surface water over time. In order to account for any possible increased toxicity to aquatic organisms as a result of an increase in the ratio of a single isomer, an UF has been applied to the risk assessment of M01, M02, M03 and M06. The UF has been calculated following the recommendations of the isomer Guidance Document and have been presented in the table below.

Table CP 10.2-2 Uncertainty Factors determined for the aquatic toxicity data with the metabolites of spiroxamine

Test item	Study reference	Test material batch number	Isomer ratio	UF [1]
Acute fish				
M01	-	-	-	4.76 [2]
M02	-	-	-	12.5 [2]
M03	-	-	-	10.0 [2]
M06	-	-	-	2.32 [2]
Acute invertebrate				
M01	-	-	-	4.76 [2]
M02	-	-	-	12.5 [2]
M03	M-006702-01-1	950209ELB01	Not available	10.0 [3]
M06	-	-	-	2.32 [2]
Algae				
M01	M-288232-01-1	921103ELB02	A:B 56:42	4.76
M02	M-680695-01-1	AE 1344303-PU-01	A:B 83:1:16	12.5
M03	M-288235-01-1	KTS 10324-1-2	D1/D2/D3/D4: 27/26/20/27	10.0
M06	M-289818-01-1	SES 10277-2-1	A:B 43.04:52.75	2.32

[1] Changes in stereoisomeric excess are unknown therefore Uncertainty Factor = 100/content of lowest stereoisomer (%) used for ecotox endpoint [as indicted in Table B.1, p.30 of isomer GD] and assumes that the toxicological effects of the mixture can be attributed to a single isomer. This assumes that all enantiomer ratios can be safely assumed to be 50:50. For example A:B ratio of 83:1:16 would be 100/(16/2) = UF of 12.5

[2] No toxicity data available for this metabolite for this organism group therefore the isomer ratio determined in the equivalent algal study has been used as a surrogate

[3] Toxicity data available with *Daphnia* for this metabolite but no isomer details available therefore the isomer ratio determined in the equivalent algal study has been used as a surrogate

- No toxicity data on metabolite available

Risk assessment

The risk assessment procedure follows the Aquatic Guidance Document (EFSA Journal 2013[Error! Bookmark not defined.]), as appropriate to the data requirements under EU Regulations 283/2013 and 284/2013.

The risk assessment has been presented using PEC/RAC ratios. Application rates of 200 g a.s./ha and 300 g a.s./ha, considering both one and two applications, have been considered in the risk assessment. Applications to early and to late growth stages have also been considered. A risk assessment for the metabolites has been presented as well as a formulation specific spray drift risk assessment.

1 x 200 g a.s./ha

Table CP 10.2-3 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Spiroxamine EC 500 to vines (1 x 200 g a.s./ha; early application)

Group		**Fish acute**	**Fish chronic**	**Invertebrate acute**	**Invertebrate chronic**
Test species		***Danio rerio***	***Danio rerio***	***Daphnia magna***	***Daphnia magna***
Endpoint (µg a.s./L)		LC_{50} 2410	EC_{10} 1.88	EC_{50} 3000	NOEC 34
AF		100	10	100	10
RAC (µg a.s./L)		24.1	0.188	30	3.4
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios			
Step 1	31.276	**1.30**	**166**	**1.04**	**9.20**
Step 2					
NEU	1.799	0.0746	**9.57**	0.0600	0.529
SEU	2.323	0.0964	**12.4**	0.0774	0.683
Step 3					
D6 Ditch	3.346	-	**17.8**	-	-
R1 Pond	0.121	-	0.644	-	-
R1 Stream	2.446	-	**13.0**	-	-
R2 Stream	3.294	-	**17.5**	-	-
R3 Stream	3.508	-	**18.7**	-	-
R4 Stream	2.455	-	**13.1**	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

Table CP 10.2-3 (continued) Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Spiroxamine EC 500 to vines (1 x 200 g a.s./ha; early application)

Group	**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**	**Aquatic macrophytes**	**Sediment dweller**	
Test species	***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**	***Lemna gibba***	***Chironomus riparius***	***Lumbriculus variegatus***
Endpoint (µg a.s./L)	E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0	EC_{50} 1910	NOEC 5600	EC_{10} 7120 µg/kg
AF	10	10	2	10	10	10
RAC (µg a.s./L)	1.2	0.63	0.5	191	560	712

FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios					
Step 1	31.276	**26.1**	**49.6**	**62.6**	0.164	0.0559	**1.28**[1]
Step 2							
NEU	1.799	**1.50**	**2.86**	**3.60**	-	-	0.0713[2]
SEU	2.323	**1.94**	**3.69**	**4.65**	-	-	0.127[2]
Step 3							
D6 Ditch	3.346	**2.79**	**5.31**	**6.69**	-	-	-
R1 Pond	0.121	0.101	0.192	0.242			
R1 Stream	2.446	**2.04**	**3.88**	**4.89**	-	-	-
R2 Stream	3.294	**2.75**	**5.23**	**6.59**	-	-	-
R3 Stream	3.508	**2.92**	**5.57**	**7.02**	-	-	-
R4 Stream	2.455	**2.05**	**3.90**	**4.91**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**
[1] Based on a Step 1 PEC_{SED} of 909.603 µg a.s./kg
[2] Based on Step 2 PEC_{SED} of 50.731 µg a.s./kg for NEU and 90.249 µg a.s./kg for SEU

Table CP 10.2-4 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1-3 calculations for application of Spiroxamine EC 500 to vines (1 x 200 g a.s./ha; late application)

Group		**Fish acute**	**Fish chronic**	**Invertebrate acute**	**Invertebrate chronic**
Test species		***Danio rerio***	***Danio rerio***	***Daphnia magna***	***Daphnia magna***
Endpoint (µg a.s./L)		LC_{50} 2410	EC_{10} 1.88	EC_{50} 3000	NOEC 34
AF		100	10	100	10
RAC (µg a.s./L)		24.1	0.188	30	3.4
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios			
Step 1	31.276	**1.30**	**166**	**1.04**	**9.20**
Step 2					
NEU	5.352	0.222	**28.5**	0.178	**1.57**
SEU	5.352	0.222	**28.5**	0.178	**1.57**
Step 3					
D6 Ditch	3.413		**18.2**	-	1.00
R4 Pond	0.122	-	0.649	-	0.0359
R1 Stream	2.503	-	**13.3**	-	0.736
R2 Stream	3.355	-	**17.8**	-	0.987

R3 Stream	3.528	-	**18.8**	-	**1.04**
R4 Stream	2.502	-	**13.3**	-	0.736

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

Table CP 10.2-4 (continued) Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Spiroxamine EC 500 to vines (1 x 200 g a.s./ha; late application)

Group		**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**	**Aquatic macrophytes**	**Sediment dweller**	
Test species		***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**	***Lemna gibba***	***Chironomus riparius***	***Lumbriculus variegatus***
Endpoint (µg a.s./L)		E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0	EC_{50} 1910	NOEC 5600	EC_{10} 7120 µg/kg
AF		10	10	2	10	10	10
RAC (µg a.s./L)		1.2	0.63	0.5	191	560	712
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios					
Step 1	31.276	**26.1**	**49.6**	**62.6**	0.164	0.0559	**1.28**[1]
Step 2							
NEU	5.352	**4.46**	**8.50**	**10.7**	-	-	0.0912[2]
SEU	5.352	**4.46**	**8.50**	**10.7**	-	-	0.113[2]
Step 3							
D6 Ditch	3.413	**2.84**	**5.42**	**6.83**	-	-	-
R1 Pond	0.122	0.102	0.194	0.244	-	-	-
R1 Stream	2.503	**2.09**	**3.97**	**5.01**	-	-	-
R2 Stream	3.395	**2.80**	**5.33**	**6.71**	-	-	-
R3 Stream	3.528	**2.94**	**5.60**	**7.06**	-	-	-
R4 Stream	2.502	**2.09**	**3.97**	**5.00**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**
[1] Based on a Step 3 PEC_{SED} of 969.603 µg a.s./kg
[2] Based on Step 2 PEC_{SED} of 64.968 µg a.s./kg for NEU and 80.775 µg a.s./kg for SEU

For the acute fish, acute invertebrate, aquatic macrophyte and sediment-dweller risk assessments, the PEC/RAC ratios are <1 using FOCUS Step 1 or Step 2 PEC_{sw} values thereby demonstrating an acceptable risk to these organism groups from exposure to spiroxamine following application of Spiroxamine EC 500 to grapes at 1 x 200 g a.s./ha (early and late applications).

For the chronic invertebrate risk assessment an acceptable risk could be demonstrated using FOCUS Step 2 PEC_{sw} values only for early application to grapes at 1 x 200 g a.s./ha.

For the chronic fish and algal risk assessments, as well as those organisms covered by the mesocosm study, some FOCUS scenarios passed the risk assessment when Step 3 PEC_{sw} values were used but

several scenarios for these groups require refinement of the risk assessment. For the chronic invertebrate risk assessment for late applications to grapes at 1 x 200 g a.s./ha, all but one scenario passed the risk assessment using Step 3 PEC_{sw} values therefore refinement is also required here. Refined risk assessments using FOCUS Step 4 PEC_{sw} values are presented later in this section.

2 x 200 g a.s./ha

Table CP 10.2-5 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Spiroxamine EC 500 to vines (2 x 200 g a.s./ha; early application)

Group		**Fish acute**	**Fish chronic**	**Invertebrate acute**	**Invertebrate chronic**
Test species		***Danio rerio***	***Danio rerio***	***Daphnia magna***	***Daphnia magna***
Endpoint (µg a.s./L)		LC_{50} 2410	EC_{10} 1.88	EC_{50} 3000	NOEC 34
AF		100	10	100	10
RAC (µg a.s./L)		24.1	0.188	30	3.4
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios			
Step 1	31.276	**1.30**	**166**	**1.04**	**9.20**
Step 2					
NEU	2.494	0.103	**13.3**	0.0831	0.734
SEU	4.284	0.178	**22.8**	0.143	**1.26**
Step 3					
D6 Ditch	3.346	-	**17.8**	-	0.984
R1 Pond	0.176	-	0.936	-	0.0518
R1 Stream	2.446	-	**13.0**	-	0.719
R2 Stream	3.294	-	**17.5**	-	0.969
R3 Stream	3.508	-	**18.7**	-	**1.03**
R4 Stream	2.455	-	**13.1**	-	0.722

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

Table CP 10.2-5 (continued) Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Spiroxamine EC 500 to vines (2 x 200 g a.s./ha; early application)

Group	**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**	**Aquatic macrophytes**	**Sediment dweller**	
Test species	***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**	***Lemna gibba***	***Chironomus riparius***	***Lumbriculus variegatus***
Endpoint	E_rC_{50}	E_rC_{50}	NOEC	EC_{50}	NOEC	EC_{10}

(µg a.s./L)		12.0	6.3	1.0	1910	5600	7120 µg/kg
AF		10	10	2	10	10	10
RAC (µg a.s./L)		1.2	0.63	0.5	191	560	712
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios					
Step 1	31.276	**26.1**	**49.6**	**62.6**	0.164	0.0559	**1.28**[1]
Step 2							
NEU	2.494	**2.08**	**3.96**	**4.99**	-	-	0.131[2]
SEU	4.284	**3.57**	**6.80**	**8.57**	-	-	0.234[2]
Step 3							
D6 Ditch	3.346	**2.79**	**5.31**	**6.69**	-	-	-
R1 Pond	0.176	0.147	0.279	0.352	-	-	-
R1 Stream	2.446	**2.04**	**3.88**	**4.89**	-	-	-
R2 Stream	3.294	**2.75**	**5.23**	**6.59**	-	-	-
R3 Stream	3.508	**2.92**	**5.57**	**7.02**	-	-	-
R4 Stream	2.455	**2.05**	**3.90**	**4.91**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**
[1] Based on a Step 1 PEC $_{SED}$ of 909.603 µg a.s./kg
[2] Based on Step 2 PEC $_{SED}$ of 93.626 µg a.s./kg for NEU and 166.878 µg a.s./kg for SEU

Table CP 10.2-6 Aquatic organisms: acceptability of risk (PEC/RAC ≥1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Spiroxamine EC 500 to vines (2 x 200 g a.s./ha; late application)

Group		**Fish acute**	**Fish chronic**	**Invertebrate acute**	**Invertebrate chronic**
Test species		***Danio rerio***	***Danio rerio***	***Daphnia magna***	***Daphnia magna***
Endpoint (µg a.s./L)		LC_{50} 2410	EC_{10} 1.88	EC_{50} 3000	NOEC 34
AF		100	10	100	10
RAC (µg a.s./L)		24.1	0.188	30	3.4
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios			
Step 1	31.276	**1.30**	**166**	**1.04**	**9.20**
Step 2					
NEU	5.735	0.238	**30.5**	0.191	**1.69**
SEU	5.735	0.238	**30.5**	0.191	**1.69**
Step 3					
D6 Ditch	3.531	-	**18.8**	-	**1.04**

R1 Pond	0.181	-	0.963	-	0.0532
R1 Stream	2.503	-	**13.3**	-	0.736
R2 Stream	3.355	-	**17.8**	-	0.987
R3 Stream	3.528	-	**18.8**	-	**1.04**
R4 Stream	2.502	-	**13.3**	-	0.736

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

Table CP 10.2-6 (continued) Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Spiroxamine EC 500 to vines (2 x 200 g a.s./ha; late application)

Group		**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**	**Aquatic macrophytes**	**Sediment dweller**	
Test species		***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**	***Lemna gibba***	***Chironomus riparius***	***Lumbriculus variegatus***
Endpoint (µg a.s./L)		E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0	EC_{50} 1920	NOEC 5600	EC_{10} 7120 µg/kg
AF		10	10	2	10	10	10
RAC (µg a.s./L)		1.2	0.63	0.5	191	560	712
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios					
Step 1	31.276	**26.1**	**49.6**	**62.6**	0.164	0.0559	**1.28**[1]
Step 2							
NEU	5.735	**4.78**	**9.10**	**11.5**	-	-	0.164[2]
SEU	5.735	**4.78**	**9.10**	**11.5**	-	-	0.205[2]
Step 3							
D6 Ditch	3.531	**2.94**	**5.60**	**7.06**	-	-	-
R1 Pond	0.181	0.151	0.287	0.362	-	-	-
R1 Stream	2.503	**2.09**	**3.97**	**5.01**	-	-	-
R2 Stream	3.355	**2.80**	**5.33**	**6.71**	-	-	-
R3 Stream	3.528	**2.94**	**5.60**	**7.06**	-	-	-
R4 Stream	2.502	**2.09**	**3.97**	**5.00**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**
[1] Based on a Step 1 PEC_{SED} of 909.603 µg a.s./kg
[2] Based on Step 2 PEC_{SED} of 116.712 µg a.s./kg for NEU and 146.012 µg a.s./kg for SEU

For the acute fish, acute invertebrate, aquatic macrophyte and sediment-dweller risk assessments, the PEC/RAC ratios are <1 using FOCUS Step 1 or Step 2 PEC_{sw} values thereby demonstrating an acceptable risk to these organism groups from exposure to spiroxamine following application of Spiroxamine EC 500 to grapes at 2 x 200 g a.s./ha (early and late applications).

For the chronic fish, chronic invertebrate and algal risk assessments, as well as those organisms covered by the mesocosm study, some FOCUS scenarios passed the risk assessment when Step 3 PEC_{sw} values were used but several scenarios for these groups require refinement of the risk assessment. Refined risk assessments using FOCUS Step 4 PEC_{sw} values are presented later in this section.

1 x 300 g a.s./ha

Table CP 10.2-7 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Spiroxamine EC 500 to vines (1 x 300 g a.s./ha; early application)

Group		**Fish acute**	**Fish chronic**	**Invertebrate acute**	**Invertebrate chronic**
Test species		***Danio rerio***	***Danio rerio***	***Daphnia magna***	***Daphnia magna***
Endpoint (µg a.s./L)		LC_{50} 2410	EC_{10} 1.88	EC_{50} 3000	NOEC 34
AF		100	10	100	10
RAC (µg a.s./L)		24.1	0.188	30	3.4
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios			
Step 1	46.914	**1.95**	**250**	**1.56**	**13.8**
Step 2					
NEU	2.699	0.112	**14.4**	0.0900	**0.794**
SEU	3.484	0.145	**18.5**	0.116	**1.02**
Step 3					
D6 Ditch	3.021	-	**26.7**	-	**1.48**
R1 Pond	0.182	-	0.968	-	0.0535
R1 Stream	3.670	-	**19.5**	-	**1.08**
R2 Stream	4.942	-	**26.3**	-	**1.45**
R3 Stream	5.263	-	**28.0**	-	**1.55**
R4 Stream	3.683	-	**19.6**	-	**1.08**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

Table CP 10.2-7 (continued) Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Spiroxamine EC 500 to vines (1 x 300 g a.s./ha; early application)

Group	**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**	**Aquatic macrophytes**	**Sediment dweller**	
Test species	***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**	***Lemna gibba***	***Chironomus riparius***	***Lumbriculus variegatus***
Endpoint	E_rC_{50}	E_rC_{50}	NOEC	EC_{50}	NOEC	EC_{10}

(µg a.s./L)		12.0	6.3	1.0	1910	5600	7120 µg/kg
AF		10	10	2	10	10	10
RAC (µg a.s./L)		1.2	0.63	0.5	191	560	712
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios					
Step 1	46.914	**39.1**	**74.5**	**93.8**	0.246	0.0838	**1.91**[1]
Step 2							
NEU	2.699	**2.25**	**4.28**	**5.40**	-	-	0.107[2]
SEU	3.484	**2.90**	**5.53**	**6.97**	-	-	0.190[2]
Step 3							
D6 Ditch	5.021	**4.18**	**7.97**	**10.0**	-	-	-
R1 Pond	0.182	0.152	0.289	0.364	-	-	-
R1 Stream	3.670	**3.06**	**5.83**	**7.34**	-	-	-
R2 Stream	4.942	**4.12**	**7.84**	**9.88**	-	-	-
R3 Stream	5.263	**4.39**	**8.35**	**10.5**	-	-	-
R4 Stream	3.683	**3.07**	**5.85**	**7.37**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**
[1] Based on a Step 1 PEC$_{SED}$ of 1360 µg a.s./kg
[2] Based on Step 2 PEC$_{SED}$ of 76.097 µg a.s./kg for NEU and 135.374 µg a.s./kg for SEU

Table CP 10.2-8 Aquatic organisms: acceptability of risk (PEC/RAC ≥1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Spiroxamine EC 500 to vines (1 x 300 g a.s./ha; late application)

Group		**Fish acute**	**Fish chronic**	**Invertebrate acute**	**Invertebrate chronic**
Test species		***Danio rerio***	***Danio rerio***	***Daphnia magna***	***Daphnia magna***
Endpoint (µg a.s./L)		LC_{50} 2410	EC_{10} 1.88	EC_{50} 3000	NOEC 34
AF		100	10	100	10
RAC (µg a.s./L)		24.1	0.188	30	3.4
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios			
Step 1	46.914	**1.95**	**250**	**1.56**	**13.8**
Step 2					
NEU	8.028	0.333	**42.7**	0.268	**2.36**
SEU	8.028	0.333	**42.7**	0.268	**2.36**
Step 3					
D6 Ditch	5.120	-	**27.2**	-	**1.51**

R1 Pond	0.183	-	0.973	-	0.0538
R1 Stream	3.755	-	**20.0**	-	**1.10**
R2 Stream	5.034	-	**26.8**	-	**1.48**
R3 Stream	5.294	-	**28.2**	-	**1.56**
R4 Stream	3.754	-	**20.0**	-	**1.10**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

Table CP 10.2-8 (continued) Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Spiroxamine EC 500 to vines (1 x 300 g a.s./ha; late application)

Group		**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**	**Aquatic macrophytes**	**Sediment dweller**	
Test species		***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**	***Lemna gibba***	***Chironomus riparius***	***Lumbriculus variegatus***
Endpoint (µg a.s./L)		E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0	EC_{50} 1920	NOEC 5600	EC_{10} 7120 µg/kg
AF		10	10	2	10	10	10
RAC (µg a.s./L)		1.2	0.63	0.5	191	560	712
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios					
Step 1	46.914	**39.1**	**74.5**	**93.8**	0.246	0.0838	**1.91**[1]
Step 2							
NEU	8.028	**6.69**	**12.7**	**16.1**	-	-	0.137[2]
SEU	8.028	**6.69**	**12.7**	**16.1**	-	-	0.170[2]
Step 3							
D6 Ditch	5.120	**4.27**	**8.13**	**10.2**	-	-	-
R1 Pond	0.183	0.153	0.290	0.366	-	-	-
R1 Stream	3.755	**3.13**	**5.96**	**7.51**	-	-	-
R2 Stream	5.034	**4.20**	**7.99**	**10.1**	-	-	-
R3 Stream	5.294	**4.41**	**8.40**	**10.6**	-	-	-
R4 Stream	3.754	**3.13**	**5.96**	**7.51**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**
[1] Based on a Step 1 PEC_{SED} of 1360 µg a.s./kg
[2] Based on Step 2 PEC_{SED} of 97.452 µg a.s./kg for NEU and 121.163 µg a.s./kg for SEU

For the acute fish, acute invertebrate, aquatic macrophyte and sediment-dweller risk assessments, the PEC/RAC ratios are <1 using FOCUS Step 1 or Step 2 PEC_{sw} values thereby demonstrating an acceptable risk to these organism groups from exposure to spiroxamine following application of Spiroxamine EC 500 to grapes at 1 x 300 g a.s./ha (early and late applications).

For the chronic fish, chronic invertebrate and algal risk assessments, as well as those organisms covered by the mesocosm study, a small number of FOCUS scenarios passed the risk assessment when Step 3 PEC_{sw} values were used but the majority of FOCUS scenarios for these groups require refinement of the risk assessment. Refined risk assessments using FOCUS Step 4 PEC_{sw} values are presented later in this section.

2 x 300 g a.s./ha

Table CP 10.2-9 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Spiroxamine EC 500 to vines (2 x 300 g a.s./ha; early application)

Group		Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic
Test species		***Danio rerio***	***Danio rerio***	***Daphnia magna***	***Daphnia magna***
Endpoint		LC_{50}	EC_{10}	EC_{50}	NOEC
(µg a.s./L)		2410	1.88	3000	34
AF		100	10	100	10
RAC (µg a.s./L)		24.1	0.188	30	3.4
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios			
Step 1	46.914	**1.95**	**250**	**1.56**	**13.8**
Step 2					
NEU	3.741	0.155	**19.9**	0.125	**1.10**
SEU	6.425	0.267	**34.2**	0.214	**1.89**
Step 3					
D6 Ditch	5.021	-	**26.7**	-	**1.48**
R1 Pond	0.265	-	**1.41**		0.0779
R1 Stream	3.670	-	**19.5**	-	**1.08**
R2 Stream	4.942		**26.3**	-	**1.45**
R3 Stream	5.263	-	**28.0**	-	**1.55**
R4 Stream	3.683		**19.6**	-	**1.08**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

Table CP 10.2-9 (continued) Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Spiroxamine EC 500 to vines (2 x 300 g a.s./ha; early application)

Group	Algae (Freshwater)	Algae (Marine)	Mesocosm	Aquatic macrophytes	Sediment dweller	
Test species	***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**	***Lemna gibba***	***Chironomus riparius***	***Lumbriculus variegatus***

Endpoint (µg a.s./L)		E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0	EC_{50} 1910	NOEC 5600	EC_{10} 7120 µg/kg
AF		10	10	2	10	10	10
RAC (µg a.s./L)		1.2	0.63	0.5	191	560	712
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios					
Step 1	46.914	**39.1**	**74.5**	**93.8**	0.246	0.0838	**1.91**[1]
Step 2							
NEU	3.741	**3.12**	**5.94**	**7.48**	-	-	0.197[2]
SEU	6.425	**5.35**	**10.2**	**12.9**	-	-	0.352[2]
Step 3							
D6 Ditch	5.021	**4.18**	**7.97**	**10.0**	-	-	-
R1 Pond	0.265	0.221	0.421	0.530	-	-	-
R1 Stream	3.670	**3.06**	**5.83**	**7.34**	-	-	-
R2 Stream	4.942	**4.12**	**7.84**	**9.88**	-	-	-
R3 Stream	5.263	**4.39**	**8.35**	**10.5**	-	-	-
R4 Stream	3.683	**3.07**	**5.85**	**7.37**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**
[1] Based on a Step 1 PEC_{SED} of 1360 µg a.s./kg
[2] Based on Step 2 PEC_{SED} of 140.439 µg a.s./kg for NEU and 250.316 µg a.s./kg for SEU

Table CP 10.2-10 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Spiroxamine EC 500 to vines (2 x 300 g a.s./ha; late application)

Group		**Fish acute**	**Fish chronic**	**Invertebrate acute**	**Invertebrate chronic**
Test species		***Danio rerio***	***Danio rerio***	***Daphnia magna***	***Daphnia magna***
Endpoint (µg a.s./L)		LC_{50} 2410	EC_{10} 1.88	EC_{50} 3000	NOEC 34
AF		100	10	100	10
RAC (µg a.s./L)		24.1	0.188	30	3.4
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios			
Step 1	46.914	**1.95**	**250**	**1.56**	**13.8**
Step 2					
NEU	8.602	0.357	**45.8**	0.287	**2.53**
SEU	8.602	0.357	**45.8**	0.287	**2.53**
Step 3					

D6 Ditch	5.309	-	**28.2**	-	**1.56**
R1 Pond	0.272	-	**1.45**	-	0.0800
R1 Stream	3.755	-	**20.0**	-	**1.10**
R2 Stream	5.034	-	**26.8**	-	**1.48**
R3 Stream	5.294	-	**28.2**	-	**1.56**
R4 Stream	3.754	-	**20.0**	-	**1.10**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

Table CP 10.2-10 (continued) Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine for each aquatic group based on FOCUS Steps 1 - 3 calculations for application of Spiroxamine EC 500 to vines (2 x 300 g a.s./ha; late application)

Group		**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**	**Aquatic macrophytes**	**Sediment dweller**	
Test species		***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**	***Lemna gibba***	***Chironomus riparius***	***Lumbriculus variegatus***
Endpoint (µg a.s./L)		E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0	EC_{50} 1910	NOEC 3600	EC_{10} 7120 µg/kg
AF		10	10	2	10	10	10
RAC (µg a.s./L)		1.2	0.63	0.5	191	360	712
FOCUS Scenario	PEC $_{max}$ (µg a.s./L)	PEC/RAC ratios					
Step 1	46.914	**39.1**	**74.5**	**93.8**	0.246	0.0838	**1.91**[1]
Step 2							
NEU	8.602	**7.17**	**13.7**	**17.2**	-	-	0.246[2]
SEU	8.602	**7.17**	**13.7**	**17.2**	-	-	0.308[2]
Step 3							
D6 Ditch	5.309	**4.42**	**8.43**	**10.6**	-	-	-
R1 Pond	0.272	0.227	0.432	0.544	-	-	-
R1 Stream	3.755	**3.13**	**5.96**	**7.51**	-	-	-
R2 Stream	5.034	**4.20**	**7.99**	**10.1**	-	-	-
R3 Stream	5.294	**4.41**	**8.40**	**10.6**	-	-	-
R4 Stream	3.754	**3.13**	**5.96**	**7.51**	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**
[1] Based on a Step 1 PEC_{SED} of 1360 µg a.s./kg
[2] Based on Step 2 PEC_{SED} of 175.067 µg a.s./kg for NEU and 219.018 µg a.s./kg for SEU

For the acute fish, acute invertebrate, aquatic macrophyte and sediment-dweller risk assessments, the PEC/RAC ratios are <1 using FOCUS Step 1 or Step 2 PEC_{sw} values thereby demonstrating an

acceptable risk to these organism groups from exposure to spiroxamine following application of Spiroxamine EC 500 to grapes at 2 x 300 g a.s./ha (early and late applications).

For the chronic fish, chronic invertebrate and algal risk assessments, as well as those organisms covered by the mesocosm study, a small number of FOCUS scenarios passed the risk assessment when Step 3 PEC_{sw} values were used but the majority of FOCUS scenarios for these groups require refinement of the risk assessment. Refined risk assessments using FOCUS Step 4 PEC_{sw} values are presented below.

Refined risk assessment using Step 4 PEC_{sw} values

For each of the proposed uses of Spiroxamine EC 500, refined risk assessments for those organism groups that did not pass the risk assessment using Step 3 PEC_{sw} values for all of the relevant FOCUS scenarios have been presented below. The exposure estimates have been refined by use of Step 4 PEC_{sw} values considering mitigation measures in the form of either *i)*: a 20 m no-spray buffer zone with a 20 m vegetated filter strip or *ii)*: a 25 m no-spray buffer zone with a 20 m vegetated filter strip.

Table CP 10.2-11 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine based on FOCUS Step 4 calculations for application of Spiroxamine EC 500 to vines (1 x 200 g a.s./ha, early application)

Group		Fish chronic	Algae (Freshwater)	Algae (Marine)	Mesocosm
Test species		***Danio rerio***	***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**
Endpoint (µg a.s./L)		EC_{10} 1.88	E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0
AF		10	10	10	2
RAC (µg a.s./L)		0.188	1.2	0.63	0.5
FOCUS Scenario	PEC_{sw} max (µg a.s./L)	PEC/RAC ratios			
Step 4 (20 m nsbz and 20 m vfs)					
D6 Ditch	0.257	**1.37**	0.214	0.408	0.514
R1 Pond	0.049	-	-		-
R1 Stream	0.232	**1.23**	0.193	0.368	0.464
R2 Stream	0.312	**1.66**	0.260	0.495	0.624
R3 Stream	0.330	**1.76**	0.275	0.524	0.660
R4 Stream	0.234	**1.24**	0.195	0.371	0.468
Step 4 (25 m nsbz and 20 m vfs)					
D6 Ditch	0.182	0.968	-	-	-
R1 Pond	0.041	-	-	-	-
R1 Stream	0.167	0.888	-	-	-
R2 Stream	0.223	**1.19**	-	-	-
R3 Stream	0.238	**1.27**	-	-	-
R4 Stream	0.168	0.894	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; nsbz: no spray buffer zone; vfs: vegetated filter strip; - not required as risk assessment already passes

Table CP 10.2-12 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine based on FOCUS Step 4 calculations for application of Spiroxamine EC 500 to vines (1 x 200 g a.s./ha; late application)

Group		**Fish chronic**	**Invertebrate chronic**	**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**
Test species		***Danio rerio***	***Daphnia magna***	***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**
Endpoint (µg a.s./L)		EC_{10} 1.88	NOEC 34	E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0
AF		10	10	10	10	2
RAC (µg a.s./L)		0.188	3.4	1.2	0.63	0.5
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios				
Step 4 (20 m nsbz and 20 m vfs)						
D6 Ditch	0.310	**1.65**	-	0.258	0.492	0.620
R1 Pond	0.049	-	-	-	-	-
R1 Stream	0.239	**1.27**	-	0.199	0.379	0.478
R2 Stream	0.318	**1.69**	-	0.265	0.505	0.636
R3 Stream	0.331	**1.76**	0.0974	0.276	0.525	0.662
R4 Stream	0.239	**1.27**	-	0.199	0.379	0.478
Step 4 (25 m nsbz and 20 m vfs)						
D6 Ditch	0.242	**1.29**	-	-	-	-
R1 Pond	0.041	-	-	-	-	-
R1 Stream	0.172	0.915	-	-	-	-
R2 Stream	0.229	**1.22**	-	-	-	-
R3 Stream	0.238	**1.27**	-	-	-	-
R4 Stream	0.172	0.915	-	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; nsbz: no spray buffer zone; vfs: vegetated filter strip; - not required as risk assessment already passes

Table CP 10.2-13 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine based on FOCUS Step 4 calculations for application of Spiroxamine EC 500 to vines (2 x 200 g a.s./ha; early application)

Group	**Fish chronic**	**Invertebrate chronic**	**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**
Test species	***Danio rerio***	***Daphnia magna***	***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**
Endpoint (µg a.s./L)	EC_{10} 1.88	NOEC 34	E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0
AF	10	10	10	10	2

RAC (µg a.s./L)		0.188	3.4	1.2	0.63	0.5
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios				
Step 4 (20 m nsbz and 20 m vfs)						
D6 Ditch	0.257	**1.37**	-	0.214	0.408	0.514
R1 Pond	0.074	-	-	-	-	-
R1 Stream	0.232	**1.23**	-	0.193	0.368	0.464
R2 Stream	0.312	**1.66**	-	0.260	0.495	0.624
R3 Stream	0.330	**1.76**	0.0971	0.275	0.524	0.660
R4 Stream	0.255	**1.36**	-	0.213	0.405	0.510
Step 4 (25 m nsbz and 20 m vfs)						
D6 Ditch	0.198	**1.05**	-	-	-	-
R1 Pond	0.062	-	-	-	-	-
R1 Stream	0.167	0.888	-	-	-	-
R2 Stream	0.223	**1.19**	-	-	-	-
R3 Stream	0.238	**1.27**	-	-	-	-
R4 Stream	0.255	**1.36**	-	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; nsbz: no spray buffer zone; vfs: vegetated filter strip; -: not required as risk assessment already passes

Table CP 10.2-14 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine based on FOCUS Step 4 calculations for application of Spiroxamine EC 500 to vines (2 x 200 g a.s./ha; late application)

Group		**Fish chronic**	**Invertebrate chronic**	**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**
Test species		***Danio rerio***	***Daphnia magna***	***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**
Endpoint		EC_{10}	NOEC	E_rC_{50}	E_rC_{50}	NOEC
(µg a.s./L)		1.88	34	12.0	6.3	1.0
AF		10	10	10	10	2
RAC (µg a.s./L)		0.188	3.4	1.2	0.63	0.5
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios				
Step 4 (20 m nsbz and 20 m vfs)						
D6 Ditch	0.318	**1.69**	0.0935	0.265	0.505	0.636
R1 Pond	0.075	-	-	-	-	-
R1 Stream	0.239	**1.27**	-	0.199	0.379	0.478
R2 Stream	0.318	**1.69**	-	0.265	0.505	0.636

R3 Stream	0.331	**1.76**	0.0974	0.276	0.525	0.662
R4 Stream	0.239	**1.27**	-	0.199	0.379	0.478
Step 4 (25 m nsbz and 20 m vfs)						
D6 Ditch	0.250	**1.33**	-	-	-	-
R1 Pond	0.063	-	-	-	-	-
R1 Stream	0.172	0.915	-	-	-	-
R2 Stream	0.229	**1.22**	-	-	-	-
R3 Stream	0.238	**1.27**	-	-	-	-
R4 Stream	0.230	**1.22**	-	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; nsbz: no spray buffer zone; vfs: vegetated filter strip; - not required as risk assessment already passes

Table CP 10.2-15 Aquatic organisms: acceptability of risk (PEC/RAC ≥1) for spiroxamine based on FOCUS Step 4 calculations for application of Spiroxamine EC 500 to vines (1 x 300 g a.s./ha; early application)

Group		**Fish chronic**	**Invertebrate chronic**	**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**
Test species		***Danio rerio***	***Daphnia magna***	***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**
Endpoint (µg a.s./L)		EC_{10} 1.88	NOEC 34	E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0
AF		10	10	10	10	2
RAC (µg a.s./L)		0.188	3.4	1.2	0.63	0.5
FOCUS Scenario	PEC_{sw-max} (µg a.s./L)	PEC/RAC ratios				
Step 4 (20 m nsbz and 20 m vfs)						
D6 Ditch	0.385	**2.05**	0.113	0.321	0.611	0.770
R1 Pond	0.074	-	-	-	-	-
R1 Stream	0.349	**1.86**	0.103	0.291	0.554	0.698
R2 Stream	0.467	**2.48**	0.137	0.389	0.741	0.934
R3 Stream	0.496	**2.64**	0.146	0.413	0.787	0.992
R4 Stream	0.351	**1.87**	0.103	0.293	0.557	0.702
Step 4 (25 m nsbz and 20 m vfs)						
D6 Ditch	0.274	**1.46**	-	-	-	-
R1 Pond	0.062	-	-	-	-	-
R1 Stream	0.251	**1.34**	-	-	-	-
R2 Stream	0.335	**1.78**	-	-	-	-
R3 Stream	0.357	**1.90**	-	-	-	-
R4 Stream	0.253	**1.35**	-	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; nsbz: no spray buffer zone; vfs: vegetated filter strip; - not required as risk assessment already passes

Table CP 10.2-16 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine based on FOCUS Step 4 calculations for application of Spiroxamine EC 500 to vines (1 x 300 g a.s./ha; late application)

Group		**Fish chronic**	**Invertebrate chronic**	**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**
Test species		***Danio rerio***	***Daphnia magna***	***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**
Endpoint		EC_{10}	NOEC	E_rC_{50}	E_rC_{50}	NOEC
(µg a.s./L)		1.88	34	12.0	6.3	1.0
AF		10	10	10	10	2
RAC (µg a.s./L)		0.188	3.4	1.2	0.63	0.5
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios				
Step 4 (20 m nsbz and 20 m vfs)						
D6 Ditch	0.466	**2.48**	0.137	0.388	0.740	0.932
R1 Pond	0.074	-	-	-	-	-
R1 Stream	0.358	**1.90**	0.105	0.298	0.568	0.716
R2 Stream	0.478	**2.54**	0.141	0.398	0.759	0.956
R3 Stream	0.496	**2.64**	0.146	0.413	0.787	0.992
R4 Stream	0.358	**1.90**	0.105	0.298	0.568	0.716
Step 4 (25 m nsbz and 20 m vfs)						
D6 Ditch	0.364	**1.94**	-	-	-	-
R1 Pond	0.062	-	-	-	-	-
R1 Stream	0.259	**1.38**	-	-	-	-
R2 Stream	0.343	**1.82**	-	-	-	-
R3 Stream	0.357	**1.90**	-	-	-	-
R4 Stream	0.259	**1.38**	-	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; nsbz: no spray buffer zone; vfs: vegetated filter strip; - not required as risk assessment already passes

Table CP 10.2-17 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine based on FOCUS Step 4 calculations for application of Spiroxamine EC 500 to vines (2 x 300 g a.s./ha; early application)

Group	**Fish chronic**	**Invertebrate chronic**	**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**
Test species	***Danio rerio***	***Daphnia magna***	***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**
Endpoint	EC_{10}	NOEC	E_rC_{50}	E_rC_{50}	NOEC

(µg a.s./L)		1.88	34	12.0	6.3	1.0
AF		10	10	10	10	2
RAC (µg a.s./L)		0.188	3.4	1.2	0.63	0.5
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios				
Step 4 (20 m nsbz and 20 m vfs)						
D6 Ditch	0.385	**2.05**	0.113	0.321	0.611	0.770
R1 Pond	0.111	0.590	-	-	-	-
R1 Stream	0.349	**1.86**	0.103	0.291	0.554	0.698
R2 Stream	0.467	**2.48**	0.137	0.389	0.741	0.934
R3 Stream	0.496	**2.64**	0.146	0.413	0.787	0.992
R4 Stream	0.399	**2.12**	0.117	0.333	0.633	0.798
Step 4 (25 m nsbz and 20 m vfs)						
D6 Ditch	0.297	**1.58**	-	-	-	-
R1 Pond	0.093	-	-	-	-	-
R1 Stream	0.251	**1.34**	-	-	-	-
R2 Stream	0.335	**1.78**	-	-	-	-
R3 Stream	0.357	**1.90**	-	-	-	-
R4 Stream	0.399	**2.12**	-	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; nsbz: no spray buffer zone; vfs: vegetated filter strip; - not required as risk assessment already passes

Table CP 10.2-18 Aquatic organisms: acceptability of risk (PEC/RAC <1) for spiroxamine based on FOCUS Step 4 calculations for application of Spiroxamine EC 500 to vines (2 x 300 g a.s./ha; late application)

Group		**Fish chronic**	**Invertebrate chronic**	**Algae (Freshwater)**	**Algae (Marine)**	**Mesocosm**
Test species		***Danio rerio***	***Daphnia magna***	***Scenedesmus subspicatus***	***Skeletonema costatum***	**Algae and invertebrates**
Endpoint (µg a.s./L)		EC_{10} 1.88	NOEC 34	E_rC_{50} 12.0	E_rC_{50} 6.3	NOEC 1.0
AF		10	10	10	10	2
RAC (µg a.s./L)		0.188	3.4	1.2	0.63	0.5
FOCUS Scenario	PEC $_{sw-max}$ (µg a.s./L)	PEC/RAC ratios				
Step 4 (20 m nsbz and 20 m vfs)						
D6 Ditch	0.478	**2.54**	0.141	0.398	0.759	0.956
R1 Pond	0.113	0.601	-	-	-	-

R1 Stream	0.358	**1.90**	0.105	0.298	0.568	0.716
R2 Stream	0.478	**2.54**	0.141	0.398	0.759	0.956
R3 Stream	0.496	**2.64**	0.146	0.413	0.787	0.992
R4 Stream	0.360	**1.91**	0.106	0.300	0.571	0.720
Step 4 (25 m nsbz and 20 m vfs)						
D6 Ditch	0.376	**2.00**	-	-	-	-
R1 Pond	0.095	-	-	-	-	-
R1 Stream	0.259	**1.38**	-	-	-	-
R2 Stream	0.343	**1.82**	-	-	-	-
R3 Stream	0.357	**1.90**	-	-	-	-
R4 Stream	0.360	**1.91**	-	-	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; nsbz: no spray buffer zone; vfs: vegetated filter strip; - not required as risk assessment already passes

For the chronic invertebrate and algal risk assessments, including those organisms covered by the mesocosm study, an acceptable risk from exposure to spiroxamine can be concluded for all proposed uses of Spiroxamine EC 500 to grapes when mitigation in the form of a 20 m no-spray buffer zone with a 20 m vegetated filter strip is applied.

For the chronic fish risk assessment, the PEC:RAC ratios for majority of FOCUS scenarios were still >1 when up to 95% total application mitigation in the form of a 25 m no-spray buffer zone with a 20 m vegetated filter strip was applied. Further refinement of the chronic fish risk assessment is therefore required and has been discussed at the end of this section.

The table below provides a summary of those FOCUS scenarios for which an acceptable risk can be demonstrated using up to 95% total application mitigation and those scenarios for which further refinement is required.

Table CP 10.2-19 Summary of aquatic risk assessment: FOCUS scenarios with an acceptable risk demonstrated and those requiring further refinement

Proposed use of Spiroxamine EC 500 to grapes (g a.s./ha)		**FOCUS scenarios for which acceptable risks have been demonstrated using a 25 m nsbz with a 20 m vfs**	**FOCUS scenarios for which further refinement is required**
1 x 200	Early	D6 Ditch, R1 Pond*, R1 Stream, R4 Stream	R2 Stream, R3 Stream
	Late	R1 Pond*, R1 Stream, R4 Stream	D6 Ditch, R2 Stream, R3 Stream
2 x 200	Early	R1 Pond*, R1 Stream	D6 Ditch, R2 Stream, R3 Stream, R4 Stream
	Late	R1 Pond*, R1 Stream	D6 Ditch, R2 Stream, R3 Stream, R4 Stream
1 x 300	Early	R1 Pond*	D6 Ditch, R1 Stream, R2 Stream, R3 Stream, R4 Stream
	Late	R1 Pond*	D6 Ditch, R1 Stream, R2 Stream, R3 Stream, R4 Stream
2 x 300	Early	R1 Pond	D6 Ditch, R1 Stream, R2 Stream, R3 Stream, R4 Stream

	Late	R1 Pond	D6 Ditch, R1 Stream, R2 Stream, R3 Stream, R4 Stream

nsbz: no spray buffer zone; vfs: vegetated filter strip
* R1 Pond scenario for this use passes the risk assessment at Step 3 therefore no mitigation required

Metabolites

Risk assessments for the metabolites of spiroxamine; KWG 4168-desethyl (M01), KWG 4168-despropyl (M02), KWG 4169-N-oxide (M03) and KWG 4168-acid (M06) have been presented below. As for spiroxamine, application rates of 200 g a.s./ha and 300 g a.s./ha (one and two applications) have been considered for both early and late growth stages.

The selection of endpoints for the metabolite risk assessment have been discussed at the start of Section 10.2. As previously discussed, to account for possible selective isomeric degradation, Uncertainty Factors (UF) have been applied to the RAC values (refer to Table CP 10.2-2).

1 x 200 g a.s./ha

Table CP 10.2-20 Aquatic organisms: acceptability of risk (PEC/RAC ≤1) for M01 and M02 based on FOCUS Steps 1 and 2 calculations for application of Spiroxamine EC 500 to vines (1 x 200 g a.s./ha; early application)

Metabolite	**M01**			**M02**		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 737	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 383
AF	100	100	10	100	100	10
RAC (µg/L)	24.1	30.0	73.7	24.1	30.0	38.3
UF	4.76	4.76	4.76	12.5	12.5	12.5
Corrected RAC (µg/L)	5.06	6.30	15.5	1.93	2.40	3.06
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC $_{sw\text{-}max}$ 4.088 µg/L			PEC $_{sw\text{-}max}$ 3.384 µg/L		
	0.807	0.649	0.264	**1.76**	**1.41**	**1.10**
Step 2						
NEU	PEC $_{sw\text{-}max}$ 0.197 µg/L			PEC $_{sw\text{-}max}$ 0.165 µg/L		
	-	-	-	0.0856	0.0688	0.0539
SEU	PEC $_{sw\text{-}max}$ 0.208 µg/L			PEC $_{sw\text{-}max}$ 0.316 µg/L		
		-	-	0.164	0.132	0.103

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor
[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; - not required as risk assessment passes using Step 1 PEC values

Table CP 10.2-21 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M03 and M06 based on FOCUS Steps 1 and 2 calculations for application of Spiroxamine EC 500 to vines (1 x 200 g a.s./ha; early application)

Metabolite	**M03**			**M06**		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} >100000	E_rC_{50} 31700	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 3200
AF	100	100	10	100	100	10
RAC (µg/L)	24.1	1000	3170	24.1	30.0	320
UF	10.0	10.0	10.0	2.32	2.32	2.32
Corrected RAC (µg/L)	2.41	100	317	10.4	12.9	138
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC_{sw-max} 9.611 µg/L			PEC_{sw-max} 78.029 µg/L		
	3.99	0.0961	0.0303	**7.51**	**6.03**	0.566
Step 2						
NEU	PEC_{sw-max} 0.478 µg/L			PEC_{sw-max} 4.308 µg/L		
	0.198	-	-	0.415	0.333	-
SEU	PEC_{sw-max} 0.870 µg/L			PEC_{sw-max} 5.340 µg/L		
	0.361	-	-	0.514	0.413	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor

[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; - not required as risk assessment passes using Step 1 PEC values

Table CP 10.2-22 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M01 and M02 based on FOCUS Steps 1 and 2 calculations for application of Spiroxamine EC 500 to vines (1 x 200 g a.s./ha; late application)

Metabolite	**M01**			**M02**		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 737	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 383
AF	100	100	10	100	100	10
RAC (µg/L)	24.1	30.0	73.7	24.1	30.0	38.3
UF	4.76	4.76	4.76	12.5	12.5	12.5

Metabolite	M01			M02		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Corrected RAC (µg/L)	5.06	6.30	15.5	1.93	2.40	3.06
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC $_{sw\text{-}max}$ 4.088 µg/L			PEC $_{sw\text{-}max}$ 3.384 µg/L		
	0.807	0.649	0.264	**1.76**	**1.41**	**1.10**
Step 2						
NEU	PEC $_{sw\text{-}max}$ 0.375 µg/L			PEC $_{sw\text{-}max}$ 0.164 µg/L		
	-	-	-	0.0851	0.0683	0.0535
SEU	PEC $_{sw\text{-}max}$ 0.268 µg/L			PEC $_{sw\text{-}max}$ 0.224 µg/L		
	-	-	-	0.116	0.0933	0.0731

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor

[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; - not required as risk assessment passes using Step 1 PEC values

Table CP 10.2-23 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M03 and M06 based on FOCUS Steps 1 and 2 calculations for application of Spiroxamine EC 500 to vines (1 x 200 g a.s./ha; late application)

Metabolite	M03			M06		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} >100000	E_rC_{50} 31700	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 3200
AF	100	100	10	100	100	10
RAC (µg/L)	24.1	1000	3170	24.1	30.0	320
UF	10.0	10.0	10.0	2.32	2.32	2.32
Corrected RAC (µg/L)	2.41	100	317	10.4	12.9	138
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC $_{sw\text{-}max}$ 9.611 µg/L			PEC $_{sw\text{-}max}$ 78.029 µg/L		
	3.99	0.0961	0.0303	**7.51**	**6.03**	0.566

Metabolite	M03			M06		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***
Step 2						
NEU	PEC $_{sw\text{-}max}$ 0.637 µg/L			PEC $_{sw\text{-}max}$ 7.746 µg/L		
	0.264	-	-	0.746	0.599	-
SEU	PEC $_{sw\text{-}max}$ 0.726 µg/L			PEC $_{sw\text{-}max}$ 6.715 µg/L		
	0.301	-	-	0.646	0.519	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor

[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; - not required as risk assessment passes using Step 1 PEC values

2 x 200 g a.s./ha

Table CP 10.2-24 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M01 and M02 based on FOCUS Steps 1 and 2 calculations for application of Spiroxamine EC 500 to vines (2 x 200 g a.s./ha; early application)

Metabolite	M01			M02		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 737	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 383
AF	100	100	10	100	100	10
RAC (µg/L)	24.1	30.0	73.7	24.1	30.0	38.3
UF	4.76	4.76	4.76	12.5	12.5	12.5
Corrected RAC (µg/L)	5.06	6.30	15.5	1.93	2.40	3.06
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC $_{sw\text{-}max}$ 4.088 µg/L			PEC $_{sw\text{-}max}$ 3.384 µg/L		
	0.807	0.649	0.264	**1.76**	**1.41**	**1.10**
Step 2						
NEU	PEC $_{sw\text{-}max}$ 0.387 µg/L			PEC $_{sw\text{-}max}$ 0.139 µg/L		
	-	-	-	0.0721	0.0579	0.0454
SEU	PEC $_{sw\text{-}max}$ 0.371 µg/L			PEC $_{sw\text{-}max}$ 0.611 µg/L		
	-	-	-	0.317	0.255	0.199

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor

[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; - not required as risk assessment passes using Step 1 PEC values

Table CP 10.2-25 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M03 and M06 based on FOCUS Steps 1 and 2 calculations for application of Spiroxamine EC 500 to vines (2 x 200 g a.s./ha; early application)

Metabolite	**M03**			**M06**		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} >100000	E_rC_{50} 31700	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 3200
AF	100	100	10	100	100	10
RAC (µg/L)	24.1	1000	3170	24.1	30.0	320
UF	10.0	10.0	10.0	2.32	2.32	2.32
Corrected RAC (µg/L)	2.41	100	317	10.4	12.9	138
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC $_{sw-max}$ 9.611 µg/L			PEC $_{sw-max}$ 78.029 µg/L		
	3.99	0.0961	0.0303	**7.51**	**6.03**	0.566
Step 2						
NEU	PEC $_{sw-max}$ 0.886 µg/L			PEC $_{sw-max}$ 8.015 µg/L		
	0.368	-		0.772	0.620	-
SEU	PEC $_{sw-max}$ 1.614 µg/L			PEC $_{sw-max}$ 9.678 µg/L		
	0.670	-		0.932	0.748	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor

[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; - not required as risk assessment passes using Step 1 PEC values

Table CP 10.2-26 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M01 and M02 based on FOCUS Steps 1 and 2 calculations for application of Spiroxamine EC 500 to vines (2 x 200 g a.s./ha; late application)

Metabolite	**M01**			**M02**		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 737	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 383
AF	100	100	10	100	100	10

Metabolite	M01			M02		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
RAC (µg/L)	24.1	30.0	73.7	24.1	30.0	38.3
UF	4.76	4.76	4.76	12.5	12.5	12.5
Corrected RAC (µg/L)	5.06	6.30	15.5	1.93	2.40	3.06
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC $_{sw\text{-}max}$ 4.088 µg/L			PEC $_{sw\text{-}max}$ 3.384 µg/L		
	0.807	0.649	0.264	**1.76**	**1.41**	**1.10**
Step 2						
NEU	PEC $_{sw\text{-}max}$ 0.723 µg/L			PEC $_{sw\text{-}max}$ 0.311 µg/L		
	-	-	-	0.161	0.130	0.102
SEU	PEC $_{sw\text{-}max}$ 0.508 µg/L			PEC $_{sw\text{-}max}$ 0.427 µg/L		
	-			0.221	0.178	0.139

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor.
[a] Endpoint has assumed equivalent toxicity of the parent material. PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; not required as risk assessment passes using Step 1 PEC values

Table CP 10.2-27 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M03 and M06 based on FOCUS Steps 1 and 2 calculations for application of Spiroxamine EC 500 to vines (2 x 200 g a.s./ha; late application)

Metabolite	M03			M06		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} >100000	E_rC_{50} 31700	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 3200
AF	100	100	10	100	100	10
RAC (µg/L)	24.1	1000	3170	24.1	30.0	320
UF	10.0	10.0	10.0	2.32	2.32	2.32
Corrected RAC (µg/L)	2.41	100	317	10.4	12.9	138
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC $_{sw\text{-}max}$ 9.611 µg/L			PEC $_{sw\text{-}max}$ 78.029 µg/L		
	3.99	0.0961	0.0303	**7.51**	**6.03**	0.566

Metabolite	M03			M06		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***
Step 2						
NEU	PEC $_{sw\text{-}max}$ 1.034 µg/L			PEC $_{sw\text{-}max}$ 14.437 µg/L		
	0.429	-	-	**1.39**	**1.12**	-
SEU	PEC $_{sw\text{-}max}$ 1.325 µg/L			PEC $_{sw\text{-}max}$ 12.247 µg/L		
	0.550	-	-	**1.18**	0.947	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor
[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; - not required as risk assessment passes using Step 1 PEC values

1 x 300 g a.s./ha

Table CP 10.2-28 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M01 and M02 based on FOCUS Steps 1 and 2 calculations for application of Spiroxamine EC 500 to vines (1 x 300 g a.s./ha; early application)

Metabolite	M01			M02		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 737	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 383
AF	100	100	10	100	100	10
RAC (µg/L)	24.1	30.0	73.7	24.1	30.0	38.3
UF	4.76	4.76	4.76	12.5	12.5	12.5
Corrected RAC (µg/L)	5.06	6.30	15.5	1.93	2.40	3.06
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC $_{sw\text{-}max}$ 6.131 µg/L			PEC $_{sw\text{-}max}$ 5.076 µg/L		
	1.21	0.973	0.396	**2.63**	**2.12**	**1.66**
Step 2						
NEU	PEC $_{sw\text{-}max}$ 0.295 µg/L			PEC $_{sw\text{-}max}$ 0.242 µg/L		
	0.0583	-	-	0.126	0.101	0.0790
SEU	PEC $_{sw\text{-}max}$ 0.562 µg/L			PEC $_{sw\text{-}max}$ 0.474 µg/L		
	0.111	-	-	0.246	0.198	0.155

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor

[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; - not required as risk assessment passes using Step 1 PEC values

Table CP 10.2-29 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M03 and M06 based on FOCUS Steps 1 and 2 calculations for application of Spiroxamine EC 500 to vines (1 x 300 g a.s./ha; early application)

Metabolite	**M03**			**M06**		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} >100000	E_rC_{50} 31700	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 3200
AF	100	100	10	100	100	10
RAC (µg/L)	24.1	1000	3170	24.1	30.0	320
UF	10.0	10.0	10.0	2.32	2.32	2.32
Corrected RAC (µg/L)	2.41	100	317	10.4	12.9	138
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC $_{sw-max}$ 14.417 µg/L			PEC $_{sw-max}$ 117.043 µg/L		
	5.98	0.144	0.0455	**11.3**	**9.05**	0.849
Step 2						
NEU	PEC $_{sw-max}$ 0.717 µg/L			PEC $_{sw-max}$ 6.462 µg/L		
	0.298	-	-	0.622	0.500	-
SEU	PEC $_{sw-max}$ 1.305 µg/L			PEC $_{sw-max}$ 11.619 µg/L		
	0.541	-	-	**1.12**	0.899	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor
[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; - not required as risk assessment passes using Step 1 PEC values

Table CP 10.2-30 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M01 and M02 based on FOCUS Steps 1 and 2 calculations for application of Spiroxamine EC 500 to vines (1 x 300 g a.s./ha; late application)

Metabolite	**M01**			**M02**		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 737	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 383
AF	100	100	10	100	100	10

Metabolite	M01			M02		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
RAC (µg/L)	24.1	30.0	73.7	24.1	30.0	38.3
UF	4.76	4.76	4.76	12.5	12.5	12.5
Corrected RAC (µg/L)	5.06	6.30	15.5	1.93	2.40	3.06
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC $_{sw\text{-}max}$ 6.131 µg/L			PEC $_{sw\text{-}max}$ 5.076 µg/L		
	1.21	0.973	0.396	**2.63**	**2.12**	**1.66**
Step 2						
NEU	PEC $_{sw\text{-}max}$ 0.313 µg/L			PEC $_{sw\text{-}max}$ 0.246 µg/L		
	0.0618	-	-	0.128	0.103	0.0803
SEU	PEC $_{sw\text{-}max}$ 0.402 µg/L			PEC $_{sw\text{-}max}$ 0.337 µg/L		
	0.0794			0.175	0.140	0.110

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor
[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; not required as risk assessment passes using Step 1 PEC values

Table CP 10.2-31 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M03 and M06 based on FOCUS Steps 1 and 2 calculations for application of Spiroxamine EC 500 to vines (1 x 300 g a.s./ha; late application)

Metabolite	M03			M06		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} >100000	E_rC_{50} 31700	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 3200
AF	100	100	10	100	100	10
RAC (µg/L)	24.1	1000	3170	24.1	30.0	320
UF	10.0	10.0	10.0	2.32	2.32	2.32
Corrected RAC (µg/L)	2.41	100	317	10.4	12.9	138
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC $_{sw\text{-}max}$ 14.417 µg/L			PEC $_{sw\text{-}max}$ 117.043 µg/L		
	5.98	0.144	0.0455	**11.3**	**9.05**	0.849

Metabolite	M03			M06		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***
Step 2						
NEU	PEC $_{sw-max}$ 0.956 µg/L			PEC $_{sw-max}$ 8.010 µg/L		
	0.397	-	-	0.771	0.619	-
SEU	PEC $_{sw-max}$ 1.09 µg/L			PEC $_{sw-max}$ 10.072 µg/L		
	0.452	-	-	0.970	0.779	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor
[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; - not required as risk assessment passes using Step 1 PEC values

2 x 300 g a.s./ha

Table CP 10.2-32 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M01 and M02 based on FOCUS Steps 1 and 2 calculations for application of Spiroxamine EC 500 to vines (2 x 300 g a.s./ha; early application)

Metabolite	M01			M02		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 737	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 383
AF	100	100	10	100	100	10
RAC (µg/L)	24.1	30.0	73.7	24.1	30.0	38.3
UF	4.76	4.76	4.76	12.5	12.5	12.5
Corrected RAC (µg/L)	5.06	6.30	15.5	1.93	2.40	3.06
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC $_{sw-max}$ 6.131 µg/L			PEC $_{sw-max}$ 5.076 µg/L		
	1.21	0.973	0.396	**2.63**	**2.12**	**1.66**
Step 2						
NEU	PEC $_{sw-max}$ 0.567 µg/L			PEC $_{sw-max}$ 0.479 µg/L		
	0.112	-	-	0.248	0.200	0.156
SEU	PEC $_{sw-max}$ 1.084 µg/L			PEC $_{sw-max}$ 0.917 µg/L		
	0.214	-	-	0.476	0.382	0.299

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor

[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; - not required as risk assessment passes using Step 1 PEC values

Table CP 10.2-33 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M03 and M06 based on FOCUS Steps 1 and 2 calculations for application of Spiroxamine EC 500 to vines (2 x 300 g a.s./ha; early application)

Metabolite	**M03**			**M06**		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} >100000	E_rC_{50} 31700	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 3200
AF	100	100	10	100	100	10
RAC (µg/L)	24.1	1000	3170	24.1	30.0	320
UF	10.0	10.0	10.0	2.32	2.32	2.32
Corrected RAC (µg/L)	2.41	100	317	10.4	12.9	138
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC_{sw-max} 14.417 µg/L			PEC_{sw-max} 117.043 µg/L		
	5.98	0.144	0.0455	**11.3**	**9.05**	0.849
Step 2						
NEU	PEC_{sw-max} 1.329 µg/L			PEC_{sw-max} 12.022 µg/L		
	0.551	-		**1.16**	0.930	-
SEU	PEC_{sw-max} 2.420 µg/L			PEC_{sw-max} 21.656 µg/L		
	1.00	-	-	**2.08**	**1.67**	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor

[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; - not required as risk assessment passes using Step 1 PEC values

Table CP 10.2-34 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M01 and M02 based on FOCUS Steps 1 and 2 calculations for application of Spiroxamine EC 500 to vines (2 x 300 g a.s./ha; late application)

Metabolite	**M01**			**M02**		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 737	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 383
AF	100	100	10	100	100	10

Metabolite	M01			M02		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
RAC (µg/L)	24.1	30.0	73.7	24.1	30.0	38.3
UF	4.76	4.76	4.76	12.5	12.5	12.5
Corrected RAC (µg/L)	5.06	6.30	15.5	1.93	2.40	3.06
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC $_{sw\text{-}max}$ 6.131 µg/L			PEC $_{sw\text{-}max}$ 5.076 µg/L		
	1.21	0.973	0.396	**2.63**	**2.12**	**1.66**
Step 2						
NEU	PEC $_{sw\text{-}max}$ 0.556 µg/L			PEC $_{sw\text{-}max}$ 0.446 µg/L		
	0.110	-	-	0.231	0.186	0.146
SEU	PEC $_{sw\text{-}max}$ 0.763 µg/L			PEC $_{sw\text{-}max}$ 0.641 µg/L		
	0.151			0.332	0.267	0.209

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor
[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; not required as risk assessment passes using Step 1 PEC values

Table CP 10.2-35 Aquatic organisms: acceptability of risk (PEC/RAC <1) for M03 and M06 based on FOCUS Steps 1 and 2 calculations for application of Spiroxamine EC 500 to vines (2 x 300 g a.s./ha; late application)

Metabolite	M03			M06		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***
Endpoint (µg/L)	LC_{50} 2410 [a]	EC_{50} >100000	E_rC_{50} 31700	LC_{50} 2410 [a]	EC_{50} 3000 [a]	E_rC_{50} 3200
AF	100	100	10	100	100	10
RAC (µg/L)	24.1	1000	3170	24.1	30.0	320
UF	10.0	10.0	10.0	2.32	2.32	2.32
Corrected RAC (µg/L)	2.41	100	317	10.4	12.9	138
FOCUS Scenario	PEC/RAC ratios					
Step 1	PEC $_{sw\text{-}max}$ 14.417 µg/L			PEC $_{sw\text{-}max}$ 117.043 µg/L		
	5.98	0.144	0.0455	**11.3**	**9.05**	0.849

Metabolite	M03			M06		
Group	**Fish acute**	**Invertebrate acute**	**Algae**	**Fish acute**	**Invertebrate acute**	**Algae**
Test species	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***	***Danio rerio***	***Daphnia magna***	***Desmodesmus subspicatus***
Step 2						
NEU	PEC $_{sw-max}$ 1.551 µg/L			PEC $_{sw-max}$ 14.517 µg/L		
	0.644	-	-	**1.40**	**1.12**	-
SEU	PEC $_{sw-max}$ 1.988 µg/L			PEC $_{sw-max}$ 18.370 µg/L		
	0.825	-	-	**1.77**	**1.42**	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; UF: Uncertainty Factor

[a] Endpoint has assumed equivalent toxicity of the parent material; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**; - not required as risk assessment passes using Step 1 PEC values

For KWG 4168-desethyl (M01), KWG 4168-despropyl (M02) and KWG 4169-N-oxide (M03) acceptable risks to aquatic organisms have been demonstrated using either FOCUS Step 1 or Step 2 PEC_{sw} values for all proposed uses of Spiroxamine EC 500 on grapes.

For KWG 4168-acid (M06) acceptable risks to aquatic organisms have been demonstrated for the proposed uses of Spiroxamine EC 500 on grapes at 1 x 200 g a.s./ha (early and late application), 2 x 200 g a.s./ha (early applications only) and 1 x 300 g a.s./ha (late application only). For late applications at 2 x 200 g a.s./ha, early application at 1 x 300 g a.s./ha and early and late applications at 2 x 300 g a.s./ha to grapes, possible risks to aquatic organisms have been identified. Further environmental fate data for this metabolite are currently being generated and the PEC_{sw} modelling will be updated and submitted as part of the top-up submission for the Renewal of Approval of spiroxamine.

Formulation risk assessment

A formulation specific risk assessment using the available formulation data with Spiroxamine EC 500 has been conducted and presented below. Formulations are considered to remain intact only for very short periods following application therefore exposure due to spray drift only has been considered here. The maximum single application rates of 200 g a.s./ha and 300 g a.s./ha have been used in the risk assessments below. Further details on the PEC_{sw} calculations can be found in Document M-CP Section 9 Environmental Fate.

Table CP 10.2.36 Aquatic organisms: acceptability of risk (PEC/RAC <1) for Spiroxamine EC 500 based on spray drift PEC_{sw} calculations for application of Spiroxamine EC 500 to vines (2 x 200 g a.s./ha)

Group		Fish acute	Invertebrate acute	Algae
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Scenedesmus subspicatus***
Endpoint (µg/L)		LC_{50} >1500	EC_{50} 10300	E_rC_{50} 29
AF		100	100	10
RAC (µg/L)		115	103	2.9
Water body type	PECsw (µg/L)	PEC/RAC ratios		
Default distance				
Ditch	6.918	0.0602	0.0672	**2.39**

Group		Fish acute	Invertebrate acute	Algae
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Scenedesmus subspicatus***
Pond	0.2456	0.00214	0.00238	0.0847
Stream	5.741	0.0499	0.0557	**1.98**
5 m distance				
Ditch	4.183	0.0364	0.0406	**1.44**
Pond	0.2851	0.00248	0.00277	0.0983
Stream	4.183	0.0364	0.0406	**1.44**
10 m distance				
Ditch	1.515	0.0132	0.0147	0.522
Pond	0.1570	0.00137	0.00152	0.0541
Stream	1.515	0.0132	0.0147	0.522

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

Table CP 10.2-37 Aquatic organisms: acceptability of risk (PEC/RAC <1) for Spiroxamine EC 500 based on spray drift PEC_{sw} calculations for application of Spiroxamine EC 500 to vines (2 x 300 g a.s./ha)

Group		Fish acute	Invertebrate acute	Algae
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Scenedesmus subspicatus***
Endpoint (µg/L)		LC_{50} 11500	EC_{50} 10300	E_rC_{50} 29
AF		100	100	10
RAC (µg/L)		115	103	2.9
Water body type	PECsw (µg/L)	PEC/RAC ratios		
Default distance				
Ditch	10.38	0.0903	0.101	**3.58**
Pond	0.3684	0.00320	0.00358	0.127
Stream	8.612	0.0749	0.0836	**2.97**
5 m distance				
Ditch	6.274	0.0546	0.0609	**2.16**
Pond	0.4277	0.00372	0.00415	0.147
Stream	6.274	0.0546	0.0609	**2.16**
10 m distance				
Ditch	2.273	0.0198	0.0221	0.784
Pond	0.2355	0.00205	0.00229	0.0812
Stream	2.273	0.0198	0.0221	0.784

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the trigger of 1 are highlighted in **bold**

At the default distance of 3 m, possible risks to aquatic organisms have been identified following application of Spiroxamine EC 500, however, acceptable risks to aquatic organisms from exposure to Spiroxamine EC 500 have been demonstrated when suitable mitigation is applied. For the proposed uses on grapes at both 200 g a.s./ha and 300 g a.s./ha a 10 m no spray buffer zone needs to be applied as an application mitigation measure in order for the risks to aquatic organisms from Spiroxamine EC 500 exposure to be acceptable. It should be noted that the proposed mitigation measures for the risk assessment of spiroxamine also cover this mitigation.

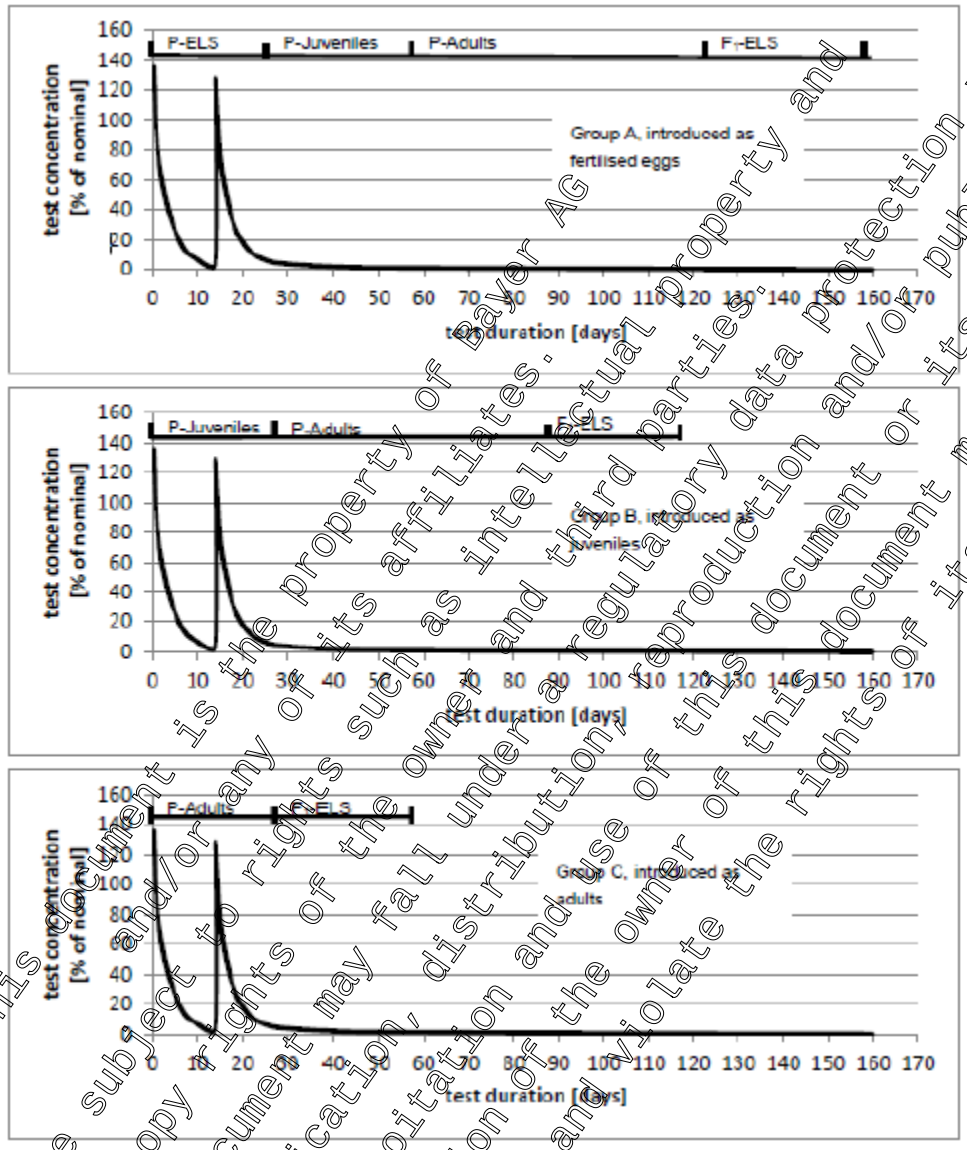
Refined risk assessment for the chronic risk to fish

Two Fish Full Life Cycle (FFLC) studies are available using spiroxamine technical. The first study (M-304458-02-1) was conducted under continuous exposure conditions (*i.e.* a flow-through test design) and provided a NOEC of 2.6 µg a.s./L. In the Assessment report for spiroxamine provided by the Rapporteur Member State (RMS) Germany in September 2009 (Volume 3, Annex B9, pp. 963-969), the RMS calculated an EC_{10} of 2 µg a.s./L. The RMS considered the EC_{10} to be an adequate endpoint for regulation, *i.e.* for Tier 1 risk assessment. This EC_{10} value has been recalculated as part of the current Renewal of Approval of spiroxamine, following an assessment of the statistical methods (M-760413-01-1), and a value of 1.88 µg a.s./L has been determined. Thus, the EC_{10} of 1.88 µg a.s./L has been used in the Tier I chronic fish risk assessment presented above.

The second FFLC study (M-467979-03-1) was conducted as a refinement study and simulated a peak-exposure scenario in the presence of sediment. This refined FFLC study provided NOEC and EC_{10} values of 15.8 and 23.3 µg a.s./L, respectively. In line with the EFSA Aquatic Guidance Document (EFSA PPR Panel 2013), refined exposure-laboratory toxicity tests can be used in the higher tier risk assessment (Tier 2C), if the exposure regime of the higher-tier effect study covers the predicted exposure regime in edge-of-field water bodies.

The objective of the refined FFLC study was to assess the effects of spiroxamine peak-exposure on different life stages of zebrafish (*Danio rerio*) under static conditions in a water-sediment system. A full summary of the study has been provided in Document M-CA, Section 8 and further details can be found in the study report (M-467979-03-1). In short, zebrafish were exposed to two successive pulses of spiroxamine separated by a 14-day interval during a full life-cycle that included F0 early life stages, juvenile growth, adult reproduction, and early life stages of the F1 generation (Figure 10.2-01). The two spiroxamine pulses therefore expose the fish at several different sensitive development stages (fertilised eggs, newly hatched larvae, 4-week old juveniles during growth, and adult during reproduction). The target nominal peak-exposure concentrations were 12, 24, 48 and 192 µg a.s./L. Mean measured peak-exposure concentrations of spiroxamine were 15.3, 30.8, 68.0 and 265.7 µg a.s./L for the first pulse, and 16.2, 30.0, 59.7 and 244 µg a.s./L for the second pulse. The overall measured test concentrations were determined by taking a mean of the two initial peak exposure concentrations.

Figure CP 10.2-01 Spiroxamine peak exposure of different life stages of zebrafish. Exposure started with fertilised eggs, juveniles and adults in Groups A, B and C respectively. (Source M-467979-03-1)



P: Parental generation, F1: Filial 1 generation, ELS: Early Life Stage

The most sensitive biological endpoint was survival of the F1-larvae of group C (parental generation exposed as adults). The corresponding NOEC was determined to be 15.8 µg a.s./L and the EC_{10} was determined to be 23.3 µg a.s./L (expressed as mean measured concentrations). The first and second pulses corresponding to the peak concentrations of 15.3 and 16.2 µg a.s./L, respectively, resulted in no mortality in the exposed fish. The NOEC value of 15.8 µg a.s./L was therefore calculated as the mean of the exposure concentrations at the first and second peaks.

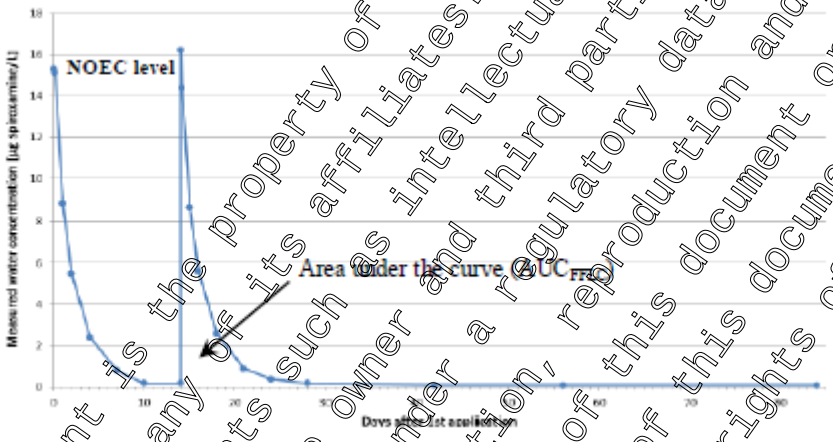
Both EC_{10} and NOEC values have been calculated for the most sensitive endpoint of the refined-exposure FFLC study as outlined in the EFSA Aquatic Guidance Document (2013) and in data requirements (Comission Regulation 283/2013). EFSA Supporting publication 2015: EN-924 states that where a reliable median EC_{10} could be calculated, then the lower between this value and the NOEC should be used. Looking at the study, it is clear that with a limited number of tested doses (4 plus controls) the study has been designed to derive a NOEC and not an EC_x. However, the dose intervals cover an adequate range for EC_x calculation with effect values of 4.4 %, 10.9 %, 50.9 %, and 94.4 % for 15.8, 30.4, 63.9, and 255 µg/L, respectively. Because the confidence limits span a broad range and taking into account the effects actually measured in the test, the EC_{10} was considered as less meaningful,

and the NOEC of 15.8 µg/L was deemed more appropriate for risk assessment. Overall, the NOEC is found more adequate to be used for the risk assessment based on the refined-exposure FFLC study.

The comparison of the two fish full life cycle studies with spiroxamine conducted under continuous and peak exposure conditions, respectively, showed in both studies the same sensitive endpoints indicating consistency of the results of both studies (*i.e.* effects on survival of F1-generation, survival of F0-generation, sex ratio of adults, weight and vitellogenin biomarker for adult females).

Questions have previously been raised regarding this study and the possibility that not all developmental life stages of the fish have been sufficiently exposed in light of the exposure profile achieved in the test. Figure 10.2-02 presents the measured concentrations of spiroxamine determined in the study.

Figure CP 10.2-02 Measured spiroxamine concentration in water samples from study M-467979-03-1, following application of 12 µg a.s./L (NOEC, nominal concentration) on Day 0 and Day 14



Mean measured peak concentrations of Spiroxamine were 15.3 µg a.s./L and 16.2 µg a.s./L for the first and second pulses, respectively. Mean measured NOEC was therefore 15.8 µg a.s./L

The study comprised three test groups in which different life stages of the fish were used at the start of the exposure test. Thus, fertilised eggs, newly hatched larvae, 4-week old juveniles during growth, and adults during reproduction would have been exposed to one or both of the exposure pulses. In a refined laboratory exposure test, such as this, the fundamental concept is to use a more representative exposure regime which mimics the situation in the field. This is achieved by using an exposure regime which is considered to be realistic to worst case when compared to the relevant FOCUS profile(s). A direct consequence of taking this modified exposure approach is that the exposure will not be 'worst case' for the duration of the exposure period. The only way that this can be achieved is under the continuous renewal conditions used in the standard Tier I test design. Thus, by deliberate design, a modified exposure test like this cannot expose every possible part of the organism's life for the duration of the test. However, what it can do is provide results which are considered to be more realistic based on what is likely to occur in the field and therefore the results determined from the test are more realistic and, hence, more relevant. The refined FFLC study has included all of the critical life stages of a fish from embryo through to adult therefore all life stages are considered to have been covered by the test design. Whilst the maximum exposure may not have coincided with, for example, the day of hatching, this critical phase has still been covered by the exposure regime and hatching embryos would have been exposed to spiroxamine. It is therefore considered that the test design was appropriate to sufficiently expose the fish at all sensitive life stages but under conditions which are much more realistic in relation to the environment following application of spiroxamine. Whilst it is accepted that the concentrations of spiroxamine reduced to below LOQ 10 days after application, ultimately this is the purpose of the study as it recreates the typical exposure that would occur in the field.

The results of the refined FFLC study can be compared to the results of the other available Tier I chronic fish data. Two flow through fish early life stage (ELS) studies with rainbow trout using continuous flow-through conditions are available in which the test concentrations were maintained from embryo addition

through to juvenile fish. In the first study (M-006232-01-1) a NOEC was not established but a 93-day EC_0 of 14 µg a.s./L was derived as a surrogate. In the second ELS study (M-006449-01-1) a 96-day NOEC of 14.2 µg a.s./L was derived. In the standard FFLC study with zebrafish, which also used continuous flow-through test conditions, a 230-day NOEC value of 2.6 µg a.s./L was achieved. Although these three studies have used two different fish species and have used different test durations, the results are considered to be largely consistent with each other and provide a good reference point for the chronic NOEC for fish following constant exposure to spiroxamine. The NOEC achieved in the refined FFLC study of 15.8 µg a.s./L is therefore considered to be at a remarkably similar level to those values already achieved. Indeed, the difference between the two FFLC studies using the same test species, but different exposure regimes, is only a factor of 6. Thus, the results achieved under the modified exposure test conditions are highly similar to those results achieved under constant exposure in which all sensitive life stages were exposed to worst case conditions. This would strongly suggest that the exposure regime of the refined FFLC study was sufficient for the toxic effects of spiroxamine to manifest themselves. It is therefore considered that the zebrafish in the refined FFLC study were adequately exposed to spiroxamine, including the most sensitive developmental stages.

The EFSA Aquatic Guidance Document stipulates certain conditions under which modified chronic exposure studies can be used to derive a Chronic RAC for use in a refined risk assessment. These are:

- The (repeated pulsed) exposure regime in the refined laboratory toxicity test is realistic to worst case when compared with the relevant predicted (modelled) field exposure profile.
- The duration of the test is long enough to allow the observation of delayed effects
- The refined chronic RAC is compared with the $PEC_{sw;max}$

In order for the refined Tier 2C RAC value of 1.58 µg a.s./L to be used in the risk assessment it is necessary to compare the exposure profile achieved in this study with the exposure profiles for each of the relevant FOCUS scenarios that did not pass the risk assessment using the Tier I RAC of 0.188 µg a.s./L. Only those FOCUS scenarios that are considered to be covered by the refinement study, in terms of the exposure profile, can use the Tier 2C RAC value in the refined risk assessment. The Tier 2C RAC value would then be compared to the $PEC_{sw;max}$ as required by the Aquatic Guidance Document.

A full analysis of each relevant FOCUS exposure profile in relation to the exposure in the refined FFLC study, along with an assessment of the applicability for use in a refined risk assessment, will be conducted and submitted as part of the top-up submission. Consideration over the length of the test in relation to assessing delayed effects will also be provided.

Illustrative refined risk assessment

The table below presents an illustrative refined risk assessment, using the Tier 2C RAC value of 1.58 µg a.s./L, for the scenarios that did not demonstrate an acceptable risk at Tier I. Note that this has been presented purely in order to demonstrate the potential that the Tier 2C RAC has to refine the risk assessment and to demonstrate an acceptable chronic risk to fish. The current proposed mitigation has been maintained here but it should also be noted that, for scenarios where the Tier 2C RAC can be used, a lower level of mitigation could possibly be used.

Table CP 10.2-38 Summary of potential refined risk assessment for the proposed uses of Spiroxamine EC 500 on grapes using the Tier 2C RAC of 1.58 µg a.s./L

Proposed use of Spiroxamine EC 500 to grapes (g a.s./ha)		FOCUS scenarios for which further refinement is required based on the Tier I RAC of 0.188 µg a.s./L	Step 4 PEC_{sw} using a 25 m nsbz with a 20 m vfs (µg a.s./L)	PEC/RAC ratio based on Tier 2C RAC of 1.58 µg a.s./L
1 x 200	Early	R2 Stream	0.223	0.141
		R3 Stream	0.238	0.151
	Late	D6 Ditch	0.242	0.153
		R2 Stream	0.229	0.145

		R3 Stream	0.238	0.151
2 x 200	Early	D6 Ditch	0.198	0.125
		R2 Stream	0.223	0.141
		R3 Stream	0.238	0.151
		R4 Stream	0.255	0.161
	Late	D6 Ditch	0.250	0.158
		R2 Stream	0.229	0.145
		R3 Stream	0.238	0.151
		R4 Stream	0.230	0.146
1 x 300	Early	D6 Ditch	0.274	0.173
		R1 Stream	0.251	0.159
		R2 Stream	0.335	0.212
		R3 Stream	0.357	0.226
		R4 Stream	0.253	0.160
	Late	D6 Ditch	0.364	0.230
		R1 Stream	0.259	0.164
		R2 Stream	0.343	0.217
		R3 Stream	0.357	0.226
		R4 Stream	0.259	0.164
2 x 300	Early	D6 Ditch	0.297	0.188
		R1 Stream	0.251	0.159
		R2 Stream	0.335	0.212
		R3 Stream	0.357	0.226
		R4 Stream	0.399	0.253
	Late	D6 Ditch	0.376	0.238
		R1 Stream	0.259	0.164
		R2 Stream	0.343	0.217
		R3 Stream	0.357	0.226
		R4 Stream	0.360	0.228

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; nsbz: no spray buffer zone; vfs: vegetated filter strip

It is clear that in situations where the Tier 2C RAC value is considered to be suitable for use in the risk assessment (*i.e.* the refined FFLC study exposure is realistic to worst case in relation to that particular FOCUS scenario exposure profile), all relevant scenarios for all of the proposed uses of Spiroxamine EC 500 could result in PEC/RAC ratios <1, thereby allowing for the demonstration of an acceptable chronic risk to fish when mitigation in the form of a 25 m no-spray buffer zone with a 20 m vegetated filter strip is applied.

Secondary poisoning risk assessment

The Log P_{ow} of spiroxamine is 2.79 and 2.98 at pH 7 for diastomers A and B, respectively but at pH 9 these value are 4.88 and 5.08, respectively therefore a specific risk assessment to address the potential risks of accumulation and biomagnification in the aquatic food chain is required. The worst case BCF value has been determined to be 87 L/kg (M-006479-01-1). With a Log P_{ow} of >3 there is the potential for accumulation of spiroxamine within the aquatic food chain, *via* secondary poisoning of birds and mammals, following consumption of contaminated fish.

In accordance with the EFSA Aquatic Guidance Document[6] and the EFSA Bird & Mammal Guidance Document[7], a secondary poisoning risk assessment has been conducted in order to assess the potential risks of transfer of lipophilic compounds, such as spiroxamine, through the food chain.

The biomagnification factor (BMF) is defined as the relative concentration in a predatory animal compared with the concentration in its prey (BMF = $C_{predator}/C_{prey}$). The Regulatory Acceptable Concentration for secondary poisoning (RAC_{sp}) is calculated for both birds and mammals using the following equations:

$$RAC_{sp} = \frac{NOAEL_{bird}}{5 \times 0.159 \times BCF_{fish} \times BMF} \quad \text{or} \quad \frac{NOAEL_{mammal}}{5 \times 0.142 \times BCF_{fish} \times BMF}$$

In accordance with the Aquatic Guidance Document, for the Tier 1 secondary poisoning risk assessment a default BMF value of 1 is used for compounds with a BCF < 2,000 L/kg. The values of 0.159 and 0.142 are multiplication factors based on a 1000 g bird eating 159 g fish per day and a 3000 g mammal eating 425 g of fish per day. The worst case BCF value of 87 L/kg has also been used in the calculations. The NOAEL for birds is 5.40 mg a.s./kg bw/day and the NOAEL for mammals is 21.0 mg a.s./kg bw/day.

The following RAC_{sp} values have been calculated:

RAC_{sp} bird = 0.0781 mg/L = **78.1 µg a.s./L**

RAC_{sp} mammal = 0.340 mg/L = **340 µg a.s./L**

According to the Aquatic Guidance Document:

If RAC_{sp} >21-day TWA PEC_{sw} - Acceptable risks; no further action necessary

If RAC_{sp} <21-day TWA PEC_{sw} - Refinement is necessary

The highest FOCUS Step 3 TWA PEC_{sw} value for grapes has been determined to be 2.627 µg a.s./L (D6 ditch, 2 x 300 g a.s./ha, late application). This value has therefore been used in the risk assessment. It is clear that the RAC_{sp} values for birds and mammals (78.1 and 340 µg a.s./L, respectively) are greater than the worst case FOCUS Step 3 TWA PEC_{sw} value for spiroxamine (2.627 µg a.s./L) following the representative uses. Thus, the concentrations of spiroxamine will not accumulate within the tissues of birds and mammals at concentrations high enough to cause possible harmful effects following consumption of contaminated fish. A low risk from bioaccumulation within the aquatic food chain is therefore concluded.

Summary of aquatic risk assessment

The acute risks to fish, acute risks to aquatic invertebrates, risks to aquatic macrophytes and to sediment dwelling organisms was demonstrated to be acceptable following all proposed uses of Spiroxamine EC 500 without the need for any mitigation measures. For the chronic risks to fish, chronic risks to invertebrates, risks to algae and to those organisms covered by the mesocosm study (algae and invertebrates) application mitigation was required.

For all organism groups, with the exception of the chronic risk to fish, acceptable risks could be demonstrated for all relevant FOCUS scenarios for all proposed uses of Spiroxamine EC 500 when a 20 m no-spray buffer zone with a 20 m vegetated filter strip is applied as a mitigation measure. For the chronic risk to fish a **25 m no-spray buffer zone with a 20 m vegetated filter strip** was necessary in

[6] Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11 (7): 3290

[7] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. Doi:10.2903/j.efsa.2009.1438

order to demonstrate an acceptable chronic risk for some of the relevant FOCUS scenarios. However, not all scenarios passed the chronic fish risk assessment, as detailed below.

Proposed use of Spiroxamine EC 500 to grapes (g a.s./ha)		**FOCUS scenarios for which acceptable risks have been demonstrated using a 25 m nsbz with a 20 m vfs**	**FOCUS scenarios for which further refinement is required**
1 x 200	Early	D6 Ditch, R1 Pond*, R1 Stream, R4 Stream	R2 Stream, R3 Stream
	Late	R1 Pond*, R1 Stream, R4 Stream	D6 Ditch, R2 Stream, R3 Stream
2 x 200	Early	R1 Pond*, R1 Stream	D6 Ditch, R2 Stream, R3 Stream, R4 Stream
	Late	R1 Pond*, R1 Stream	D6 Ditch, R2 Stream, R3 Stream, R4 Stream
1 x 300	Early	R1 Pond*	D6 Ditch, R1 Stream, R2 Stream, R3 Stream, R4 Stream
	Late	R1 Pond*	D6 Ditch, R1 Stream, R2 Stream, R3 Stream, R4 Stream
2 x 300	Early	R1 Pond	D6 Ditch, R1 Stream, R2 Stream, R3 Stream, R4 Stream
	Late	R1 Pond	D6 Ditch, R1 Stream, R2 Stream, R3 Stream, R4 Stream

nsbz: no spray buffer zone; vfs: vegetated filter strip
* R1 Pond scenario for this use passes the risk assessment at Step 3 therefore no mitigation required

At 1 x 200 g a.s./ha and 2 x 200 g a.s./ha at least one full FOCUS scenario passed the risk assessment. At the 1 x 300 g a.s./ha and 2 x 300 g a.s./ha uses, no complete FOCUS scenario passed the risk assessment, thereby requiring refinement of the chronic fish risk assessment. Justification to use a refined chronic fish RAC value to further refine the risk assessment will be provided in the top-up submission later this year.

Following a formulation specific risk assessment, considering spray drift only, the risks to aquatic organisms were demonstrated to be acceptable when a 10 m no-spray buffer zone is used as application mitigation. This mitigation is covered by the mitigation of a **25 m no-spray buffer zone with a 20 m vegetated filter strip** already proposed above.

The risks to aquatic organisms from exposure to the metabolites KWG 4168-desethyl (M01), KWG 4168-despropyl (M02) and KWG 4169-N-oxide (M03) was demonstrated to be acceptable following all proposed uses of Spiroxamine EC 500. For KWG 4168-acid (M06) acceptable risks to aquatic organisms have been demonstrated for the proposed uses of Spiroxamine EC 500 on grapes at 1 x 200 g a.s./ha (early and late application), 2 x 200 g a.s./ha (early applications only) and 1 x 300 g a.s./ha (late application only). For late applications at 2 x 200 g a.s./ha, early application at 1 x 300 g a.s./ha and early and late applications at 2 x 300 g a.s./ha to grapes, possible risks to aquatic organisms have been identified following exposure to M06. Further environmental fate data for this metabolite are currently being generated and the PEC_{sw} modelling will be updated and submitted as part of the top-up submission later this year.

The potential risks from bioaccumulation of spiroxamine within the aquatic food chain have been demonstrated to be low.

Biodiversity

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on aquatic organisms. Therefore, it is considered that the potential impact of the active substance on biodiversity and the ecosystem, including potential

indirect effects *via* alteration of the food web, are covered by the risk assessment for aquatic organisms in this section and in the ED hazard assessment.

Sumaries of the available data with Spiroxamine EC 500 are presented below.

CP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Data Point:	KCP 10.2.1/01
Report Author:	[redacted]
Report Year:	1994
Report Title:	KWG 4168 500 EC - Acute toxicity (96 h) to rainbow trout in a static test
Report No:	DOM 93057
Document No:	M-006610-01-1
Guideline(s) followed in study:	OECD 203 (1992)
Deviations from current test guideline:	The loading rate was 1.2 g fish/L (the current guidance recommends a loading of <0.85 g/L), however, the health and survival of the fish in the control would suggest this had no impact on the test Only one set of observations in the first 24 hours of the test
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The acute toxicity of KWG 4168 500 EC to Rainbow trout (*Oncorhynchus mykiss*) was determined in a static 96-hour test. Six test concentrations were assessed, along with a control. Each treatment group contained 20 rainbow trout.

At test termination, mortalities of 0, 0, 0, 0, 100, 100 and 100% were observed in the control, 3.16, 5.62, 10.0, 17.8, 31.6 and 56.2 mg/L test concentrations.

No sub-lethal effects were observed in the control, 3.16 and 5.62 mg/L test concentrations, whereas, fish were observed showing irregular swimming behaviour in the 17.8, 31.6 and 56.2 mg/L test groups at the 4 hour observations.

Measured test concentrations of 2.28, 4.83, 6.70, 19.8, 29.1 and 57.9 mg/L were achieved in the test.

Based on measured test concentrations the LC_{50} (96-hour) of KWG 4168 500 EC (active ingredient 494 g/L) to rainbow trout (*Oncorhynchus mykiss*) in a static 96-hour test was determined to be 11.5 mg test substance/L with a 95% confidence interval from 6.70 - 19.8 mg test substance/L (binomial probability).

The lowest lethal concentration (LLC) was 19.8 mg test substance/L, the lowest-observed-effect-concentration (LOEC) was 6.70 mg test substance/L and the no-observed-effect-concentration (NOEC) was 4.83 mg test substance/L.

I. Materials and Methods

Materials

Test Material	KWG 4168 500 EC
Lot/Batch #:	04023/0021
Purity (a.s.):	494 g/L

Description:	Clear yellow liquid
Stability of test compound:	Not reported
Reanalysis/Expiry date:	Not reported
Density:	Not reported

Treatments

Test rates:	Nominal:	3.16, 5.62, 10.0, 17.8, 31.6 and 56.2 mg test substance/L
	Measured:	2.28, 4.83, 6.70, 19.8, 29.1 and 57.9 mg test substance/L
Solvent/vehicle:	Test water	
Analysis of test concentrations:	Yes, mean measured concentrations 67 to 111% of nominal	

Test organisms

Species:	Rainbow Trout (*Oncorhynchus mykiss*)
Source:	G.Mueller, D-37186 Moringen
Acclimatisation period:	All test fish were held in culture tanks on a 16/8 hour light/dark photoperiod and observed for at least 14 days prior to testing. Less than 3 % mortality as noted prior to the test initiation. In the 48 hour acclimation period before testing less than 5 percent of fish died.
Feeding:	Not fed during test
Treatment for disease:	There was no treatment of the fish from this lot until used in this test.

Test design

Test vessel:	The aquaria were made of glass and had a size of 32 x 36 x 38 cm containing 40 L test solution
Test medium:	Reconstituted water prepared by adding salt stock solutions
Replication:	Single replicate
No. of animals/vessel:	Twenty fish per vessel
Duration of test:	96 hours

Environmental test conditions

Temperature:	11 °C
Dissolved oxygen:	5.6 to 10.3 mg/L
pH:	6.8 to 7.4
Photoperiod:	16 hours light : 8 hours dark

Study Design

An acute 96-hour toxicity test was conducted to estimate the toxicity of KWG 4168 500 EC to rainbow trout *(Oncorhynchus mykiss)*. The primary measure for acute toxicity was mortality. Sublethal and behavioral responses were also observed during the course of the study. Results of the test were expressed as a 96-hour median lethal concentration (LC_{50}) which is the concentration of KWG 4168 500 EC estimated to be lethal to 50 percent of the test population of fish at the specified time.

Nominal test concentrations were 3.16, 5.62, 10.0, 17.8, 31.6 and 56.2 mg/L. Mean measured concentrations were 2.28, 4.83, 6.70, 19.8, 29.1 and 57.9 mg/L.

Test vessels were glass aquaria and had a size of 32 x 36 x 38 cm (l x d x h). The test volumes amounted to 40L. To each vessel 20 fish were added, which were observed after four hours and then daily for mortalities and symptoms of intoxication.

Dissolved oxygen, temperature and pH values were determined daily in each aquarium.

Analytical determinations of the active substance were made in the test medium at test initiation and termination.

Analytical method

Samples of water were analysed using the validated analytical method 00252 M001, report reference M-008490-02-2 **(see Doc MCP Section 5)**.

II. Results and Discussion

Validity criteria were not assessed in the study report.

At Day 0 measured concentrations ranged between 59 and 111 % of nominal and at Day 4 measured concentrations ranged between 60 and 78 % of nominal. The test substance was quite stable under test conditions as shown by the percent ranges for measured analytical values observed between Day 0 and Day 4 for the individual test levels.

Table CP 10.2.1/01-1 Measured concentrations during the test

Nominal concentration (mg test substance/L)	Measured concentration of nominal (%)[1]		Mean calculated concentration Day 0 - Day 4 (%)	Recalculated mean measured concentration (mg test substance/L)
	Day 0	Day 4		
0 (Control)**	<LOD	<LOD	<LOD	0
3.16	84	60	72	2.28
5.62	94	78	86	4.83
10.0	59	75	67	6.70
17.8	111	-	111	19.8
31.6	92	-	92	29.1
56.2	103	-	103	57.9

[1] Average of two measurements, corrected by the recoveries

Limit of detection: 0.01 mg a.s./L

No mortalities or observable symptoms of intoxication were observed in the control, 2.28 or 4.83 mg/L mean measured groups. All fish in the 6.70 mg/L test group were observed with toxic symptoms and swimming behaviour slightly irregular (slight symptom). All the fish in the 19.8, 29.1 and 57.9 mg/L groups died during the 96-hour test period.

Table CP 10.2.1/01-2 Mortalities and symptoms of intoxication (number dead / number affected with symptoms) (description of observed symptoms)

Mean measured concentration (mg test substance/L)	4 hours	24 hours	48 hours	72 hours	96 hours
0 (Control)	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
2.28	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
4.83	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
6.70	0 / 20 (SN)	0 / 20 (SN)	0 / 20 (SN)	0 / 20 (SN)	0 / 20 (SN)
19.8	20 / 20	-	-	-	-
29.1	20 / 20	-	-	-	-
57.9	20 / 20	-	-	-	-

Dead fish are added to the sum of fish with symptoms

SN: Swimming behaviour slightly irregular (slight symptom)

Table CP 10.2.1/01-3 Study end-points based on mean measured concentrations (mg test substance/L)

End point	24 hours	48 hours	72 hours	96 hours
LC_{50}	11.5	11.5	11.5	11.5
LOEC	-	-	-	6.70
NOEC	-	-	-	4.83

III. Conclusion

The LC_{50} (96-hour) of KWG 4168 500 EC (active ingredient 484 g/L) to rainbow trout (*Oncorhynchus mykiss*) in a static 96-hour test was determined to be 11.5 mg test substance/L (equivalent to 5.7 mg a.s./L) with a 95% confidence interval from 6.70 - 19.8 mg test substance/L.

The lowest lethal concentration (LLC) was 19.8 mg test substance/L, the lowest-observed-effect-concentration (LOEC) was 6.70 mg test substance/L and the no-observed-effect-concentration (NOEC) was 4.83 mg test substance/L.

All reported results are related to mean measured concentrations of the test substance.

Assessment and conclusion by applicant:

The study was conducted to the original 1992 version of the OECD 203 test guideline. Validity criteria according to the current OECD 203 (2019) guideline have been assessed and were met:

- Control mortality must not exceed 10% at the end of the test (actual: 0%)
- Dissolved oxygen concentration in all test vessels to be ≥60% of the air saturation value (actual: 9.6 to 10.3 mg/L)
- Analytical measurement of test concentrations is compulsory (analysis was performed)

The study is therefore considered acceptable.

The LC_{50} (96-hour) was determined to be 11.5 mg test substance/L (equivalent to 5.7 mg a.s./L).

Data Point:	KCP 10.2.1/02
Report Author:	[redacted]
Report Year:	1994
Report Title:	Acute toxicity of KWG 4168 EC 500 to waterfleas (Daphnia magna)
Report No:	HBF/DM 123
Document No:	M-006630-01-1
Guideline(s) followed in study:	OECD 202 (1984)
Deviations from current test guideline:	Daphnids were 10/vessel instead of the recommended 5/vessel Temperature was measured in only one vessel and at the end of the study Three replicate vessels used, 4 required by current guidance
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The 48-hour acute toxicity of KWG 4168 EC 500 to *Daphnia magna* was assessed under static conditions. Test species were exposed to nominal test concentrations of 0.65, 2.03, 3.62, 6.42, 11.4, 20.3 and 64.2 mg/L for 48 hours. Immobilisation and sub-lethal effects were observed after 24 and 48 hours.

The 48-hour EC_{50} value was 10.3 mg formulation/L and the no observed effect concentration (NOEC) (48 hours) was 3.62 mg formulation/L. The lowest observed effect concentration (LOEC) was 6.42 mg formulation/L and the threshold effect concentration was 4.82 mg formulation/L (TEC, geometric mean of NOEC and LOEC).

I. Materials and Methods

Materials

Test Material KWG 4168 EC 500

Lot/Batch #: 089-A according to 94023/0021

Purity: 49.2% (a.s. content)

Description: Clear yellowish liquid

Stability of test compound: Stable for the duration of the test, as shown by the results of the 48-hr analytical determination

Reanalysis/Expiry date: 17 March 1994

Density: 1.004 g/mL

Treatments

Test rates: 0.65, 2.03, 3.62, 6.42, 11.4, 20.3 and 64.2 mg formulation/L

Solvent/vehicle: None

Analysis of test concentrations: Yes, mean measured concentrations 67.7 to 148.7% of nominal

Test organisms

Species: *Daphnia magna*, first instar (6 – 24 hrs old)

Source:	Bundesgesundheitsamt in Berlin
Acclimatisation period:	This strain has been maintained in the laboratory (2 litre containers), the water in which they are kept is changed weekly (dilution water was M7-medium), 2 0 ± 1 °C, 16 : 8 hour light-dark cycle); the animals were fed single cell green algae *Scenedesmus subspicatus* and occasionally some commercial ornamental fish feed (trade name: TetraMinR) (aqueous suspension)
Feeding:	Not fed during the test
Treatment for disease:	None reported
Test design	
Test vessel:	100-mL beakers with plexi glass plates for lids
Test medium:	M7-medium
Replication:	Three replicates
No. of animals/vessel:	Ten daphnids per vessel
Duration of test:	48 hrs
Environmental test conditions	
Temperature:	Test end: 19.9°C
Dissolved oxygen:	Test start: 8.7 – 8.8 mg/L Test end: 8.3 – 8.7 mg/L
pH:	Test start: 7.98 – 8.03 Test end: 7.93 – 8.06
Photoperiod:	16 h light : 8 h dark

Study Design

This study was conducted to assess the acute toxicity of KWG 4168 EC 500 to the water flea *Daphnia magna* over 48 hours. Test concentrations were based on the results of a preliminary non-GLP test.

Daphnia magna were used in the test from an in-house culture, aged 6 to 24 hours. First instar daphnids were separated from older daphnids by sequential mesh screening.

Test vessels were 100-mL beakers containing 50 mL test solution, covered with a plexi glass plate. Beakers were held in a climatic chamber for 48 hours at 20 ± 1°C under a photoperiod of 16 hours light to 8 hours dark.

For the test, 32.1 mg and 40.6 mg of the test substance were weighed into 500 and 2000 mL test water, respectively, in order to prepare the nominal concentrations of 64.2 and 20.3 mg formulation/L. The solutions were stirred for 10 minutes with a magnetic stirrer. Lower concentrations were prepared from the concentration 20.3 mg formulation/L by dilution. Using a pipette, ten 6 - 24 hour old first instars were carefully transferred into each beaker. The animals were randomly distributed to the single beakers. Three beakers, each containing ten water fleas in 50 mL test water, were used for each concentration. Nominal concentrations were 0.65, 2.03, 3.62, 6.42, 11.4, 20.3 and 64.2 mg/L.

To each test concentration were added ten first instar *Daphnia magna* using a pipette. Three replicates were used per concentration.

After 24 and 48 hours, water fleas were assessed visually by counting survivors, *i.e.* animals with swimming movements within 15 seconds of gentle agitation of the test vessel, and any uncertainty was checked using a stereomicroscope.

Temperature, oxygen content and pH of the test water was determined using electronic measuring equipment. Temperature was determined at test end, and oxygen content and pH were determined both at test start and test end.

EC_{50} determination was probit-analysis after the maximum likelihood method using a calculator.

Analytical method

Samples of water were analysed using the validated analytical method 00252 M001, report reference M-008490-02-2 **(see Doc MCP Section 5)**.

II. Results and Discussion

The study was deemed valid in the report as control mortalities were less than 10%.

The active substance content analysed at the beginning of the test showed recoveries of 93.0 to 148.7% of the nominal concentrations (for an average: 114.9%). These results indicate that the test concentrations prepared in this test correspond well to the nominal concentrations.

Moreover, the active substance content in the test concentrations of 2.03, 6.42 and 20.3 mg formulation/L were analysed at the end of the 48 hours exposure period. The active substance contents at the end of the test are slightly lower (19%) than those at the start of the test.

The results have been expressed in terms of nominal concentrations.

Table CP 10.2.1/02-1 Measured concentrations of KWG 4168 during the test

Nominal concentration		Analysed concentration (mg a.s./L)			
(mg formulation/L)	(mg a.s./L)	0 hours	% of nominal value	48 hours	% of 0-hour values
0.65	0.32	0.40	125.0		-
2.03	1.00	0.93	93.0	0.82	88.2
3.62	1.78	1.7	95.5	-	-
6.42	3.16	3.1	98.1	2.7	87.1
11.4	5.61	6.4	114.1	-	-
20.3	10.0	13.0	130.0	8.8	67.7
64.2	31.6	47.0	148.7	-	-

The number of immobilised water fleas after 24 and 48 hours are presented below along with observed abnormalities.

Table CP 10.2.1/02-2 Immobility of *Daphnia magna* after 48-hr exposure to KWG 4168

Measured concentrations (mg a.s./L)	Rep.	Number of living animals after		Immobilised water fleas (%) after	
		24 hours	48 hours	24 hours	48 hours
Control	1	10	10	0	0
	2	10	10		
	3	10	10		

Measured concentrations (mg a.s./L)	Rep.	Number of living animals after		Immobilised water fleas (%) after	
		24 hours	48 hours	24 hours	48 hours
0.65	1	10	10	0	0
	2	10	10		
	3	10	10		
2.03	1	10	10	0	0
	2	10	10		
	3	10	10		
3.62	1	10	9	0	3 ± 6
	2	10	10		
	3	10	10		
6.42	1	9	6 [3] [6, 4]	3 ± 6	23 ± 15
	2	10	9 [4] [6, 4]		
	3	10	8 [3] [6, 4]		
11.4	1	9	8 [3] [6, 4]	10 ± 0	37 ± 15
	2	9	6 [4] [6, 4]		
	3	9 [2] [5, 4]	5 [5] [6, 4]		
20.3	1	7 [2] [6, 4]	0	43 ± 12	100
	2	5 [3] [6, 4]	0		
	3	5 [2] [6, 4]	0		
64.2	1	0	0	100	100
	2	0	0		
	3	0	0		

[ ] number of living animals showing symptoms (if observed
* given as mean ± standard deviation (n = 1 method)
Symptoms:

1 Quick, trembling antennae movements
2 Frequency of antennae movements clearly increased
3 Frequency of antennae movements clearly decreased
4 Hardly any loveients perceivable
5 Swimming movements show coordination disturbances
6 Animals lay at the bottom
7 Animals cling to the water surface
8 Animals cling together in clusters

Table CP 10.2.1/02-3 Summary of endpoints after 48-hour exposure to KWG 4168 EC 500

Endpoint (mg formulation/L)	EC_{50}	NOEC	LOEC	TEC
24-hour	21.4	6.42	11.4	8.55
48-hour	10.3	3.62	6.42	4.82

TEC, geometric mean of NOEC and LOEC

III. Conclusion

The 48-hour EC_{50} value for *Daphnia magna* exposed to KWG 4168 EC 500 was 10.3 mg formulation/L (equivalent to 5.07 mg a.s./L) and the no observed effect concentration (NOEC) was 3.62 mg formulation/L. The lowest observed effect concentration (LOEC) was 6.42 mg formulation/L.

Assessment and conclusion by applicant:

The study was conducted to an older version of the OECD 202 test guideline. The study has therefore been assessed against the most recent version (April 2004).

Validity criteria according to OECD 202 (2004) were met:

- Mortality/immobilisation in the control to not exceed 10% (actual: 0.0%)
- Dissolved oxygen concentration at test termination to be ≥3 mg/L in all test vessels (actual: 8.3 to 8.8 mg/L)

This study used three replicates of 10 organisms which is a deviation from current guideline requirements of four replicates of 5 organisms but as the total number of organisms used in this study was greater than that required, this deviation is not considered to have had a detrimental impact and the results are still considered to be valid.

The study is therefore considered acceptable.

The 48-hour EC_{50} value was determined to be 10.3 mg formulation/L (equivalent to 5.07 mg a.s./L).

Data Point:	KCP 10.2.1.03
Report Author:	[redacted]
Report Year:	1994
Report Title:	Influence of KWG 4168 500 EC on the growth of the green alga, Scenedesmus subspicatus
Report No:	AJO/122094
Document No:	M-086617-01-1
Guideline(s) followed in study:	OECD-Guideline No. 201 (1984), "OECD-Guideline for Testing of Chemicals", "Alga, Growth Inhibition Test" (7 June 1984).
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In a 72-hour toxicity study, cultures of green alga, *Scenedesmus subspicatus*, were exposed to KWG 4168 EC 500 at nominal test concentrations of 0.00032, 0.00056, 0.0010, 0.0018, 0.0032, 0.0056, 0.010, 0.018 and 0.032 mg/L.

The growth rate inhibition in the treated algal culture as compared to the control ranged from -11.8% to 52.4%. The biomass inhibition in the treated algal culture compared to the control ranged from -20.9% to 85.6%.

Based on nominal test concentrations, the E_rC_{50} and E_bC_{50} values were determined to be 0.029 and 0.012 mg/L, respectively.

I. Materials and Methods

Materials

Test Material	KWG 4168 500 EC
Lot/Batch #:	089A
Purity:	491.4 g/L
Description:	Clear, yellow liquid
Stability of test compound:	Not reported
Reanalysis/Expiry date:	16 December 1994
Density:	Not reported
Treatments	
Test rates:	Nominal 0.00032, 0.00056, 0.0010, 0.0018, 0.0032, 0.0056, 0.010, 0.018 and 0.032 mg/L
Solvent/vehicle:	None
Analysis of test concentrations:	Yes, measured concentrations 76.6 to 96.6% of nominal. The average measured concentration was 88.5% of nominal.
Test organisms	
Species:	Green alga, *Scenedesmus subspicatus*
Source:	Collection of Algal Cultures, Inst. Plant Physiology, Universitat Göttingen, Nikolausberger Weg 18, 37077 Göttingen, Germany
Treatment for disease:	None reported
Test design	
Test vessel:	Cotton plugged, 300-mL Erlenmeyer flasks
Test medium:	Sterile, deionised water and nutrient solution
Replication:	6 replicates for the control, 3 per test concentration
Initial cell density:	1 x 10^4 cells/mL
Duration of test:	72 hours
Environmental test conditions	
Temperature:	23 ± 2°C
Photoperiod:	24-hr a day at 8000 lux

Study Design

Green alga (*Scenedesmus subspicatus*) were exposed to KWG 4168 EC 500 over 72 hours to determine inhibition of growth of biomass and inhibition of the growth rate.

Stock cultures and pre-cultures of the alga were grown in nutrient solutions. The pre-cultures were inoculated with 1 x 10^4 cells/mL. Water, nutrient solution and stock solution of the product were mixed together and split into two parts. One part was used for growth inhibition tests by inoculating it with

enough pre-culture to give a density of 1 x 10^4 cells/mL. The second part was used for quantitative analyses and was not mixed with algal pre-culture.

The tests were conducted in 300mL Erlenmeyer flasks sealed with cotton wool plugs and placed in an incubator at 23 ± 2°C and 8000 lux 24 h/day. Temperature and pH was determined using electronic equipment throughout the study.

The nominal concentration of KWG 4168 EC 500 tested were 0.00032, 0.00056, 0.0010, 0.0018, 0.0032, 0.0056, 0.010, 0.018 and 0.032 mg/L. Three replicates of each test concentration were conducted and six replicates of the control. The reference chemical $K_2Cr_2O_7$ was tested at 0.10, 0.18, 0.32, 0.56, 1.00 and 1.80 mg/L.

Algal cell numbers were determined microscopically at 400x magnification using a Thoma counting chamber.

Analytical method

Samples of water were analysed using the validated analytical method 00252/M001, report reference M-008490-02-2 **(see Doc MCP Section 5)**.

II. Results and Discussion

Validity criteria were not assessed in the study report.

The analytical results are presented below and confirmed the correct dosing of the test system. All results have been expressed in terms of the nominal concentration.

Table CP 10.2.1/03-1 Initial measured concentrations of KWG 4168 500 EC

Nominal concentration (µg test item/L)	Nominal concentration (µg a.s./L)	Initial measured concentration (µg a.s./L)	Difference to the control (%)
0.32	0.16	0.15	93.8
0.56	0.27	0.26	96.3
1.0	0.49	0.41	83.7
1.8	0.88	0.85	96.6
3.2	1.56	1.5	96.2
5.6	2.74	2.1	76.6
10	4.89	4.3	87.9
18	8.80	7.2	81.8
32	15.6	13	83.3
		Mean:	88.5

The highest deviation from the control in growth rate was observed in the test with 0.032 mg/L KWG 4168 EC 500 after 72 hrs with growth rates inhibited by 52.4% when compared to the control. The greatest percentage inhibition in biomass was observed after 72 hrs in the test vessel treated with the highest dose of 0.032 with 85.6%.

At a concentration of 0.0056 mg/L, some cells were deformed. At concentrations of 0.010, 0.018 and 0.032 mg/L, some cells were observed to be swollen with deformed cell walls.

Table CP 10.2.1/03-2 Inhibition of growth rate and biomass (relative to control) in *Scenedesmus subspicatus* exposed to KWG 4168 500 EC

Nominal concentration (mg/L)	% inhibition in growth rate			% inhibition in biomass		
	24 h	48 h	72 h	24 h	48 h	72 h
Control	-	-	-	-	-	-
0.00032	-11.8	4.1	-0.6	-20.9	7.6	2.1
0.00056	17.7	2.3	-2.1	25.6	12.0	-2.8
0.0010	0.1	8.7	0.0	2.3	18.7	7.3
0.0018	-3.9	-4.2	-1.2	-11.6	-14.7	-9.1
0.0032	-7.1	3.2	-1.6	-11.6	7.1	-1.7
0.0056	0.4	5.8	-1.3	2.3	14.2	4.4
0.010	48.3*	24.7*	8.4	58.1*	58.2	42.5*
0.018	26.4*	31.8*	30.7*	44.2	64.0	72.5*
0.032	17.7*	44.8*	52.4*	30.2	71.6	85.6*

* t-values analysed in "Dunnett's Test" that are statistically different from the control

A summary of the relevant endpoints determined in the report are presented below.

Table CP 10.2.1/03-3 Summary of derived endpoints

Growth rate	
E_rC_{50}:	0.029 mg/L
LOE_rC:	0.018 mg/L
NOE_rC:	0.010 mg/L
Effect threshold:	0.013 mg/L
Biomass	
E_bC_{50}:	0.012 mg/L
LOE_bC:	0.010 mg/L
NOE_bC:	0.006 mg/L
Effect threshold:	0.007 mg/L

III. Conclusion

The growth rate values (72 hour) determined for *Scenedesmus subspicatus* in KWG 4168 500 EC - treated nutrient medium were, E_rC_{50} 0.029 mg/L (equivalent to 0.0143 mg a.s./L), LOE_rC 0.018 mg/L and NOE_rC 0.010 mg/L.

The biomass values (72 hour) determined for *Scenedesmus subspicatus* in KWG 4168 500 EC - treated nutrient medium were an E_bC_{50} of 0.012 mg/L (equivalent to 0.0059 mg a.s./L), a LOE_bC of 0.010 mg/L and a NOE_bC of 0.006 mg/L.

Assessment and conclusion by applicant:

The study was conducted to the OECD 201 test guideline (1984), the current version of which is the OECD 201 "Freshwater alga and cyanobacteria, growth inhibition test", adopted 28 July 2011.

Validity criteria have therefore been re-assessed against the criteria in the current 2011 version of the guideline and have been met.

- The biomass in the control cultures should have increased exponentially by a factor of ≥16 within the 72-hour test period (actual: 118);
- The mean coefficient of variation for section-by-section specific growth rates in the control cultures <35% (actual: 22.9%);
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures <10% (actual: 2.04%).

It is noted that chemical analysis of the test media only took place at 0 hours therefore, although it can be confirmed that nominal test concentrations were achieved at the start of the test, there is no information on the stability of the test concentations over the course of the 72-hour test period. The results have been based on nominal test concentrations but there is the possibility that this may under-estimate the E_rC_{50} which could be lower if it were based on mean measured test concentrations based on recoveries from 0 hours and 72 hours. However, several other algal studies with technical spiroxamine are available and demonstrate that spiroxamine is relatively stable over this 72-hour period under algal test conditions. It is therefore considered that the results determined in this test, based on nominal test concentrations, are likely to be reflective of the mean concentration achieved over the course of the test.

Furthermore, the results of this formulation study are considered to be highly consistent with the results of the technical material studies with several species of green algae and therefore there is no indication that the formulation has any greater toxicity when compared to the active substance alone. It is considered that the formulation reflects the toxicity of the active substance.

It is also noted that this study does not provide the most conservative endpoint for the Tier I algal risk assessment and, as a result, is not directly used in the risk assessment.

For these reasons the study is considered to be valid and acceptable.

The E_rC_{50} was determined to be 0.029 mg/L (equivalent to 0.0143 mg a.s./L).

The data have been subjected to statistical re-evaluation and the results have been presented in the following study summary.

Data Point:	KCP 10.2.1/04
Report Author:	[redacted]
Report Year:	2020
Report Title:	Calculation of EC10, EC20 and EC50 values for Scenedesmus subspicatus with KWG 4168, 500 EC in an algal growth inhibition test
Report No:	0471836-ECO28
Document No:	M-761431-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted None
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The report M-006617-01-1 on the effects of exposure to KWG 4168 500 EC on the growth of algae (*Scenedesmus subspicatus*) did not provide estimates of EC_{10} or EC_{20} values. Therefore, these values as well as EC_{50} values have been calculated in accordance with the Annex to Com. Reg. 283/2013.

The resulting EC_{10}, EC_{20} and EC_{50} values for yield at 72 h were 3.20, 4.02 and 6.22 µg a.s./L, respectively. For growth rate after 72 h, the EC_{10}, EC_{20} and EC_{50} values were 4.90, 7.09 and 14.41 µg a.s./L, respectively.

I. Methods

The statistical evaluation was performed with statistical software ToxRatPro v3.3.0.

Effect concentrations with 10, 20 and 50% from the test item treatment when compared to the control were determined for yield and growth rate after 72 hours exposure. A Probit regression was performed for both measures, with confidence limits estimated according to Fieller's theorem.

II. Results and Discussion

Yield at 72 hours

Regarding the calculation of EC_{10}, EC_{20} and EC_{50} values for yield at 72 h, a statistically significant concentration/response was found (p(F) <0.001) for this parameter.

The resulting EC_{10}, EC_{20} and EC_{50} values and the respective confidence intervals are represented in the following table below.

Table CP 10.2.1/04-1 Results of the Probit analysis of yield at 72 h: Selected effective concentrations (EC_x) of the test item and their 95%-confidence limits

Parameter	Yield		
	EC_{10} (95 % confidence interval) [µg a.s./L]	EC_{20} (95 % confidence interval) [µg a.s./L]	EC_{50} (95 % confidence interval) [µg a.s./L]
Effect on yield at 72 h	3.20 (2.70 – 3.62)	4.02 (3.54 – 4.42)	6.22 (5.79 – 6.67)

The resulting EC_{10}, EC_{20} and EC_{50} values of 3.20 (95%CL: 2.70 – 3.62), 4.02 (95%CL: 3.54 – 4.42) and 6.22 (95%CL: 5.79 – 6.67) µg a.s./L, respectively, meet the goodness of fit criteria showing a significant concentration/response relationship and therefore the estimated EC_x values are considered reliable.

Growth rate at 72 hours

Regarding the calculation of EC_{10}, EC_{20} and EC_{50} values for growth rate at 72 h, a statistically significant concentration/response was found (p(F) <0.001) for this parameter.

The resulting EC_{10}, EC_{20} and EC_{50} values and the respective confidence intervals are represented in the following table below.

Table CP 10.2.1/04-2 Results of the Probit analysis with growth rate at 72 h: Selected effective concentrations (EC_x) of the test item and their 95%-confidence limits

Parameter	Growth rate		
	EC_{10} (95 % confidence interval) [µg a.s./L]	EC_{20} (95 % confidence interval) [µg a.s./L]	EC_{50} (95 % confidence interval) [µg a.s./L]
Effect on growth rate at 72 h	4.90 (3.70 – 5.87)	7.09 (5.94 – 8.03)	14.41 (12.95 – 16.56)

The resulting EC_{10}, EC_{20} and EC_{50} values of 4.90 (95%CL: 3.70 – 5.87), 7.09 (95%CL: 5.94 – 8.03) and 14.41 (95%CL: 12.95 – 16.56) µg a.s./L, respectively, meet the goodness of fit criteria showing a

significant concentration/response relationship and therefore the estimated ECx values are considered reliable.

III. Conclusion

The resulting EC_{10}, EC_{20} and EC_{50} values for yield at 72-hours were determined to be 3.20, 4.02 and 6.22 µg a.s./L, respectively. The EC_{10}, EC_{20} and EC_{50} values for growth rate at 72-hours were determined to be 4.90, 7.09 and 14.41 µg a.s./L.

Assessment and conclusion by applicant:

The statistical re-evaluation of the data has determined reliable EC_{10}, EC_{20} and EC_{50} values for both growth rate and yield. The E_rC_{50} determined in this re-evaluation work of 14.4 µg a.s./L is considered to be the same as the E_rC_{50} determined in the original study report of 0.029 mg/L (14.3 µg a.s./L) therefore the original E_rC_{50} from the study report remains the critical endpoint determined from this study.

The values determined in the re-evaluation work are considered to be fully valid.

CP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

Data Point:	KCP 10.2.2/01
Report Author:	[redacted]
Report Year:	1994
Report Title:	Influence of KWG 4168 EC 500 on development and emergence of larvae of Chironomus riparius
Report No:	HBF/CH 03
Document No:	M-006626-01-1
Guideline(s) followed in study:	Proposed method for sediment toxicity tests developed by the BBA/IVA ad hoc working group "sediment toxicity tests" (February 1994)
Deviations from current test guideline:	Replication of vessels not as per current guidance which recommends at least four replicates per control and test group. However, there were only 5 too few larvae per group, therefore the impact was considered minimal 3 L glass beakers were used as test vessels, however, 600 mL glass beakers are recommended by the current guidance. However, the larger vessels were not likely to have a negative impact on the organisms, based on space available per larvae The composition of the artificial sediment is not as per current guidance, however, it was prepared following OECD 207 guidance at the time of testing. Based on the control emergence rate this did not impact the validity of the test
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Exposure to KWG 4168 EC 500 was tested to assess the potential impact on the maturation of the sediment dwelling life stage of *Chironomus riparius*. Test species were exposed to nominal concentrations of 0.05, 0.5 and 5.0 µg/L (equivalent to 0.025, 0.25 and 2.5 µg a.s./L). A control group was also tested. The test was conducted with both natural sediment and artificial sediment.

In test containers with natural sediment, 45 and 76% of nominal concentrations were analysed in the concentrations 0.5 and 5.0 µg/L respectively, one day after application. In beakers with artificial

sediment, 33% of nominal concentrations were analysed at 5.0 µg/L. The content of a.s. at 0.5 µg/L was lower than the detection limit of 0.1 µg a.s./L. Assuming that the behaviour of the test substance was analogous to 5.0 µg/L (artificial sediment), only 0.08 µg a.s./L could be expected at this concentration which is well below the detection limit.

The percentage emergence of midges in the natural sediment controls in relation to the number of inserted larvae was 92%. In the controls with artificial sediment, the emergence was 87%. The number of emerged midges was not influenced by the treatment at any dosage as revealed by the statistical comparison of emerged midges from all test concentrations with those of the control (X^2-Test, $p = 0.05$).

Also, the day of first emergence was not influenced at any concentration at both types of sediment, as well as the time of emergence (emergence peak) (U-Test, $p = 0.05$). Only the concentration of 5.0 µg/L caused a slight but statistically significant delay of emergence of male midges of about 1.1 days (artificial sediment only), which is not considered to be caused by the test substance.

Thus the application rates of 0.05, 0.5 and 5.0 µg/L of KWG 4168 EC 500 (related to the water volume) did not affect development and growth of larvae of *Chironomus riparius* in both types of sediment.

I. Materials and Methods

Materials

Test Material	KWG 4168 EC 500
Lot/Batch #:	089A
Active substance content:	494.0 g/L
Description:	Clear yellow liquid
Stability of test compound:	Sufficient based on expiration date
Reanalysis/Expiry date:	17 March 1994
Density:	1.004 g/mL

Treatments

Test rates:	0.05, 0.5 and 5.0 µg/L (0.025, 0.25 and 2.5 µg a.s./L)
Solvent/vehicle:	Test water
Analysis of test concentrations:	Day 1, 15 and 28 after application

Test organisms

Species:	*Chironomus riparius*
Source:	Obtained from a culture maintained at the University of Sheffield (UK)
Acclimatisation period:	The L1 larvae used in the study were obtained by laying some fresh egg masses in small crystallising dishes with culture medium. After 2 to 3 days the L1-larvae hatched and were transferred to the test vessels.
Feeding:	During the study the test organisms were fed two to three times per week with appropriate amounts of fish food extract, as used for the breeding.

Test design

Test vessel:	2-L glass beakers

Test medium:	Natural sediment, artificial sediment and the test water was Elendt M7 medium
Replication:	Three for the control, 0.05 and 0.5 µg/L and duplicate vessels for the 5.0 µg/L test group per sediment type
No. of animals/vessel:	25
Duration of test:	28 days
Environmental test conditions	
Temperature:	17.9 to 20.1 °C
Dissolved oxygen:	7.0 to 9.9 mg/L
pH:	7.29 to 8.16
Photoperiod:	16:8 hours light-dark cycle (including half hour dusk and dawn) mean light intensity – 1200 lux

Study Design

KWG 4168 EC 500 was tested to assess the potential impact on the maturation of the sediment dwelling life stage of *Chironomus riparius*.

The test concentrations were selected on the basis of estimated environmental concentrations based on drift rates caused by normal agricultural use. As the lowest concentration of 0.05 µg/L (0.025 µg a.s./L) was below the detection limit of 0.1 µg a.s./L, the concentration of 5.0 µg/L, which is the tenfold of the highest environmental concentration, was also tested for better validation of analysis.

The following test concentrations were selected: 0.05 µg/L, 0.5 µg/L and 5.0 µg/L (0.025, 0.25 and 2.5 µg a.s./L). The test concentrations were set up as follows: 95 mg test formulation was added to 1000 mL test water (stock solution). Of this stock solution, 10, 1.0 and 0.1 mL were each made up to 1000 mL with test water (dilution I, II and III).

In order to reach the concentration of 0.05 µg/L, 10 mL of dilution III were applied to the overlying water column of beakers containing 1.99 L water and 10.79 mL to those containing 2.05 L. The suspension was applied just below the water surface by using a pipette and gently mixing to ensure homogeneous distribution without disturbing the sediment. For the test concentration of 0.5 µg/L, 10 mL or 10.79 mL of dilution II and for the concentration of 5.0 µg/L, 10 mL or 10.79 mL of dilution I, respectively, were applied each in the same way. For the biological examinations three replicates were prepared for the control, 0.05 µg/L and 0.5 µg/L and two replicates for the highest concentration of 5.0 µg/L per sediment type.

For analysis of the active ingredient content about 2 litres of test medium were needed. Thus, additional parallel replicates were prepared for analytical purposes only (control: 1 replicate, 0.5 µg/L and 5.0 µg/L: 2 replicates of each sediment).

During the study the test organisms were fed two to three times per week with a commercial ornamental fish food extract as used for the breeding.

The animals in the test containers were exposed to a temperature of 20 ± 2°C and a 16 : 8 hour light-dark cycle (additionally a 30 minute hour dusk and dawn) in a climatic chamber. Light intensity was determined to be on average approximately 1200 lux.

The test vessels were observed at least three times per week to make a visual assessment of any behavioural differences compared to the control. The sex, time and number of emerged adults was recorded at the same dates.

To determine number and sex of emerged adults, the covering plates of each test container were carefully partly removed and the midges which mostly stayed at the glass sides of the aquaria were enumerated; after identification of the sex (male midges have feathered antennae) midges were removed.

The bottom of the test containers was covered with a 2 cm high layer of natural or artificial sediment, respectively. Beakers were filled with 1.90 to 2.05 L test media. The height of the overlying water corresponded to 20 cm. The overlying water level was marked outside on the test vessel. Gentle aeration was provided through a glass pasteur pipette situated about 2.5 cm above the sediment layer.

The nominal test concentrations were 0.05, 0.5 and 5.0 mg formulation/litre. An aqueous suspension of the test substance was applied to the test containers just beneath the water surface with a pipette on day 0 and mixed in the overlying water by gently aeration.

The test containers (2 1-glass beaker) were filled with a layer of 2 cm of sediment and 20 cm reconstituted overlying water ("M 7" according to ELENDT). During the study, number, sex and time of emergence of emerged midges were determined daily. Emerged midges were removed from test systems. The test period was 28 days.

Test containers were 2-L glass beakers with an average diameter of 11 to 12 cm each labelled indicating study number, concentration and replicate. Test and breeding water was prepared Elendt M7 medium.

Two different kinds of test sediment were used: a natural sediment and an artificial sediment. One half of the test vessels were filled with the natural sediment, the other with the artificial one. The natural sediment originates from the Honninger Weiher, a water body close to the "Oberbergisches Land". The sediment was taken there after draining the pond by means of a track-laying digger, transported in containers to Monheim. The Honninger Weiher, being about 0.1 km^2 in size, lies about 4.5 km north-east of Wipperfurth (Rheinisch-Bergischer Kreis) and is fed by the brook Honnige. The pond can be characterised as oligo- to mesotrophic. It lies in a protected area and serves as a drinking water reservoir (Wupperverband). Three experimental ponds located in the "Pflanzenschutzzentrum Monheim" were filled with a layer of this sediment and ground water in 1990. The test sediment for the study which is reported here was collected from one of these experimental ponds. Only the top 5 - 10 cm of the sediment were taken, sieved (mesh size: 2.0 mm), mixed and deep frozen until the test container were prepared.

The artificial sediment was prepared on day -12 and directly distributed over the test vessels. It consists of 69% fine quartz sand (84% of the sand has a particle size of 0.06 - 0.2 mm), 10% dried, finely ground peat (sphagnum peat; pH 2 - 4), 20% kaolin (kaolinite content of about 36%, pH value ca 7, "Kaolin W", from Erbsloh / Geisenheim) and around 1% calcium carbonate (pure) to adjust the pH value to 6 ± 0.5 (figures refer to dry weight).

Analytical method

Samples of water were analysed using the validated analytical method 00252 M001, report reference M-008490-02-2 **(see Doc MCP Section 5)**.

II. Results and Discussion

No validity criteria assessment was included in the report.

The analytical results of the four stock solutions (day 0) were between 79 % and 133 % of the nominal values (on average 94.8 %). Based on these findings, initial concentrations were assumed to be the nominal values.

In test containers with natural sediment, 45 and 76% of nominal concentrations were analysed in the concentrations 0.5 and 5.0 µg/L respectively, one day after application. In beakers with artificial sediment, 33% of nominal concentrations were analysed at 5.0 µg/L; the content of a.s. at 0.5 µg/L was lower than the detection limit of 0.1 µg a.s./L. Assuming that the behaviour of the test substance was analogous to 5.0 µg/L (artificial sediment), only 0.08 µg a.s./L could be expected at this concentration which is well below the detection limit.

These findings are caused by the high absorptivity of KWG 4168. A major part of the test substance adsorbed at the surface of the sediment and only fractions of it could be found in the overlying water after some days. The artificial sediment absorbed the test substance at a slightly higher amount than the natural one, probably caused by the slightly higher content of organic matter.

The analytical results indicate a continuous decrease of active ingredient concentrations in the overlying water during the 28 day study period at the concentration of 2.46 µg a.s./L (natural sediment) (analysed concentrations were measured at 1.54 µg/L on day 1 decreasing to 0.15 µg/L by day 28). At all other concentrations the analysed results fell below the detection limit after day one of the study in both natural and artificial sediment. Results are given in the table below.

Table CP 10.2.2/01-1 Summary of analysis of active ingredient contents in overlying water

Nominal concentration (µg/L)	Analytical results on sampling days, mean of two analyses (µg a.s./L)								
	Trials with natural sediment								
	Day 1	Corrected by the recovery*		Day 15	Corrected by the recovery*		Day 28	Corrected by the recovery*	
	Analysed conc.	Analysed conc.	% of nominal	Analysed conc.	Analysed conc.	% of nominal	Analysed conc.	Analysed conc.	% of nominal
Control	<0.1	-	-	na	na	na	na	na	na
0.25	0.12	0.11	45	<0.1	-	<40	<0.1	-	<40
2.46	1.54	1.88	076	0.2	0.26	10.6	0.15	0.18	7.3
	Trials with artificial sediment								
Control	<0.1	-	-	na	na	na	na	na	na
0.25	<0.1	-	<40	<0.1	-	<40	<0.1	-	<40
2.46	0.67	0.82	33	<0.1	-	<4.0	<0.1	-	<4.0
na = not analysed									

*The test concentration of 0.25 µg a.s./L is corrected with the recovery of 106%, the test concentration of 2.46 µg a.s./L is corrected with the recovery of 82%

The %-emergence of midges in the controls in relation to the number of inserted larvae was high: 92 % of the inserted larvae maturated to adults in control with natural sediment and 87 % in the control with artificial sediment. The number of emerged midges was not influenced by the treatment at any dosage as revealed by the statistical comparison of emerged midges from all test concentrations with those of the control (X2-Test, p = 0.05).

The data indicate that the emergence of the female started with" a small delay compared to male midges in all treatments. As numbers of female midges are only slightly higher than those of males by 8.5% for an average, the relation of females and males is considered to be about the same at all test concentrations.

Table CP 10.2.2/01-2 Summary of numbers of emerged midges

Concentration (µg/L)	Number of emerged midges (3 replicates)	Emergence (%) of inserted larvae	% male emergence	% female emergence
Natural sediment				
Control	69	92.0	46.4	53.6
0.05	67	89.3	46.3	53.7

0.5	66	88.0	54.5	45.5
5.0	44	88.0*	47.7	52.3
Artificial sediment				
Control	65	86.7	41.5	58.5
0.05	66	88.0	48.5	51.5
0.5	57	76.0	42.1	57.9
5.0	41	82.0*	39.0	61.0

*related to 2 beakers with 25 larvae each

Thus the application rates of 0.05, 0.5 and 5.0 µg/L of KWG 4168 EC 500 (related to the water volume) did not affect development and growth of larvae of *Chironomus riparius* at both types of sediment. The NOEC was determined to be ≥5.0 µg/L (≥2.5 µg a.s./L).

III. Conclusion

The percentage emergence of midges in the controls in relation to the number of inserted larvae was 92% with natural sediment and 87% in the control with artificial sediment. The number of emerged midges was not influenced by the treatment at any dosage as revealed by the statistical comparison of emerged midges from all test concentrations with those of the control (X^2-Test, p = 0.05).

Also, the day of first emergence was not influenced at any concentration at both types of sediment, as well as the time of emergence (emergence peak) (U-Test, p = 0.05). (Only the concentration of 5.0 µg/L caused a slight but statistically significant delay of emergence of male midges of about 1.1 days (artificial sediment only), which is not considered to be caused by the test substance).

Thus the application rates of 0.05, 0.5 and 5.0 µg/L of KWG 4168 EC 500 (related to the water volume) did not affect development and growth of larvae of *Chironomus riparius* at both types of sediment.

Assessment and conclusion by applicant:

No validity criteria assessment was included in the report, therefore, an assessment has been made against the current OECD 219 (2004):

- The emergence in the control and solvent control must be at least 70% at the end of the test (actual: 92.0 and 86.7%, for the artificial and natural sediments, respectively)
- *C. riparius* emergence to adults from control vessels should occur between 12 and 23 days after their insertion into the vessels (actual: between days 17 and 23)
- At the end of the test, pH and the dissolved oxygen concentration should be measured in each vessel. The oxygen concentration should be at least 60% of the air saturation value (ASV) at the temperature used, and the pH of overlying water should be in the 6-9 range in all test vessels (actual: 7.29 to 8.16 mg/L)
- The water temperature should not differ by more than ± 1.0°C. The water temperature could be controlled by isothermal room and in that case the room temperature should be confirmed in an appropriate test vessel (actual: 2.2°C, however, the control emergence (in both sediment types) would suggest that this temperature variation had no impact on the organisms).

The validity criteria according to the current OECD test guideline are considered to have been met. The temperature deviation was slightly larger than that stated by the criterion but this is not considered to have affected the validity of the study.

The study was conducted in 1994 and therefore followed the BBA test guideline in place at the time. Several differences exist between this test guideline and the current OECD 218 and 219 test

guidelines, most notably the number of replicates used and the size of the test vessels. This study tested only a single replicate of 25 organisms at each test substance concentration as opposed to the four replicates of 20 organisms (total: 80 organisms per treatment). The artificial sediment is also different to that currently recommended.

All these points taken into consideration, the results are still considered to be suitable for use in the risk assessment as the study met the requirements of the test guideline at the time and the results largely fulfil the validity criteria of the current OECD test guideline. The study is therefore considered acceptable.

The NOEC has been determined to be ≥5.0 µg/L (≥2.5 µg a.s./L) but it is noted that there was a slight but statistically significant delay of emergence of male midges of about 1.1 days in the artificial sediment only. This was not considered to be a treatment-related effect as it was not seen in the natural sediment. It is also noted that there is a *Chironomus* study using spiroxamine technical (M-006549-01-1) which has a NOEC more than three orders of magnitude greater than this NOEC value which supports the lack of any treatment-related effects at 5.0 µg/L.

The data have been subjected to statistical re-evaluation and the results have been presented in the following study summary.

Data Point:	KCP 10.2.2.02
Report Author:	[redacted]
Report Year:	2020
Report Title:	Calculation of EC10 and EC20 values for Chironomus riparius with spiroxamine EC 500 in a chronic study
Report No:	0471836-ECO7
Document No:	M-760408-01-1
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

The report M-006626-01-1 on the effects of Spiroxamine EC 500 on the development and emergence of the non-biting midge (*Chironomus riparius*) study did not provide estimates of EC_{10} or EC_{20} values. Therefore, these values have been calculated in accordance with the Annex to Com. Reg. 283/2013. Due to a lack of dose-response, it was not possible to calculate reliable EC_x values for either of the parameters tested in either sediment types (natural or artificial).

I. Methods

The statistical evaluation was performed with statistical software ToxRat Professional v3.3.0.

Effect concentrations with 10 and 20% from the test item treatment when compared to the pooled controls were calculated for cumulative emergence and development rate in both sediment types, but due to lack of a dose response, these could not be determined for either parameter.

II. Results and Discussion

Cumulative emergence at 28 days (natural sediment)

Due to the lack of a significant dose response on the emergence, when compared to the control, it was not possible to calculate EC_{10} and EC_{20} values.

Development rate at 28 d (natural sediment)

Due to the lack of a significant dose response on the development rate, when compared to the control, it was not possible to calculate EC_{10} and EC_{20} values.

Development rate at 28 d in males (natural sediment)

Due to the lack of a significant dose response on the development rate, when compared to the control, it was not possible to calculate EC_{10} and EC_{20} values.

Development rate at 28 d in females (natural sediment)

Due to the lack of a significant dose response on the development rate, when compared to the control, it was not possible to calculate EC_{10} and EC_{20} values.

Sex ratio at 28 d (natural sediment)

According to the obtained results due to the p(Chi2) being above the chosen alpha, no effects were detected on sex ratio differences at the study termination.

Cumulative emergence at 28 days (artificial sediment)

Due to the lack of a significant dose response on the emergence, when compared to the control, it was not possible to calculate EC_{10} and EC_{20} values.

Development rate at 28 d (artificial sediment)

Due to the lack of a significant dose response on the development rate, when compared to the control, it was not possible to calculate EC_{10} and EC_{20} values.

Development rate at 28 d in males (artificial sediment)

Due to the lack of a significant dose response on the development rate, when compared to the control, it was not possible to calculate EC_{10} and EC_{20} values.

Development rate at 28 d in females (artificial sediment)

Due to the lack of a significant dose response on the development rate, when compared to the control, it was not possible to calculate EC_{10} and EC_{20} values.

Sex ratio at 28 d (artificial sediment)

According to the obtained results due to the p(Chi2) being above the chosen alpha, no effects were detected on sex ratio differences at the study termination.

III. Conclusion

Due to a lack of dose response, it was not possible to calculate reliable EC_r values for either of the parameters tested in either sediment types (natural or artificial).

Assessment and conclusion by applicant:

The statistical re-evaluation of the data confirmed that due to a lack of a significant dose-response it was not possible to determine reliable EC_{10} and EC_{20} values for emergence and development rate.

The NOEC of 5.0 µg/L (2.5 µg a.s./L) from the original study report shall remain the critical endpoint determined from this study.

The values determined in the re-evaluation work are considered to be fully valid.

CP 10.2.3 Further testing on aquatic organisms

A mesocosm study using Spiroxamine EC 500 (M-304557-01-1) is available and has been summarized below. A re-analysis study has also been conducted in order to assess the mesocosm data against current requirements. A summary of this report has been presented following the summary for the original report.

Data Point:	KCP 10.2.3/01
Report Author:	[redacted]
Report Year:	2008
Report Title:	Biological effects and fate of Spiroxamine EC 500 in outdoor mesocosm ponds simulating actual exposure conditions in agricultural use
Report No:	EBKWX091
Document No:	M-304557-01-1
Guideline(s) followed in study:	OECD Guidance Document Simulated Freshwater Lentic Field Tests (Outdoor Microcosms and Mesocosms), April 2006 Guidance Document on Testing Procedures for Pesticides in Freshwater Microcosms (SETAC Europe Workshop, Monks Wood, UK, July 1991) Community-Level Aquatic System Studies Interpretation Criteria (2002) (Proceeding from the CLASSIC Workshop)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The aim of the study was to determine the ecological effects of a simulated contamination with Spiroxamine EC 500 on different trophic levels (phytoplankton, zooplankton, macroinvertebrates and periphyton) in outdoor mesocosms as an aquatic model ecosystem for lentic aquatic freshwater systems with different trophic levels. The fate of the compound in the individual compartments (water body, sediment and macrophytes) was monitored simultaneously.

Three applications of the test substance (with a 7-day interval) were made and the study ran for 14 weeks post-application.

Analysis of the test substance (Spiroxamine EC 500) in the water column 4 hours after each of the three fungicide applications indicated that all microcosms received the intended doses. On average 82.7% of the intended dose were measured by the accompanying chemical analysis.

Due to the fact that the variability in concentration levels probably is higher during the first hours post application due to incomplete mixing, and the concentration of higher treatment levels can be measured with a higher accuracy, it was decided to express the treatment-related responses observed in terms of the intended treatment levels (1.0, 2.1, 4.4, 9.3, 19.4 µg a.s./L).

At the end of the experiment (Day 84) approximately all measured concentrations were below the Limit of quantification (0.1 µg a.s./L).

The estimated DT_{50} of Spiroxamine in the water phase was determined as 3.8 days.

The DT_{50}-value for the whole system of 7.2 days (water + macrophytes + sediment) has to be considered with caution, as the fate of the test substance in the sediment showed a fluctuating pattern.

The overall NOEAEC for this mesocosm study was set at 9.3 µg/L. All effects observed up to the highest dose level of 19.4 µg a.s. /L showed a fast recovery. Taking the fact into consideration that the highest concentration was investigated in one replicate only, the overall NOEAEC for this mesocosm study on Spiroxamine was set at 9.3 µg a.s./L.

The taxa and species, on which the most pronounced effects were observed at this concentration are the Rotatoria. The most sensitive Rotatorian species were Asplancha, Polyarthra and Keratella quadrata. Similar sensitivities were observed for some species from several Phytoplankton families, for example the Cryptophyceae Chroomonas and Cryptomonas, the Diatomeae Achnantes and the Chlorophyceae Ankya judai and Characium, as well as the Chlorophyll a levels as a measure for the periphyton. The effects are statistically reflected in low indices for diversity, similarity and PRC community response.

Heterogeneous occurrence of filamentous algae was shown to be not compound dependent at concentrations up to 4.4 µg a.s./L by an additional laboratory study. Periphyton chlorophyll-a determinations revealed slight differences to the control at sampling days 28 to 42 in all dose levels. These differences to the control were not statistically significant at two consecutive measurement points. No indirect effects by potential adverse effects on periphyton were observed neither in zooplankton nor in phytoplankton.

Investigations by ASS and litter cages did not reveal any adverse effects for all dose levels within the entire test period.

No effects could be observed on the three macrophytes species, which were introduced into the ponds or occurred naturally.

First significant effects were detectable at 4.4 µg a.s./L. The study revealed a clear dose/response relationship. The highest observed effect classes belong to class 3B. No pronounced effects without recovery were observed within this study.

The overall NOEAEC for this mesocosm study (including the laboratory study with *filamentous algae*) was set at 9.3 µg/L.

I. Materials and Methods

Materials

Test Material	Spiroxamine EC 500
Lot/Batch #:	PF90087683
Purity:	49.8%, 501 g/L (content of a.s.)

Description:	Clear brown liquid
Stability of test compound:	Not reported
Reanalysis/Expiry date:	Not reported
Density:	1.006 g/mL
Treatments	
Test rates:	1.0, 2.1, 4.4, 9.3 and 19.4 µg a.s./L
Solvent/vehicle:	Water
Analysis of test concentrations:	Yes - days 0 7, 14, 18, 21, 28, 35, 42, 49, 56, 63, 70, 77 and 84
Test organisms	
Species:	Phytoplankton, zooplankton, macrozoobenthos and periphyton
Source:	Naturally occuring
Acclimatisation period:	Mesocosms prepared 5 months prior to application
Test design	
Test vessel:	Cylindrical tanks made of black Polyethylene - each tank is 2.75 m in diameter and 1.55 m in depth, the surface is 5.94 m²
Test medium:	Tanks were filled with sediment to a level of about 14 cm and with water up to 1 m depth (The water was composed of 80 % local ground water and 20 % water from a nearby uncontaminated pond)
Replication:	3 replicates for the control, 2 replicates for the 1.0 – 9.3 µg a.s./L treatments and one replicate for the 19.4 µg a.s./L treatment.
Duration of test:	84 days
Environmental test conditions	
Temperature:	10.21 – 21.48 °C
Dissolved oxygen:	2.35 – 20.4 mg/L
pH:	Not reported
Photoperiod:	Natural. 0 – 9.70 hours sunshine per day

Study Design

The aim of the study was to determine the ecological effects of a simulated contamination with spiroxamine EC 500 on different trophic levels (phytoplankton, zooplankton, macroinvertebrates and periphyton) in outdoor mesocosms as an aquatic model ecosystem for lentic aquatic freshwater systems with different trophic levels. The fate of the compound in the individual compartments (water body, sediment and macrophytes) was monitored simultaneously.

The mesocosms used were twelve cylindrical tanks made of black Polyethylene (PE). They are installed next to the Institute for Ecotoxicology at the Agricultural Research Centre of BayerCropScience in Monheim am Rhein, Germany (geographical position: 51° 4′ N, 6° 55′ E). Each tank is 2.75 m in

diameter and 1.55 m in depth, the surface is 5.94 m² (Figure 1 and Figure 2). When filled to the nominal operating depth of 1.0 m, each tank contains 5.94 m³ water. The respective water level is obtained from a gauge inside the tank. A plastic tray (depth: 0.2 m) is located on the bottom of each tank. The trays are filled with natural sediment up to a height of about 14 cm. The tanks are arranged with three basins each in four rows. All basins are connected with a separate 13th tank by pipes. During the months before the start of the study the water was pumped from the separate tank into the 12 study basins forwards and backwards, guaranteeing a homogenous mixing in the complete system in order to adjust the same chemical and biological conditions before study start. The ponds were disconnected from each other one week before the first application.

Twelve test tanks (6 m^3 water, 1 m water depth) which were used in this study are especially designed systems which allow the establishment of almost identical conditions at the start of a study. The bottoms of the artificial tanks were covered with natural sediment (approximately 14 cm in height) five months prior to the study start. The water was composed of local ground water and water from a nearby uncontaminated pond which was inoculated several times with zooplankton from a natural pond nearby. Natural communities developed spontaneously from seeds and roots of aquatic plants as well as from air borne and naturally transferred stages of planktonic, benthic and filamentous algae organisms during the months before study start. Additionally one and two weeks respectively before application plants of three macrophyte species (Callitriche palustris, Myriophyllum spicatum and Potamogeton crispus) were inserted into the ponds to initiate a heterogeneous habitat. In general, the artificial ponds are representative of a small stagnant water body.

The test substance was applied three times during the early growing season in May 2007 three times at an interval of 7 days onto the water surface of nine test ponds. The treatment levels were 1.0, 2.1, 4.4, 9.3 and 19.4 µg a.s./L (two replicates of 1.0 to 9.3 µg a.s./L, one replicate for 19.4 µg a.s./L). Three further tanks were used as untreated controls.

The mesocosms were investigated for a period of two weeks before and 14 weeks after treatment. Several times during the study period water, sediment and macrophyte samples were taken and analysed to investigate the concentration of the test substance in water and sediment. Further parameters evaluated were the taxonomic composition of phytoplankton, zooplankton and macroinvertebrates at different days before and after the applications.

All ponds had six predetermined sampling positions to cover the whole expanse. At these positions samples for biological samples and water were taken. The sediment samples were taken at different places which were chosen by chance each time. Separate equipment was used to sample controls, ponds with lower and ponds with higher concentrations of the test substance.

The water samples were taken with a commercial water-proof vacuum cleaner (Elektrostar, Starmix Zyklon HG 81). The suction tube (length: 120 cm, diameter 5 cm) was introduced vertically from the water surface down to about 15 cm above the sediment and withdrawn within a few seconds. During this time approximately 12 L of water were filled into the sampling container.

Sediment samples were taken by means of a corer according to MILBRINK (1971) (sampled sediment surface: 19.6 cm², height about 10 cm). Previously it has been shown that chemicals primarily adsorb to the upper layer of the sediment, thus only the top about 2 cm of sediment were used for analysis. The sampled sedimentcolumn was filled into a glass-beaker having the dimension of the corer. The upper 2 cm of four separate sediment samples per pond were mixed.

During the study the abundance of macrophytes (% coverage) and filamentous algae was assessed visually. A qualitative statement on the development during the study is available in this report. At the end of the study all macrophytes and filamentous algae were harvested. The species were identified. For the determination of the biomass each species was dried in a dryer at 50°C.

250 mL of the mixed water sample was preserved with Lugol´s solution. For evaluation of these samples, a fixed volume of the thoroughly shaken phytoplankton sample were emptied into a sedimentation chamber (Utermöhlkammer) for phytoplankton identification in the laboratory and allowed to stand for

at least 12 hours. The identification and enumerating of the cells was made by means of a reversed microscope within five days after the filling. The number of enumerating fields (at least 5 fields) was chosen according to the actual concentration of algae. The number of individuals was calculated according to Utermöhl. Until identification and counting, the samples were stored at room temperature in the dark.

3.3 litre of water were sampled and merged from six sampling positions in each pond, resulting in 20 L water samples. The merged samples were filtered through a plankton sieve (mesh size: 56 µm) and preserved in a fixation solution (70% Ethanol containing 40 g Sucrose/L and 40 mL Glycerine/L). For species identification (see 4.1.2), the thoroughly shaken samples were filtered through a plankton sieve (mesh size: 30 to 50 µm). The sample bottles were rinsed again with fixation solution and emptied into the plankton sieve. The zooplankton was transferred into a petri-dish with enumeration lines, containing fixation solution. The zooplankton samples were evaluated by use of a reversed microscope, identifying and enumerating the individual organisms. To prevent evaporation the petri-dishes were covered. After determination, all samples were filled back into the bottles and stored at room temperature in the dark. If the density of organisms was too high, two methods were used to evaluate the sample. First, the individual sample was divided for enumerating by full evaluation of all sub-samples and summing up the results. Second, only a few sub samples were taken from a thoroughly homogenised sample and the enumerated results were projected for the total sample. Not all organisms were identified at the species level, only most abundant species and/or those which could be identified within a reasonable time frame.

Two artificial substrate samplers (ASS) per mesocosm were placed on the sediment surface. The ASS was pulled up at each sampling, placed in a bucket, and the animals were washed into the bucket with tap water. The water was passed through a 0.5 mm sieve, and the residue was fixed as described above for the zooplankton. The fixed samples were macroscopically examined (see 4.1.2) and enumerated under a binocular. Not all organisms were identified at the species level, only most abundant species and/or those which could be identified within a reasonable time frame.

Within this mesocosm study possible adverse effects on the detrivorous biocoenosis were investigated using litter cages. Small litter cages were filled with leaves of *Populus spec.* (about 5 g dry weight) and offered as habitat (cage size: diameter: 10 cm; high: 7 cm; mesh size 1 cm; stainless steel). The cages were exposed onto the sediment surface on small plates made of stainless steel mesh (diameter: 20 cm mesh size 0.2 cm). Three weeks before the first application eight cages were exposed in each pond, thus a natural detrivorous biocoenosis could develop onto the leaves until application. The dry weight of the Populus leaves was determined before exposure by weighing. Two and four weeks after the last application one cage per pond and six, eight and ten weeks after the last application two cages each respectively, were taken out of the ponds. The leaves were poured shortly with clean water, thereafter the water was passed through a 0.5 mm sieve, and the residue was fixed as described above for the zooplankton. The leaves were dried in a dryer at 50°C for at least 20 hours and the dry weight was determined.

One Emergence trap was fixed with lines at the center of each pond. The traps had a diameter of 56 cm (= 0.25 m²) In the traps, emerged organisms were fixed with 1,2-Ethandiol. The samples were preserved in fixation solution see above. As the ASS samples clearly show no effects on macroinvertebrates and as emerging organisms were not expected to be the most sensitive group, the samples of the emergence were not evaluated anymore.

The determination of chlorophyll-a was performed in accordance with NUSCH (DIN 3842 L 16 (DEV 1987)). Water samples were filtered through Whatman GF/C glass fibre filters (pore size 1 µm). The filters were folded, placed in aluminium foil and deep-frozen (-18°C) until extraction with ethanol. During the 2 hour extraction period the samples were agitated. The final extinction measurement was made in a 1-ray photometer (type MPM 1500, WTW) at a wavelength of 665 nm.

For the determination of the periphyton eight racks (made of stainless steel) per pond with ten glass slides each were placed in the water column (about 20 cm beneath the water surface. After a defined exposure time one slide per rack was taken out of the mesocosm and the periphyton on the glass slides

was wiped off with a glassfaser filter. The filters were folded, placed in aluminium foil and deep-frozen (- 18°C) until extraction with ethanol. During the 2 hour extraction period the samples were agitated. The final extinction measurement was made in a 1-ray photometer (type MPM 1500, WTW) at a wavelength of 665 nm.

The physico-chemical water parameters were measured several times during the study at an interval of about 7 days in mixed water samples.

For water analysis, 2 x 20 mL of a mixed water sample (see 4.3.4.2) was poured into a 50 mL amber glass bottle. On some occasions the water samples were obtained from three depths (ca. 10 – 30, 30 – 60 and 60 – 90 cm beneath water surface), to reveal the distribution of the test substance in the water column during the first 4days after application. For this purpose the water samples were obtained with a flask attached to a metal rod. The flask (1.0 L glass bottle) was moved around in the pond during filling to obtain water from different sites. Samples of the water in the control mesocosms were taken one hour before and 1 day after application to ensure that no cross contamination has entered the control ponds. Additionally to the water samples, the application suspensions were analysed on day 0, 7 and 14. For sediment analysis the upper 2 cm of four sediment samples (see 4.3.4.3) were mixed and about 320 g were taken for analysis.

As the heterogeneous occurrence of filamentous algae could not be sufficiently resolved within the mesocosm study, a separate laboratory study with focus on the filamentous algae only was performed in April 2008 using the same test regimes; three applications of 1.0, 2.1, 4.4, 9.3 and 19.4 µg a.s./L on Day 0, 7 and 14. The test was run for 21 days.

The biological data were analysed as follows: For each taxon (species up to phylum if appropriate; total counts per sample (e.g., zooplankton, phytoplankton, sediment organisms) univariate statistics were used to test on differences between treatments and controls and to calculate a NOEC (No Observed Effect Concentration). At the community level, diversity and similarity indices as well as Principal Response Curves were used for analysis. The program Community Analysis V4.25 was used for all of the calculations, except for the Principal Response Curves. A former version of the CA program is described in ([redacted] et al. 1992). The PRC analysis was performed with CANOCO 4.02 (DLO, Wageningen, NL), which represents the original program used in published papers describing the method.

Analytical method

Samples of water were analysed using the validated analytical method 00623, report reference M-031628-01-1 (**see Doc MCP Section 5**).

Samples of sediment were analysed using the validated analytical method 01088, report reference M-298750-01-1 (**see Doc MCP Section 5**).

Samples of macrophytes were analysed using the validated analytical method 00721, report reference M-304557-01-1 (**see Doc MCP Section 5**).

II. Results and Discussion

The analyzed concentrations of the application solutions gave an average of 92.1 % of the nominal concentrations for the three applications (minimum: 86 %, maximum: 97 %), thereby confirming nominal concentrations were achieved.

All analytical results correspond very well to each other (average over all concentrations at days 0/+4h, 7/+4h, 14/+4h: 82.7 %). The results demonstrate, that nominal concentrations had been initially applied at each of the three treatments. The concentration of the test substance in the pond water declined continuously. Four weeks after the last application the concentration at the two lowest treatment levels was below the limit of detection (= 0.102 µg a.s./L). In the highest treatment level the concentration in the water phase was below the limit of detection on day 70.

Stratified samples of three different water heights were taken to determine the a.s. distribution in the water column four hours, one and four days after each application. The results show that four hours after the second and third application the major part of the test substance was still in the upper water layer. 24 hours after the second and 4 days after the third application it was homogeneously distributed in the total water column. At the first application no stratification of the concentration of the test substance was found. Probably the rainfall after this application caused a faster distribution.

The measurement of centrifuged water samples did not reveal any significant difference to non-centrifuged water samples. Therefore it can be assumed, that the total amount of test substance was bio available.

The concentration of the test substance in the sediment increased until two weeks after the last application. Thereafter the concentrations decreased very slowly with a fluctuating pattern. The highest measured amount of the test substance in this matrix was 20.8 % of the nominal concentration on day 28 at the highest test concentration. At the end of the study the portion of the test substance in sediment was below 10 % of the initial applied amount for all treatments.

A very small portion of the applied substance was attributed to the macrophytes only (4 % as a maximum). Comparable to the water analysis the concentrations in the low dosages fall below the limit of quantification on day 28 and at the highest dosage on day 70.

The mean DT_{50}-value for dissipation of spiroxamine in the water is 3.77 days. The mean DT_{50}-value for the whole system (water plus macrophytes plus sediment) was 7.22 days. This latter value has to be considered with caution, as the fate of the test substance in the sediment was very heterogeneous.

Table CP 10.2.3/01-1 Summary of analysis of spray solutions used to dose the test system

Experimental day	Nominal concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)	% nominal concentration
Day 0 [1 hour]	6.25	5.86	94
	13.13	12.07	92
	27.51	25.07	91
	58.15	52.06	90
	124.30	114.11	94
Day 7 [1 hour]	6.25	5.88	94
	13.13	12.42	95
	27.51	25.01	91
	58.15	49.95	86
	121.30	117.23	97
Day 14 [1 hour]	6.25	5.87	94
	13.13	12.50	95
	27.51	24.19	88
	58.15	50.95	88
	121.30	112.95	93
		Mean of %	92.1
		SD	1.9

Table CP 10.2.3/01-2 Mass balance of spiroxamine in the test system during the study – 1.0 µg a.s./L treatment

Experimental day	Measured concentration (as % of the total applied nominal amounts)			
	Water	Sediment	Macrophytes	Sum
0 [+ 4 hours]	72.5	-	-	72.5
1	53.7	-	-	53.7
4	24.9	-	-	24.9
7 [-1 hour]	15.5	12.0	0.003	27.5
7 [+ 4 hours]	94.5	-	-	94.5
8	62.2	-	-	62.2
11	35.7	-	-	35.7
14 [-1 hour]	28.6	12.4	0.404	41.2
14 [+ 4 hours]	76.5	-	-	76.5
15	60.5	-	-	60.5
18	21.9	-	-	21.9
21	12.3	6.33	0.000	19.6
28	6.51	13.4	0.000	20.9
42	3.74	12.2	0.000	16.3
56	3.82	5.85	0.000	9.71
70	3.84	12.1	0.000	16.0
84	3.78	6.61	0.000	10.5

Table CP 10.2.3/01-3 Mass balance of spiroxamine in the test system during the study – 2.1 µg a.s./L treatment

Experimental day	Measured concentration (as % of the total applied nominal amounts)			
	Water	Sediment	Macrophytes	Sum
0 [+ 4 hours]	67.2	-	-	67.2
1	53.4	-	-	53.4
4	25.3	-	-	25.3
7 [-1 hour]	16.0	3.24	0.420	19.7
7 [+ 4 hours]	83.7	-	-	83.7
8	63.4	-	-	63.4
11	33.4	-	-	33.4
14 [-1 hour]	21.2	12.2	1.18	34.5
14 [+ 4 hours]	68.8	-	-	68.8
15	67.2	-	-	67.2
18	21.6	-	-	21.6

Experimental day	Measured concentration (as % of the total applied nominal amounts)			
	Water	Sediment	Macrophytes	Sum
21	12.3	8.60	1.40	22.3
28	3.84	12.1	0.725	16.7
42	1.90	9.00	0.030	10.9
56	1.94	8.41	0.037	10.4
70	1.97	4.72	0.038	6.73
84	1.91	6.14	0.038	8.09

Table CP 10.2.3/01-4 Mass balance of spiroxamine in the test system during the study – 4.4 µg a.s./L treatment

Experimental day	Measured concentration (as % of the total applied nominal amounts)			
	Water	Sediment	Macrophytes	Sum
0 [+ 4 hours]	71.3	-	-	71.3
1	57.1	-	-	57.1
4	26.1	-	-	26.1
7 [-1 hour]	15.9	6.37	0.168	22.5
7 [+ 4 hours]	94.6	-	-	94.6
8	67.4	-	-	67.4
11	38.3	-	-	38.3
14 [-1 hour]	35.2	13.8	1.11	50.0
14 [+ 4 hours]	68.9	-	-	68.9
15	64.4	-	-	64.4
18	30.8	-	-	30.8
21	21.6	10.9	2.09	34.6
28	11.9	15.2	1.29	28.4
42	1.33	11.7	0.162	13.2
56	0.82	8.14	0.130	9.09
70	0.83	8.36	0.015	9.20
84	0.81	9.36	0.015	9.19

Table CP 10.2.3/01-5 Mass balance of spiroxamine in the test system during the study – 9.3 µg a.s./L treatment

Experimental day	Measured concentration (as % of the total applied nominal amounts)			
	Water	Sediment	Macrophytes	Sum
0 [+ 4 hours]	80.3	-	-	80.3
1	60.9	-	-	60.9

Experimental day	Measured concentration (as % of the total applied nominal amounts)			
	Water	Sediment	Macrophytes	Sum
4	28.4	-	-	28.4
7 [-1 hour]	17.6	4.37	0.288	22.2
7 [+ 4 hours]	98.7	-	-	98.7
8	77.8	-	-	77.8
11	43.0	-	-	43.0
14 [-1 hour]	33.5	6.08	1.48	41.0
14 [+ 4 hours]	87.4	-	-	87.4
15	70.6	-	-	70.6
18	36.3	-	-	36.3
21	21.2	14.2	2.26	37.7
28	14.5	8.49	1.19	24.2
42	1.51	4.15	0.196	5.86
56	0.392	7.94	0.196	8.53
70	0.396	9.34	0.050	9.79
84	0.387	5.38	0.051	5.82

Table CP 10.2.3/01-6 Mass balance of spiroxamine in the test system during the study – 19.4 µg a.s./L treatment

Experimental day	Measured concentration (as % of the total applied nominal amounts)			
	Water	Sediment	Macrophytes	Sum
0 [+ 4 hours]	85.3	-	-	85.3
1	62.9	-	-	62.9
4	29.2	-	-	29.2
7 [-1 hour]	19.3	6.80	0.16	26.3
7 [+ 4 hours]	115.7	-	-	115.7
8	80.1	-	-	80.1
11	45.1	-	-	45.1
14 [-1 hour]	36.4	9.54	1.23	46.2
14 [+ 4 hours]	86.0	-	-	86.0
15	87.9	-	-	87.9
18	53.8	-	-	53.8
21	46.3	5.85	3.68	55.9
28	12.1	20.8	1.92	34.9
42	1.83	13.9	0.15	15.9
56	0.377	13.0	0.089	13.4

Experimental day	Measured concentration (as % of the total applied nominal amounts)			
	Water	Sediment	Macrophytes	Sum
70	0.185	12.4	0.029	12.6
84	0.180	8.30	0.030	8.51

Direct effects of the applications of Spiroxamine EC 500 to the overall community metabolism could not be observed. In the second half of the study the development of the filamentous algae caused, especially for one replicate of the control and one replicate of 1.0 µg/L, significant lower pH values and oxygen concentrations. The other treated ponds displayed slight higher values as compared to the control range during this period. All other investigated chemical physical parameters did not show any difference between treated ponds and the controls.

The macrophytes showed a strong growth in all ponds without any sign of a treatment related effect.

The measurements of the chlorophyll a content of the pelagial water revealed a short term effect for the three highest test concentrations between days 7 to 14. These findings are in agreement with the observed effects of the phytoplankton during this time period.

The periphyton was determined indirectly via chlorophyll a measurement. The total development was comparable for all ponds with a strong increase during the pre-treatment and a decrease after the application phase. A transient effect between days 28 and 42 cannot be excluded for the treated ponds, although one replicate of 4.4 and 9.3 µg/L each, was always in the range of the control. A statistical significant difference was calculated for day 42 only. A full recovery could be stated for all treatments on day 56 (= 6 weeks after last application). Periphyton consists of epiphytic algae, which to a lower amount can be detected in the pelagial water as well. The epiphytic algae, which were detected in the pelagial water in this study revealed a NOEC of 2.1 µg a.s./L for Achnantes spec., which was one of the dominant taxa in this study. For two other epiphytic taxa, which occurred in minor abundances only, a slight benefit was found at the two highest test concentrations.

The heterogeneous development of the filamentous algae in the mesocosm did not allow a reliable determination of this organism, as the values within the treated ponds were very inconsistent. Although the abundance of filamentous algae was lower in most of the treated ponds, a clear dose related effect could not be stated. Taking the heterogeneous growth of filamentous algae into consideration still a potential recovery for these algae up to the second highest concentration can be stated. A laboratory test performed to further investigate possible effects on the filamentous algae revealed a NOEC of 4.4 µg a.s./L. The results of this study are considered for the assessment of short term effects of the test substance to filamentous algae. Considering the results of the mesocosms and the additional laboratory study the NOEAEC was set to 9.3 µg/L.

The total results of the chlorophyll a, periphyton and filamentous algae result in a NOEAEC of 9.3 µg/L. The development of the periphyton and the filamentous algae obviously had no major influence on the dynamics of phyto- and zooplankton and the macroinvertebrates. The observed biological results for these groups are summarised in the following Table and in respective assessment for each treatment level. Where statistically significant differences between treatments and controls were observed, and these were considered to be treatment-related and biologically significant, the responses were categorized in effect classes as mentioned in Working Document SANCO/3268/2001 rev.4(final), 2002, but following the adapted effect classes as described by [redacted] et al (2006) and De Jong et al. (2008). These effect classes are presented below.

Table CP 10.2.3/01-7 Effect classes used to evaluate the treatment-related responses of spiroxamine in the mesocosm study

Effects class	Definition of effects
1	Effect could not be demonstrated
2	Slight effect (minor in duration and magnitude)
3 A	Pronounced short-term effects and recovery within 8 weeks after the first application or total period of effects <8 weeks followed by recovery
3 B	Pronounced short-term effects and recovery within 8 weeks after the last application, followed by recovery
4	Pronounced effect but study/measurement too short to demonstrate that treatment related effects last less than 8 weeks
5 A	Clear long-term effects lasting longer than 8 weeks but full recovery observed at end of experiment
5 B	Clear long-term effects lasting longer than 8 weeks but full recovery not observed at the end of the experiment

In the summary table (see below) trends of treatment-related effects are indicated by placing the effect class. A trend of an effect on a certain endpoint does not need to be statistically significant on consecutive sampling days or at the end of the experiment, but is considered relevant in connection with the overall effects observed.

Treatment level of 1.0 µg a.s./L: No consistent treatment-related effect could be observed for the population and community endpoints of the benthic macroinvertebrates sampled in the ASS and the phyto- and zooplankton studied.

Treatment level of 2.1 µg a.s./L: No consistent treatment-related effect could be observed for the population and community endpoints of the benthic macroinvertebrates sampled in the ASS and the phyto- and zooplankton studied.

Treatment level of 4.4 µg a.s./L: The benthic macroinvertebrates were not affected by the treatments. For the zooplankton a slight adverse treatment related effect became apparent for one taxonomic group the Rotatoria. Regarding the single species, only weak adverse effects which were statistically not significant were found for the dominant taxa *Polyarthra species* and *Asplanchna species*. But for the sum of Rotatoria a Class 2 effect resulted for a very short period after the third application (days 14 to 18). Another Rotatoria species (*Keratella quadrata*) showed higher densities as compared to the control after day 28. But it is questionable, if this finding is caused by lower competition of other zooplankter, as no biological significant adverse effect was found for the zooplankton anymore after day 21. Effects on the zooplankton community were not found. For the phytoplankton a Class 2 effect was found for one Diatomeae taxa (*Achnantes species*) and a Class 3 effect for one Cryptophyceae taxa (*Cryptomonas species 20-30 m*). These taxa mainly contributed to the total algae abundance, these effects were also reflected on the community level. However already 4 weeks after the last application a full recovery could be stated for all these endpoints.

Treatment level of 9.3 µg a.s./L: Again no treatment related effect existed for the macroinvertebrates. The pronounced effects on the Rotatoria species *Asplanchna species* and *Polyarthra species* and thus on the sum of Rotatoria are regarded as Class 3 effect. But a recovery was obvious already 2 weeks after the last application. The rotatoria dynamics caused a transient lower similarity of the zooplankton between the control and this dosage. A class 3 effect could also be derived from the PRC calculations. Nevertheless a recovery on the species level and the zooplankton community can be stated. The effects on single phytoplankton species and algae community, which were observed at 4.4 µg/L were also seen in this dosage although slightly more pronounced (Class 3 effect). The dynamics of the respective single taxa and the Community indices show a very high similarity between the control and this dosage from

day 42 onwards. Regarding the results of the acroinvertebrates and the zoo- and phytoplankton this treatment can be considered as the overall $NOEAEC_{mesocosm}$.

Treatment-level of 19.4 µg a.s./L: The treatment-related responses observed in the microcosms treated with 19.4 µg a.s./L, and the species involved, were similar to the previous treatment but more pronounced. The strongest effects observed at this treatment level belong to class 3 thus this treatment could be considered as NOEAEC as well, but since only one replicate exist at this dose level, the concentration 9.3 µg a.s./l was chosen as the overall $NOEAEC_{mesocosm}$.

Table CP 10.2.3/01-8 Summary of treatment related effects observed in the mesocosm study in terms of effect classes

	Number of detected taxa *)	Test concentrations (µg a.s./L)				
		1.0	2.1	4.4	9.3	19.4
Zooplankton						
Cladocera	10 taxa	1	1	1	1	1
	Sum of Cladocera	1	1	1	1	2 ↑
Copepoda	2 taxa	1	1	1	1	1
	Nauplii	1	1	1	1	2 ↓
Ostracada	1 taxa	1	1	1	1	1
Diptera	1 taxa	1	1	1	1	1
Rotatoria	14 taxa	1	1	1	1	1
	Asplanchna spec.	1	1	1	2 ↓	2 ↓
	Polyarthra spec.	1	1	1	3A ↓	3A ↓
	Keratella quadrata	1	1	3B ↓	3B ↑	3B ↑
	Sum of Rotatoria	1	1	2 ↓	3A ↓	3A ↓
Taxa richness		1	1	1	1	1
Diversity		1	1	1	1	1
Similarity		1	1	1	3B ↓	3B ↓
PRC community response		1	1	1	3B ↓	3B ↓
Macroinvertebrates						
(ASS)	46 taxa	1	1	1	1	1
Taxa richness		1	1	1	1	1
Diversity		1	1	1	1	2 ↑
Similarity		1	1	1	1	1
PRC community response		1	1	1	1	1
Litterbags		1	1	1	1	1
Phytoplankton						
Cryptophyceae	1 taxa	1	1	1	1	1
	Chroomonas spec.	1	1	1	2 ↓	3A ↓
	Cryptomonas spec.	1	1	3A ↓	3A ↓	3A ↓

	Number of detected taxa *)	Test concentrations (µg a.s./L)				
		1.0	2.1	4.4	9.3	19.4
Diatomeae	8 taxa	1	1	1	1	1
	Achnantes spec.	1	1	2 ↓	3A ↓	3B ↓
Chlorophyceae	6 taxa	1	1	1	1	1
	Ankyra judai	1	1	1	2 ↑	2 ↑
	Characium spec.	1	1	2 ↑	2 ↑	2 ↑
Chrysophycea	2 taxa	1	1	1	1	1
Conjugatophyceae	3 taxa	1	1	1	1	1
Cyanobacteria	3 taxa	1	1	1	1	1
Euglenophyta	4 taxa	1	1	1	1	1
Taxa richness		1	1	1	1	2 ↓
Diversity		1	1	1	1	2 ↓
Similarity		1	1	2 ↓	3A ↓	3A ↓
PRC Community response		1	1	2 ↓	3A ↓	3A ↓
Chlorophyll a		1	1	2 ↓	2 ↓	2 ↓

*) only affected taxa are named (other taxa are summed up)

↓ = decrease

↑ = increase

III. Conclusion

Analysis of the test substance (Spiroxamine EC 500) in the water column 4 hours after each of the three fungicide applications indicated that all microcosms received the intended doses.

On average 82.7% of the intended dose were measured by the accompanying chemical analysis.

Due to the fact that the variability in concentration levels probably is higher during the first hours post application due to incomplete mixing, and the concentration of higher treatment levels can be measured with a higher accuracy, it was decided to express the treatment-related responses observed in terms of the intended treatment levels (1.0, 2.1, 4.4, 9.3, 19.4 µg a.s./L).

At the end of the experiment (Day 84) approximately all measured concentrations were below the Limit of quantification (0.1 µg a.s./L).

The estimated DT_{50} of Spiroxamine in the water phase was determined as 3.8 days.

The DT_{50}-value for the whole system of 7.2 days (water + macrophytes + sediment) has to be considered with caution as the fate of the test substance in the sediment showed a fluctuating pattern.

The overall NOEAEC for this mesocosm study was set at 9.3 µg a.s./L. All effects observed up to the highest dose level of 19.4 µg a.s./L showed a fast recovery. Taking the fact into consideration that the highest concentration was investigated in one replicate only, the overall NOEAEC for this mesocosm study on spiroxamine was set at 9.3 µg a.s./L.

The taxa and species, on which the most pronounced effects were observed at this concentration are the Rotatoria. The most sensitive Rotatorian species were Asplancha, Polyarthra and Keratella quadrata.

Similar sensitivities were observed for some species from several Phytoplankton families, for example the Cryptophyceae Chroomonas and Cryptmonas, the Diatomeae Achnantes and the Chlorophyceae *Ankya judai* and Characium, as well as the Chlorophyll a levels as a measure for the periphyton.

The effects are statistically reflected in low indices for diversity, similarity and PRC community response.

Heterogeneous occurrence of filamentous algae was shown to be not compound dependent at concentrations up to 4.4 µg a.s./L by an additional laboratory study.

Periphyton chlorophyll-a determinations revealed slight differences to the control at sampling days 28 to 42 in all dose levels. These differences to the control were not statistically significant at two consecutive measurement points.

No indirect effects by potential adverse effects on periphyton were observed neither in zooplankton nor in phytoplankton.

Investigations by ASS and litter cages did not reveal any adverse effects for all dose levels within the entire test period.

No effects could be observed on the three macrophytes species, which were introduced into the ponds or occurred naturally.

First significant effects were detectable at 4.4 µg a.s./L. The study revealed a clear dose/response relationship. The highest observed effect classes belong to class 3B. No pronounced effects without recovery were observed within this study.

The overall NOEAEC for this mesocosm study (including the laboratory study with filamentous algae) was set at 9.3 µg a.s./L.

Assessment and conclusion by applicant:

The study was conducted to the guidance in place at the time of conduct.

Analytical measurements taken on the days of application demonstrate that the nominal test concentrations were achieved.

In accordance with the Aquatic Guidance Document, the study data has been re-evaluated to take account of MDD analysis as well as re-assessment of the effect classifications. The results of this re-assessment are presented in the subsequent summary of study (M-690576-01-1). Further commentary on the reliability and acceptability of these mesocosm data is also included at the end of the following study summary.

Data Point:	KCP 10.2.3/03
Report Author:	[redacted]
Report Year:	2019
Report Title:	Re-evaluation of a mesocosm study with spiroxamine
Report No:	E 413 3295-9
Document No:	M-690576-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The effects of the fungicide active substance spiroxamine on aquatic organisms of different trophic levels (phytoplankton, periphyton, zooplankton and macroinvertebrates), were investigated in an outdoor mesocosm study (Bayer CropScience AG Report ID EBKWX091) conducted in 2007, in accordance with the guidance available at that time. In the current 'Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters' EFSA (2013), it is suggested to report minimum detectable differences (MDD) in connection with the NOECs for taxa assessed in a micro- or mesocosm study. To derive a regulatory acceptable concentration (RAC), it is recommended that for at least eight populations of the sensitive taxonomic groups, the MDDs should be sufficiently low for an evaluation of direct effects. Therefore, the objective of this re-evaluation work was to calculate MDDs for the biological data sets, to determine for how many populations of sensitive groups a reliable evaluation of direct effects was possible, and to re-evaluate the effects according to the Aquatic Guidance Document (EFSA 2013).

Due to the fungicide mode of action of the test item, all taxonomic groups present in the mesocosms were considered for the re-evaluation. Therefore, the analysis was performed for the data sets of phytoplankton, periphyton, zooplankton and macroinvertebrates. NOECs and MDDs for the taxa considered for the evaluation were calculated using the one-sided Williams-test following the proposal outlined in Brock *et al.* (2015). In addition, the effects of the most relevant taxa were classified according to the current guidance and recommendations in order to allow an estimation of ETO- and ERO-RAC.

For phytoplankton, macroinvertebrates and zooplankton, 19 taxa plus pooled data on higher taxonomic levels fulfil the MDD criterion proposed by Brock *et al.* (2015). Furthermore, the chlorophyll a measurements of phytoplankton and periphyton as well as the macrophyte coverage could be evaluated. If a more strict criterion is applied, *e.g.* that the MDD should be at least once <70% within the first four weeks after the first application, 13 taxa representing populations of macroinvertebrates, zooplankton and phytoplankton allow a statistical evaluation of direct effects: Tubificidae, Chironomini, *Chaoborus spec.*, *Simocephalus vetulus*, *Chydorus sphaericus*, *Eucercus lamellatus*, cyclopoid copepods (and nauplia larvae), *Polyarthra spec.*, *Chlamydomonas spec.*, coccoid Chlorophyceae, *Chroomonas spec.*, *Cryptomonas spec.* (20-30 µm), and Pennales (30-40 µm). Thus, the requirement of the Aquatic Guidance Document (EFSA PPR 2013) that the MDDs should be sufficiently low to allow the analysis of direct effect for at least 8 potential sensitive populations is met by the study.

The following effect classes were assigned to the different test concentrations:

- At the lowest test concentration of 1.0 µg/L, no treatment effects were found (class 1).
- At 2.1 µg/L, a slight direct effect on total phytoplankton abundance and a pronounced short-term promoting effect on the rotifer *Keratella quadrata* were detected (class 2 for the direct effect on the phytoplankton and 3A if the potential temporary promotion of a rotifer species is considered an adverse effect). Other taxa showed no effects.

- At 4.4 µg/L, class 3A effects for total rotifers, total phytoplankton, chlorophyll a and *Cryptomonas spec*. were observed while some other taxa were slightly affected. Thus, the total effect class for 4.4 µg/L is considered 3A.
- At 9.3 µg/L, more taxa were affected, effects were more pronounced or prolonged and for two algae species, significantly higher abundances than in the controls were found at the end of the study. However, since in general both species were rare and no algae bloom was found at the end of the study, this was not considered to be ecologically relevant. Thus effect class 3A was chosen as the overall effect class for 9.3 µg/L.
- At 19.4 µg/L the effect classification was similar to the one for 9.3 µg/L. For leeches, higher abundances at the end of the study could not be excluded, which was considered as class 2/4A for the highest test concentration.

According to EFSA (2013) the ETO-RAC can be derived from the overall class 1 concentration of 1 µg/L (nominal for three applications) and would be 0.5 µg/L using the recommended assessment factor of 2 in EFSA (2013).

However, if the potential temporary promotion of the rotifer Keratella and the slight effect (class 2) on total phytoplankton is considered acceptable, the 2.1 µg/L concentration could be used to derive the ETO-RAC, considering that the observed effects here are of low ecological relevance.

Since rotifers and algae seem to be the most sensitive taxa while taxa with a lower recovery potential were found to be less sensitive (Cladocera, Copepoda, Chironomidae, Chaoboridae, Hirudinea, Oligochaeta) the study can also be used to derive an ERO-RAC. At 9.3 µg/L, no, slight or only effects with recovery within 8 weeks were found. Thus, the ERO-RAC can be derived using this concentration and would be 3.1 µg/L using an assessment factor of 3.

No clear long-term effects were found at the highest test concentration of 19.4 µg/L, only for a potential promotion of leeches the effect duration could not be assessed. Thus, the ERO-RAC would be 4.85 µg/L applying an assessment factor of 4 to consider the higher uncertainty due to data on leeches.

Table CP 10.2.3/03-1 Summary of the effect classification

Taxon or endpoint		1.0 µg/L	2.1 µg/L	4.4 µg/L	9.3 µg/L	19.4 µg/L
Zooplankton	Cladocera	1	1	2	2	2
	Daphnia Longispina	1	1	1	1	2+
	Simocephalus vetulus	1	1	1	1	2+
	Chydorus sphaericus	1	1	1	2+	2+
	Copepoda	1	1	1	1	2
	Cyclopoid Copepods	1	1	1	1	1
	Nauplii	1	1	1	1	3A
	Rotifera	1	1	3A	3A	3A
	Keratella quadrata	1	3A+	3A+	3A+	3A+
	Polyarthra spec.	1	1	1	3A+	3A+
	Asplancha spec.	1	1	1	2	2
	Chaoborus spec. (larvae)	1	1	1	1	1
	Synchaeta spec.	1	1	2	2	2
Macroinvertebrates	Chironomidae	1	1	1	1	2
	Chironominae	1	1	1	1	2
	Chironomini gen spec.	1	1	1	1	2

Taxon or endpoint		1.0 µg/L	2.1 µg/L	4.4 µg/L	9.3 µg/L	19.4 µg/L
	Chaoborus crystallinus	1	1	1	1	1
	Hirundinea	1	1	1	1	2+/4A+
	Oligochaeta	1	1	1	1	1
Primary producers	Total phytoplankton	1	2	3A	3A	3A
	Cryptophyceae	1	1	2	3A	3A
	Chroomonas spec.	1	1	1	3A	3A
	Cryptomonas spec. 20-30 µm	1	1	3A	3A	3A
	Chlorophyceae	1	1	1	2	3A+
	Chlamydomonas spec.	1	1	1	1	1
	coccoid Chlorophyceae	1	1	2	2	2
	Ankyra judayi	1	1	2A	3A+	3A+
	*Characium spec.**	1	1	1	2+/4A+	2+/4A+
	Closterium cf leibleinii*	1	1	2	3A+/4A+	3A+/4A+
	Diatoms (Bacillariophyceae)	1	1	1	1	2
	Achnanthes spec.	1	1	2	3A	3A
	Pennales 20-30 µm	1	1	1	1	1
	Cyanobacteria	1	1	1	1	1
	Pseudoanabaena spec.	1	1	1	1	1
	Phytoplankton chlorophyll a	1	1	3A	3A	3A
	Periphyton chlorophyll a	1	1	1	1	1
	Total macrophyte coverage	1	1	1	2	2
Proposed total effect class		**1**	**2/3A**	**3A**	**3A***	**3A/4A+**

Taxa set in bold indicate MDD category 1 taxa which are considered to present a potentially sensitive population.

A sign '+' indicates a promoting effect o

* Increased abundances at the end of the study on the two algae species were not considered an adverse effect, i.e. an algae bloom, since the species were not abundant

I. Materials and Methods

In the outdoor mesocosm study, the test item (Spiroxamine EC 500) was applied three times (on day 0, 7 and 14 of the study). Effects were monitored for 84 days after the first application. The following biological examinations were performed during the study: the abundance of macrophytes (% coverage) and filamentous algae was assessed visually. Moreover, for the determination of the biomass, all macrophytes and filamentous algae were harvested at the end of the study. Determination of chlorophyll *a* (chl *a*) was performed for phytoplankton and periphyton. Phytoplankton was also sampled for identification and enumerating. The zooplankton was counted and identified at the species level, if possible. Emergence of insects was assessed by means of emergence traps. Macroinvertebrates were sampled by artificial substrates samplers (ASS). Additionally, macroinvertebrates were investigated using litter cages.

The test item was analysed in the water column and in the sediment during the study period. The limit of quantification (LOQ) of spiroxamine in the pond water was 0.102 µg/L. After each application (on day 0, 7 and 14), the test item dissipated continuously from the pond water. For the two lowest nominal

test concentrations of 1.0 and 2.1 µg/L, the actual concentrations were below LOQ from day 42 onwards. At the higher nominal test concentrations of 4.4 and 9.3 µg/L, the measured concentration was below LOQ from day 56 onwards. After day 70, the actual concentration of the test item was below LOQ in all tanks (Figure 1).

For the re-evaluation analysis described in this report, the data sets used for the original statistical evaluation in the study results were provided by the sponsor as excel files. The input data sets are given in Annex 1. The following data sets were re-evaluated: zooplankton, macroinvertebrates in ASS, phytoplankton, phytoplankton and periphyton chlorophyll *a*, and macrophytes (% coverage). The development of filamentous algae was quantified using categories as described in the original study report. Since the Williams test used for calculation of NOEC and MDD assumes normal distributed data on an interval or ratio scale, these data were not re-evaluated. Note that for a better assessment of filamentous algae, a laboratory bioassay was performed and included in the original study report. In this laboratory study, short term effects occurred at 9.3 µg/L; thus the overall NOEC for filamentous algae in the laboratory study was stated to be 4.4 µg/L.

In this re-evaluation the same taxonomic groups were used, as in the original data sets following the recommendations of the guidance document (EFSA, 2013). Univariate statistics was performed on single species level (or the lowest taxonomic level identified) as well as on aggregated data like total abundances of organisms at a higher taxonomic level (*e.g.* family or order level) if these were provided.

The NOECs and related MDDs were calculated by means of the multiple t-tests of Williams (Williams, 1971, 1972). This test is similar to the well-known Dunnett-test (Dunnett 1955, 1964), but has slightly more power to detect differences to the controls (Jaki and Hothorn, 2013).

If the data did not show a monotonous dose-response relationship, the Williams´ test uses a moving average procedure before testing to achieve monotony. The assumption of a monotonous concentration-response can be made here because the focus is on direct effects on sensitive taxa.

The Williams' tests were performed one-sided with $\alpha = 0.05$ (5% level of significance).

The abundance data of the organisms were log-transformed [y' = ln(a y+1)] before analysis, in order to approximate normality and homoscedasticity (homogeneity of variances) requirements (van den Brink *et al*. 2000). The factor 'a' was selected to achieve a logtransformed value close to 1 for the lowest non-zero value in the data set which results in log data sets scaled in a comparable way.

According to Brock *et al.* (2015), MDDs were calculated for the NOECs derived by the Williams´ test.

As abundance data were log-transformed for statistical testing, this MDD was also related to the transformed data, *i.e.* on a log-scale. Because % effects on a log-scale are difficult to interpret, the MDDs were transformed back to the abundance scale and these MDDs were used for evaluation (see Brock *et al*. 2015). Thus, for example, an MDD of 80 % means that the geometric mean abundance at the NOEC would have to be more than 80 % lower than the geometric mean of the controls to become statistically significant.

In the EFSA guidance document (EFSA 2013) no clear criteria are given for MDDs to be sufficiently low for a reliable analysis but classes for effect magnitudes have been proposed.

Table CP 10.2.3/03-2 Proposal on classes of MDDs due to treatment-related declines in abundance/biomass

Class	MDD	Comment
0	>100 %	No effect can be determined
I	90 – 100 %	Only large effects can be determined
II	70 – 90 %	Large to medium effects can be determined
III	50 – 70 %	Medium effects can be determined

IV	<50 %	Small effects can be determined

Based on this MDD classification by EFSA, Brock *et al*. (2015) proposed a criterion for evaluating the MDDs per taxon. They suggest that for a reliable analysis of direct effects on a specific taxon, the following should be given:

- MDD <100 % on at least 5 samplings or
- MDD <90 % on at least 4 samplings or
- MDD <70 % on at least 3 samplings or
- MDD <50 % on at least 2 samplings after the application of the test item.

To apply this criterion, it was counted for each taxon how often (after the first application) the MDD fell in the MDD classes suggested by EFSA, 2013 *i.e*. how often the MDD was below 50 %, 70 %, 90 % and 100 %. Based on this count, it was decided if the MDD criterion proposed by Brock *et al*. (2015) was fulfilled.

Each taxon (or endpoint) was assigned to one of the three following categories, as suggested by Brock *et al*. (2015):

Category 1: The MDDs are sufficiently low for a reliable analysis according to the criterion proposed by Brock *et al*. 2015 (see above). These taxa are considered for the effect classification.

Category 2: The MDD criterion is not met, but on at least one sampling date (after application) a significant difference (negative or positive) to controls is found. These taxa are checked whether the statistical results indicate an effect of the treatment. If yes, the effects are classified

Category 3: The MDD criterion is not met and no significant deviation to control is found. These taxa are not further considered for evaluation.

The biological effects were classified according to the recommendations of the EFSA aquatic guidance document (2013) and Brock *et al.* (2015), which are a modification of the scheme of De Jong *et al.* (2008) now considering also the MDD. In order to differentiate cases when recovery is clearly not shown from cases when recovery cannot be demonstrated because of *e.g.* the taxon is declining or absent in the controls during the recovery period, or the effect was found at the end of the study, or the MDD was too large to demonstrate recovery, such cases were also put into effect class 4 (originally used for cases when the study was too short to test recovery within 8 weeks). Therefore, class 4 was differentiated into 4A (study too short to analyse recovery) and 4B (recovery could not be assessed due to high MDD or decline of abundance also in the controls). If potential treatment effects were found at the end of the study, these were indicated as 2 - 4A or 3A - 4A because the duration of the effect could not be assessed.

Class 0 (treatment related effects cannot be statistically evaluated) does not fit well with the other effect classes because this is a property of the full data set for a taxon over all treatment levels including the controls while the other effect classes are related to the effect at the different treatment levels. Thus, if treatment-related effects on a taxon cannot be statistically evaluated, class 0 would apply for each treatment level. This is however covered already by the MDD categorisation: all taxa of MDD category 3 are the ones with effect class 0. Therefore, no effect classification was conducted for category 3 taxa.

With respect to the demonstration of 'full' or 'complete' recovery, EFSA (2013) states in footnote 33 (p. 121) that 'An endpoint is considered as recovered if the MDD allows statistical evaluation during the relevant recovery period (so excluding MDD class 0) and the conclusion of no statistically significant effect between treated systems and controls is not caused by a decline of that endpoint in controls (*e.g.* at the end of the growing season). If these criteria are violated, a higher effect class has to be selected'.

This would mean that a difference to controls of *e.g.* 95 % can demonstrate full recovery as long as the MDD is <100 % and the difference to control is not significant. This is in contradiction to the specific protection goals listed in the same document where only small effects over months, medium effects over

weeks or large effects over days are considered acceptable for aquatic invertebrates (Table 14, p. 54 in EFSA 2013) while with respect to the MDD, small effects are defined as <50 %, and large effects as 90 – 100 % (Table 31, p. 118 in EFSA (2013)).

Therefore, the more stringent recovery criterion of Brock *et al.* (2015) that 'recovery from treatment-related declines in abundance can only be considered if the MDDabu values during the relevant recovery period are <70 % on at least one sampling, or <90 % on at least two samplings, or if the % deviation from controls is less than 20 %' was used in this report.

The aim of this re-evaluation work was to provide data for deriving an ETO-RAC and an ERO-RAC according to the current EFSA aquatic guidance document (2013), *i.e.* to identify the treatment levels with effect classes up to 3A only based on the identification of the most sensitive taxa. Therefore, the focus of the effect evaluation was on the MDD category 1 taxa. Taxa of category 2 were only discussed if, based on the statistical finding, they were more sensitive than category 1 taxa. Category 3 taxa were not considered further because of high MDD values and missing statistical significance, and, in most cases, their low abundances.

Note that the MDD evaluation is related to direct effects, *i.e.* reduction of abundances. If a test item has an indirect effect shown as a treatment-related increase of abundance, the MDD classification is not applicable because the effects can be larger than 100 %. Thus, MDD category 2 taxa can be used for the assessment of indirect effect, even if MDDs are high. A promotion effect is indicated by a '+' sign added to the effect class, *e.g.* 3A+ indicates a pronounced but temporary promotion.

With hundreds of taxa and many sampling dates, a large number of statistical tests was conducted. Using an error level of 5 % means that a lot of positive findings were to be expected just by chance. In addition, by the default use of the Williams´ test as a most conservative multiple test, low NOECs can be obtained also in cases where there was no monotonous (or almost monotonous) concentration-response relation – just by the moving average procedure used by the Williams´ test to achieve a monotonous concentration response before the testing. Therefore, the statistical findings were evaluated for their ecotoxicological relevance based on different criteria.

Table CP 10.2.3/03-3 Definition of effect classes based on EFSA (2013) and Brock *et al.* (2015)

Effect class	Description	Criteria
1	No treatment-related effects demonstrated	No (statistically and/or ecologically significant) effects observed as a result of the treatment Observed differences between treatment and controls show no clear causal relationship
2	Slight effects	Effects concern short-term and/or quantitatively restricted responses usually observed at individual samplings only.*
3A	Pronounced short-term effects (effect period < 8 weeks), followed by recovery	Clear response of sensitive endpoints, but full recovery within 8 weeks after the last application, or in the case of delayed responses and repeated applications, the duration of the effect period is less than 8 weeks and followed by full recovery.* Treatment-related effects demonstrated on consecutive samplings
3B	Pronounced effects longer than 8 weeks but recovery within 8 weeks after last application	Clear response of the endpoint in micro-/mesocosm experiment repeatedly treated with the test substance and that lasts longer than eight weeks (responses already start in treatment period), but full recovery of affected endpoint within eight weeks post last application.*
4A	Significant effects in short-term study	Clear effects (e.g. large reductions in densities of the population) observed, but the study is too short to demonstrate complete recovery within eight weeks after the (last) application. If delayed response is observed on the last sampling(s) only, this may be indicated as effect class 2-4A or 3A-4A.
4B	Significant short-term effects but MDD too high in recovery period	Significant short-term effects demonstrated but recovery cannot be properly evaluated due to high %MDD values in recovery period or the population in the controls is declining or even absent. If significant treatment-related response is demonstrated on one sampling but recovery cannot be interpreted due to high MDD this may be indicated as class 2-4B, in other case it can be 3A-4B.
5A	Pronounced long-term effect followed by recovery	Clear response of sensitive endpoint, effect period longer than 8 weeks and recovery did not yet occur within 8 weeks after the last application but full recovery is demonstrated to occur in the year of application.*
5B	Pronounced long-term effects without recovery	Clear response of sensitive endpoints (> 8 weeks post last application) and full recovery cannot be demonstrated before termination of the experiment or before the start of the winter period.

*Note that following Brock *et al.* (2015) recovery can only be considered if the MDDs during the recovery period are < 70 % on at least one sampling or < 90 % on at least two samplings or if the deviation to controls is less than 20 %. If this is not the case, an appropriate higher class has to be selected.

The program Community Analysis (CA) V4 was used for NOEC, MDD and diversity calculations. A former version of the CA program is described in Hommen *et al.* (1994). Calculations of the CA program have been validated by means of example data and of calculations using MS-Excel™ (Microsoft® Corp.) and ToxRat® (Vers. 2.09).

II. Results and Discussion

Zooplankton

Total zooplankton, the sums for cladocerans, daphnids, copepods and rotifers but also several lower taxa fulfil the MDD criteria proposed by Brock *et al*. (2015). In total eight taxa representing potentially sensitive populations (*Daphnia longispina, Simocephalus vetulus, Chydorus sphaericus, Cyclopoida* (adults and copepods as well as nauplia larvae which could not be further determined), *Keratella quadrata, Polyarthra spec*., *Asplanchna spec*. and *Chaoborus spec*.) could be evaluated for direct effects according to the MDD criterion proposed by Brock *et al*. (2015). Ten other taxa belonged to MDD category 2, *i.e*. the MDDs did not meet the criterion but significant deviations to controls were found at least once after the first application.

Table CP 10.2.3/03-4 % MDDs for the taxa in the zooplankton data set which met the criterion proposed by Brock *et al*. (2015). MDD category 1

Zooplankton	Summary			
	Min	Max	Mean	MDD Cat
Sum of Cladocerans	45	90	76	1
Sum of Daphnids	78	167	105	1
Sum of Copepods	47	88	73	1
Sum of rotifers	30	96	69	1
Daphnia longispina	84	167	106	1
Simocephalus vetulus	63	104	85	1
Chydorus sphaecirus	56	114	94	1
Cyclopoid Copepods	58	91	80	1
Copepod Nauplii	50	88	72	1
Keratella quadrata	79	102	89	1
Polyarthra spec.	45	104	88	1
Asplanchna spec.	73	107	86	1
Chaoborus spec. larvae	62	213	103	1

MDD cat = category based on MDD evaluation according to Brock et al. (2015)

Table CP 10.2.3/03-5 % MDDs for the taxa in the zooplankton data set which met the criterion proposed by Brock *et al*. (2015). MDD category 2

Zooplankton	Summary			
	Min	Max	Mean	MDD Cat
Graptoleberis testudinaria	71	165	123	2
Acroperus harpae	105	166	122	2
Eucercus lamellatus	34	157	101	2
Ceriodaphnia reticulate	n.c.	n.c.	n.c.	2
Ostracodes	77	145	107	2
Testudinella patina	87	158	127	2
Lepadella patella	84	166	112	2

Zooplankton	Summary			
	Min	Max	Mean	MDD Cat
Synchaeta spec.	85	105	98	2
Hexarthra spec.	105	171	126	2
Chaoborus spec. pupae	225	246	232	2

MDD cat = category based on MDD evaluation according to Brock et al. (2015)

Significantly lower abundances than in the controls on at least two consecutive sampling dates were found for two rotifer species and also for the sum of rotifers. The most sensitive taxa according to the statistical analysis were *Asplanchna spec*. and *Polyarthra spec*. For *Asplanchna spec*., NOECs of 4.4 and 2.1 µg/L were found on day 11 and 14 indicating potential short-term effects. However, abundance of *Asplanchna* collapsed in all mesocosms, including the controls during the first three weeks after the day of the first application. After the first and the third application, the decline in 19.4 µg/L was not stronger than in the controls and on day 14 there was no clear concentration response relation. Thus, effects of 9.3 and 19.4 µg/L were considered slight (conservatively, class 2 effects are proposed). The findings of *Asplanchna* later in the mesocosm treated with 19.4 µg/L supports that the interpretation of no pronounced effects on this species up to the highest test concentration.

Table CP 10.2.3/03-6 NOECs [µg/L] and related % MDDs (in brackets) for the taxa in the zooplankton data set

Macrozoobenthos		Days after application													
MDD cat	Taxa / day	-14	-7	0	4	7	11	14	18	21	28	42	56	70	84
1	Sum of Cladocerans	≥19.4 (84)	≥19.4 (91)	≥19.4 (89)	≥19.4 (73)	≥19.4 (85)	2.1-(47)	≥19.4 (83)	≥19.4 (85)	≥19.4 (77)	1+ (53)	9.3+ (78)	≥19.4 (90)	≥19.4 (85)	≥19.4 (89)
1	Sum of Daphnids	≥19.4 (121)	≥19.4 (103)	≥19.4 (100)	≥19.4 (100)	≥19.4 (100)	≥19.4 (101)	9.3-(98)	≥19.4 (99)	≥19.4 (93)	≥19.4 (84)	2.1+ (78)	9.3-(84)	≥19.4 (85)	≥19.4 (85)
1	Sum of Copepods	≥19.4 (63)	≥19.4 (65)	≥19.4 (66)	≥19.4 (82)	≥19.4(81)	≥19.4(88)	≥19.4(72)	≥19.4 (78)	≥19.4(75)	≥19.4(76)	≥19.4(69)	9.3-(47)	≥19.4(73)	≥19.4(64)
1	Sum of Rotifers	≥19.4 (27)	≥19.4 (74)	1-(48)	≥19.4 (77)	4.4-(46)	4.4-(55)	2.1-(30)	2.1-(66)	≥19.4 (71)	≥19.4 (96)	<1+ (76)	≥19.4 (84)	≥19.4 (84)	≥19.4 (79)
1	Daphnia longispina	≥19.4 (121)	≥19.4 (103)	≥19.4 (100)	≥19.4 (101)	≥19.4 (100)	≥19.4 (101)	≥19.4 (98)	≥19.4 (99)	≥19.4 (93)	≥19.4 (84)	2.1+ (84)	9.3-(84)	≥19.4 (155)	≥19.4 (167)
1	Simocephalus vetulus	≥19.4 (95)	≥19.4 (99)	≥19.4 (97)	9.3-(69)	≥19.4 (81)	2.1-(63)	≥19.4 (88)	≥19.4 (92)	≥19.4 (97)	≥19.4 (80)	≥19.4 (97)	≥19.4 (89)	≥19.4 (104)	≥19.4 (74)
1	Chydorus sphaericus	≥19.4 (85)	≥19.4 (90)	≥19.4 (95)	≥19.4 (93)	≥19.4 (85)	≥19.4 (56)	4.4+(80)	≥19.4 (98)	≥19.4 (98)	≥19.4 (101)	≥19.4 (107)	≥19.4 (114)	≥19.4 (103)	≥19.4 (104)
1	Cyclopoid Copepods	≥19.4 (78)	≥19.4 (55)	≥19.4 (63)	≥19.4 (86)	≥19.4 (88)	≥19.4 (85)	≥19.4 (91)	≥19.4 (89)	≥19.4 (74)	≥19.4 (77)	≥19.4 (87)	≥19.4 (61)	≥19.4 (83)	≥19.4 (58)
1	Copepod Nauplii	≥19.4 (63)	≥19.4 (74)	≥19.4 (75)	≥19.4 (82)	≥19.4 (72)	≥19.4 (88)	4.4+(58)	≥19.4 (80)	≥19.4 (81)	≥19.4 (79)	9.3-(52)	9.3-(50)	≥19.4 (71)	≥19.4 (77)

Macrozoobenthos		Days after application													
MDD cat	Taxa / day	-14	-7	0	4	7	11	14	18	21	28	42	56	70	84
1	Keratella quadrata	≥19.4 (29)	≥19.4 (70)	≥19.4 (44)	≥19.4 (82)	≥19.4 (90)	≥19.4 (99)	≥19.4 (79)	≥19.4 (87)	≥19.4 (87)	≥19.4 (102)	1+ (92)	1+ (86)	1+ (90)	≥19.4 (85)
1	Polyarthra spec.	≥19.4 (95)	≥19.4 (86)	≥19.4 (72)	≥19.4 (72)	4.4+ (45)	4.4+ (67)	4.4+ (93)	9.3- (98)	4.4+ (95)	≥19.4 (101)	≥19.4 (104)	≥19.4 (97)	4.4+ (98)	≥19.4 (99)
1	Asplanchna spec.	≥19.4 (99)	≥19.4 (82)	<1- (50)	≥19.4 (85)	≥19.4 (80)	4.4+ (73)	2.1- (85)	≥19.4 (107)		9.3+ (n.c.)	9.3+ (n.c.)			
1	Chaoborus spec. larvae	9.3+ (87)	≥19.4 (169)	≥19.4 (145)	≥19.4 (62)	≥19.4 (114)	≥19.4 (80)	≥19.4 (111)	≥19.4 (92)	≥19.4 (89)	≥19.4 (106)	≥19.4 (93)	≥19.4 (89)	≥19.4 (213)	≥19.4 (79)
2	Graptoleberis testudinaria	≥19.4 (274)	≥19.4 (n.c.)	≥19.4 (159)	≥19.4 (137)	≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (165)	≥19.4 (146)	≥19.4 (160)	≥19.4 (113)	1+ (71)	≥19.4 (97)	≥19.4 (98)	≥19.4 (100)
2	Acroperus harpae		≥19.4 (142)	≥19.4 (161)		9.3+ (n.c.)	≥19.4 (n.c.)				≥19.4 (166)		≥19.4 (110)	≥19.4 (105)	≥19.4 (106)
2	Eucercus lamellatus		9.3+ (n.c.)	≥19.4 (163)	≥19.4 (119)	≥19.4 (127)	4.4+ (75)	≥19.4 (101)	≥19.4 (103)	9.3+ (34)	≥19.4 (114)	≥19.4 (103)	<1- (80)	≥19.4 (100)	≥19.4 (157)
2	Ceriodaphnia reticulata			≥19.4 (n.c.)					9.3+ (n.c.)		≥19.4 (n.c.)	9.3+ (n.c.)			
2	Ostracodes	≥19.4 (198)	≥19.4 (n.c.)	≥19.4 (n.c.)		≥19.4 (n.c.)	≥19.4 (n.c.)	9.3+ (n.c.)	≥19.4 (145)	≥19.4 (167)	≥19.4 (112)	≥19.4 (77)	≥19.4 (94)	≥19.4 (94)	≥19.4 (93)
2	Testudinella patina						≥19.4 (n.c.)	≥19.4 (158)	≥19.4 (n.c.)	2.1+ (n.c.)	≥19.4 (137)	<1+ (148)	<1+ (125)	≥19.4 (105)	≥19.4 (87)
2	Lepadella patella			≥19.4 (n.c.)					≥19.4 (n.c.)	≥19.4 (166)	≥19.4 (137)	1+ (91)	≥19.4 (84)	≥19.4 (98)	≥19.4 (94)
2	Synchaeta spec.			9.3- (98)	≥19.4 (105)	≥19.4 (101)	≥19.4 (100)		4.4- (85)	≥19.4 (99)	≥19.4 (99)	≥19.4 (n.c.)	≥19.4 (n.c.)		≥19.4 (n.c.)
2	Hexarthra spec.										9.3- (n.c.)	≥19.4 (171)	≥19.4 (108)	≥19.4 (121)	≥19.4 (105)
2	Chaoborus spec. pupae		≥19.4 (n.c.)		≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (246)		≥19.4 (n.c.)	≥19.4 (n.c.)		≥19.4 (n.c.)	≥19.4 (225)	≥19.4 (225)	9.3+ (n.c.)

Signs indicate the direction of a significant effect and colours indicate the different NOECs. Blank fields: taxon not present. n.c.: MDD could not be calculated because of absence in the controls. Empty cells indicate absence in all samples of that day. Cat = MDD category according Brock et al. (2015).

Figure CP 10.2.3/03-1 ***Asplanchna*** **spec.: Geometric means per treatment level and range of controls**

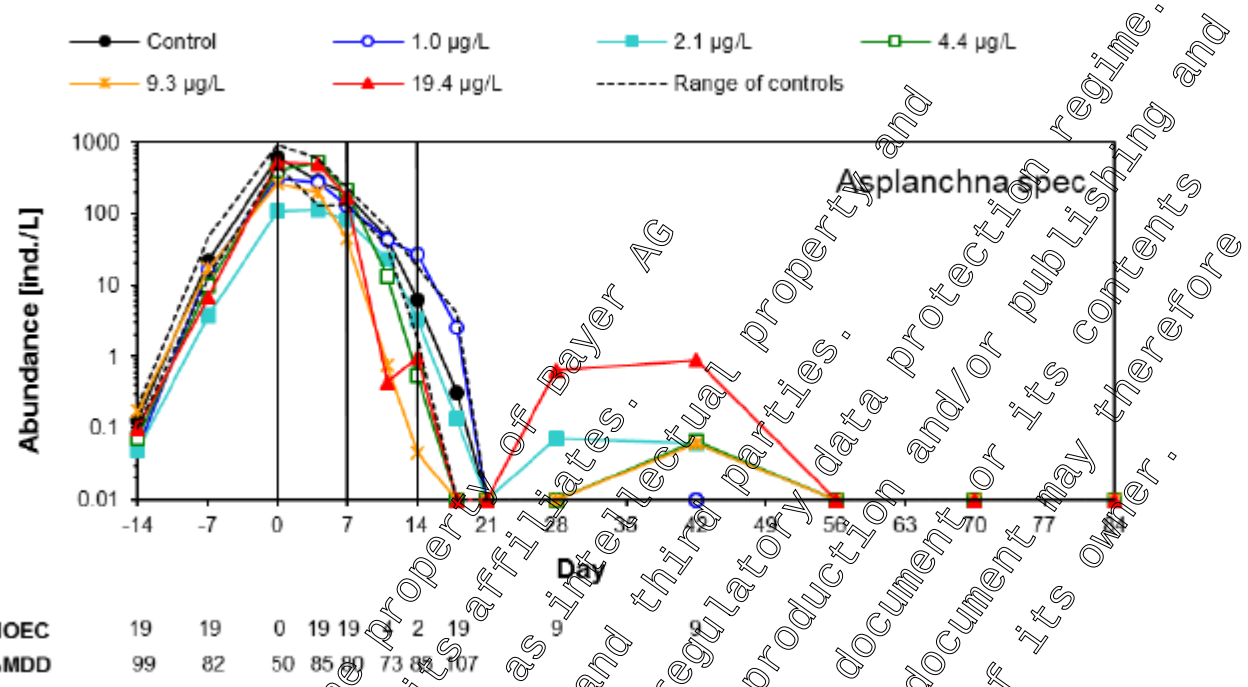


Figure CP 10.2.3/03-2 ***Polyarthra*** **spec.: Geometric means per treatment level and range of controls**

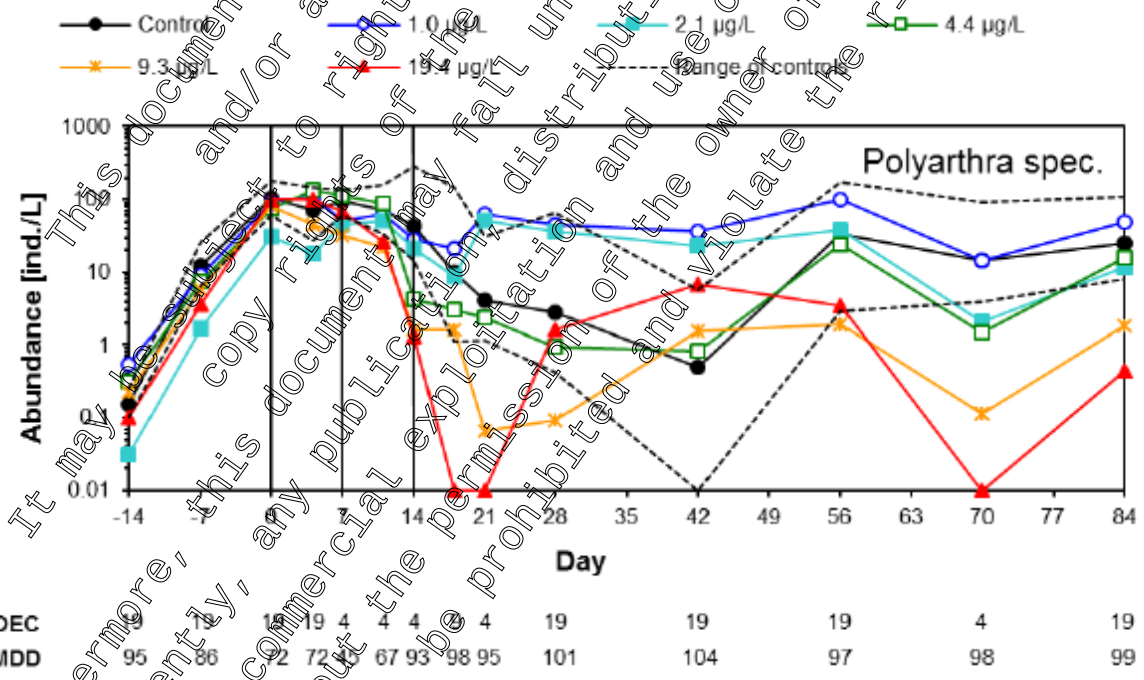
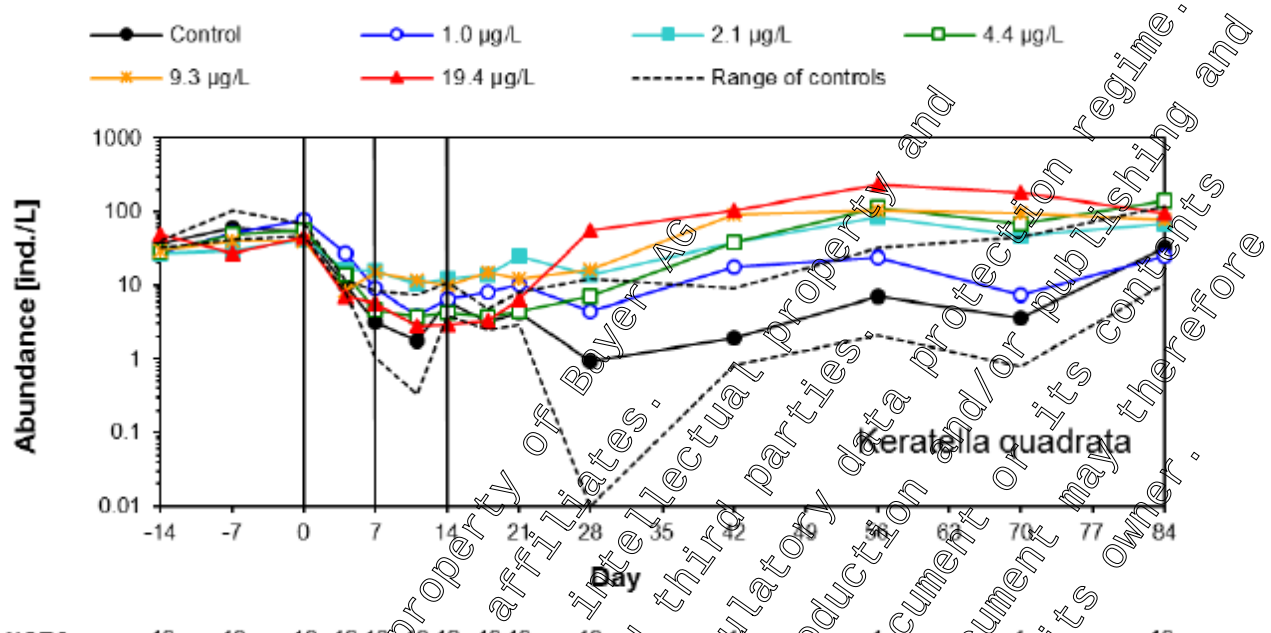
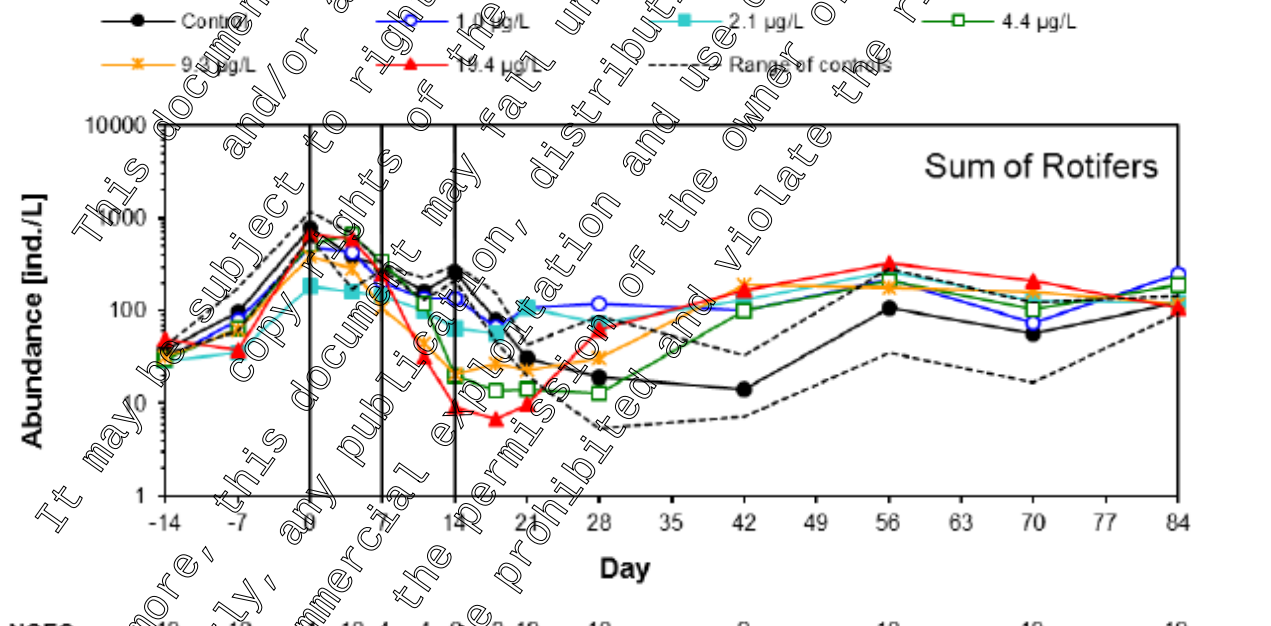


Figure CP 10.2.3/03-3 ***Keratella quadrata*: Geometric means per treatment level and range of controls**



NOEC	19	19	19	19	19	19	19	19	19			1	19
%MDD	29	70	44	82	80	99	79	87	87	102	92	86	85

Figure CP 10.2.3/03-4 **Sum of rotifers: Geometric means per treatment level and range of controls**



NOEC	19	19	19	4	4	4	2	19	19	0	19	19	19
%MDD	27	74	48	77	46	55	30	66	71	96	76	84	84	79

Figure CP 10.2.3/03-5 Copepod nauplii: Geometric means per treatment level and range of controls

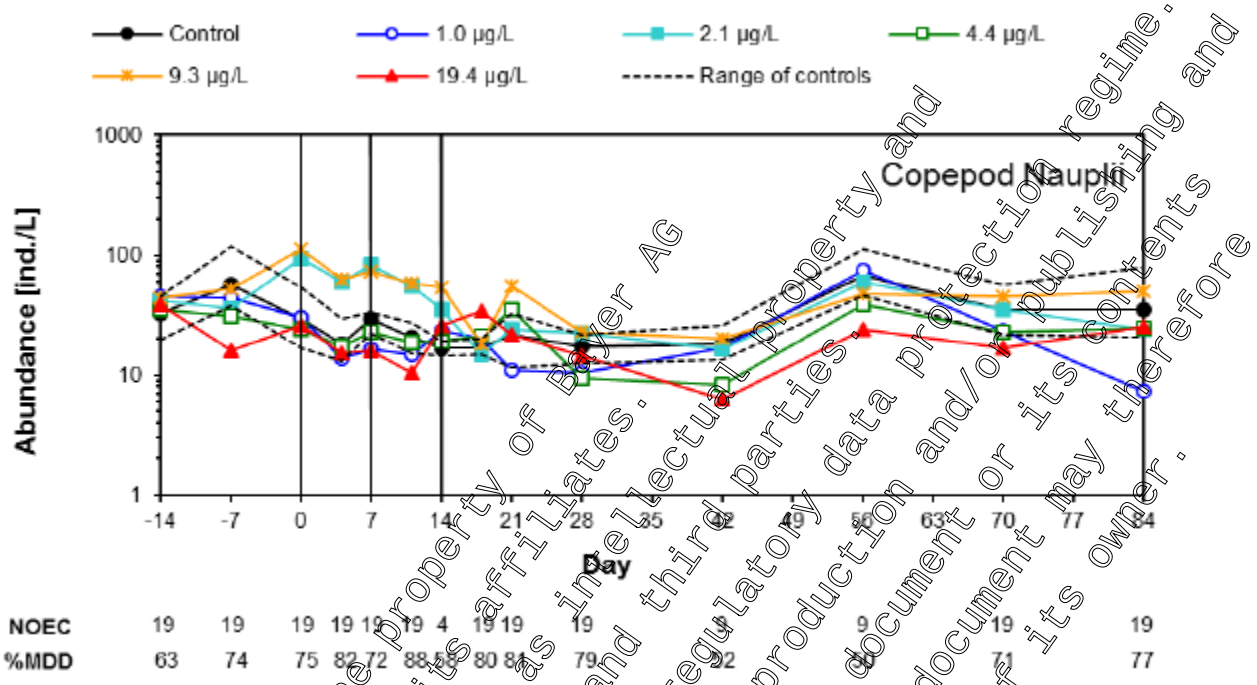


Figure CP 10.2.3/03-6 Sum of Copepods: Geometric means per treatment level and range of controls

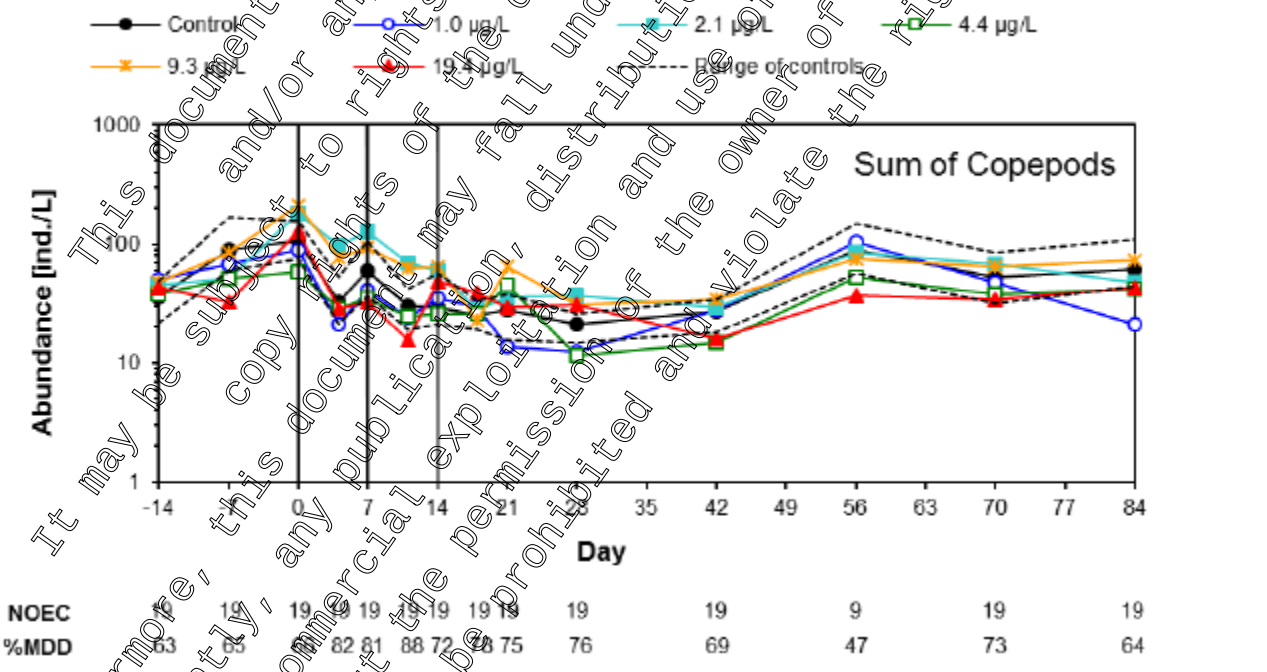


Figure CP 10.2.3/03-7 Sum of Cladocerans: Geometric means per treatment level and range of controls

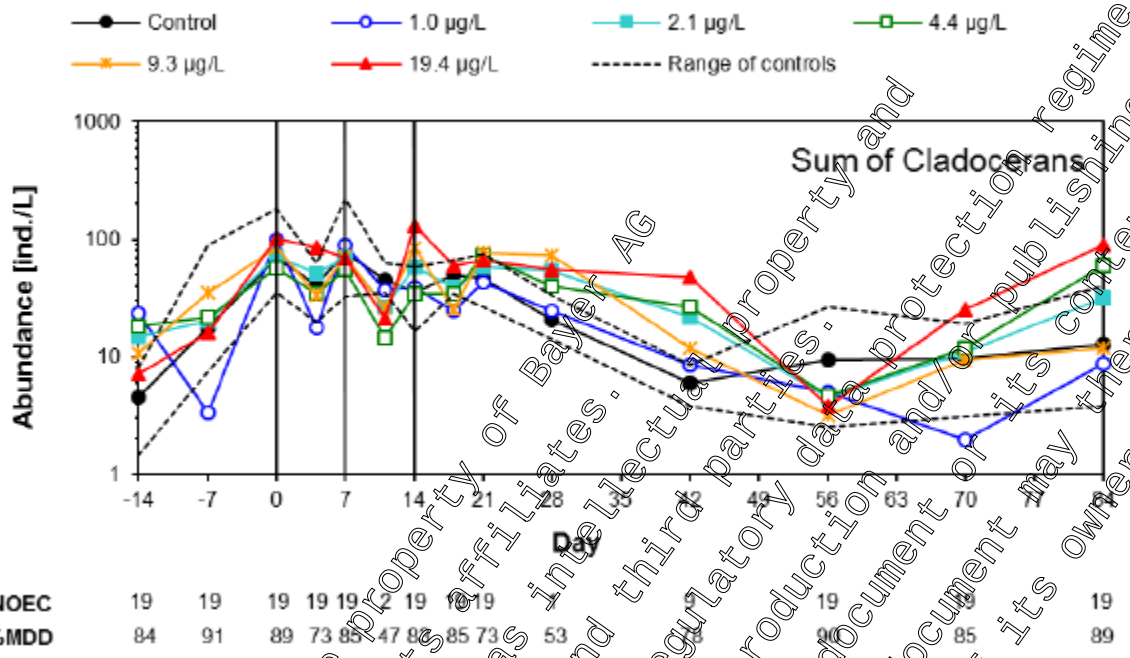


Figure CP 10.2.3/03-8 *Daphnia longispina*: Geometric means per treatment level and range of controls

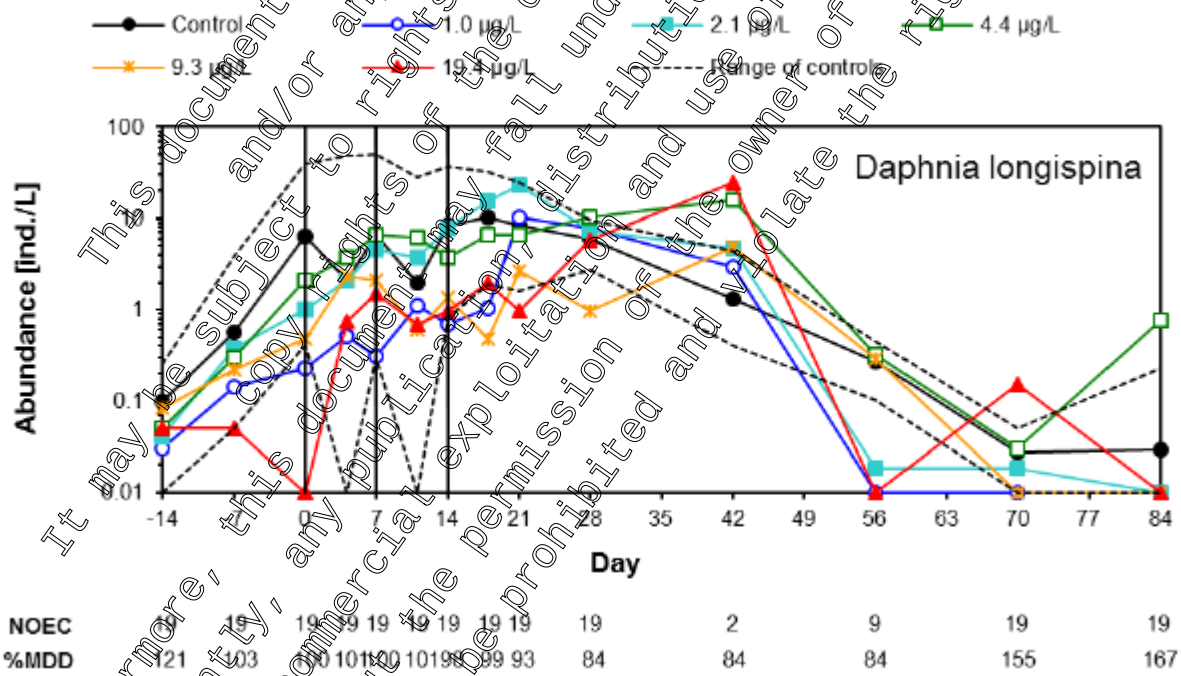
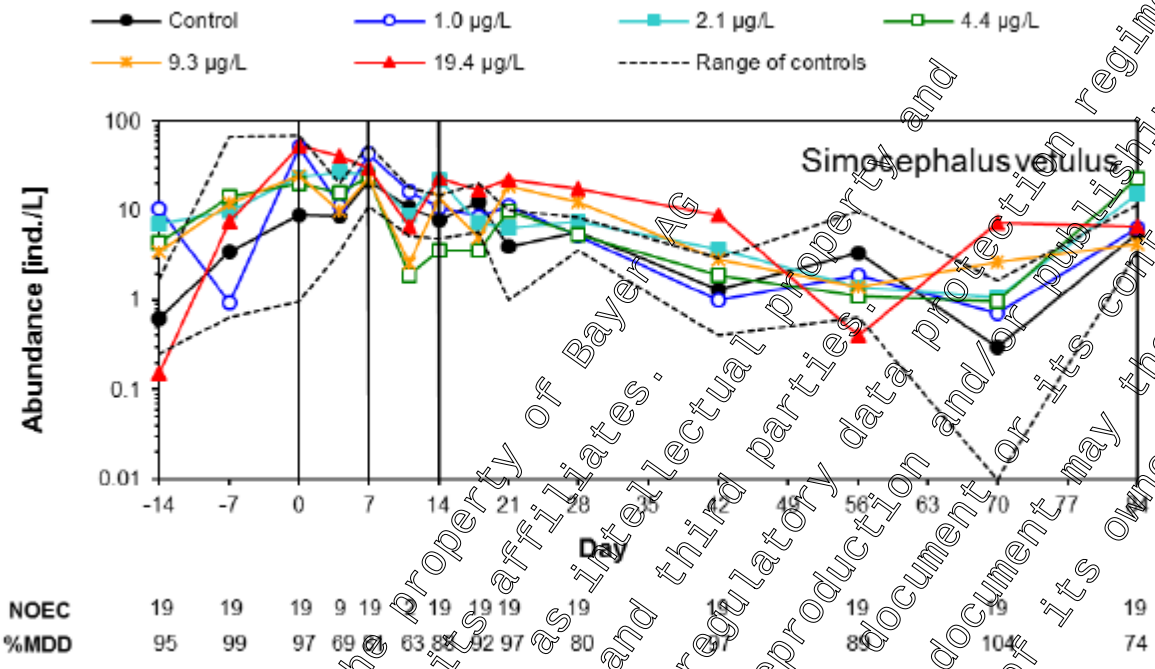
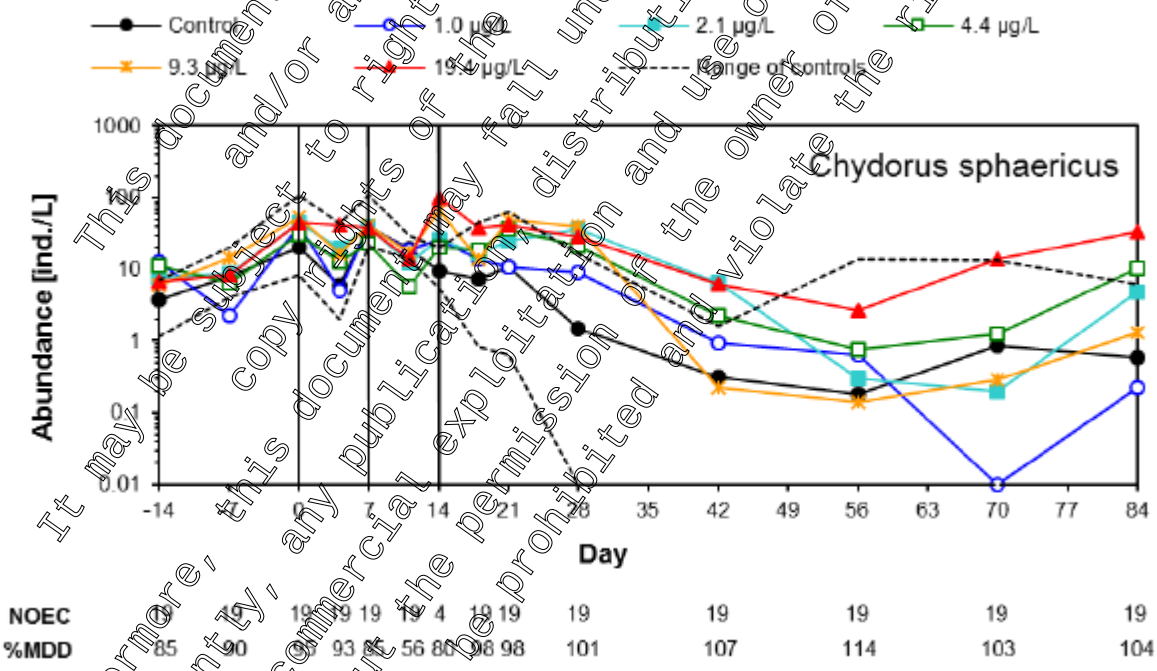


Figure CP 10.2.3/03-9 ***Simocephalus vetulus*: Geometric means per treatment level and range of controls**



NOEC	19	19	19	9	19	19	19	19	19	19	19	19	19
%MDD	95	99	97	69	61	63	88	92	97	80	87	89	104	74

Figure CP 10.2.3/03-10 ***Chydorus sphaericus*: Geometric means per treatment level and range of controls**



NOEC	19	19	19	19	19	19	4	19	19	19	19	19	19
%MDD	85	90	90	93	85	56	80	98	98	101	107	114	103	104

Figure CP 10.2.3/03-11 Sum of Daphnids: Geometric means per treatment level and range of controls

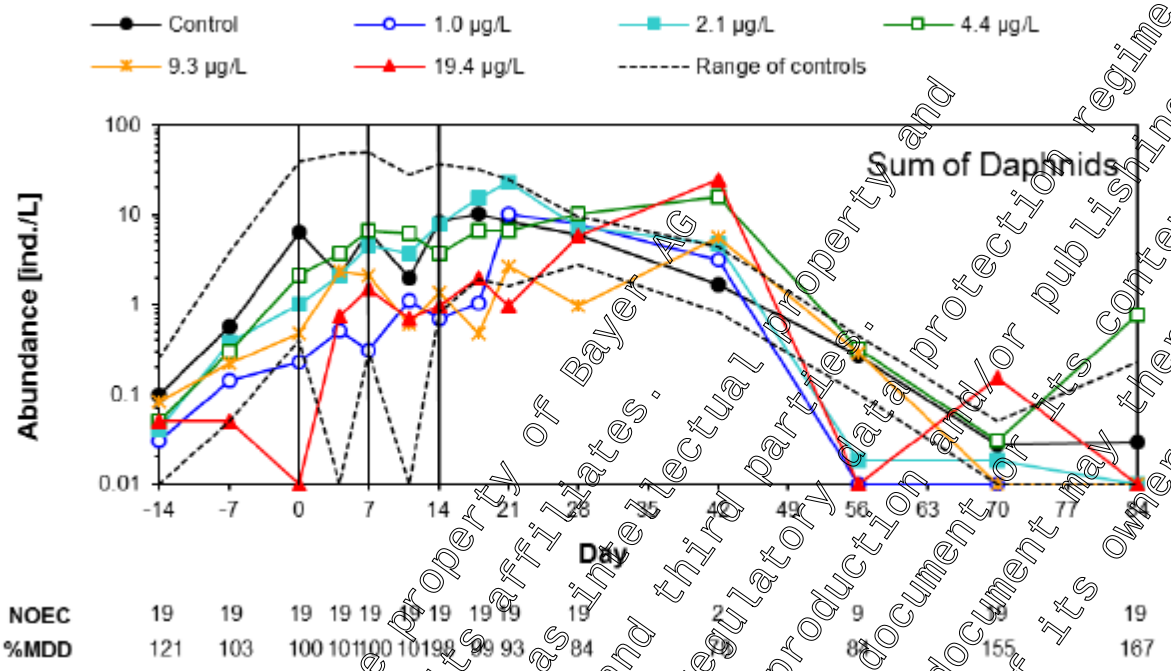


Figure CP 10.2.3/03-12 *Synchaeta* spec.: Geometric means per treatment level and range of controls

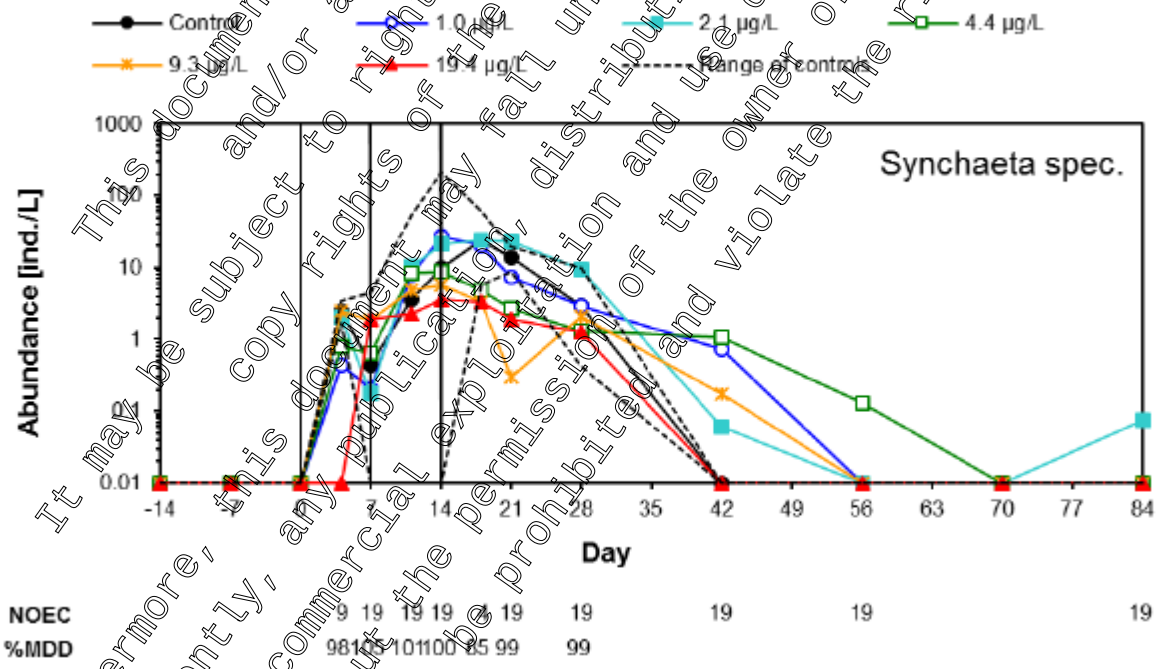
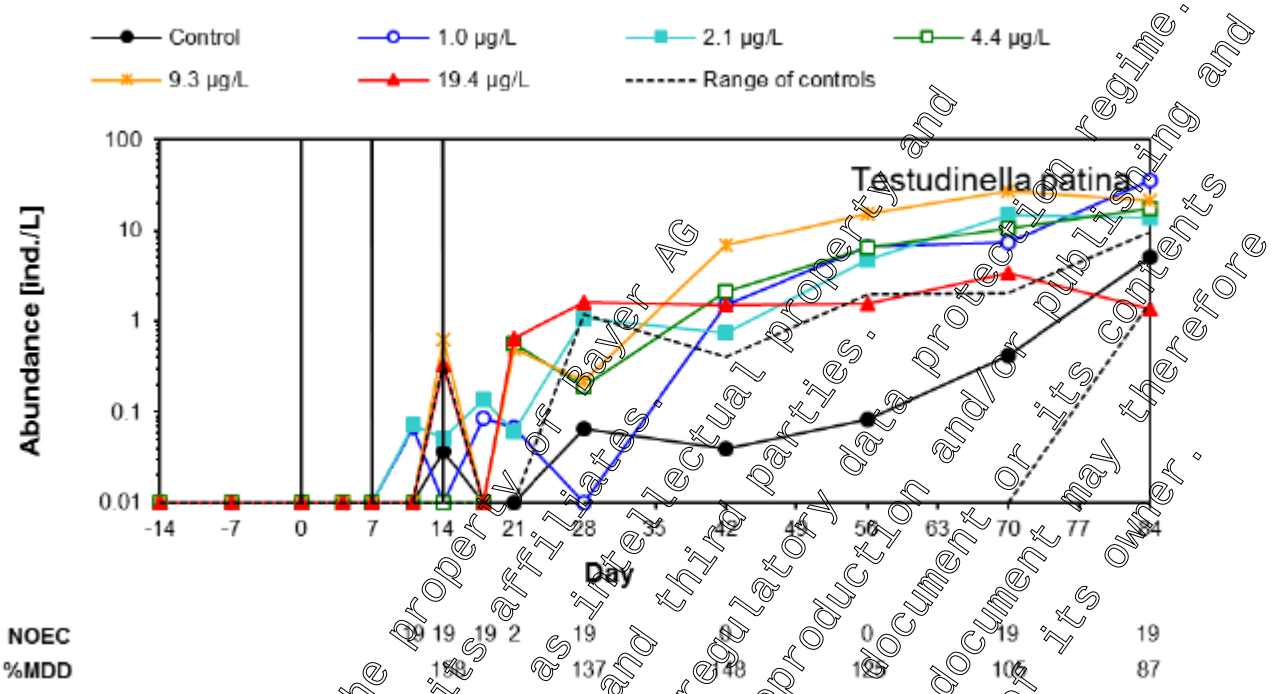


Figure CP 10.2.3/03-13 ***Testudinella patina*: Geometric means per treatment level and range of controls**



Macrozoobenthos

Macrozoobenthos organisms were sampled using artificial substrate samplers (ASS). In the data set, six taxa fulfil the MDD criterion defined by Brock *et al.* (2015): Chironomidae in total, Chironominae, *Chironomini gen spec*. *Chaoborus crystallinus* larvae, leeches (Hirudinea) and Oligochaeta.

Table CP 10.2.3/03-7 % MDDs for the taxa in the macroinvertebrate data set which met the criterion proposed by Brock *et al.* (2015). MDD category 1

Zooplankton	Summary			
	Min	Max	Mean	MDD Cat
Sum of Chironomids	55	262	112	1
Sum of Chironominae	55	174	95	1
Sum of leeches	73	226	107	1
Sum of Oligochaeta	64	138	91	1
Chironomini gen spec.	52	225	108	1
Chaoborus crystallinus larvae	82	148	101	1

MDD cat = category based on MDD evaluation according to Brock et al. (2015).

Furthermore, for 13 taxa, the MDD criterion was not met, but on at least one sampling date after application, a significant difference to the controls was found (MDD category 2 taxa).

Table CP 10.2.3/03-8 % MDDs for the taxa in the macroinvertebrate data set which met the criterion proposed by Brock *et al*. (2015). MDD category 2

Zooplankton	Summary			
	Min	Max	Mean	MDD Cat
Sum of Tanypodinae	71	225	115	2
Stylaria lacustris	72	122	97	2
Herpobdella octoculata	71	262	131	2
Gastropoda non det.	n.c.	n.c.		2
Gyraulus albus	97	186	136	2
Musculium lacustre	94	218	167	2
Pisidium spec	117	225	186	2
Caenis spec	65	112	89	2
Tanypodinae gen spec	71	225	115	2
Tanytarsini gen spec.	186	192	189	2
Culex spec	93	93	93	2
Hydrophilidae gen spec	92	92	92	2
Zygoptera Gen spec	n.c.	n.c.		2
Dugesia gonocephala	84	272	153	2

MDD cat = category based on MDD evaluation according to Brock et al. (2015)

No consistent significant differences to controls over at least two consecutive sampling dates were found for the taxa in the MASS data set, only on isolated sampling occasions NOECs <19.4 µg/L were detected.

Sums of Chironomidae as well as Chironominae were significantly reduced in the highest treatment on day 28 while similar reductions were observed on day 21 however without statistical significance. Later, the abundance in the mesocosm treated with 19.4 µg/L was close to control level again. The deviation of the mean abundance in the 2.1 µg/L mesocosms from controls on day 28 was not considered treatment related, because no concentration response relation was given. Thus, only the slight temporary lower abundances at 19.4 µg/L were considered a class 2 effect. For Chironomini, a NOEC of 1 µg/L was calculated but since the mean abundances at 4.4 and 9.3 µg/L were very close to the control and while the mean abundance at 2.1 µg/L showed the lowest abundance, also here only the slightly reduced abundance at 19.4 µg/L was considered to indicate a slight treatment effect.

Table CP 10.2.3/03-9 NOECs [µg/L] and related % MDDs (in brackets) for the taxa in the macrozoobenthos data set

Macrozoobenthos		Days after application									
MDD cat	Taxa / day	-14	-7	0	7	14	21	28	42	56	70	84
1	Sum of Chironomids ASS	≥19.4 (57)	≥19.4 (82)	≥19.4 (80)	≥19.4 (60)	≥19.4 (64)	≥19.4 (85)	9.3-(55)	≥19.4 (93)	≥19.4 (115)	≥19.4 (262)	≥19.4 (161)
1	Sum of Chironominae ASS	1-(43)	≥19.4 (85)	≥19.4 (81)	≥19.4 (61)	≥19.4 (66)	≥19.4 (86)	9.3-(55)	≥19.4 (94)	≥19.4 (171)	≥19.4 (n.c.)	≥19.4 (133)
1	Sum of Leeches ASS	≥19.4 (109)	≥19.4 (145)	≥19.4 (128)	≥19.4 (226)	9.3+ (101)	≥19.4 (83)	<1+ (119)	≥19.4 (85)	≥19.4 (79)	≥19.4 (92)	9.3+ (73)

Macrozoobenthos		Days after application										
MDD cat	Taxa / day	-14	-7	0	7	14	21	28	42	56	70	84
1	Sum of Oligochaeta ASS	≥19.4 (86)	≥19.4 (58)	≥19.4 (61)	≥19.4 (68)	≥19.4 (88)	≥19.4 (80)	≥19.4 (74)	≥19.4 (138)	≥19.4 (114)	<1-(64)	≥19.4 (100)
1	Chironomini gen spec.	1-(46)	≥19.4 (85)	≥19.4 (81)	≥19.4 (62)	≥19.4 (68)	≥19.4 (87)	1-(52)	≥19.4 (92)	≥19.4 (171)	≥19.4 (n.c.)	≥19.4 (225)
1	Chaoborus crystallinus larvae	≥19.4 (165)	≥19.4 (134)	≥19.4 (210)	4.4+ (148)	≥19.4 (96)	≥19.4 (104)	≥19.4 (93)	≥19.4 (82)	≥19.4 (89)	≥19.4 (90)	≥19.4 (112)
2	Sum of Tanypodinae ASS	≥19.4 (97)	≥19.4 (195)	≥19.4 (89)	≥19.4 (98)	≥19.4 (102)	9.3-(71)	≥19.4 (79)	≥19.4 (118)	≥19.4 (114)	≥19.4 (225)	≥19.4 (n.c.)
2	Stylaria lacustris	≥19.4 (88)	≥19.4 (61)	≥19.4 (63)	≥19.4 (72)	≥19.4 (93)	≥19.4 (108)	1+ (n.c.)	≥19.4 (n.c.)	≥19.4 (122)	≥19.4 (81)	≥19.4 (107)
2	Herpobdella octoculata	≥19.4 (106)	≥19.4 (147)	≥19.4 (127)	≥19.4 (262)	≥19.4 (223)	≥19.4 (100)	<1+ (129)	≥19.4 (71)	≥19.4 (79)	≥19.4 (97)	≥19.4 (87)
2	Gastropoda non det.		≥19.4 (n.c.)									9.3+ (n.c.)
2	Gyraulus albus	≥19.4 (169)	≥19.4 (246)	9.3+ (n.c.)	9.3+ (138)	≥19.4 (97)	≥19.4 (130)	≥19.4 (186)	≥19.4 (169)	≥19.4 (128)	≥19.4 (113)	≥19.4 (131)
2	Musculium lacustre		≥19.4 (225)	≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (165)	≥19.4 (218)	≥19.4 (146)	≥19.4 (183)	≥19.4 (94)	≥19.4 (195)
2	Pisidium spec	≥19.4 (133)	≥19.4 (128)	≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (197)	≥19.4 (225)	≥19.4 (117)	≥19.4 (203)	≥19.4 (n.c.)	≥19.4 (n.c.)
2	Caenis spec				≥19.4 (n.c.)				≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (112)	<1-(65)
2	Tanypodinae gen spec	≥19.4 (97)	≥19.4 (193)	≥19.4 (89)	≥19.4 (98)	≥19.4 (102)	9.3+ (71)	≥19.4 (79)	≥19.4 (118)	≥19.4 (114)	≥19.4 (225)	≥19.4 (n.c.)
2	Tanytarsini gen spec	≥19.4 (99)	≥19.4 (113)	≥19.4 (160)	≥19.4 (186)	4.4+ (n.c.)	≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (n.c.)			≥19.4 (192)
2	Culex spec							<1-(93)				
2	Hydrophilidae gen spec		≥19.4 (n.c.)							<1-(92)		
2	Zygoptera Gen spec							9.3+ (n.c.)	4.4+ (n.c.)	≥19.4 (n.c.)		
2	Dugesia gonocephala		≥19.4 (192)	9.3+ (n.c.)		≥19.4 (n.c.)	9.3+ (n.c.)	≥19.4 (272)	≥19.4 (n.c.)	≥19.4 (134)	≥19.4 (121)	1-(84)

Signs indicate the direction of a significant effect and colours indicate the different NOECs. Blank fields: taxon not present. n.c.: MDD could not be calculated because of absence in the controls. Empty cells indicate absence in all samples of that day. Cat = MDD category according Brock et al. (2015).

Oligochaeta were clearly not affected until day 42. The deviations form controls later and the single NOEC of <1 µg/L are assumed to be not caused by the treatment but by chance due to the low numbers in general. Thus, effect class 1 was used up to 19.4 µg/L.

Hirudinea (leeches) and Chaoborus crystallinus showed significantly higher abundances than in controls on single samplings. The NOEC of <1 µg/L for leeches on day 28 is caused by a lower abundance in the control on that single date rather than by an increase in abundance in all the treated mesocosms. Therefore, this is not considered as a promotion in all treated mesocosms. However, due to a trend of higher abundances in the mesocosm treated with 19.4 µg/L over several sampling dates, a potential slight promotion is considered for 19.4 µg/L. Because significantly higher abundance was also found at the last sampling day, class 2+/4A+ was assumed.

Numbers of Chaoborus crystallinus in the ASS were relatively small (<5 / sample before day 14) and therefore the calculated NOEC of 4 µg/L on day 7 was not considered to indicate an effect (class 1).

Figure CP 10.2.3/03-14 Sum of Chironomids: Geometric means per treatment level and range of controls

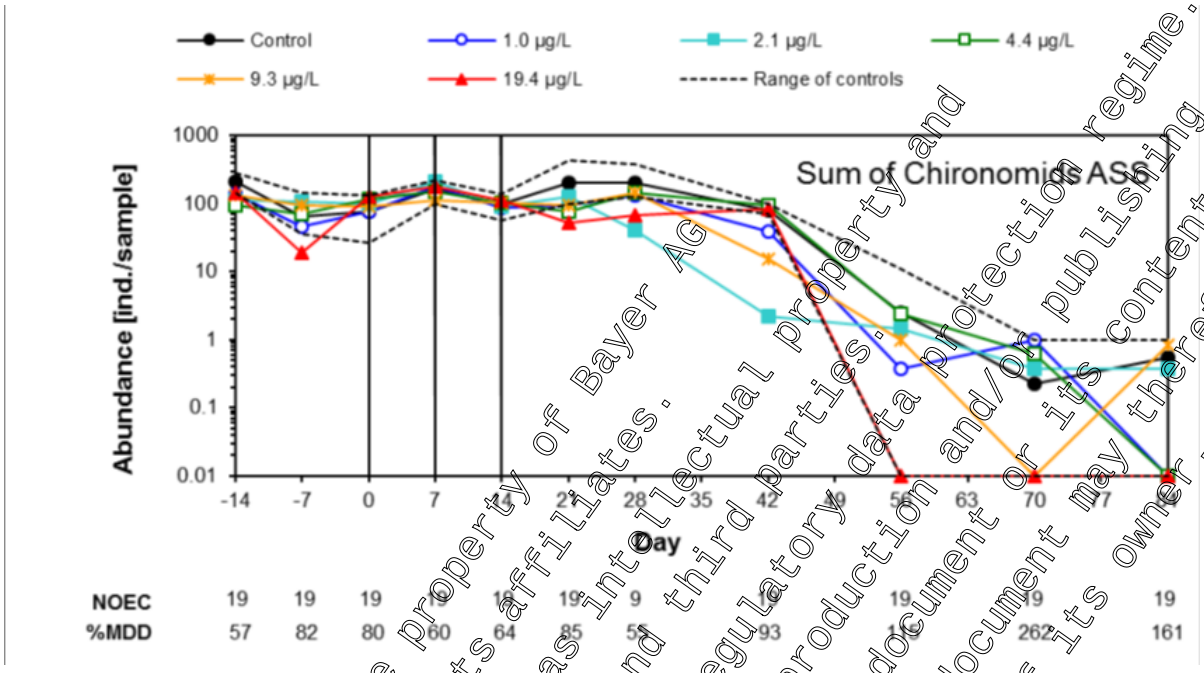


Figure CP 10.2.3/03-15 Sum of Chironominae: Geometric means per treatment level and range of controls

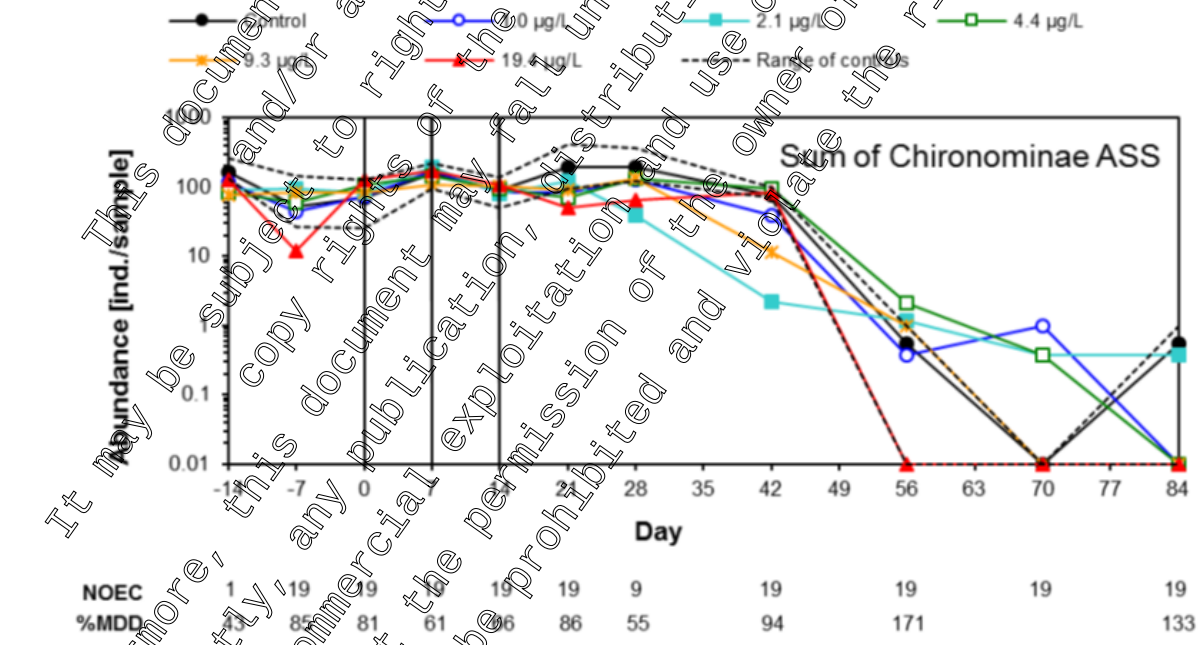


Figure CP 10.2.3/03-16 Chironomini gen spec.: Geometric means per treatment level and range of controls

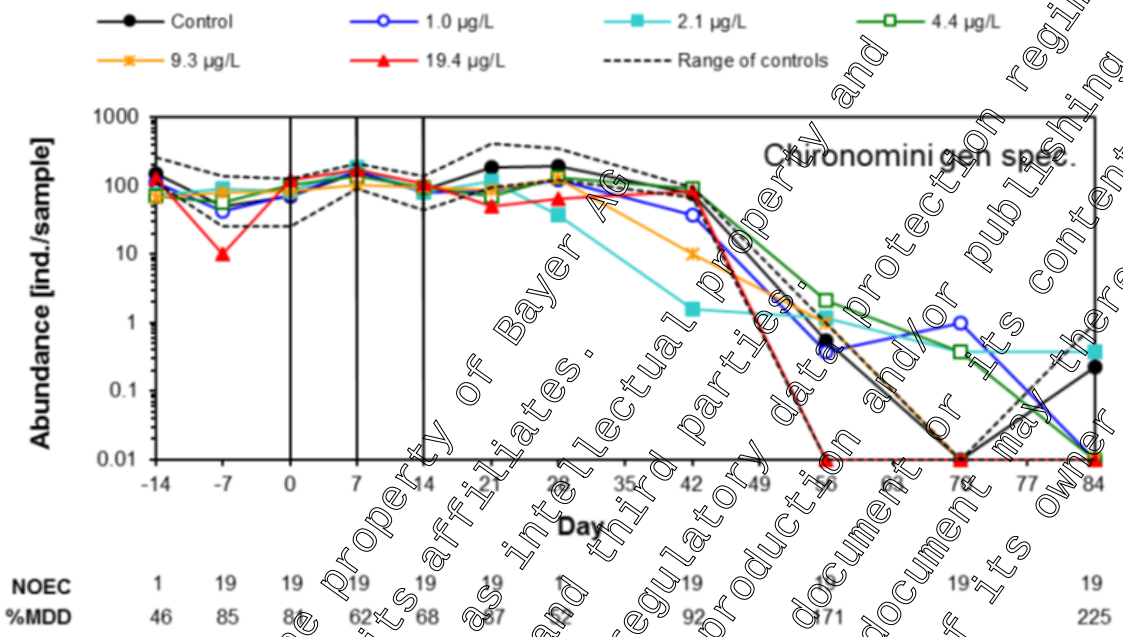


Figure CP 10.2.3/03-17 Sum of Oligochaeta: Geometric means per treatment level and range of controls

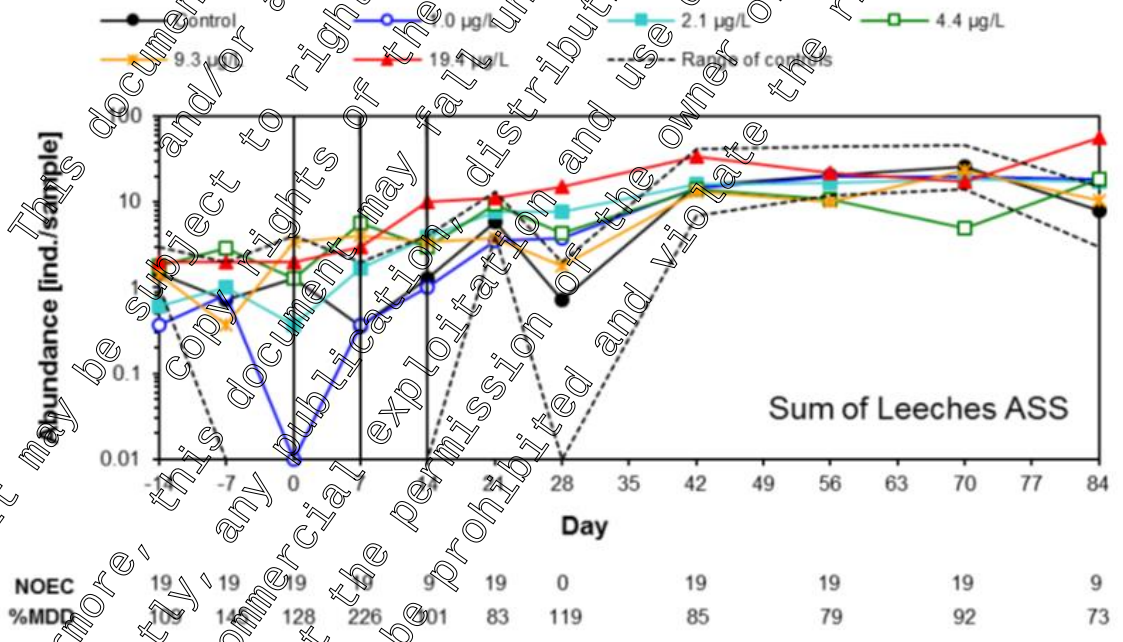
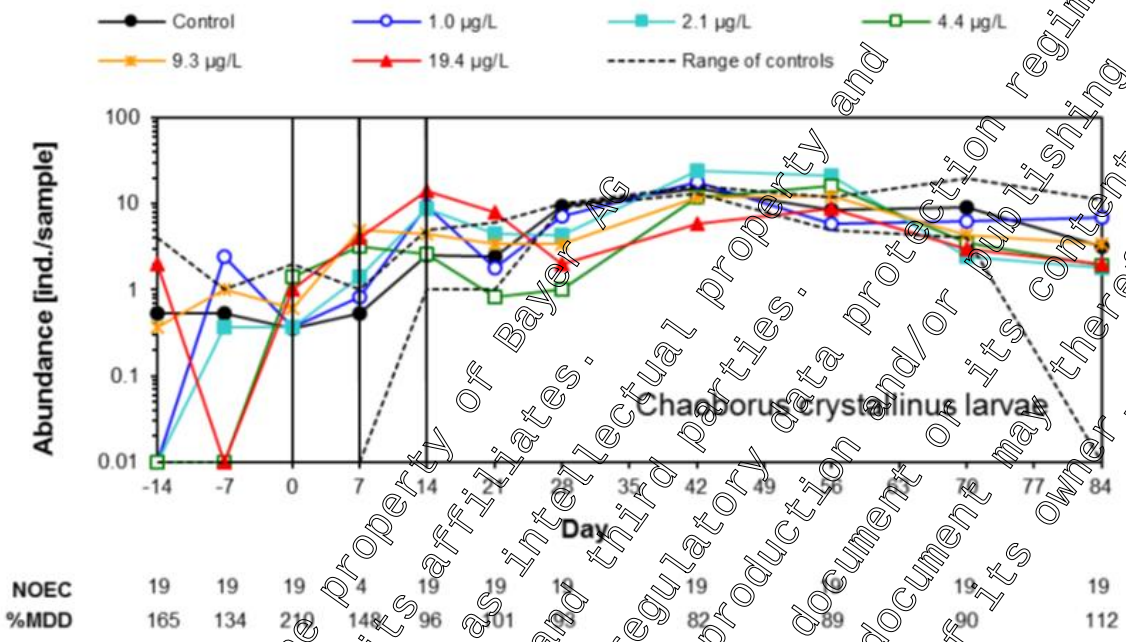


Figure CP 10.2.3/03-18 Sum of Hirudinea (leeches): Geometric means per treatment level and range of controls



Most of the taxa in MDD category 2 showed a short promotion on single samplings without a difference to the control at the end of the study (e.g. *Herpobdella octoculata, Gyraulus albus, Pisidium spec and Tanytarsini gen spec.*). Others were extremely rare (*e.g. Culex spec*, Hydrophilidae). For two taxa (*Caenis spec* and *Dugesia gonocephala*) a significant decrease with a NOEC of 1.0 µg/L was observed on the last sampling on day 84. However, no clear concentration response could be detected and the taxa were found only on isolated samplings. As also these statistical differences are not characterized by a clear concentration-response, these taxa were not considered for effect classification.

Phytoplankton

The effects on phytoplankton were evaluated by means of identification and enumerating of the cells using a reversed microscope and by means of measurements of chlorophyll a content.

Phytoplankton counts

Algae of seven classes were identified in the outdoor mesocosm study: Cryptophyceae, Diatomeae, Chlorophyceae, Chrysophyceae, Conjugatophyceae, Cyanobacteria and Euglenophyceae. For four of them plus the total sum of algae, and seven of the differentiated taxa, the MDDs were sufficiently low to allow an evaluation of direct effects. For several other taxa, significant differences were detected despite the MDDs did not meet the criterion defined by Brock *et al.* (2015) (MDD category 2 taxa).

Table CP 10.2.3/03-10 % MDDs for the taxa in the phytoplankton counts which met the criterion proposed by Brock *et al.* (2015). MDD category 1

Phytoplankton	Summary			
	Min	Max	Mean	MDD Cat
Sum algae	34	81	60	1
Sum Chlorophyceae	42	95	75	1
Sum Chlorophyceae	67	95	79	1
Sum Diatoms	48	101	84	1

Phytoplankton	Summary			
	Min	Max	Mean	MDD Cat
Sum Cyanobacteria	75	101	90	1
Pseudoanabaena spec.	75	101	90	1
Chlamydomonas spec.	42	115	94	1
coccoid Chlorophyceae	59	182	101	1
Chroomonas spec.	67	136	95	1
Cryptomonas spec. 20-30 µm	45	93	76	1
Achnanthes spec.	75	99	90	1
Pennales 20-30 µm	72	230	117	1

MDD cat = category based on MDD evaluation according to Brock et al. (2015).

Table CP 10.2.3/03-11 % MDDs for the taxa in the phytoplankton data set which met the criterion proposed by Brock *et al.* (2015). MDD category 2

Zooplankton	Summary			
	Min	Max	Mean	MDD Cat
Sum_Euglenophyceae	111	208	158	2
Merismopedia spec.	n.c.	n.c.		2
Phacus pleuronectis	92	92	92	2
Euglena spec.	119	221	170	2
Trachelomonas spec.	119	249	178	2
Scenedesmus cf Dimorphus	104	136	120	2
Characium spec.	84	223	134	2
Ankyra judayi	90	189	125	2
Closterium cf leiblenii	146	248	190	2
Cocconeis spec.	102	269	179	2
Synedra ulna	156	161	159	2
Pennales 30-40 µm	64	172	116	2
Pennales 70-80 µm	168	203	186	2

MDD cat = category based on MDD evaluation according to Brock et al. (2015).

For the sum of algae, significantly reduced abundances in comparison to controls were detected on four consecutive samplings (day 4 – day 14), with NOEC of 1 µg/L on day 11, 2.1 µg/L on day 7 and 4.4 µg/L on day 4 and 14. Upon the last application, on day 14, the sum of algae recovered quickly and no significant effects were detected (effect class 3A for 4.4 µg/L and higher test concentrations; effect class 2 for 2.1 µg/L).

Cryptophyceae were the most abundant and one of the most affected groups. Prolonged effects (day 4 – day 28) were detected on total Cryptophyceae, in particular on Cryptomonas spec. (20-30 µm) and Chroomonas spec. Until day 28, the Cryptophyceae were affected in the two highest treatments of 9.3 and 19.4 µg/L (effect class 3A), while the lower abundances at 4.4 µg/L were only significant on day18 (class 2). However, for Cryptomonas spec. (20-30 µm) significantly lower abundances were detected already in the mesocosms treated with 4.4 µg/L (day 11 – day 21) with recovery until day 56 (effect

class 3A). Chroomonas spec. was less sensitive, with pronounced short-term effects at 9.3 and 19.4 µg/L (effect class 3A).

For total Chlorophyceae, significantly reduced abundances were detected on day 4 at the two highest treatments of 9.3 and 19.4 µg/L and on day 11 in all treatments. However, it seems unlikely that on day 11 all treatment levels had a direct effect since there was clearly no effect after the first and the third application. After the third application, the strongest growth was found in the mesocosm treated with 19.4 µg/L leading to significantly higher abundances over 2 weeks. Thus, this promoting effect of the three applications was considered more relevant for the effect classification (effect class 3A at 19.4 and 2 at 9.3 µg/L). On day 7, a NOEC of 1 µg/L was calculated for the green algae Chlamydomonas spec.. This isolated NOEC is considered not to be treatment related as in the higher treatments, the abundances were in the range of controls. Thus, effect class 1 was used for all treatment levels for this species. For the coccoid Chlorophyceae, a significant reduction was detected on two sampling occasions (day 11 and 21) for the three, respectively two highest test concentrations of 19.4, 9.3 and 4.4 µg/L and was considered to be an effect class 2.

The total Diatoms were not affected except on day 18, when significant differences to the control were detected for the highest test concentration of 19.4 µg/L (effect class 2). For the diatom Achnanthes spec., significantly reduced abundances were detected on day 11, 14, 18, 28 and 42 which was considered as an effect class 2 for 4.4 µg/L and an effect class 3A for 9.3 and 19.4 µg/L. For small Pennales (20-30 µm), significantly higher abundances with a NOEC of 1 µg/L were detected on day 56. Since these increases showed not concentration-dependent response, it was not considered as an indication of a promoting effect, and class 1 was assumed for all test concentrations.

The cyanobacteria in total and the taxon Pseudoanabaena spec. were not affected during the whole study (effect class 1).

Table CP 10.2.3/03-12: NOECs [µg/L] and related % MDDs (in brackets) for the phytoplankton counts

Macrozoobenthos		Days after application													
MDD cat	Taxa / day	-14	-7	0	4	7	11	14	18	21	28	42	56	70	84
1	Sum algae	≥19.4 (49)	≥19.4 (36)	≥19.4 (39)	4.4- (45)	2.1- (34)	1- (52)	4.4- (50)	9.3 (46)	≥19.4 (78)	≥19.4 (51)	≥19.4 (69)	≥19.4 (79)	≥19.4 (79)	≥19.4 (81)
1	Sum chlorophyceae	≥19.4 (71)	≥19.4 (95)	≥19.4 (80)	4.4- (54)	19.4 (71)	1- (52)	≥19.4 (87)	9.3+ (67)	9.3+ (84)	4.4+ (69)	≥19.4 (79)	≥19.4 (88)	≥19.4 (92)	≥19.4 (95)
1	Sum cryptophyceae	≥19.4 (56)	≥19.4 (37)	≥19.4 (37)	4.4- (77)	4.4- (72)	4.4- (92)	4.4- (76)	2.1- (67)	9.3- (70)	4.4- (72)	≥19.4 (68)	≥19.4 (95)	≥19.4 (92)	≥19.4 (84)
1	Sum diatoms	≥19.4 (61)	9.3+ (48)	≥19.4 (76)	≥19.4 (69)	≥19.4 (48)	≥19.4 (81)	≥19.4 (101)	9.3- (86)	≥19.4 (89)	≥19.4 (87)	≥19.4 (95)	≥19.4 (89)	≥19.4 (88)	≥19.4 (96)
1	Sum cyanobacteria	≥19.4 (128)	≥19.4 (100)	≥19.4 (102)	≥19.4 (98)	≥19.4 (81)	≥19.4 (101)	≥19.4 (95)	≥19.4 (99)	≥19.4 (98)	≥19.4 (75)	≥19.4 (82)	≥19.4 (84)	≥19.4 (83)	≥19.4 (91)
1	Pseudoanabaena spec.	≥19.4 (128)	≥19.4 (108)	≥19.4 (102)	≥19.4 (98)	≥19.4 (81)	≥19.4 (101)	≥19.4 (95)	≥19.4 (99)	≥19.4 (98)	≥19.4 (75)	≥19.4 (82)	≥19.4 (84)	≥19.4 (83)	≥19.4 (91)
1	Chlamydomonas spec.	≥19.4 (157)	≥19.4 (145)	≥19.4 (70)	≥19.4 (100)	1 (42)	≥19.4 (n.c.)	≥19.4 (102)	≥19.4 (99)	≥19.4 (99)	≥19.4 (78)	≥19.4 (97)	≥19.4 (98)	≥19.4 (115)	≥19.4 (114)
1	coccoid Chlorophyceae	≥19.4 (110)		≥19.4 (95)	≥19.4 (67)	≥19.4 (79)	2.1- (59)	≥19.4 (101)	≥19.4 (95)	4.4- (83)	≥19.4 (113)	≥19.4 (117)	≥19.4 (182)	≥19.4 (99)	≥19.4 (118)
1	Chroomonas spec.	≥19.4 (119)	≥19.4 (77)	≥19.4 (119)	≥19.4 (132)	9.3- (81)	4.4- (93)	9.3- (81)	4.4- (67)	≥19.4 (95)	9.3- (78)	≥19.4 (89)	≥19.4 (136)	≥19.4 (112)	2.1+ (85)
1	Cryptomonas spec. 20-30 µm	≥19.4 (55)	≥19.4 (55)	≥19.4 (67)	4.4- (49)	4.4- (58)	2.1- (81)	2.1- (45)	2.1- (80)	2.- (86)	4.4- (84)	≥19.4 (82)	≥19.4 (93)	≥19.4 (91)	≥19.4 (91)
1	Achnanthes spec.	≥19.4 (157)	≥19.4 (121)	≥19.4 (99)	≥19.4 (90)	≥19.4 (79)	4.4- (94)	≥19.4 (99)	4.4- (84)	≥19.4 (95)	2.1- (75)	9.3- (91)	≥19.4 (94)	≥19.4 (91)	≥19.4 (91)

Macrozoobenthos		Days after application													
MDD cat	Taxa / day	-14	-7	0	4	7	11	14	18	21	28	42	56	70	84
1	Pennales 20-30 µm	≥19.4 (81)	≥19.4 (80)	≥19.4 (71)	≥19.4 (89)	≥19.4 (78)	≥19.4 (83)	≥19.4 (230)	≥19.4 (226)	≥19.4 (72)	≥19.4 (87)	≥19.4 (119)	1+ (101)	≥19.4 (98)	≥19.4 (99)
2	Sum Euglenophyceae	≥19.4 (177)		≥19.4 (175)	≥19.4 (208)	≥19.4 (189)		≥19.4 (119)	≥19.4 (173)	≥19.4 (150)	9.3+ (125)	≥19.4 (n.c.)	≥19.4 (155)	≥19.4 (189)	≥19.4 (111)
2	Merismopedia spec.				9.3+ (n.c.)					≥19.4 (n.c.)					
2	Phacus pleuronectis				≥19.4 (n.c.)										<1- (92)
2	Euglena spec.									9.3+ (n.c.)		≥19.4 (n.c.)	≥19.4 (221)	≥19.4 (n.c.)	≥19.4 (119)
2	Trachelomonas spec.	≥19.4 (177)		≥19.4 (175)	≥19.4 (193)	≥19.4 (189)	≥19.4 (149)	≥19.4 (173)	≥19.4 (224)		≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (249)	≥19.4 (175)	≥19.4 (153)
2	Scenedesmus cf dimorphus	≥19.4 (123)	≥19.4 (45)	≥19.4 (126)	≥19.4 (122)	4.4+ (n.c.)	≥19.4 (130)	≥19.4 (136)	≥19.4 (n.c.)	≥19.4 (125)	≥19.4 (120)	≥19.4 (104)	≥19.4 (117)	≥19.4 (108)	≥19.4 (n.c.)
2	Characium spec.		≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (142)		≥19.4 (113)	≥19.4 (185)	≥19.4 (108)	≥19.4 (105)	4.4+ (84)	≥19.4 (132)	≥19.4 (112)	2.1+ (223)	4.4+ (118)
2	Ankyra judayi	≥19.4 (n.c.)			≥19.4 (n.c.)		≥19.4 (112)	≥19.4 (90)	9.3+ (93)	4.4+ (90)	2.1+ (128)	≥19.4 (n.c.)	≥19.4 (189)		≥19.4 (170)
2	Closterium cf leibleinii			≥19.4 (n.c.)	≥19.4 (n.c.)					≥19.4 (248)	≥19.4 (n.c.)	≥19.4 (146)	4.4+ (n.c.)	4.4+ (204)	4.4+ (162)
2	Cocconeis spec.	≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (n.c.)	≥19.4 (162)		2.1+ (n.c.)	≥19.4 (244)	≥19.4 (n.c.)	≥19.4 (199)	≥19.4 (152)	≥19.4 (179)	≥19.4 (269)	≥19.4 (122)	≥19.4 (102)
2	Synedra ulna							9.3+ (156)	≥19.4 (161)	≥19.4 (n.c.)	≥19.4 (n.c.)				
2	Pennales 30-40 µm	≥19.4 (89)		≥19.4 (92)	≥19.4 (61)	≥19.4 (110)	≥19.4 (97)	≥19.4 (161)	≥19.4 (172)	≥19.4 (89)	≥19.4 (115)	≥19.4 (122)	9.3+ (n.c.)	≥19.4 (128)	≥19.4 (103)
2	Pennales 70-80 µm			≥19.4 (167)	≥19.4 (186)	≥19.4 (168)	≥19.4 (203)				9.3+ (n.c.)				

Signs indicate the direction of a significant effect and colours indicate the different NOECs. Blank fields: taxon not present. n.c.: MDD could not be calculated because of absence in the controls. Cat = MDD category according Brock et al. (2015).

Figure CP 10.2.3/03-19 Total phytoplankton: Geometric means per treatment level and range of controls

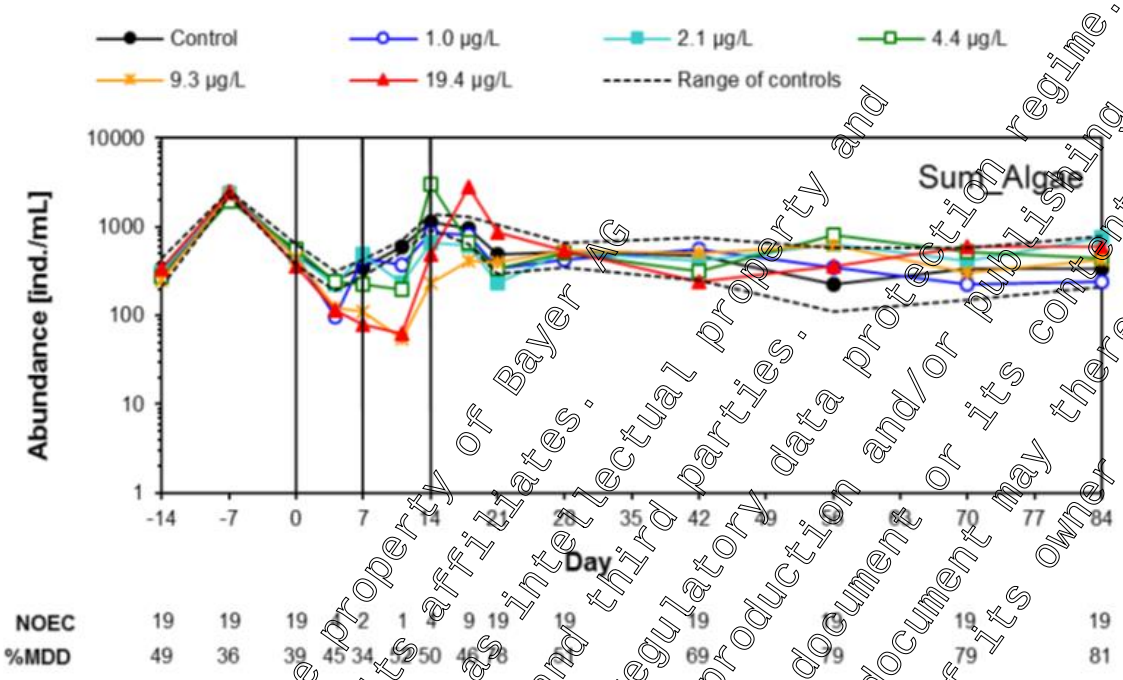


Figure CP 10.2.3/03-20 Sum of Cryptophyceae: Geometric means per treatment level and range of controls

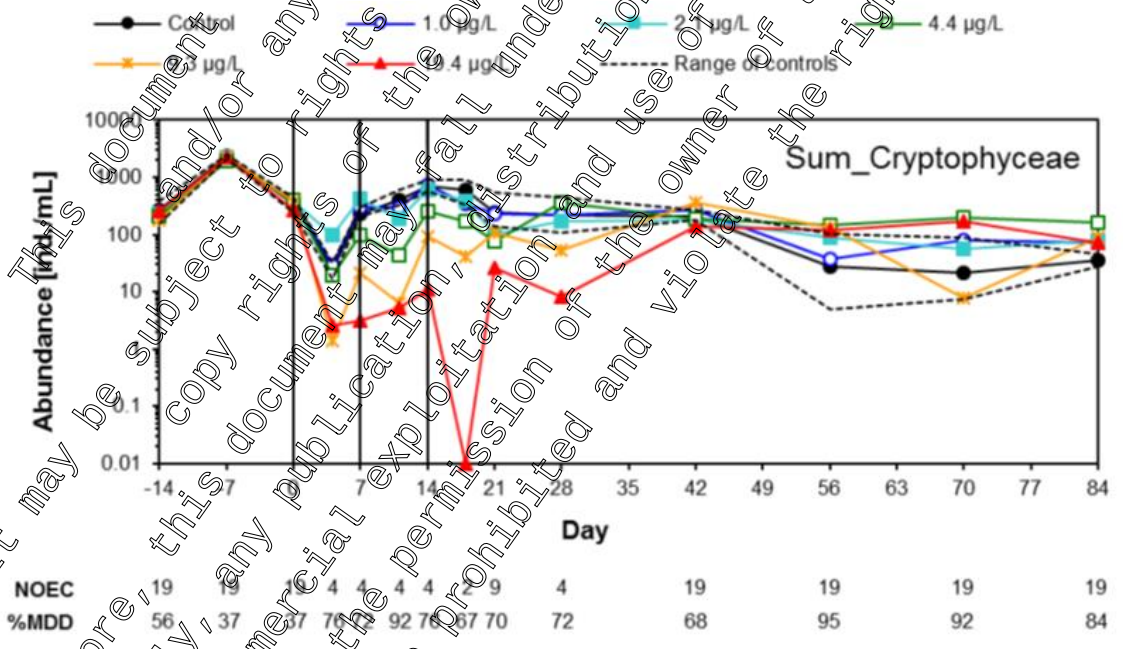


Figure CP 10.2.3/03-21 Cryptomonas spec. 20-30 µm: Geometric means per treatment level and range of controls

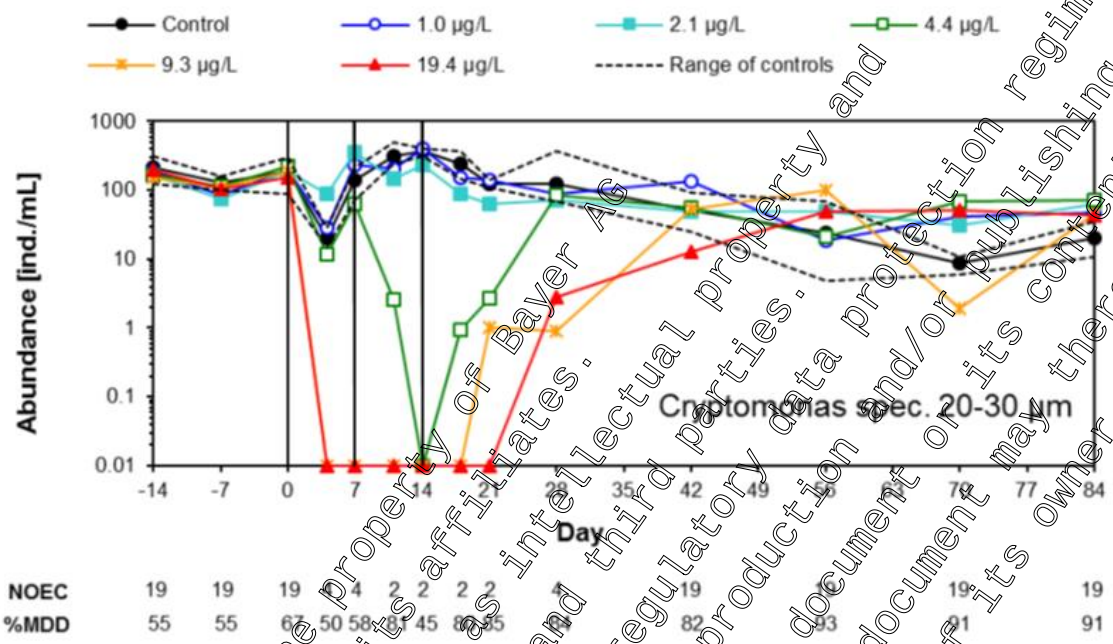


Figure CP 10.2.3/03-22 Chroomonas spec.: Geometric means per treatment level and range of controls

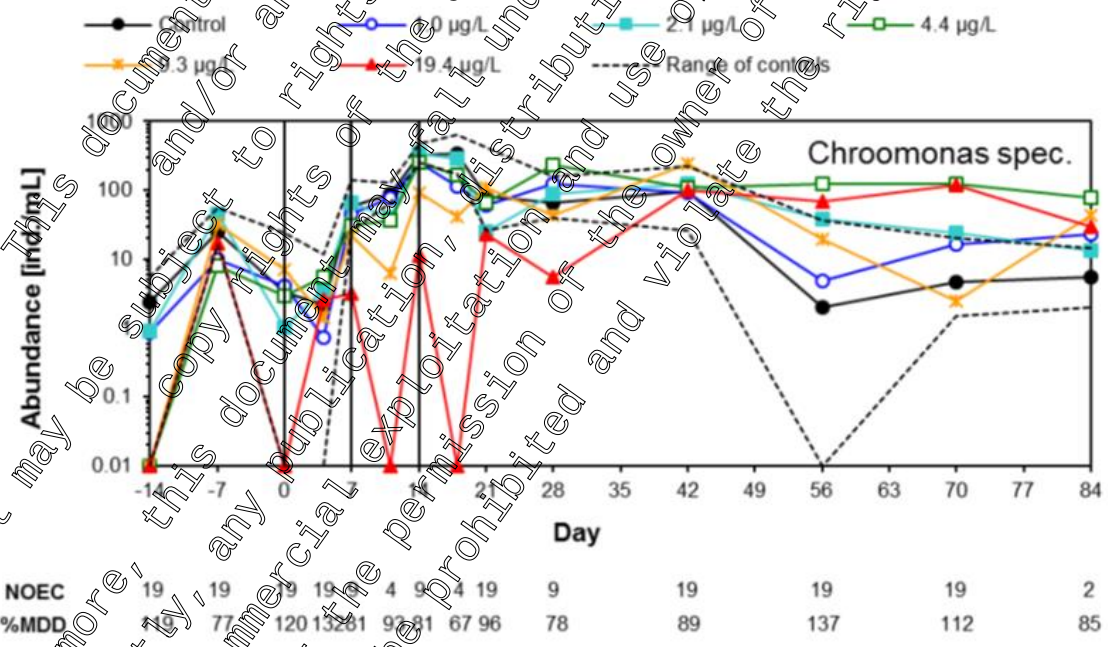


Figure CP 10.2.3/03-23 Sum of Chlorophyceae: Geometric means per treatment level and range of controls

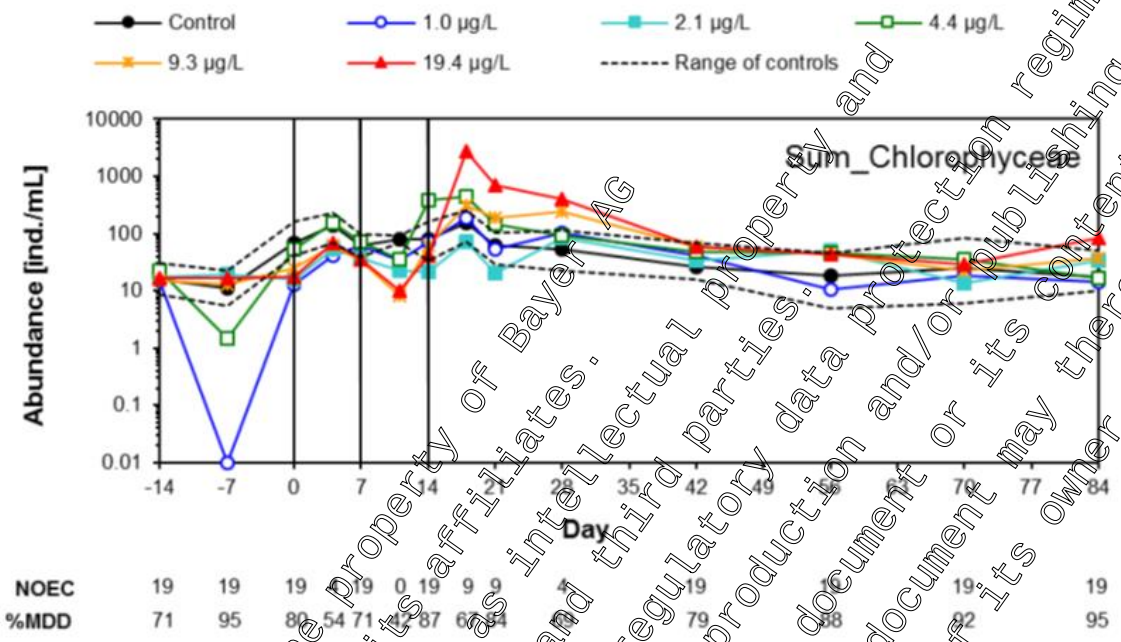


Figure CP 10.2.3/03-24 Chlamydomonas spec.: Geometric means per treatment level and range of controls

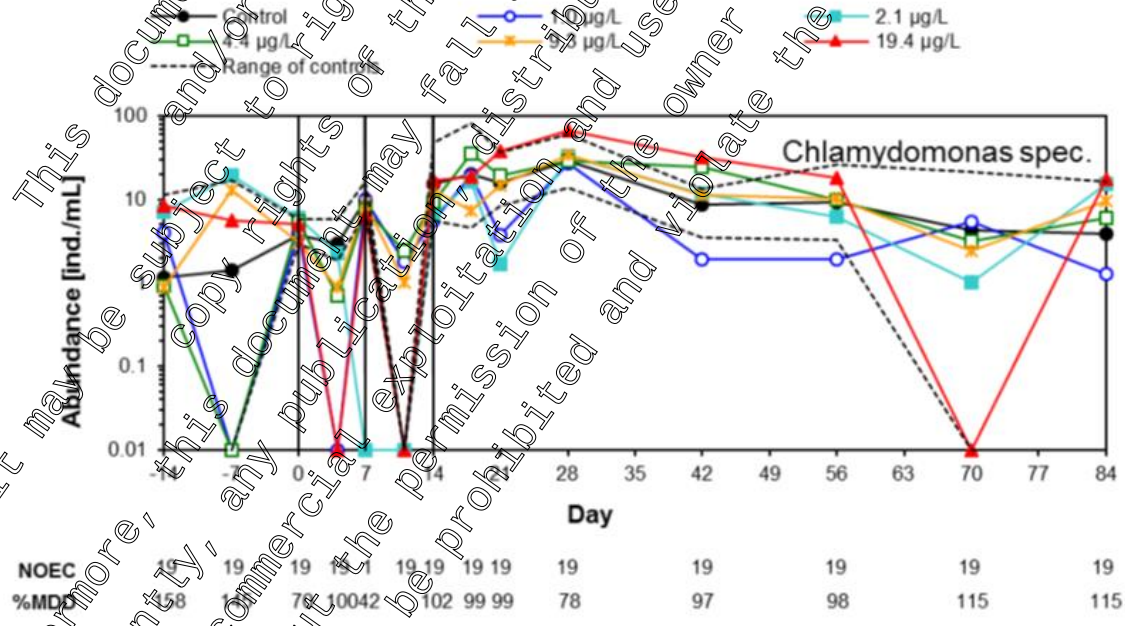


Figure CP 10.2.3/03-25 Sum of coccoid Chlorophyceae: Geometric means per treatment level and range of controls

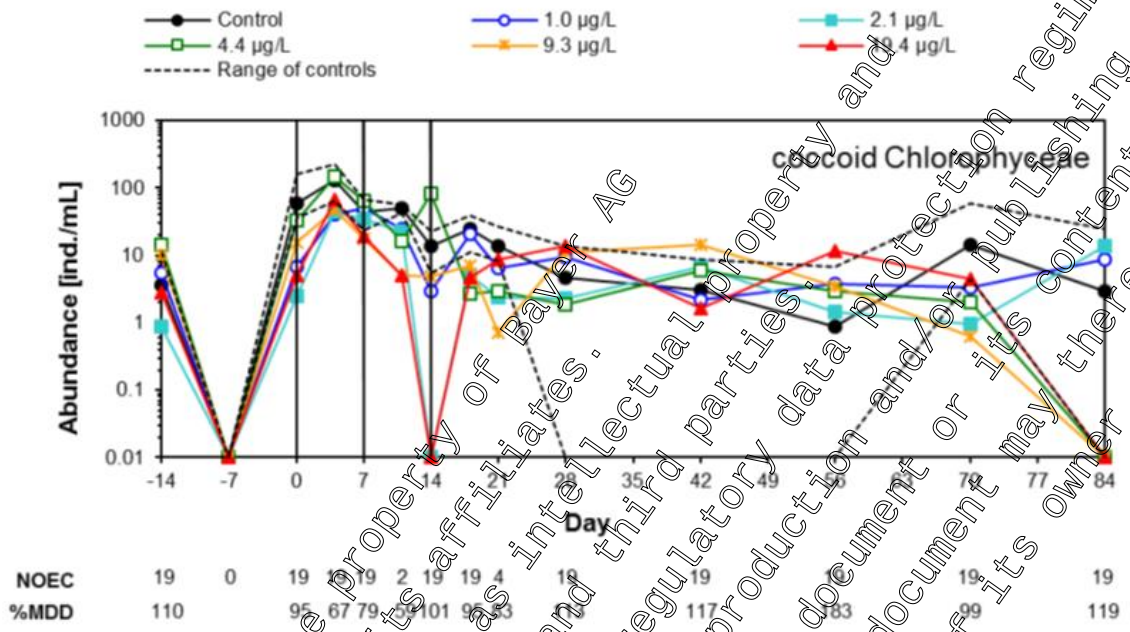


Figure CP 10.2.3/03-26 Sum of Diatoms: Geometric means per treatment level and range of controls

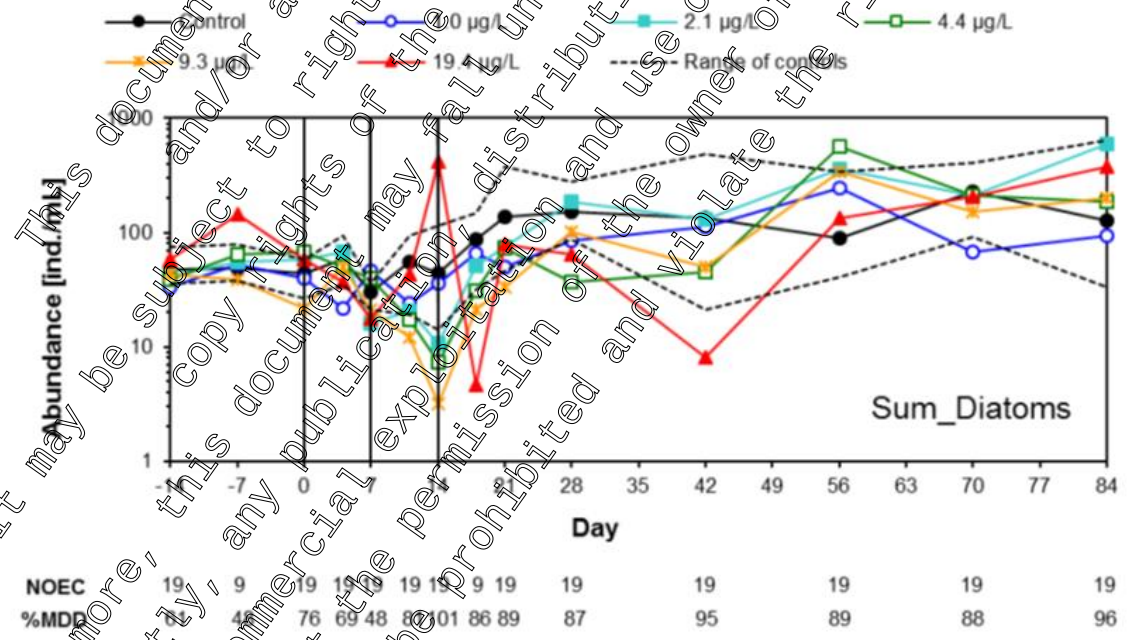


Figure CP 10.2.3/03-27 Achnanthes spec.: Geometric means per treatment level and range of controls

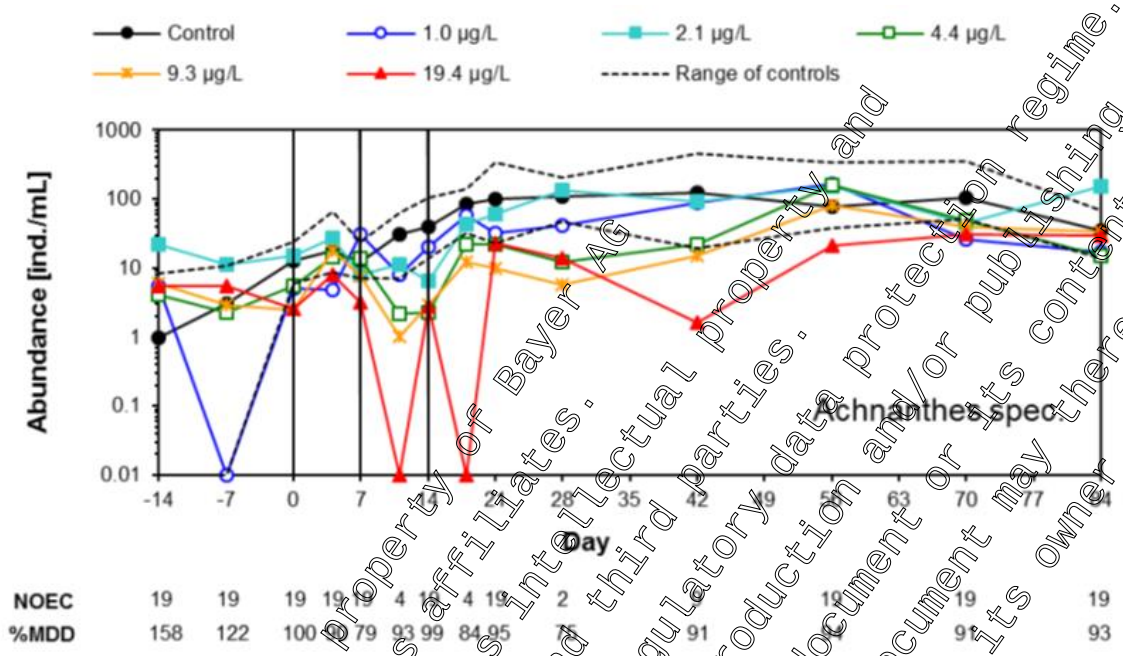


Figure CP 10.2.3/03-28 Pennales (20-30 µm): Geometric means per treatment level and range of controls

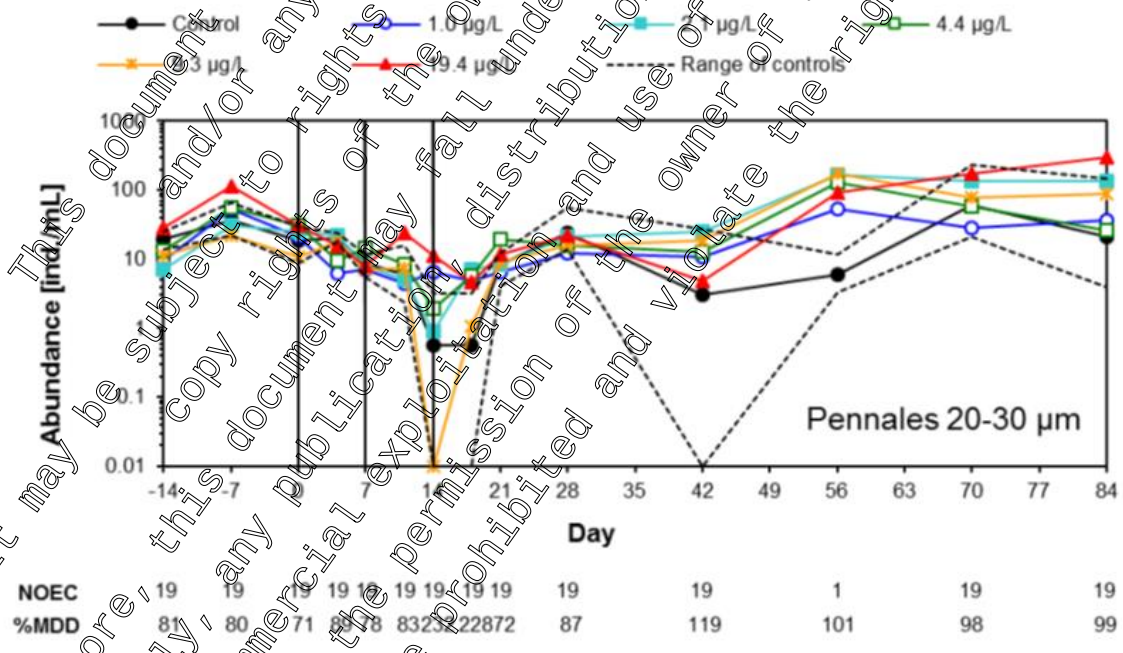


Figure CP 10.2.3/03-29 Cyanobacteria: Geometric means per treatment level and range of controls

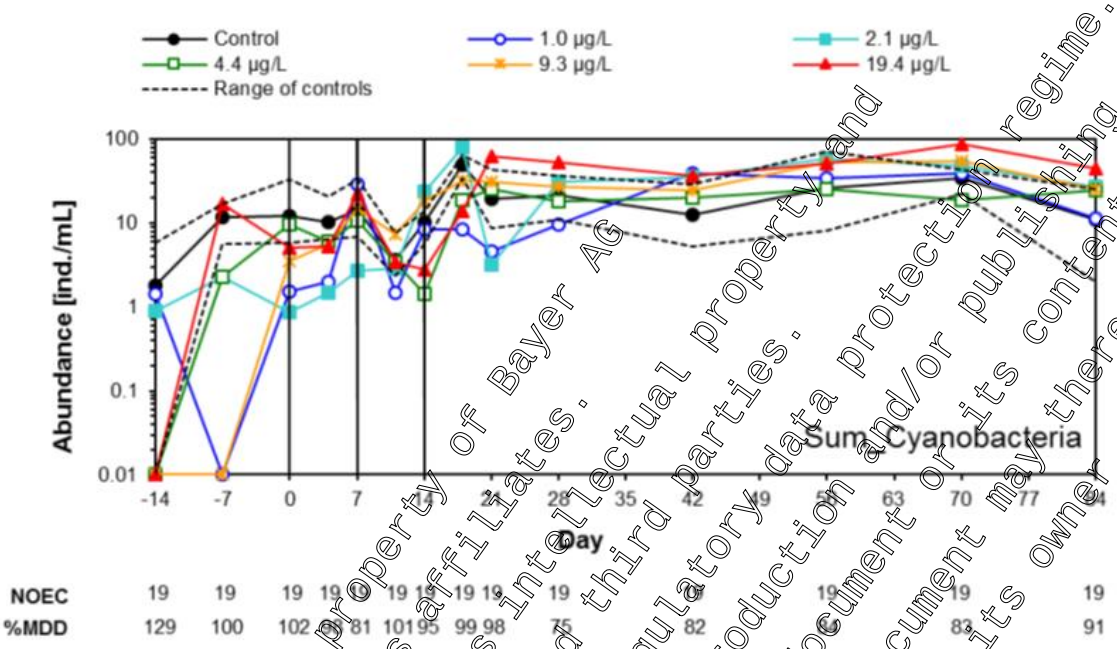
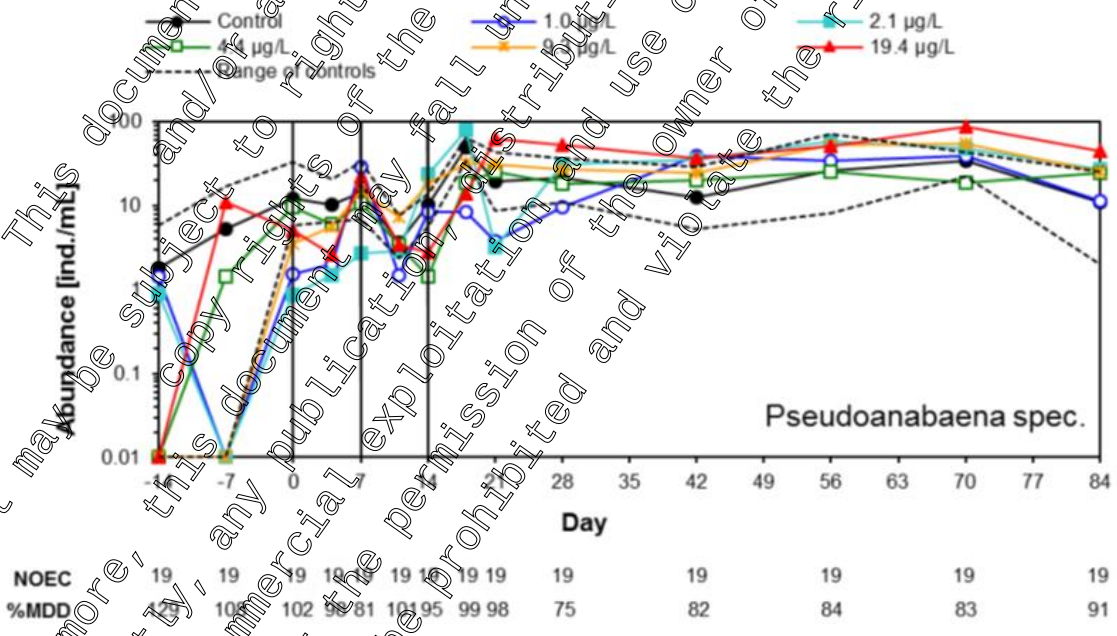


Figure CP 10.2.3/03-30 Pseudoanabaena spec.: Geometric means per treatment level and range of controls



Taxa of MDD category 2 showed increased abundances on single samplings with NOECs of 2.1 µg/L and higher such as *Merismopedia spec., Euglena spec., Trachelomonas spec. Scenedesmus dimorphus, Cocconeis spec,* Synedra ulna and Pennales. Since this would not affect the final risk assessment, these taxa were not considered further. However, the green algae *Characium spec., Ankyra judayi, Closterium leibleinii* showed significantly higher abundances than in the controls on 2 or 3 consecutive samplings.

Characium spec. showed significantly higher abundances in the mesocosms treated with 9.3 ad 19.4 µg/L on day 28 (considered as class 2+), and again on day 70 and 84. Since it could not be assessed if the statistical findings at the end of the study indicate a promotion, class 2+/4A+ was considered for this species and the two highest test concentrations.

Higher abundances were also found for Clostridium leibleinii over the last three samplings in the two highest test concentrations. To be conservative, this was interpreted as a potential promotion, effect class 3A+/4A+. However, both species were relatively rare and the higher abundances found at the end of the study did not result in a bloom of algae. Thus, the ecological relevance of the observations is considered to be low.

In contrast, Ankyra judayi showed a significant promotion of abundance on day 18, 21 and 28 with respective NOEC of 9.3, 4.4 and 2.1 µg/L (effect class 3A+ for 9.3 and 19.4 and effect class 2+ for 4.4 µg/L).

Figure CP 10.2.3/03-31 Characium spec.: Geometric means per treatment level and range of controls

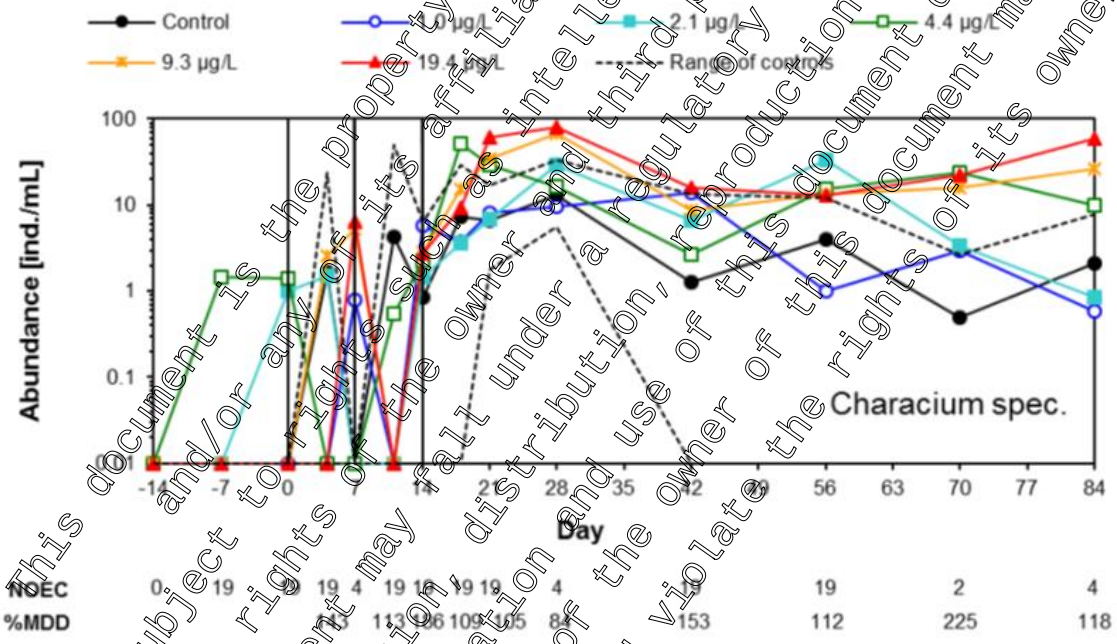


Figure CP 10.2.3/03-32 Closterium cf leibleinii: Geometric means per treatment level and range of controls

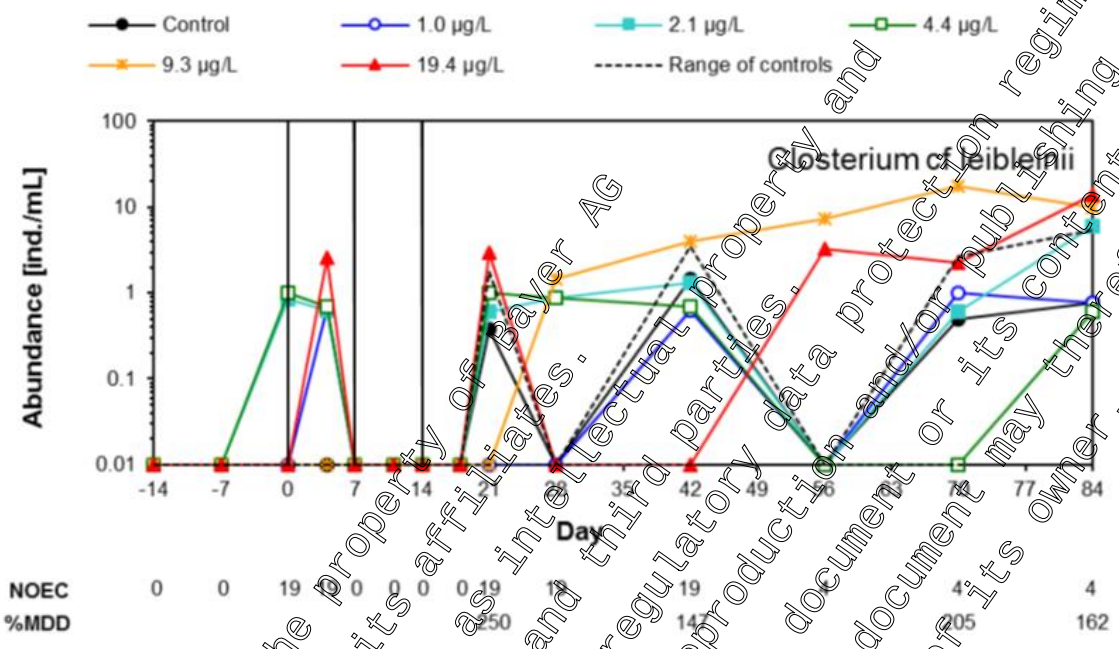
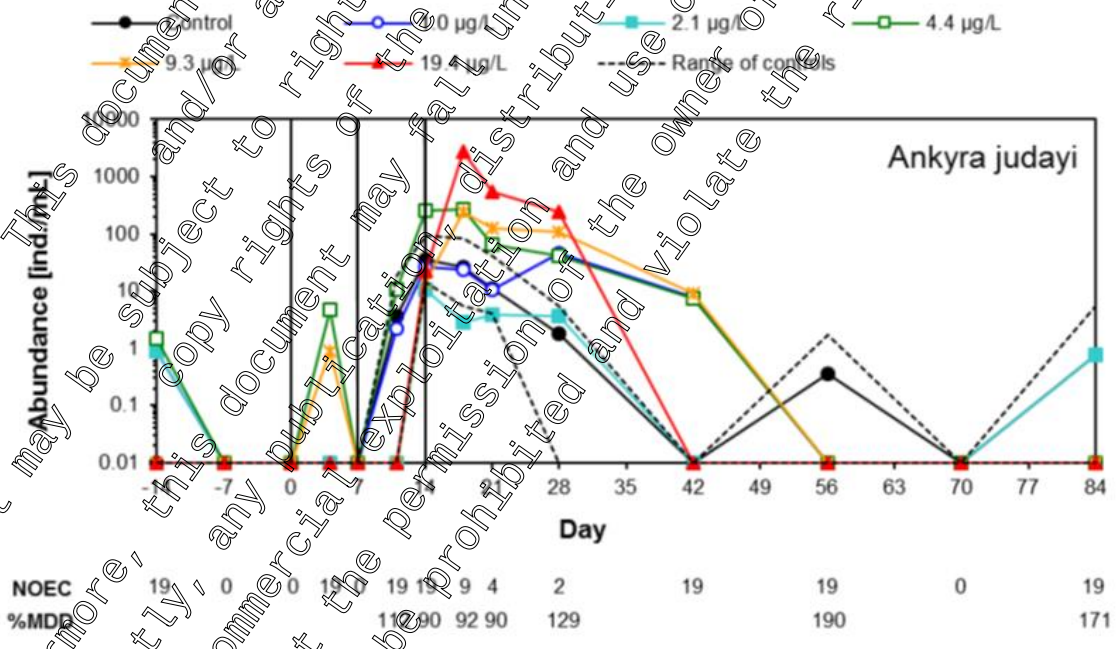


Figure CP 10.2.3/03-33 Ankyra judayi: Geometric means per treatment level and range of controls



Phytoplankton chlorophyll a

The chlorophyll a concentration was measured on several sampling occasions. The calculated MDD demonstrated that small to medium effects could be determined.

Table CP 10.2.3/03-13 % MDDs for phytoplankton chlorophyll a

Phytoplankton	Summary			
	Min	Max	Mean	MDD Cat
Chl-a Phytoplankton	32	108	68	1

MDD cat = category based on MDD evaluation according to Brock et al. (2015)

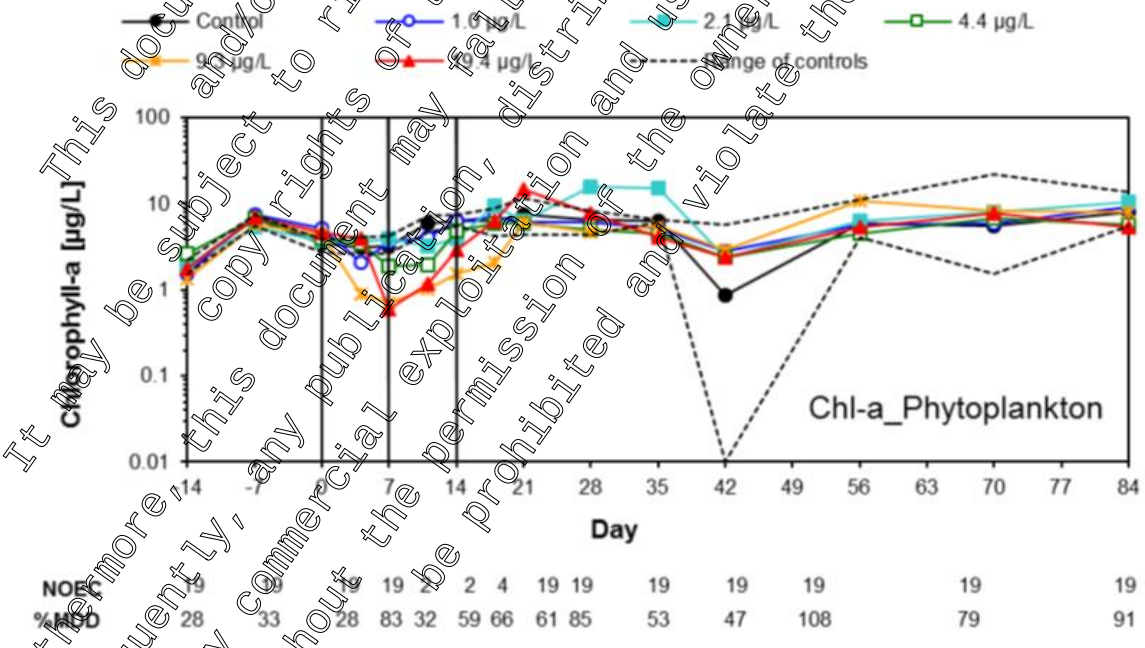
Significantly reduced chlorophyll a concentrations in comparison to the controls were detected on three consecutive samplings, from day 7 to day 14. Therefore, effects at a test concentration of 4.4 µg/L or higher were considered effect class 3A.

Table CP 10.2.3/03-14 NOECs [µg/L] and related % MDDs (in brackets) for phytoplankton chlorophyll a

Phytoplankton		Days after application													
MDD cat	Taxa / day	-14	-7	0	4	7	11	14	18	21	28	42	56	70	84
1	Chl-a Phytoplankton	≥19.4 (28)	≥19.4 (33)	≥19.4 (28)	4.4- (83)	2.1- (32)	2.1- (59)	4.4- (66)	9.3 (46)	≥19.4 (85)	≥19.4 (47)	≥19.4 (108)	≥19.4 (79)	≥19.4 (91)	≥19.4 (56)

Signs indicate the direction of a significant effect and colors indicate the different NOECs. Cat = MDD category according Brock et al. (2015).

Figure CP 10.2.3/03-34 Phytoplankton chlorophyll a: Geometric means per treatment level and range of controls



Periphyton

The effects on periphyton were examined by measurements of chlorophyll a concentrations. The MDDs calculated for periphyton fulfil the criteria according to Brock *et al*. (2015). On most of the sampling occasions, the MDDs were low enough for small to medium effects to be detected. The periphyton

chlorophyll a determinations showed in all test concentrations a slight but not significant difference to the control on day 28 and a significant difference to the control only on day 42. On both dates, the response was not concentration-related since the deviation of the 1.0 µg/L mesocosms from control was very similar to the one of the 19.3 µg/L. Thus, a direct effect of the test item seems to be unlikely (class 1).

Table CP 10.2.3/03-15 % MDDs for periphyton chlorophyll a

Periphyton	Summary			
	Min	Max	Mean	MDD Cat
Chl-a Periphyton	45	99	74	1

MDD cat = category based on MDD evaluation according to Brock et al. (2015)

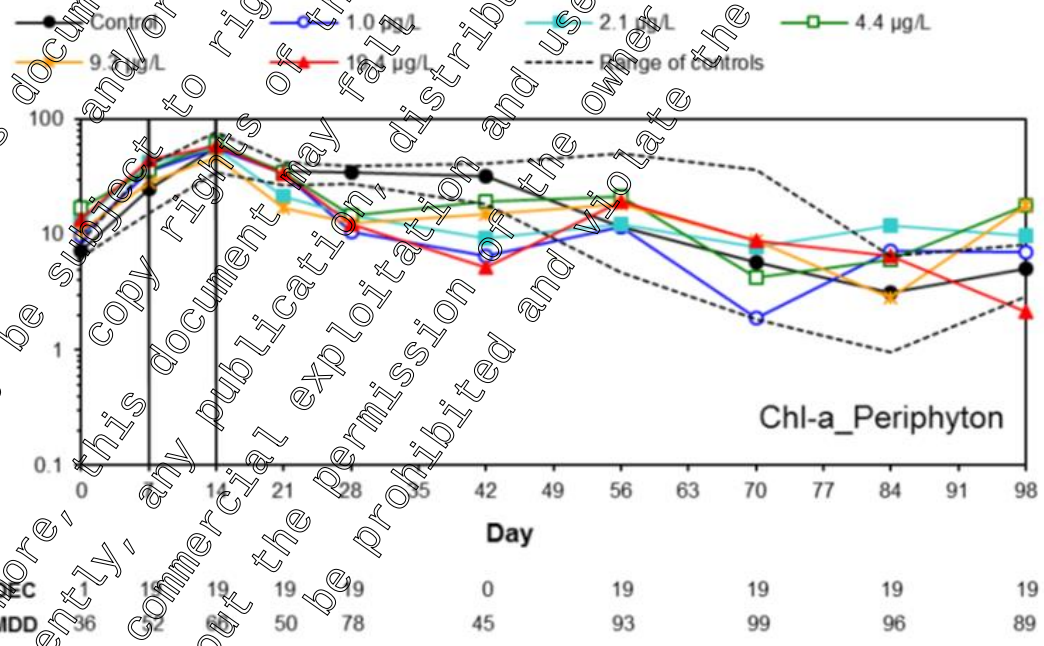
Table CP 10.2.3/03-16 NOECs [µg/L] and related % MDDs (in brackets) for periphyton chlorophyll a

Peryphyton		Days after application									
MDD cat	Taxa / day	0	7	14	21	28	42	56	70	84	98
1	Chl-a Phytoplankton	1+ (36)	≥19.4 (52)	≥19.4 (66)	≥19.4 (50)	≥19.4 (78)	1- (45)	≥19.4 (93)	≥19.4 (99)	≥19.4 (96)	≥19.4 (89)

Signs indicate the direction of a significant effect and colors indicate the different NOECs

Cat = MDD category according Brock et al. (2015).

Figure CP 10.2.3/03-35 Periphyton chlorophyll a: Geometric means per treatment level and range of controls



Macrophytes

The calculated MDDs for the total macrophytes coverage were sufficiently low to allow an evaluation of direct effects, small to medium effects (MDD 13-60 %) could be determined. No adverse effects on the macrophytes coverage were detected during the outdoor mesocosm study. On day 64 of the study,

significantly increased macrophytes coverage was determined at two highest treatments, resulting in a NOEC of 4.4 µg/L.

Table CP 10.2.3/03-17 % MDDs for the taxa in the macrophytes coverage

Macrophytes coverage	Summary			
	Min	Max	Mean	MDD Cat
Coverage %	13	60	27	1

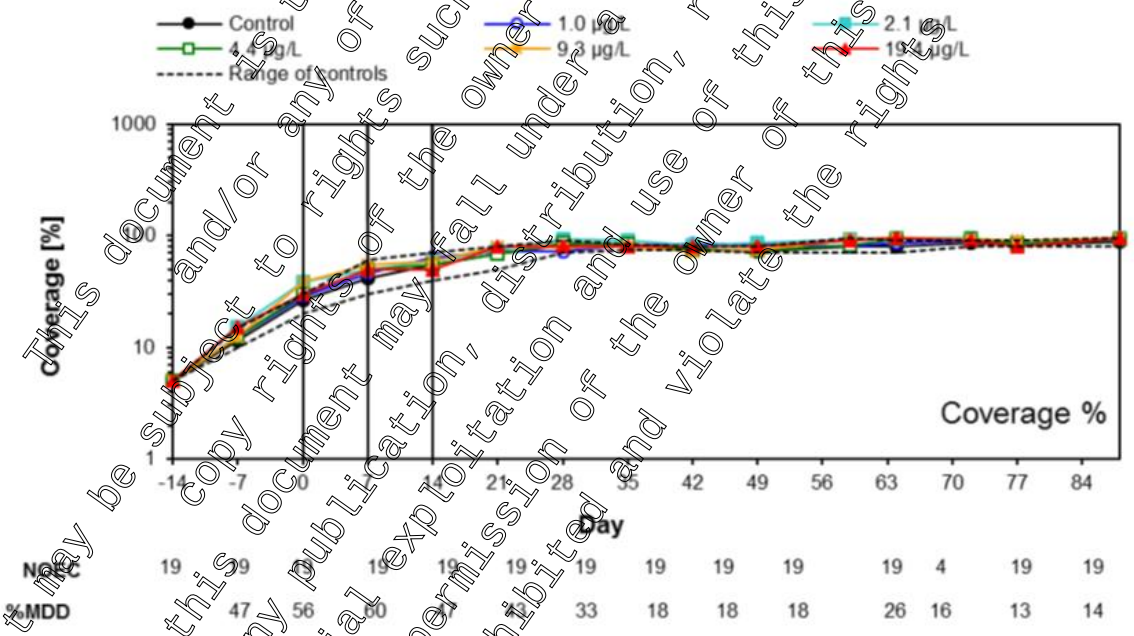
MDD cat = category based on MDD evaluation according to Brock et al. (2015).

Table CP 10.2.3/03-18 NOECs [µg/L] and related % MDDs (in brackets) for macrophytes coverage

Macrophytes coverage		Days after application													
MDD cat	Taxa / day	-14	-7	0	7	14	21	28	35	42	49	59	64	72	77	88
1	Chl-a Phytoplankton	≥19.4 (0)	≥19.4 (47)	≥19.4 (56)	≥19.4 (0)	≥19.4 (47)	≥19.4 (43)	≥19.4 (33)	≥19.4 (18)	≥19.4 (18)	≥19.4 (18)	≥19.4 (26)	4.4+ (16)	≥19.4 (13)	≥19.4 (14)	≥19.4 (13)

Signs indicate the direction of a significant effect and colors indicate the different NOECs. Cat = MDD category according Brock et al. (2015).

Figure CP 10.2.3/03-36 Macrophytes sum of coverage: Geometric means per treatment level and range of controls



III. Conclusions

An outdoor mesocosm study to investigate the effects of three applications of spiroxamine EC 500 was re-analysed with respect to MDDs and effect classification according to the most recent guidance (EFSA 2013).

The MDDs of eleven invertebrate and seven algae taxa (plus combined data on higher taxonomic levels) fulfil the criterion proposed by Brock et al. (2015). If a more strict criterion is applied, *e.g.* that the MDD should be at least once <70% within the first four weeks after the first application, 13 taxa representing populations of macroinvertebrates, zooplankton and phytoplankton allow a statistical evaluation of direct effects: Tubificidae, Chironomini, *Chaoborus* spec *Simocephalus vetulus*, *Chydorus sphaericus*,

Eucercus lamellatus, cyclopoid copepods (and nauplia larvae), *Polyarthra spec.*. *Chlamydomonas spec*., coccoid Chlorophyceae, *Chroomonas spec*., *Cryptomonas spec*. (20-30 µm), and Pennales (30-40 µm). Thus, despite the fact that only 12 test systems were used in the study, a reliable statistical analysis of direct effects was possible for at least eight taxa considered to represent potentially sensitive populations as requested by EFSA PPR (2013).

The following effects were found at the different test concentrations:

- At the lowest test concentration of 1.0 µg/L, no treatment effects were found (class 1).
- At 2.1 µg/L, a slight direct effect on total phytoplankton abundance and a pronounced short-term promoting effect on the rotifer Keratella quadrata were detected (class 2 for the direct effect on the phlytoplankton and 3A if the potential temporary promotion of a rotifer species is considered an adverse effect). Other taxa showed no effects.
- At 4.4 µg/L, class 3A effects for total rotifers, total phytoplankton, chlorophyll a and Cryptomonas spec. were observed while some other taxa were slightly affected. Thus, the total effect class for 4.4 µg/L is considered 3A.
- At 9.3 µg/L, more taxa were affected, effects were more pronounced or prolonged and for two algae species, significantly higher abundances than in the controls were found at the end of the study. However, since in general both species were rare and no algae bloom was found at the end of the study, this was not considered to be ecologically relevant. Thus effect class 3A was chosen as the overall effect class for 9.3 µg/L.
- At 19.4 µg/L the effect classification was similar to the one for 9.3 µg/L. For leeches, higher abundances at the end of the study could not be excluded which was considered as class 2/4A for the highest test concentration.

According to EFSA (2013) the ETO-RAC can be derived from the overall class 1 concentration of 1 µg/L (nominal for three applications) and would be 0.5 µg/L using the recommended assessment factor of 2 in EFSA (2013).

However, if the potential temporary promotion of the rotifer *Keratella* and the slight effect (class 2) on total phytoplankton is considered acceptable, the 2.1 µg/L concentration could be used to derive the ETO-RAC considering that the observed effects here are of low ecological relevance.

Since rotifers and algae seem to be the most sensitive taxa while taxa with a lower recovery potential were found to be less sensitive (Cladocera, Copepoda, Chironomidae, Chaoboridae, Hirudinea, Oligochaeta) the study can also be used to derive an ERO-RAC. At 9.3 µg/L, no, slight or only effects with recovery within 8 weeks were found. Thus, the ERO-RAC can be derived using this concentration (9.3 µg/L) and would be 3.1 µg/L applying an assessment factor of 3.

No clear long-term effects were found at the highest test concentration of at 19.4 µg/L, only for a potential promotion of leeches the effect duration could not be assessed. Thus, the ERO-RAC would be 4.85 µg/L applying an assessment factor of 4 to consider the higher uncertainty due to data on leeches.

Assessment and conclusion by applicant:

The mesocosm study and the re-assessment study has been assessed using the checklist presented in the Aquatic Guidance Document (adapted from De Jong *et al*., 2008) in order to confirm the reliability of the data. The outcome of the assessment is presented below.

Table CP 10.2.3/03-19 Reliability assessment of the mesocosm study according to EFSA (2013)

Items	Notes	Reliability index 1–3
Methodology and test description		
1. Substance	**Properly characterised and reported?**	
1.1 Concentration	Identity and amount of a.s. per litre test water?	**1**

		Fully reported (p 19 and 28 of study report0)
1.2 Formulation and purity	Substances in the formulation influencing the working action of the a.s. should be reported	**1** The rep. formulation Spiroxamine EC 500 was tested
1.3 Vehicle	In case a vehicle (other than in the formulation) is used, identity and concentration?	n/a
1.4 Chemical analyses	Method, LOQ, LOD, recovery	**1** Fully reported (p248-262) Refer also to analytical methods section of dossier)
1.5 Properties	Relevant for potential fate and effects in test system	**1**
2. Test site, duration	**Properly characterised and reported?**	
2.1 Location	Necessary to make a link between the effects and local environmental conditions, representativeness	**1** Fully reported (p 21)
2.2 Test date/duration	Application dates and experimental period?	**1** Fully reported (p 25)
2.4 General climatic conditions	Necessary to make a link between the effects and local climatic conditions	**1** Fully reported (p 41)
3. Application	**Properly characterised and reported?**	
3.1 Mode of application	Exposure route, spraying or homogenising the a.s. into the test medium?	**1** Fully reported (p 27)
3.2 Dosage and exposure	Actual concentrations during the test? Chemical analysis of dosing solution?	**1** Fully reported. Chemical analysis of water, sediment and macrophytes
3.3 Application scheme	Necessary to make a link between the test and the intended use of the PPP	**1** Fully reported (p 27)
3.4 Conditions during application	Weather conditions during application, wind speed and temperature	**1** Fully reported (p 41)
4. Test design	**Properly designed and reported?**	
4.1 Type and size	e.g. outdoor microcosm, outdoor pond or mesocosm, dimensions	**1** Fully reported (p 21 - 22)
4.2 Pre-treatment	Proper equilibration?	**1** 5 months of acclimation prior to dosing
4.3 Treatment period	Number and spacing of treatments?	**1** 3 applications with a 7-day interval
4.3 Post-treatment	Period long enough to allow expression of effects and recovery?	**1** 14 week (84-day) duration sufficient to assess effects and potential recovery
4.4 Untreated control	Sufficient number; solvent applied?	**1** 3 control reps; no solvent required

4.5 Replications	Sufficient replications for proper statistical analysis?	**1** 3 control reps; 2 reps for 1.0, 2.1, 4.4 and 9.3 µg/L; a single rep for 19.4 µg/L
4.6 Statistics	Univariate and multivariate techniques applied	**1** Fully reported. Also refer to mesocosm re-assessment report)
4.8 Dose–response	Number of test concentrations for finding a dose relationship (excluding controls)	**1** 5 test concentrations used in the study
4.9 Quality assurance	Study conducted under GLP?	**1** GLP study
5. Biological system	**Representative and properly reported?**	
5.1 Populations	Enough sensitive/vulnerable species of the relevant taxonomic group?	**1** MDD criteria fulfilled (at least 8 sensitive taxa). Refer to re-assessment report
5.2 Community	The community/ecosystem representative and complete?	**1** Aquatic ecosystem considered sufficiently represented
6. Sampling	**Is sampling adequate for risk assessment?**	
6.1 General features	Relevance selected measurement endpoints	**1** Fully reported (p 29 – 32)
6.2 Actual concentration	Actual concentrations measured in medium and other compartments or biota?	**1** Fully reported; concentrations measured in overlying water, sediment and in macrophytes
6.3 Biological sampling	Appropriate methods and frequency?	**1** Suitable sampling techniques used for zooplankton, macrozoobenthos, chlorophyll a, algae and macrophytes (p 29 – 32)
Results		
7. Endpoints	**Properly reported?**	
7.1 Type	Reported endpoints relevant for objective of study?	**1** Fully reported. Also refer to re-assessment report
7.2 Value	Are measured data consistently presented?	**1**
7.3 Verification of endpoint	Test results are verifiable and source data reported	**1** Fully reported (refer to re-assessment report)
8. Elaboration of results	**Are conclusions based on measured data?** **Methodology correct?**	
8.1 Statistical comparison	Data meet requirements for method used?	**1** (refer to re-assessment report)
8.2 Dose–effect relationship	Minimal detectable difference; consistence of response	**1** MDD analysis performed (refer to re-assessment report)

8.3 Population-level responses	Sufficiently reported?	**1** Sufficiently reported
8.4 Community-level responses	Sufficiently reported?	**1** Sufficiently reported
9. Control		
9.1 Untreated control	Unexpected effects or disappearance of species?	**1** No unexpected effects
9.2 Solvent control	Possible effects caused by solvent?	**1** No solvent used
10. Classification of effects	**Properly derivable?**	**1** Refer to re-assessment report where effects are classified in accordance with the Aquatic Guidance Document
11. Biological meaning of statistically significant differences	**Sufficiently explained?**	**1** Fully reported

Relevant page numbers from the mesocosm report (FYF 379) have been added for information purposes.

1 Reliable - All data are reported, the methodology and the description are in accordance with internationally accepted test guidelines and/or the instructions, all other requirements fulfilled

2 Less Reliable - Not all data reported, the methodology and/or the description are slightly deviating from internationally accepted test guidelines or the instructions, without motivation, or not all other requirements fulfilled

3 Not reliable - Essential data missing, the methodology and/or the description are not in accordance with internationally accepted test guidelines and/or the instructions without motivation, or not reported, or important other requirements are not fulfilled

Based on the reliability assessment above, it is considered that the mesocosm study was conducted to recognised test methodology and was sufficiently reported. Taking the results of this study as well as the results of the re-assessment study into account, it is considered that the data are robust and reliable and of sufficient quality to be able to derive an endpoint for use in the aquatic risk assessment.

For phytoplankton, macroinvertebrates and zooplankton, 19 taxa plus pooled data on higher taxonomic levels fulfilled the MDD criterion proposed by Brock *et al*. (2015). Furthermore, the chlorophyll a measurements of phytoplankton and periphyton as well as the macrophyte coverage could be evaluated. If a more strict criterion is applied, e.g. that the MDD should be at least once <70% within the first four weeks after the first application, 13 taxa representing populations of macroinvertebrates, zooplankton and phytoplankton allow a statistical evaluation of direct effects: Tubificidae, Chironomini, *Chaoborus spec*, *Simocephalus vetulus, Chydorus sphaericus, Eucercus lamellatus*, cyclopoid copepods (and nauplia larvae), *Polyarthra spec*., *Chlamydomonas spec*., coccoid Chlorophyceae, *Chroomonas spec*., *Cryptomonas spec*. (20-30 µm), and Pennales (30-40 µm). Thus, the requirement of the Aquatic Guidance Document (EFSA PPR 2013) that the MDDs should be sufficiently low to allow the analysis of direct effect for at least 8 potential sensitive populations is met by the study.

The study is considered to be acceptable and sufficiently robust to derive an ETO-RAC of 0.5 µg a.s./L (Class 1 effects at 1.0 µg a.s./L and an assessment factor of 2). The data are also considered sufficient to derive an ERO-RAC of 3.1 µg a.s./L (Class 3A effects at 9.3 µg a.s./L and an assessment factor of 3).

Data Point:	KCP 10.2.3/02
Report Author:	[redacted]
Report Year:	2000
Report Title:	Fate of spiroxamine in enclosures of an experimental ditch
Report No:	HBF/MT 12
Document No:	M-030336-01-1
Guideline(s) followed in study:	OECD Guidance Document "Freshwater Lentic Field Tests", July 1996 (Draft)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

The aim of the study was to assess the fate and partitioning of spiroxamine in an environmentally realistic aquatic system. The study was carried out using four enclosures in an experimental ditch at Renkum, the Netherlands. Two enclosures contained macrophytes and had an upper layer of sediment rich in organic matter, whereas in the two other enclosures macrophytes and the upper layer of detritus were removed.

A single application of the test substance was introduced into the enclosures at two different concentrations of (nominal) 3.5 and 35 µg a.s./L, respectively. The test lasted for 56 days after application of the test substance. The concentrations of the active substance in the water phase, in sediment and in macrophytes were followed over time.

In the enclosure study it was demonstrated that macrophytes affect the initial dissipation of spiroxamine from the water column, by sorbing a large proportion of the dose applied (up to 32 - 41%). In the two enclosures with macrophytes and an organic-rich detritus layer the DT_{50} values for spiroxamine in water ranged from 0.9 - 2.0 days. In one of these systems a DT_{50} value of 10.1 days was measured for the total system.

In the two enclosures without macrophytes and an organic-rich detritus layer the DT_{50} values of Spiroxamine in water ranged from 3.1 - 5.9 days. Despite the somewhat slower dissipation from water, the DT_{50} value for the total system (9.6 days) was very similar to that in the other type of enclosure.

I. Materials and Methods

Materials

Test Material Spiroxamine EC 500

Lot/Batch #: TOX No. 05232-00

Purity (a.s.): 48.3% w/w

Description: Brown liquid

Stability of test compound: Sufficient based on expiration date

Reanalysis/Expiry date: 17 August 2000

Density: 1.006 g/mL

Treatments

Test rates:	3.5 and 35 µg a.s./L
Solvent/vehicle:	Tap water
Analysis of test concentrations:	Days 0.17, 1, 3, 7, 14, 28, 56 and 68
Test organisms	
Species:	Macrophytes
Source:	Wild collected
Acclimatisation period:	Not applicable
Feeding:	Not applicable
Treatment for disease:	Not reported
Test design	
Test vessel:	Cylinder (diameter 1.05 m, height 0.90 m) made of polycarbonate pervious to light, which is pushed approx. 0.15 m into the sediment. The height of the enclosures above water surface is approx. 0.15 m, water depth is approx. 0.50 m
Test medium:	Water
Replication:	Duplicate
No. of animals/vessel:	-
Duration of test:	56 days
Environmental test conditions	
Temperature:	13.8 – 21.4°C
Dissolved oxygen:	8.26 – 11.52 mg/L
pH:	7.97 – 9.64
Photoperiod:	Natural light (88 - 524 uE cm^{-2} s^{-1})

Study Design

The aim of the study was to assess the fate and partitioning of spiroxamine in an environmentally realistic aquatic system. The study was carried out using four enclosures in an experimental ditch at Renkum, the Netherlands. Two enclosures contained macrophytes and had an upper layer of sediment rich in organic matter, whereas in the two other enclosures macrophytes and the upper layer of detritus were removed. On May 15, 2000 a single application of the test substance was introduced into the enclosures at two different concentrations of (nominal) 3.5 and 35 µg a.s./L, respectively.

The test lasted for 56 days after application of the test substance. The concentrations of the active substance in the water phase, in sediment and in macrophytes were followed over time.

II. Results and Discussion

Residue analysis of water

The achieved initial water concentrations in the enclosures were 58 - 94% of the nominal target concentrations. Concentrations measured 4 hour (0.17 days) after application ranged from 58 -

64% and 114 - 116% of calculated initial concentrations in systems with and without macrophytes, respectively. This difference can be explained by fast sorption of spiroxamine to macrophytes. The fact that concentrations observed in enclosures 1 and 2 (without macrophytes) at 4 hours after application were above 100% of the initial concentrations was most probably the result of inhomogeneous distribution of the chemical in the water column during the first hours after application.

Concentrations of spiroxamine in the water of the two enclosures containing macrophytes and an organic-rich upper sediment layer decreased faster ($DT_{50water}$ = 0.9 - 2.0 days) than in the water of macrophyte-free enclosures ($DT_{50water}$ = 3.1 - 5.9 days).

Table CP 10.2.3/02-1 Mean spiroxamine concentrations of the water samples

Days after application	Mean concentrations in water (µg a.s./L)			
	Enclosure 1 (3.5 µg/L)	Enclosure 3 (3.5 µg/L)	Enclosure 2 (35 µg/L)	Enclosure 4 (35 µg/L)
-0.04	<0.2	<0.2	<0.2	<0.2
0	2.56	3.28	20.3	29.5
0.17	2.93	2.10	23.6	17.2
1	2.02	1.13	19.7	10.1
3	1.16	0.38	15.5	4.1
7	0.55	<0.2	10.8	1.43
14	<0.2	<0.2	3.47	0.40
28	<0.2	<0.2	<0.2	<0.2
56	<0.2	<0.2	<0.2	<0.2

Residue Analysis of Sediment

Residues in the sediment of enclosures treated with (nominal) 3.5 µg/L spiroxamine were on all sampling days below detection limits. Residues in sediments of enclosures treated with (nominal) 35 µg/L spiroxamine tended to increase during the first days after treatment. Maximum values of 20.3 - 32.3 mg/kg were found. Starting approximately 1 week after treatment, a steady decline was observed during the remainder of the observation period. Four weeks after treatment residues in the sediment were less than 25% of the observed maximum residues. At the end of the experiment (day 56) residues in the sediment had declined to 13- 15% of the observed maximum values.

Overall, the organic-rich sediment in the enclosure with macrophytes contained higher concentrations of spiroxamine than the organic-poor sediment of the enclosure without macrophytes.

Table CP 10.2.3/02-2 Mean spiroxamine concentrations of the sediment samples

Days after application	Mean concentrations in dry sediment (µg/kg)			
	Enclosure 1 (3.5 µg/L)	Enclosure 3 (3.5 µg/L)	Enclosure 2 (35 µg/L)	Enclosure 4 (35 µg/L)
1	<LOQ	<LOQ	12.6	32.3
3	<LOQ	<LOQ	20.3	14.4
7	<LOQ	<LOQ	16.8	26.7

14	<LOQ	<LOQ	15.5	16.3
28	<LOQ	<LOQ	3.59	7.4
56	<LOQ	<LOQ	3.14	4.3

<LOQ = concentrations of KWG4168 below the limit of detection of the analytical method of 2 µg/kg

Residue Analysis of Macrophytes

Concentrations of spiroxamine in macrophytes increased during the first days after treatment of the enclosures, reaching a maximum concentration approximately 3 days after treatment. Maximum values in the enclosures treated with (nominal) 3.5 µg/L and 35 µg/L were 760 and 8900 µg per kg fresh weight, respectively. From 1 week after treatment spiroxamine concentrations in macrophytes steadily decreased. Two weeks after treatment the concentrations in macrophytes were slightly less than 25% of the maximum concentrations observed 3 days after treatment. At the end of the experiment (day 56) these concentrations had declined to less than 1% of the maximum concentration.

Table CP 10.2.3/02-3 Mean spiroxamine concentrations of the macrophyte samples

Days after application	Mean concentrations in macrophytes (µg a.s./kg)	
	Enclosure 3 (3.5 µg/L)	Enclosure 4 (35 µg/L)
1	530	6500
3	760	8900
7	410	6200
14	180	2100
28	<10	110
56	<10	<10

Overall Mass Budget

In the enclosure containing macrophytes and a top-layer of sediment rich organic material up to 41 % of the total amount of spiroxamine applied could be found in the macrophyte compartment and up to 19 % in the sediment compartment. The amount of spiroxamine in water decreased most rapidly, whereas the amounts in sediment took longest to decrease. The overall DT_{50} of spiroxamine in the total system was 10.1 days.

In enclosures where the upper detritus layer and macrophytes were removed the sediment compartment contained up to 19 % of the spiroxamine applied. The overall DT_{50} in the total system was with 9.5 days very similar to that in the enclosure with macrophytes.

III. Conclusion

In the enclosure study it is demonstrated that macrophytes affect the initial dissipation of spiroxamine from the water column, by sorbing a large proportion of the dose applied (up to 32 - 41%). In the two enclosures with macrophytes and an organic-rich detritus layer the DT_{50} values for spiroxamine in water ranged from 0.9 - 2.0 days. In one of these systems a DT_{50} value of 10.1 days was measured for the total system.

In the two enclosures without macrophytes and an organic-rich detritus layer the DT_{50} values of spiroxamine in water ranged from 3.1 - 5.9 days. Despite the somewhat slower dissipation from water, the DT_{50} value for the total system (9.6 days) was very similar to that in the other type of enclosure.

Assessment and conclusion by applicant:

The study was conducted in accordance with the guidance in place at the time for mesocosm studies. Essentially this study was a dissipation trial under realistic conditions in order to provide information on the fate of spiroxamine following application to a water-sediment system.

The study is considered acceptable in its own right but is not used directly in the risk assessment and has therefore been submitted as supporting information only.

CP 10.3 Effects on arthropods

CP 10.3.1 Effects on bees

The available data for spiroxamine technical and Spiroxamine EC 500 with bees are presented in the table below. Several acute oral and contact toxicity studies are available for honeybees and an acute toxicity data are also available for bumblebees. Two honey bee chronic adult oral LDD_{50} studies are available using Spiroxamine EC 500 and a 22-day larval repeated exposure toxicity study is also available using spiroxamine technical. Furthermore, two cage studies and a field study are also available using the representative formulation Spiroxamine EC 500.

Table CP 10.3.1-1 Summary of bee toxicity studies with spiroxamine and Spiroxamine EC 500

Organism	Test item	Test type	Endpoints		Reference
Adult honey bee (*Apis mellifera*)	Spiroxamine	Acute oral	48 h LD_{50} >100 µg a.s./bee	EU	M-008208-01-1
Adult honey bee (*Apis mellifera*)	Spiroxamine EC 500	Acute oral	**48 h LD_{50} 84.3 µg a.s./bee**	NEW	M-680761-01-1
Adult honey bee (*Apis mellifera*)	Spiroxamine EC 500	Acute oral	48 h LD_{50} >12.5 µg/bee (>6.1 µg a.s./bee)	EU	M-008241-01-1
Adult honey bee (*Apis mellifera*)	Spiroxamine EC 500	Acute oral	**48 h LD_{50} >77 µg/bee (>39 µg a.s./bee)**	EU	M-008222-01-1
Adult bumble bee (*Bombus terrestris*)	Spiroxamine	Acute oral	48 h LD_{50} >50.9 µg a.s./bumblebee	NEW	M-688128-01-1
Adult honey bee (*Apis mellifera*)	Spiroxamine	Acute contact	**48 h LD_{50} 4.2 µg a.s./bee**	EU	M-008208-01-1
Adult honey bee (*Apis mellifera*)	Spiroxamine EC 500	Acute contact	**72 h LD_{50} 59.7 µg a.s./bee**	NEW	M-680761-01-1
Adult honey bee (*Apis mellifera*)	Spiroxamine EC 500	Acute contact	48 h LD_{50} 30 µg/bee (15 µg a.s./bee)	EU	M-008241-01-1
Adult honey bee (*Apis mellifera*)	Spiroxamine EC 500	Acute contact	48 h LD_{50} >200 µg/bee (>100 µg a.s./bee)	EU	M-008222-01-1
Adult bumble bee (*Bombus terrestris*)	Spiroxamine	Acute contact	48 h LD_{50} >100 µg a.s./bumblebee	NEW	M-510841-01-1

Organism	Test item	Test type	Endpoints		Reference
Adult honey bee larva (*Apis mellifera*)	Spiroxamine EC 500	Chronic oral	10-day LC_{50} 1864 mg a.s./kg feeding solution 10-day LDD_{50} 27.3 µg a.s./bee/day **NOEDD 10.6 µg a.s./bee/day**	NEW	M-704650-01-1
Adult honey bee larva (*Apis mellifera*)	Spiroxamine EC 500	Chronic oral	10-day LC_{50} >100 mg a.s./kg feeding solution 10-day LDD_{50} >4.86 µg a.s./bee/day NOEDD 4.86 µg a.s./bee/day	NEW	M-538628-01-1
Honey bee larva (*Apis mellifera*)	Spiroxamine	Chronic larva (22 day repeated exposure)	LD_{50} >33 µg a.s./larva **NOED 33 µg a.s./larva**	NEW	M-623462-01-1
Honey bee (*Apis mellifera*)	Spiroxamine EC 500	Semi-field (cage) study. Application to flowering *Phacelia tanacetifolia* in England	Following a single application of KWG 4168 EC 500 to Phacelia at 7.5 L product/ha, there were no significant effects on honeybee mortality, foraging activity, behavior, brood and colony size when compared to the control	EU	M-008239-01-1
Honey bee (*Apis mellifera*)	Spiroxamine EC 500	Semi-field (cage) study. Application to flowering *Phacelia tanacetifolia* in Germany	Following a single application of KWG 4168 EC 500 to Phacelia at 3.0 L product/ha, there were no significant effects on honeybee mortality, flight intensity or brood when compared to the control	EU	M-008244-01-1

Organism	Test item	Test type	Endpoints		Reference
Honey bee (*Apis mellifera*)	Spiroxamine EC 500	Field study. Application to flowering *Phacelia tanacetifolia* in Germany	Following a single application of KWG 4168 EC 500 to Phacelia at 1.5 L product/ha, there were no significant effects on honeybee mortality, foraging activity, behavior, colony strength and brood development when compared to the control	EU	M-008232-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
NEW: new study or data generated since the previous EU review or previously not submitted
Values in **bold** have been used in the risk assessment

Exposure

The highest single application rate of Spiroxamine EC 500 to grapes is 300 g a.s./ha. This rate has been considered below in the risk assessment for bees and covers the risk assessment for the use of Spiroxamine EC 500 at 2 x 200 g a.s./ha.

Selection of endpoints

Several acute oral and contact LD_{50} values are available which have been generated using either spiroxamine technical or Spiroxamine EC 500. For the acute oral toxicity to honey bees, the lowest available LD_{50} is >6.1 µg a.s./bee (M-008241-01-1) but concerns over the reliability of this study have been raised therefore this value has not been considered in the risk assessment. Two other studies (M-008208-01-1 and M-008222-01-1) generated 'greater than' values (>100 and >39 µg a.s./bee, respectively) and are considered to be valid endpoints but the studies were relatively old and not specifically conducted to OECD guidelines. For this reason the new acute oral study (M-680761-01-1), which has generated a bound LD_{50} value of 84.3 µg a.s./bee, is considered to be the most suitable endpoint for risk assessment. However, the lowest of the valid endpoints (>39 µg a.s./bee) has also been considered here in order to take a conservative approach.

For the acute contact toxicity to honeybees, the lowest available LD_{50} is 4.2 µg a.s./bee which has been taken from an old study (M-008208-01-1) using spiroxamine technical which was not conducted to OECD guidelines but is still considered to be valid. One available study gave an LD_{50} of >15 µg a.s./bee (M-008241-01-1) but concerns over the reliability of this study have been raised therefore this value has not been considered further. Another available study gave an LD_{50} of >100 µg a.s./bee (M-008222-01-1) but the most suitable endpoint for risk assessment is considered to be the bound LD_{50} of 59.7 µg a.s./bee from the new acute contact study (M-680761-01-1). However, in order to take a conservative approach, the lowest endpoint of 4.2 µg a.s./bee has also been considered in the risk assessment.

For the chronic adult oral endpoint, two valid studies with Spiroxamine EC 500 are available. In the first study (M-538628-01-1) the LDD_{50} was determined to be >4.86 µg a.s./bee/day as there was 0% mortality recorded at the single dose tested of 100 mg a.s./kg feeding solution (equivalent to 4.86 µg a.s./bee/day). The NOEDD was therefore 4.86 µg a.s./bee/day. In a second study (M-704650-01-1) a bound LDD_{50} value of 27.3 µg a.s./bee/day was established with a corresponding NOEDD of 10.6 µg a.s./bee/day. It is considered justified to take the study with the LDD_{50} of 27.3 µg a.s./bee/day (NOEDD of 10.6 µg a.s./bee/day) as the chronic adult oral endpoint because this is a bound LDD_{50} which represents the 50% mortality level whereas the study with the LDD_{50} value of >4.86 µg a.s./bee/day was simply the only treatment level tested and one which gave rise to no mortality.

For larval toxicity a 22-day repeated exposure study with spiroxamine technical is available which gave a NOED of 33 µg a.s./larva.

Isomers

The risk assessments for bees involves potential chronic exposure of these organisms to residues in plants therefore it may be necessary to apply an uncertainty factor (UF) to the chronic risk assessments. The acute risk assessment need not have an UF applied as exposure in this scenario is immediate. However, the chronic risk assessment considers exposure over a prolonged period therefore potential changes in isomeric ratio needs to be considered. For the bee risk assessments the same approach taken in the residues section for the consumer risk assessment, with respect to isomers, has been followed. Based on the current residues data set for spiroxamine, there are no indications of a significant change in isomer ratios therefore no additional factor need be applied to the risk assessments below (*i.e.* an UF of 1.0 has been used).

Risk assessment for bees

The EFSA[8] guidance on bee risk assessment has not been noted at the EU level and is currently under revision. The notifier has therefore presented an acute risk assessment in accordance with the current SANCO[9] terrestrial guidance document. However, in order to consider the chronic risks to bees, an illustrative assessment of chronic risk has been presented using the existing EU community guidance provided by EPPO (2010)[10].

CP 10.3.1-2

Calculation of HQ_{oral} for honey bees exposed to Spiroxamine EC 500

Test substance	Crop Group	Species	App. rate (g a.s./ha)	LD_{50} oral (µg a.s./bee)	HQ_{oral}	Trigger
Spiroxamine EC 500	Grapes BBCH 13-85	Honey bee	300	>39	<7.69	50
				84.3	3.56	

HQ (Hazard Quotient) for adult oral exposure

CP 10.3.1-3

Calculation of $HQ_{contact}$ for honey bees exposed to Spiroxamine EC 500

[8] European Food Safety Authority, 2013 (*updated 04 July 2014*). EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2013;11(7):3295, 268 pp., doi:10.2903/j.efsa.2013.3295

[9] SANCO/10329/2002 rev 2 final (17 October 2002). Guidance Document on Terrestrial Ecotoxicology Under council Directive 91/414/EEC

[10] EPPO 2010: EPPO Standard PP 3/10 (3) Environmental risk assessment scheme for plant protection products. Chapter 10: honeybees. Bulletin OEPP/EPPO Bulletin 40: 323-331

Test substance	Crop Group	Species	App. rate (g a.s./ha)	LD_{50} contact (µg a.s./bee)	$HQ_{contact}$	Trigger
Spiroxamine	Grapes BBCH 13-85	Honey bee	300	4.2	**71.4**	50
Spiroxamine EC 500				59.7	5.03	

HQ (Hazard Quotient) for adult contact exposure. HQ values shown in bold breach the relevant trigger

For oral exposure the HQ values are below the trigger value of 50 thereby demonstrating no unacceptable acute risk to honey bees, *via* oral exposure, following the proposed uses of Spiroxamine EC 500 to grapes at 300 g a.s./ha.

For contact exposure the HQ value is below the trigger value of 50 when the LD_{50} of 59.7 µg a.s./bee is considered thereby demonstrating no unacceptable acute risk to honey bees, *via* contact exposure, following the proposed uses of Spiroxamine EC 500 to grapes at 300 g a.s./ha. When the technical material LD_{50} of 4.2 µg a.s./bee is considered the HQ value is >50 thereby indicating possible acute risks. However, it should be noted that the data with the formulation are considered to be most relevant and that there are three acute contact endpoints available for Spiroxamine EC 500 with LD_{50} values of 15, 59.7 and >100 µg a.s./bee, respectively and all of these values would result in HQ values below 50. Thus, the weight of evidence would suggest that the acute risks to honey bees, *via* contact exposure, is acceptable. Furthermore, higher tier data are available which can be used as supporting information to demonstrate acceptable risks to honey bees following the proposed uses of Spiroxamine EC 500.

It should also be noted that when the rate of 200 g a.s./ha is considered in the risk assessment, all HQ values are below the trigger of 50 even when the contact LD_{50} value of 4.2 µg a.s./bee is used.

Chronic toxicity to honeybees

Spiroxamine EC 500 is intended to be used in grape vines during a period when the vines may be flowering. However, vines are not highly attractive to bees that will seldom visit the crop due to the fact that it does not produce nectar. The reason for this is that vines are wind pollinated and do not require animal pollination for fruit set or production and, as such, vine growers do not employ beekeepers to locate their hives at the edge of vineyards. Consequently, there is no agricultural or apicultural necessity for bees to be placed in or near vines.

Bees may collect pollen from vines but cannot collect nectar and, as there is no sugar stimulus, means that returning bees will not perform a waggle dance to inform other bees of the location of such plants. The fact that vines are not highly attractive to bees means that they will only visit to collect pollen if there is a lack of other pollen sources nearby which also offer a nectar reward. As vines do not provide a source of nectar there is negligible exposure to foraging bees and exposure may only be possible *via* pollen. In the colony there is only a limited storage of pollen as pollen foraging is stimulated by brood demands so consequently there is a rapid turnover of pollen with fresh supplies being provided from a wide range of plants. These factors therefore severely limit the long-term exposure of bees to applications of Spiroxamine EC 500.

A chronic risk assessment has been presented below in accordance with the EPPO scheme. Although the EPPO guidance gives procedures based on systemic product applied by soil or seed treatments, the methodology is also suitable for the chronic risk by spray application.

The chronic risk assessment below considers exposure *via* pollen only as nectar is not considered to be a viable route of exposure to bees from vines.

The chronic risk assessment for adult honeybees and honeybee larvae is based on the generic worst-case residue value of 1 mg a.s./kg plant matrix in pollen, as specified in the revised EPPO scheme (2010) and determines the ratio between the NOEDD (oral) and the exposure by means of a TER calculation. For adult honeybees, the exposure was assessed through the amount of residues that may be ingested by a bee in one day. The ratio between the NOEDD (µg a.s./bee/day) and the exposure (in µg a.s./bee/day) was calculated using the following formula:

$$TER_{chronic,adult} = \frac{NOEDD_{oral}\ (\mu g\ a.s./bee/day)}{Residues\ ingested\ by\ a\ bee\ in\ one\ day\ (\mu g\ a.s./bee/day)}$$

As the available endpoint for larvae is expressed over the total developmental period, the exposure for larvae was assessed through the amount of residues that may be ingested by the larvae over that period. For larvae, the ratio between the NOED (in µg a.s./larva) and the exposure (in µg a.s./larva) was calculated using the following formula:

$$TER_{chronic,larvae} = \frac{NOED_{oral}\ (\mu g\ a.s./larva)}{Residues\ ingested\ by\ a\ larva\ (\mu g\ a.s./larva)}$$

Data for consumption of nectar and pollen by adult honey bees and honey bee larvae are given in the EFSA Opinion on bees (2012)[11]. According to the EFSA Opinion the maximum amount of pollen an adult bee consumes per day is 300 mg/bee/day. For honey bee larvae the maximum amount of pollen consumed by a larva is stated as 2 mg/5 days.

To calculate the residue intake of spiroxamine by adult honey bees and honey bee larvae, the consumed amounts of pollen are multiplied with the generic residue value in nectar and pollen of 1.0 mg a.s./kg (equivalent to 0.001 µg a.s./mg). Thus, adult honeybees that consume 300 mg bee/day of pollen will therefore be exposed to 0.3 µg a.s./bee/day (300 mg/bee/day x 0.001 µg a.s./mg). Larvae will be exposed to 0.002 µg a.s./larva through the consumption of pollen.

TER values have been calculated in the table below and are compared to the TER trigger value of 1, as stated in the EPPO scheme.

CP 10.3.1-4

Chronic risk assessment for honeybee adults and larvae from exposure to spiroxamine *via* pollen

Stage of development	Route of exposure	NOEDD / NOED	Residue intake	TER	Trigger value
Adult	Oral	10.6 µg a.s./bee/day	0.3 µg a.s./bee/day	35.3	1
Larvae		33 µg a.s./larva	0.002 µg a.s./larva	16500	

The TER values are both greater than the trigger value of 1 therefore the chronic risks to honeybee adults and larvae, from consumption of potentially contaminated pollen, are considered to be acceptable. Thus, the chronic risks to bees following the proposed uses of Spiroxamine EC 500 on grapes are considered to be acceptable.

Higher-tier risk assessment

A higher-tier risk assessment is not considered to be necessary as acceptable acute and chronic risks have been demonstrated in the risk assessments above. However, reference is made to the available data which support the conclusion that the risk to bees following the use of Spiroxamine EC 500 are acceptable.

Higher-tier data in the form of two cage tests and a field study are available using Spiroxamine EC 500. Although these three studies do not meet some of the current requirements for higher-tier bee studies, they are considered to be valid in their own right and can be used to help support the risk assessment

[11] EFSA Panel on Plant Protection Products and their Residues (PPR) (2012). Scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (Apis mellifera, Bombus spp. and solitary bees). EFSA Journal 2012; 10(5) 2668

and to demonstrate an acceptable risk to bees following the proposed use of Spiroxamine EC 500. All three studies used the bee attractive crop, *Phacelia* and all three studies tested at rates far exceeding the proposed rate of 300 g a.s./ha. All three studies demonstrated that there were no significant effects on honey bees following application of Spiroxamine EC 500.

Residues decline data in nectar and pollen are available for Spiroxamine (M-763122-01-1). In the study, Spiroxamine EC 500 was applied twice to *Phacelia tanacetifolia* (at pre-flowering and flowering growth stages) in semi-field tunnel conditions at a rate of 300 g a.s./ha with a 10-day interval. Two trials were conducted in Germany and three trials in Spain. Sampling occurred shortly after the second application, 8 hours, 1, 2, 3, 5 and 7 days after the second application. The study has been summarized in Section CP 10.3.1.5 below. The results confirm that residues of spiroxamine dissipated relatively quickly following application. These results are considered to be suitable for use in a refined risk assessment, where required.

Bumble bee data

Acute oral and contact toxicity data are available for bumble bees. The acute oral LD_{50} has been established to be >59 µg a.s./bumble bee and the acute contact LD_{50} has been established to be >100 µg a.s./bumble bee. The data demonstrates that bumble bees are no more acutely sensitive than honey bees to the effects of spiroxamine.

Biodiversity

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on bees. Therefore, it is considered that the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects *via* alteration of the food web, are covered by the risk assessment for bees in this section.

The risk assessment for bees does not indicate a need for higher tier assessment nor mitigation measures. Therefore, the applicant concludes that the use of the representative lead formulation Spiroxamine EC 500 has low potential to cause unacceptable effects on biodiversity and the ecosystem *via* trophic interactions. To the best of our knowledge and with the presented safety profile of the active substance spiroxamine and the representative lead formulation, the applicant does not foresee any effects on biodiversity and the ecosystem.

CP 10.3.1.1 Acute toxicity to bees

CP 10.3.1.1.1 Acute oral toxicity to bees

Data Point:	KCP 10.3.1.1.1/03
Report Author:	[redacted]
Report Year:	2020
Report Title:	Spiroxamine EC 500: Effects (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No:	143091035
Document No:	M-680761-01-1
Guideline(s) followed in study:	Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.3020, 850.supp OECD 213 and 214 (1998)
Deviations from current test guideline:	Yes (refer below) The maximum temperature: during exposure phase of the experiment in both tests was 27.7 °C for 2 hours and one minute which was outside of the range of 25 ± 2 °C
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Honeybees (*Apis mellifera* L.) were exposed to Spiroxamine EC 500 in a 72-hour contact toxicity study and a 48-hour oral toxicity study. Exposure was at dose levels up to 400 µg a.s./bee in the contact study and up to 89.7 µg a.s./bee in the oral study. A reference rate of 12 µg dimethoate/bee was used along with a water control. This was in accordance with the amended OECD 213 and 214 (1998) guidelines.

The 72-hour NOED and LD_{50} values for the contact test were 17.1 and 59.7 µg a.s./bee, respectively.

The 48-hour NOED and LD_{50} values for the oral test were 53.0 and 84.3 µg a.s./bee, respectively.

I. Materials and Methods

Materials

Test Material Spiroxamine EC 500

Lot/Batch #: LM4L018425

Purity: 501.6g/L

Description: Liquid

Stability of test compound: Not reported

Reanalysis/Expiry date: 9th May 2020

Density: 1.004g/mL (20°C)

Treatments

Test rates: Contact (nominal): 400.0, 181.8, 82.6, 37.6 and 17.1 µg a.s./bee
Oral (nominal): 200.0, 100.0, 50.0, 25.0 and 12.5 µg a.s./bee

	Actual dose levels in the oral test: 89.7, 81.3, 53.0, 26.8 and 13.3 µg a.s./bee
Solvent/vehicle:	Contact: Adhäsit (0.5%) in water Oral: 50% w/v sucrose solution
Analysis of test concentrations:	None
Test organisms	
Species:	Adult female worker honeybees, *Apis mellifera* L.
Source:	Honey bee colonies, disease-free and queen-right, bred by ibacon
Acclimatisation period:	None reported
Feeding:	50% w/v sucrose solution (500g/L tap water) *ad libitum* given directly after treatment using syringes.
Treatment for disease:	None reported
Test design	
Test vessel:	Stainless steel chambers, 8cm x 6cm x 4cm (length x height x width), front plate is a removable glass sheet. Bottom plate is perforated with 98 ventilation holes of diameter 1mm
Replication:	3 test units per test item dose level, control and reference item dose level
No. animals/vessel:	10 per test unit
Duration of test:	Contact: 72 hours Oral: 48 hours
Environmental test conditions	
Temperature:	25.2 - 27.7°C
Relative humidity:	58.7 - 65.3%
Photoperiod:	Darkness (except during observation)

Study Design

Honeybees were exposed to Spiroxamine EC 500 in acute contact and oral tests over 72 and 48 hours, respectively.

The test organisms were adult female worker *Apis mellifera* L. bred in house. The honeybees were kept in test units and the contact application was conducted outside of the test unit. Temperature and relative humidity were kept at 25.2 to 27.7 °C and 58.7 to 65.3%, respectively throughout the test period. The bees were kept in darkness except during observation.

The bees were housed in stainless steel chambers, 8cm x 6cm x 4cm with a front plate made from a removable glass sheet. The bottom plate was perforated with 98 ventilation holes 1.0 mm in diameter. Ten bees were housed in each unit and three test units were used per test item dose level, control and reference item dose level.

A preliminary contact toxicity test with 100, 10 and 1.0 µg a.s./bee was performed. Based on the results the dose levels for the definitive test were adjusted appropriately. Bees were anaesthetised for 20 seconds

with CO_2 until they were completely immobilised immediately before application. Thirty bees were treated with each concentration of the test item of 400.0, 181.8, 82.6, 37.6 and 17.1 µg a.s./bee by topical application for 72 hours.

The test item was applied as one 5µL droplet of spiroxamine EC 500, dissolved in tap water with 0.5 % Adhäsit, to the dorsal bee thorax using a calibrated pipette. The reference item was applied as one 5 µL droplet of dimethoate, dissolved in tap water with 0.5 % Adhäsit. For the control, one 5 µL droplet of tap water containing 0.5 % Adhäsit was used. A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item;

In the oral test, the bees were starved for 20 minutes prior to test. Thirty worker bees were exposed for 48 hours to target doses of 200.0, 100.0, 50.0, 25.0 and 12.5 µg a.s./bee by feeding. The test item and reference item were applied in 50% w/v sucrose solution, which was used as carrier in the oral test. For the control, pure 50 % w/v sucrose solution was offered to the bees.

The treated food was offered in syringes, which were weighed before and after introduction into the cages. In practice, uptake of the treated 50 % w/v sucrose solutions differs slightly from the nominal 20 mg/bee and results are therefore given based on the measured consumption.

In both tests, the number of dead bees were observed at 4 (± 0.5), 24 and 48 (± 2) hours and also at 72 (± 2) hours for the contact test. Behavioural abnormalities (*e.g.* vomiting, apathy, intensive cleaning) were observed at 4 (± 0.5), 24 and 48 (± 2) hours in both tests and at 72 (± 2) hours in the contact test.

II. Results and Discussion

Validity criteria according to the OECD 213 (1998) and OECD 214 (1998) guidelines were met:

- Average mortality for the total number of controls must not exceed 10% at the end of the test (actual: 0% in the control in both contact and oral tests)
- The 24-hr LD_{50} of the toxic standard to be 0.10 to 0.30 µg a.s./bee for the contact test and 0.10 to 0.35 µg a.s./bee for the oral test (actual: 24-hr LD_{50} 0.26 and 0.15 µg a.s./bee in the contact and oral tests, respectively)

In the contact test dose levels of 400.0, 181.8, 82.6, 37.6 and 17.1 µg a.s./bee led to dose dependent mortality of 100.0, 96.7, 56.7, 33.3 and 3.3% at test termination (72 hours). No mortality occurred in the control group. The contact toxicity test was prolonged for further 24 hours up to 72 hours due to increasing mortality between 24 and 48 hours.

Table CP 10.3.1.1.1/03-1 Mortality and behaviour of honeybees exposed to Spiroxamine EC 500 for 72 hours (contact test)

Nominal concentration (µg a.s./bee)	Mean mortality (%)				Mean number of bees displaying abnormal behaviour (%)			
	4 hours	24 hours	48 hours	72 hours	4 hours	24 hours	48 hours	72 hours
Test item								
400.0	13.3	80.0	100.0	100.0	86.7	20.0	0.0	0.0
181.8	0.0	76.7	96.7	96.7	86.7	16.7	0.0	0.0
82.6	0.0	40.0	53.3	56.7	56.7	13.3	0.0	0.0
37.6	0.0	26.7	33.3	33.3	30.0	0.0	0.0	0.0
17.1	0.0	3.3	3.3	3.3	3.3	0.0	0.0	0.0

Nominal concentration (µg a.s./bee)	Mean mortality (%)				Mean number of bees displaying abnormal behaviour (%)			
	4 hours	24 hours	48 hours	72 hours	4 hours	24 hours	48 hours	72 hours
Control								
water + 0.5 % Adhäsit	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Reference								
0.30	13.3	56.7	66.7	70.0	6.7	0.0	0.0	0.0
0.20	6.7	40.0	43.3	46.7	10.0	0.0	0.0	0.0
0.15	6.7	33.3	33.3	40.0	0.0	0.0	3.3	0.0
0.10	0.0	3.3	6.7	6.7	0.0	0.0	0.0	0.0

Actual dose levels achieved in the oral test were 89.7, 81.3, 53.0, 26.8 and 13.3 µg a.s./bee. The maximum nominal dose levels of the test item could not be achieved, because the bees did not ingest the full volume of treated sugar solution even when offered over a period of six hours. Mortality occurred at the two highest dose levels with 60.0% mortality at the 89.7 µg a.s./bee and 46.7% at the 81.3 µg a.s./bee dose groups at test end. No behavioural abnormalities were found in the test item treated groups.

Table CP 10.3.1.1.1/03-2 Mortality and behaviour of honeybees exposed to Spiroxamine EC 500 for 48 hours (oral test)

Nominal concentration (µg a.s./bee)	Mean mortality (%)			Mean number of bees displaying abnormal behaviour (%)		
	4 hours	24 hours	48 hours	4 hours	24 hours	48 hours
Test item						
89.7	3.3	60.0	60.0	23.3	0.0	0.0
81.3	6.7	46.7	46.7	6.7	0.0	0.0
53.0	0.0	0.0	0.0	0.0	0.0	0.0
26.8	0.0	0.0	0.0	0.0	0.0	0.0
13.3	0.0	0.0	0.0	0.0	0.0	0.0
Control						
water + 0.5 % Adhäsit	0.0	0.0	0.0	0.0	0.0	0.0
Reference						
0.32	33.3	96.7	96.7	36.7	0.0	0.0
0.16	0.0	53.3	60.0	6.7	0.0	0.0
0.08	0.0	3.3	6.7	3.3	0.0	0.0
0.06	0.0	0.0	0.0	0.0	0.0	0.0

The following LD_{10}, LD_{20}, LD_{50} and NOED values were determined in this study:

Table CP 10.3.1.1.1/03-3 LD_x and NOED values for honeybees exposed to Spiroxamine EC 500 in contact and oral tests

Parameter	Value (µg a.s./bee)		
	24 hours	**48 hours**	**72 hours**
Contact LD_{50}	102.9	61.1	59.7
Contact LD_{20}	35.9	33.0	32.5
Contact LD_{10}	20.7	23.9	23.7
Contact NOED	17.1	17.1	17.1
Oral LD_{50}	84.3	84.3	-
Oral LD_{20}	72.5	72.5	-
Oral LD_{10}	67.0	67.0	-
Oral NOED	53.0	53.0	-

III. Conclusion

In an acute contact and oral toxicity test with honeybees using Spiroxamine EC 500, the 72-hour contact LD_{50} value was 59.7 µg a.s./bee and the 48-hour oral LD_{50} value was 84.3 µg a.s./bee.

Assessment and conclusion by applicant:

Validity criteria according to the OECD 213 (1998) and OECD 214 (1998) guidelines, to which the study was conducted, were met:

- Average mortality for the total number of controls must not exceed 10% at the end of the test (actual: 0% in the control in both contact and oral tests)
- The 24-hr LD_{50} of the toxic standard to be 0.10 to 0.30 µg a.s./bee for the contact test and 0.10 to 0.35 µg a.s./bee for the oral test (actual: 24-hr LD_{50} 0.26 and 0.15 µg a.s./bee in the contact and oral tests respectively)

The study is therefore considered to be acceptable.

The 72-hour contact LD_{50} value was 59.7 µg a.s./bee and the 48-hour oral LD_{50} value was 84.3 µg a.s./bee.

Data Point:	KCP 10.3.1.1.1/01
Report Author:	[redacted]
Report Year:	1997
Report Title:	Testing toxicity to honeybee - Apis mellifera L. (laboratory) according to EPPO Guideline No. 170 (1992) KWG 4168 EC 500
Report No:	97 10 48 001
Document No:	M-008222-01-1
Guideline(s) followed in study:	EPPO 170 (1992)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Honeybees (*Apis mellifera* L.) were exposed to KWG 4168 EC 500 in a 48-hour oral and contact toxicity study.

Test rates of 77 and 191 µg product/bee were used for the oral test and rates of 100 and 200 µg product/bee were used for the contact test.

In the oral toxicity test, bees were fed a defined quantity of a 50% aqueous sucrose solution containing 0.5 or 1.0% w/v test item in *ca.* 0.2 mL test solution. In the contact toxicity test, 1 µL test solution with 10 or 20% test item w/v in acetone were applied topically to the thorax of anaesthetised bees.

KWG 4168 EC 500 caused an overall low to moderate mortality up to the top dose levels of 191 µg product/bee in the oral toxicity test (67 %) and of 200 µg product/bee in the contact toxicity test (37 %).

LD_{50} calculation was not performed due to the limited number of doses tested but has been considered to be >77 µg product/bee (>39 µg a.s./bee) for the oral test and >200 µg product/bee (>100 µg a.s./bee) for the contact test.

I. Materials and Methods

Materials

Test Material	KWG 4168 EC 500
Lot/Batch #:	04931025
Purity:	505.5 g/L
Description:	Clear yellow liquid
Stability of test compound:	Not reported
Reanalysis/Expiry date:	23 July 1997
Density:	1.007 g/cm^3

Treatments

Test rates:	Oral:	77 and 191 µg product/bee
	Contact:	100 and 200 µg product/bee
Solvent/vehicle:	Sucrose solution / Acetone	
Analysis of test concentrations:		

Test organisms

Species:	Honeybees (*Apis mellifera* L.)
Source:	Mr H. Weimann, 04448 Gottscheina, Germany
Acclimatisation period:	1 – 2 hours
Feeding:	Fed continuously a 1:1 mixture of sugar and honey

Test design

Test vessel:	80 x 45 x 65 mm cardboard cages with a glass plate for observation
Replication:	Three
No. animals/vessel:	10 bees
Duration of test:	48 hours

Environmental test conditions

Temperature:	25 – 26°C
Relative humidity:	58 – 75%
Photoperiod:	8 h light : 16 h dark at *ca.* 100 lux

Study Design

This study was conducted in order to assess the effects of exposure to KWG 4168 EC 500 to the honeybee (*Apis mellifera* L.) in a 48-hour oral and contact toxicity test.

Test vessels were 80 x 45 x 65 mm cardboard cages with a glass plate for observation, to which were added ten bees for each of the three replicates per test.

In the oral toxicity test, bees were fed a defined quantity of a 50% aqueous sucrose solution containing 0.5 or 1.0% w/v test item in *ca.* 0.2 mL test solution. The feeding tube was re-weighed at most three hours after introduction in order to ascertain the exact quantity of test solution consumed. The actual dose applied was 77 and 191 µg product/bee, respectively.

In the contact toxicity test, 1 µL test solution with 10 or 20% test item w/v in acetone were applied topically to the thorax of anaesthetised bees. This is equivalent to 100 and 200 µg test item/bee, respectively.

Dimethoate was used as a positive control at rates of 0.20, 0.24, 0.28, 0.32, 0.36 and 0.40 µg Dimethoate EC 400/bee.

Bees were assessed for mortality and sub-lethal effects after 24 and 48 hours.

II. Results and Discussion

Validity criteria were not assessed as part of the study report.

In the oral toxicity test, mortality of 27 and 67% was observed 48 hours post-exposure to 77 and 191 µg product/bee, respectively.

Table CP 10.3.1.1.1/01-1 Bee mortality over 48 hours after oral exposure to KWG 4168 EC 500

Test item concentration (%)	Dose applied (µg formulation/bee)	Mortality (%)	
		24 h	48 h
Control (sucrose)	-	0	0
0.5	77	23	27
1.0	191	67	67

In the contact toxicity test, mortality of 33 and 37% was observed 48 hours post-exposure to 100 and 200 µg product/bee, respectively.

Table CP 10.3.1.1.1/01-2 Bee mortality over 48 hours after contact exposure to KWG 4168 EC 500

Test item concentration (%)	Dose applied (µg formulation/bee)	Mortality (%)	
		24 h	48 h
Control (sucrose)	-	0	7
10	100	7	23
20	200	33	37

LD_{50} calculation was not performed due to the limited number of doses tested but has been considered to be >77 µg product/bee (>39 µg a.s./bee) for the oral test and >200 µg product/bee (>100 µg a.s./bee) for the contact test.

In the reference test oral LD_{50} values of 0.31 and 0.30 µg Dimethoate EC 400/bee were determined after 24 and 48 hours, respectively. Contact LD_{50} values of 0.22 and 0.16 µg Dimethoate EC 400/bee were determined after 24 and 48 hours, respectively.

III. Conclusion

Honeybees (*Apis mellifera* L.) were exposed to KWG 4168 EC 500 in a 48-hour oral and contact toxicity study.

LD_{50} calculation was not performed due to the limited number of doses tested but has been considered to be >77 µg product/bee (>39 µg a.s./bee) for the oral test and >200 µg product/bee (>100 µg a.s./bee) for the contact test.

Assessment and conclusion by applicant:

The study was conducted in 1997 and therefore pre-dates the OECD 213 and 214 test guidelines. Validity criteria according to the OECD 213 (1998) and OECD 214 (1998) guidelines have been assessed:

- Average mortality for the total number of controls must not exceed 10% at the end of the test (actual: 0% in the oral test and 7% in the contact test)
- The 24-hr LD_{50} of the toxic standard to be 0.10 to 0.30 µg a.s./bee for the contact test and 0.10 to 0.35 µg a.s./bee for the oral test (actual: 24-hr LD_{50} 0.22 and 0.31 µg Dimethoate EC 400/bee in the contact and oral tests, respectively)

The test methodology and procedures used in this study are consistent with current OECD test guidelines. The reference test results (24-hr LD_{50} 0.22 and 0.31 µg Dimethoate EC 400/bee in the contact and oral tests, respectively) have been expressed in terms of the formulation but are considered to be equivalent to 0.08 and 0.12 µg a.s./bee in the contact and oral tests, respectively. Thus, the oral test result is within range and the contact test result is ever so slightly below the range but is considered to still demonstrate sufficient sensitivity of the test organism.

The study is therefore considered to be acceptable.

The LD_{50} was considered to be >77 µg product/bee (>39 µg a.s./bee) for the oral test and >200 µg product/bee (>100 µg a.s./bee) for the contact test.

Data Point:	KCP 10.3.1.1.1/02
Report Author:	[redacted]
Report Year:	1994
Report Title:	KWG 4168 EC 500 - Acute toxicity to honey bees (Apis mellifera)
Report No:	BAY 170/942957
Document No:	M-008241-01-1
Guideline(s) followed in study:	Pesticides Regulations 1986, working document 7/3; EPA Guideline 1989, Subdivision L, Series 141-1; EPPO Guideline 1992, No. 170
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

Honey bees (*Apis mellifera*) were exposed to KWG 4168 EC 500 in 48-hour acute contact and oral toxicity tests. For the contact and oral toxicity tests KWG 4168 EC 500 was administered either topically to the ventral thorax or as part of a feeding solution at an application rate of 6.25, 12.5, 25, 50 and 100 µg product/bee. The reference item, dimethoate, was administered in doses of 0.025, 0.050, 0.10, 0.20 and 0.40 µg a.s./bee. A solvent control, acetone, was also administered to a group of the test organisms.

The 48-hour LD_{50} values for contact and oral administration were 30 and >12.5 µg product/bee, respectively (equivalent to 15 and >6.1 µg a.s./bee, respectively).

1. Materials and Methods

Materials

Test Material: KWG 4168 EC 500

Lot/Batch #: 089A

Purity: 491.4 g KWG 4168/litre

Description: Transparent yellow liquid

Stability of test compound: Not reported

Reanalysis/Expiry date:	16 December 1994
Density:	Not reported

Treatments

Test rates:	Oral: 6.25, 12.5, 25, 50 and 100 µg product/bee Contact: 6.25, 12.5, 25, 50 and 100 µg product/bee
Solvent/vehicle:	Acetone
Analysis of test concentrations:	No

Test organisms

Species:	Honey bee, *Apis mellifera*
Source:	Mr. R. Baker, 19 Abbotts Crescent, St Ives, Cambridgeshire, UK.
Acclimatisation period:	None. Bees were dosed within 2 hours of removal from hive.
Feeding:	In oral test, after the test solutions had been taken the bees were provided with 20% sucrose solution
Treatment for disease:	None reported

Test design

Test vessel:	Cylindrical wire mesh cages 11.5 cm long x 4.0 cm in diameter
Replication:	5 test concentrations, 1 control, 1 solvent control. All in triplicate. This was the same for the reference item.
No. animals/vessel:	10
Duration of test:	48 hours

Environmental test conditions

Temperature:	25 - 26 °C
Photoperiod:	Darkness except during essential procedures

Study Design

This study was conducted in order to assess the acute toxicity of KWG 4168 EC 500 on honeybees in oral and contact tests over 48 hours.

Female honeybees were collected from the hive and kept in cylindrical wire mesh cages 11.5 cm long x 4.0 cm in diameter. Ten bees per cage were tested.

For the contact test, one cage at a time, the bees were anaesthetised with CO_2 and a 1.0 µL droplet of the appropriate dilution of the test substance was applied to the ventral thorax of each bee using a micrometer syringe.

A 50 µL aliquot of the test solution for the oral test was added to 950 µL of 20% w/v sucrose solution. This solution was then administered as a single 0.2 mL dose per cage (10 bees). The bees were assumed to have received similar doses of 20 µL per bee.

The test vessels were kept at 25 °C with a relative humidity of 53 - 27%.

Five concentrations of the test item (6.25, 12.5, 25, 50 and 100 µg product/bee) were administered to each group of 10 bees in triplicate. The solvent control (acetone) and control groups were also replicated in triplicate. The same study design was kept for the reference item (dimethoate) which was tested at concentrations of 0.025, 0.050, 0.10, 0.20 and 0.40 µg a.s./bee with a solvent control and control, all tested in triplicate.

The bees were observed after 24 and 48 hours for incidence of mortality.

II. Results and Discussion

Validity criteria were not assessed as part of the study report.

For the oral test, concentrations greater than 12.5 µg a.s./bee were not fully consumed as the test organisms found these doses impalatable.

Mortality at the highest dose administered (100 µg a.s./bee) was 17% and 93% after 48 hours for oral and contact administrations, respectively.

Table CP 10.3.1.1.1/02-1 Cumulative mortality for honey bees exposed to KWG 4168 EC 500 for 48 hours

Nominal concentration (µg product/bee)	Cumulative mortality per replicate (%)			
	24 hours		48 hours	
	Oral	Contact	Oral	Contact
Control	0	0	0	0
Solvent control	6.7	3.3	6.7	10
6.25	6.7	13.3	6.7	20
12.5	10	33.3	10	43.3
25	23.3	50	23.3	50
50	16.7	50	16.7	50
100	16.7	90	16.7	93.3

Initial population: 10 bees per replicate

In the reference test mortality at the highest dose administered (0.40 µg a.s./bee) was 93.3% and 100% of bees after 48 hours for oral and contact administration, respectively.

Table CP 10.3.1.1.1/02-2 Cumulative mortality for honey bees exposed to dimethoate for 48 hours

Nominal concentration (µg a.s./L)	Cumulative mortality per replicate (%)			
	24 hours		48 hours	
	Oral	Contact	Oral	Contact
Control	0	0	10	10
Solvent control	0	10	3.3	13.3
0.025	33.3	40	36.6	46.6
0.050	36.6	86.6	43.3	86.6
0.10	66.6	96.6	73.3	100
0.20	93.3	96.6	100	100
0.40	90	100	93.3	100

Nominal concentration (µg a.s./L)	Cumulative mortality per replicate (%)			
	24 hours		48 hours	
	Oral	Contact	Oral	Contact

Initial population: 10 bees per replicate

Table CP 10.3.1.1.1/02-3 48-hour LD_{50} for honeybees

	LD_{50} (µg/bee)	
	Oral	Contact
KWG 4168 EC 500	>12.5 (>6.1 µg a.s./bee)	30 (15 µg a.s./bee)
Dimethoate (reference item)	0.040	0.027

III. Conclusion

In an acute contact and oral toxicity test with honeybees using Spiroxamine EC 500, the 48-hour oral LD_{50} was >12.5 µg product/bee (>6.1 µg a.s./bee) and the 48-hour contact LD_{50} was 30 µg product/bee (15 µg a.s./bee).

Assessment and conclusion by applicant:

The study was conducted in 1994 and therefore pre-dates the OECD 213 and 214 test guidelines. Validity criteria according to the OECD 213 (1998) and OECD 214 (1998) guidelines have been assessed:

- Average mortality for the total number of controls must not exceed 10% at the end of the test (actual: 0% in the control in both contact and oral tests)
- The 24-hr LD_{50} of the toxic standard to be 0.10 to 0.30 µg a.s./bee for the contact test and 0.10 to 0.35 µg a.s./bee for the oral test (actual: 24-hr LD_{50} 0.027 and 0.040 µg a.s./bee in the contact and oral tests, respectively)

The test methodology and procedures used in this study are consistent with current OECD test guidelines. However, it is clear that the criterion for survival has been met but the LD_{50} values achieved in the reference test are almost one order of magnitude lower than the recommended ranges in the current OECD guidelines. The results of the study are therefore considered to be potentially unreliable and as a result the study has been submitted as supporting information only.

The 48-hour oral LD_{50} was >12.5 µg product/bee (>6.1 µg a.s./bee) and the 48-hour contact LD_{50} was 30 µg product/bee (15 µg a.s./bee).

CP 10.3.1.1.2 Acute contact toxicity to bees

Please refer to Section CP 10.3.1.1.1 for summaries of the available acute contact toxicity tests.

CP 10.3.1.2 Chronic toxicity to bees

Data Point:	KCP 10.3.1.2/01
Report Author:	[redacted]; [redacted]
Report Year:	2020
Report Title:	Spiroxamine EC 500: Chronic oral toxicity test on the honey bee (Apis mellifera L.) in the laboratory
Report No:	143091136
Document No:	M-704650-01-1
Guideline(s) followed in study:	Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.SUPP OECD Guideline 245 (2017)
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

Honeybees (*Apis mellifera* L.) were exposed to five concentrations of Spiroxamine EC 500 by continuous and *ad libitum* feeding over a period of 10 days. Nominal test concentrations were 5000, 2000, 800, 320 and 128 mg a.s./kg feeding solution (equivalent to 100, 40.0, 16.0, 6.40, 2.56 µg a.s./bee/day).

The analytical recovery rates of the active substance spiroxamine in the feeding solutions were within a range of 57 % to 120%. The results have been corrected for the analytical recovery rate. When adjusted for analytical recovery the test concentrations were 5005, 1480, 646, 224 and 91 mg a.s./kg feeding solution. In the oral toxicity test actual consumed doses were determined to be 39.1, 34.1, 13.1, 7.70 and 3.98 µg a.s./bee/day. When adjusted for analytical recovery the actual consumed doses were 39.1, 25.2, 10.6, 5.39 and 2.84 µg a.s./bee/day.

Mortality levels of 100% and 26.7% occurred in the concentration groups of 5000 and 2000 mg a.s./kg feeding solution (corresponding to a mean dietary dose of 39.1 and 34.1 µg a.s./bee/day).

The active substance dimethoate was used as a toxic standard. When applied at a concentration rate of 1 mg a.s./kg feeding solution (corresponding to an actual mean dietary dose of 0.016 µg a.s./bee/day), this caused a continuous increase in morality leading to 100% mortality at study day 6.

The NOEC was determined to be 646 mg a.s./kg feeding solution. The NOEDD was determined to be 10.6 µg a.s./bee/day.

The LC_{50} was determined to be 1864 mg a.s./kg feeding solution and the LDD_{50} was determined to be 27.3 µg a.s./bee/day.

I. Materials and Methods

Materials

Test Material	Spiroxamine EC 500
Lot/Batch #:	EM4L018425
Purity:	50.0%
Description:	Yellow liquid

Reanalysis/Expiry date:	09 May 2020
Density:	1.004 g/mL
Treatments	
Test rates:	Nominal: 5000, 2000, 800, 320 and 128 mg a.s./kg feeding solution, corresponding to: 100, 40.0, 16.0, 6.40, 2.56 µg a.s./bee/day
Solvent/vehicle:	50% w/v sucrose solution
Analysis of test concentrations:	Yes. Analysis of feeding solutions on each day of the test
Test organisms	
Species:	*Apis mellifera* L. Hymenoptera, Apidae 2 days old from a queen-right colony
Source:	In-house culture
Acclimatisation period:	At least 1 day
Feeding:	*Ad libitum* 50% (w/v) sucrose solution containing the test item, the reference item or untreated
Treatment for disease:	None
Test design	
Test vessel:	Stainless steel cages (*ca.* 8 x 6 x 4 cm)
Replication:	3 replicates per test item dose level, controls and reference item dose
No. animals/vessel:	10 per test vessel
Duration of test:	10 days
Environmental test conditions	
Temperature:	33°C
Relative humidity:	57 – 69%
Photoperiod:	Darkness (except during observation)

Study Design

Young worker honey bees (2 days old at test initiation) from *Apis mellifera* L. were exposed to a control treatment, one reference item treatment and five concentrations on Spiroxamine EC 500 by continuous and *ad libitum* feeding over a period of 10 days.

Four brood combs with sealed brood from four hives in which bees were visibly starting to emerge were used in the test. These combs contained pollen which was used as a first feeding source for the freshly hatched bees. The combs were taken from the hive and adult bees were removed. The combs were transferred to the laboratory and placed into a hatching box. The box was placed into an incubator for one day to let the bees hatch under test conditions. The next day the hatched bees were collected and randomly assigned into cages (test units) in groups of 10 bees. The following day the test was initiated (Day 0, first dose administration) with 1-2 days old worker honey bees. Moribund bees were rejected and replaced by healthy bees prior to first feeding.

The bees were housed in cages made of stainless steel (*ca*. 8 x 6 x 4 cm) and incubated at 33ºC. Each treatment group consisted of 30 organisms (divided into 3 replicates, containing 10 test organisms each).

The control group were fed with untreated aqueous sucrose solution and the treatment groups were fed with sucrose solution containing the test item. Spiroxamine EC 500 was administered at nominal concentrations of 5000, 2000, 800, 320 and 128 mg a.s./kg feeding solution, equivalent to 100, 40.0, 16.0, 6.40 and 2.56 µg a.s./bee/day, respectively. The reference item group were exposed to 1 mg a.s./kg feeding solution of BAS 152 11 I (dimethoate 400 g/L).

The bees were fed *ad libitum* with a 50 % (w/v) sucrose solution containing the test item (test item group), the reference item (reference item group) or the sugar solution only (control group). The feeding solutions were provided in syringes and daily replaced by freshly prepared solutions.

In order to adjust for possible evaporation of test solutions from the feeders, 3 cages were set up containing pre-weighted syringes filled with sugar solution in absence of bees. The syringes were weighted and replaced daily. The evaporation figure was determined daily by weighting feeders from separate cages without honey bees. The measured difference was subtracted from the measured uptake to adjust the values for the loss by evaporation.

The daily food consumption per bee was calculated by the number of surviving bees per assessment and the amount of food consumed on the following assessment day.

Duplicate samples of the feeding solutions of the test item (5 concentrations) and control were taken for chemical analysis on day 0 – 9.

Mortality and behavioural abnormalities were recorded daily after application (start of feeding) during the 10-day exposure period. The chronic effects of Spiroxamine EC 500 were evaluated by comparing the results of the test item group to those of the treatment groups. The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1 ® ToxRat Solutions GmbH.

Analytical method

Samples of feeding solution were analysed using the validated analytical method M-704650-01-1, report reference M-704650-01-1 (**see Doc MCP Section 5**).

II. Results and Discussion

Validity criteria according to the OECD 245 guideline (2017) were met.

- The average mortality across replicates for the untreated control should be ≤15% (actual: 6.7% on Day 10)
- The average mortality across replicates for the reference substance should be ≥50% (actual: 100% by Day 6)

The analytical recovery rates of the active substance, spiroxamine in the feeding solutions were within a range of 57 % to 120%. The results have been corrected for the analytical recovery rate.

When adjusted for analytical recovery the test concentrations were 5005, 1480, 646, 224 and 91 mg a.s./kg feeding solution.

In the oral toxicity test actual consumed doses were determined to be 39.1, 34.1, 13.1, 7.70 and 3.98 µg a.s./bee/day. When adjusted for analytical recovery the actual consumed doses were 39.1, 25.2, 10.6, 5.39 and 2.84 µg a.s./bee/day.

Mortality levels of 100% and 26.7% occurred at the nominal concentration groups of 5000 and 2000 mg a.s./kg feeding solution (corresponding to a mean dietary dose of 39.1 and 34.1 µg a.s./bee/day), respectively, at test termination. This effect was statistically significant compared to the control (Step-down Cochran-Armitage Test Procedure, α = 0.05).

No mortality occurred in the 800, 320 and 128 mg a.s./kg feeding solution (corresponding to a mean dietary dose of 13.1, 7.7 and 3.98 µg a.s./bee/day, respectively). There was 6.7% mortality in the untreated control group (50% w/v sucrose solution) at the end of the 10 day testing period.

The reference item (dimethoate) at a concentration of 1 mg a.s./kg feeding solution (corresponding to an actual mean dietary dose of 0.016 µg a.s./bee/day) caused a continuously increasing mortality leading to 100% mortality at day 6.

Table CP 10.3.1.2/01-1 Summary of mortality data following exposure to Spiroxamine EC 500

Treatment group (mg a.s./kg)	Mean mortality (%)									
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
5000	0	56.7	80	96.7	100	100	100	100	100	100
2000	0	0	0	3.3	6.7	10	10	16.7	20	26.7
800	0	0	0	0	0	0	0	0	0	0
320	0	0	0	0	0	0	0	0	0	0
128	0	0	0	0	0	0	0	0	0	0
Reference item: 1.0	0	0	20	73.3	96.7	100	100	100	100	100
Control	0	0	0	3.3	3.3	3.3	3.3	3.3	3.3	6.7

On day 2, behavioural abnormalities (e.g. moribund and affected bees) were observed in the highest dose level group of 5000 mg a.s./kg feeding solution. On day 10, a single bee in the 2000 mg a.s./kg feeding solution treatment group was affected. No behavioural abnormalities occurred in the treatment groups of 800, 320 and 128 mg a.s./kg feeding solutions. On day 9, one bee in the control group was affected.

Table CP 10.3.1.2/01-2 Summary of behavioural abnormalities following exposure to Spiroxamine EC 500

Treatment group (mg a.s./kg)	Behavioural abnormalities (%)									
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
5000	0	16.7	0	0	0	0	0	0	0	0
2000	0	0	0	0	0	0	0	0	0	3.3
800	0	0	0	0	0	0	0	0	0	0
320	0	0	0	0	0	0	0	0	0	0
128	0	0	0	0	0	0	0	0	0	0
Reference item: 1.0	0	0	3.3	3.3	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	3.3	0

Table CP 10.3.1.2/01-3 Summary of mortality and endpoints following exposure to Spiroxamine EC 500

Test Object	*Apis mellifera carnica*

Treatment Group	Concentration [mg a.s./kg feeding solution]	Dietary Dose [1] [µg a.s./bee/day]	Mortality at day 10 [2], [% Mean]	Corrected Mortality at day 10 [3], [% Mean]
Spiroxamine EC 500	5000 (5005)[4]	39.1 (39.1)[5]	100.0 (*)	100.0
Spiroxamine EC 500	2000 (1480)[4]	34.1 (25.2)[5]	26.7 (*)	21.4
Spiroxamine EC 500	800 (646)[4]	13.1 (10.6)[5]	0.0 (n.s.)	- 7.2
Spiroxamine EC 500	320 (224)[4]	7.70 (5.39)[5]	0.0 (n.s.)	- 7.2
Spiroxamine EC 500	128 (91)[4]	3.98 (2.84)[5]	0.0 (n.s.)	- 7.2
Water control	-	-	6.7	-
Reference Item	1.0	0.016	100.0	100.0

Endpoint at test termination (day 10)			
LC_{50}	LDD_{50}	LC_{20}	LDD_{20}
1864 mg a.s./kg feeding solution	27.3 µg a.s./bee/day	1377 mg a.s./kg feeding solution	22.2 µg a.s./bee/day
LC_{10}	LDD_{10}	NOEC	NOEDD
1127 mg a.s./kg feeding solution	19.9 µg a.s./bee/day	646 mg a.s./kg feeding solution	10.6 µg a.s./bee/day

[1]mean dose per bee per day; dose measured based on consumed feeding solution
[2]Mortality at study termination 10 days after start of first feeding
[3]Corrected mortality was calculated by control mortality using Abbott's formula (1925)
[4]Values in parentheses were corrected dietary concentrations based on the mean values of the dose verification
[5]Values in parentheses were corrected dietary doses based on the mean values of the dose verification
Statistics:
$LC_{50/20/10}$: using the Weibull analysis. The LD_{50} calculation was carried out taking into account the mortality data corrected by control mortality using Abbott's formula (1925).
$LDD_{50/20/10}$: using the Probit Analysis. The LD_{50} calculation was carried out taking into account the mortality data corrected by control mortality using Abbott's formula (1925).
NOEC/NOEDD: Step-down Cochran-Armitage Test Procedure, one-sided greater, $\alpha = 0.05$
n.s. = no statistical significant difference compared to the control, * = statistically significant different compared to the control ($\alpha = 0.05$),

III. Conclusion

Adult honeybees (*Apis mellifera* L.) were exposed to Spiroxamine EC 500 in a 10-day chronic feeding test.

After 10 days exposure, the NOEC and NOEDD were determined to be 646 mg a.s./kg feeding solution and 10.6 µg a.s./bee/day, respectively.

The LC_{50} was determined to be 1864 mg a.s./kg feeding solution and the LDD_{50} was determined to be 27.3 µg a.s./bee/day.

Assessment and conclusion by applicant:

Validity criteria according to the OECD 245 guideline (2017), to which the study was conducted, were met.

- The average mortality across replicates for the untreated control should be ≤15% (actual: 6.7% on Day 10)

- The average mortality across replicates for the reference substance should be ≥50% (actual: 100% by Day 6)

The study is therefore considered to be acceptable.

The LDD_{50} was determined to be 27.3 µg a.s./bee/day. The NOEDD was determined to be 10.6 µg a.s./bee/day.

Data Point:	KCP 10.3.1.2/02
Report Author:	[redacted]
Report Year:	2015
Report Title:	Spiroxamine EC 500E G - Assessment of effects on the adult honey bee, Apis mellifera L. in a 10 days chronic feeding test under laboratory conditions
Report No:	S14-00173
Document No:	M-538628-01-1
Guideline(s) followed in study:	GLP compliant study based on OECD 213 (1998) and CEB No. 230 with modifications and current recommendations of the ring test group (2014)
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 objective of this study was to determine the effects of the test item Spiroxamine EC 500E G on the honey bee, *Apis mellifera* L., in a 10-day chronic feeding test under laboratory conditions. The No Observed Effect Concentration (NOEC) and the No Observed Effect Dietary Dose (NOEDD) were determined at the end of the test period. Furthermore it was demonstrated that the median Lethal Concentration (LC_{50}) and the median lethal dietary dose (LDD_{50}) was greater than the tested dose.

Honey bees were exposed to a 50 % aqueous sucrose solution containing one concentration of Spiroxamine EC 500E G by continuous and *ad libitum* feeding over a period of 10 days. The control group was fed with untreated sucrose solution. Mortality and sub-lethal effects were assessed during the 10-day exposure period. The chronic effects of Spiroxamine EC 500E G were evaluated by comparing the results of the test item group to those of the control group.

The LC_{50} after 10 days of continuous exposure was determined to be higher than 100 mg a.s. spiroxamine/kg feeding solution. The corresponding LDD_{50} (Lethal Dietary Dose), based on the actual consumption of the respective feeding solutions, was determined to be >4.86 µg a.s./bee/day. The NOEDD was determined to be 4.86 µg a.s./bee/day.

I. Materials and Methods

Materials

Test Material Spiroxamine EC 500E G

Lot/Batch #: EDFL021571

Purity analysed: Content of active substance (analysed) = 501.1 g/L

Description: Liquid / yellow to brown

Stability of test compound:	Sufficient for the test purposes
Reanalysis/Expiry date:	04 Feb 2015
Density:	1.00 g/cm³
Treatments	
Test rates:	100 mg a.s. spiroxamine/kg feeding solution
Solvent/vehicle:	50% (w/v) aqueous sucrose solution
Analysis of test concentrations:	Yes. Mean measured concentration of spiroxamine in the larval diet was 74%
Test organisms	
Species:	*Apis mellifera*
Source:	Colonies located at testing facility – originally obtained from Klaus Hampel, Mühlhausenerstr. 1 I, 75233 Tiefenbronn, Germany
Feeding:	*Ad libitum* - The definitive test item feeding solution was prepared every day by diluting the stock solution with 50 % (w/v) aqueous sucrose solution
Test design	
Test vessel:	Cages made of stainless steel (base: 8 cm x 4 cm; height: 6 cm)
Test medium:	Bees exposed *via* the diet
Replicates	4
Number of organisms per vessel:	10
Duration of test:	10 days
Environmental test conditions	
Temperature:	32.0 to 33.8°C
Relative humidity:	53.5 to 67.1%
Photoperiod:	Constant darkness except during the assessments

Study Design

The objective of this study was to determine the effects of the test item Spiroxamine EC 500E G on the honey bee, *Apis mellifera* L, in a 10-day chronic feeding test under laboratory conditions. The No Observed Effect Concentration (NOEC) and the No Observed Effect Dietary Dose (NOEDD) were determined at the end of the test period.

Honey bees were exposed to a 50 % aqueous sucrose solution containing one concentration of Spiroxamine EC 500E G by continuous and *ad libitum* feeding over a period of 10 days. The control group was fed with untreated sucrose solution. Mortality and sub-lethal effects were assessed during the 10-day exposure period. The chronic effects of Spiroxamine EC 500E G were evaluated by comparing the results of the test item group to those of the solvent control group.

In the control group the honey bees received an untreated 50 % (w/v) aqueous sucrose solution, *ad libitum*. Over a test period of 10 days, honey bees were fed continuously and *ad libitum* with a 50 % (w/v) aqueous sucrose solution, containing the test item Spiroxamine EC 500E G at the concentration level of 100 mg a.s. spiroxamine /kg feeding solution. The abbreviation a.s. refers to the analysed content of the active substance spiroxamine (501.1 g/L). A reference rate (dimethoate; Tradename Perfekthion) at 0.9 mg a.s./kg feed was also tested.

The test organism was the honey bee, *Apis mellifera* (Hymenoptera, Apoidea). The test was carried out with young adult worker bees (newly hatched; 1 to 4 days old) from a healthy colony descended from a breeding line of a beekeeper in Tiefenbronn, Germany (Klaus Hampel, Mühlhausenerstr. 1/1, 75233 Tiefenbronn, Germany). The colonies were examined for a reportable bee epidemic by an authorised bee specialist and were inspected periodically according to the standard bee-keeping practices by an experienced apiarist. The hives used for honey bee collection for this test was adequately fed, healthy, as far as possible disease-free and queen-right.

For the preparation of the test item feeding solutions, the test item Spiroxamine EC 500E G was first dissolved in tap water in order to obtain a stock solution. The amount of test item needed for the daily preparation of the stock solution was weighed in advance and then stored tightly closed under cool and dark conditions in the refrigerator (6 ± 2 °C) until use. The definitive test item feeding solution was prepared every day by diluting the stock solution with 50 % (w/v) aqueous sucrose solution.

The feeding solutions were offered *ad libitum* to each cage of 10 bees in plastic syringes (Omnifix®, 5 mL, B. Braun, Melsungen, Germany). The tip of each syringe was removed so that the bees had access to the feeding solution.

The number of dead bees in the individual test units was recorded every 24 h (± 1h) during the 10 days test period. Sub-lethal effects as symptoms of poisoning or any abnormal behaviour in comparison to the control were recorded according to the following categories; moribund, affected, cramps, apathy and vomiting.

The daily consumption of feeding solution per bee was calculated by dividing the total daily consumption per replicate by the number of living bees at the beginning of the respective feeding interval.

For the statistical comparison of the food consumption, non-rounded mean values per replicate over the entire test period were taken. Data of food consumption were statistically analysed by using the Student-t-test (left-sided, $\alpha = 0.05$), depending on the results of the pre-test of Shapiro Wilks and F-Test ($\alpha = 0.05$). Statistical calculations were made by using the statistical program TOXRAT Professional 2.10.

Samples of the feeding solutions prepared freshly every day throughout the 10 days continuous feeding period were taken daily for subsequent chemical analysis in order to reveal the actual concentration of the test item.

Analytical method

Samples of feeding solution were analysed using the validated analytical method MR-15/063, report reference M-527552-01-2 (**see Doc MCP Section 5**).

II. Results and Discussion

The study was conducted to the OECD Guideline proposal: Honey bee (*Apis mellifera* L.), chronic toxicity test (10-day feeding test in the laboratory). Submitted to OECD for Evaluation, 20 November 2013.

The study was considered valid because:

- The mean mortality in the control was ≤ 15 % at the end of the test (actual: 2.5%).
- The mean mortality in the reference item group was ≥ 50 % at the end of the test (actual: 100%).

The average actual concentration of Spiroxamine EC 500E G over a period of 10 consecutive days per individual test item treatment level was 74 % of the nominal concentration. No residues of Spiroxamine EC 500E G above the LOQ (10 µg/kg) were found in any of the control samples.

The mortality in the control group was 2.5 % at the final assessment after 10 days and thus remained within bounds of the validity criterion of 15 % mortality.

Mortality in the reference item treatment group increased during the test period and reached 100 % (corrected 100 %) after 10 days. Exceeding the 50 % mortality threshold set as validity criterion the reference item treatment group showed that the test design is suitable to determine toxic effects in a chronic exposure scenario.

After 10 days of continuous exposure to the concentration level of 100 mg a.s./kg feeding solution, no mortality could be observed (corrected -2.6 %). A LDD_{50} value of >4.86 µg a.s./bee/day and a NOEDD at 4.86 µg a.s./bee/day could be determined.

In the control group and at the concentration level of 100 mg a.s./kg feeding solution no remarkable sub-lethal effects could be observed.

Table CP 10.3.1.2/02-1 Cumulative mortality and sub-lethal effects

Treatment (mg a.s./kg)	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10
Cumulative mortality (%)										
Control	0.0	0.0	0.0	0.0	0.0	2.5	2.5	2.5	2.5	2.5
Spiroxamine EC 500E G[1]										
100[2]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Perfekthion										
0.9	0.0	0.0	0.0	22.5	57.5	67.5	82.5	97.5	97.5	100.0
Corrected cumulative mortality (%)										
Spiroxamine EC 500E G[1]										
100	0.0	0.0	0.0	0.0	0.0	-2.6	-2.6	-2.6	-2.6	-2.6
Perfekthion										
0.9	0.0	0.0	0.0	22.5	57.5	66.7	82.1	97.4	97.4	100.0

E Assessment

C Control; 50 % (w/v) aqueous sucrose solution containing Spiroxamine EC 500E G

[1] Feeding solution: 50 % (w/v) aqueous sucrose solution containing Spiroxamine EC 500E G

[2] Determined to be the NOEC based on mortality (a statistical evaluation according to the Fisher's exact test was not conducted, as no increased mortality in the test item treatment group compared to the control group was observed).

After 10 days of continuous exposure, by considering the actual food consumption of the honeybees, the accumulated nominal uptake of Spiroxamine EC 500E G at the treatment level of 100 mg a.s./kg feeding solution was 48.6 µg a.s./bee.

The corresponding average daily dose was therefore 4.86 µg a.s./bee. The overall mean daily consumption of feeding solution (i.e. the average consumption/bee over 10 days) was not statistically significantly different (lower) when compared to the untreated control group (48.6 mg/bee/day at 100 mg a.s. spiroxamine/kg feeding solution, compared to 41.2 mg/bee/day in the control group).

Table CP 10.3.1.2/02-2 Mean consumption of feeding solution per treatment group per day during the test period

Treatment (mg a.s./kg)	Mean consumption of feeding solution (mg/bee/day)										
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	x̄
C	38.7	34.5	39.2	43.1	40.3	40.1	42.8	41.7	49.0	43.1	41.2
Spiroxamine EC 500E G[1]											
100	63.6	39.2	42.2	43.0	50.6	40.8	64.9	47.7	46.0	47.6	48.6
Perfekthion											
0.9	33.0	38.5	25.2	22.7	30.0	27.7	40.3	39.6	103	75.4	35.2

A Application

C Control; 50 % (w/v) aqueous sucrose solution

[1] Feeding solution: 50 % (w/v) aqueous sucrose solution containing Spiroxamine EC 500E G

x̄ Overall mean consumption of feeding solution (calculation based on replicate values)

Table CP 10.3.1.2/02-3 Mean uptake of test item during the test period

Treatment (mg a.s./kg)	Mean uptake of test item (µg a.s./bee/day)										
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	DD
Spiroxamine EC 500E G[1]											
100	6.36	3.92	4.22	4.30	5.06	4.08	6.49	4.77	4.60	4.76	4.86
Perfekthion											
0.9	0.03	0.04	0.02	0.02	0.03	0.03	0.04	0.04	0.09	0.07	0.03

A Application

[1] Feeding solution: 50 % (w/v) aqueous sucrose solution containing Spiroxamine EC 500E G

|DD Dietary dose in µg a.s./bee/day

Table CP 10.3.1.2/02-4 Accumulated mean uptake of test item (µg a.s./bee) over test days

Treatment (mg a.s./kg)	Accumulated mean uptake of test item (µg a.s./bee/day)									
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
Spiroxamine EC 500E G[1]										
100	6.36	10.3	14.5	18.8	23.9	27.9	34.4	39.2	43.8	48.6
Perfekthion										
0.9	0.03	0.07	0.09	0.11	0.14	0.17	0.21	0.25	0.34	0.41

A Application

[1] Feeding solution: 50 % (w/v) aqueous sucrose solution containing Spiroxamine EC 500E G

Conclusion

The continuous *ad libitum* feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item Spiroxamine EC 500E G at the concentration level of 100 mg a.s. spiroxamine/kg feeding solution resulted with no adverse effects regarding mortality and behaviour.

The cumulative control mortality was 2.5 %, as determined at the final assessment after 10 days. The cumulative mortality at the treatment level of 100 mg a.s./kg feeding solution was 0.0 % (corrected -2.6 %) at the final assessment.

The overall mean daily consumption of feeding solution (*i.e.* the average consumption/bee over 10 days) was not statistically significantly different (lower) when compared to the untreated control group (48.6 mg/bee at 100 mg a.s./kg feeding solution compared to 41.2 mg/bee in the control group).

The NOEC for mortality after 10 days of continuous exposure was determined to be 100 mg a.s. spiroxamine/kg feeding solution. The corresponding NOEDD, based on the actual consumption of the respective feeding solutions, was determined to be 4.86 µg a.s./bee/day.

The LC_{50} after 10 days of continuous exposure was determined to be >100 mg a.s./kg feeding solution. The corresponding LDD_{50} (Lethal Dietary Dose), based on the actual consumption of the respective feeding solutions, was determined to be >4.86 µg a.s./bee/day.

Assessment and conclusion by applicant:

The study was conducted to the OECD Guideline proposal: Honey bee (*Apis mellifera* L.), chronic toxicity test (10 day feeding test in the laboratory). Submitted to OECD for Evaluation, 20 November 2013. The validity criteria of the current OECD 245 (2017) guideline are the same as those used in this study and have been met:

- The average mortality across replicates for the untreated control should be ≤15% (actual: 2.5% on Day 10)
- The average mortality across replicates for the reference substance should be ≥50% (actual: 100% by Day 10)

The study is therefore considered to be acceptable.

The LDD_{50} was determined to be >4.86 µg a.s./bee/day. The NOEDD was determined to be 4.86 µg a.s./bee/day.

Data Point:	KCP 10.3.1.2/03
Report Author:	[redacted]; [redacted]
Report Year:	2015
Report Title:	Determination of spiroxamine in feeding solutions from a chronic feeding test of spiroxamine EC 500E G on the honey bee (Apis mellifera L.) in the laboratory (Study number: S14-00173; Eurofins)
Report No:	MR-15/063
Document No:	M-527552-01-1
Guideline(s) followed in study:	not specified
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 purpose of the study was to determine the effects of the test item Spiroxamine EC 500E G on the honey bee (*Apis mellifera* L.) in an oral feeding test in the laboratory. Bees were exposed to 50% aqueous sugar solution containing the test item Spiroxamine EC 500E G or to an untreated sugar solution by continuous and *ad libitum* feeding over a period of 10 days. The objective of this study was to determine the residue levels of spiroxamine in the feeding solution.

For the purpose of this study, it was only necessary to take an aliquot (1 g) of the feeding solutions and to dilute the samples with acetonitrile/water (4/1) including 1.6 mL of a 250 g/L cysteine hydrochloride solution. Thereafter, aliquots of the diluted samples enriched with internal standard solution were subjected to reversed phase - High Performance Liquid Chromatography (HPLC), coupled with electrospray and mass spectrometry (MS/MS) detection without a further clean-up step. Method validation was done with a full set of recoveries at the LOQ and 10 x LOQ level at 150 mg/kg.

The mean actual concentration of spiroxamine in the feeding solution was 74% of the nominal. No residues of spiroxamine above the LOD were found in any of the control samples.

I. Study Design

The analytical method 01013/M001 was developed for the determination of residues of BYF00587, Prothioconazole, Tebuconazole, Trifloxystrobin, Spiroxamine (KWG4168) and the metabolites BYF00587-desmethyl, JAU 6476-desthio (SXX0665) and CGA321113 in/on plant materials.

Due to the fact that the concentration level of spiroxamine is very high it was only necessary to dilute the sugar solutions before the measurement.

For recovery, control and treated samples, 1 g of the corresponding feeding solution was filled up to 40 mL with acetonitrile/water (4/1) including 1.6 mL of a 250 g/L cysteine hydrochloride solution. An aliquot of 200 µL was filled up to 1 mL final volume with 100 µL ISTD solution (10 µg/L) and 700 µL methanol/water (4/6). If necessary dilute the samples again and fill up with ISTD solution (1 µg/L) to final volume.

An aliquot of the solutions was subjected to HPLC-MS/MS without a further clean-up step. Spiroxamine was detected using electrospray ionization in the positive ion mode (ESI+). Residues were quantified using internal stable labelled standards.

The limit of quantitation (LOQ) for spiroxamine is 0.01 mg/kg (= 10 µg/kg) for the sample material aqueous sugar solution, corresponding to the lowest fortification level of successfully conducted recovery experiments. The limit of detection (LOD) was estimated to be at least 30% of the LOQ.

For spiroxamine recoveries were performed in feeding solution at the LOQ (0.01 mg/kg), at 10-fold LOQ level (0.1 mg/kg) and at 15000-fold LOQ level (150 mg/kg). Recovery experiments were performed by spiking control samples with defined amounts of spiroxamine. Fortification levels and recovery data are given in the following tables. As a control material for feeding solution Milli-Q-Water/Api Invert Solution (1/1; 2015-03-30) was used for validation.

II. Results and Discussion

All residues in control samples used for recovery determination were below LOD. Residues in the control and treated samples of feeding solution are shown in the table below.

Table CP 10.3.1.2/03-1 Actual concentrations of spiroxamine in the feeding solutions of the control group

Sample ID	Spiroxamine Nominal (mg/kg)	Spiroxamine Actual (mg/kg)	Spiroxamine Actual (% of nominal)
C-0DBA1-A1	-	<LOD	-
C-0DBA2-A1	-	<LOD	-
C-0DBA3-A1	-	<LOD	-
C-0DBA4-A1	-	<LOD	-
C-0DBA5-A1	-	<LOD	-
C-0DBA6-A1	-	<LOD	-
C-0DBA7-A1	-	<LOD	-
C-0DBA8-A1	-	<LOD	-
C-0DBA9-A1	-	<LOD	-
C-0DBA10-A1	-	<LOD	-

LOQ = Limit of Quantification = 0.01 mg/kg for spiroxamine

LOD = Limit of Detection = 0.003 mg/kg for spiroxamine

DBA = days before application

Table CP 10.3.1.2/03-2 Actual concentrations of spiroxamine in the feeding solutions of the test item treatment group

Sample ID	Spiroxamine Nominal (mg/kg)	Spiroxamine Actual (mg/kg)	Spiroxamine Actual (% of nominal)
T1-0DBA1-A1	100	75.3	75
T1-0DBA2-A1	100	71.6	72
T1-0DBA3-A1	100	70.4	70
T1-0DBA4-A1	100	67.0	67
T1-0DBA5-A1	100	79.9	80
T1-0DBA6-A1	100	77.0	77
T1-0DBA7-A1	100	73.8	74

Sample ID	Spiroxamine Nominal (mg/kg)	Spiroxamine Actual (mg/kg)	Spiroxamine Actual (% of nominal)
T1-0DBA8-A1	100	75.9	76
T1-0DBA9-A1	100	76.7	77
T1-0DBA10-A1	100	70.2	70

LOQ = Limit of Quantification = 0.01 mg/kg for spiroxamine

LOD = Limit of Detection = 0.003 mg/kg for spiroxamine

DBA = days before application

*For the calculation of "% of nominal concentration" as it appears in the result table above, unrounded values were used. Therefore, minor deviations may occur between the values shown above and when the values given in the residue results column are used for calculation.

III. Conclusion

The purpose of the study was to determine the effects of the test item Spiroxamine EC 500E G on the honey bee (*Apis mellifera* L.) in an oral feeding test in the laboratory. Bees were exposed to 50% sugar solution containing the test item Spiroxamine EC 500E G or to an untreated sugar solution by continuous and *ad libitum* feeding over a period of 10 days. The objective of this study was to determine the residue levels of spiroxamine in the feeding solution.

Regarding spiroxamine, analysis of the feeding solution followed the provisions of the Bayer CropScience method 01013/M001.

For the purpose of this study, it was only necessary to take an aliquot (1 g) of the feeding solutions and to dilute the samples with acetonitrile/water (4/1) including 1.6 mL of a 250 g/L cysteine hydrochloride solution. Thereafter, aliquots of the diluted samples enriched with internal standard solution were subjected to reversed phase High Performance Liquid Chromatography (HPLC), coupled with electrospray and mass spectrometry (MS/MS) detection without a further clean-up step. Method validation was done with a full set of recoveries at the LOQ and 10 x LOQ level at 150 mg/kg.

The Limit of Quantification (LOQ), defined as the lowest validated fortification level, of spiroxamine was 0.01 mg/kg in the feeding solution, respectively; the corresponding Limit of Detection (LOD) was always 0.003 mg/kg.

All results of the method validations were in accordance with the general requirements for residue analytical methods; therefore, the employed method was validated successfully.

The mean actual concentration of spiroxamine in the feeding solution was 74% of the nominal.

No residues of spiroxamine above the LOD were found in any of the control samples.

Assessment and conclusion by applicant:

The study report presents the analytical method and the results of the analysis for the 10-day oral toxicity study with honeybees (M-538628-01-1). As such the study is considered to be acceptable.

Please refer to the Analytical Methods section of the dossier for a full assessment of the analytical method used.

CP 10.3.1.3 Effects on honey bee development and other honey bee life stages

No larval toxicity data are available with Spiroxamine EC 500. However, a study is available using spiroxamine technical. Please refer to Document M-CA Section 8 for full details.

CP 10.3.1.4 Sub-lethal effects

Additional studies on sub-lethal effects have not been conducted and are not considered to be necessary.

CP 10.3.1.5 Cage and tunnel tests

Residues decline trials in nectar and pollen have been conducted under semi-field tunnel test conditions. A summary of the study has been provided below.

Data Point:	KCP 10.3.1.5/03
Report Author:	[redacted]
Report Year:	2021
Report Title:	Determination of residues of spiroxamine in nectar and pollen of Phacelia tanacetifolia after two applications of spiroxamine EC 500 in a semi-field tunnel residue study in Central and Southern Europe in 2020
Report No:	S20-02289
Document No:	M-763122-01-1
Guideline(s) followed in study:	Commission Regulation (EU) No 283/2013 and 284/2013 (Mar. 2013) in accordance with Regulation (EC) No 1107/2009 (Oct. 2009), SANCO/825/00 (2010), SANCO/3029/99 rev. 4 (2000), EC (2018) Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey (SANTE/11956/2016 rev. 9)
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

Residues of spiroxamine were determined in nectar and pollen from *Phacelia tanacetifolia* plants under semi-field conditions. The study comprised five separate semi-field residue trials conducted in Germany (2 sites) and Spain (3 sites) in 2020. Each site comprised two plots (one untreated and one treated with Spiroxamine EC 500). The tunnels used per plot had an area of 200 m^2 each. Two bee hives were placed at the end of each tunnel. Spiroxamine EC 500 was applied at the nominal application rate of 0.6 L product/ha (corresponding to 300 g a.s./ha) in 200 L water/ha for both applications and all trials. On each sampling day forager bees were collected for the preparation of nectar from their honey stomachs for residue analysis. On each sampling day pollen from *Phacelia tanacetifolia* retrieved by the bees was collected using pollen traps. Sampling occurred shortly after application, 8 hours after application, 1, 2, 3, 5 and 7 days after application. Residues of spiroxamine enantiomers A1, A2, B1 and B2 were determined by HPLC-MS/MS detection. The Limit of Quantitation (LOQ), defined as the lowest validated fortification level, was 0.01 mg/kg (10 µg/kg, sum of four enantiomers) for all analysed samples. The corresponding respective Limit of Detection (LOD) was determined to be 0.003 mg/kg (3 µg/kg, sum of four enantiomers). Residues in control samples of pollen ranged between <0.01 mg/kg and 0.0110 mg/kg (sum of four enantiomers). No residues of the analytes above the LOQ were found in any of the control samples of nectar. The residues found in pollen and nectar on the 5 tested sites are presented in the following tables.

Table CP 10.3.1.5/03-1 Residues of spiroxamine in pollen (sum of four enantiomers) found on each trial site [mg /kg]

Sample ID L20-02289	Sample type	Sample weight [g]	Residues of spiroxamine (sum of four enantiomers) [mg/kg]
Trial S20-02289-01 (Germany)			
01-C-S1-P-A	C	0.220	<0.01
01-C-S1-P-R	C	0.198	<0.01
01-T-S1-P-A	T	0.200	87.0
01-T-S2-P-A	T	0.202	5.22
01-T-S3-P-A	T	0.201	3.40
01-T-S5-P-A	T	0.200	0.697
01-T-S6-P-A	T	0.250	2.10
01-T-S7-P-A	T	0.202	0.797
Trial S20-02289-02 (Germany)			
02-C-S1-P-A	C	0.202	<0.01
02-C-S1-R-A	C	0.217	<0.01
02-T-S1-P-A	T	0.201	71.9
02-T-S2-P-A	T	0.238	20.6
02-T-S3-P-A	T	0.202	3.63
02-T-S5-P-A	T	0.245	8.95
02-T-S6-P-A	T	0.201	0.193
02-T-S7-P-A	T	0.200	0.172
Trial S20-02289-03 (Spain)			
03-C-S1-P-A	C	0.211	0.0110
03-T-S1-P-A	T	0.201	37.9
03-T-S2-P-A	T	0.229	22.1
03-T-S3-P-A	T	0.201	4.59
03-T-S5-P-A	T	0.237	2.25
03-T-S6-P-A	T	0.201	1.13

03-T-S7-P-A	T	0.217	1.10
Trial S20-02289-04 (Spain)			
04-C-S1-P-A	C	0.201	<0.01
04-T-S3-P-A	T	0.232	21.4
04-T-S5-P-A	T	0.200	1.35
04-T-S6-P-A	T	0.201	1.13
04-T-S7-P-A	T	0.200	0.280
Trial S20-02289-05 (Spain)			
05-C-S1-P-A	C	0.197	<0.01
05-T-S1-P-A	T	0.192	54.7
05-T-S2-P-A	T	0.130	14.1
05-T-S3-P-A	T	0.251	9.32
05-T-S5-P-A	T	0.200	1.23
05-T-S6-P-A	T	0.194	1.48
05-T-S7-P-A	T	0.191	0.569

C = Control, T = Treatment, LOQ = Limit of Quantification = 0.01 mg/kg (= 10 µg/kg = 10 ppb, sum of four enantiomers) for spiroxamine, LOD = Limit of Detection = 0.003 mg/kg (= 3 µg/kg = 3 ppb, sum of four enantiomers) for spiroxamine

Table CP 10.3.1.5/03-2 Residues of spiroxamine in nectar (sum of four enantiomers) found on each trial site [mg/kg]

Sample ID **L20-02289**	**Sample type**	**Sample weight [g]**	**Residues of Spiroxamine (sum of four enantiomers) [mg/kg]**
Trial S20-02289-01 (Germany)			
01-C-S1-NFB-A	C	0.200	<0.01
01-T-S1-NFB-A	T	0.200	0.700
01-T-S2-NFB-A	T	0.200	0.252
01-T-S3-NFB-A	T	0.200	0.116
01-T-S4-NFB-A	T	0.200	0.0268
01-T-S5-NFB-A	T	0.200	0.00943
01-T-S6-NFB-A	T	0.186	<0.01

01-T-S7-NFB-A	T	0.200	<0.01
Trial S20-02289-02 (Germany)			
02-C-S1-NFB-A	C	0.200	<0.01
02-T-S1-NFB-A	T	0.200	0.163
02-T-S2-NFB-A	T	0.201	0.195
02-T-S3-NFB-A	T	0.200	0.0313
02-T-S4-NFB-A	T	0.200	<0.01
02-T-S5-NFB-A	T	0.200	<0.01
02-T-S6-NFB-A	T	0.200	<0.01
02-T-S7-NFB-A	T	0.200	<0.01
Trial S20-02289-03 (Spain)			
03-C-S1-NFB-A	C	0.200	<0.01
03-T-S1-NFB-A	T	0.200	0.117
03-T-S2-NFB-A	T	0.200	0.0757
03-T-S3-NFB-A	T	0.200	0.0562
03-T-S4-NFB-A	T	0.199	0.0247
03-T-S5-NFB-A	T	0.200	<0.01
03-T-S6-NFB-A	T	0.200	<0.01
03-T-S7-NFB-A	T	0.200	<0.01
Trial S20-02289-04 (Spain)			
04-C-S1-NFB-A	C	0.183	<0.01
04-T-S1-NFB-A	T	0.200	0.221
04-T-S2-NFB-A	T	0.200	0.445
04-T-S3-NFB-A	T	0.200	0.104
04-T-S4-NFB-A	T	0.200	0.0193
04-T-S5-NFB-A	T	0.200	<0.01
04-T-S6-NFB-A	T	0.200	<0.01
04-T-S7-NFB-A	T	0.200	<0.01

Trial S20-02289-05 (Spain)			
05-C-S1-NFB-A	C	0.200	<0.01
05-T-S1-NFB-A	T	0.200	0.0471
05-T-S2-NFB-A	T	0.133	0.0777
05-T-S3-NFB-A	T	0.167	0.0704
05-T-S4-NFB-A	T	0.200	0.0128
05-T-S5-NFB-A	T	0.200	<0.01
05-T-S6-NFB-A	T	0.200	<0.01
05-T-S7-NFB-A	T	0.168	<0.01

C = Control, T = Treatment, LOQ = Limit of Quantification = 0.01 mg/kg (= 10 µg/kg = 10 ppb, sum of four enantiomers) for spiroxamine, LOD = Limit of Detection = 0.003 mg/kg (= 3 µg/kg = 3 ppb, sum of four enantiomers) for spiroxamine

I. Materials and Methods

Materials

Study code:	S20-02289-01	S20-02289-02	S20-02289-03	S20-02289-04	S20-02289-05
Test Material	Spiroxamine EC 500				
Lot/Batch #:	EM4L027093				
Actual content of active ingredients:	49.8 % w/w, 500 g/L (nominal) 49.2 % w/w, 494.1 g/L (analysed)				
Description:	Dark yellow, clear liquid				
Stability of test compound:	Sufficient for the test purpose				
Reanalysis/Expiry date:	January 22, 2024				
Density:	1.004 g/cm³ (analysed)				

Treatments

Test rates:	Nominal: 0.6 L product/ha (corresponding to 300 g a.s./ha) in 200 L water/ha	
Vehicle:	Tap water	
Application:	Calibrated boom sprayer (2.5 m) - 5 / flat fan, 50 cm spacing (XR 110 01 VS)	Calibrated bar sprayer sprayer - 5 / HYPRO green (F110-015)

Test design

Test system:	*Phacelia tanacetifolia*	*Phacelia tanacetifolia*	*Phacelia tanacetifolia*	*Phacelia tanacetifolia*	*Phacelia tanacetifolia*
Cultivar / Variety:	Balo	Natra	Stala	Stala	Stala
Location:	76297, Stutensee, Baden-Württemberg, Germany	75177, Pforzheim, Baden-Württemberg, Germany	46220, Picassent, Valencia, Spain	46820, Anna, Valencia, Spain	02640 Almansa, Albacete, Spain
Distance between trials:	>20 km	>20 km	>20 km	>20 km	>20 km
Planting or seeding date:	2020-03-19	2020-06-08	2020-02-11	2020-02-05	2020-05-26
Seeds per ha:	15 kg seeds/ha	15 kg seeds/ha	10 kg seeds/ha	10 kg seeds/ha	10 kg seeds/ha
Plot size (width x length):	5 m x 40 m	5 m x 40 m	5 m x 40 m	5 m x 40 m	5 m x 40 m
Treated area:	185 m^2	185 m^2	169.4 m^2	169.4 m^2	169.4 m^2
Closest distance between control and treated plot(s):	10 m	10 m	20 m	25.7 m	27.3 m
Minimum distance to the edge of the field:	3 m	3 m	6.7 m	2 m	12.45 m
Soil type (USDA):	Loamy sand	Silt	Sandy loam	Sandy clay loam	Sandy loam
Test organisms:	Honeybee colonies (*Apis mellifera* L.)				

Environmental test conditions

Temperature (°C):	2.0 - 29.2	7.9 - 36.9	9.7 - 32.3	8.7 - 35.9	11.8 - 40.8
Humidity (%):	26.2 - 93.7	20.1 - 99.8	0.0 - 100.0	23.0 - 100.0	15.6 - 100.0
Daily precipitation (mm):	0.0 - 7.1	0.0 - 24.0	0.0 - 4.0	0.0 - 14.06	0.0 - 0.8

Study Design

Residues of spiroxamine were determined in nectar and pollen from *Phacelia tanacetifolia* plants under semi-field conditions. The study comprised five separate semi-field residue trials conducted in Germany (2 sites) and Spain (3 sites) in 2020 (please refer to the table above for details on the location and field sites). Each site comprised two plots (one untreated and one treated with Spiroxamine EC 500). Specifications of the plots were provided in the above table per site. The tunnels used per plot had an area of 200 m^2 each, with 2 rows of treated *P. tanacetifolia* (2.2 m x 37.0 m) divided by a 0.6 m uncultivated inter-row. Two bee hives and a water supply were placed at the end and middle of each tunnel respectively. Weather data (air temperature, humidity and precipitation) were recorded at the field site of each trial. During sowing and residue sampling the climatic conditions were measured with portable equipment or weather stations at the trial sites (GLP data). For the period between sowing and start of measurement at the field site weather data from an official weather station were taken (non-GLP).

Spiroxamine EC 500 was applied using a calibrated sprayer. The nominal application rate was 0.6 L product/ha (corresponding to 300 g a.s./ha) in 200 L water/ha for both applications and all trials. Before application, the sprayer was calibrated and the duration of spraying per plot was calculated according to the output. The actual amounts of the product and spray volume applied were determined by recording the amount of spray solution prepared and the amount remaining after the application. The application rate of the active substance was calculated based on the nominal content and density. No additional adjuvants, surfactants or mixing partners were used for the application. Actual applied spray volume was within a spray tolerance of ±10 %. For all trials and both applications the deviations ranged between -2.23 % and +3.66 %.

Sample processing:

On each sampling day forager bees were collected for the preparation of nectar from their honey stomachs for residue analysis. The hive entrances were sealed before the sampling and the forager bees were subsequently collected as they returned to the hive using modified hoovers ("bee vac"), or using tweezers if only few bees are returning. After sampling, the hives were re-opened. On each sampling day an A-sample of at least 150 bees was collected. If possible an R-sample of at least 150 bees was taken on each sampling day, too.

For the preparation of nectar from honey stomachs for determination of sugar content, forager bees were sampled in the control on each sampling day. One sample of at least 50 bees was taken per sampling day. No R-sample was taken.

On each sampling day pollen from *Phacelia tanacetifolia* retrieved by the bees was collected using pollen traps. The hives in each tunnel were equipped with pollen traps. Bees strip off the pollen when passing a grid. This pollen grid was only inserted on sampling days. After collection of the pollen the grid was removed. On each sampling day an A-sample and an R-sample of at least 0.2 g pollen was collected.

Control samples were taken before the test item treatment samples or were taken by different personnel, and different equipment was used.

All samples were transported on dry ice from the field to the test facility/test site. Samples were stored deep frozen within 12 hours after sampling. The field samples were stored in a freezer at -18 °C or below until preparation of the examination samples. The forager bees were shipped to the Study Director for preparation of honey stomachs and sugar content determination. The maximum storage interval from sampling to extraction was 164 days. Storage at the Analytical Test Site from sample receipt until lab sample preparation was at ≤ -18 C. The maximum interval from extraction to analysis at 1 °C to 10 °C with given exceptions was 2 days.

For the preparation of honey stomachs from forager bees for residue analysis the total amount of bees per sample was counted. At least 75 bees of the A-sample were prepared. If the minimum amount of prepared nectar was not obtained from the sub-sample A, sub-sample R was prepared and added to sub-sample A, until the requested amount of 200 mg nectar was achieved. The duration of any samples

remaining outside of the freezer did not exceed 2 hours. Honeybees from the control group (C) were processed first. Once this task was completed, then the process was started with the honeybees from the test item treatment group (T). The total number of prepared honeybees and the sub-samples used was recorded. For the preparation of honey stomachs from forager bees for sugar content determination the total amount of honeybees per sample was counted. The amount of at least 12 forager bees was prepared. The sugar content was determined immediately after preparation by a digital refractometer in the laboratory.

Sample schedule

Sampling of the different matrices was performed according to the following schedule:

Table CP 10.3.1.5/03-3 Matrices sampling schedule for trials S20-02289-01 to -05:

Sampling code	Timing	Treatment/ Plot	Commodity	Quantity (min) per subsample		Sample type
				A	R	
S1	0DAA2 (shortly after application)	C, T	Forager bees	150	150	Residue
			Forager bees	50	-	Sugar content
			Pollen	0.2 g	0.2 g	Residue
S2	0DAA2 (8 h after application)	T	Forager bees	150	150	Residue
			Forager bees	50		Sugar content
			Pollen	0.2 g	0.2 g	Residue
S3	1DAA2	T	Forager bees	150	150	Residue
			Forager bees	50	-	Sugar content
			Pollen	0.2 g	0.2 g	Residue
S4	2DAA2	T	Forager bees	150	150	Residue
			Forager bees	50	-	Sugar content
S5	3(+1)DAA2	T	Forager bees	150	150	Residue
			Forager bees	50	-	Sugar content
			Pollen	0.2 g	0.2 g	Residue
S6	5(±1)DAA2	T	Forager bees	150	150	Residue
			Forager bees	50	-	Sugar content
			Pollen	0.2 g	0.2 g	Residue
S7	7(±1)DAA2	T	Forager bees	150	150	Residue
			Forager bees	50	-	Sugar content
			Pollen	0.2 g	0.2 g	Residue

DAA: days after application

Residue analysis and analytic methods

The analytical method M01480/M001 was developed to determine the residues of spiroxamine (AE 1344293) in/on honey, pollen and nectar as sum of its four enantiomers A1, A2, B1 and B2 by HPLC–MS/MS detection.

The samples were diluted/extracted with a methanol/water mixture (3/1, v/v). After filtration of the raw extract, an aliquot was analyzed by high performance liquid chromatography chromatographed under chiral reverse phase column chromatography and detected by Tandem Mass Spectrometry with electrospray ionisation. Residues were quantified using solvent standards with an isotopic stable-labelled internal standard. For details on sample preparation for pollen and nectar please refer to the study report.

The method validation was done with a set of recoveries at the LOQ (5 x 0.01 mg/kg, sum of four enantiomers) and 10 x LOQ (5 x 0.10 mg/kg, sum of four enantiomers) level.

Full validation data is documented within the method 01480/M001 (chiral method) for pollen and nectar. A full set of validation recoveries (one control sample, at least 5 repetitions each at two fortification levels) at the LOQ (0.01 mg/kg, sum of four enantiomers) and at the 10-fold LOQ level (0.10 mg/kg, sum of four enantiomers) was also performed within this study, corresponding to 0.0027 mg/kg and 0.027 mg/kg for enantiomers A1 and A2 and 0.0023 mg/kg and 0.023 mg/kg for enantiomers B1 and B2 (chiral method). In order to check the performance of the methods, concurrent recovery determinations were included in each set of analyses (at least one recovery for ten study samples).

Recoveries were performed by spiking pollen and nectar with the test items. For control material pollen provided by the laboratory and synthetic nectar (prepared by dissolving 24.0 g glucose and 12.0 g fructose in water and filling up to 100 mL with water) was used for validation and concurrent recoveries.

The apparent residues in the control samples used for the performance of recoveries were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries.

The mean recovery values (method validation) of the spiroxamine enantiomers (chiral method) in pollen ranged between 98% and 107% with relative standard deviations between 3.3% and 17.3%. The overall mean recoveries of the analytes ranged between 98% and 104% and the corresponding overall relative standard deviation (RSD) ranged between 10.9% and 12.6% (n = 10 for each analyte).

The mean recovery values (method validation) of the spiroxamine enantiomers (chiral method) in nectar ranged between 91% and 105% with relative standard deviations between 2.6% and 12.5%. The overall mean recoveries of the analytes ranged between 94% and 100% and the corresponding overall relative standard deviation (RSD) ranged between 6.4% and 11.2% (n = 10 for each analyte).

The Limit of Quantitation (LOQ), defined as the lowest validated fortification level, was 0.01 mg/kg (10 µg/kg, sum of four enantiomers) for all analysed samples. The corresponding respective Limit of Detection (LOD) was determined to be 0.003 mg/kg (3 µg/kg, sum of four enantiomers). Therefore, all results of the concurrent recoveries were in accordance with the general requirements for residue analytical methods.

Analytical method

Samples of nectar and pollen were analysed using the validated analytical method 01480/M001, report reference M-763138-01-1 (**see Doc MCA Section 4**).

II. Results and Discussion

Residues in control samples of pollen ranged between <0.01 mg/kg and 0.0110 mg/kg (sum of four enantiomers). No residues of the analytes above the LOQ were found in any of the control samples of nectar. The residues found in the control and treated nectar and pollen samples are shown in the following tables. The results were not corrected for concurrent recoveries.

Table CP 10.3.1.5/03-4 Summary of residues of spiroxamine in pollen (sum of four enantiomers) found on each trial site [mg /kg]

Sample ID L20-02289	Sample type	Sample weight [g]	Residues of Spiroxamine (sum of four enantiomers) [mg/kg]
Trial S20-02289-01 (Germany)			
01-C-S1-P-A	C	0.220	<0.01
01-C-S1-P-R	C	0.198	<0.01
01-T-S1-P-A	T	0.200	87.0
01-T-S2-P-A	T	0.202	5.22
01-T-S3-P-A	T	0.201	3.40
01-T-S5-P-A	T	0.200	0.697
01-T-S6-P-A	T	0.250	2.10
01-T-S7-P-A	T	0.202	0.797
Trial S20-02289-02 (Germany)			
02-C-S1-P-A	C	0.202	<0.01
02-C-S1-R-A	C	0.217	<0.01
02-T-S1-P-A	T	0.201	71.9
02-T-S2-P-A	T	0.238	20.6
02-T-S3-P-A	T	0.202	3.63
02-T-S5-P-A	T	0.245	8.95
02-T-S6-P-A	T	0.201	0.193
02-T-S7-P-A	T	0.200	0.172
Trial S20-02289-03 (Spain)			
03-C-S1-P-A	C	0.211	0.0110
03-T-S1-P-A	T	0.201	37.9
03-T-S2-P-A	T	0.229	22.1
03-T-S3-P-A	T	0.201	4.59
03-T-S5-P-A	T	0.237	2.25
03-T-S6-P-A	T	0.201	1.13

03-T-S7-P-A	T	0.217	1.10
Trial S20-02289-04 (Spain)			
04-C-S1-P-A	C	0.201	<0.01
04-T-S3-P-A	T	0.232	21.4
04-T-S5-P-A	T	0.200	1.35
04-T-S6-P-A	T	0.201	1.13
04-T-S7-P-A	T	0.200	0.280
Trial S20-02289-05 (Spain)			
05-C-S1-P-A	C	0.197	<0.01
05-T-S1-P-A	T	0.192	54.7
05-T-S2-P-A	T	0.130	14.1
05-T-S3-P-A	T	0.251	9.32
05-T-S5-P-A	T	0.200	1.23
05-T-S6-P-A	T	0.194	1.48
05-T-S7-P-A	T	0.191	0.569

C = Control, T = Treatment, LOQ = Limit of Quantification = 0.01 mg/kg (= 10 µg/kg = 10 ppb, sum of four enantiomers) for spiroxamine, LOD = Limit of Detection = 0.003 mg/kg (= 3 µg/kg = 3 ppb, sum of four enantiomers) for spiroxamine

Table CP 10.3.1.5/03-5 Summary of residues of spiroxamine in nectar (sum of four enantiomers) found on each trial site [mg /kg]

Sample ID **L20-02289**	**Sample type**	**Sample weight [g]**	**Residues of Spiroxamine (sum of four enantiomers) [mg/kg]**
Trial S20-02289-01 (Germany)			
01-C-S1-NFB-A	C	0.200	<0.01
01-T-S1-NFB-A	T	0.200	0.700
01-T-S2-NFB-A	T	0.200	0.252
01-T-S3-NFB-A	T	0.200	0.116
01-T-S4-NFB-A	T	0.200	0.0268
01-T-S5-NFB-A	T	0.200	0.00943
01-T-S6-NFB-A	T	0.186	<0.01

01-T-S7-NFB-A	T	0.200	<0.01
Trial S20-02289-02 (Germany)			
02-C-S1-NFB-A	C	0.200	<0.01
02-T-S1-NFB-A	T	0.200	0.163
02-T-S2-NFB-A	T	0.201	0.195
02-T-S3-NFB-A	T	0.200	0.0313
02-T-S4-NFB-A	T	0.200	<0.01
02-T-S5-NFB-A	T	0.200	<0.01
02-T-S6-NFB-A	T	0.200	<0.01
02-T-S7-NFB-A	T	0.200	<0.01
Trial S20-02289-03 (Spain)			
03-C-S1-NFB-A	C	0.200	<0.01
03-T-S1-NFB-A	T	0.200	0.117
03-T-S2-NFB-A	T	0.200	0.0757
03-T-S3-NFB-A	T	0.200	0.0562
03-T-S4-NFB-A	T	0.199	0.0247
03-T-S5-NFB-A	T	0.200	<0.01
03-T-S6-NFB-A	T	0.200	<0.01
03-T-S7-NFB-A	T	0.200	<0.01
Trial S20-02289-04 (Spain)			
04-C-S1-NFB-A	C	0.183	<0.01
04-T-S1-NFB-A	T	0.200	0.221
04-T-S2-NFB-A	T	0.200	0.445
04-T-S3-NFB-A	T	0.200	0.104
04-T-S4-NFB-A	T	0.200	0.0193
04-T-S5-NFB-A	T	0.200	<0.01
04-T-S6-NFB-A	T	0.200	<0.01
04-T-S7-NFB-A	T	0.200	<0.01

Trial S20-02289-05 (Spain)			
05-C-S1-NFB-A	C	0.200	<0.01
05-T-S1-NFB-A	T	0.200	0.0471
05-T-S2-NFB-A	T	0.133	0.0777
05-T-S3-NFB-A	T	0.163	0.0704
05-T-S4-NFB-A	T	0.200	0.0128
05-T-S5-NFB-A	T	0.200	<0.01
05-T-S6-NFB-A	T	0.200	<0.01
05-T-S7-NFB-A	T	0.168	<0.01

C = Control, T = Treatment, LOQ = Limit of Quantification = 0.01 mg/kg (= 10 µg/kg = 10 ppb, sum of four enantiomers) for spiroxamine, LOD = Limit of Detection = 0.003 mg/kg (= 3 µg/kg = 3 ppb, sum of four enantiomers) for spiroxamine

Sugar content determination

The sugar content of nectar sampled from forager bees was determined by a digital refractometer. The sugar content was in a range from 30.1% to 44.4% for Trial 01, from 21.1% to 61.0% for Trial 02, from 11.9% to 42.2% for Trial 03, from 12.3% to 44.1% for Trial 04 and from 8.7% to 32.3% for Trial 05.

III. Conclusion

The mean recovery values (method validation) of the spiroxamine enantiomers (chiral method) in pollen ranged between 98% and 107% with relative standard deviations between 3.3% and 17.3%. The overall mean recoveries of the analytes ranged between 98% and 104% and the corresponding overall relative standard deviation (RSD) ranged between 10.9% and 12.6% (n = 10 for each analyte).

The mean recovery values (method validation) of the spiroxamine enantiomers (chiral method) in nectar ranged between 91% and 105% with relative standard deviations between 2.6% and 12.5%. The overall mean recoveries of the analytes ranged between 94% and 100% and the corresponding overall relative standard deviation (RSD) ranged between 6.4% and 11.2% (n = 10 for each analyte).

The Limit of Quantitation (LOQ), defined as the lowest validated fortification level, was 0.01 mg/kg (10 µg/kg, sum of four enantiomers) for all analysed samples. The corresponding respective Limit of Detection (LOD) was determined to be 0.003 mg/kg (3 µg/kg, sum of four enantiomers).

Assessment and conclusion by applicant:

The study followed the analytical Guidance Document, SANCO/3029/99 rev. 4 and the criteria for method validation were all met. Thus, the analytical results are considered to be valid and acceptable for use in the risk assessment.

The study was conducted taking into consideration the requirments of modern guidance on residues decline trials. The sampling regime was considered to be suitable (0 hours, 8 hours, 1, 2, 3, 5 and 7 days after application) as frequent sampling timepoints were used which spanned the estimated DT_{50} value therefore the results are considered suitable for kinetic modelling for DT_{50} values.

Five trials were conducted therefore it is considered that a sufficient number of trials are available in order to derive mean DT_{50} values for spiroxamine in pollen and in nectar for use in a risk assessment.

The study was conducted in *Phacelia* which was chosen as it is known to be a bee attractive crop and one that produces both nectar and pollen.

The study is considered to be acceptable.

Two cage tests using Spiroxamine EC 500 have been conducted which are summarized below.

Data Point:	KCP 10.3.1.5/01
Report Author:	[redacted]
Report Year:	1995
Report Title:	Field evaluation of the toxicity of KWG 4168 EC 500 to foraging honey bees (Apis mellifera) under cage test field conditions
Report No:	BAY 171
Document No:	M-008239-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

In a semi-field cage test the effects of KWG 4168 EC 500 on honeybees were assessed. The behaviour and mortality of the colonies was observed over 8 days after treatment.

The flowering crops (*Phacelia tanacetifolia*) were treated with 1.5 L product/ha of the test substance and the hives were placed adjacent to the plot within mesh cages.

KWG 4168 EC 500 did not significantly affect bee behaviour when compared to the control group. Mortality in the test item group was not significantly different to the control group.

I. Materials and Methods

Materials

Test Material: KWG 4168 EC500

Lot/Batch #: 089A

Purity: 491.4 g/L

Description: Not reported

Stability of test compound: Not reported

Reanalysis/Expiry date: 16th December 1994

Density: Not reported

Treatments

Test rates: 1.5 L product/ha (750 g a.s./ha)

Solvent/vehicle: None

Analysis of test concentrations:	No
Test organisms	
Species:	Honey bee, *Apis mellifera,* queen-right colonies
Source:	Not reported
Acclimatisation period:	Colonies were introduced to the cage and crops 3 days prior to treatment
Treatment for disease:	Bees were reported to be in good condition at test start.
Test design	
Test vessel:	Mesh cage 4 x 2 x 2 m
Replication:	4 per treatment
No. animals/vessel:	One colony per cage
Duration of test:	8 days
Environmental test conditions	
Temperature:	11.8 - 23.3 °C

Study Design

This study was conducted in order to assess the toxicity of KWG 4168 EC 500 on honeybees under semi-field cage conditions over 8 days.

Bee hives were placed on the edge of the test plot of the flowering crop, *Phacelia tanacetifolia*. The hives and crop plot were within fine mesh cages (4 x 2 x 2 m) to keep out foreign bees and ensure test bees did not escape. Temperature and humidity were measured at a nearby weather station and temperatures ranged between 11.8 and 23.3°C. One nucleus hive colony including bees and brood was set up in each tent three days before application.

One plot was treated with the test substance, another with a reference item, Hostathion (420 g a.s./ha) and a further untreated control was used.

The test substance was sprayed on the flowering crop during bee flight, at a dose of 1.5 L/ha (750 g a.s./ha) using a plot sprayer.

The cage size was 8 m².

Assessments for mortality, foraging activity, behavior, brood and colony size were made before and up to 7 days after application. The effects of the treatment on the bees were assessed in order to include a potential influence on the brood. Bee foraging activity and bee behaviour was measured at 2-hr intervals on the day of treatment, 3 times a day on day 1 and 2 after treatment, twice a day 3 days after and once a day on day 5 and 7 after treatment. Dead bee traps were placed at the entrances of three hives to collect dead bees which were counted at the same time as the behaviour observations. Colony condition was observed one day before treatment and 8 days after.

II. Results and Discussion

The guidelines according to EPPO 170 (1992) were followed in this study.

KWG 4168 EC 500 did not significantly affect bee behaviour when compared to the control group.

Table CP 10.3.1.5/01-1 Number of honeybees observed foraging after exposed to KWG 4168 EC 500

Treatment group	Timing	Number of bees/plot
Water	Before application	73.3
	1 day after application	98.0
	7 days after application	78.3
Test item	Before application	85.5
	1 day after application	15.8
	7 days after application	69.5
Reference	Before application	92.3
	1 day after application	119.5
	7 days after application	125.0

The number of deaths recorded in the treatment group was higher than the number of bee deaths observed in the control but not significantly different.

Table CP 10.3.1.5/01-2 Mortality of honeybees exposed to KWG 4168 EC 500 under semi-field conditions after 8 days

Treatment group	Timing	Number of bees/plot
Water	Before application	183.8
	1 day after application	10.0
	7 days after application	98.3
Test item	Before application	241.8
	1 day after application	21.8
	7 days after application	187.8
Reference	Before application	254.0
	1 day after application	75.5
	7 days after application	240.5

There was no effect on colony size or condition when assessed over 8 days after application.

Table CP 10.3.1.5/01-3 Colony size after 8 days of exposure to KWG 4168 EC 500 under semi-field conditions

Treatment group	No. live bees/hive		No dead bees/hive	
	-1 DAT	8 DAT	-1 DAT	8 DAT
Water	2804.8	2715.8	325.3	11.9
Test item	2745.5	3031.8	1800.5	66.5
Reference	2508.8	3367.5	584.3	23.3

A very slight increase in mortality after application of the test substance could be observed compared to the negative control, but not when compared to pre-application mortality. Therefore this effect was not considered to be treatment-related. Regarding the other test parameters no negative effects could be observed. In contrast the toxic reference caused strong mortality, reduction of bee flight and behavioural impact.

III. Conclusion

Following application of KWG 4168 EC 500 to *Phacelia* at 1.5 L product/ha, there were no significant effects on honeybee mortality, foraging activity, behavior, brood and colony size when compared to the control.

Assessment and conclusion by applicant:

The study appears to have been conducted in accordance with EPPO 170 although no guideline is specifically referenced in the study report. The study no longer meets many of the required test parameters currently expected from a higher tier bee study as detailed in OECD 75 as well as the recommendations of the EFSA (2013) Bee Guidance Document. The most notable deficiency is the absence of any analysis of residues in the crop plants and in the nectar and pollen collected from the forager bees and/or from the hives themselves. The area of the cages used in the study was relatively small and, although colony assessments were made, specific assessments for the development of the brood were not performed. Thus, the test design adopted in this study is not considered to meet current requirements.

The crop used in this study was *Phacelia* which, although not the specific crop in question, is accepted to be a bee attractive crop and is therefore considered to be a suitable surrogate for arable crops. The application rate used in this study (1.5 L product/ha; equivalent to nominally 750 g a.s./ha) is greater than the proposed application rate of 300 g a.s./ha for Spiroxamine EC 500 and therefore covers the potential risks, in terms of application rate, following this use.

On balance, this study is not considered to be sufficient on its own to address the potential risks from exposure to Spiroxamine EC 500 and has therefore been submitted as supporting information only. However, as a bee attractive crop was used and because the tested rate exceeds that proposed for Spiroxamine EC 500, the results have been considered as part of a weight of evidence argument.

Data Point:	KCP 10.3.1.5/02
Report Author:	[redacted]
Report Year:	1994
Report Title:	Toxicity testing of KWG 4168 EC 500 to honey bees (Apis mellifera L.) (Hymenoptera, Apidae) semi field study
Report No:	459900
Document No:	M-008244-01-1
Guideline(s) followed in study:	BBA, VI, 1991
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

In a semi-field cage test the effects of KWG 4168 EC 500 on honeybees were assessed. The behaviour and mortality of the colonies was observed for 72 hours following application.

The flowering crops (*Phacelia tanacetifolia*) were treated with 3.0 L product/ha of the test substance during daily bee flight and two tests, one week apart, were conducted. Additional cages were exposed to crops treated with water and with Perfektthion, a.s. 400g/L dimethoate, were used as control and reference treatments, respectively.

KWG 4168 EC 500 did not significantly affect bee behaviour when compared to the control group. Mortality in the test item group was not significantly different to the control group.

The results of the two experiments of this study led to the same conclusion that application of KWG 4168 EC 500 at 3.0 L product/ha did not cause a reduction of flight intensity or mortality to honey bees. Further to this, no impact on the brood was observed.

I. Materials and Methods

Materials

Test Material KWG 4168 EC 500

Lot/Batch #: FL 089 A

Purity: 491.4 g/L

Description: Amber liquid

Stability of test compound: Not reported

Reanalysis/Expiry date: 16 December 1994

Density: Not reported

Treatments

Test rates: 3.0 L product/ha (1500 g a.s./ha)

Solvent/vehicle: None

Analysis of test concentrations:	No
Test organisms	
Species:	Honey bee, *Apis mellifera*
Source:	Christoph Mohr, Goethestr. 19, D-64354 Reinheim
Acclimatisation period:	Bees were allowed to acclimatise to their new home range prior to test substance application
Treatment for disease:	None reported
Test design	
Replication:	2
Test vessel:	3 cages, 4m x 3m x 2m (length x width x height), metal frame covered with synthetic gauze (hole diameter 2mm)
No. animals/vessel:	One colony of bees per test cage in a hive with three elaborated honeycombs, containing about 5000 bees.
Duration of test:	72 hours
Environmental test conditions	
Temperature:	First experiment: 8 - 32°C Second experiment: 13.8 - 31.6°C
Relative humidity	40 – 100%
Wind velocity	<2 m/s

Study Design

This study was conducted in order to assess the effects of KWG 4168 EC 500 on honeybees under semi-field cage conditions over 72 hours.

The test was performed twice at different times in three big cages ('tents') placed in the field. One colony of bees was present per test cage.

The study was performed with *Phacelia tanacetifolia* Benth. as the crop plant. The cages were placed over the flowering *Phacelia* a few days before the experiment began. The bees were allowed to acclimatise to their new home range. Daily mortality and bee flight intensity was recorded prior to application of the test item.

Temperature, relative humidity, cloud cover and wind velocity were recorded with a MICROMEC 4-channel datalogger. One measurement of temperature, relative humidity and wind speed was taken every 10 minutes.

The first cage was treated with the test substance at 3.0 L product/ha (equivalemt to 1500 g a.s./ha), the second with reference substance (Perfekthion, a.s. 400 g/L dimethoate) and the third with water as a negative control.

A portable compression sprayer with an extension tube porting four spraying nozzles was used. Spray was administered whilst the bees were in flight. KWG 4168 EC 500 was applied in an amount of 40 mL/m^2 of a 7.5 mL/L dilution corresponding to 3.0 L/ha in a spray volume of 400 L/ha water. The

reference substance Perfekthion was applied in an amount of 40 mL/m^2 of a 2.5 mL/L dilution corresponding to 1.0 L/ha in a spray volume of 400 L/ha water. The negative control was sprayed with common tap water (40 mL/m^2), corresponding to a spray volume of 400 L/ha.

The bees were exposed for 72 hours. The second test was preformed one week after the first experiment.

After start of the experiment observation and recording of mortality, behaviour and flight of the bees was continuous for the first 60 minutes. Further observations and recording of dead bees in the traps attached to the hives as well as recording of the flight intensity and behaviour took place every two hours until the evening of the first day. Observations and recording were continued at Days 1, 2 and 3 for three times a day. The mortality at the edges of the cages was recorded every day in the evening. Any observable cases of poisoning or behavioural abnormalities of the bees were recorded.

II. Results and Discussion

After application in both tests, the number of foraging bees in the test substance treated and negative control tent were comparable. The flight intensity of the bees under the impact of the positive control substance was relatively low and had almost ceased by the end of the tests.

In the first test, by the third day flight intensity was higher in the test substance treated cage than in the negative control. The fact that the highest number of foraging bees were observed in the test substance treated tent was probably caused by a relatively high population density of the corresponding colony. However, in comparison to the days prior to application, no effect on the flight density can be related to the test substance application in either test.

Table CP 10.3.1.5/03-1 Flight density of honeybees exposed to KWG 4168 EC 500 under field conditions for 72 hours

Test 1	Number of foraging worker bees observed		
	Before application	Directly after application	72 hours after application
Control	10	3	15
Test item	13	5	51
Reference item	10	6	0
Test 2			
Control	25	15	20
Test item	25	15	21
Reference item	20	11	1

In the first test, after 24 hours exposure 10200% more bees died in the reference item tent than on the day before the test began, this was 2740% in the second test. Mortality of the bees exposed to KWG 4168 EC 500 only increased a small amount compared to the negative controls and remarkably less than compared to the reference item cages.

Table CP 10.3.1.5/03-2 Mortality of honeybees exposed to KWG 4168 EC 500 under field conditions for 72 hours

Test 1	Dead honeybees (% of dead bees observed 1 day prior to test)	
	24 hours after application	72 hours after application
Control	67	17
Test item	100	200

	Dead honeybees (% of dead bees observed 1 day prior to test)	
Test 1	**24 hours after application**	**72 hours after application**
Reference item	10200	7200
Test 2		
Control	100	1050
Test item	129	214
Reference item	2740	310

The assessment of the bees and brood of the colonies involved in this study did not give any indications for intoxications or other impacts of the test substance.

III. Conclusion

The results of the two experiments of this study led to the same conclusion that application of KWG 4168 EC 500 at 3.0 L product/ha (1500 g a.s./ha) did not cause a reduction of flight intensity or mortality to honey bees. Further to this, no impact on the brood was observed.

Assessment and conclusion by applicant:

The study was conducted to an older BBA test guideline which has since been superseded by more up to date test guidelines. The study no longer meets many of the required test parameters currently expected from a higher tier bee study as detailed in OECD 75 as well as the recommendations of the EFSA (2013) Bee Guidance Document. The most notable deficiency is the absence of any analysis of residues in the crop plants and in the nectar and pollen collected from the forager bees and/or from the hives themselves. The area of the cages used in the study was relatively small and, although colony assessments were made, specific assessments for the development of the brood were not performed. Thus, the test design adopted in this study is not considered to meet current requirements.

The crop used in this study was *Phacelia* which, although not the specific crop in question, is accepted to be a bee attractive crop and is therefore considered to be a suitable surrogate for arable crops. The application rate used in this study (3.0 L product/ha; equivalent to nominally 1500 g a.s./ha) is greater than the proposed application rate of 300 g a.s./ha for Spiroxamine EC 500 and therefore covers the potential risks, in terms of application rate, following this use.

On balance, this study is not considered to be sufficient on its own to address the potential risks from exposure to Spiroxamine EC 500 and has therefore been submitted as supporting information only. However, as a bee attractive crop was used and because the tested rate exceeds that proposed for Spiroxamine EC 500, the results have been considered as part of a weight of evidence argument.

CP 10.3.1.6 Field tests with honeybees

Data Point:	KCP 10.3.1.6/01
Report Author:	[redacted]
Report Year:	1995
Report Title:	Testing toxicity to honeybee - Apis mellifera L. under field conditions - KWG 4168 EC 500, fungicid (BAY 12260 F)
Report No:	95 10 48 500
Document No:	M-008232-01-1
Guideline(s) followed in study:	BBA-Richtlinie VI, 23-1 (1991)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

In a field study the effects of KWG 4168 EC 500 on honeybees were assessed. Hives of honey bees (*Apis mellifera*) were placed adjacent to fields of flowering crops treated with KWG 4168 EC 500. The bees were allowed to forage over 3 weeks and behaviour and mortality of the colonies was observed.

The flowering crops (*Phacelia tanacetifolia*) were treated with 1.5 L product/ha (750 g a.s./ha) of the test substance during daily bee flight and four hives were placed adjacent to the field. Four hives situated in an apiary were used as a control.

The test substance did not cause any mortality in the field test in comparison to the control. In particular, the test substance did not affect the proper larval development. The collected pollen proved that foraging took place in the treated field.

The results and recorded observations did not provide evidence that an application of KWG 4168 EC 500 to flowering *Phacelia* at 1.5 L product/ha affected bee colonies in terms of mortality, foraging activity, behavior, colony strength and brood development under field conditions.

I. Materials and Methods

Materials

Test Material KWG 4168 EC 500, Fungicid (BAY 12260 F)

Lot/Batch #: Not reported

Purity: Not reported

Description: Not reported

Stability of test compounds: Not reported

Reanalysis/Expiry date: Not reported

Density: 1.0g/cm^3

Treatments

Test rates: 1.5 L product/ha 8750 g a.s./ha)

Solvent/vehicle:	None
Analysis of test concentrations:	No
Test organisms	
Species:	Honey bee, *Apis mellifera*
Source:	purchase from the bee-keeper Mr. Mehlhorn (Seidewitz) on 09.06.95
Acclimatisation period:	Bees were allowed to sufficiently forage prior to test substance application
Treatment for disease:	None reported
Test design	
Replication:	4 hives were used for exposure to the test item
No. animals/vessel:	Each hive had a colony with well-developed combs. 10-12 frames with 4-10 broods
Duration of test:	3 weeks
Plot size:	0.25 ha
Environmental test conditions	
Temperature:	8 - 29°C treated field 6 - 27°C control apiary
Relative humidity	Treated field: 31 – 100% Control apiary: 28 – 100%
Wind velocity	1 - 2 m/s

Study Design

This study was conducted in order to assess the effects of KWG 4168 EC 500 on honeybees under field conditions over 3 weeks.

Bee colonies were placed on the edge of the test field of the flowering crop, *Phacelia tanacetifolia*. The field was chosen so that bees could mainly forage in the field in which their hive was placed. Four hives of well-established colonies were placed at the edge of the crop field 5 days prior to application of the test substance. The control was located in an apiary. Temperature and humidity were measured continuously and temperatures ranged between 8 and 29°C at the treated field site and 6 and 27°C at the apiary.

The test substance was sprayed on the flowering crop during daily bee flight at a dose of 1.5 L product/ha (750 g a.s./ha) in 300 L/ha water using a tractor-mounted sprayer in favourable weather. Hives were protected from direct contamination during spraying. Actual volume sprayed was 95.3% of nominal.

The effects of the treatment on the bees were assessed over a period of 3 weeks in order to include a potential influence on the brood. 1.5 m gauze strips were placed in front of the hives and dead bee traps were placed at the entrances of three hives to collect dead bees. Observations were made at the entrance of hives to assess the foraging activity of the bees.

II. Results and Discussion

The guidelines according to EPPO 170 (2010) were followed in this study.

Foraging activities within the treated plots were quite high at the beginning of the experiment with the proportion of collected pollen from the plot at 77%. This decreased to just 17% of collected pollen by the end of the 3-week study.

Table CP 10.3.1.6/01-1 Pollen collected by honeybees exposed to KWG 4168 EC 500 under field conditions

	Proportion collected pollen from *Phacelia* (%)
Before application	77
1 week after application	54
2 week after application	26
3 week after application	17

No impacts on honeybee behaviour were observed during the 3-week study period. There was no excessive deadfall observed in the hives exposed to the test substance.

Table CP 10.3.1.6/01-2 Mortality of honeybees exposed to KWG 4168 EC 500 under field conditions after 22 days

Concentration of test substance (L/ha)	**Dead honeybees/trap**
Control	578.4
1.5	491.8

All colonies, both the control and the test hives, showed well-filled beeways before application of the experiment. Three weeks after application in one case a deviation of the population density regarding the beeways could be observed, because the queen swarmed some days before the end of the experiment.

Table CP 10.3.1.6/01-3 Population density of honeybees exposed to KWG 4168 EC 500 under field conditions

Concentration of test substance (L/ha)	**Average number of well filled beeways**	
	6 days before application	**22 days after application (23 days after for control)**
Control	9.25	9.25
1.5	9.0	8.5*

* The Queen swarmed 1 week before evaluation in one hive

Neither in the dead-bee traps nor at the final evaluation of the comb brood damaged larvae or pupae of the bees were discovered. Eggs and young larvae, which were present in high number at the beginning of the experiment, developed very well. No damaged stages of honeybees or malformed young bees could be observed. The beeways between the frames of all hives were well filled at the pre- and the final evaluation.

Table CP 10.3.1.6/01-4 Brood status of honeybees exposed to KWG 4168 EC 500 under field conditions

Concentration of test substance (L/ha)	Average number of brood combs	
	6 days before application	22 days after application (23 days after for control)
Control	7.5	7.5*
1.5	5.75	7.75

* The Queen swarmed a few days before evaluation in one hive

III. Conclusion

The results and recorded observations did not provide evidence that an application of KWG 4168 EC 500 to flowering *Phacelia* at 1.5 L product/ha affected bee colonies in terms of mortality, foraging activity, behavior, colony strength and brood development under field conditions.

Assessment and conclusion by applicant:

The study was conducted in accordance with EPPO but no longer meets many of the required test parameters currently expected from a higher tier bee study (including the recommendations of the EFSA (2013) Bee Guidance Document). The most notable deficiency is the absence of any analysis of residues in the crop plants and in the nectar and pollen collected from the forager bees and/or from the hives themselves. The plot size used in the study was relatively small and, although colony assessments were made, specific assessments for the development of the brood were not performed. Thus, the test design adopted in this study is not considered to meet current requirements.

The crop used in this study was *Phacelia* which, although not the specific crop in question, is accepted to be a bee attractive crop and is therefore considered to be a suitable surrogate for arable crops. The application rate used in this study (1.5 L product/ha, equivalent to nominally 750 g a.s./ha) is greater than the proposed application rate of 300 g a.s./ha for Spiroxamine EC 500 and therefore covers the potential risks, in terms of application rate, following this use.

On balance, this study is not considered to be sufficient on its own to address the potential risks from exposure to Spiroxamine EC 500 and has therefore been submitted as supporting information only. However, as a bee attractive crop was used and because the tested rate exceeds that proposed for Spiroxamine EC 500, the results have been considered as part of a weight of evidence argument.

CP 10.3.2 Effects on non-target arthropods other than bees

The table below summarises the available data for non-target arthropods, all of which has been conducted using the representative formulation Spiroxamine EC 500. Glass plate, extended test and semi-field data are available with a range of foliar and soil NTA species. Furthermore, six field trials are available for mites.

Table CP 10.3.2-1 Summary of NTA studies with Spiroxamine EC 500

Organism	Test item	Test type	Endpoints		Reference
Typhlodromus pyri	Spiroxamine EC 500	Tier I Laboratory test glass plates (2D)	LR_{50} 240 g a.s./ha ER_{50} >180 g a.s./ha	EU	M-025030-01-1

Organism	Test item	Test type	Endpoints		Reference
Typhlodromus pyri	Spiroxamine EC 500	Tier I Laboratory test glass cages (coffin cells) (2D)	LR_{50} <741 g a.s./ha	EU	M-008573-01-1
Aphidius rhopalosiphi	Spiroxamine EC 500	Tier I Laboratory test glass plates (2D)	LR_{50} 80.1 g a.s./ha ER_{50} >30 g a.s./ha	EU	M-030680-01-1
Coccinella septempunctata	Spiroxamine EC 500	Tier I Laboratory test glass plates (2D)	LR_{50} <731 g a.s./ha	EU	M-008516-01-1
Coccinella septempunctata	Spiroxamine EC 500	Tier I Laboratory test glass plates (2D)	LR_{50} >750 g a.s./ha ER_{50} >750 g a.s./ha	EU	M-027529-01-1
Chrysoperla carnea	Spiroxamine EC 500	Tier I Laboratory test glass plates (2D)	LR_{50} <737 g a.s./ha	EU	M-008545-01-1
Chrysoperla carnea	Spiroxamine EC 500	Tier I Laboratory test glass plates (2D)	LR_{50} >1600 g a.s./ha ER_{50} >1600 g a.s./ha	EU	M-033924-01-1
Pardosa spp.	Spiroxamine EC 500	Tier I Laboratory test (2D)	LR_{50} <731 g a.s./ha ER_{50} >731 g a.s./ha	EU	M-008519-01-1
Bembidion tetracolum	Spiroxamine EC 500	Tier I Laboratory test (2D)	LR_{50} >741 g a.s./ha ER_{50} >741 g a.s./ha	EU	M-008726-01-1
Typhlodromus pyri	Spiroxamine EC 500	Tier II Extended laboratory test; natural substrate (2D) - grapevine	LR_{50} >510 g a.s./ha ER_{50} >510 g a.s./ha	EU	M-462852-01-1
Aphidius rhopalosiphi	Spiroxamine EC 500	Tier II Extended laboratory test; natural substrate (3D) – barley seedlings	LR_{50} >741 g a.s./ha ER_{50} >741 g a.s./ha	EU	M-008715-01-1
Aphidius rhopalosiphi	Spiroxamine EC 500	Tier II Extended laboratory test; natural substrate (3D) – barley seedlings	LR_{50} >900 g a.s./ha ER_{50} >900 g a.s./ha	EU	M-289317-01-1
Pardosa spp.	Spiroxamine EC 500	Tier II Extended laboratory test; (2D); two applications	LR_{50} >737 g a.s./ha ER_{50} >737 g a.s./ha	EU	M-008522-02-1

Organism	Test item	Test type	Endpoints		Reference
Bembidion tetracolum	Spiroxamine EC 500	Tier II Extended laboratory test; (2D); two applications	LR_{50} >1482 g a.s./ha ER_{50} >1482 g a.s./ha	EU	M-008528-01-1
Coccinella septempunctata	Spiroxamine EC 500	Semi-field conditions (3D); two treatments on bean plants	No effects on metamorphosis, fecundity and hatch rate at 2x 737 g a.s./ha	EU	M-008547-01-1
Aphidius rhopalosiphi	Spiroxamine EC 500	Semi-field conditions (3D); winter wheat	No effects on mortality at 737 g a.s./ha	EU	M-008539-01-1
Typhlodromus pyri	Spiroxamine EC 500	Field trial in vines, Germany	Four applications (totaling 1297 g a.s./ha) with an interval of approximately two weeks did not lead to a reduction of predatory mite populations	EU	M-008494-01-1
Typhlodromus pyri	Spiroxamine EC 500	Field trial in vines, Germany	Six applications (216, 426, 550, 667, 754 and 889 mL/ha) with an interval of approximately two weeks did lead to populations of the predatory mites reducing to 59% of the control level	EU	M-008496-01-1
Typhlodromus pyri	Spiroxamine EC 500	Field trial in vines, Germany	Four applications (165, 275, 330 and 440 g/a.s.ha) led to 25% effect after the 3rd application	EU	M-008505-01-1
Typhlodromus pyri	Spiroxamine EC 500	Field trial in vines, Germany	Four applications (300, 721, 738 and 732 mL/ha) with an interval of approximately two weeks led to populations of the predatory mites that were 12% lower than the control level	EU	M-024960-01-1

Organism	Test item	Test type	Endpoints		Reference
Typhlodromus pyri	Spiroxamine EC 500	Field trial in vines, Germany	Six applications (302, 283, 756, 762, 735 and 769 mL product/ha) with an interval of approximately two weeks led to populations of the predatory mites being reduced by 59% when compared to the control level	EU	M-024963-01-1
Amblyseius abberans	Spiroxamine EC 500	Field trial in vines, Southern Italy	Three applications of 300 g a.s./ha led to an 18.5% effect on the predatory mites four weeks after the final treatment	EU	M-008498-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2. As required, a risk assessment for both in-field and off-field exposure have been conducted.

Isomers

In terms of organism exposure, the critical highest predicted concentrations in the environment will occur immediately or very shortly after application therefore the effects of potential changes in the isomer ratios over time in the environment are not considered to be relevant to the NTA risk assessment. However, even if exposure to residues over a prolonged period of time were to occur, according to the current residues data set for spiroxamine there are no indications of a significant change in isomer ratios therefore no additional factor need be applied to the risk assessments below (*i.e.* an UF of 1.0 has been used).

Risk assessment for in-field exposure

The representative GAP for Spiroxamine EC 500 includes both a single or two applications of either 200 g a.s./ha or 300 g a.s./ha to grapes therefore all of these uses have been considered in the risk assessment.

The in-field exposure (predicted environmental rate, PER) is calculated according to ESCORT 2 using the following equation:

$$PER_{IN\text{-}FIELD} = \text{Application rate (g a.s./ha)} \times MAF$$

The MAF is a generic multiple application factor which is used to take in to account the potential build-up of applied substances between applications based on the application interval, DT_{50} value and number of applications.

The maximum in-field exposure (Predicted Environmental Rate, $PER_{IN\text{-}FIELD}$) to foliar-dwelling or soil-dwelling organisms assumes the worst-case scenarios of 100% crop interception and 0% crop interception, respectively.

The predicted exposure rate (PER) for in-field exposure of both foliar and soil-dwelling non-target arthropods for the uses in grapes (1 x 200 g a.s./ha, 2 x 200 g a.s./ha, 1 x 300 g a.s./ha and 2 x 300 g a.s./ha) are summarised in the table below.

Table CP 10.3.2-2 PER for in-field exposure following the uses of Spiroxamine EC 500

Crop	Application rate (g a.s./ha)	Foliar		Soil	
		MAF[1]	$PER_{IN-FIELD}$ (g a.s./ha)	MAF[1]	$PER_{IN-FIELD}$ (g a.s./ha)
Grapes	1 x 200	1.0	200	1.0	200
	2 x 200	1.7	340	1.9	380
	1 x 300	1.0	300	1.0	300
	2 x 300	1.7	510	1.9	570

[1]: MAF = Multiple Application Factor (Appendix III of ESCORT II)

The potential risk to in-field non-target arthropods based on Tier I studies is assessed by calculation of the hazard quotient (HQ = exposure/toxicity) with the predicted environmental rate (PER) and the lowest lethal rate (LR_{50}) values according to the following formula:

$$\text{In-field HQ} = \frac{PER_{IN-FIELD}\ (\text{g a.s./ha})}{LR_{50}\ (\text{g a.s./ha})}$$

The HQ trigger for Tier I laboratory studies is 2. For the extended laboratory Tier II studies the risk is considered acceptable if the $PER_{IN-FIELD}$ concentrations are below the test concentrations resulting in 50% effects.

For *Chrysoperla* and *Coccinella* there are more than one standard laboratory study available and these have potentially conflicting results. For completeness, all available standard laboratory data have been considered in the Tier I risk assessment below.

The Tier I in-field risk assessments for one and two applications of 200 g a.s./ha to grapes are presented in Table 10.3.2-3 and Table 10.3.2-4, respectively. The Tier I in-field risk assessments for one and two applications of 300 g a.s./ha to grapes are presented in Table 10.3.2-5 and Table 10.3.2-6, respectively.

Table CP 10.3.2-3 Tier I in-field risk assessment for the proposed use of Spiroxamine EC 500 in grapes (1 x 200 g a.s./ha)

Intended use	Grapes
Product	Spiroxamine EC 500
Application rate (g a.s./ha)	1 × 200
MAF	1.0

Test species Tier I	LR_{50} (g a.s./ha)	Foliar $PER_{IN-FIELD}$ (g a.s./ha)	Foliar $HQ_{IN-FIELD}$	Soil $PER_{IN-FIELD}$ (g a.s./ha)	Soil $HQ_{IN-FIELD}$
Aphidius rhopalosiphi	80.1	200	**2.50**	200	**2.50**
Typhlodromus pyri	240		0.833		0.833
Bembidion tetracolum	>741		<0.270		<0.270

Pardosa spp.	<731		**>0.274**		**>0.274**
Chrysoperla carnea	<737		**>0.271**		**>0.271**
Chrysoperla carnea	>1600		<0.125		<0.125
Coccinella septempunctata	<731		**>0.274**		**>0.274**
Coccinella septempunctata	>750		**<0.267**		**<0.267**

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient
Criteria values shown in **bold** breach the relevant trigger (criterion HQ ≤ 2). Some values shown in bold do not breach the trigger but they are marked in bold as the endpoint which originated the HQ is a "smaller than" value, showing some intrinsic uncertainty.

Table CP 10.3.2-4 Tier I in-field risk assessment for the proposed use of Spiroxamine EC 500 in grapes (2 x 200 g a.s./ha)

Intended use	Grapes				
Product	Spiroxamine EC 500				
Application rate (g a.s./ha)	2 × 200				
MAF	1.7 (foliar), 1.9 (soil)				
Test species Tier I	**LR_{50} (g a.s./ha)**	**Foliar $PER_{IN-FIELD}$ (g a.s./ha)**	**Foliar $HQ_{IN-FIELD}$**	**Soil $PER_{IN-FIELD}$ (g a.s./ha)**	**Soil $HQ_{IN-FIELD}$**
Aphidius rhopalosiphi	80.1		**4.24**		**4.74**
Typhlodromus pyri	240		1.42		1.58
Bembidion tetracolum	>741		**<0.459**		**<0.513**
Pardosa agricola	<731		**>0.465**		**>0.520**
Chrysoperla carnea	<737	340	**>0.461**	380	**>0.516**
Chrysoperla carnea	>1600		**<0.213**		**<0.238**
Coccinella septempunctata	<731		**>0.465**		**>0.520**
Coccinella septempunctata	>750		**<0.453**		**<0.507**

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient
Criteria values shown in **bold** breach the relevant trigger (criterion HQ ≤ 2). Some values shown in bold do not breach the trigger but they are marked in bold as the endpoint which originated the HQ is a "smaller than" value, showing some intrinsic uncertainty.

Table CP 10.3.2-5 Tier I in-field risk assessment for the proposed use of Spiroxamine EC 500 in grapes (1 x 300 g a.s./ha)

Intended use	Grapes
Product	Spiroxamine EC 500
Application rate (g a.s./ha)	1 × 300
MAF	1.0

Test species Tier I	**LR_{50} (g a.s./ha)**	**Foliar $PER_{IN-FIELD}$ (g a.s./ha)**	**Foliar $HQ_{IN-FIELD}$**	**Soil $PER_{IN-FIELD}$ (g a.s./ha)**	**Soil $HQ_{IN-FIELD}$**
Aphidius rhopalosiphi	80.1	300	**3.75**	300	**3.75**
Typhlodromus pyri	240		1.25		1.25
Bembidion tetracolum	>741		<0.405		<0.405
Pardosa agricola	<731		**>0.410**		**>0.410**
Chrysoperla carnea	<737		**>0.407**		**>0.407**
Chrysoperla carnea	>1600		<0.188		<0.188
Coccinella septempunctata	<731		**>0.410**		**>0.410**
Coccinella septempunctata	>750		<0.400		<0.400

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient
Criteria values shown in **bold** breach the relevant trigger (criterion HQ ≥ 2). Some values shown in bold do not breach the trigger but they are marked in bold as the endpoint which originated the HQ is a "smaller than" value, showing some intrinsic uncertainty.

Table CP 10.3.2-6 Tier I in-field risk assessment for the proposed use of Spiroxamine EC 500 in grapes (2 x 300 g a.s./ha)

Intended use	Grapes
Product	Spiroxamine EC 500
Application rate (g a.s./ha)	2 × 300
MAF	1.7 (foliar); 1.9 (soil)

Test species Tier I	**LR_{50} (g a.s./ha)**	**Foliar $PER_{IN-FIELD}$ (g a.s./ha)**	**Foliar $HQ_{IN-FIELD}$**	**Soil $PER_{IN-FIELD}$ (g a.s./ha)**	**Soil $HQ_{IN-FIELD}$**
Aphidius rhopalosiphi	80.1	510	**6.37**	570	**7.12**
Typhlodromus pyri	240		**2.13**		**2.38**
Bembidion tetracolum	>741		<0.688		<0.769
Pardosa agricola	<731		**>0.698**		**>0.780**
Chrysoperla carnea	<737		**>0.692**		**>0.773**
Chrysoperla carnea	>1600		<0.319		<0.356
Coccinella septempunctata	<731		**>0.698**		**>0.780**

Coccinella septempunctata	>750		<0.680		<0.760

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient
Criteria values shown in **bold** breach the relevant trigger (criterion HQ ≤ 2). Some values shown in bold do not breach the trigger but they are marked in bold as the endpoint which originated the HQ is a "smaller than" value, showing some intrinsic uncertainty.

For all of the proposed GAP uses of Spiroxamine EC 500 the HQ values based on the indicator species, *Aphidius rhopalosiphi*, are greater than the trigger value of 2 therefore further risk assessment is necessary. Furthermore, several of the HQ values for the other species are also greater than the trigger value of 2. A Tier II risk assessment has therefore been conducted and presented below.

Tier II risk assessment

Extended laboratory test data are available for two foliar-dwelling and two soil-dwelling species. This includes the two standard indicator species and then at least one additional species therefore the requirements of ESCORT 2 have been fulfilled. For *Aphidius rhopalosiphi*, two extended laboratory tests are available as well as a semi-field study. All available extended laboratory test data have been considered in the Tier II risk assessment.

The Tier II in-field risk assessments for one and two applications of 200 g a.s./ha to grapes are presented in Table 10.3.2-7 and Table 10.3.2-8, respectively. The Tier II in-field risk assessments for one and two applications of 300 g a.s./ha to grapes are presented in Table 10.3.2-9 and Table 10.3.2-10, respectively.

Table CP 10.3.2-7 Tier II in-field risk assessment for the proposed use of Spiroxamine EC 500 in grapes (1 x 200 g a.s./ha)

Intended use	Grapes		
Product	Spiroxamine EC 500		
Application rate (g a.s./ha)	1 x 200		
MAF	1.0		
Species	**LR_{50}/ER_{50} (g a.s./ha)**	**$PER_{IN-FIELD}$ (g a.s./ha)**	**<50% effects at predicted rate?**
Aphidius rhopalosiphi	>900	200	Y
Aphidius rhopalosiphi	>741		Y
Aphidius rhopalosiphi (semi-field)	>737		Y
Typhlodromus pyri	>510		Y
Bembidion tetracolum	>1482 (2 x apps)		Y
Pardosa spp	>737 (2 x apps)		Y
Coccinella septempunctata (semi-field)	>737 (2 x apps)		Y

MAF: Multiple application factor; PER: Predicted environmental rate

Table CP 10.3.2-8 Tier II in-field risk assessment for the proposed use of Spiroxamine EC 500 in grapes (2 x 200 g a.s./ha)

Intended use	Grapes		
Product	Spiroxamine EC 500		
Application rate (g a.s./ha)	2 × 200		
MAF	1.7 (foliar); 1.9 (soil)		
Species	**LR_{50}/ER_{50} (g a.s./ha)**	**$PER_{IN-FIELD}$ (g a.s./ha)**	**<50% effects at predicted rate?**
Aphidius rhopalosiphi	>900	Foliar: 340 Soil: 380	Y
Aphidius rhopalosiphi	>741		Y
Aphidius rhopalosiphi (semi-field)	>737		Y
Typhlodromus pyri	>510		Y
Bembidion tetracolum	>1482 (2 x apps)		Y
Pardosa spp	>737 (2 x apps)		Y
Cocinella septempunctata (semi-field)	>737 (2 x apps)		Y

MAF: Multiple application factor; PER: Predicted environmental rate

Table CP 10.3.2-9 Tier II in-field risk assessment for the proposed use of Spiroxamine EC 500 in grapes (1 x 300 g a.s./ha)

Intended use	Grapes		
Product	Spiroxamine EC 500		
Application rate (g a.s./ha)	1× 300		
MAF	1.0		
Species	**LR_{50}/ER_{50} (g a.s./ha)**	**$PER_{IN-FIELD}$ (g a.s./ha)**	**<50% effects at predicted rate?**
Aphidius rhopalosiphi	>900	300	Y
Aphidius rhopalosiphi	>741		Y
Aphidius rhopalosiphi (semi-field)	>737		Y
Typhlodromus pyri	>510		Y
Bembidion tetracolum	>1482 (2 x apps)		Y
Pardosa spp	>737 (2 x apps)		Y
Cocinella septempunctata (semi-field)	>737 (2 x apps)		Y

MAF: Multiple application factor; PER: Predicted environmental rate

Table CP 10.3.2-10 Tier II in-field risk assessment for the proposed use of Spiroxamine EC 500 in grapes (2 x 300 g a.s./ha)

Intended use	Grapes		
Product	Spiroxamine EC 500		
Application rate (g a.s./ha)	2 × 300		
MAF	1.7 (foliar); 1.9 (soil)		
Species	**LR_{50}/ER_{50} (g a.s./ha)**	**$PER_{IN-FIELD}$ (g a.s./ha)**	**<50% effects at predicted rate?**
Aphidius rhopalosiphi	>900	Foliar: 510 Soil: 570	Y
Aphidius rhopalosiphi	>741		Y
Aphidius rhopalosiphi (semi-field)	>737		Y
Typhlodromus pyri	>510		**N**
Bembidion tetracolum	>1482 (2 x apps)		Y
Pardosa spp	>737 (2 x apps)		Y
Cocinella septempunctata (semi-field)	>737 (2 x apps)		Y

MAF: Multiple application factor; PER: Predicted environmental rate
Criteria values shown in **bold** breach the relevant trigger

For both uses at 200 g a.s./ha and for the single application of 300 g a.s./ha the PER values are below the LR/ER_{50} values therefore it can be concluded that there will be <50% effects at the predicted in-field exposure rates and that the in-field risk to NTA populations is therefore acceptable for these uses. No further in-field risk assessment is considered to be necessary for these uses of Spiroxamine EC 500.

For two applications of 300 g a.s./ha an acceptable in-field risk has been demonstrated for all species with the exception of *Typhlodromus pyri* which indicates potential risks to NTA populations based on the soil $PER_{in-field}$ value. The LR_{50} and ER_{50} in the extended *T. pyri* study is >510 g a.s./ha and the maximum PER value for this proposed use is 570 g a.s./ha. In the *T.pyri* study there was 0% mortality (control corrected) and only a 20.9% reduction in reproduction, relative to the control, at the highest rate tested of 510 g a.s./ha. Thus, it is likely that the true LR_{50} and ER_{50} value would be greater than 570 g a.s./ha, thereby demonstrating an acceptable risk. However, this cannot be confirmed therefore a refined in-field risk assessment has been presented below.

Refined risk assessment for *T.pyri* at 2 x 300 g a.s./ha to grapes

In accordance with ESCORT 2, acceptable in-field risks can be concluded if the potential for recovery (*i.e.* LR/ER_{50} >$PER_{IN-FIELD}$) can be demonstrated within one year. In order to do this the degradation of spiroxamine residues has been taken into account in order to show that the $PER_{IN-FIELD}$ will reduce rapidly to below the toxicity threshold value of 510 g a.s./ha.

Dissipation of spiroxamine residues have been demonstrated to be relatively rapid. In several foliar decline studies on cereals the overall geomean DT_{50} value has been determined to be 3.03 days (M-759383-01-1) and in study M-090880-01-1, residues were measured on potential food items associated with vineyards which gave a DT_{50} of *ca.* 4 days for weed heads. These values are considered to demonstrate the rapid dissipation of spiroxamine residues in the environment.

Acceptable risk to foliar NTAs has already been demonstrated in the Tier II risk assessment but potential concern was indicated for soil-dwelling NTAs. The DT_{50} of 4 days has therefore been taken as an illustrative example of the decline in residues that soil-dwelling organisms may be exposed to following the application of Spiroxamine EC 500. The table below presents the PER values for soil over time considering a DT_{50} of 4 days.

Table CP 10.3.2-11 Decline in $PER_{in\text{-}field}$ values for soil following application of Spiroxamine EC 500

Day from last application	Soil PER (g a.s./ha)	Risk acceptable? (PER <LR/ER_{50})
0	570	N
1	479	Y
2	403	Y
3	339	Y
4	285	Y
5	240	Y
6	202	Y
7	169	Y

It is clear that after only one day following the last application of Spiroxamine EC 500 (2 x 300 g a.s./ha) that the predicted exposure will have reduced to a level below the ER_{50} and LR_{50} value of 510 g a.s./ha which would thereby allow for an acceptable risk to NTA populations, based on the extended *T. pyri* test results, to be concluded as the potential for recovery has been demonstrated in well under one year.

Even if the worst case reported soil DT_{50} value of 56.6 days from the Environmental Fate Document M-CA Section 7 is used in the calculations, the $PER_{in\text{-}field}$ value reduces to below 510 g a.s./ha after only a 10-day period. Thus, it is considered that the potential for recovery has been demonstrated here and that the in-field risk to NTA populations following two applications of 300 g a.s./ha are acceptable.

Risk assessment for off-field exposure

Effects on non-target terrestrial plants are of concern in the off-field environment, where plants may be exposed to spray drift. The amount of spray drift reaching off-crop habitats is calculated using the appropriate percentile estimates, which depends on the number of applications, and is derived from the BBA (2000[12]) values from the spray drift predictions of Ganzelmeier & Rautmann (2000[13]).

Off-field foliar PER values have been calculated from in-field foliar PERs in conjunction with drift values as shown in the following equation:

$$\text{Off-field PER} = \frac{PER_{IN\text{-}FIELD} \times (\%\ \text{drift}/100)}{\text{Vegetation distribution factor}}$$

The model used to estimate spray drift was developed for drift onto a two-dimensional water surface and, as such, does not account for interception and dilution by three-dimensional vegetation in off-crop areas. Therefore, a vegetation distribution or dilution factor is incorporated into the equation when calculating PERs to be used in conjunction with toxicity endpoints derived from two-dimensional (glass plate or leaf disc) studies. A dilution factor of 10 is recommended by ESCORT 2. For 3-dimensional studies, *i.e.* where spray treatment is applied onto whole plants, the dilution factor of 10 is not used, as any dilution over the 3-dimensional vegetation surface is accounted for in the study design.

[12] BBA (2000) Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Public domain.

[13] Ganzelmeier H., Rautmann D. (2000) Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application. Public domain.

The drift rate (predicted environmental rate, $PER_{off\text{-}field}$) associated with grapes has been calculated based on spray drift predictions for one application using 90th percentile drift values and for two applications using 82nd percentile drift values. This gives drift rates of 8.02% at 3 m for grapes (late) and 7.23% at 3 m for grapes (late) for one and two applications, respectively. These equate to drift factors of 0.0802 and 0.0723 for one and two applications, respectively.

The predicted exposure rates for off-field exposure ($PER_{off\text{-}field}$) have been calculated according to ESCORT II and summarised in the table below. The default distance of 3 m for vines has been considered in the calculation of the PER, both with and without a vegetation distribution factor (to be used in conjunction with two dimensional and three dimensional exposure test data, respectively).

Table CP 10.3.2-12 PER for off-field exposure following the uses of Spiroxamine EC 500

Crop	App. rate (g a.s./ha)	$PER_{IN\text{-}FIELD}$ (foliar) (g a.s./ha)	% Drift (percentile)	Drift factor (% drift/100)	Vegetation distribution factor (VDF)	$PER_{OFF\text{-}FIELD}$ (g a.s./ha)	
						without VDF [1]	with VDF [2]
Grapes	1 x 200	200	8.02 (90th)	0.0802	10	16.0	1.60
	2 x 200	340	7.23 (82nd)	0.0723		24.6	2.46
	1 x 300	300	8.02 (90th)	0.0802		24.1	2.41
	2 x 300	510	7.23 (82nd)	0.0723		36.9	3.69

[1] For lab test endpoints obtained with 3-D exposure, directly comparable to the distribution of spray drift deposit in 3-D vegetated off-field environment

[2] With dilution factor of 10 for lab test endpoints obtained with 2-D exposure, to adjust for distribution of spray drift deposit in 3-D vegetated off-field environment

For the risk assessment, the predicted environmental rate is compared with the toxicity endpoints according to the following formula:

$$\text{Off-field HQ} = \frac{PER_{OFF\text{-}FIELD}\ (g\ a.s./ha)}{ER_{50}\ (g\ a.s./ha)} \times \text{Correction factor}$$

The HQ trigger for Tier I laboratory studies is 2. For the extended laboratory Tier II studies, the risk is considered acceptable if the $PER_{OFF\text{-}FIELD}$ concentrations are below the test concentrations resulting in 50% effects.

ESCORT 2 recommends that a correction factor of 5 be used when assessing extended laboratory studies, or 10 for Tier I data, to account for extrapolation from testing just two representative species, to the species diversity expected in off-crop areas.

The Tier I off-field risk assessments for one and two applications of 200 g a.s./ha to vines are presented in Table 10.3.2-12 and Table 10.3.2-13, respectively. The Tier I off-field risk assessments for one and two applications of 300 g a.s./ha to grapes are presented in Table 10.3.2-14 and Table 10.3.2-15, respectively.

Table CP 10.3.2-13 Tier I off-field risk assessment for the proposed use of Spiroxamine EC 500 in grapes (1 x 200 g a.s./ha)

Intended use	Grapes			
Product	Spiroxamine EC 500			
Application rate (g a.s./ha)	1 × 200			
MAF	1.0			
Test species Tier I	**LR_{50} (g a.s./ha)**	**$PER_{OFF-FIELD}$ (g a.s./ha)**	**Corrected $PER_{OFF-FIELD}$[1] (g a.s./ha)**	**$HQ_{OFF-FIELD}$**
Aphidius rhopalosiphi	80.1	1.60	16.0	0.200
Typhlodromus pyri	240			0.0667
Bembidion tetracolum	>741			<0.0216
Pardosa agricola	<731			**>0.0219**
Chrysoperla carnea	<737			>**0.0217**
Chrysoperla carnea	>1600			<0.0100
Coccinella septempunctata	<731			**>0.0219**
Coccinella septempunctata	>750			<0.0213

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient
Criteria values shown in **bold** potentially breach the relevant trigger (criterion HQ ≥ 2). Some values shown in bold do not breach the trigger but they are marked in bold as the endpoint which originated the HQ is a "smaller than" value, showing some intrinsic uncertainty.
[1] Correction factor of 10 applied for use with standard laboratory data to account for the inter-species variability in sensitivity (ESCORT 2)

Table CP 10.3.2-14 Tier I off-field risk assessment for the proposed use of Spiroxamine EC 500 in grapes (2 x 200 g a.s./ha)

Intended use	Grapes			
Product	Spiroxamine EC 500			
Application rate (g a.s./ha)	2 × 200			
MAF	1.7 (foliar)			
Test species Tier I	**LR_{50} (g a.s./ha)**	**$PER_{OFF-FIELD}$ (g a.s./ha)**	**Corrected $PER_{OFF-FIELD}$[1] (g a.s./ha)**	**$HQ_{OFF-FIELD}$**
Aphidius rhopalosiphi	80.1	2.46	24.6	0.307
Typhlodromus pyri	240			0.103
Bembidion tetracolum	>741			<0.0332
Pardosa agricola	<731			**>0.0337**
Chrysoperla carnea	<737			>**0.0334**
Chrysoperla carnea	>1600			<0.0154
Coccinella septempunctata	<731			**>0.0337**
Coccinella septempunctata	>750			<0.0328

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient

Criteria values shown in **bold** potentially breach the relevant trigger (criterion HQ ≤ 2). Some values shown in bold do not breach the trigger but they are marked in bold as the endpoint which originated the HQ is a "smaller than" value, showing some intrinsic uncertainty.
[1] Correction factor of 10 applied for use with standard laboratory data to account for the inter-species variability in sensitivity (ESCORT 2)

Table CP 10.3.2-15 Tier I off-field risk assessment for the proposed use of Spiroxamine EC 500 in grapes (1 x 300 g a.s./ha)

Intended use	Grapes			
Product	Spiroxamine EC 500			
Application rate (g a.s./ha)	1 × 300			
MAF	1.0			
Test species Tier I	**LR_{50} (g a.s./ha)**	**$PER_{OFF-FIELD}$ (g a.s./ha)**	**Corrected $PER_{OFF-FIELD}$[1] (g a.s./ha)**	**$HQ_{OFF-FIELD}$**
Aphidius rhopalosiphi	80.1	2.41	24.1	0.301
Typhlodromus pyri	240			0.100
Bembidion tetracolum	>741			<0.0325
Pardosa agricola	<731			**>0.0330**
Chrysoperla carnea	<737			>**0.0327**
Chrysoperla carnea	>1600			<0.0151
Coccinella septempunctata	<731			**>0.0330**
Coccinella septempunctata	>750			<0.0321

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient
Criteria values shown in **bold** potentially breach the relevant trigger (criterion HQ ≤ 2). Some values shown in bold do not breach the trigger but they are marked in bold as the endpoint which originated the HQ is a "smaller than" value, showing some intrinsic uncertainty.
[1] Correction factor of 10 applied for use with standard laboratory data to account for the inter-species variability in sensitivity (ESCORT 2)

Table CP 10.3.2-16 Tier I off-field risk assessment for the proposed use of Spiroxamine EC 500 in grapes (2 x 300 g a.s./ha)

Intended use	Grapes			
Product	Spiroxamine EC 500			
Application rate (g a.s./ha)	2 × 300			
MAF	1.7 (foliar)			
Test species Tier I	**LR_{50} (g a.s./ha)**	**$PER_{OFF-FIELD}$ (g a.s./ha)**	**Corrected $PER_{OFF-FIELD}$[1] (g a.s./ha)**	**$HQ_{OFF-FIELD}$**
Aphidius rhopalosiphi	80.1	3.69	36.9	0.461
Typhlodromus pyri	240			0.154
Bembidion tetracolum	>741			<0.0498
Pardosa agricola	<731			**>0.0505**
Chrysoperla carnea	<737			>**0.0501**
Chrysoperla carnea	>1600			<0.0231

Coccinella septempunctata	<731			**>0.0505**
Coccinella septempunctata	>750			<0.0492

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient
Criteria values shown in **bold** potentially breach the relevant trigger (criterion HQ ≤ 2). Some values shown in bold do not breach the trigger but they are marked in bold as the endpoint which originated the HQ is a "smaller than" value, showing some intrinsic uncertainty.
[1] Correction factor of 10 applied for use with standard laboratory data to account for the inter-species variability in sensitivity (ESCORT 2)

For all of the proposed GAP uses of Spiroxamine EC 500 the HQ values based on the *Pardosa*, *Chrysoperla* and *Coccinella* data cannot confirm acceptable risks due to the 'less than' toxicity values. A Tier II risk assessment has therefore been conducted and presented below.

Tier II risk assessment

The Tier II off-field risk assessments for one and two applications of 200 g a.s./ha to grapes are presented in Table 10.3.2-16 and Table 10.3.2-17, respectively. The Tier II off-field risk assessments for one and two applications of 300 g a.s./ha to grapes are presented in Table 10.3.2-18 and Table 10.3.2-19, respectively.

Table CP 10.3.2-17 Tier II off-field risk assessment for the proposed use of Spiroxamine EC 500 in grapes (1 x 200 g a.s./ha)

Intended use	Grapes			
Product	Spiroxamine EC 500			
Application rate (g a.s./ha)	1 × 200			
MAF	1.0			
Species	**LR_{50}/ER_{50} (g a.s./ha)**	**$PER_{OFF-FIELD}$ (g a.s./ha)**	**Corrected $PER_{OFF-FIELD}$[2] (g a.s./ha)**	**<50% effects at predicted rate?**
Aphidius rhopalosiphi	>900	16.0	80.0	Y
Aphidius rhopalosiphi	>741	16.0[1]	80.0	Y
Aphidius rhopalosiphi (semi-field)	>737	16.0[1]	80.0	Y
Typhlodromus pyri	>510	1.60	8.0	Y
Bembidion tetracolum	>1482 (2 x apps)	1.60	8.0	Y
Pardosa spp	>737 (2 x apps)	1.60	8.0	Y
Cocinella septempunctata (semi-field)	>737 (2 x apps)	16.0[1]	80.0	Y

MAF: Multiple application factor; PER: Predicted environmental rate
[1] Study incorporated 3-Dimensional exposure therefore no vegetation distribution factor (VDF) applied
[2] Correction factor of 5 applied for use with extended laboratory data to account for the inter-species variability in sensitivity (ESCORT 2)

Table CP 10.3.2-18 Tier II off-field risk assessment for the proposed use of Spiroxamine EC 500 in grapes (2 x 200 g a.s./ha)

Intended use	Grapes			
Product	Spiroxamine EC 500			
Application rate (g a.s./ha)	2 × 200			
MAF	1.7 (foliar); 1.9 (soil)			
Species	**LR_{50}/ER_{50} (g a.s./ha)**	**$PER_{OFF-FIELD}$ (g a.s./ha)**	**Corrected $PER_{OFF-FIELD}$[2] (g a.s./ha)**	**<50% effects at predicted rate?**
Aphidius rhopalosiphi	>900	24.6[1]	123	Y
Aphidius rhopalosiphi	>741	24.6[1]	123	Y
Aphidius rhopalosiphi (semi-field)	>737	24.6[1]	123	Y
Typhlodromus pyri	>510	2.46	12.3	Y
Bembidion tetracolum	>1482 (2 x apps)	2.46	12.3	Y
Pardosa spp	>737 (2 x apps)	2.46	12.3	Y
Cocinella septempunctata (semi-field)	>737 (2 x apps)	24.6[1]	123	Y

MAF: Multiple application factor; PER: Predicted environmental rate
[1] Study incorporated 3-Dimensional exposure therefore no vegetation distribution factor (VDF) applied
[2] Correction factor of 5 applied for use with extended laboratory data to account for the inter-species variability in sensitivity (ESCORT 2)

Table CP 10.3.2-19 Tier II off-field risk assessment for the proposed use of Spiroxamine EC 500 in grapes (1 x 300 g a.s./ha)

Intended use	Grapes			
Product	Spiroxamine EC 500			
Application rate (g a.s./ha)	1 × 300			
MAF	1.0			
Species	**LR_{50}/ER_{50} (g a.s./ha)**	**$PER_{OFF-FIELD}$ (g a.s./ha)**	**Corrected $PER_{OFF-FIELD}$[2] (g a.s./ha)**	**<50% effects at predicted rate?**
Aphidius rhopalosiphi	>900	24.1[1]	121	Y
Aphidius rhopalosiphi	>741	24.1[1]	121	Y
Aphidius rhopalosiphi (semi-field)	>737	24.1[1]	121	Y
Typhlodromus pyri	>510	2.41	12.1	Y
Bembidion tetracolum	>1482 (2 x apps)	2.41	12.1	Y
Pardosa spp	>737 (2 x apps)	2.41	12.1	Y
Cocinella septempunctata (semi-field)	>737 (2 x apps)	24.1[1]	121	Y

MAF: Multiple application factor; PER: Predicted environmental rate
[1] Study incorporated 3-Dimensional exposure therefore no vegetation distribution factor (VDF) applied
[2] Correction factor of 5 applied for use with extended laboratory data to account for the inter-species variability in sensitivity (ESCORT 2)

Table CP 10.3.2-20 Tier II off-field risk assessment for the proposed use of Spiroxamine EC 500 in grapes (2 x 300 g a.s./ha)

Intended use	Grapes			
Product	Spiroxamine EC 500			
Application rate (g a.s./ha)	2 × 300			
MAF	1.7 (foliar); 1.9 (soil)			
Species	**LR_{50}/ER_{50} (g a.s./ha)**	**$PER_{OFF-FIELD}$ (g a.s./ha)**	**Corrected $PER_{OFF-FIELD}$[2] (g a.s./ha)**	**<50% effects at predicted rate?**
Aphidius rhopalosiphi	>900	36.9[1]	185	Y
Aphidius rhopalosiphi	>741	36.9[1]	185	Y
Aphidius rhopalosiphi (semi-field)	>737	36.9[1]	185	Y
Typhlodromus pyri	>510	3.69	18.5	Y
Bembidion tetracolum	>1482 (2 x apps)	3.69	18.5	Y
Pardosa spp	>737 (2 x apps)	3.69	18.5	Y
Cocinella septempunctata (semi-field)	>737 (2 x apps)	36.9[1]	185	Y

MAF: Multiple application factor; PER: Predicted environmental rate
[1] Study incorporated 3-Dimensional exposure therefore no vegetation distribution factor (VDF) applied
[2] Correction factor of 5 applied for use with extended laboratory data to account for the inter-species variability in sensitivity (ESCORT 2)

It is clear that for all proposed uses of Spiroxamine EC 500 that the $PER_{OFF-FIELD}$ values are below the LR_{50} and ER_{50} values for all species for which data are available. Thus, the off-field risk to NTA populations following use of Spiroxamine EC 500 is acceptable.

Biodiversity

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on non-target arthropods (other than bees). Therefore, it is considered that the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects *via* alteration of the food web, are covered by the risk assessment for non-target arthropods (other than bees) in this section.

With respect to the NTA in-field and NTA off-field risk assessments, which demonstrated acceptable in-field risks at tier 1 and tier 2 level for NTA and acceptable off-field risks for NTA without the need for risk mitigation, the applicant concludes that the use of the representative lead formulation (Spiroxamine EC 500) has a low potential to cause unacceptable effects on biodiversity and the ecosystem *via* trophic interactions. To the best of our knowledge and with the presented safety profile of the spiroxamine a.s. and the representative lead formulation, the applicant does not foresee any unacceptable effects on biodiversity and the ecosystem with spiroxamine.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

Data Point:	KCP 10.3.2.1/01
Report Author:	[redacted]; [redacted]
Report Year:	2000
Report Title:	KWG 4168 EC 500: A laboratory dose-response study to evaluate the effects on the predaceous mite Typhlodromus pyri Scheuten (Acari: Phytoseiidae) in ventilated glass cages
Report No:	B020TPL
Document No:	M-025030-01-1
Guideline(s) followed in study:	Bakker et al. (1992), Baier et al., ungtested guideline in prep, Overmeer (1988), SETAC/ESCORT (1994).
Deviations from current test guideline:	3 replicates used per test item treatment instead of 5
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The effects of a repeated spray application of 80 to 600 g a.s./ha KWG 4168 EC 500 on the mortality and reproduction of *Typhlodromus pyri* Scheuten were tested over 7 and 14 days, respectively.

Statistically significant increases were observed in mortality, when compared to the control, at dose concentrations of 180, 250, 400 and 600 g a.s./ha. These doses resulted in corrected mortalities of 25, 59, 90 and 94%, respectively. At a dose rate of 180 g a.s./ha a statistically significant reduction of 46% in the mean number of eggs/capita/day was observed, when compared to the control.

The LR_{50} of KWG 4168 EC 500 to the predatory mite, *Typhlodromus pyri*, was 240 g a.s./ha. The ER_{50} was considered to be >180 g a.s./ha.

The NOER for mortality and fecundity was 120 g a.s./ha KWG 4168 EC 500.

I. Materials and Methods

Materials

Test Material KWG 4168 EC 500

Lot/Batch #: 04931025/ 0697

Purity: 509.5 g/L

Description: EC

Stability of test compound: Not reported

Reanalysis/Expiry date: 3 August 1999

Density: Not reported

Treatments

Test rates: 80, 120, 180, 250, 400 and 600 g a.s./ha

Solvent/vehicle: Water

Analysis of test concentrations: No

Test organisms

Species:	*Typhlodromus pyri* Scheuten (Acari: Phytoseiidae)
Source:	Strain bred in the lab, originally from apple in the Province of Zeeland, The Netherlands
Acclimatisation period:	1-day old protonymphs were kept under same conditions as breeding cultures
Feeding:	Pollen of broad bean. Test organisms fed every 2-4 days throughout.
Treatment for disease:	None reported

Test design

Test vessel:	Ventilated glass cage consisting of a bottom glass plate (10 x 5 x 0.3 cm; 3 holes = 0.6cm), a top glass plate (10 x 5 x 0.15 cm), a middle part of inert material (10 x 5 x 0.3 cm, inner open surface approximately 2.4 x 7.5 cm, slope of inner side 45°). Test units for determining effects on oviposition rates were glass plates (9 cm) of which an inner circle (2.8 cm) was treated on day 0 together with the glass cages. These glass plates were stored under similar conditions as the glass cages during the mortality phase
Test medium:	Water
Replication:	3 (6 test concentrations, water control, toxic standard)
No. animals/vessel:	20
Duration of test:	14 days

Environmental test conditions

Temperature:	26.3 ± 0.5°C during exposure phase, 25.7 ± 0.4°C during fecundity phase.
Photoperiod:	Photoperiod of 18 hour light : 6 hour dark

Study Design

This study was conducted in order to assess the effects of KWG 4168 EC 500 on the reproduction and mortality of *Typhlodromus pyri* over 14 days.

The test organisms used were one day old at the start of the study and both male and female mites were used.

Glass test vessels and glass oviposition vessels were sprayed with dilutions of the test concentration (80, 120, 180, 250, 400 and 600 g a.s./ha), and the toxic standard. The mean spray deposit on the glass surface was 2.09 mg/cm^2. After the residues had dried 20 *T. pyri* were added to each replicate vessel. A water control group was used. For the toxic standard, dimethoate was used at a rate of approximately 0.6 mL a.s./ha.

The test was split into two phases. After 7 days, mortality was recorded within the groups of *T. pyri* (exposure phase). The surviving *T. pyri* were then transferred to the oviposition vessels where reproduction was calculated after a further 7 days (fecundity phase). The fecundity phase was conducted at the 120 and 180 g a.s./ha rates only.

Temperature was measured at 8-hour intervals during the exposure phase and continuously during the fecundity phase to ensure that test vessels were kept at 26.5 ± 0.5°C and 25.7 ± 0.4°C, respectively. Light intensity was measured once during each phase.

II. Results and Discussion

Validity criteria according to the guidelines to which the study was conducted were met:

- Mean mortality (dead and escaped) to be <20% in the control on day 7 (actual: 15%);
- Cumulative mean number of eggs produced per female in the control between days 7 and 14 to be ≥4 (actual: 9.94);
- Cumulative mean mortality (corrected) of organisms exposed to the toxic standard to be ≥50% on day 7 (actual: 100%).

Corrected mortality refers to mortality due to the test item and excludes other causes of mortality, such as escaped mites. At the highest dose rate, 94% of test organisms died due to exposure to KWG 4168 EC 500. Statistically significant increases were observed in mortality at dose concentrations of 180, 250, 400 and 600 with mortalities of 25, 59, 90 and 94%, respectively.

Table CP 10.3.2.1/01-1 Summary of mortality

KWG 4168 EC 500 (g a.s./ha)	Mean (%)	Standard deviation (%)	Corrected mortality (%)
Water control	15	10	0
Toxic standard	100	0	100
80	17	13	2
120	15	5	0
180	37	15	25*
250	65	18	59*
400	92	10	90*
600	95	5	94*

* Statistically different from water control performance

At a dose rate of 180 g a.s./ha a statistically significant reduction of 46% in the mean number of eggs/capita/day was observed, when compared to the control.

Table CP 10.3.2.1/01-2 Summary of mean number eggs per capita, produced daily

KWG 4168 EC 500 (g a.s./ha)	Observation day					
	7	9	12	14	Cumulative total per female	Reduction relative to control
Water control	0.28	1.10	1.37	1.68	9.94	-
120	0.00	0.57	1.08	1.25	6.87	31%
180	0.00	0.20	0.90	1.12	5.37	46%*

* Statistically different from water control performance

III. Conclusion

The LR_{50} of KWG 4168 EC 500 to the predatory mite *Typhlodromus pyri* is 240 g a.s./ha with 95% confidence limits of 216 to 266 g a.s./ha.

The NOER for mortality and fecundity of KWG 4168 EC 500 on the predatory mite *Typhlodromus pyri* was 120 g a.s./ha. At a dose rate of 180 g a.s./ha a statistically significant reduction of 46% in the mean number of eggs/capita/day was observed, when compared to the control. The ER_{50} was therefore considered to be >180 g a.s./ha.

Assessment and conclusion by applicant:

Validity criteria according to the current IOBC test method by Blümel *et al*. (2000) "Laboratory, residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products" were met:

- Mean mortality (dead and escaped) to be <20% in the control on day 7 (actual: 13%);
- Cumulative mean number of eggs produced per female in the control between days 7 and 14 to be ≥4 (actual: 9.94);
- Cumulative mean mortality (corrected) of organisms exposed to the toxic standard to be ≥50% on day 7 (actual: 100%).

The study was conducted before the IOBC test methods were formalised but the test method used in this study is highly consistent with the method of Blümel *et al*. (2000) and the current validity criteria have all been met. The study is therefore considered acceptable. It is noted that this test used only three replicates per test item treatment whereas the current test method recommends five replicates. The results are still considered to be suitable for use in the Tier I risk assessment. It should also be noted that a more recent extended laboratory study is also available.

The LR_{50} was determined to be 240 g a.s./ha. The ER_{50} was considered to be >180 g a.s./ha.

Data Point:	KCP 10.3.2.1/02
Report Author:	[redacted]
Report Year:	1994
Report Title:	Testing the effect of KWG 4168 on the predaceous mite Typhlodromus pyri Scheuten (Acari: Phytoseiidae) using ventilated glass cages (coffin cells)
Report No:	MB007
Document No:	M-008573-01-1
Guideline(s) followed in study:	IOBC-Working Group "Pesticides and Beneficial Arthropods"
Deviations from current test guideline:	This study does not comply with current guidelines.
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

Two strains of *Typhlodromus pyri* were exposed to KWG 4168 to assess the effects on reproduction and mortality. One strain had a degree of OP-resistance and the other was sensitive to organophaphates (OP).

Both strains were exposed to residues equivalent to a rate of 741 g a.s./ha and the tolerant strain was also exposed to the reference substance (parathion at 2.67 g a.s./ha) as well as a water only control.

Mean juvenile mortality in the KWG 4168-treated group of OP-tolerant and OP-sensitive individuals were 99.0 and 95.7%, respectively. Juvenile mortality of the water treated group is significantly different

from the juvenile mortality in all the three other treatments. The average number of eggs/female/sampling period was 0.7 for the OP-sensitive females exposed to KWG 4168.

The LR_{50} was therefore determined to be <741 g a.s./ha.

I. Materials and Methods

Materials

Test Material	KWG 4168 EC 500
Lot/Batch #:	089A
Purity:	494.0 g/L
Description:	Not reported
Stability of test compound:	Not reported
Reanalysis/Expiry date:	17 March 1994
Density:	1.004 g/mL
Treatments	
Test rates:	741 g a.s./ha (7410 ppm a.s. (w/v)
Solvent/vehicle:	water
Analysis of test concentrations:	No
Test organisms	
Species:	Two strains of *Typhlodromus pyri*
Source:	OP-resistant strain collected from apple in the Province of Zeeland, The Netherlands. OP-sensitive strain originates from individuals collected from chestnut in Amsterdam.
Acclimatisation period:	None reported
Feeding:	Pollen of broad bean, *Vicia faba* I.
Treatment for disease:	None reported
Test design	
Test vessel:	'Coffin cells': bottom glass plate (10 x 5 x 0.3 cm; 3 holes 0 = 0.6 cm), a top glass plate (10 x 5 x 0.15 cm), two long glass sides (7.6 x 1.2 x 0.3 cm), two short glass sides
Replication:	4
No. animals/vessel:	20 to 25
Duration of test:	2 weeks
Environmental test conditions	
Temperature:	25.2 ± 0.51°C

Photoperiod: Continuous darkness

Study Design

This study was conducted in order to assess the effects on mortality and reproduction of residues of KWG 4168 EC 500 on *T. pyri* over 14 days.

Two strains of *T. pyri* were used, one organo-phosphate (OP)-resistant and one OP-sensitive and a mixture of larvae and protonymphs that differed maximally 24 hours of age were used in the study.

These test organisms were placed in 'coffin cells' that had been sprayed with 7410 ppm KWG 4168 EC 500, with 5 replicates per treatment group. The cells were kept at 25.2 ± 0.42°C during the mortality phase of the study and at 25.2 ± 0.51°C during the reproduction phase, measured at 8-h intervals and kept in continuous darkness. Food (broad bean pollen) was administered throughout the study.

Mortality was assessed after 3 and 7 days. Eggs from surviving females were counted on day 7, 10 and 14.

II. Results and Discussion

No control validity criteria have been reported or assessed in the study report. The study report d Validity criteria according to guidelines by Blümel *et al.* (2000) "laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products". Some of the criteria were met:

No female individuals of the tolerant strain survived the juvenile exposure to KWG 4168. Therefore, no effect on adult reproduction could be determined.

Mean juvenile mortality in the KWG 4168-treated group of OP-tolerant and OP-sensitive individuals were 99.0 and 95.7% respectively. Juvenile mortality of the water treated group is significantly different from the juvenile mortality in all the three other treatments. The average number of eggs/female/sampling period was 0.7 for the OP-sensitive females exposed to KWG 4168.

Table CP 10.3.2.1/02-1 Mortality and productivity of *T. pyri* exposed to residues of KWG 4168 EC 500 residues

Test item	Mean mortality (%)	± Standard deviation	Eggs/female
Water control	14.2	9.7	1.9
KWG 4168 OP-Tolerant (741 g a.s./ha)	99.0*	1.8	-
KWG 4168 OP-Sensitive (741 g a.s./ha)	95.7*	3.0	0.7
Reference item	54.2*	17.4	-

* statistically significant

III. Conclusion

The LR_{50} of *T. pyri* exposed to KWG 4168 EC 500 was determined to be <741 g a.s./ha.

Assessment and conclusion by applicant:

Validity criteria according to the current IOBC test guideline by Blümel *et al*. (2000) "laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products" have been assessed. Some of the criteria were met:

- Cumulative mean mortality (corrected) of organisms exposed to the toxic standard ≥50% on day 7 (actual: 54.2%)

- Mean mortality (dead and escaped) <20% in the control on day 7 (actual: 14.2%)

The following criterion from the guidelines were not met:

- Cumulative mean number of eggs produced per female, in the control between days 7 and 14, ≥ 4 (actual: 1.9)

Not all of the validity criteria have been met but, according to Blümel *et al*. (2000), reproductive assessments should not be conducted if the mortality is >50%. Thus, the reproductive criterion is not considered appropriate here therefore the study is considered to be valid. This study pre-dates the IOBC test guidelines and the test method used in this study is no longer the current test method. However, the results are still considered suitable for use in the risk assessment, as supporting information, to demonstrate the fact that the Tier I LR_{50} is <741 g a.s./ha.

Data Point:	KCP 10.3.2.1/03
Report Author:	[redacted]
Report Year:	2000
Report Title:	A laboratory test to determine the effects of spiroxamine EC 500 on the parasitic wasp, Aphidius rhopalosiphi
Report No:	BAY499-17
Document No:	M-030680-01-1
Guideline(s) followed in study:	ESCORT (Barrett al.), 1994
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Parasitic wasps, *Aphidius rhopalosiphi*, were exposed to Spiroxamine EC 500 at concentrations of 30, 60, 120, 240 and 480 g a.s./ha. The wasps were exposed for 48-hours to assess the effects on mortality and reproduction.

Mortalities compared to the control were observed at 0, 33, 89, 100 and 100% in the 30, 60, 120, 240 and 480 g a.s./ha treatment rates of Spiroxamine EC 500, respectively. Statistically significant reductions in fecundity were observed at concentrations of 30 and 60 g a.s./ha.

The LR_{50} after 48 hours exposure was calculated to be equivalent to 80.1 g a.s./ha (with 95% confidence limits of 63.7 and 100.4 g a.s./ha). The ER_{50} was considered to be >30 g a.s./ha.

I. Materials and Methods

Materials

Test Material	Spiroxamine EC 500
Lot/Batch #:	0778
Purity:	498.5 g/L
Description:	Not reported

Stability of test compound:	Not reported
Reanalysis/Expiry date:	21 April 2000
Density:	1.005 g/mL
Treatments	
Test rates:	30, 60, 120, 240 and 480 g a.s./ha (60, 120, 240, 480 and 960 mL product/ha)
Solvent/vehicle:	Water
Analysis of test concentrations:	No
Test organisms	
Species:	Wasp, *Aphidius rhopalosiphi*, (Hymenoptera: Braconidae)
Source:	PK Niitzlingszuchten, Industriestrasse 38, 73642 Welzheim, Germany
Acclimatisation period:	Not reported
Feeding:	Exposure phase: 1:3 honey/water solution Fecundity phase: *Rhopalosiphum padi* and *Metopolophium dirhodum* Wlk
Treatment for disease:	None reported
Test design	
Test vessel:	Treated glass plates fitted to a square frame (10 x 10 cm external dimensions) made from aluminium casing (1.8 x 0.5 cm in cross-section)
Replication:	3
No. animals/vessel:	10
Duration of test:	72 hours
Environmental test conditions	
Temperature:	19 - 22°C
Photoperiod:	4700 - 6500 lux for a 16 h photoperiod.

Study Design

This study was conducted in order to assess the effects on mortality and fecundity of Spiroxamine EC 500 on wasps (*Aphidius rhopalosiphi*) over 48 hours.

The adult wasps used in the bioassay were less than 24 hours old. These were placed individually onto glass in steel frames that had been treated and dried. Ten wasps were exposed to each treatment concentration per replicate.

The test item was applied to glass plates at concentrations equivalent to 30, 60, 120, 240 and 480 g a.s./ha (60, 120, 240, 480 and 960 mL product/ha). A water control was used as was a reference item, Dimethoate 40 (400 g/L dimethoate) applied at 0.12 g a.s./ ha (0.3 mL product/ha).

The condition of the wasps was assessed at 2, 24 and 48 h. They were recorded as being:

- Live - alive and apparently unaffected
- Affected - still upright and attempting to walk but showing signs of reduced co-ordination
- Moribund - on their back or side, still twitching
- Dead - no longer moving

An assessment was then made on the fecundity of the surviving wasps, using females from the control and from the treatment groups of the test item at which <50% mortality was seen. The wasps (10 per treatment) were confined individually with aphid-infested barley plants (untreated) for a further 24 hours and then removed. The numbers of parasitised aphids that developed were recorded 11 days later.

II. Results and Discussion

Validity criteria according to the study report were met.

- Mortality in the control group ≤13.3% (actual: 10%)
- Mortality in the reference item group ≥50% (actual: 100%)
- Mummies per female produced in the control group ≥5 (actual: 92.6)
- Number of female wasps producing no mummies ≤2 (actual: 0)

In the control treatment, three wasps were dead at 48 hours (10% mortality). This compared with mortalities of 0, 33, 89, 100 and 100% in the 30, 60, 120, 240 and 480 g a.s./ha treatment rates of Spiroxamine EC 500, respectively. The 48-h LR_{50} for Spiroxamine EC 500 against *Aphidius rhopalosiphi* was calculated to be 80.1 g a.s./ha (95% CL: 63.7 - 100.1 g a.s./ha).

All of the wasps in the toxic reference treatment were dead at 48 hours (100% mortality).

Table CP 10.3.2.1/03-1 Mortality (%) of wasps after exposure to Spiroxamine EC 500 for 48 hours

Concentrations (g a.s./ha)	**Mortality (%)**
Control	10
Concentrations (g a.s./ha)	**Corrected mortality (%)**
480	100
240	100
120	89
60	33
30	0
Reference item	100

Fecundity was assessed for the 30 and 60 g a.s./ha treatment groups as these had mortality less than 50%.

The mean number of mummies produced per female was 92.6 in the control and 60.7 and 35.3 in the 30 and 60 g a.s./ha treatment rates of Spiroxamine EC 500, respectively. Thus, reduction in reproduction relative to the control was 34% and 62% for the 30 and 60 g a.s./ha treatments, respectively. These were both statistically significant. The ER_{50} was considered to be >30 g a.s./ha.

Table CP 10.3.2.1/03-2 Fecundity of wasps after exposure to Spiroxamine EC 500 for 48- hours.

Concentrations (g a.s./ha)	**Mean number mummies produced per female**	**Standard deviation**
Control	92.6	30.6

Concentrations (g a.s./ha)	Mean number mummies produced per female	Standard deviation
30	60.7*	15.6
60	35.3*	11.3

* statistically significant

III. Conclusion

The 48-h LR_{50} for Spiroxamine EC 500 against *Aphidius rhopalosiphi* was calculated to be 80.1 g a.s./ha.

Both of the treatment rates of Spiroxamine EC 500 (30 and 60 g a.s./ha) that were evaluated for sub-lethal effects showed significantly reduced fecundity, relative to the control treatment wasps. The ER_{50} was considered to be >30 g a.s./ha.

Assessment and conclusion by applicant:

Validity criteria according to the current IOBC test method by Mead-Brigs *et al.* (2000) "A laboratory test for evaluating the effects of plant protection products on the parasitic wasp *Aphidius rhopalosiphi* (DeStephani-Perez) (Hymenoptera: Braconidae)" were met.

- Mortality in the control group ≤13% (actual: 10%)
- Mortality in the reference item group ≥50% (actual: 100%)
- Mummies per female produced in the control group ≥5 (actual: 92.6)
- Number of female wasps producing no mummies ≤2 (actual: 0)

The study was conducted before the IOBC test methods were formalised but the test method used in this study is highly consistent with the method of Mead-Brigs *et al.* (2000) and the current validity criteria have all been met. The study is therefore considered acceptable. It is noted that this test used only three replicates per test item treatment whereas the current test method recommends four replicates. The results are still considered to be suitable for use in the Tier I risk assessment. It should also be noted that a more recent extended laboratory study is also available.

The LR_{50} was calculated to be 80.1 g a.s./ha. The ER_{50} was considered to be >30 g a.s./ha.

Data Point:	KCP 10.3.2.1/04
Report Author:	[redacted]
Report Year:	1995
Report Title:	Effects of KWG 4168 EC 500 on the life cycle of ladybird beetles (Coccinella septempunctata) under laboratory conditions
Report No:	SXR/CS 08
Document No:	M-008516-01-1
Guideline(s) followed in study:	BBA guideline VI, 23-2.1.5 from April 1989 (Pinsdorf 1989) with minor modification
Deviations from current test guideline:	Yes (refer below) Test organisms were not individually housed during exposure
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

The aim of this laboratory study was to examine, under laboratory conditions, the effects of KWG 4168 EC 500 on the life cycle of the seven pointed ladybird beetle (*Coccinella septempunctata* L.) in comparison with the reference standard Metasystox R EC 250 blue containing the a.s. oxydemeton-methyl.

On average, control larvae entered the pupal stage between day 6 and day 10 (average 7.9 days) after application. Adult beetles emerged between day 10 and day 15 (average: 12.9 days) following the application. Out of the 50 control larvae, 35 could successfully complete the metamorphosis. Thus, the total pre-imaginal mortality in the control groups was 26%.

All but 2 larvae exposed to spray residues of KWG 4168 EC 500 applied at 1.5 L/ha died prior pupation. Only 1 larva could complete the metamorphosis. Total pre-imaginal mortality of this group was 98%.

No larva survived a spray treatment of either 3.0 L/ha KWG 4168 EC 500 or 1.0 L/ha Metasystox R EC 500.

The LR_{50} and ER_{50} were considered to be > 731 g a.s./ha.

I. Materials and Methods

Materials

Test Material KWG 4168 (Formulation: EC 500)

Lot/Batch #: 089A based on form. no. 04023/0021

Active substance content: 487.5 g/L

Description: Clear yellow liquid

Stability of test compounds: Sufficient based on expiration date, however it should be noted that the study was initiated on the date of expiration

Reanalysis/Expiry date: 25 October 1994

Density: Not reported

Treatments

Test rates: 1.5 and 3.0 L/ha

Solvent/vehicle:	Test water
Analysis of test concentrations:	NA

Test organisms

Species:	*Coccinella septempunctata* L
Source:	Peter Katz (Nutzlingszuchten), Industriestr. 38, Germany - 73642 Welzheim
Acclimatisation period:	Eggs were stored in petri dishes until hatch. The storage room was ventilated by the air-conditioning system of the building. The ambient conditions were 16 h photoperiod at temperatures between 24 and 29°C and relative air humidity ranging from 40 to 80%
Feeding:	Pea aphids (*Acyrthosiphon pisum*) were obtained from a continuously running stock culture. To improve the feeding success of larvae, oversized aphids were sampled with the help of a sieve (2mm mesh). Only freshly caught aphids were used as food.
Treatment for disease:	Not reported

Test design

Test vessel:	Test units consisted of a bottom glass plate (40 cm x 18 cm and 0,6 cm high) to which the products were applied and a safety glass plate of the same size with 10 recesses (d = 5,3 cm) which was placed on the top of the bottom plate once the spray deposit had dried. Safety glass cylinders (d = 5,0 cm and 4 cm high) were then placed into each recess and served as confinement of the ladybird larvae during the test. The glass cylinders were sealed on the top with fine mesh gauze to prevent aphids and emerging beetles from escape. In addition, the inner walls of the glass cylinders were coated with talcum to prevent aphids and ladybird larvae from climbing, thus warranting full exposure to the dried spray deposits over the entire test period.
Test medium:	The test product was diluted in drinking water and were applied to fat free glass plates.
Replication:	Five replicate units were prepared for the active treatments (test and reference treatments) and the control
No. of animals/vessel:	10
Duration of test:	15 days

Environmental test conditions

Temperature:	23 to 25°C
Relative humidity:	55 to 60%
Photoperiod:	Not reported

Study Design

The aim of this laboratory study was to examine, under laboratory conditions, the effects of KWG 4168 EC 500 on the life cycle of the seven pointed ladybird beetle (*Coccinella septempunctata* L.) in comparison with Metasystox R EC 250 blue.

Glass plates received treatments with either drinking water (control), 1.5 and 3.0 L/ha KWG 4168 EC 500 (equivalent to 731 and 1463 g a.s./ha, respectively) or 1 L/ha Metasystox R EC 250 blau (reference treatment).

The test product and the toxic standard were diluted in drinking water and were applied to the fat free glass plates using a spray cabinet with an automatically driven spray boom (spray nozzles; spray pressure). This was calibrated beforehand to confirm an application rate equivalent to 300 L/ha (*i.e.* 3 mg deposit/cm^2). Recorded mass changes of glass plates after treatment were 2.84 - 3.11 mg/cm^3.

Five replicate units were prepared for the active treatments (test and reference treatments) and the control. The glass plates from each unit were sprayed on one surface and left to dry on a laboratory bench. After about 1 hour, the units were assembled. The ladybird larvae (10 per replicate unit) were then transferred into each glass cylinder and provided with pea aphids in excess as feed. The test units were then placed in a controlled environment room until emergence of the adult beetles.

Four of the five glass plates intended for the treatment with 3.0 L/ha KWG 4168 EC 500 had to be treated with a glass atomiser since the overdose proved to be too viscous for the used spray nozzles. Since a higher water volume had to be used for the glass atomiser, glass plates required more than 1 day to dry up and larvae were placed 24 hours after treatment on the glass plates. Although all larvae died within 24 hours after exposure, the temporal pattern of the effects are not in line with the results from the regular spray treatment due to the delay in exposure. Thus, to avoid confusion to the reader, these results were discarded. In this study, effects on reproductive performance were not examined due to the high pre-imaginal mortality in the active treatments.

The condition of the ladybird beetle larvae was recorded at approximately 2 hours after application. They were classed as being:

- Live - alive and apparently unaffected
- Affected - still attempting to crawl, showing signs of reduced coordination
- Moribund/Dead - either immobile or twitching slightly
- Pupated - larva which started pupation
- Hatched - beetle emerged

Further checks were conducted daily until the last larva had completed the metamorphosis.

II. Results and Discussion

No validity criteria assessment was included in the study report.

On average, control larvae entered the pupal stage between day 6 and day 10 (average 7.9 days) after application. Adult beetles emerged between day 10 and day 15 (average: 12.9 days) following the application. Out of the 50 control larvae, 35 could successfully complete the metamorphosis. Thus, the total pre-imaginal mortality in the control groups was 26%.

All but 2 larvae exposed to spray residues of KWG 4168 EC 500 applied at 1.5 L/ha died prior pupation. Only 1 larva could complete the metamorphosis. Total pre-imaginal mortality of this group was 98%.

No larva survived a spray treatment of either 3.0 L/ha KWG 4168 EC 500 or 1.0 L/ha Metasystox R EC 500.

Table CP 10.3.2.1/04-1 Acute effects of dried spray deposits of KWG 4168 EC 500 in comparison to reference treatments with drinking water or 1.0 L/ha Metasystox R EC 250 on development and survival rate of larvae of the seven-pointed ladybird beetle (*Coccinella septempunctata*)

Replicate No.	**1**	**2**	**3**	**4**	**5**
(A) Control plates					
Initial No. of larvae	10	10	10	10	10
Technical losses	0	0	0	1	1
No. of larvae found dead	3	4	3	0	2
No. of pupae	7	6	7	9	7
No. of emerged beetles	7	6	7	9	6
Pre-imaginal mortality (%)	30.0	40.0	30.0	0.0	30.0
Average mortality (%)	26.0				
(B) glass plates treated with 1.5 L/ha KWG 4168 EC 500					
Initial No. of larvae	10	10	10	10	10
Technical losses	0	0	0	0	0
No. of larvae found dead	10	10	10	8	10
No. of pupae	0	0	0	2	0
No. of emerged beetles	0	0	0	1	0
Pre-imaginal mortality (%)	100.0	100.0	100.0	90.0	100.0
Average mortality (%)	98.0				
(C) glass plates treated with 3.0 L/ha KWG 4168 EC 500					
Initial No. of larvae	10	-	-	-	-
Technical losses	0	-	-	-	-
No. of larvae found dead	10	-	-	-	-
No. of pupae	0	-	-	-	-
No. of emerged beetles	0	-	-	-	-
Pre-imaginal mortality (%)	100.0	-	-	-	-
Average mortality (%)	100.0				
(D) glass plates treated with 1.0 L/ha metasystox R EC 250					
Initial No. of larvae	10	10	10	10	10
Technical losses	0	0	0	0	0
No. of larvae found dead	10	10	10	10	10
No. of pupae	0	0	0	0	0
No. of emerged beetles	0	0	0	0	0
Pre-imaginal mortality (%)	100.0	100.0	100.0	100.0	100.0
Average mortality (%)	100.0				

Fourteen males and 21 females emerged from the control pupae. Since only one male was obtained from the 1.5 L/ha KWG 4168 EC 500 treatment group, reproductive performance of the descendants was not monitored.

III. Conclusion

Spray deposits of either 1.5 or 3.0 L/ha KWG 4168 EC 500 (equivalent to 731 and 1463 g a.s./ha, respectively) did strongly affect larvae of the seven-pointed ladybird beetle (*Coccinella septempunctata*) confined on treated glass plates.

The LR_{50} and ER_{50} were considered to be <1.5 L product/ha (<731 g a.s./ha).

Assessment and conclusion by applicant:

No validity criteria assessment was included in the report, therefore, an assessment has been made against Schmuck R. *et al*., 2000 (A laboratory test system for assessing effects of plant protection products on the plant dwelling insect *Coccinella septempunctata* L. (Coleoptera: Coccinellidae)) from the IOBC test guidelines.

- The average pre-imaginal mortality of the water treated larvae should not exceed 30% (actual: 26%)
- The level of pre-imaginal mortality of the larvae exposed to the reference item should result in a pre-imaginal mortality of at least 40% (actual: 100%)
- The number of eggs laid by control females should be ≥2 fertile eggs per viable female per day (not possible to assess this from the data as reproductive assessments were not performed).

The study was conducted prior to the issue of the current IOBC test methods and the test methodology used differs from the current recommended procedures (*e.g.* test organisms not individually housed during exposure) therefore the study has been submitted as supporting information only. However, the basic test design is consistent with current methods therefore the results are considered suitable for use in the risk assessment despite the deviations from the current test method.

The LR_{50} and ER_{50} were considered to be <1.5 L product/ha (<731 g a.s./ha).

Data Points:	KCP 10.3.2.1/05
Report Author:	[redacted]
Report Year:	2000
Report Title:	Spiroxamine EC 500: A laboratory study to evaluate the effects on the ladybird Coccinella septempunctata (Cucujoidea: Coccinellidae)
Report No:	B023CSL
Document No:	M-027529-01-1
Guideline(s) followed in study:	(1) W. Pinsdorf (1989) Auswirkung von Pfanzenschutzmitteln auf Coccinella septempunctata L.; (2) R. Schmuck et al. (1997). Ringtest protocol for laboratory toxicity test with Coccinella septempunctata L. (in prep.); (3) Barrett et al. (1994). Guidance document for regulatory testing procedures for pesticides with nontarget arthropods (SETAC/ESCORT 1994).
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Ladybirds (*Coccinella septempunctata*) were exposed to Spiroxamine EC 500 to assess effects on mortality and hatching rate.

The test item was applied in concentrations ranging from 180 to 3000 mL product/ha and Curamil was used as a toxic standard.

Significant decreases in mortality were seen at application rates of 3000 mL product/ha and no significant reductions on hatching rate were observed at any concentration. An LC_{50} value could not be calculated from the data gained in this study.

I. Materials and Methods

Materials

Test Material	Spiroxamine EC500
Lot/Batch #:	0697
Purity:	502.5 g/L
Description:	Not reported
Stability of test compound:	Not reported
Reanalysis/Expiry date:	18 February 2000
Density:	Not reported
Treatments	
Test rates:	180, 750, 1500 and 3000 mL/ha (equivalent to 90, 375, 750 and 1500 g a.s./ha)
Solvent/vehicle:	Demineralised water
Analysis of test concentrations:	No
Test organisms	
Species:	Ladybird, *Coccinella septempunctata*, 4-5 day old larvae, male and female
Source:	In-house culture
Acclimatisation period:	Not reported
Feeding:	Larvae were fed with *Acyrtosyphon pisum* and *Megoura viciae.* During the reproduction phase, adult ladybirds had continuous access to a source of sugar water and aphid infested bean plants potted in plastic containers.
Treatment for disease:	None reported
Test design	
Test vessel:	Cohort preparation: transparent untreated plastic boxes (about 15.5 x 9 x 10.5 cm), covered with gauze and a grated plastic lid. Exposure phase: a square bottom glass plate, a Plexiglas plate of the same size with 1 or 4 cylindrical holes (about 4.3 cm, but 3.5 cm at bottom to provide a support of about 3 mm high), which was placed on top of the bottom glass plate. The inner walls of the cylinder were coated with Fluon™ to prevent ladybird larvae from escaping.

	Reproductive phase: plastic containers 32 cm x 17 cm x 18 cm with a total volume of about 10 litres. The top was covered with a plastic lid, containing a surface grid to allow for ventilation. A gauze cloth was kept between the cage and the lid to prevent the beetles and aphids from escaping.
Replication:	40 repliactes for the exposure phase and 4 replicates (3 for 180 mL/ha fecundity assessment) for the reproductive phase
No. animals/vessel:	Individually housed for the exposure phase. 3-5 females and 3-6 males per vessel for the reproductive phase
Duration of test:	42 days
Environmental test conditions	
Temperature:	24 - 26°C
Photoperiod:	Photoperiod not reported. Recorded 545 – 1970 Lux.

Study Design

This study was conducted in order to assess the effects on mortality and reproduction of Spiroxamine EC 500 on ladybirds (*Coccinella septempunctata*) over 42 days.

Spiroxamine EC 500 was applied at concentrations of 180, 750, 1500 and 3000 mL/ha (equivalent to 90, 375, 750 and 1500 g a.s./ha). The reference item was Curamil (Afugan 30EC) was applied at 1 L product/ha and a water control was used. The test items were sprayed onto glass plates and once the residues had dried these were used in the test vessels in the exposure phase. Larvae were added to the treated vessels after 2 to 4 hours.

During the exposure phase, four to five day old ladybird larvae were confined individually to treated glass plates, 40 larvae per treatment. Pre-imaginal mortality was assessed during the first 12 days of the test. A larva was considered dead when it did not move after being gently touched with a small brush.

The reproduction phase began three days after all viable pupae in the water control had hatched. Beetles were divided into groups of about 3 to 6 males and 3 to 5 females and were divided over plastic breeding cages. Adults from all treatment groups that survived the exposure period with mortality <40% were observed for effects on fecundity. Reproduction was assessed four times a week for 3 weeks, beginning at the start of oviposition of the water control group. Egg hatch success was determined by incubating sub-batches of eggs for about 3 to 6 days after removal of eggs from the breeding cages.

Throughout the study, the test organisms were kept between 24 to 26°C, measured continuously with a thermohydrograph. Larvae were fed with *Acyrtosyphon pisum* and *Megoura viciae* during the exposure phase. The adults had continuous access to a source of sugar water and aphid infested bean plant.

II. Results and Discussion

Validity criteria according to the study report were met:

- Pre-imaginal mortality of the water treated control <30% (actual: 20%)
- Pre-imaginal mortality of the reference item treatment group 50-100% (actual: 100%)
- Number hatched eggs per viable female per day in the control group >3 (actual: 27.6 eggs)

The study was therefore considered acceptable.

The experiment was conducted twice as the first test organisms had high mortality rates in the control group. The study was repeated with in-house cultures of ladybird.

Due to mortality in the 3000 mL product/ha group and the reference item group exceeding 40%, these were excluded from fecundity assessments, as per the guidelines.

Mortality was 20% in the water control, corrected mortality in the Spiroxamine EC500 180 mL product/ha treatment was 9%, in the 750 mL product/ha -6%, in the 1500 mL product/ha -3% and in the 3000 mL product/ha treatment 56%. Mortality in the 180, 750 and 1500 mL product/ha Spiroxamine EC500 treatments did not differ statistically from the water control (P=0.600, 0.770 and 1.000, respectively). Mortality in the Spiroxamine EC500 3000 mL/ha treatment was statistically significantly different from the water control (P<0.001).

The LR_{50} was considered to be >1500 mL product/ha.

The average number of eggs produced per female per day during 12 daily observations were 37.4, 33.8, 37.1 and 35.5 eggs per female per day in the water control, at 180 mL product/ha, 750 mL product/ha and 1500 mL product/ha, respectively. The ER_{50} was considered to be >1500 mL product/ha.

Table CP 10.3.2.1/05-1 Mortality and egg production of ladybirds exposed to Spiroxamine EC 500

Concentration (mL/ha)	**Mortality (%)**	**Eggs/female/day**	**Hatching rate (%)**
Control	20	37.4	74
Concentration (mL/ha)	**Corrected mortality compared to the control (%)**	**Reduction of reproduction relative to the control (%)**	**Reduction of hatching rate relative to the control (%)**
180	9	10	4
750	-6	1	3
1500	-3	5	1
3000	56*	Not assessed	Not assessed
Reference item	100*	Not assessed	Not assessed

* statistically significant

III. Conclusion

A statistically significant reduction in mortality was observed in ladybirds exposed to 3000 mL/ha of Spiroxamine EC 500. An LC_{50} value could not be calculated from the data in this study but the LR_{50} was considered to be >1500 mL product/ha (equivalent to >750 g a.s./ha).

No statistically significant reductions in hatching rate were observed in treatment groups exposed to Spiroxamine EC 500. The ER_{50} was considered to be >1500 mL product/ha (equivalent to >750 g a.s./ha).

Assessment and conclusion by applicant:

Validity criteria according to the current IOBC test method by Schmuck *et al.* (2000) "A laboratory test system for assessing effects of plant protection products on the plant dwelling insect *Coccinella septempunctata* L. (Coleoptera: Coccinellidae)" have been met:

- Pre-imaginal mortality of the water treated control <30% (actual: 20%)
- Pre-imaginal mortality of the reference item treatment group >40% (actual: 100%)
- Number of fertile eggs laid per viable female per day in the control group >2 (actual: 27.6 eggs)

The study was conducted before the IOBC test methods were formalised but the methodology used was the same as that described by Schmuck *et al.* (2000). The validity criteria according to Schmuck *et al.* (2000) have been met therefore the study is considered acceptable.

The LR_{50} and ER_{50} was considered to be >1500 mL product/ha (equivalent to >750 g a.s./ha).

Data Point:	KCP 10.3.2.1/06
Report Author:	[redacted]
Report Year:	1994
Report Title:	Testing toxicity to beneficial arthropods - green lacewing - Chrysopa carnea Steph. according to modified IOBC guideline (Bigler 1988) KWG 4168 EC 500
Report No:	94 10 48 049
Document No:	M-008545-01-1
Guideline(s) followed in study:	IOBC Guideline (Bigler 1988)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

First instar larvae of green lacewings (*Chrysoperla carnea*) were exposed to KWG 4168 EC 500 to assess the effects on mortality and reproduction.

Test organisms were exposed to concentrations of 1.5 L/ha and 3.0 L/ha (equivalent to 737 and 1464 g a.s./ha). A water control was used and ME 605 Spritzpulver (a.s.: Parathion-methyl / 40%) was applied at 0.2 kg/ha as the reference substance. Mortality was assessed daily up to the hatch of adults. Fecundity was monitored during the 5 weeks.

The test substance KWG 4168 EC 500 caused 100% mortality of the green lacewing at the high dose (3 L/ha) and 75.6% mortality at the lower dose (1.5 L/ha).

Reproductive performance in the 1.5 L/ha treatment group was reduced by 62.2% relative to the control.

The LR_{50} and ER_{50} were considered to be <1.5 L/ha (equivalent to <737 g a.s./ha).

I. Materials and Methods

Materials

Test Material KWG 4168 EC 500

Lot/Batch #: 089-A

Purity: 491.4 g/L

Description: Not reported

Stability of test compound: Not reported

Reanalysis/Expiry date: 16 December 1994

Density: 1.005 g/cm^3

Treatments

Test rates:	Nominal: 1.5 and 3.0 L/ha (equivalent to 737 and 1464 g a.s./ha)in 200L/ha water
Solvent/vehicle:	Water

Test organisms

Species:	Green lacewings, *Chrysopa carnea* STEPH.
Source:	Purchase from the firm Sautter und Stepper
Feeding:	Daily with aphids
Treatment for disease:	None reported

Test design

Test vessel:	Glass plates (25 cm x 60 cm) with glass rings (4 cm Ø, 4 cm height) with gauze covers
Replication:	5 per treatment
No. animals/vessel:	10
Duration of test:	67 days

Environmental test conditions

Temperature:	20-26 °C
Photoperiod:	16:8 h light:dark

Study Design

Green lacewings (*Chrysoperia carnea*) were exposed to KWG 4168 EC 500 over a 67 days period to assess the effect on mortality and reproduction.

Test vessels for the exposure phase were sprayed with dilutions of the test item at concentrations of 1.5 and 3.0 L/ha and 0.2 kg/ha of the reference substance. Application rates were checked by weighing of filter paper, and determined to be 98.3 to 104.4% of the nominal spray deposit. A water control was used and ME 605 Spritzpulver (a.s.: Parathion-methyl / 40%) was applied at 0.2 kg/ha as the reference substance.

Test vessels for the exposure phase consisted of treated glass plates with 10 glass rings placed on each plate. After air drying of the spray deposit, one larva was transferred to each ring. There were five replicates per treatment group.

After hatch, the adults were transferred to rearing cages for the control of oviposition. All eggs laid were counted and transferred to hatching cages two times per week and the hatch of the larvae was recorded.

Temperature and humidity were measured continuously with a thermo hygrograph. Light intensity was *ca.* 1000 lux.

II. Results and Discussion

The validity criteria according to the study report were met:

- Maximum cumulative mortality in water control ≤20% (actual: 18%);

Mortality was 100% in both the reference substance and 3.0 L/ha treatment groups, meaning that there were no females to lay eggs for the fertility stage of the study. Mortality was 75.6% in the 1.5 L/ha treatment group.

In the 1.5 L/ha treatment group <50% of the eggs hatched. This was not significantly different to the hatching rate in the control group. The relative decrease of the reproductive performance compared to the control was 62% at the lower dose as extrapolated from 3 surviving females.

Table CP 10.3.2.1/06-1 Mortality and fertility of green lacewings exposed to KWG 4168 EC 500

Concentration (L/ha)	Mortality (corrected) (%)	Number of eggs/female	Hatched larvae/eggs (%)
Control	18	200.4	59.6
Reference substance	100	-	-
1.5	80 (75.6)	91.3	49.3
3.0	100	-	-

III. Conclusion

The test substance KWG 4168 EC 500 caused 100% mortality of the green lacewing at the high dose (3 L/ha) and 75.6% at the lower dose (1.5 L/ha). The relative decrease of the reproductive performance was 62% at 1.5 L/ha.

The LR_{50} and ER_{50} were considered to be <1.5 L/ha (equivalent to <737 g a.s./ha).

Assessment and conclusion by applicant:

The study was assessed against the current IOBC test method by Vogt *et al*. (2000) guideline "Laboratory method to test effects of plant protection products on larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae)". Two of the validity criteria was met were met:

- Maximum cumulative mortality in water control ≤20% (actual: 18%);
- Mortality in the reference item is ≥50% (actual: 100%).

However, other criteria were not met:

- Fecundity (mean number eggs per female per day) in water control ≥15 (actual: 6.5);
- Fertility (mean hatching rate) in water control ≥70% (actual: 59.6%).

The study was conducted before the IOBC test methods were formalised. Whilst the underlying principles of the test method used in this study may be consistent with the current methods, the validity criteria of the current guideline have not been met. The study has therefore been submitted as supporting information only but the results are still considered to be acceptable for use as part of the risk assessment.

The LR_{50} and ER_{50} were considered to be <1.5 L/ha (equivalent to <737 g a.s./ha).

Data Point:	KCP 10.3.2.1/07
Report Author:	[redacted]
Report Year:	2001
Report Title:	KWG 4168 EC 500: A laboratory study to evaluate the effects on the green lacewing, Chrysoperla carnea (Neuroptera: Chrysopidae)
Report No:	B051CCL
Document No:	M-033924-01-1
Guideline(s) followed in study:	(1) Prufung der Einwirkung von Pflanzenschutzmitteln auf die Nutzarthropodenart Chrysopa carnea Steph. (Neuroptera, Chrysopidae) (Suter, 1978); (2) A laboratory method for testing side-effects on larvae of the green lacewing, Chrysoperia carnea Steph. (Neuroptera, Chrysopidae) (Bigler 1988); (3) Laboratory method to test effects of pesticides on larvae of Chrysoperia carnea. Method description according to OECD form, in prep. (Vogt et al. 1997), and (4) Guidance Document on Regulatory Testing Procedures for Pesticides with Non-Target Arthropods (Barrett et al. SETAC/ESCORT1994)
Deviations from current test guideline:	Test larvae were 2 – 4 days old, guideline recommends 2 – 3. Relative humidity dropped below 70% Mortality in the toxic reference was below 50% No species identification was performed at the end of the test
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Green lacewings (*Chrysoperla carnea*) were exposed to KWG 4168 EC 500 in order to assess the effects on mortality and reproduction.

In accordance with the test guidelines, test organisms were exposed to concentrations from 250 to 1600 g a.s./ha and mortality was assessed after 7 days. Fecundity was monitored during the following 4 weeks. A water control was used and Danadim (dimethoate) at 40 mL product/ha was used as a toxic standard.

Residues of KWG 4168 EC 500, when applied to glass substrate at rates of 1000 and 1600 g a.s./ha, had no adverse effects on mortality, fecundity or egg hatch success of the lacewing *Chrysoperla carnea*. LR_{50} and ER_{50} values could not be determined from this data set but were considered to be >1600 g a.s./ha.

I. Materials and Methods

Materials

Test Material KWG 4168 EC 500

Lot/Batch #: 04931025/0697

Purity: 509.5 g/L

Description: Not reported

Stability of test compound: Not reported

Reanalysis/Expiry date: 3 August 1999

Density: Not reported

Treatments

Test rates:	250, 400, 640, 1000 and 1600 g a.s./ha
Solvent/vehicle:	Water

Test organisms

Species:	*Chrysoperla carnea*, Neuroptera: Chrysopidae
Source:	'Bioplanet' Martorano di Cesena, Italy.
Acclimatisation period:	2 days
Feeding:	Exposure phase: Ephestia eggs. 3 times a week. Fecundity phase: small quantities of paste made from 15 mL milk, 1 egg, 1 egg yolk, 50 g BeeFit™, 30 g Baker's yeast, 50 g wheatgerm, ~45 mL water. Administered at least twice a week.
Treatment for disease:	None reported

Test design

Test vessel:	Exposure phase: square bottom glass plate (about 10 x10 cm), a Plexiglas plate of the same size with 1 or 4 cylindrical holes (about 4.3 cm, but 3.5 cm at bottom to provide a support of about 3 mm high), which was placed on top of the bottom glass plate. Plexiglas cylinders (approximate dimensions: width 4 cm and 4 cm high) were subsequently placed on the supports in each recess to confine the lacewing larvae during the test. Inner walls of the cylinder were coated with Fluon™ to prevent larvae from escaping. Fecundity phase: untreated plastic boxes (about 15.5 x 9 x 10.5 cm), covered with gauze and closed with a plastic lid, containing holes. Egg viability phase: transparent plastic boxes measuring about 17 x 14 x 8 cm, with 2 gauze covered ventilation holes on a side. Upper inner side of the brim was treated with Fluon™ to prevent hatched larvae from escaping.
Replication:	48 for water control, 32 per test item treatment
No. animals/vessel:	1
Duration of test:	1 week exposure phase and a 4 week fecundity phase

Environmental test conditions

Temperature:	25 - 25.5°C
Photoperiod:	16 h light: 8 h dark

Study Design

Green lacewings (*Chrysoperla carnea*) were exposed to KWG 4168 EC 500 over 5 weeks to assess the effect on mortality and fecundity.

Test vessels for the exposure phase were sprayed with dilutions of the test item at concentrations of 250, 400, 640, 1000 and 1600 g a.s./ha or Danadim at 40 mL product/ha. Green lacewing eggs were hatched in the laboratory and 1 larvae (2-4 day old) was placed in each test vessel. There were 32 replicates per treatment group and 48 for the water control.

Test vessels for the exposure phase consisted of treated glass bottom plates with plexiglass cylinders and top plate. Adult emergence was checked 3 times a week and any adults were transferred to untreated plastic boxes with other adults of the same treatment group. Fecundity assessments started 6 days after the last adult of the water control group had emerged. The eggs present were counted twice a week (8 times total during the 4 weeks). Once a week the egg viability was assessed by storing the eggs in untreated plastic boxes for 6 days and recording the number of eggs that hatched.

Temperature and humidity were measured continuously with a thermo hygrograph. Light intensity was measured once during the exposure phase of the bioassay (900 to 1900 lux) and no light intensity was measured during fecundity phase.

II. Results and Discussion

Validity criteria according to the study report were assessed and the following criteria were met:

- Mortality of larvae after 7 days in water control ≤20% (actual: 0%);
- Fecundity (mean number eggs per female per day) in water control ≥10 (actual: 21);

The following criterion was not met:

- Mortality in the reference item is ≥50% (actual: 35%).

The criteria for mortality in the reference item was not met however it is plausible to assume that the test animals were sufficiently sensitive and that potential adverse effects of exposure to test substance residues could be detected with the set-up used in this experiment. This was checked by exposing the same strain of *Chrysoperla carnea* used in the KWG 4168 EC 500 study to several concentrations of Danadim. Exposure to 10% of the highest recommended field rate (HRFR) of Danadim resulted in a 100% mortality, whereas 5% (the concentration used as a toxic standard during this study) and 7.5% of the HRFR gave mortality rates between 35% and 80%. All concentrations were significantly different from the water control, so the test method must have been sufficiently sensitive.

Table CP 10.3.2.1/07-1 Mean mortality, fecundity and fertility of green lacewings

Treatment (g a.s./ha)	Mortality (%)	Eggs/female/day	Hatching rate (%)
Control	0	21	71
	Corrected mortality (%)	**Reduction of reproduction relative to the control (%)**	**Reduction of hatching rate relative to the control (%)**
250	0	-	-
400	0	-	-
640	0	-	-
1000	0	9	7
1600	9	-4*	2
Danadim, 5% field rate (dimethoate)	35	*	-

* Negative value means higher reproduction relative to the water control.

Residues of KWG 4168 EC 500, when applied to glass substrate at rates of 1000 and 1600 g a.s./ha, had no adverse effects on mortality, fecundity or egg hatch success of the lacewing *Chrysoperla carnea*. LR_{50} and ER_{50} values could not be determined from this data set but were considered to be >1600 g a.s./ha.

III. Conclusion

Low mortality and sufficient reproductive performance in the water control treatment indicated that test animals were in good condition. Results of an additional test on the toxic standard showed that the test set-up was adequately sensitive to detect potential adverse effects.

Residues of KWG 4168 EC500, when applied to glass substrate at rates of 1000 and 1600 g a.s./ha, had no adverse effects on mortality, fecundity or egg hatch success of the lacewing *Chrysoperla carnea*.

The LR_{50} and ER_{50} values were considered to be >1600 g a.s./ha.

Assessment and conclusion by applicant:

Validity criteria according to the current IOBC test method by Vogt *et al.* (2000) "laboratory method to test effects of plant protection products on larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae)" guideline were met.

- Maximum cumulative mortality in water control ≤20% (actual: 0%);
- Fecundity (mean number eggs per female per day) in water control ≥15 (actual: 21);
- Fertility (mean hatching rate) in water control ≥70% (actual: 71%).

The following criteria were not met:

- Mortality in the reference item is ≥50% (actual: 35%).

This study was conducted at the time that the IOBC test methods were formalised but did not follow the current guidelines. However, the test methods used are largely consistent with the current test method for this species.

The reference substance did not cause ≥50% mortality but additional work, as reported in the final report confirms that the strain of organism used was sensitive. The deviations to the guideline are also not considered to have had a detrimental effect on the study and the results are still considered to be valid.

The study is therefore considered acceptable.

The LR_{50} and ER_{50} values were considered to be >1600 g a.s./ha.

Data Point:	KCP 10.3.2.1/08
Report Author:	[redacted]
Report Year:	1995
Report Title:	Acute effects of a repeated spray treatment with the fungicide KWG 4168 EC 500 on lycosid spiders (Pardosa agricola) under laboratory conditions
Report No:	SXR/SP 03
Document No:	M-008519-01-1
Guideline(s) followed in study:	Draft guideline of the Federal Biological Research Centre for Agriculture and Forestry of Germany (BBA) (Wehling & Heimbach, 1994).
Deviations from current test guideline:	Test conducted for 18 days, whereas, the guideline recommends 14 (with possible extension to 21 days) Fewer replicates used than currently recommended Application rate of test substance was 300 L/ha, guideline states a water volume according to an application rate of 400 L/ha should be applied One concentration was tested, guideline requires that at least three treatment groups are tested
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

The aim of this study was to evaluate the potential side-effects of KWG 4168 EC 500 on lycosid spiders under worst case exposure conditions. For this, adult spiders were exposed to a repeated spray application of the test product whilst confined on quartz sand. Their survival rate and feeding activity were assessed.

Even at worst case exposure conditions, a single spray treatment with 1.5 L/ha KWG 4168 EC 500 (equivalent to 731 g a.s./ha) had no significant adverse effects on ground - dwelling spiders *(Pardosa* spp.). Under identical test conditions, a reference treatment with 2 L/ha Metasystox R EC 250 blau caused a 100% mortality. However, if the treated spiders were exposed to a second spray treatment at the same rate, 14 out of the 18 survivors died within the subsequent 72 hours.

Thus, the LR_{50} was considered to be <1.5 L product/ha (<731 g a.s./ha) when two applications are considered. The ER_{50} was considered to be >1.5 L product/ha (>731 g a.s./ha) as there was little effect on the feeding activity.

I. Materials and Methods

Materials

Test Material KWG 4168 (Formulation: EC 500)

Lot/Batch #: 089A based on form. no. 04023/0021

Active substance content: 487.5 g/L

Description: Clear yellow liquid

Stability of test compound: Sufficient based on expiration date

Reanalysis/Expiry date: 25 October 1994

Density: Not reported

Treatments

Test rates:	1.5 L/ha

Test organisms

Species:	*Pardosa* ssp
Source:	untreated fields within the Bayer AG's experimental farmland "Laacher Hof', approximately 3 km south of Monheim (Germany, NRW 41m above sea level)
Acclimatisation period:	Until testing commenced, spiders were stored individually in plastic boxes (17 x 12.5 x 6 cm) on a thin layer of quartz sand for a minimum of 3 days and a maximum of 3 weeks. kept in a climate control cabinet at a temperature of 20 + 2 C, 80 ± 10 % relative humidity and a 16 h photoperiod >1000 lux
Feeding:	The spiders were fed with onion flies (*Delia antiqua*)
Treatment for disease:	Not reported

Test design

Test vessel:	10 x 10 x 5.5 cm plastic boxes covered with plastic screens (mesh size 1 mm).
Test medium:	Quartz sand
Replication:	20 per treatment
No. of animals/vessel:	One
Duration of test:	18 days

Environmental test conditions

Temperature:	20 to 23°C (the range of these conditions deviated slightly from those detailed in the study protocol (20 ± 1° C, 80 ± 10 % relative humidity) but it was not considered that this affected the outcome of the study)
Relative humidity:	73 to 88% (the range of these conditions deviated slightly from those detailed in the study protocol (20 ± 1° C, 80 ± 10 % relative humidity) but it was not considered that this affected the outcome of the study)
pH:	NA
Photoperiod:	Not reported

Study Design

The aim of this study was to evaluate the potential side-effects of KWG 4168 EC 500 on lycosid spiders under worst case exposure conditions. For this, adult spiders were exposed to a repeated spray application of the test product whilst confined on quartz sand. Their survival rate and feeding activity were assessed.

The test boxes were set up 3 days before starting the study with 20 replicates per treatment. One *Pardosa* ssp. was placed into each test box without food supply. After this acclimation period, the highest proposed field rate of KWG 4168 EC 500 (1.5 L/ha) was applied two times within one week to each of the 20 test boxes. The reference boxes received a single spray treatment with 2 L/ha Metasystox R EC

250 blau. The control boxes were treated two times with 300 litres of drinking water/ ha which was equivalent to the spray volume of both active treatments.

The treatments were performed on days 0 and 7. A laboratory spray equipment simulating field operation procedures was used for spray application. A motor-driven spray boom (spray nozzle: TeeJet 800 15 E - SS) was moved at 3.5 m/sec in a distance of 45 cm over the plastic boxes. All 20 boxes of each treatment group were set up in a group and treated concurrently. The applied spray fluid volume was 300 L/ha (30 mL per application). The 30 mL spray fluid were held in reservoir containers. Spray fluid was delivered at a constant pressure of 3.0 bar. The spray apparatus was calibrated beforehand to obtain the desired amount of spray fluid in each test box (0.285 mL). The actual measured amount of applied fluid was between 0.27 and 0.34 ml per test box (n = 5). The control vessels were sprayed with drinking water in the same way.

The test boxes were maintained in a light thermostat at 20 - 23 °C and 73 - 88% relative humidity. The range of these conditions deviated slightly from those detailed in the study protocol (20 ± 1° C, 80 ± 10 % relative humidity) but it was not considered that this affected the outcome of the study.

The condition of the spiders was recorded on days 0 (4h, 6h), 3, 4, 5, 6, 7 (4h, 6h), 10, 11, 12, 13, 14, and 18 days following the application. They were classified as affected (still upright and attempting to walk; showing signs of reduced coordination) or moribund (on their back or side, either immobile or twitching slightly). Spiders which showed no movements after mechanical stimulation were regarded as dead. Dead spiders were removed and not replaced. The number of provided onion flies eaten were recorded and removed together with the flies left intact.

Significant differences in mortality rates were tested with the chi square test (Sachs 1992). The number of onion flies eaten by lycosid spiders were statistically compared by means of an ANOVA-test (Statgraphics, Version 5.5 D, Serial No.: 4552182).

II. Results and Discussion

No validity criteria assessment was included in the study report.

In the control boxes, 17 out of 20 spiders survived the 18-day test period unaffected. Nine records of behavioural impacts were made after the second spray treatment.

All but 2 of the exposed lycosid spiders survived a single spray treatment with 1.5 L/ha KWG 4168 EC 500. However, 14 spiders (70%) were killed by the repeat spray. At the end of the 18 day exposure period, only 20% of the exposed spiders were still alive.

The first spray treatment with 1.5 L/ha KWG 4168 EC 500 caused only reversible impacts on behavior in 6 spiders but no significantly increased mortality. However, after the repeat treatment, all of the 18 survivors were severely impacted.

The reference treatment caused a 100% mortality within 72 hours after application.

The individual feeding activity was not statistically affected by the test compound. If the total number of onion flies eaten per viable spider per day in the control plots of 0.47 is used as a basis for comparison and assumed to be 100%, then the feeding rates for the 2 times 1.5 L/ha KWG 4168 EC 500 treatment were 94% (0.44). In contrast, beetles of the reference group showed virtually no feeding activity.

Even at worst case exposure conditions, a single spray treatment with 1.5 L/ha KWG 4168 EC 500 had no significant adverse effects on ground-dwelling spiders *(Pardosa* spp.). Under identical test conditions, a reference treatment with 2 L/ha Metasystox R EC 250 blau caused a 100% mortality. However, if the treated spiders were exposed to a second spray treatment at the same rate, 14 out of the 18 survivors died within the subsequent 72 hours.

The results of the present study indicate that ground-dwelling spiders may be impacted when exposed to a repeated spray treatment with 1.5 L/ha KWG 4168 EC 500. Thus, the LR_{50} was considered to be <1.5 L product/ha (<731 g a.s./ha) when two applications are considered. The ER_{50} was considered to be >1.5 L product/ha (>731 g a.s./ha) as there was little effect on the feeding activity.

Table CP 10.3.2.1/08-1 Effects of a repeated spray treatment with either drinking water or KWG 4168 EC 500 (1.5 L/ha) or Metasystox R EC 250 (2 L/ha) on survival rate and health condition of lycosid spiders (*Pardosa* spp.) during a subsequent 18 day period

Replicate No.	Mortality (%)			Total mortality (%)
	Week 1	Week 2	Week 3	
Control	5	10	0	15
KWG 4168 EC 500 (1.5 L/ha)	10	70	0	80*
Metasystox R EC 250 blau (2 L/ha)	100	0	0	100*

*Significantly different from control treatment

Table CP 10.3.2.1/08-2 Effects of a repeated spray treatment with either drinking water or KWG 4168 EC 500 (1.5 L/ha) or Metasystox R EC 250 (2 L/ha) on the feeding activity of lycosid spiders (*Pardosa* spp.) during a subsequent 18 day period

Treatment	Average feeding activity by day [Number of onion flies eaten]										
	3	4	5	6	10	11	12	13	14	18	Total
Control	2.05	1.11	0.58	1.05	0.89	0.38	0.24	0.35	0.58	1.24	0.47
KWG 4168 EC 500 (1.5 L/ha)	1.55	0.72	0.72	0.56	0.45	0.00	0.25	0.50	0.50	0.50	0.44
Metasystox R EC 250 blau (2 L/ha)	0.00	-	-	-	-	-	-	-	-	-	0.00

III. Conclusion

Even at worst case exposure conditions, a single spray treatment with 1.5 L/ha KWG 4168 EC 500 had no significant adverse effects on ground - dwelling spiders (*Pardosa* spp.). Under identical test conditions, a reference treatment with 2 L/ha Metasystox R EC 250 blau caused a 100% mortality. However, if the treated spiders were exposed to a second spray treatment at the same rate, 14 out of the 18 survivors died within the subsequent 72 hours.

The results of the present study indicate that ground-dwelling spiders may be impacted when exposed to a repeated spray treatment with 1.5 L/ha KWG 4168 EC 500. Thus, the LR_{50} was considered to be <1.5 L product/ha (<731 g a.s./ha) when two applications are considered. The ER_{50} was considered to be >1.5 L product/ha (>731 g a.s./ha) as there was little effect on the feeding activity.

Assessment and conclusion by applicant:

No validity criteria assessment was included in the report, therefore, an assessment has been made against the current IOBC test method for testing effects of plant protection products on spiders of the genus *Pardosa* (Araneae, Lycosidae) under laboratory conditions (Heimbach *et al*, 2000).

- The guideline suggests a maximum mortality of two spiders (6.7%) with 30 replicates after three weeks or four spiders (13.3%) if the test is extended. The current study has only 20 replicates, and a control mortality of three spiders (15%), therefore the criterion is considered not to be met.
- The reference item to result in mortality of 65 ± 35% (actual: 100%)

The study was conducted prior to the IOBC test methods being published and therefore the test method used in this study, although largely consistent, does deviate from the current test method. Furthermore, the validity criteria from the current IOBC test method have not all been met therefore

the study has been submitted as supporting information only. However, the results are still considered valid and suitable for consideration in the risk assessment.

The LR_{50} was considered to be <1.5 L product/ha (<731 g a.s./ha). The ER_{50} was considered to be >1.5 L product/ha (>731 g a.s./ha).

Data Point:	KCP 10.3.2.1/09
Report Author:	[redacted]
Report Year:	1994
Report Title:	Acute effects of a multiple spray application of the fungicide KWG 4168 (500 EC) on carabid beetles (Bembidion tetracolum) under laboratory conditions
Report No:	SXR/CA 119
Document No:	M-008726-01-1
Guideline(s) followed in study:	BBA guideline 23-2.1.8 (1991)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Carabid beetles (*Bembidion tetracolum*) were exposed to KWG 4168 EC 500 in a spray application test to assess the effects on mortality and feeding habits.

The carabid beetles were exposed to KWG 4168 EC 500 at concentrations of 0.75, 1.5 and 3.0 L product/ha (equivalent to 371, 741 and 1482 g a.s./ha), to the reference item (Methylparathion) at 20 g/ha and to a water control.

The highest dose, 3.0 L/ha KWG 4168 EC 500, resulted in 100% mortality in the test organisms and mortality was statistically increased at concentrations of 1.5 L/ha and greater. Feeding activity was statistically significantly affected at 3.0 L/ha test item.

The LR_{50} and ER_{50} were considered to be >1.5 L product/ha (>741 g a.s./ha).

I. Materials and Methods

Materials

Test Material KWG 4168 EC 500

Lot/Batch #: 089A

Purity: 494.0 g/L

Description: Yellow liquid

Stability of test compound: Maximum expected loss of a.s. by hydrolysis <0.2%

Reanalysis/Expiry date: 17 March 1994

Density: Not reported

Treatments

Test rates:	0.75, 1.5 and 3.0 L/ha
Solvent/vehicle:	Water
Analysis of test concentrations:	None

Test organisms

Species:	Carabid beetle, *Bembidion tetracolum*, Carabidae (Arthropoda: Coleoptera). 4-8 weeks old, male and female
Source:	Dr. Thieme, D-18184 Sagerheide
Acclimatisation period:	Not reported.
Feeding:	Fly pupae (*Delia antiqua*)
Treatment for disease:	None reported.

Test design

Test vessel:	1.2 L plastic boxes: 16.8 x 11.8 x 6.2 cm, covered with plastic screens (mesh size 1 mm).
Test medium:	Water
Replication:	5 per test concentration (3 for the 0.75 L/ha test item group)
No. animals/vessel:	6 (3 male and 3 female)
Duration of test:	35 days

Environmental test conditions

Temperature:	21 - 22°C
Photoperiod:	Not reported

Study Design

This study was conducted in order to assess the effects of KWG 4168 EC 500 on carabid beetles over 5 weeks.

Carabid beetles were exposed to KWG 4168 EC 500 at concentrations of 0.75, 1.5 and 3.0 L/ha (equivalent to 371, 741 and 1482 g a.s./ha), to the reference item, methylparathion at 20 g/ha, and to a water control. The beetles were aged 4-8 weeks and three male and three female were used in each replicate. Five replicates were conducted per test item concentration (except 0.75 L/ha test item, for which three replicates were conducted). The beetles were exposed to two treatments of each concentration.

The beetles were contained in 1.2 L plastic boxes: 16.8 cm length, 11.8 cm width and 6.2 cm height, covered with plastic screens (mesh size 1 mm), filled with quartz sand. The test vessels were kept at 21 to 22°C.

Shortly before application, beetles were sorted out of the sand and placed back on the sand surface, and then they were directly exposed to the spray fluid of test item.

The condition of the beetles was recorded at days 0, 1, 2, 4, 7, 8, 9, 11, 14, 17, 21, 24, 28, 31 and 35 days following the first application. The effects on the beetles were categorised as the following:

- Affected: Still upright and attempting to walk; showing signs of reduced coordination
- Moribund: On their back or side, either immobile or twitching slightly
- Dead: Beetles that showed no movements after mechanical stimulation. Moribund beetles that did not recover before the next assessment were also recorded as dead.

The beetles were fed with six pupae on days 0, 2, 4, 7, 9, 11, 14, 17, 21, 24, 28, 31 and 35. The number of pupae eaten were recorded and removed together with the pupae left intact.

II. Results and Discussion

Validity criteria were not assessed as part of the study.

A multiple exposure to a spray application of spiroxamine at a rate of 371 g a.s./ha had no sublethal or lethal effects on the test beetles. 741 g a.s./ha led to the death of approximately 45 %, whilst at 1482 g a.s./ha all beetles died. A single spray of the reference standard methylparathion also gave total mortality.

The mortality rate (35 d) of the test beetles for Spiroxamine EC 500 applied twice a week at 371 g a.s./ha was 5.6 %, at 741 g a.s./ha (maximum recommended field rate) 43.3 %, and at 1482 g a.s./ha 100 %, respectiveliy. The individual feeding activity was only temporarily affected at 371 g a.s./ha as well as at 741 g a.s./ha. If the total number of pupae consumed per viable beetle per day in the control plots of 0.04 is used as a basis for comparison and assumed to be 100 %, then the feeding rates for the 371 g a.s./ha and the 741 g a.s./ha treatment were 100 % (0.04) and 175 % (0.07), respectively. Beetles which had been exposed to a spray application of 1482 g a.s./ha spiroxamine or 8 g a.s./ha Methyiparathion WP 40 (reference treatment) revealed no feeding activity at all. Therefore, the effect on feeding rate was 100 % when 1482 g a.s./ha was sprayed twice.

The LR_{50} and ER_{50} were considered to be >1.5 L product/ha (>741 g a.s./ha).

Table CP 10.3.2.1/09-1 % mortality and feeding rate of Carabid beetles exposed to KWG 4168 EC 500

Test item concentration (L/ha)	% mortality	Feeding rate (number of fly pupae eaten per beetle per day)
Control	0	0.04
0.75	5.6	0.04
1.5	43.3*	0.07
3.0	100*	0*
Reference item	100*	0*

* statistically significant

III. Conclusion

Mortality of *Bembidion tetracolum* was statistically increased at concentrations of 1.5 L/ha and above. Feeding was reduced at concentrations of 3.0 L/ha of KWG 4168 EC 500.

The LR_{50} and ER_{50} were considered to be >1.5 L product/ha (>741 g a.s./ha).

Assessment and conclusion by applicant:

Validity criteria were not assessed in the study and there is currently no formalised test method for *Bembidion tetracolum*. Validity criteria from the IOBC test method for another beetle species (*Poecilus cupreus*) has therefore been used as a substitute. Thus, according to Heimbach *et al.* (2000) "A method for testing effects of plant protection products on the carabid beetle *Poecilus cupreus* (Coleoptera, Carabidae) under laboratory and semi-field conditions" the validity criteria were met.

- Mortality to be less than 6.7% in the water control (actual: 0%)

- Mortality to be 65% (± 35%) in the reference item within 2 weeks (actual: 100% mortality after 7 days)

Based on this the study is therefore considered acceptable and the results suitable for use in the risk assessment.

The LR_{50} and ER_{50} were considered to be >1.5 L product/ha (>741 g a.s./ha).

CP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

Data Point:	KCP 10.3.2.2/05
Report Author:	[redacted]
Report Year:	2013
Report Title:	Toxicity to the predatory mite Typhlodromus pyri (Acari: Phytoseiidae) using an extended laboratory test on grapevine - Spiroxamine EC 500 g/L
Report No:	CW13/030
Document No:	M-462852-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC, Regulation (EC) No. 1107/2009; US EPA OCSPP not applicable; BLÜMEL ET AL. (2000) modified. Use of natural substrate (detached grapevine leaves) instead of glass plate; CANDOLFI ET AL. (2001)
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 objective of this study was to investigate the lethal and sub-lethal toxicity of Spiroxamine EC 500 g/L to the predatory mite *Typhlodromus pyri* when exposed to treated leaf surfaces.

The corrected mortality at all test item rates was below 10%. The LR_{50} was estimated to be >510 g a.s./ha.

Reproduction was assessed for all rates of Spiroxamine EC 500 g/L. At 51 g a.s./ha, the reproduction was reduced by 19.1%. No reduction (-5.3%) occurred at 91 g a.s./ha. At the higher test item rates of 161, 287 and 510 g a.s./ha, the reduction of reproduction was 24.2, 23.7 and 20.9%, respectively. The ER_{50} was estimated to be >510 g a.s./ha.

I. Materials and Methods

Materials

Test Material KWG 4168 EC 500 g/L

Lot/Batch #: EDFL021371

Purity: 49.9% w/w (501.1 g/L)

Description: Clear yellow-brown liquid

Stability of test compound: Not applicable

Reanalysis/Expiry date: 04 February 2015

Density:	1.004 g/mL
Treatments	
Test rates:	51, 91, 161, 287 and 510 g a.s./ha in 200 L deionised water
Solvent/vehicle:	Deionised water
Analysis of test concentrations:	None
Test organisms	
Species:	*Typhlodromus pyri*
Source:	Katz Biotech AG, D-15837 Baruth, Germany
Feeding:	Apple pollen
Test design	
Test vessel:	A treated *Vitis vinifera* leaf disc was laid on a layer of wet filter paper on top of a water soaked floral foam. A circle of insect glue (ø approx. 40 mm) was formed on the leaves. Sets of such units were placed on a plastic tray such that the filter paper was constantly provided with deionised water.
Test medium:	As above
Replication:	5 replicates per treatment and control group
No. animals/vessel:	20
Duration of test:	14 days
Environmental test conditions	
Temperature:	23.5 – 26.0°C
Relative humidity:	60 – 71%
Lighting:	Light:dark cycle = 16:8 hour (851 – 1483 lux)

Study Design

The objective of this study was to investigate the lethal and sub lethal toxicity of Spiroxamine EC 500 g/L to the predatory mite *Typhlodromus pyri* when exposed to treated leaf surfaces.

Eggs of the predatory mite *Typhlodromus pyri* were supplied by Katz Biotech AG, D-15837 Baruth, Germany. The original source of the mites was Staatliche Lehr- und Versuchsanstalt, Weinsberg, Germany; the rearing in the laboratory of Katz Biotech started 1992 (rearing conditions: 20 - 25°C, 60 - 80% relative humidity, day length 16:8 h with a light intensity of >3000 lux, food: apple pollen). The grapevine plants (*Vitis vinifera*), were provided by the horticultural group of BCS-R&D-SMR-Weed Control.

Test vessels contained a treated *Vitis vinifera* leaf disc, laid on a layer of wet filter paper on top of a water soaked floral foam. A circle of insect glue (ø approx. 40 mm) was formed on the leaves. Sets of such units were placed on a plastic tray such that the filter paper was constantly provided with deionised water.

The test item was applied onto detached grapevine leaves (*Vitis vinifera*) at rates of 51, 91, 161, 287 and 510 g a.s./ha and the effects on the predatory mite *Typhlodromus pyri* were compared to those of a deionised water treated control. A toxic reference (active substance: Dimethoate) applied at 14 g a.s./ha was included to indicate the relative susceptibility of the test organisms and the test system.

Mortality of 100 predatory mites, protonymphs at study start (5 replicates with 20 individuals per test group), was assessed 1, 3, 7, 10, 12 and 14 days after exposure by counting the number of living and dead mites. The number of escaped mites was calculated as the difference from the total number exposed.

The reproduction rate of surviving mites was then evaluated from day 7 until day 14 after treatment by counting the total number of offspring (eggs and larvae) produced.

The climatic test conditions during the study were 23.5 - 26.0°C temperature and 60 - 71% relative humidity. The light / dark cycle was 16:8 h with a light intensity range of 851 – 1483 lux.

II. Results and Discussion

Validity criteria according to Blümel *et al*. (2000) "Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products" were met:

- Mortality in the control group after 7 days to be ≤20% (actual: 17%)
- Cumulative mean number of eggs per female to be ≥4 (actual: 6.14)
- Corrected mortality of the reference item to be ≥50% (actual 100%)

The mortality / escaping rate in the control group up to day 7 after treatment was 17%.

No statistically significant mortality compared to the control group occurred in all test item rates. The corrected mortality was below 10%. The LR_{50} was therefore estimated to be >510 g a.s./ha.

In the reference item group all mites were dead on day 7 of the study.

The mean number of offspring produced per female in the control group was 6.14. This compared to 4.97 eggs/female in the 51 g a.s./ha rate of the test item, 6.46 eggs/female in the 91 g a.s./ha rate, 4.65 eggs/female in the 161 g a.s./ha rate, 4.68 eggs/female in the 287 g a.s./ha rate and 4.86 eggs/female in the 510 g a.s./ha rate.

No statistically significant reduction in reproductive success compared to the control occurred at all test item rates.

At the 51 g a.s./ha rate, the reduction of reproduction was 19.1%. At the rate of 91 g a.s./ha, no reduction was found (-5.3%). At the higher rates of 161, 287 and 510 g a.s./ha, a reduction of reproduction of 24.2, 23.7 and 20.9% was found, respectively. The ER_{50} was therefore estimated to be >510 g a.s./ha.

Table CP 10.3.2.2/05-1 Summary of mortality and reproductive effects

Test concentration (g a.s./ha)	Mortality after 7 days (%)		Reproduction	
	Uncorrected	Corrected	Rate (eggs/female)	Reduction relative to control (%)
Control	17.0		6.14	-
51	15.0	-2.4	4.97	19.1
91	17.0	0.0	6.46	-5.3
161	22.0	6.0	4.65	24.2
287	25.0	9.6	4.68	23.7
510	17.0	0.0	4.86	20.9
Reference item	100.0	100.0	-	-

III. Conclusion

In this extended laboratory test the effects of Spiroxamine EC 500 g/L residues on the survival of the predatory mite *Typhlodromus pyri* were determined at the rates of 51, 91, 161, 287 and 510 g a.s./ha applied to detached grapevine leaves (*Vitis vinifera*).

The corrected mortality at all test item rates was below 10%. The LR_{50} was estimated to be >510 g a.s./ha.

Reproduction was assessed for the all rates of Spiroxamine EC 500 g/L. At 51 g a.s./ha the reproduction was reduced by 19.1%. No reduction (-5.3%) occurred at 91 g a.s./ha. At the higher test item rates of 161, 287 and 510 g a.s./ha, the reduction of reproduction was 24.2%, 23.7% and 20.9% respectively. The ER_{50} was estimated to be >510 g a.s./ha.

Assessment and conclusion by applicant:

Validity criteria according to Blümel *et al.* (2000) "Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) for regulatory testing of plant protection products" were met:

- Mortality in the control group after 7 days to be ≤20% (actual: 1.7%)
- Cumulative mean number of eggs per female to be ≥4 (actual: 6.14)
- Corrected mortality of the reference item to be ≥50% (actual: 100%)

The study was conducted in accordance with the test method of [redacted] *et al.* (2000), which is the current test guideline for this study type, and followed the recommended methods and procedures. The study is therefore considered acceptable.

The LR_{50} and ER_{50} were estimated to be >510 g a.s./ha.

Data Point:	KCP 10.3.2.2/01
Report Author:	[redacted]
Report Year:	1994
Report Title:	An laboratory evaluation of the side-effects of Fungicide KWG 4168, on the parasitic wasp Aphidius rhopalosiphi, when applied to barley seedlings
Report No:	BAY-93-16
Document No:	M-008715-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Parasitic wasps, *Aphidius rhopalosiphi*, were exposed to a KWG 4168 EC formulation to assess the effects on mortality and fecundity after a 48-hour exposure period.

The wasps were exposed to the test item at 1.5 L product/ha (equivalent to 741 g a.s./ha), a control and to the reference item, dimethoate.

KWG 4168 had no effect on either the survival or the fecundity, and little effect on the activity of adult *Aphidius rhopalosiphi* when they were exposed to fresh residues on plants. However topical application of the product to pupae within their mummified host resulted in the death of most of the wasps that were exposed.

I. Materials and Methods

Materials

Test Material	KWG 4168 00500 EC
Lot/Batch #:	089A
Purity:	494.0 g/L
Description:	
Stability of test compound:	Not reported
Reanalysis/Expiry date:	17 March 1994
Density:	Not reported
Treatments	
Test rates:	1.5 L product/200L water/ha (7.5 mL product/L water)
Solvent/vehicle:	Water
Analysis of test concentrations:	No
Test organisms	
Species:	Parasitic wasp, *Aphidius rhopalosiphi*, Hymenoptera: Braconidae
Source:	In-house culture
Acclimatisation period:	None
Feeding:	1:2 honey:water solution prior to the bioassay. 25% fructose solution during the test
Treatment for disease:	None reported
Test design	
Test vessel:	Clear acrylic cylinders (9 cm diameter, 20 cm high), the tops of which were sealed with nylon netting
Replication:	3
No. animals/vessel:	5 for mortality assessment, 3 for fecundity assessment
Duration of test:	Mortality assessment: 48 hours Fecundity assessment: 24 hours Juvenile emergence: 10 days
Environmental test conditions	
Temperature:	20 ± 1°C

Photoperiod: 16 hour light : 8 hour dark at >4000 lux

Study Design

This study was conducted in order to assess the effetcs of a KWG 4168 EC formulation on parasitic wasps (*Aphidius rhopalosiphi*) in a mortality study over 48 hours followed by fecundity assessments for 24 hours and juvenile emergence over a further 10 days.

The wasps used in the study were up to 48 hours old and were deprived of food for approx. 17 hours prior to the test.

Pots of barley seedlings were treated with the test product at a rate of 1.5 L product/ha in 200L of water. Three pots of barley seedlings were used for the control, three for the KWG 4168 treatment and one for dimethoate. Once the product had dried on the plants, the treated seedlings were enclosed within clear acrylic cylinders (9 cm diameter, 20 cm high) the tops of which were sealed with nylon netting.

Fifteen mated females were used for each of the control and KWG 4168 treatments and 5 females for the dimethoate treatment (340 g a.s./ha). The wasps were confined over the seedlings.

The pots were stored in a controlled environment room maintained at 20 ± 1°C, with a 16 h photoperiod.

The condition of the wasps was recorded at approximately 1, 2, 4, 24 & 48 h after their introduction. They were classed as live, affected or moribund/dead. To assess sub-lethal effects, the behaviour of the confined wasps was assessed after 30 mins and 2 h, and assessments of their condition were made up to 48 h after treatment.

After the 48 hours, wasps surviving on the treated and control plants were transferred to additional untreated pots of barley seedlings. The fecundity of the surviving wasps was then assessed over a further 24 h period in clear acrylic cylinders (9 cm diameter, 20 cm high), the tops of which were sealed with nylon netting. After 24-hours the wasps were removed and the numbers of mummies produced were counted after a further 10 days.

In the second part of the study, the test item was also applied topically to the mummies produced in the first part of the study. Mummies that were produced were attached to glass plates and sprayed with either the test product at 1.5 L product/ha, or water (control). The mummies were then observed over a 10-day period to assess emergence.

II. Results and Discussion

No specific assessment of validity criteria was made in the study report.

First part of the test: During the two day exposure period, no wasps died in either the control or the spiroxamine treatments. All of the wasps in the toxic standard treatment (dimethoate) died within 24 hours. The residues of spiroxamine appeared to have a slight effect on the behaviour of the confined wasps with evidence of short term repellency from the treated plants resulting in fewer insects resting compared to the control. However, there was no treatment effect on the fecundity of the wasps following exposure to residues of spiroxamine. The LR_{50} and ER_{50} for adult wasps is therefore considered to be >741 g a.s./ha.

In the second part, most of the mummies (78.6 %) sprayed with spiroxamine failed to emerge, whereas 25 of the 26 (96.2 %) control mummies did develop to adults. This test has indicated that spiroxamine had no effect on either the survival or the fecundity, and little effect on the activity of adult *Aphidius rhopalosiphi* when they were exposed to fresh residues on plants. However topical application of the product to pupae within their mummified host resulted in the death of most of the wasps that were exposed.

Table CP 10.3.2.2/01-1 Mortality following exposure to residues of KWG 4168

Treatment	48-hour mortality (%)
Control	0
KWG 4168 at 741 g a.s./ha	0
Dimethoate at 340 g a.s./ha	100 (after 24 hours)

Most of the mummies (78.6 %) sprayed with KWG 4168 failed to emerge, whereas 25 of the 26 (96.2%) control mummies did develop to adults.

Table CP 10.3.2.2/01-2 Mummies per wasp and emergence following exposure to residues of KWG 4168

Treatment	Mean no. mummies/wasp	Emergence (% of mummies produced)
Control	7.4	96.2
KWG 4168 at 741 g a.s./ha	11.6	21.4

III. Conclusion

In conclusion, in this laboratory study spiroxamine applied at the maximum recommended rate (741 g as/ ha) had no effect on either the survival (adult mortality 0 %) or the fecundity, and little effect on the activity (avoidance) of adult *Aphidius rhopalosiphi* when they were exposed to fresh residues on plants. However topical application of the product to pupae within their mummified host resulted in the death of most of the wasps that were exposed (78 % mortality). The fecundity of the adult was not negatively affected. The LR_{50} and ER_{50} for adult wasps is therefore considered to be >741 g a.s./ha.

Assessment and conclusion by applicant:

The study has been assessed against the current IOBC Mead-Briggs *et al*. guideline (2000) "A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (DeStephani-Perez) (Hymenoptera: Braconidae)". Validity criteria were met:

- Mortality in the control group ≤13% (actual: 0%)
- Mortality in the reference item group ≥50% (actual: 100%)
- Mummies per female produced in the control group ≥5 (actual: 7.4)
- Number of female wasps producing no mummies ≤2 (insufficient data to make assessment)

The study was conducted prior to the issue of the current IOBC test methods but the first part of the study uses a test method which is largely consistent with that of Mead-Briggs *et al*. guideline (2000). The number of replicates used in the mortality assessment was fewer than the recommended four but on the whole the test design is considered to be valid and the current validity criteria have been met, where these can be assessed. The results are therefore considered suitable for use in the risk assessment. The second part of the study was non-standard and the current risk assessment scheme does not require effects on the pupae to be assessed. However, consideration of these results will be given in the risk assessment.

Based on the standard parameters assessed in this study type, the LR_{50} and ER_{50} for adult wasps are considered to be >741 g a.s./ha. Effects were seen following application to the mummies themselves which is not a standard parameter of this study type. Some uncertainty may be created as a consequence of this but other available data with this species, most notably a semi-field study (M-008539-01-1). In this study aphid mummies, containing the parasitoid pupae, were exposed to Spiroxamine EC 500 at 737 g a.s./ha which led to 95% emergence. Thus, there was a clear lack of

effects following application to the mummies therefore there should be no uncertainty over possible effects on *Aphidius* pupae.

Data Point:	KCP 10.3.2.2/02
Report Author:	[redacted]
Report Year:	2007
Report Title:	Toxicity to the parasitoid wasp Aphidius rhopalosiphi (DESTEPHANI-PEREZ) (Hymenoptera: Braconidae) using an extended laboratory test Spiroxamine EC 500 g/l
Report No:	CW07/012
Document No:	M-289317-01-1
Guideline(s) followed in study:	MEAD-BRIGGS ET AL. (2000), MEAD-BRIGGS ET AL. (draft 2006), CANDOLFI ET AL. (2001)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The objective of this extended laboratory study was to investigate the lethal and sublethal toxicity of Spiroxamine EC 500 g/L to the parasitoid wasp *Aphidius rhopalosiphi* when exposed on a plant surface.

The effects of residues of Spiroxamine EC 500 g/L on the survival of *Aphidius rhopalosiphi* were determined at 100, 173, 300, 520 and 900 g a.s./ha applied to barley plants. In the dose rates of 173 and 900 g a.s./ha 3.3% corrected mortality was observed. At the rates of 100, 300 and 520 g a.s./ha no mortality was detected.

The reduction in reproductive success relative to the control at the 100, 300, 520 and 900 g a.s./ha rate was 37.5, 18.1, 19 and 5.2%. No reduction (-43.1%) was detected at the 173 g a.s./ha rate.

A statistically significant dose related repellent effect of the test item at 900 g a.s./ha was observed.

The LR_{50} and ER_{50} were estimated to be >900 g a.s./ha.

I. Materials and Methods

Materials

Test Material	KWG 4168 EC 500 g/L
Lot/Batch #:	PF90087683
Purity:	49.8%
Description:	Not reported
Stability of test compound:	Not applicable
Reanalysis/Expiry date:	31 January 2010
Density:	1.006 g/mL

Treatments

Test rates:	100, 173, 300, 520 and 900 g a.s./ha in 400 L water
Solvent/vehicle:	Deionised water
Analysis of test concentrations:	None

Test organisms

Species:	*Aphidius rhopalosiphi*
Source:	Katz Biotech AG, D-13837 Baruth, Germany
Feeding:	10% fructose solution

Test design

Test vessel:	The test units consisted of a pot with treated barley plants which were enclosed within a clear polyacrylic cylinder (195 mm high and 100 mm in diameter) with a hole (approximately 5 mm in diameter) for the introduction of the parasitoids. After introduction the hole was closed by a stopper. The top of the cylinder was closed with a fine mesh gauze.
Test medium:	As above
Replication:	6 replicates per treatment and control group
No. animals/vessel:	5
Duration of test:	48 hours

Environmental test conditions

Temperature:	18 – 22.0°C
Relative humidity:	60 – 90%
Lighting:	The light / dark cycle was 16:8 hours. The light intensity was 467 - 1115 lux in the mortality phase, 545 - 1716 lux in the parasitation phase and 1560 – 10650 lux in the reproduction phase of the study (measured once per phase).

Study Design

The objective of this extended laboratory study was to investigate the lethal and sub-lethal toxicity of Spiroxamine EC 500 g/L on the parasitoid wasp *Aphidius rhopalosiphi* when exposed on a plant surface.

Test rates of 100, 173, 300, 520, 900 g a.s./ha, a control and reference item (dimethoate at 3 g a.s./ha) groups were tested.

The mummies obtained from the breeder were distributed to several glass tubes which were tapered at the end, obliquely ground and to be closed with silicone stoppers. Until the start of the study the mummies were stored at the temperature given by the breeder. Two days prior to the start of the study all hatched animals were removed from the tubes to ensure that the age of the test animals was below 48 hours. The animals were fed via feeding tubes inserted into the boring of silicone stoppers. The feeding solution consisted of 3 parts of water + 1 part of honey.

For the mortality assessment seven days prior to the start of the study the barley plants were sown (10-12 seed grains each). For the reproduction assessment five days prior to the start of the study the barley plants required were sown (18- 20 seed grains each). One day after the start of the study the plants were infected with *Rhopalosiphum padi*, and the soil surface of the pots was covered with quartz sand.

The test units consisted of a pot with treated barley plants which were enclosed within a clear polyacrylic cylinder (195 mm high and 100 mm in diameter) with a hole (approximately 5 mm in diameter) for the introduction of the parasitoids. After introduction the hole was closed by a stopper. The top of the cylinder was closed with a fine mesh gauze.

Prior to application the plants were sprayed with a 10% fructose solution and were left to dry for at least one hour. After spraying the plants with fructose solution the soil surface was covered with a thin layer of quartz sand before treatment.

The suspensions for the test and reference items were prepared at the day of application group at concentrations of 100, 173, 300, 520, 900 g a.s./ha, a control and reference item (3 g a.s./ha) groups were also tested. They were applied to the test plants using a sprayer specially constructed, permitting area application.

After the spray coating had dried, the potted plants were enclosed within the polyacrylic cylinder.

Within the first hour after application the test animals were introduced from prepared test tubes by slightly blowing them.

If all wasps were in the test unit, the glass tube was removed and the orifice closed with a stopper. During the exposure phase of 2 days the test animals had access to the sugar solution on the treated plants.

To determine whether residues of the test item were repellent to the wasps, observations on the position of the individual insects were made during the initial 3 hours after their release. Five separate observations were made at approximately 30-minute intervals starting 0-15 minutes after the introduction of all wasps. Each wasp was recorded as being on either the plants, cylinder or soil.

Wasps on the plants were not counted directly, but the number of wasps on the cylinder and soil were subtracted from the number of introduced wasps.

At the end of the exposure period the condition of the test animals was recorded as either, live and unaffected, affected, moribund or dead.

Subsequently 15 healthy females were transferred and kept individually in untreated acrylic cylinders with aphid-infested barley plants. One day later the wasps were removed and the plants kept for 12 more days. The cylinders were aerated to avoid the formation of condensed water on the walls. The parasitation rate was determined by counting the number of mummies for each individual wasp.

At 2, 24 and 48 hours of the test, the number of moribund and dead wasps was summed up for each replicate and calculated as percentages. A mean value of the six replicates was calculated.

The percentage of observations of wasps settled on the plants over the whole assessment period was calculated for each treatment. The calculation was based on the parasitoids on the plants and the cylinder because under normal circumstances the wasps do not naturally visit the sand surface beneath the plants. Any individuals observed to be on the sand were inevitably there because they have been affected by the treatment to such an extent that they cannot alight on the plants or the cylinder. Therefore these individuals were not included in the statistical analysis, since their position in the arenas was not a direct consequence of any potential repellent effect. Data of the control and the test item were assessed with suitable statistical procedures.

Reproductive performance was calculated for each replicate and expressed as mummies per female.

The experiment was performed in a controlled environment room at a temperature of 18 - 22°C and a relative humidity of 60 – 90%. The light dark cycle was 16:8 hours. The light intensity was 467 - 1115 lux in the mortality phase, 545 - 1716 lux in the parasitation phase and 1560 - 10650 lux in the reproduction phase of the study (measured once per phase).

II. Results and Discussion

The validity criteria used in the study have been met:

- Mortality in the control group ≤13% (actual: 0%)
- Mortality in the reference item group ≥50% (actual: 53.3%)
- Mummies per female produced in the control group ≥5 (actual: 16.5)
- Number of female wasps producing no mummies ≤2 (actual: 0%)

After 48 hours of the test all wasps were found alive in the control group and in the group treated with 100, 300 and 520 g a.s. test item/ha. In the 173 and 900 g a.s. test item/ha rates 96.67% of the wasps survived and 3.3% were found dead or moribund. In the reference item group 23.3% of the wasps were dead and 30% were moribund after 48 hours of exposure.

Table CP 10.3.2.2/02-1 A summary of the effects of Spiroxamine 500 g/L on mortality of *Aphidius rhopalosiphi* on barley plants

Test rate (g a.s./ha)	Mortality (%)
Control	0.0
100	0.0
173	3.3
300	0.0
520	0.0
900	3.3
Reference item	53.3

The mean number of mummies per female in the control group was 16.5. This compared to 10.3 mummies/female in the 100 g a.s. /ha rate of the test item, 23.7 and 13.5 mummies/female in the 173 and 300 g a.s./ha rate and 13.4 and 15.7 mummies/female in the 520 and 900 g a.s./ha rate of Spiroxamine 500 g/L.

The reduction in reproductive success relative to the control at the 100, 300, 520 and 900 g a.s./ha rate was 37.5, 18.1, 19 and 5.2%. No reduction (-43.1%) was detected at the 173 g a.s./ha rate.

Table CP 10.3.2.2/02-2 A summary of the effects of Spiroxamine 500 g/L on reproduction of *Aphidius rhopalosiphi* on barley plants

Test rate (g a.s./ha)	Mean mummies/female	Reduction relative to control [%]
Control	16.5	0
100	10.3	37.5
173	23.7	-43.1
300	13.5	18.1
520	13.4	19
900	15.7	5.2
Reference item	n.d.	n.d.

n.d. Not detected

During the observations in the initial 3 hours of the test a mean of 32% of the wasps settled on the plants in the control group. In the groups treated with 100, 173, 300, 520 and 900 g a.s./ha a mean of 34.7, 30.5, 24.2, 23.5 and 4.7% of the wasps settled on the plants. In the toxic reference group 39.3% of the wasps were found on the plants.

Table CP 10.3.2.2/02-3 A summary of the mean effects of Spiroxamine 500 g/L on repellency of *Aphidius rhopalosiphi* on barley plants

Test rate (g a.s./ha)	% wasps on plant	Relative to control [%]
0	32	0
100	34.7	-8.3
173	30.5	4.7
300	24.2	24.5
520	23.5	26.6
900	4.7	85.4*
Reference item	39.3	-22.9

*Statistically significant p <0.0001. One-way ANOVA, p-values are adjusted according to Dunnet

III. Conclusion

In this extended laboratory test the effects of residues of Spiroxamine EC 500 g/L on the survival of *Aphidius rhopalosiphi* were determined at 100, 173, 300, 520 and 900 g a.s./ha applied to barley plants. In the dose rates of 173 and 900 g a.s./ha 3.3% corrected mortality was observed. At the rates of 100, 300 and 520 g a.s./ha no mortality was detected.

The reduction in reproductive success relative to the control at the 100, 300, 520 and 900 g a.s./ha rate was 37.5, 18.1, 19 and 5.2%. No reduction (-43.1%) was detected at the 173 g a.s./ha rate.

A statistically significant dose related repellent effect of the test item at 900 g a.s./ha was observed.

The LR_{50} and ER_{50} were estimated to be >900 g a.s./ha.

Assessment and conclusion by applicant:

Validity criteria have been assessed according to the current extended *Aphidius rhopalosiphi* test method by Mead-Briggs *et al*. (2009) and have been met:

- Mortality in the control group ≤10% (actual: 0%)
- Mortality in the reference item group ≥50% (actual: 53.3%)
- Mummies per female produced in the control group ≥5 (actual: 16.5)
- Number of female wasps producing no mummies ≤2 (actual: 0%)

It is noted that this study pre-dates the issue of the formal test method by Mead-Briggs *et al*. (2009) for the extended test design but was conducted in accordance with a draft (2006) version as well as the standard glass plate test design method (Mead-Briggs *et al*. (2000)). The methods used in this study are consistent with the 2009 version and followed the recommended methods and procedures. The study is therefore considered acceptable.

The LR_{50} and ER_{50} were estimated to be >900 g a.s./ha. A significant repellent effect of the test item at 900 g a.s./ha was observed.

Data Point:	KCP 10.3.2.2/03
Report Author:	[redacted]
Report Year:	2001
Report Title:	Acute effects of a repeated spray treatment with the fungicide KWG 4168 EC 500 on lycosid spiders (Pardosa spp., mainly P. agricola) under extended laboratory conditions
Report No:	SXR/SP 04
Document No:	M-008522-02-1
Guideline(s) followed in study:	Auswirkungen von Pflanzenschutzmitteln auf Spinnen der Gattung Pardosa (Araneae, Lycosidae) im Laboratorium. Richtlinienvorschlag für die Prüfung von Pflanzenschutzmitteln im Zulassungsverfahren Teil VI, 23-2.1.9 (Wehling, A and Heimbach, U., 1994)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The effects of a repeated spray treatment with KWG 4168 EC 500 on lycosid spiders were examined under extended laboratory conditions over 15 days. Spiders were exposed to the test substance *via* a spray delivering nominally 1.5 L product/ha (equivalent to 737 g a.s./ha) on test days 0 and 8.

No significant effects on mortality, behaviour or feeding were observed over the test period.

The results of this study indicate that ground-dwelling spiders as represented by *Pardosa* spp. (mainly *P. agricola*) will not be impacted when exposed to a repeated spray treatment with 1.5 L/ha KWG 4168 EC 500. The LR_{50} and ER_{50} were considered to be >1.5 L product/ha (>737 g a.s./ha).

I. Materials and Methods

Materials

Test Material KWG 4168 EC 500

Lot/Batch #: 089A based on Form. No. 04023/0021

Purity: 491.4 g/L

Description: Clear yellow liquid

Stability of test compound: Maximum expected loss of a.s. by hydrolysis <0.1%

Reanalysis/Expiry date: 16 December 1994

Density: Not reported

Treatments

Test rates: Two applications of 1.5 L/ha (equivalent to 737 g a.s./ha) in 300 L water, equivalent to 0.285 mL per test vessel per application

Solvent/vehicle: Water

Analysis of test concentrations: No

Test organisms	
Species:	*Pardosa* spp. (mainly *Pardosa agricola*)
Source:	Collected from untreated fields approximately 3 km south of Monheim, Germany
Feeding:	Fed onion flies (*Delia antiqua*)
Test design	
Test vessel:	Plastic boxes of approx. 10 x 10 x 5.5 cm, covered with plastic screens of mesh size 1 mm containing 125 g soil.
Test medium:	Natural “silty sand” soil
Replication:	20 replicates per treatment
No. animals/vessel:	Individually housed
Duration of test:	15 days
Environmental test conditions	
Temperature:	20 – 23°C
Relative humidity:	63 – 86%

Study Design

The effects of a repeated spray treatment with KWG 4168 EC 500 on lycosid spiders were examined under extended laboratory conditions over 15 days.

Test species were *Pardosa* spp. spiders (mainly *Pardosa agricola*) collected from untreated fields approximately 3 km south of Monheim, Germany. Spiders were held until for three days to acclimate them to test conditions. Spiders were fed six times during the test period with 2 to 6 onion flies (*Delia antiqua*), with a total of 20 flies introduced over the duration of the test.

Test vessels were plastic boxes of approximately 10 x 10 x 5.5 cm (surface area approximately 95 cm^2), covered with plastic screens of mesh size 1 mm. To each test vessel was added 125 g natural “silty sand” soil.

Application of the spray solution was performed twice, on days 0 and 8, delivering nominally 1.5 L/ha test substance in 300 L water to the test vessels. Application of the test substance was conducted with a motor-driven spray boom, delivering nominally 0.285 mL spray fluid to each vessel per application. Control vessels were sprayed with drinking water only. Measured amounts of applied test fluid were between 0.27 and 0.40 mL per box.

A single spray application of 2 L/ha of Metasystox R EC 250 blau (containing the a.s. oxydemeton – methyl) served as reference treatment.

Each treatment condition included 20 individually housed spiders.

Assessments of spider condition were made on days 0 (2h, 4h, 6h), 1, 4, 5, 7, 8 (2h, 4h, 6h), 11, 13 and 15 following application.

Spiders were assessed as being affected (upright and attempting to walk, showing signs of reduced coordination), moribund (on their back or side, either immobile or twitching slightly) or dead (showing no movements after mechanical stimulation). The number of provided onion flies eaten was also recorded.

Test boxes were maintained in a light thermostat at 20 to 23°C with a relative humidity of 63 to 86%.

II. Results and Discussion

Validity criteria were not assessed in the study report.

In the control boxes, all spiders survived the 15-day test period unaffected. None of the 20 spiders revealed any impacts on behaviour during the study period.

All 20 of the exposed spiders survived the repeated spray treatment with 1.5 L/ha KWG 4168 EC 500. Only one spider showed reversible impacts on behaviour.

The reference treatment caused a 100% mortality following application.

Table CP 10.3.2.2/03-1 Mortality after exposure to spray treatments

Nominal concentration (L/ha)	Cumulative mortality by week (%)			Total mortality (%)
	1	2	3	
Control	0	0	0	0
2 x 1.5	0	0	0	0
Reference	100	100	100	100*

* Significantly different to the control

Control spiders consumed an average of 0.33 onion flies per individual per day over the 15-day exposure period. Those spiders exposed to the test substance consumed an average of 0.35 onion flies per individual per day. The individual feeding activity was not statistically significantly affected by exposure to the test compound.

Table CP 10.3.2.2/03-2 Feeding activity during exposure to spray treatments

Nominal concentration (L/ha)	Average number of flies eaten by day							
	1	4	5	7	11	13	15	Total
Control	1.0	1.0	0.5	0.65	1.1	0.2	0.53	4.95
2x 1.5	1.1	0.9	0.55	0.35	1.2	0.12	1.0	4.55
Reference	0.00	-	-	-	-	-	-	0.00

III. Conclusion

The results of this study indicate that ground-dwelling spiders as represented by *Pardosa* spp. (mainly *P. agricola*) will not be impacted when exposed to a repeated spray treatment with 1.5 L/ha KWG 4168 EC 500. The LR_{50} and ER_{50} were considered to be >1.5 L product /ha (>737 g a.s./ha).

Assessment and conclusion by applicant:

No validity criteria assessment was included in the report, therefore, an assessment has been made against the current IOBC test method for testing effects of plant protection products on spiders of the genus *Pardosa* (Araneae, Lycosidae) under laboratory conditions (Heimbach *et al*, 2000).

- The guideline suggests a maximum mortality of two spiders (6.7%) with 30 replicates after three weeks or four spiders (13.3%) if the test is sextended. The current study has only 20 replicates but the control mortality was 0% therefore the criterion is considered to have been met.
- The reference item to result in mortality of 65 ± 35% (actual: 100%)

The study was conducted prior to the IOBC test methods being published and therefore the test method used in this study, although largely consistent, does deviate from the current test method. However, the validity criteria from the current IOBC test method are considered to have been met therefore the study is considered to be acceptable, the results valid and suitable for consideration in the risk assessment.

The LR_{50} and ER_{50} were considered to be >1.5 L product /ha (>737 g a.s./ha).

Data Point:	KCP 10.3.2.2/04
Report Author:	[redacted]
Report Year:	1994
Report Title:	Acute effects of repeated spray application of KWG 4168 on carabid beetles (Bembidion tetracolum) under extended laboratory conditions
Report No:	SXR/CA 115
Document No:	M-008528-01-1
Guideline(s) followed in study:	BBA guideline 23-2.1.8
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The effects of a repeated spray treatment with KWG 4168 EC 500 on carabid beetles were examined under extended laboratory conditions over 21 days. Beetles were exposed to two treatments of 1.5 or 3.0 L product/ha (equivalent to 741 and 1482 g a.s./ha) test substance *via* a spray.

No significant effects on mortality, behaviour or feeding were observed over the test period.

The results of this study indicate that carabid beetles will not be impacted when exposed to a repeated spray treatment of 1.5 or 3.0 L product/ha. Thus, the LR_{50} and ER_{50} were considered to be >3.0 L product/ha (>1482 g a.s./ha).

I. Materials and Methods

Materials

Test Material	KWG 4168 EC 500
Lot/Batch #:	089A based on Form. 04023/0021
Purity:	494 g/L
Description:	Yellow liquid
Stability of test compound:	Maximum expected loss of a.s. by hydrolysis <0.2%
Reanalysis/Expiry date:	17 March 1994
Density:	Not reported

Treatments

Test rates:	Two applications of 1.5 or 3.0 L product/ha (equivalent to 741 and 1482 g a.s./ha) in 400 L water, equivalent to 0.8 mL solution per test vessel per application
Solvent/vehicle:	Water
Analysis of test concentrations:	None

Test organisms

Species:	Carabid beetle *Bembidion tetracolum* aged 3 – 5 weeks
Source:	Commercial supplier (Dr. Thieme, D-18184 Sagerheide)
Feeding:	Fed onion fly (*Delia antiqua*) pupae
Acclimation:	Three days

Test design

Test vessel:	1.2 L plastic boxes of 16.8 x 11.8 x 6.2 cm covered with 1 mm plastic mesh screens containing 250 g natural soil
Test medium:	Loamy sand soil sieved to 2 mm from "Laacher Hof", Monheim, which had been untreated with pesticides for several years
Replication:	Five replicates per treatment
No. animals/vessel:	Three males and three females per vessel
Duration of test:	21 days

Environmental test conditions

Temperature:	20 – 23°C
Relative humidity:	60 – 80% (five days with deviations, ranging from 40 – 85% RH)

Study Design

This study was conducted in order to evaluate the effects of KWG 4168 exposure to carabid beetles under more realistic conditions *via* a repeated spray application over 21 days.

Test species were *Bembidion tetracolum* beetles, obtained from a commercial supplier (Dr. Thieme, D-18184 Sagerheide) and aged between 3 and 5 weeks. Beetles were fed six pupae of *Delia antiqua* periodically throughout the test period.

Test vessels were 1.2-L plastic boxes of approximately 16.8 x 11.8 x 6.2 cm, covered with plastic screens of mesh size 1 mm. To each test vessel was added 250 g loamy sand soil sieved to 2 mm. Test soil was collected from "Laacher Hof", Monheim, from a field that had been untreated with pesticides for several years.

Application of the spray solution was performed twice, on days 0 and 7, delivering nominally 1.5 or 3.0 L product/ha (equivalent to 741 and 1482 g a.s./ha) test substance in 400 L water to the test vessels. Application of the test substance was conducted under constant air flow through a fine nozzle, delivering nominally 0.8 mL spray fluid to each vessel per application. Control vessels were sprayed with drinking water only.

Assessments of beetle condition were made on days 0 (2h, 4h), 1, 2, 4, 7, 8, 11, 14, 17 and 21 following application. Beetles were assessed as being affected (upright and attempting to walk, showing signs of

reduced coordination), moribund (on their back or side, either immobile or twitching slightly) or dead (showing no movements after mechanical stimulation). The number of pupae consumed was also recorded.

Test boxes were maintained in a light thermostat at 20 to 23°C with a relative humidity of 60 to 80%. Humidity varied between 40 and 85% on five days during the test, however this was not considered to affect the outcome of the study.

II. Results and Discussion

Validity criteria were not assessed as part of the study.

In the control boxes, 29 out of 30 beetles were still alive at the end of the 21 day exposure period (mortality rate: 3.3%). No behavioural impacts were recorded during the course of the study. A repeated exposure to a spray application of KWG 4168 EC 500 at a rate of 1.5 or 3.0 L/ha did not result in sublethal or lethal impacts on the test beetles (mortality rates: 3.3 and 6.6% for the 1.5 and 3.0 L/ha treatment, respectively). Under identical test conditions, the reference formulation (50 and 100 g/ha Methylparathion WP 40) caused a 50 % mortality within the 21 day test period.

Table CP 10.3.2.2/04-1 Mortality after exposure to spray treatments

Nominal concentration (L/ha)	Cumulative mortality by week (%)			Total mortality (%)
	1	2	3	
Control	3.3	3.3	3.3	3.3
2 x 1.5	3.3	3.3	3.3	3.3
2 x 3.0	3.3	3.3	6.6	6.6
Reference	10.0	40.0	0.0	50.0%

The individual feeding activity was also statistically not affected by the test compound. If the total number of pupa consumed per viable beetle per day in the control plots of 0.25 is used as a basis for comparison and assumed to be 100%, then the feeding rates for the 1.5 and the 3.0 L/ha KWG 4168 EC 500 treatment were 84% (0.21). In contrast, the reference treatment caused a statistically significant reduction of the feeding activity which corresponded to 44% (0.11) of that of the controls.

Table CP 10.3.2.2/04-2 Feeding activity after exposure to spray treatments

Nominal concentration (L/ha)	Feeding activity			Mean feeding rate
	Week 1	Week 2	Week 3	
Control	0.23	0.28	0.23	0.25
2 x 1.5	0.17	0.27	0.20	0.21
2 x 3.0	0.16	0.26	0.21	0.21
Reference	0.12	0.12	0.10	0.11

III. Conclusion

In this extended laboratory test, application of spiroxamine twice a week resulted in corrected mortality rates of 0 % for 1.5 L product/ha and 3.5 % for 3.0 L product/ha. The feeding activity was not significantly affected (16 %).

The LR_{50} and ER_{50} were considered to be >3.0 L product/ha (>1482 g a.s./ha).

Assessment and conclusion by applicant:

Validity criteria were not assessed in the study and there is currently no formalised test method for *Bembidion tetracolum*. Validity criteria from the IOBC test method for another beetle species (*Poecilus cupreus*) has therefore been used as a substitute. Thus, according to Heimbach *et al.* (2000) "A method for testing effects of plant protection products on the carabid beetle *Poecilus cupreus* (Coleoptera, Carabidae) under laboratory and semi-field conditions" the validity criteria were met.

- Mortality to be less than 6.7% in the water control (actual: 3.3%)
- Mortality to be 65% (± 35%) in the reference item within 2 weeks (actual: 50% mortality after 21 days)

Based on this the study is therefore considered acceptable and the results suitable for use in the risk assessment.

The LR_{50} and ER_{50} were considered to be >3.0 L product/ha (>1482 g a.s./ha).

CP 10.3.2.3 Semi-field studies with non-target arthropods

Two semi-field studies using Spiroxamine EC 500 are available and have been summarized below.

Data Point:	KCP 10.3.2.3/01
Report Author:	[redacted]
Report Year:	1995
Report Title:	Effects of KWG 4168 EC 500 on the life cycle of ladybird beetles (Coccinella septempunctata) under semifield conditions
Report No:	SXR/CS 06
Document No:	M-008547-01-1
Guideline(s) followed in study:	(BBA guideline 23 - 2.1.5 (Pinsdorf, 1989)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997) RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The effects of a repeated spray application of 1.5 L/ha KWG 4168 EC 500 on the life cycle of the seven pointed ladybird beetle (*Coccmeila septempunctata* L.) were tested under outdoor conditions for 61 days. Ladybird beetles were exposed to two treatments of 1.5 L/ha test substance *via* a spray.

The fecundity of the ladybird beetle females did not statistically significantly differ between the control and the KWG 4168 treatment. Hatch rate of the eggs laid by the KWG 4168 treated beetles was even statistically significantly higher than of those laid by the control beetles.

The results of this study indicate that, under normal agricultural practice, ladybird beetles as represented by the seven pointed ladybird beetle will not be impacted by a repeated spray application of KWG 4168 EC 500 up to 1.5 L/ha. Moreover, there were no indications of treatment – related effects on the reproductive performance of this non-target beneficial species.

I. Materials and Methods

Materials

Test Material	KWG 4168 EC 500
Lot/Batch #:	089A based on Form. 04023/0021
Purity:	491.4 g/L
Description:	Clear yellow liquid
Stability of test compound:	Not reported
Reanalysis/Expiry date:	16 December 1994
Density:	Not reported
Treatments	
Test rates:	Two applications of 1.5 L/ha
Solvent/vehicle:	Drinking water
Analysis of test concentrations:	None
Test organisms	
Species:	4 to 5 day old ladybird larvae (*Coccinella septempunctata*)
Source:	Commercial supplier (PK-Nützlingszuchten, 73642 Welzheim)
Feeding:	Pea aphids
Acclimation:	Test species were purchased from a commercial supplier as eggs laid between July 4 and 6, 1994. The eggs were transferred to petri dishes (9 cm diameter) and stored until hatch. Larvae appeared between July 9 and 10. They were fed with aphids (*Myzus persicae*) and kept in a climate control cabinet at a temperature of 23 - 25°C, 40 - 60% relative humidity and in a light-dark-cycle of 16:8 hours until one day before application
Test design	
Test vessel:	Tray housing infested bean plants, with stainless steel frames 52 x 33 x 14 cm. nylon gauze was added to prevent escape and unwanted predation or parasitisation
Test medium:	Garden soil
Replication:	Four replicates per treatment
No. animals/vessel:	20
Duration of test:	61 days
Environmental test conditions	
Temperature:	Outdoor part of study: 13 – 33°C Indoor part of the study: 22 – 26°C
Relative humidity:	50 – 60% with very occasional drops up to 45% and peaks up to 70%

Study Design

The aim of this semi-field study was to evaluate the side-effects of KWG 4168 EC 500 exposure under conditions more realistic to the field. For this, 2nd instar larvae were confined over aphid-infested broad bean seedlings treated with the test product and their survival, differentiation rates and the reproductive capacity of the descendants were assessed.

Test species were *Coccinella septempunctata,* obtained from a commercial supplier (PK-Nützlingszuchten, 73642 Welzheim) and aged between 4 and 5 days. Beetles were fed pea aphids.

Twenty randomised beetle larvae were carefully put onto the aphid-infested broad beans within the centre of each planted replicate tray. The broad bean plants were sealed by a rectangular enclosure made of nylon gauze to prevent the larvae from escaping. After approximately 1 hour, the nylon gauze enclosure was removed and the four replicate trays of each treatment (control, test and reference compound) were arranged along a line close to the study site and jointly sprayed. Immediately after spraying, stainless steel frames (52 x 33 cm = 0.17 m²) of 14 cm height were firmly pressed onto the planted trays and the planted central area enclosed between the frame walls.

The upper borders of the steel frames were twisted to the inner side to prevent beetle larvae from escape. Then, rectangular enclosures made of nylon gauze (47 x 28 x 50 cm) were put on the upper site of the steel frames to keep predators like birds and small mammals as well as parasites out of the test system. The entire cage system was put into larger trays which were provided with an outlet and could be filled with water if required. Subsequently, all cage devices were transferred to the study site where they were arranged in a block design. *Via* drainage holes in the bottom of the inner trays, the plants could be supplied with water during precipitation free intervals without influencing the spray deposit on the leaf surfaces by filling the outer tray with water.

When prey aphids were harmed by either treatment, they were replaced regularly until day 10 after application which allowed to discriminate between direct and indirect adverse effects.

A hand-operating spray boom was used to apply both active treatments and the drinking water. All replicate cages of a treatment were set up in a line within an area of 1.5 x 10m in size, to ensure even application. The sprayer was carried on the back while walking along the treatment area. Spray application rate was controlled by adjusting walking speed. This was tested before and recorded during the application. The applied spray fluid volume was 300 L/ha (450 ml per 15 m² plot). The 450 ml spray fluid were held in reservoir containers. Spray fluid was advanced to an Agrotop spray boom (I red with Lurmark 03F110 nozzles), via dead-space free ball-valves, which delivered the spray at a constant pressure of 2.0 bar.

Since KWG 4168 EC 500 will be repeatedly applied in the field, a second exposure of the same generation was simulated by exposing adult beetles to treated plants during the fecundity assay in that week which was considered for hatching rate. The bean plants were treated in a spray cabinet. A motor-driven spray boom (spray nozzle: Teejet 8001 5 E - SS) was moved at 3.5 m/second in a distance of 45 cm over the bean plants. All replicate plants of a treatment were set up in a line to ensure even application. The applied spray fluid volume was 300 L/ha (30 mL per treatment). The 30 mL spray fluid were held in reservoir containers. Spray fluid was delivered at a constant pressure of 3.0 bar.

The conditions of the ladybird beetle larvae was recorded at approximately 24 hours after application. They were classed as being live, affected moribund/dead and pupated. Further checks were conducted on days 4 and 5. After appearance of the first pupa at day 5, checks were made daily until the last visible larva had been pupated.

The pupal and prepupal stages were sampled on a daily basis by cutting off the leaves on which pupae were clinging. They were stored in plastic petri dishes (9 cm diameter) under controlled laboratory conditions (22 - 26°C; 45 - 65% air humidity; 16:8 hour light:dark cycle). After emergence, beetles were transferred into uncontaminated breeding containers. Observations on test animals were continued to assess potential effects of the active treatments on fecundity. The sex of the beetles was determined by

microscope 4 to 8 days after emergence. Then, all surviving animals from one treatment were pooled and subdivided into groups of up to 20 individuals with equalized sex ratios.

The rearing containers consisted of macrolon with side and rear walls of nylon netting. The front wall was removable. The floor was lined with filter paper. Breeding cages were maintained at 24 - 29°C, 16 h photoperiod >1000 lux and 40 - 80% air humidity. The laboratory cabinet was ventilated by the air-conditioning system of the building. During the bioassay, beetles were fed on aphids that were held on various plants which were exchanged regularly. Every 7 days, the beetles were transferred to clean disinfected cages. To support oviposition, uncontaminated fan-folded filter papers were placed between the aphid - infested plants. Filter papers on which eggs had been deposited were removed from the cage daily (excluding weekends) and replaced against fresh filter papers. After removal from the cage, eggs were counted and disposed off. Starting with day 14 relative to that date when the last test beetle had emerged, all eggs which were laid during the subsequent 7 days were sampled and stored in a petri dish (1 egg-batch per dish) until hatching. The hatching rate of this week was determined and extrapolated to the total amount of eggs laid.

Weather conditions during the outdoor part of the study were continuously recorded at an established weather station located approximately 0.5 km apart from the study site. During the indoor phase, temperature and relative air humidity were monitored by hygrometers.

II. Results and Discussion

In the control and the test substance cages, more than 50% of the added larvae were easily visible during most of the assessment periods. In contrast, only one larva was observed in the reference cages. However, only a marginal number of dead larvae were recorded in the reference cages, which is most presumably related to the small size of the test larvae at that time.

On average, control beetles entered the pupal stage 7.7 days after application. In the cages which were treated with the test substance, larvae pupated on average a bit earlier (7.3 days after application) than in the control cages. In the control cages, 3 out of the 80 added larvae were not recovered at the end of the outdoor part of the study. Including the mortality in the pupal stage, the control mortality was 15.0%. The corrected preimaginal mortality for the KWG 4168 treatment was calculated to be 2.9%.

Table CP 10.3.2.3/01-1 The average number of mortalities recorded at each developmental stage in the test cages

Test parameter	Treatment[1]		
	Control	KWG 4168 EC 500	Reference item
Initial No. of larvae	20	20	20
Technical losses	0	0	0
No. of dead larvae found	0	1	0
No. of pupae found	18.5	17.5	0
No. of emerged beetles	17	16.5	0
% total mortality	15	17.5	100

[1] Mean of three replicates in the control and KWG 4168 EC 500, two in the reference item

The results of the fecundity/fertility test are presented below. The controls laid on average 379.4 eggs per female. Females which emerged from KWG 4168 EC 500 treated larvae produced 413.5 eggs on a per female basis. The hatching rate of eggs laid by treated females averaged 88.6% whereas in the controls a significantly lower hatching rate of 82.1% was recorded. The reproductive performance R [%] of the ladybird beetles which were exposed to KWG 4168 EC 500 was 117.6 % relative to the controls.

Table CP 10.3.2.3/01-2 The total number of eggs laid and larvae hatched in relation to treatment in the control cages (fertility was only tested on eggs laid between day 34 and day 40 after application)

Test parameter	Treatment[1]	
	Control	KWG 4168 EC 500
Initial No. of larvae	22.7	22.0
Initial No. of females	14.7	14.0
Total No. of eggs laid	5356.7	5283.7
No. of eggs laid per female	1268.0	413.5
No. of hatched larvae	4630 of 5631	4841 of 5463
% hatching rate	82.1	88.6
Total No. fertilised eggs/female	312.0	368.0

[1] Mean of four replicates

III. Conclusion

For larval ladybird beatles, direct overspray and fresh residues of Spiroxamine 500 EC under semi-field conditions did not have harmful effects on completion of metamorphoses, fecundicy and hatch rate when applied at rates of 737 g a.s./ha in the field followed by a second spraying in reproduction phase in the lab. The effect on preimaginal mortality (2.9 %) is not significant. Moreover, there were no indications of treatment related effects on the reproductive performance of this non-target beneficial species.

Assessment and conclusion by applicant:

The study was conducted to an old BBA guideline but with some modifications.

Although not directly applicable to this study, an assessment of validity has been made against the current IOBC test method by Schmuck R *et al.* (2000) for *Coccinella* in order to determine if the results achieved are valid:

- The average pre-imaginal mortality of the water treated larvae should not exceed 30% (actual: average = 15%)
- The level of pre-imaginal mortality of the larvae exposed to the reference item should result in a pre-imaginal mortality of at least 40% (actual: average = 100%)
- The number of eggs laid by control females should be ≥2 fertile eggs per viable female per day (actual: 1268 eggs per female were laid over 61 days which equals 20.8 eggs per viable female per day).

According to guidance on testing and interpretation of studies with non-target arthropods by Candolfi *et al.* (2000), the recommended model crop for vineyard uses is apple or vines whereas this study used broad beans which is the recommended crop for arable crop uses. The study has not been conducted to the current test methodology but the parameters assessed are consistent and the validity criteria of the current laboratory test method are considered to have been met. The study is therefore considered acceptable and the results suitable for use in the risk assessment.

Data Point:	KCP 10.3.2.3/02
Report Author:	[redacted]
Report Year:	1994
Report Title:	A semi-field evaluation of the side-effects of the fungicide KWG 4168 500 EC, applied to winter wheat, on the robust life-stage of the parasitic wasp Aphidius rhopalosiphi
Report No:	BAY-94-3
Document No:	M-008539-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

A semi-field study was carried out to determine the side-effects of the fungicide KWG 4168 500 EC on the robust pupal stage of the aphid parasitoid, *Aphidius rhopalosiphi*.

In the control, 98% of the wasps emerged successfully compared with 95% in the spiroxamine treatment and 69% in the dimethoate treatment. Over half (71%) of the wasps that emerged in the dimethoate treatment were found dead inside the chip cages by the time assessments were made, probably due to their contact with product residues on the leaf surface. This compared with wasp mortalities of 11% in the spiroxamine treatment and 2% in the control.

The results indicate that KWG 4168 500 EC applied at a rate of 737 g a.s./ha had no relevant effects on mortality (9.2%) to pupal *A. rhopalosiphi* under semi-field conditions.

I. Materials and Methods

Materials

Test Material KWG 4168 500 EC

Lot/Batch #: 089A

Purity: 491.4 g/L

Description: Not reported

Stability of test compound: Not reported

Reanalysis/Expiry date: Not reported

Density: Not reported

Treatments

Test rates: 200 L/ha

Solvent/vehicle: Drinking water

Analysis of test concentrations: None

Test organisms

Species:	*Aphidius rhopalosiphi*
Source:	Not reported (reared in culture)
Feeding:	Not reported
Acclimation:	Mummies containing pupating wasps were collected and stored in lidded plastic pots for two days prior to being treated in the field. storage conditions were 12 – 19°C, 16 hour photoperiod.

Test design

Test vessel:	Three blocks of three plots (each 2 x 5 m) were marked out in the crop using "flexicanes". Each separated from the next with a 2 m buffer strip. The three treatments (test product, toxic standard and a water treated control) were randomly assigned to the plots
Test medium:	Chalky loam soil (moist, with a medium tilth and firm compaction)
Replication:	Three
No. animals/vessel:	15
Duration of test:	7 days in field with additional 3 days in laboratory as required

Environmental test conditions

Temperature:	3 – 26°C
Rainfall:	0.0 mm (excluding day 3, 1.0 mm of rainfall was observed)

Study Design

A semi-field study was carried out to determine the side-effects of the fungicide KWG 4168 500 EC on the robust pupal stage of the aphid parasitoid, *Aphidius rhopalosiphi*.

Laboratory-reared aphid mummies containing parasitoid pupae of a uniform age were attached to the upper surfaces of leaves at three heights in a crop of mature winter wheat. Three replicate plots of 15 mummies were prepared for each treatment and these were then sprayed. Plots were treated with the test product (KWG 4168 500 EC at 1.5 L/ha), a toxic standard (Dimethoate 40 at 0.85 L/ha) or with water as a control.

Clip-cages were placed over the mummies following treatment to prevent their being dislodged. The success of emergence of adult wasps from the mummies was then assessed every 1-2 days over a 7 day period.

The trial was carried out in a crop of winter wheat var. Hunter, on an arable farm in southern England. The crop had not previously been treated with plant protection products but had received fertiliser treatments in February, April and May 1994. At application the crop was at the end of flowering.

Three blocks of three plots (each 2 m x 5 m) were separated from the next by a 2 m buffer strip. The three treatments (test product, toxic standard and a water treated control) were assigned to the plots in a randomised block design.

Whilst still attached to short lengths of leaf, the laboratory-reared mummies were attached to the upper surfaces of leaves in the crop using small staples. The mummies were positioned so that when the leaf was released, they were still visible when viewed from above, *i.e.* so as to be directly exposed to spray. Three groups of five mummies were placed in each plot, the groups being at separate heights of approximately 75, 65 and 55 cm above the ground. To achieve this, the mummies were fixed to the flag leaves and first leaves of the crop plants.

The plots were then treated using an AZO compressed-air sprayer with a 1-m-wide spray boom fitted with flat-fan nozzles (Teejet 8002). The sprayer was calibrated beforehand so that all treatments were applied at a volume rate equivalent to 200 L/ha. Products were diluted in tap water immediately prior to application and tap water was applied to the control treatment plots.

The chalky loam soil was moist, with a medium tilth and firm compaction.

During spraying, water-sensitive papers were fixed to leaves in the crop, at similar heights to the aphid mummies to record the droplet deposition patterns obtained within the crop.

The emergence of the wasps from the mummies was assessed by carefully opening the clip cages at 1, 2, 3, 5, 6 and 7 days after treatment (DAT). It was recorded whether any adult wasps found were alive or dead, and whether any had died during emergence.

After 7 days any intact mummies were collected in and returned to the laboratory. These were kept for a further 3 days and a final assessment of emergence made 10 DAT. Storage conditions for these mummies were 21 - 24°C.

Temperature and rainfall during the study period were monitored using a weather station (Delta-T Instruments data logger fitted with a tipping-bucket rain gauge and air temperature probe) placed at the field margin.

II. Results and Discussion

In the control treatment, 43 out of 44 wasps (98%) emerged successfully, although one of these had died in the clip-cage before being assessed. In the KWG 4168 500 EC treatment, 42 out of 44 wasps (95%) emerged successfully but 5 had died before they were assessed. In the dimethoate treatment, 31 out of 45 wasps (69%) emerged but 22 had died before they were assessed. Fourteen mummies in the dimethoate treatment failed to develop, compared with two in the KWG 4168 500 EC treatment and none in the control. None of the mummies removed to the laboratory between 7 and 10 DAT gave rise to any further adults.

These results would suggest that the toxic standard treatment of dimethoate had affected a proportion of the wasps within the protective pupal cocoon, preventing them from developing into adults. No such effect was seen in the KWG 4168 500 EC treatment, despite the fact that the mummies would almost certainly have been hit by spray droplets – as indicated by the water-sensitive papers. These papers also suggest that the exact position of mummies within the upper parts of the crop did not appear to have a marked influence on their risk of exposure.

In addition, 71% of the wasps emerging in the dimethoate treatment died before being assessed (*i.e.* mostly within 24 hour of emergence). Since the survival of wasps was poorest in the first few days after treatment, it is probably that they were killed by being exposed to product residues on the leaf surface. The results do not suggest that residues of KWG 4168 500 EC were harmful to the emerging adult wasps.

III. Conclusion

A semi-field study was carried out to determine the side-effects of the fungicide KWG 4168 500 EC on the robust pupal stage of the aphid parasitoid, *Aphidius rhopalosiphi*.

In the control, 98% of the wasps emerged successfully compared with 95% in the spiroxamine treatment and 69% in the dimethoate treatment. Over half (71%) of the wasps that emerged in the dimethoate treatment were found dead inside the clip cages by the time assessments were made, probably due to their contact with product residues on the leaf surface. This compared with wasp mortalities of 11% in the spiroxamine treatment and 2% in the control.

The results indicate that KWG 4168 500 EC applied at a rate of 737 g a.s./ha has no relevant effects on mortality (9.2%) to pupal *A. rhopalosiphi* under semi-field conditions.

Assessment and conclusion by applicant:

The study was not conducted to any relevant guideline and as such it is not assessed against any specific validity criteria.

The results of the study are considered to be valid in their own right but it is noted that the crop used in the study was winter wheat and therefore has limited relevance to the crop for which this representative formulation is used on (vines). According to guidance on testing and interpretation of studies with non-target arthropods by Candolfi *et al.* (2000), the recommended model crop for vineyard uses is apple or vines whereas this study used cereals which is the recommended crop for arable crop uses.

The study has therefore been submitted as supporting information only.

CP 10.3.2.4 Field studies with non-target arthropods

Field studies using Spiroxamine EC 500 are available and have been summarized below.

Data Point:	KCP 10.3.2.4/01
Report Author:	[redacted]
Report Year:	1998
Report Title:	A field study to evaluate the effects of a KWG 4168 EC 500 containing 500 g/l Spiroxamine against the predatory mite, Typhlodromus pyri in vines (one location in Germany)
Report No:	98062/G1-NFTP
Document No:	M-008494-01-1
Guideline(s) followed in study:	BBA-guideline Teil VI, 23-2.3.4 (1991)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and classified RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

This study was conducted in order to assess the effects on population development of KWG 4168 EC 500 on predatory mites (*Typhlodromus pyri*) in a study over 4 weeks.

The mites were exposed to residues of the test item at a total rate of 1297 g a.s./ha over four treatments, a control and to the reference item for 4 weeks.

Whilst there was a statistically significant difference in the number of mite eggs after the first two applications, by the end of the study the number of predatory mite eggs observed in plots treated with the test substance was again similar to the number of eggs in the untreated control. At no observation was the number of adult predatory mites in plots treated with the test substance statistically different from the control.

Four applications of KWG 4168 EC 500 with an interval of approximately two weeks did not lead to a reduction of predatory mite populations.

I. Materials and Methods

Materials

Test Material	KWG 4168 EC 500
Lot/Batch #:	04023/0627
Purity:	510.5 g/L
Description:	Liquid
Stability of test compound:	Not reported
Reanalysis/Expiry date:	17 August 1998
Density:	1.003 g/cm^3
Treatments	
Test rates:	1297 g a.s./ha
Solvent/vehicle:	Water
Analysis of test concentrations:	Amounts applied did not differ more than 10% from nominal dose.
Test organisms	
Species:	Predatory mites, *Typhlodromus pyri*
Source:	GAB Biotechnolgie GmbH & IFU Umweltanalytik GmbH,
Acclimatisation period:	None
Feeding:	pollen
Treatment for disease:	None reported
Test design	
Replication:	4
Duration of test:	4 weeks
Environmental test conditions	
Temperature:	11 - 26°C
Relative humidity:	33 - 70%

Study Design

This study was conducted in order to assess the effects on population development of KWG 4168 EC 500 on predatory mites (*Typhlodromus pyri*) in a study over 4 weeks.

Riesling vines were planted at a density of 3570 plants per ha and plots of 10 vines were used for testing. Vines were treated with four spray treatments with 14 ± 4 days interval between each treatment. All treatments were applied using a knapsack sprayer and the amounts applied did not differ more than 10% from nominal dose. The first application was 0.075% of the product application rate followed by 3 applications of 0.15% of the recommended application rate. The total dose applied to the crops was 1297

g a.s./ha. A control treatment (water) and toxic reference treatment (Mancozeb, 750 g/kg) were also used as part of the study.

Population development of the predatory mites was assessed in all three treatments. Leaf samples were taken 6 times throughout the study and the number of mites on the sample was determined. The first assessment was a few days before the first application; the second, third and fourth took place max. 2 days before the second, third and fourth applications, respectively; the 5th assessment was 7 days after the last application and the 6th assessment was approximately 4 weeks after the last application. At all samplings in all plots 25 fully developed leaves, if possible of the same size, were selected randomly from the 8 vines in the centre of the middle row of each plot.

Temperature and relative humidity at the test site varied between 11 - 26 °C and 33 - 70%, respectively throughout the study.

II. Results and Discussion

Four weeks after the spraying sequence the number of adult predatory mites was quite similar between plots treated with the test substance and the untreated control. At no observation was the number of predatory mites in plots treated with the test substance statistically different from the control.

Table CP 10.3.2.4/01-1 Effect on adult predatory mites following exposure to residues of KWG 4168 EC 500

Treatment	% reduction compared to the control at each assessment					
	1	2	3	4	5	6
KWG 4168 EC 500	-56.5	24.2	6.3	24.2	-23.6	8.3
Reference item	-20.4	26.8	45.4	77.0	67.1	86.3

Table CP 10.3.2.4/01-2 Population density following exposure to residues of KWG 4168 EC 500

Treatment	Average number of predatory mites per 25 leaves at each assessment					
	1	2	3	4	5	6
Control	74.8	88.8	142.8	133.5	99.5	96.8
KWG 4168 EC 500	117.0	67.3	133.8	101.3	123.0	88.8
% reduction compared to the control	-56.5	24.2	6.3	24.2	-23.6	8.3

The numbers of eggs in the plots treated with KWG 4168 EC 500 was statistically different from the control plots on two occasions, at the third and fifth evaluation. On the third evaluation date, it was significantly lower in the KWG 4168 EC 500 treated plots whereas on the fifth evaluation date, the number of eggs in the treated plots was significantly higher than in control plots. At the final evaluation four weeks after the last treatment, the number of eggs was again quite similar between the control and the plots treated with KWG 4168 EC 500.

Table CP 10.3.2.4/01-3 Effect on predatory mite eggs following exposure to residues of KWG 4168 EC 500

Treatment	% reduction compared to the control at each assessment					
	1	2	3	4	5	6
KWG 4168 EC 500	-133.3	14.6	51.7	42.6	-116.7	36.4
Reference item	-34.8	41.5	66.0	77.5	60.0	100.0

III. Conclusion

Four applications of KWG 4168 EC 500 with an interval of approximately two weeks did not lead to a reduction of predatory mite populations.

Assessment and conclusion by applicant:

The study was conducted in 1998 and therefore pre-dates much of the current guidance for NTA field testing as detailed in Candolfi *et al*. (2000), ESCORT 3 and de Jong *et al*. (2010)[14]. Analytical dose verification of spray solutions and more advanced statistical techniques, including MDD analysis, are now used as standard in field studies and this study did not employ either of these.

There is a formalised IOBC test method for predatory mite field trials by Blümel *et al*. (2000) "*Guidance document to detect side effects of plant protection products on predatory mites (Acari: Phytoseiidae) under field conditions: vineyards and orchards*". The study was not specifically conducted to this test method as it again pre-dates this guidance but the test methodology used in the study is largely consistent.

Despite the age and lack of recognised guidance being referenced by the study, the results are considered to be valid in their own right and can be used to support the risk assessment by demonstrating an absence of long term effects to mites in vines following application of Spiroxamine EC 500. The study report concluded that the results were valid on the basis that the toxic standard gave the expected level of effect when compared to the control.

There was significant inconsistency in the mite distribution between replicates and before treatment the control mite density was much lower than in the treatment plots therefore the results should be treated with caution.

The study is therefore considered to be supporting information only.

[14] Guidance for summarising and evaluating field studies with non-target arthropods. A guidance document of the Dutch Platform for the Assessment of Higher Tier Studies. De Jong *et al.* (2010)

Data Point:	KCP 10.3.2.4/02
Report Author:	[redacted]
Report Year:	1998
Report Title:	Effects on Typhlodrdomus pyri predatory mites of 'KWG 4168 EC 500' under typical vine culture conditions on grape vines, Germany 1997
Report No:	BAY23
Document No:	M-008496-01-1
Guideline(s) followed in study:	BBA-guideline VI, 23-2.3.4 (1990)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

This study was conducted in order to assess the efficacy of KWG 4168 EC 500 on predatory mites (*Typhlodromus pyri*) in a study over 4 weeks.

The application rates were 216, 426, 550, 667, 754 and 889 mL/ha in the 1st, 2nd, 3rd, 4th, 5th, and 6th applications, respectively. A control treatment (water) and toxic reference treatment (Topas 100 EC, 105 g/L penconazole) were also used as part of the study. The number of mites on leaf samples was assessed after each treatment and 4 weeks after the final treatment.

Six applications of KWG 4168 EC 500 with an interval of approximately two weeks did lead to populations of the predatory mites reducing to 59% of the control level.

I. Materials and Methods

Materials

Test Material KWG 4168 EC 500

Lot/Batch #: 04023/0627

Purity: 505.5 g/L

Description: Clear yellow liquid

Stability of test compound: Not reported

Reanalysis/Expiry date: November 1997

Density: 1.007 g/ml

Treatments

Test rates: 216, 426, 550, 667, 754 and 889 mL/ha in the 1st, 2nd, 3rd, 4th, 5th, and 6th applications, respectively

Solvent/vehicle: Water

Analysis of test concentrations: None

Test organisms

Species:	Predatory mites, *Typhlodromus pyri*
Source:	Staatliche Lehr- und Forschungsanstalt fiir Landwirtschaft, Weinbau und Gartenbau
Acclimatisation period:	None
Feeding:	Pollen
Treatment for disease:	None reported

Test design

Replication:	4
Duration of test:	4 weeks

Environmental test conditions

Temperature:	2.4 – 32.9°C
Precipitation:	Monthly average 6.9 – 90.0 mm

Study Design

This study was conducted in order to assess the efficacy of KWG 4168 EC 500 on predatory mites (*Typhlodromus pyri*) in a study over 4 weeks.

Rows of 15 vines from plots of 120 m^2 Riesling vines were used for testing. Vines were treated with six spray treatments with 14 ± 2 days interval between each treatment. All treatments were applied using a plot tunnel sprayer. The application rates were 216, 426, 550, 667, 754 and 889 mL/ha in the 1st, 2nd, 3rd, 4th, 5th, and 6th applications, respectively. A control treatment (water) and toxic reference treatment (Topas 100 EC, 105 g/L penconazole) were also used as part of the study.

Leaf samples were taken 6 times throughout the study and the number of mites on the sample was determined. The population of predatory mites was assessed by determining the number of mites on leaf samples, using the washing method. Leaf samples consisting of 25 leaves were taken 1 day before the first treatment, after the first to fifth treatment, 1 week and 4 weeks after the final treatment. At all samplings in all plots 25 fully developed leaves, if possible of the same size, were selected randomly from each test plot.

Temperature at the test site varied between 2.4 – 32.9°C and the monthly average precipitation was 6.9 – 90.0 mm.

II. Results and Discussion

Table CP 10.3.2.4/02-1 KWG 4168 EC 500 actual and nominal application rates

	Nominal application rate (ml/ha)	Actual application rate (ml/ha)
KWG 4168 EC 500	220	216
	440	426
	550	550
	660	667

	Nominal application rate (ml/ha)	Actual application rate (ml/ha)
	770	754
	880	889
Reference item	60	58
	120	115
	150	147
	180	174
	210	218
	240	237

During a multiple spray treatment of KWG 4168 EC 500, the populations of predatory mites in the test plots were close to those in the control plots up to 1 week after the final treatment. At the final assessment, populations of the predatory mites were reduced to 59% of the control level. The decrease of predatory mite populations in all plots during the study is consistent with expectations from natural population dynamics.

Table CP 10.3.2.4/02-2 Effect on predatory mites following exposure to residues of KWG 4168 EC 500

Treatment	Number of mites per 25 leaves							
	Pre-assessment	After 1st treatment	After 2nd treatment	After 3rd treatment	After 4th treatment	After 5th treatment	1 week after 6th treatment	4 weeks after 6th treatment
Control	223	229	187	371	270	179	120	190
KWG 4168 EC 500	232	257	232	396	293	204	114	112
Reference substance	220	241	223	368	260	206	134	116
	% reduction compared to the control							
KWG 4168 EC 500	-	-12	-27	-7	-9	-14	5	41
Reference substance	-	-5	-19	1	4	-15	-12	39

III. Conclusion

Six applications of KWG 4168 EC 500 with an interval of approximately two weeks were made to vines. Predatory mites were not impacted by a multiple spray treatment with 0.055 % Spiroxamine EC 500 on any assessment day except on the day of the final evaluation (41 % effect).

Assessment and conclusion by applicant:

The study was conducted in 1997 and therefore pre-dates much of the current guidance for NTA field testing as detailed in Candolfi *et al*. (2000), ESCORT 3 and de Jong *et al*. (2010)[15]. Analytical dose verification of spray solutions and more advanced statistical techniques, including MDD analysis, are now used as standard in field studies and this study did not employ either of these.

There is a formalised IOBC test method for predatory mite field trials by Blümel *et al.* (2000) "*Guidance document to detect side effects of plant protection products on predatory mites (Acari: Phytoseiidae) under field conditions: vineyards and orchards*". The study was not specifically conducted to this test method as it again pre-dates this guidance but the test methodology used in the study is largely consistent.

Despite the age and lack of recognised guidance being referenced by the study, the results are considered to be valid in their own right and can be used to support the risk assessment by demonstrating an absence of long term effects to mites in vines following application of Spiroxamine EC 500. Predatory mites were not impacted by a multiple spray treatment with 0.055 % Spiroxamine EC 500 on any assessment day except on the day of the final evaluation (41 % effect). That value, however, might be a not treatment-related effect but an untypical increase in the population density on the control plots. This conclusion is based on the following observations: (1) the population density of predatory mites typically decreases towards the end of the growing season as it is recorded in the treated study plots, (2) there was no short-term impact at any treatment day and no long-term response up to the last treatment which indicates that Spiroxamine EC 500 has no impact on reproduction, and (3) the population density in the soft standard plots follows very closely that in the treated plot (Topas 100 EC is known to be harmless to predatory mites). It is therefore considered that the measured 41% reduction at the final timepoint is an artefact of the data and the fact that the control numbers were higher on the occasion than typically expected. However, it is recognised that there is some uncertainty here therefore the results should be treated with caution.

The study is therefore considered to be supporting information only.

[15] Guidance for summarising and evaluating field studies with non-target arthropods. A guidance document of the Dutch Platform for the Assessment of Higher Tier Studies. De Jong *et al.* (2010)

Data Point:	KCP 10.3.2.4/03
Report Author:	[redacted]; [redacted]
Report Year:	1996
Report Title:	A field experiment to determine the effects of KWG 4168 EC 500 on the predatory mite Typhlodromus pyri (Acari, Phytoseiidae) in vines in Germany
Report No:	ER-95-29
Document No:	M-008505-01-1
Guideline(s) followed in study:	Boller, E. (1983) and Heimann-Detlefsen (1991)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

This study was conducted in order to assess the effects of KWG 4168 EC 500 on predatory mites (*Typhlodromus pyri*) in a study over 6 weeks.

The application rates were 600, 1000, 1200 and 1600 L/ha in the 1st, 2nd, 3rd and 4th applications, respectively (equivalent to 165, 275, 330 and 440 g a.s./ha). A control treatment (water) and toxic reference treatment (Rody 11% fenpropathrin) were also used as part of the study. The number of mites on leaf samples was assessed after one, four and six weeks after the final treatment.

The effect of the test substance on *Typhlodromus pyri* was calculated at 25% after the third application. However, due to the very low numbers of *T. pyri* present towards the end of the study it was not possible to obtain a realistic percent effect.

I. Materials and Methods

Materials

Test Material KWG 4168 EC 500

Lot/Batch #: 04023/0021

Purity: 501 g/L

Description: Not reported

Stability of test compound: Not reported

Reanalysis/Expiry date: 23rd September 1995

Density: Not reported

Treatments

Test rates: 600, 1000, 1200 and 1600 L/ha

Solvent/vehicle: Water

Analysis of test concentrations: None

Test organisms

Species:	Predatory mites, *Typhlodromus pyri*
Source:	Not reported
Acclimatisation period:	None
Feeding:	None reported
Treatment for disease:	None reported

Test design

Replication:	4
Duration of test:	6 weeks

Environmental test conditions

Temperature:	5.7 – 33.6°C
Precipitation:	Average 2.9 mm

Study Design

This study was conducted in order to assess the effects of KWG 4168 EC 500 on predatory mites (*Typhlodromus pyri*) in a study over 6 weeks.

Experimental plots were established in rows approximately 39 - 45 m. Vines were treated with four spray treatments. All treatments were applied using a sprayer calibrated to deliver 600 L/ha. The test item was applied at a concentration of 0.055% at rates of 600, 1000, 1200 and 1600 L/ha in the 1st, 2nd, 3rd, and 4th applications, respectively (equivalent to 165, 275, 330 and 440 g a.s./ha). A control treatment (water) and toxic reference treatment (Rody, 11% fenpropathrin applied at a rate 20 g a.s./ha) were also used as part of the study.

The predatory mites were assessed shortly before each application and approximately one, four and six weeks after the final treatment. Leaf samples consisting of 25 leaves were taken one, four and six weeks after the final treatment and the number of adults, juveniles and eggs were recorded. All leaves were examined within 24 h of their collection and populations of mites were assessed by visual observation of leaves under a binocular microscope.

Temperature at the test site varied between 5.7 - 33.6°C and the average precipitation was 2.9 mm.

II. Results and Discussion

There was a gradual decline in the number of T. *pyri* present in the control plots from a mean of 306.75 motiles per 25 leaves at the beginning of the study to 2.25 by the end. *T. pyri* numbers in samples from the KWG 4168 treated plots were very similar to those in control plots with a mean of 331.25 motiles per 25 leaves at the beginning of the study declining to zero by the end. No statistically significant differences between the numbers of any stage of *T. pyri* sampled in the test substance and control plots were detected on any sampling occasion (P=0.05 in Anova). Rody was harmful to all stages of *T. pyri.*

In Rody (reference standard) treated plots *T. pyri* motiles were virtually eliminated after the first application of test substances and remained absent for the remainder of the study. Statistically significant differences in the number of T. *pyri* motiles observed in the toxic reference and control treatments were observed (p =0.05 in Anova and Tukeys) after the first and second applications.

Evaluating the product according to Henderson & Tilton, the effect of the test substance on *Typhlodromus pyri* was calculated at 25% after the third application of treatments.

Table CP 10.3.2.4/03-1 Number of predatory mites following exposure to residues of KWG 4168 EC 500

Treatment	Pre-assessment	9 days after 1st treatment	4 days after 2nd treatment	18 days after 3rd treatment	7 days after 4th treatment	32 days after 4th treatment	46 days after 4th treatment
	Number of adult mites per 25 leaves						
Control	114.5	52.57	17.50	5.00	2.00	0.50	1.25
KWG 4168 EC 500	126.25	41.50	11.25	2.75	0.50	0.25	0.00
Reference item	108.25	0.00	0.00	0.00	0.00	0.00	0.00
	Number of juvenile mites per 25 leaves						
Control	75.75	8.50	6.25	1.50	0.35	0.00	1.00
KWG 4168 EC 500	62.75	12.50	6.25	1.75	0.00	0.00	0.00
Reference item	52.25	0.00	0.00	0.00	0.00	0.00	0.00
	Number of eggs per 25 leaves						
Control	116.50	12.50	7.25	0.00	0.00	0.25	0.00
KWG 4168 EC 500	142.25	36.75	3.50	0.75	0.00	0.00	0.00
Reference item	102.50	0.00	0.00	0.00	0.00	0.00	0.00

III. Conclusion

The effect of the test substance on *Typhlodromus pyri* was calculated at 25% (Henderson & Tilton) after the third application. No statistically significant differences between the numbers of any stage of *T. pyri* sampled in the test substance and control plots were detected on any sampling occasion. However, due to the very low numbers of *T. pyri* present towards the end of the study it was not possible to obtain a realistic percent effect.

Assessment and conclusion by applicant:

The study was conducted in 1996 and therefore pre-dates much of the current guidance for NTA field testing as detailed in Candolfi *et al.* (2000), ESCORT 3 and de Jong *et al.* (2010)[16]. Analytical dose verification of spray solutions and more advanced statistical techniques, including MDD analysis, are now used as standard in field studies and this study did not employ either of these.

There is a formalised IOBC test method for predatory mite field trials by Blümel *et al.* (2000) "*Guidance document to detect side effects of plant protection products on predatory mites (Acari:*

[16] Guidance for summarising and evaluating field studies with non-target arthropods. A guidance document of the Dutch Platform for the Assessment of Higher Tier Studies. De Jong *et al.* (2010)

Phytoseiidae) under field conditions: vineyards and orchards". The study was not specifically conducted to this test method as it again pre-dates this guidance but the test methodology used in the study is largely consistent.

Despite the age and lack of recognised guidance being referenced by the study, the results are considered to be valid in their own right and can be used to support the risk assessment by demonstrating an absence of long term effects to mites in vines following application of Spiroxamine EC 500. The results demonstrated that the toxic standard gave the expected level of effect when compared to the control therefore the study is considered to be valid.

The mite density was very low in the samples taken in the second half of the study in the control and test item treatment, therefore there is much uncertainty in drawing any firm conclusions over the potential long-term effects and recovery of mites following treatment of Spiroxamine EC 500. The results should therefore be treated with caution.

The study is therefore considered to be supporting information only.

Data Point:	KCP 10.3.2.4/04
Report Author:	[redacted]
Report Year:	1999
Report Title:	Effects of 'spiroxamine EC 500' on predatory mites (Typhlodromus pyri) under typical vine culture conditions on grape vines, Germany 1999
Report No:	BAY43
Document No:	M-024960-01-1
Guideline(s) followed in study:	BBA-Richtlinie VI, 23-2.3.4 (1991)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

This study was conducted in order to assess the effects of residues of Spiroxamine EC 500 on predatory mites (*Typhlodromus pyri*) over 4 weeks.

The application rates were 300, 721, 738 and 732 ml product/ha in the 1st, 2nd, 3rd and 4th applications, respectively. A control treatment (water), soft reference treatment (Bayfidan spezial WG, a.s. triadimenol) and toxic reference treatment (Antracol WG, a.s. probineb) were also used as part of the study. The number of mites on leaf samples was assessed after each treatment and 4 weeks after the final treatment.

Four applications of Spiroxamine EC 500 with an interval of approximately two weeks led to populations of the predatory mites that were 12% lower than the control level.

I. Materials and Methods

Materials

Test Material Spiroxamine EC 500

Lot/Batch #: 04023/0627

Purity:	508.5 g/L
Description:	Orange/brown
Stability of test compound:	Not reported
Reanalysis/Expiry date:	10th November 1997
Density:	1.005 g/ml
Treatments	
Test rates:	300, 721, 738 and 732 mL product/ha in the 1st, 2nd, 3rd and 4th applications, respectively
Solvent/vehicle:	Water
Analysis of test concentrations:	Yes
Test organisms	
Species:	Predatory mites, *Typhlodromus pyri*
Source:	Staatliche Lehr- und Forschungsanstalt fiir Landwirtschaft, Weinbau und Gartenbau
Acclimatisation period:	None
Feeding:	Not reported
Treatment for disease:	None reported
Test design	
Replication:	4
Duration of test:	4 weeks
Environmental test conditions	
Temperature:	5.5 - 34.2°C
Precipitation:	Monthly average 38.4 - 68.2 mm

Study Design

This study was conducted in order to assess the effects of Spiroxamine EC 500 residues on predatory mites (*Typhlodromus pyri*) in a study over 4 weeks.

Rows of 15-20 vines from plots of 159 - 169 m^2 vines were used for testing. Four plots per test were used. Vines were treated with four spray treatments with 12 - 14 days interval between each treatment. All treatments were applied using a plot tunnel sprayer. The application rates were 300, 721, 738 and 732 mL product/ha in the 1st, 2nd, 3rd and 4th applications, respectively. A control treatment (water), soft reference treatment (Bayfidan spezial WG, a.s. triadimenol) and toxic reference treatment (Antracol WG, a.s. probineb) were also used as part of the study.

Leaf samples were taken 6 times throughout the study and the number of mites on the sample was determined. The population of predatory mites was assessed by determining the number of mites on leaf

samples, using the washing method. Leaf samples consisting of 25 leaves were taken before the first treatment, 1 week after each application and 4 weeks after the final treatment.

Temperature at the test site varied between 5.5 – 34.2°C and the monthly average precipitation was 38.4 – 68.2 mm.

II. Results and Discussion

Table CP 10.3.2.4/04-1 Spiroxamine EC 500 actual and nominal application rates

	Nominal application rate (ml/ha)	Actual application rate (ml/ha)
Spiroxamine EC 500	300	300
	750	721
	750	738
	750	732

After exposure to a multiple spray treatment of Spiroxamine EC 500, the populations of predatory mites at the final assessment were reduced by 12% (Henderson & Tilton) when compared to the control. There was no statistically significant difference between the population of the control and the test substance plots.

The toxic standard caused a 89% reduction (H &T) relative to the control 4 weeks after the final treatment.

Table CP 10.3.2.4/04-2 Effect on predatory mites following exposure to residues of Spiroxamine EC 500

Treatment	Number of mites per 25 leaves					
	Pre-assessment	6 days after 1st treatment	7 days after 2nd treatment	7 days after 3rd treatment	8 days after 4th treatment	4 weeks after 4th treatment
Control	586	161	207	186	84	75
Spiroxamine EC 500	551	154	212	140	77	62
Soft reference standard	519	140	203	120	80	64
Toxic reference	493	98	113	54	12	7

Treatment	Number of mites per 25 leaves					
	Pre-assessment	6 days after 1st treatment	7 days after 2nd treatment	7 days after 3rd treatment	8 days after 4th treatment	4 weeks after 4th treatment
	% reduction of Spiroxamine EC 500 compared to the control					
Effect (Abbot)	-	4	-2	25	8	17
Effect (H & T)	-	-2	-9	20	3	12
	% reduction of soft reference standard compared to the control					
Effect (Abbot)	-	13	2	35	5	15
Effect (H & T)	-	2	-11	21	-8	4
	% reduction of toxic standard compared to the control					
Effect (Abbot)	-	39	45	71	86	91
Effect (H & T)	-	28	35	65	83	89

H & T: Henderson & Tilton

III. Conclusion

Four applications of Spiroxamine EC 500 with an interval of approximately two weeks there was no statistically significant difference between the population of the control and the test substance plots.

Assessment and conclusion by applicant:

The study was conducted in 1999 and therefore pre-dates much of the current guidance for NTA field testing as detailed in Candolfi *et al.* (2000), ESCORT 3 and de Jong *et al.* (2010)[17]. Analytical dose verification of spray solutions and more advanced statistical techniques, including MDD analysis, are now used as standard in field studies and this study did not employ either of these.

There is a formalised IOBC test method for predatory mite field trials by Blümel *et al.* (2000) "*Guidance document to detect side effects of plant protection products on predatory mites (Acari: Phytoseiidae) under field conditions: vineyards and orchards*". The study was not specifically conducted to this test method as it again pre-dates this guidance but the test methodology used in the study is largely consistent.

Despite the age and lack of recognised guidance being referenced by the study, the results are considered to be valid in their own right and can be used to support the risk assessment by demonstrating an absence of long term effects to mites in vines following application of Spiroxamine EC 500. The results demonstrated that the toxic standard gave the expected level of effect when compared to the control therefore the study is considered to be valid.

The study is considered to be supporting information only.

[17] Guidance for summarising and evaluating field studies with non-target arthropods. A guidance document of the Dutch Platform for the Assessment of Higher Tier Studies. De Jong *et al.* (2010)

Data Point:	KCP 10.3.2.4/05
Report Author:	[redacted]
Report Year:	1999
Report Title:	Effects of 'spiroxamine EC 500' on predatory mites (Typhlodromus pyri) under typical vine culture conditions on grape vines, Germany 1999
Report No:	BAY42
Document No:	M-024963-01-1
Guideline(s) followed in study:	BBA-Richtlinie VI, 23-2.3.4 (1991)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

This study was conducted in order to assess the effects of Spiroxamine EC 500 on predatory mites (*Typhlodromus pyri*) in a study over 4 weeks.

The application rates were 302, 283, 756, 762, 735 and 769 mL product/ha in the 1st, 2nd, 3rd, 4th, 5th, and 6th applications, respectively. A control treatment (water), soft reference treatment (Bayfidan spezial WG, a.s. triadimenol) and toxic reference treatment (Antracol WG, a.s. probineb) were also used as part of the study. The number of mites on leaf samples was assessed after each treatment and 4 weeks after the final treatment.

Six applications of Spiroxamine EC 500 with an interval of approximately two weeks led to populations of the predatory mites reducing to 41% of the control level.

I. Materials and Methods

Materials

Test Material Spiroxamine EC 500

Lot/Batch #: 04023/0627

Purity: 508.5 g/L

Description: Orange/brown

Stability of test compound: Not reported

Reanalysis/Expiry date: 10th November 1997

Density: 1.005 g/ml

Treatments

Test rates: 302, 283, 756, 762, 735 and 769 mL product/ha in the 1st, 2nd, 3rd, 4th, 5th, and 6th applications, respectively

Solvent/vehicle: Water

Analysis of test concentrations: Yes

Test organisms

Species:	Predatory mites, *Typhlodromus pyri*
Source:	Staatliche Lehr- und Forschungsanstalt fiir Landwirtschaft, Weinbau und Gartenbau
Acclimatisation period:	None
Feeding:	Not reported
Treatment for disease:	None reported

Test design

Replication:	4
Duration of test:	4 weeks

Environmental test conditions

Temperature:	6.4 – 34.2°C
Precipitation:	Monthly average 38.4 – 68.2 mm

Study Design

This study was conducted in order to assess the effects of Spiroxamine EC 500 on predatory mites (*Typhlodromus pyri*) in a study over 4 weeks.

Rows of 12-16 vines from plots of 137 - 139 m^2 Riesling vines were used for testing. Four plots per test was used. Vines were treated with six spray treatments with 10 - 14 days interval between each treatment. All treatments were applied using a plot funnel sprayer. The application rates were 302, 283, 756, 762, 735 and 769 mL product/ha in the 1st, 2nd, 3rd, 4th, 5th, and 6th applications, respectively. A control treatment (water), soft reference treatment (Bayfidan spezial WG, a.s. triadimenol) and toxic reference treatment (Antracol WG, a.s. probineb) were also used as part of the study.

Leaf samples were taken 8 times throughout the study and the number of mites on the sample was determined. The population of predatory mites was assessed by determining the number of mites on leaf samples, using the washing method. Leaf samples consisting of 25 leaves were taken before the first treatment, 1 week after each application and 4 weeks after the final treatment.

Temperature at the test site varied between 6.4 – 34.2°C and the monthly average precipitation was 38.4 – 68.2 mm.

II. Results and Discussion

Table CP 10.3.2.4/05-1 Spiroxamine EC 500 actual and nominal application rates

	Nominal application rate (ml/ha)	Actual application rate (ml/ha)
Spiroxmine EC 500	300	302
	300	283
	750	756
	750	762
	750	735

	Nominal application rate (ml/ha)	Actual application rate (ml/ha)
	750	769

After exposure to a multiple spray treatment of Spiroxamine EC 500, the populations of predatory mites at the final assessment, were reduced by 59% (H & T) when compared to the control.

The toxic standard caused a 93% reduction (H &T) relative to the control 4 weeks after the final treatment.

Table CP 10.3.2.4/05-2 Effect on predatory mites following exposure to residues of Spiroxamine EC 500

Treatment	Number of mites per 25 leaves							
	Pre-assessment	7 days after 1st app.	8 days after 2nd app.	6 days after 3rd app.	7 days after 4th app.	7 days after 5th app.	7 days after 6th app.	4 weeks after 6th app.
Control	142	88	77	57	60	37	53	50
Spiroxamine EC 500	214	130	73	65	51	38	39	31
Soft reference standard	159	101	95	54	71	37	49	36
Toxic reference	167	75	59	35	25	14	14	4
	% reduction compared to the control							
Effect (Abbot)	-	-48	-5	-14	15	-3	26	38
Effect (H & T)	-	2	37	24	44	32	51	59
	% reduction of soft reference standard compared to the control							
Effect (Abbot)	-	-15	-23	5	-18	0	8	28
Effect (H & T)	-	-3	-10	15	-6	11	17	36
	% reduction of toxic standard compared to the control							
Effect (Abbot)	-	15	23	39	58	62	79	92
Effect (H & T)	-	28	35	48	65	68	82	93

App. = application
H & T: Henderson & Tilton

III. Conclusion

Six applications of Spiroxamine EC 500 with an interval of approximately two weeks led to populations of the predatory mites being reduced by 59% when compared to the control level.

Assessment and conclusion by applicant:

The study was conducted in 1999 and therefore pre-dates much of the current guidance for NTA field testing as detailed in Candolfi *et al*. (2000), ESCORT 3 and de Jong *et al*. (2010)[18]. Analytical dose verification of spray solutions and more advanced statistical techniques, including MDD analysis, are now used as standard in field studies and this study did not employ either of these.

There is a formalised IOBC test method for predatory mite field trials by Blümel *et al.* (2000) "*Guidance document to detect side effects of plant protection products on predatory mites (Acari: Phytoseiidae) under field conditions: vineyards and orchards*". The study was not specifically conducted to this test method as it again pre-dates this guidance but the test methodology used in the study is largely consistent.

Despite the age and lack of recognised guidance being referenced by the study, the results are considered to be valid in their own right. The results demonstrated that the toxic standard gave the expected level of effect when compared to the control therefore the study is considered to be valid.

The study is considered to be supporting information only.

Data Point:	KCP 10.3.2.4/06
Report Author:	[redacted]; [redacted]
Report Year:	1996
Report Title:	A field experiment to determine the effects of KWG 4168 EC 500, on the predatory mite Amblyseius aberrans (Acari: Phytoseiidae) in vines in Southern Italy
Report No:	ER-95-24
Document No:	M-008498-01-1
Guideline(s) followed in study:	Boller, E. (1983) and Heimann-Detlefsen (1991)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

This study was conducted in order to assess the effects of KWG 4168 EC 500 on predatory mites (*Amblyseius abberans*) on vines in a study over 4 weeks.

The application rate of the test item was 300 g a.s./ha for three applications. A control treatment (water) and toxic reference treatment (Danitol, 91 g/L fenpropathrin) were also used as part of the study. The number of mites on leaf samples was assessed one and four weeks after the final treatment.

The effect of the test substance on *Amblyseius abberans* was calculated to be 18.5% 4 weeks after the third application.

[18] Guidance for summarising and evaluating field studies with non-target arthropods. A guidance document of the Dutch Platform for the Assessment of Higher Tier Studies. De Jong *et al.* (2010)

I. Materials and Methods

Materials

Test Material	KWG 4168 EC 500
Lot/Batch #:	04023/0021
Purity:	501 g/L
Description:	Emulsifiable concentrate
Stability of test compound:	Not reported
Reanalysis/Expiry date:	23rd September 1992
Density:	Not reported
Treatments	
Test rates:	300 g a.s./ha
Solvent/vehicle:	Water
Analysis of test concentrations:	None
Test organisms	
Species:	Predatory mites, *Amblyseius abberans*
Source:	Not reported
Acclimatisation period:	None
Feeding:	None reported
Treatment for disease:	None reported
Test design	
Replication:	4
Duration of test:	4 weeks
Environmental test conditions	
Temperature:	11.5 – 38°C

Study Design

This study was conducted in order to assess the effects of KWG 4168 EC 500 on predatory mites (*Amblyseius abberans*) in vines in a study over 4 weeks.

Experimental plots were established in blocks of 36 m^2, consisting of 22-30 vines. Vines were treated with three spray treatments at 14-day intervals. All treatments were applied using a sprayer calibrated to deliver 1000 L/ha. The test item was applied at a rate of 300 g a.s./ha in a volume of 1000 L/ha. A control treatment (water) and toxic reference treatment (Danitol, 91 g/L fenpropathrin applied at a rate 20 g a.s./ha) were also used as part of the study.

The predatory mites were assessed shortly before each application and approximately one and four weeks after the final treatment. For each assessment leaf samples consisting of 25 leaves were taken and the number of adults, juveniles and eggs were recorded. All leaves were examined within 24 hours of their collection and populations of mites were assessed by visual observation of leaves under a binocular microscope.

Temperature at the test site varied between 11.5 – 38°C. There was very little rainfall during the study. Approximately 50% of the total rainfall occurred over a two-day period shortly before the second application of test substances. There was no rainfall during or shortly after any of the application occasions.

II. Results and Discussion

By the final observation four weeks after the third application of the test substance, numbers of adults, nymphs, larvae and eggs in the control plots had increased from a mean of 23.00 motiles per 25 leaves one week after the third application to 61.00 by the end of the study.

In the treated plots the mean number of motiles observed per 25 leaves was 31.00 at the pre-treatment observation increasing to a mean of 67.00 per 25 leaves by the end of the study. There were no statistically significant differences between the numbers of mites observed in test substance treated plots and control on any sampling occasion.

Table CP 10.3.2.4/06-1 Number of predatory mites following exposure to residues of KWG 4168 EC 500

Treatment	Pre-assessment	2 weeks after 1st treatment	2 weeks after 2nd treatment	1 week after 3rd treatment	4 weeks after 3rd treatment
	Number of adult mites per 25 leaves				
Control	11.75	17.50	15.25	9.50	19.50
KWG 4168 EC 500	14.75	16.00	12.00	8.25	15.50
Reference item	12.25	0.75	0.00	0.00	0.00

Treatment	Pre-assessment	2 weeks after 1st treatment	2 weeks after 2nd treatment	1 week after 3rd treatment	4 weeks after 3rd treatment
	Number of adult mites per 25 leaves				
	Number of juvenile mites per 25 leaves				
Control	9.00	11.75	6.75	7.00	18.75
KWG 4168 EC 500	11.50	10.75	5.25	9.00	27.50
Reference item	9.25	0.25	0.00	0.00	0.00
	Number of eggs per 25 leaves				
Control	1.00	1.25	2.50	9.50	19.25
KWG 4168 EC 500	2.25	1.00	2.00	8.25	25.50
Reference item	1.75	1.00	0.25	0.00	0.00
	Total motiles				
Control	23.00	33.00	28.50	24.75	61.00
KWG 4168 EC 500	31.00	31.00	24.00	25.50	67.00
	Total prey				
Control	89.75	89.25	59.00	45.50	36.00
KWG 4168 EC 500	91.25	91.75	48.75	30.00	33.25

III. Conclusion

The overall effect of KWG 4168 EC 500 on predatory mites (*Amblyseius abberans*) in vines was 18.5% four weeks after the final application.

Assessment and conclusion by applicant:

The study was conducted in 1996 and therefore pre-dates much of the current guidance for NTA field testing as detailed in Candolfi *et al.* (2000), ESCORT 3 and de Jong *et al.* (2010)[19]. Analytical dose verification of spray solutions and more advanced statistical techniques, including MDD analysis, are now used as standard in field studies and this study did not employ either of these.

There is a formalised IOBC test method for predatory mite field trials by Blümel *et al.* (2000) "*Guidance document to detect side effects of plant protection products on predatory mites (Acari: Phytoseiidae) under field conditions: vineyards and orchards*". The study was not specifically conducted to this test method as it again pre-dates this guidance but the test methodology used in the study is largely consistent.

Despite the age and lack of recognised guidance being referenced by the study, the results are considered to be valid in their own right and can be used to support the risk assessment by

[19] Guidance for summarising and evaluating field studies with non-target arthropods. A guidance document of the Dutch Platform for the Assessment of Higher Tier Studies. De Jong *et al.* (2010)

demonstrating an absence of long term effects to mites in vines following application of Spiroxamine EC 500. The results demonstrated that the toxic standard gave the expected level of effect when compared to the control therefore the study is considered to be valid.

The study is considered to be supporting information only.

CP 10.3.2.5 Other routes of exposure for non-target arthropods

Acceptable risks have been demonstrated in the risk assessments for non-target arthropods following application of Spiroxamine EC 500 following the proposed uses. It is therefore considered that other routes of exposure, *e.g. via* systemic activity, do not need to be specifically investigated. The standard species risk assessments for the in-field and off field exposure *via* contact to foliar residues were acceptable, therefore additional studies investigating other routes of exposure were not considered necessary.

CP 10.4 Effects on non-target soil meso- and macrofauna

CP 10.4.1 Earthworms

The avaialable earthworm toxicity data for Spiroxamine EC 500 and the metabolites of spiroxamine are summarised in the table below.

Table CP 10.4.1-1 Summary of earthworm toxicity studies with spiroxamine metabolites and Spiroxamine EC 500

Organism	Test item	Test type	Endpoints		Reference
Earthworm (*Eisenia fetida*)	Spiroxamine EC 500	56 d Chronic toxicity; 10% peat	NOEC 4.0 mg a.s./kg soil dw; $NOEC_{corr}$ 2.0 mg a.s./kg soil dw[1]	EU	M-008843-01-1
		Statistical Re-analysis	EC_{10}/EC_{20} not determinable	NEW	M-760439-01-1
Earthworm (*Eisenia fetida*)	Spiroxamine EC 500	56 d Chronic toxicity; 5% peat	NOEC 5.0 mg a.s./kg soil dw;	EU	M-026522-01-1
		Statistical Re-analysis	EC_{10}/EC_{20} not determinable	NEW	M-760441-01-1
Earthworm (*Eisenia fetida*)	Spiroxamine EC 500 G	56 d Chronic toxicity; 5% peat	**NOEC 158.40 mg/kg soil dw (equivalent to 80 mg a.s./kg soil dw);**	NEW	M-416761-01-1
		Statistical Re-analysis	EC_{10}/EC_{20} not determinable	NEW	M-761531-01-1
Earthworm (*Eisenia fetida*)	KWG 4168-desethyl (M01)	56 d Chronic toxicity; 5% peat	NOEC 100 mg/kg soil dw;	EU	M-281615-01-1
		Statistical Re-analysis	**EC_{10} 93.8 mg/kg soil dw** EC_{20} 120 mg/kg soil dw	NEW	M-760435-01-1

Organism	Test item	Test type	Endpoints		Reference
Earthworm (*Eisenia andrei)*	KWG 4168-despropyl (M02)	56 d Chronic toxicity; 10% peat	NOEC 100 mg/kg soil dw; **$NOEC_{corr}$ 50 mg/kg soil dw[1];** EC_{10} >100 mg/kg soil dw; $EC_{10\ corr}$ >50 mg/kg soil dw	NEW	M-680755-01-1
Earthworm (*Eisenia fetida)*	KWG 4168-N-oxide (M03)	56 d Chronic toxicity; 5% peat	**NOEC 100 mg/kg soil dw**	EU	M-281677-01-1
		Statistical Re-analysis	EC_{10} 245 mg/kg soil dw EC_{20} 287 mg/kg soil dw	NEW	M-760434-01-1
Earthworm (*Eisenia fetida)*	KWG 4168-acid (M06)	56 d Chronic toxicity; 10% peat	**NOEC 100 mg/kg soil dw;** EC_{10} >100 mg/kg soil dw;	NEW	M-727023-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
NEW: new study or data generated since the previous EU review or previously not submitted
Values in **bold** have been used in the risk assessment
[1] The NOEC from the study, which was conducted using soil with a 10% peat content, has been divided by 2 to account for lipophilic effects from compounds with a Log Pow>2

Toxicity endpoints

In accordance with the Terrestrial Guidance Document (SANCO/10329/2002), to account for the high organic matter content in the test soils, the effect concentrations are corrected by a factor of 2 for lipophilic substances with log P_{ow} >2. Note that endpoints have only been corrected for studies in which artificial soil with a 10% peat content was used. The Log P_{ow} of spiroxamine is 2.79 and 2.98 at pH 7 for diastomers A and B, respectively and at pH 9 these value are 4.88 and 5.08, respectively. Thus, correction of the endpoint is necessary where artificial soil with a 10% peat content has been used.

The Log P_{ow} of spiroxamine-desethyl (M01) is 2.41, 1.97 and >3.64 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-despropyl (M02) is 1.95, 1.41 and >3.44 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-N-oxide (M03) is 2.82, 2.59 and 2.63 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-carboxylic acid (M06) is -0.45, -0.25 and 0.10 at pH 4, 7 and 9, respectively. Thus, correction of the endpoint for the studies using M01, M02 and M03 would be necessary but only where artificial soil with a 10% peat content has been used.

Three reproduction studies are available using Spiroxamine EC 500. However, the older two studies which provided NOEC values of ≥3 kg a.s./ha ($NOEC_{corr}$ equivalent to 2.0 mg a.s./kg soil dw) and ≥3.75 kg a.s./ha (NOEC equivalent to 5.0 mg a.s./kg soil dw) adpopted an application method in which the soil surface was oversprayed with the test material and therefore this was not evenly mixed within the soil. This application is no longer an acceptable method and these studies have been presented as supporting information only. The reproductive risk assessment for earthworms has therefore been conducted using the more recent study in which the test material was incorporated into the soil which provided a NOEC of 158.4 mg product/kg soil dw (equivalent to 80 mg a.s./kg soil dw).

Earthworm reproduction data for spiroxamine technical are not available. However, the available formulation data are considered to be more relevant for the risk assessment as this has been generated using the representative formulation itself and also represents the toxicity to spiroxamine.

Acute earthworm toxicity data are available for spiroxamine technical and have been summarised in Document M-CA Section 8. However, no acute risk assessment has been presented because acute earthworm data are no longer a data requirement under EU Regulations 283/2013 and 284/2013.

Exposure

Full details of the PEC_{soil} calculations have been provided in Document M-CP Section 9 Environmental Fate. The maximum initial and accumulation PEC_{soil} values for spiroxamine and its metabolites, as calculated using FOCUS equations, are given in the table below for the highest application rate of 300 g a.s./ha.

Table CP 10.4.1-2 PEC_{soil} for spiroxamine and its metabolites

Substance	1 x 300 g a.s./ha		2 x 300 g a.s./ha	
	Max PEC_{soil} (mg/kg)	$PEC_{soil\ accumulation}$ (mg/kg)	Max PEC_{soil} (mg/kg)	$PEC_{soil\ accumulation}$ (mg/kg)
Vines				
Spiroxamine	0.200	0.277	0.372	**0.555**
M01	0.022	0.032	0.044	**0.064**
M02	0.016	0.020	0.032	**0.040**
M03	0.017	0.018	0.033	**0.037**
M06	0.012	0.052	0.023	**0.104**

PEC_{soil} values used in the risk assessment are highlighted in **bold**

For the risk assessment below the risk envelope approach has been applied in which the PEC_{soil} values for the proposed use with the highest application rate has been used. Thus, the risk assessment has been conducted for the use on grapes at 2 x 300 g a.s./ha. Furthermore, the $PEC_{soil\ accumulation}$ values were greater than the maximum initial PEC_{soil} values therefore the risk assessment has been conducted using the worst case $PEC_{soil\ accumulation}$ values.

For Spiroxamine EC 500 the formulation PEC_{soil} was determined to be 0.401 mg/kg soil for the maximum application rate of 300 g a.s./ha. Please refer to Document M-CP Section 9 Environmental Fate for further details.

Isomers

For parent spiroxamine the environmental fate soil degradation data currently suggest that there is no significant selective degradation of isomers over time. As a result, the toxicity data generated using the mixture of the isomers (*i.e.* Spiroxamine EC 500) are considered to represent the toxicity of the isomers in the ratios that would occur in the soil following application. In accordance with the isomer Guidance Document[20] it is therefore not necessary to apply any additional Uncertainty Factor (UF) to the risk assessment (*i.e.* a UF of 1.0 is used).

For the metabolites of spiroxamine there are no chiral data available to be able to make this assessment therefore there is a possibility that selective degradation of isomers could occur in the soil over time. In order to account for any possible increased toxicity to soil organisms as a result of an increase in the ratio of a single isomer, an UF has been applied to the risk assessment of M01, M02, M03 and M06.

[20] 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

The UF have been calculated following the recommendations of the isomer Guidance Document and have been presented in the table below.

Table CP 10.4.1-3 Uncertainty Factors determined for the earthworm toxicity data with the metabolites of spiroxamine

Test item	Study reference	Test material batch number	Isomer ratio	UF[1]
Spiroxamine	-	-	-	1.0[2]
M01	M-281615-01-1	921103ELB02	A:B 56:42	4.76
M02	M-680755-01-1	AE 1344303-PU-01	A:B 83.1:16.0	12.5
M03	M-281617-01-1	KTS 10324-1-2	D1:D2:D3:D4 27:26:20:27	10.0
M06	M-727123-01-1	AE 1344313-01-03	A:B 47:53	4.26

[1] Changes in stereoisomeric excess are unknown therefore Uncertainty Factor = 100/content of lowest stereoisomer (%) used for ecotox endpoint [as indicted in Table B.1, p.30 of isomer GD] and assumes that the toxicological effects of the mixture can be attributed to a single isomer. This assumes that all enantiomer ratios can be safely assumed to be 50:50. For example A:B ratio of 83.1:16 would be 100/(16/2) = UF of 12.5

[2] No additional UF required for parent as no significant change in isomeric ratios has been demonstrated

This assumes that all enantiomer ratios can be safely assumed to be 50:50

Risk assessment

The risk assessment has been conducted in accordance with the Terrestrial Guidance Document (SANCO/10329/2002).

The effect concentrations for spiroxamine (Spiroxamine EC 500) and for the metabolites are compared to the PEC$_{soil}$ values in the following table.

Table CP 10.4.1-4 Earthworm risk assessment for spiroxamine and relevant metabolites following application of Spiroxamine EC 500 to vines

Intended use	**Vines 2 x 300 g a.s./ha**			
Chronic effects on earthworms				
Test item	**NOEC/EC$_{10}$**	**PEC$_{soil}$**	**UF[1]**	**TER$_{LT}$[2] (criterion TER ≥ 5)**
Spiroxamine EC 500	158.4 mg product/kg soil	0.401 mg product/kg soil	1.0	395
	(80 mg a.s./kg soil dw)	0.555 mg a.s./kg soil		144
M01	93.8 mg/kg soil dw	0.064 mg/kg soil	4.76	308
M02	50 mg/kg soil dw	0.040 mg/kg soil	12.5	100
M03	100 mg/kg soil dw	0.037 mg/kg soil	10.0	270
M06	100 mg/kg soil dw	0.104 mg/kg soil	4.26	226

[1] Uncertainty Factor applied to account for the unknown effect of a possible change in isomer ratios over time

[2] TER calculated as follows: Toxicity endpoint/(PEC$_{soil}$ × UF)

The TER$_{LT}$ values for spiroxamine and the metabolites M01, M02, M03 and M06 all exceed the trigger value of 5, therefore acceptable risks to earthworms, following the proposed uses of Spiroxamine EC 500, can be concluded.

Biodiversity

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on earthworms. Therefore, it is considered that the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects *via* alteration of the food web, are covered by the risk assessment for earthworms in this section.

With respect to the earthworm risk assessment, which demonstrated an acceptable outcome with large margins of safety and without the need for risk mitigation, the applicant concludes that the use of the representative lead formulation (Spiroxamine EC 500) has a low potential to cause unacceptable effects on biodiversity and the ecosystem *via* trophic interactions. To the best of our knowledge and with the presented safety profile of the spiroxamine a.s., metabolites, and the representative lead formulation, the applicant does not foresee any unacceptable effects on biodiversity and the ecosystem with spiroxamine.

CP 10.4.1.1 Earthworms sub-lethal effects

Data Point:	KCP 10.4.1.1/03
Report Author:	[redacted]
Report Year:	2011
Report Title:	Spiroxamine EC 500 G: Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil
Report No:	KRA-RG-R-120/11
Document No:	M-416761-01-1
Guideline(s) followed in study:	ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004
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 purpose of this study was to assess the effect of Spiroxamine EC 500 G on survival, growth and reproduction on the earthworm *Eisenia fetida*.

In an 8 week study, earthworms were exposed to Spiroxamine EC 500 G at nominal concentrations of 50.00, 89.00, 158.40, 282.00 and 502.00 mg test item/kg dry weight artificial soil. There were 40 earthworms per treatment group, at test initiation they had a mean weight range of 0.25 to 0.47 g.

Exposure to Spiroxamine EC 500 G did not show significant lethal effects to the earthworm *Eisenia fetida* in artificial soil up to the highest test concentration of 502.00 mg test item/kg dry weight soil.

There were no statistically significant differences in growth or reproduction data at test concentrations of up to 158.40 and 282.00 mg test item/kg dry weight soil, respectively. There were statistically significant differences in growth and reproduction data at ≥282.00 and 502.00 mg test item/kg dry weight soil, respectively. The overall NOEC and LOEC values related for growth were therefore determined to be 158.40 and 282.00 mg test item/kg dry weight soil. The overall NOEC and LOEC values related to reproduction were determined to be 282.00 and 502.00 mg test item/kg dry weight soil.

I. Materials and Methods

Materials

Test Material	Spiroxamine EC 500 G
Lot/Batch #:	EDFL013642
Purity:	508.1 g/L
Description:	Yellow-brown liquid
Reanalysis/Expiry date:	8 August 2014
Density:	1.006 g/mL
Treatments	
Test rates:	Nominal: 50.00, 89.00, 158.40, 282.00 and 502.00 mg/kg soil
Analysis of test concentrations:	No
Test organisms	
Species:	Earthworm, (*Eisenia fetida*)
Source:	Prof. Graff, 38104 Braunschweig, Germany
Acclimatisation period:	Four days prior to test initiation
Feeding:	Finely ground animal manure
Test design	
Test vessel:	Plastic boxes (16.5 x 12 x 6 cm) covered with perforated plastic lids
Test medium:	Artificial soil: 500 g dry weight
Replication:	Four per treatment group
No. animals/vessel:	Ten animals per test vessel
Duration of test:	Eight weeks
Environmental test conditions	
Temperature:	20 ± 2°C
pH:	5.64 – 6.94
Photoperiod:	16 hours light, 8 hours dark (light intensity: 400 – 800 lux)

Study Design

This study was conducted in order to assess the effect of Spiroxamine EC 500 G on the survival, growth and reproduction on the earthworm *Eisenia fetida* during an exposure into an artificial soil at five different test concentrations.

Ten earthworms were added to each of the four replicate test vessels. Test vessels were plastic boxes (length x width x height *ca*. 16.5 x 12 x 6 cm) with perforated plastic lids.

The test soil consisted of 73.82% industrial quartz sand, 20% kaolinite clay, 5% sphagnum peat, 1% dried cattle manure and 0.18% calcium carbonate. Prepared soil consisted of approximately 500 g of dry weight.

The earthworms were exposed to nominal concentrations of 50.00, 89.00, 158.40, 282.00 and 502.00 mg test item/kg dry weight artificial soil.

Incubation was at 20 ± 2°C with a photoperiod of 16 hours light and 8 hours dark at approximately 400 to 800 lux.

After 4 weeks of exposure, the content of each test vessel was emptied and the adult worms were counted, removed and weighed before the substrate was returned to the respective test units minus the worms. Mortality and behavioural abnormalities (*e.g.* lack of movement and rigidity) were observed at this stage (28 days after application). For the LC_{50} calculation, probit analysis was used with ToxRatPro Version 2.10 statistical software.

At test initiation and after 4 weeks of exposure, the adult test organisms of each vessel were weighed (individually at initiation and together after 4 weeks exposure). Weights were determined by washing the worms and placing them on filter paper to absorb surplus water. The data were statistically evaluated using Williams multiple sequential t-test. For the EC_{50} calculation, probit analysis was used with ToxRatPro Version 2.10 statistical software.

At test termination, the number of surviving juveniles per test vessel was determined by placing the test vessels in a water bath of 50 to 60 °C and counting all emerging worms. Reproduction was determined by the number of alive juveniles at test termination. The data were statistically evaluated using Welch-t test for inhomogeneous variances with Bonferroni-Holm-Adjustment. For the EC_{50} calculation, probit analysis was used with ToxRatPro Version 2.10 statistical software.

The earthworms were fed finely ground cattle manure throughout the test which was added to the soil weekly. At each feeding date, the amount of food consumed by the adult earthworms was visually estimated for each test vessel.

II. Results and Discussion

Validity criteria according to the OECD 222 version of the guideline to which the study was performed were met:

- Each replicate (containing 10 adults) to have produced ≥30 juveniles by the end of the test (actual: 328 to 468)
- The coefficient of variation of reproduction to be ≤30% (actual: 11.6%)
- Adult mortality over the initial 4 weeks of the test to be ≤10% (actual: 0%)

No mortality of adult earthworms were observed after 28 days test duration either in the control group or at any test concentration.

Table CP 10.4.1.1/03-1 Mortality and survival data observed after 28 days exposure

mg test item/kg dry weight artificial soil	Number of surviving worms	Number of dead worms	Mortality (%)
Control			
Mean	10	0	0
S.D.	0	0	0
50.00			
Mean	10	0	0
S.D.	0	0	0
89.00			

mg test item/kg dry weight artificial soil	Number of surviving worms	Number of dead worms	Mortality (%)
Mean	10	0	0
S.D.	0	0	0
158.40			
Mean	10	0	0
S.D.	0	0	0
282.00			
Mean	10	0	0
S.D.	0	0	0
502.00			
Mean	10	0	0
S.D.	0	0	0

Statistically significant differences in growth relative to the control were observed at the two highest test concentrations of 282.00 and 502.00 mg test item/kg dry weight soil (results of a Williams multiple sequential t-test, two-sided, α=0.05).

Table CP 10.4.1.1/03-2 Body weight data observed after 28 days exposure

mg test item/kg dry weight artificial soil	Number of surviving worms	Weight of worms (Day 0)	Weight of worms (Day 28)	Weight change (%)
Control				
Mean	10	0.32	0.58	80.87
S.D.	0	0.01	0.03	4.76
50.00				
Mean	10	0.30	0.55	81.02
S.D.	0	0.01	0.02	9.49
89.00				
Mean	10	0.33	0.58	76.84
S.D.	0	0.02	0.01	8.94
158.40				
Mean	10	0.34	0.59	74.07
S.D.	0	0.02	0.03	2.60
282.00				
Mean	10	0.31	0.52	66.05*
S.D.	0	0.01	0.03	8.78
502.00				
Mean	10	0.31	0.48	56.21*
S.D.	0	0.01	0.02	1.80

* Statistically significantly different compared to the control

No statistically significant differences in the number of juveniles were observed at test concentrations of 50.00, 89.00, 158.40 and 282.00 mg test item/kg dry weight soil. Statistically significant differences were observed at the highest test concentration of 502.00 mg test item/kg dry weight soil (results of a

Welch-t test for inhomogeneous variances with Bonferroni-Holm adjustment, one-sided smaller, α = 0.05.

Table CP 10.4.1.1/03-3 Juvenile earthworms per test vessel observed after 56 days exposure

mg test item/kg dry weight artificial soil	Mean	S.D.	Coefficient variation	% of control
Control	397.9	46.1	11.6	-
50.00	355.8	38.0	10.7	89.4
89.00	343.3	42.7	12.4	86.3
158.40	373.5	95.0	25.4	93.9
282.00	347.3	40.7	11.7	87.3
502.00	294.3	12.6	4.3	74.0*

* Statistically significantly different compared to the control

A reference item, Derosal (active substance: 36% carbendazim) was tested from 31 January 2011 to 5 April 2011 in a dose response study. Dosages of 0, 1.25, 2.5 and 5.0 mg a.s./kg dry weight soil were tested by application into the artificial soil at test initiation. Mortality of adult earthworms as compared to control organisms was not observed throughout the test. Observed body weight changes at the application rates of 2.5 and 5.0 mg a.s./kg dry weight soil were statistically significantly reduced in comparison to the control group (α=0.05). Reproduction data at all application rates were statistically significantly reduced in comparison to the control group (α=0.05). The EC_{50} for reproduction was determined to be 1.66 mg a.s./kg dry weight soil with 95% confidence limits between 1.62 – 1.69 mg a.s./kg dry weight soil.

III. Conclusion

Based on the biological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the overall NOEC for this study is 158.40 mg test item/kg dry weight soil (equivalent to 80 mg a.s./kg soil). Thus, the overall LOEC is determined to be 282.00 mg test item/kg dry weight soil (equivalent to 142 mg a.s./kg soil).

Assessment and conclusion by applicant:

The study was conducted to an older version of the current test guideline but the validity criteria remain the same in the current OECD 222 (2016) version. The validity criteria have been met:

- Each replicate (containing 10 adults) to have produced ≥30 juveniles by the end of the test (actual: 328 to 468)
- The coefficient of variation of reproduction to be ≤30% (actual: 11.6%)
- Adult mortality over the initial 4 weeks of the test to be ≤10% (actual: 0%)

The reference substance produced significant effects with an EC_{50} of 1.66 mg a.s./kg soil. This is in line with the values given in the OECD 222 guideline of 1 - 5 mg a.s./kg soil. Thus, the sensitivity of the organisms was confirmed.

The test substance was incorporated into the soil as is now required.

The study is therefore considered to be acceptable.

The NOEC was 158.40 mg test item/kg dry weight soil (equivalent to 80 mg a.s./kg soil).

The results from this study have been statistically re-analysed and a summary of these results is presented below.

Data Point:	KCP 10.4.1.1/04
Report Author:	[redacted]
Report Year:	2020
Report Title:	Calculation of EC10 and EC20 values for Eisenia fetida with spiroxamine EC 500 in a reproduction study
Report No:	0471836-ECO17
Document No:	M-761531-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The report M-416761-01-1 on the effects of Spiroxamine EC 500 in the earthworm (*Eisenia fetida*) reproduction study did not provide estimates of EC_{10} or EC_{20} values. Therefore, these values have been calculated in accordance with the Annex to Com. Reg. 284/2013. Due to the lack of a significant dose response, the determination of EC_{10} and EC_{20} values for reproduction was not possible.

I. Methods

The statistical evaluation was performed with statistical software ToxRat Standard v3.3.0. Due to the lack of a significant dose response between treatment and control, the determination of EC_{10} and EC_{20} values for reproduction was not possible.

II. Results

Due to the lack of a significant dose response, the determination of EC_{10} and EC_{20} values for reproduction was not possible.

III. Conclusion

Due to the lack of a significant dose response between treatment and control, the determination of EC_{10} and EC_{20} values for reproduction was not possible.

Assessment and conclusion by applicant:

The statistical re-evaluation of the reproduction data could not determine reliable EC_{10} and EC_{20} values due to a lack of a dose-response.

The NOEC based on growth of 158.40 mg/kg dws remains the most critical endpoint from this study and has been used in the risk assessment.

The conclusions made in the re-evaluation work are considered to be fully valid.

Data Point:	KCP 10.4.1.1/01
Report Author:	Heim [redacted]
Report Year:	1994
Report Title:	Influence of KWG 4168 EC 500 on the reproduction of earthworms (Eisenia fetida)
Report No:	HBF/RG 186
Document No:	M-008843-01-1
Guideline(s) followed in study:	ISO draft ISO/DIS 11268-2 (1993)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (1997), RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

The purpose of this study was to investigate the toxicity of KWG 4168 EC 500 exposure to earthworms and its influence on their reproduction.

In an 8-week study, earthworms were exposed to KWG 4168 EC 500 at nominal concentrations of 1.5 L product/ha and the 4-fold rate of 6.0 L product/ha (equivalent to 0.75 and 3.0 kg a.s./ha, respectively). There were 40 earthworms per treatment group with a mean weight at test initiation of 0.43 g.

Exposure to KWG 4168 EC 500 did not show significant lethal effects to the earthworm *Eisenia fetida* in artificial soil up to the highest test concentration of 6.0 L product/ha.

There were no statistically significant reductions in earthworm body weight or reproduction data. The overall NOEC was therefore determined to be ≥3 kg a.s./ha, corresponding to ≥4.00 mg a.s./kg soil.

I. Materials and Methods

Materials

Test Material RWG 4168 EC 500

Lot/Batch #: 089 A according to 04023/0021

Purity: 494 g/L

Description: Clear yellow liquid

Reanalysis/Expiry date: 17 March 1994

Treatments

Test rates: Nominal: 1.5 L product/ha and the 4-fold rate of 6.0 L product/ha (equivalent to 0.75 and 3.0 kg a.s./ha, respectively)

Test organisms

Species: Earthworm, (*Eisenia fetida*)

Source: Prof Graff, D 3300-Braunschweig

Acclimatisation period: One day prior to test initiation

Feeding: Finely ground cattle manure

Test design

Test vessel:	1.2 L plastic boxes (16.5 x 12 x 6 cm) covered with a fine mesh gauze (0.5 mm)
Test medium:	Artificial soil: 500 g dry weight; 725 g wet weight. 10% peat content used
Replication:	Four per treatment group
No. animals/vessel:	Ten animals per test vessel
Duration of test:	Eight weeks
Environmental test conditions	
Temperature:	20 ± 2°C
Water content:	Test start: 27.9 – 28.1% (53.0 – 51.8% of WHC_{max}) Test end: 28.7 – 28.9% ( )
pH:	6.04 – 6.20
Photoperiod:	16 hours light, 8 hours dark (light intensity: 400 – 800 lux)

Study Design

This study was conducted in order to assess the effect of KWG 4168 EC 500 on the reproduction of earthworms during an exposure into an artificial soil at two different test concentrations.

Ten earthworms were added to each of the four replicate test vessels. Test vessels were 1.2 litre plastic boxes (length x width x height *ca.* 16.5 x 12 x 6 cm) covered with a fine mesh gauze (mesh size 0.5 mm).

The test soil consisted of 69% fine quartz sand, 10% dried, finely ground peat, 20% kaolin clay and 1% calcium carbonate. Each vessel was filled with 725 g of prepared soil. Prepared soil consisted of approximately 500 g of dry weight artificial soil and 125 g of water.

The earthworms were exposed to nominal concentrations of 1.5 L product/ha and the 4-fold rate of 6.0 L product/ha (equivalent to 0.75 and 3.0 kg a.s./ha, respectively).

Incubation was at 20 ± 2°C with a photoperiod of 16 hours light and 8 hours dark at approximately 400 to 800 lux.

The moisture content and maximum water capacity of the test substrate was determined with a hydrometer at 105°C and the pH was measured using an electronic measuring instrument.

After 4 weeks of exposure, the content of each test vessel was emptied and the adult worms were counted, removed and weighed before the substrate was returned to the respective test units minus the worms. Mortality and behavioural abnormalities (*e.g.* lack of movement and rigidity) were observed at this stage (28 days after application).

At test termination, the number of surviving juveniles per test vessel was determined by placing the test vessels in a water bath of 50 to 60 °C and counting all emerging worms. Reproduction was determined by the number of alive juveniles at test termination.

The earthworms were fed finely ground cattle manure throughout the test which was added to the soil weekly. At each feeding date, the amount of food consumed by the adult earthworms was visually estimated for each test vessel.

At test termination, reproduction and body weight data were statistically evaluated by the Mann-Whitney-Wilcoxon U-Test.

II. Results and Discussion

No assessment of validity criteria were made in the study report.

No mortality of adult earthworms were observed after 28 days test duration at the test concentration of 6.0 L product/ha and in the control group. At the test concentration of 1.5 L product/ha a slight mortality (5%) was observed. There was no significant difference in weight alteration data between the control and the treatment groups (U-Test, p = 0.05).

Table CP 10.4.1.1/01-1 Mortality and weight observation data after 28 days exposure

Number of applications and application rate (L/ha)	Mortality (%)	Weight alteration of the survivors (%)
Control	0	+ 24 ± 5
1 x 1.5	5 ± 6	+ 30 ± 4
1 x 6.0	0	+ 26 ± 3

Offspring data were observed following 56 days of exposure at the test concentrations of 1.5 and 6.0 L product/ha. There was no significant difference between the control and the treatment groups (U-Test, p=0.05).

Table CP 10.4.1.1/01-2 Reproduction data per surviving adult earthworm after 56 days exposure

Number of applications and application rate (L/ha)	Numbers per adult	% of control	Weight (g)	% of control
Control	9.2 ± 0.9	-	2.0 ± 0.3	-
1 x 1.5	10.0 ± 0.9	109	1.8 ± 0.2	90
1 x 6.0	9.5 ± 1.9	103	1.7 ± 0.3	85

A reference item, Derosal (active substance: 36% carbendazim) was tested from 20 December 1993 to 10 February 1994 in a dose response study. Dosages of 0.10, 0.25 and 0.50 kg formulation/ha were tested by application onto the soil surface at test initiation. Mortality of adult earthworms as compared to control organisms was not observed throughout the test. The application rate of 0.5 kg/ha slightly reduced the biomass increase of adult earthworms and the final biomass of juvenile earthworms. The application rates of 0.25 and 0.5 kg/ha reduced the number of juveniles by 49 and 31%, respectively. The NOEC and LOEC values were 0.10 and 0.25 kg/ha, respectively (equivalent to 0.032 and 0.08 kg a.s./ha, respectively).

III. Conclusion

There were no effects of KWG 4168 EC 500 on earthworm mortality, bodyweight or reproduction when applied at rates of 1.5 and 6.0 L product/ha. The NOEC has been determined to be 6.0 L product/ha (≥3 kg a.s./ha) corresponding with ≥4.00 mg a.s./kg soil (considering a soil density of 1.5 g/cm^3 and a depth of 5 cm).

Assessment and conclusion by applicant:

The study has been assessed against the validity criteria according to the OECD 222 (2016) Guideline "Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*)" and these criteria have been met:

- Each replicate (containing 10 adults) to have produced ≥30 juveniles by the end of the test (actual: 86 to 105 juveniles)
- The coefficient of variation of reproduction to be ≤30% (actual: 9.72%)

- Adult mortality over the initial 4 weeks of the test to be ≤10% (actual: 0%)

The coefficient of variation for reproduction in the control was calculated from the total number of juveniles in the four control replicates (105, 86, 87 and 89, respectively). This gave a mean of 92 per replicate, the SD was 8.9 and the CV was 9.72%.

The reference substance produced significant effects at concentrations of 0.08 kg a.s./ha) which is lower than the values given in the OECD 222 guideline. However, the sensitivity of the organisms was still confirmed as effects were seen at lower rates than those stated in the guideline.

It is noted that the test substance was applied to the surface of the test soils and was not mixed into the soil as is now required. For this reason the results should be treated with caution and, as a result, the study has therefore been submitted as supporting information only.

The NOEC has been determined to be 6.0 L product/ha (≥3 kg a.s./ha) corresponding with ≥4.00 mg a.s./kg soil.

The results from this study have been statistically re-analysed and a summary of these results is presented below.

Data Point:	KCP 10.4.1.1/05
Report Author:	[redacted]
Report Year:	2020
Report Title:	Calculation of EC10 and EC20 values for Eisenia fetida with spiroxamine EC 500 in a reproduction study
Report No:	0471836-ECO8
Document No:	M-760459-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The report M-008843-01-1 on the effects of Spiroxamine EC 500 in the earthworm (*Eisenia fetida*) reproduction study did not provide estimates of EC_{10} or EC_{20} values. Therefore, these values have been calculated in accordance with the Annex to Com Reg. 284/2013. Due to the test design and the lack of effects above 10% between treatment and control, the determination of EC_{10} and EC_{20} values for reproduction was not possible. Therefore, EC_{10} and EC_{20} values were estimated to be above the test rate of 6.0 L/ha.

I. Methods

The statistical evaluation was performed with statistical software ToxRat Standard v3.3.0. Due to the test design and the lack of effects above 10% between treatment and control, the determination of EC_{10} and EC_{20} values for reproduction was not possible.

II. Results and Discussion

Due to the test design, and the lack of effects above 10% between treatments and the control, the determination of EC_{10} and EC_{20} values for reproduction was not possible. Therefore, EC_{10} and EC_{20} values are estimated to be above the test rate of 6.0 L/ha.

III. Conclusion

Due to the test design and the lack of effects above 10% between treatment and control, the determination of EC_{10} and EC_{20} values for reproduction was not possible. Therefore, EC_{10} and EC_{20} values were estimated to be above the test rate of 6.0 L/ha.

Assessment and conclusion by applicant:

A reliable EC_{10} and EC_{20} could not be determined.

The NOEC of 6.0 L/ha (equivalent to 4.00 mg a.s./kg soil) remains the most critical endpoint from this study and has been used in the risk assessment.

The values determined in the re-evaluation work are considered to be fully valid.

Data Point:	KCP 10.4.1.1/02
Report Author:	[redacted]
Report Year:	2000
Report Title:	Influence of Spiroxamine EC 500 on the reproduction of earthworms (Eisenia fetida) tested with 5 % peat in the test substrate
Report No:	MPE/RG 349/00
Document No:	M-026522-01-1
Guideline(s) followed in study:	ISO/DIS 11268-2 (1996), BBA Guideline, Part VI-2-2 (1994)
Deviations from current test guideline:	Yes (refer below) 5% peat in the test substrate
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

The purpose of this study was to investigate the effects of Spiroxamine EC 500 on the reproduction of earthworms in artificial soil with 5% peat in the test substrate.

In an 8 week study, earthworms were exposed to Spiroxamine EC 500 at nominal concentrations of 750, 1500 and 3750 g a.s./ha. There were 40 earthworms per treatment group.

Exposure to Spiroxamine EC 500 did not show significant lethal effects to the earthworm *Eisenia fetida* in artificial soil up to the highest test concentration of 3750 g a.s./ha.

There were no statistically significant reductions in earthworm body weight or reproduction data. The overall NOEC is therefore determined to be ≥3750 g a.s./ha, corresponding to ≥5.00 mg a.s./kg soil.

I. Materials and Methods

Materials

Test Material	Spiroxamine EC 500
Lot/Batch #:	04023/0778(0627)
Purity:	488.9 g
Description:	Clear brown liquid
Reanalysis/Expiry date:	3 June 2001
Treatments	
Test rates:	Nominal: 750, 1500 and 3750 g a.s./ha
Test organisms	
Species:	Earthworm (*Eisenia fetida*)
Source:	Prof. Graff, 38104 Braunschweig, Germany
Acclimatisation period:	One day prior to test initiation
Feeding:	Finely ground cattle manure
Test design	
Test vessel:	1.2 L plastic boxes (16.5 x 12 x 6 cm) covered with a fine mesh gauze (0.5 mm)
Test medium:	Artificial soil: 500 g dry weight; added water: 121 g. 5% peat used
Replication:	Four per treatment group
No. animals/vessel:	Ten animals per test vessel
Duration of test:	Eight weeks
Environmental test conditions	
Temperature:	20 ± 2°C
pH:	6.45 – 6.51
Photoperiod:	16 hours light, 8 hours dark (light intensity: 400 – 800 lux)

Study Design

This study was conducted in order to assess the effect of Spiroxamine EC 500 on the reproduction of earthworms during an exposure into an artificial soil at three different test concentrations.

Ten adult earthworms were added to each of the four replicate test vessels. Test vessels were plastic boxes (length x width x height *ca*. 16.5 x 12 x 6 cm) covered with a fine mesh gauze (mesh size 0.5 mm).

The test soil consisted of 74% fine quartz sand, 20% kaolin clay, 5% dried, finely ground peat, 1% dried, finely ground cattle manure and 1% calcium carbonate. Prepared soil consisted of approximately 500 g of dry weight soil and approximately 121 g of water.

The earthworms were exposed to nominal concentrations of 750, 1500 and 3750 g a.s./ha.

Incubation was at 20 ± 2°C with a photoperiod of 16 hours light and 8 hours dark at approximately 400 to 800 lux.

After 4 weeks of exposure, the content of each test vessel was emptied and the adult worms were counted, removed and weighed before the substrate was returned to the respective test units minus the worms. Mortality and behavioural abnormalities (*e.g.* lack of movement and rigidity) were observed at this stage (28 days after application).

At test termination, the number of surviving juveniles per test vessel was determined by placing the test vessels in a water bath of 50 to 60 °C and counting all emerging worms. Reproduction was determined by the number of alive juveniles at test termination.

The earthworms were fed finely ground cattle manure throughout the test which was added to the soil weekly. At each feeding date, the amount of food consumed by the adult earthworms was visually estimated for each test vessel.

At test termination, reproduction and body weight data were statistically evaluated by the Mann-Whitney-Wilcoxon U-Test.

II. Results and Discussion

No assessment of validity criteria were made in the study report.

No mortality of adult earthworms were observed after 28 days test duration at the test concentrations of 750 and 1500 g a.s./ha. In the control group and the test concentration of 3750 g a.s./ha a slight mortality (3%) was observed. There was no significant difference in weight alteration data between and the control and the treatment groups (U-Test, p=0.05).

Table CP 10.4.1.1/02-1 Mortality and weight observation data after 28 days exposure

Concentration (g a.s./ha)	Mortality (%)	Weight alteration of the survivors	
		%	U-Test*)
Control	3	+ 39 ± 9	
750	0	+ 34 ± 3	-
1500	0	+ 34 ± 10	-
3750	3	+ 42 ± 9	-

*) Results of the U-test: - = weights of control and the treatment do not differ significantly (p=0.05). + = weights of control and the treatment do not differ significantly (p=0.05)

Offspring data were observed following 56 days of exposure at the test concentrations of 750, 1500 and 3750 g a.s./ha. There was no significant difference between the control and the treatment groups (U-Test, p=0.05).

Table CP 10.4.1.1/02-2 Reproduction data per surviving adult earthworm after 56 days exposure

Application rate (g a.s./ha)	Numbers per adult	Variation coefficient (%)	Juvenile worms	
			%	U-Test*)
Control	17 ± 3	20	100	

Application rate (g a.s./ha)	Numbers per adult	Variation coefficient (%)	Juvenile worms	
			%	U-Test*)
750	18 ± 2	11	101	-
1500	17 ± 2	12	97	-
3750	17 ± 5	28	98	-

*) Results of the U-test: - = numbers of control and the treatment do not differ significantly (p=0.05) + = weights of control and the treatment do not differ significantly (p=0.05)

A reference item, Derosal (active substance: 36% carbendazim) was tested from 12 July 2000 to 6 September 2000 in a dose response study. Dosages of 0.10, 0.25 and 0.50 kg/ha were tested by application onto the soil surface at test initiation. Mortality of adult earthworms as compared to control organisms was not observed throughout the test. The test showed significant reduction in body weight at 0.25 and 0.5 kg/ha. The highest dosage of 0.5 kg a.s./ha reduced the number of juvenile earthworms by 46%. The NOEC and LOEC values were 0.10 and 0.25 kg/ha, respectively (equivalent to 0.016 kg a.s./ha and 0.032 kg a.s./ha, respectively).

III. Conclusion

There were no effects of KWG 4168 EC 500 on earthworm mortality, bodyweight or reproduction when applied at rates of 750, 1500 and 3750 g a.s./ha. The NOEC was determined to be ≥3750 g a.s./ha corresponding to ≥5.00 mg a.s./kg (considering a soil density of 1.5 g/cm^3 and a depth of 5 cm).

Assessment and conclusion by applicant:

The study has been assessed against the validity criteria according to the OECD 222 (2016) Guideline "Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*)" and these criteria have been met:

- Each replicate (containing 10 adults) to have produced ≥30 juveniles by the end of the test (actual: 140 to 198 juveniles)
- The coefficient of variation of reproduction to be ≤30% (actual: 14.6%)
- Adult mortality over the initial 4 weeks of the test to be ≤10% (actual: 3%)

The coefficient of variation for reproduction in the control was calculated from the total number of juveniles in the four control replicates (198, 159, 174 and 140, respectively). This gave a mean of 168 per replicate, the SD was 24.5 and the CV was 14.6%.

The reference substance produced significant effects at concentrations of 0.032 kg a.s./ha) which is lower than the values given in the OECD 222 guideline. However, the sensitivity of the organisms was still confirmed as effects were seen at lower rates than those stated in the guideline.

It is noted that the test substance was applied to the surface of the test soils and was not mixed into the soil as is now required. For this reason the results should be treated with caution and, as a result, the study has therefore been submitted as supporting information only.

The NOEC was determined to be ≥3750 g a.s./ha corresponding to ≥5.00 mg a.s./kg.

The results from this study have been statistically re-analysed and a summary of these results is presented below.

Data Point:	KCP 10.4.1.1/06
Report Author:	[redacted]
Report Year:	2020
Report Title:	Calculation of EC10 and EC20 values for Eisenia fetida with spiroxamine EC 500 in a reproduction study
Report No:	0471836-ECO9
Document No:	M-760441-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted -
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The report M-026522-01-1 on the effects of Spiroxamine EC 500 in the earthworm (*Eisenia fetida*) reproduction study did not provide estimates of EC_{10} or EC_{20} values. Therefore, these values have been calculated in accordance with the Annex to Com. Reg. 283/2013. Due to the test design, reduced number of concentrations tested, a lack of a significant dose response and lack of effects above 10% between treatment and control, the determination of EC_{10} and EC_{20} values for reproduction was not possible. Therefore, EC_{10} and EC_{20} values are estimated to be above the test rate of 3750 g a.s./ha.

I. Methods

The statistical evaluation was performed with statistical software ToxRat Standard v3.3.0. Due to the test design, reduced number of concentrations tested, a lack of a significant dose response and lack of effects above 10% between treatment and control, the determination of EC_{10} and EC_{20} values for reproduction was not possible.

II. Results and Discussion

Due to the test design, reduced number of tested concentrations, lack of a significant dose response and the lack of effects above 10% between treatment and control, the determination of EC_{10} and EC_{20} values for reproduction is not possible. Therefore, EC_{10} and EC_{20} values are estimated to be above the test rate of 3750 g a.s./ha.

III. Conclusion

Due to the test design, reduced number of concentrations tested, a lack of a significant dose response and lack of effects above 10% between treatment and control, the determination of EC_{10} and EC_{20} values for reproduction was not possible. Therefore, EC_{10} and EC_{20} values are estimated to be above the test rate of 3750 g a.s./ha.

Assessment and conclusion by applicant:

The statistical re-evaluation of the reproduction data could not determine reliable EC_{10} and EC_{20} values.

The NOEC of 3750 g a.s./ha (equivalent to 5.00 mg a.s./kg soil) remains the most critical endpoint from this study and has been used in the risk assessment.

The conclusions made in the re-evaluation work are considered to be fully valid.

CP 10.4.1.2 Earthworms field studies

No data are available. Field data with Spiroxamine EC 500 are not considered necessary as an acceptable risk following the proposed uses has been demonstrated using the available laboratory data.

CP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

The avaialable soil meso- and macro-fauna (other than earthworms) toxicity data for spiroxamine, Spiroxamine EC 500 and the metabolites of spiroxamine are summarised in the table below.

Table CP 10.4.2-1 Summary of soil macro-organism (other than earthworm) toxicity studies with spiroxamine, Spiroxamine EC 500 and metabolites

Organism	Test item	Test type	Endpoints		Reference
Folsomia candida	Spiroxamine	28 d Chronic toxicity; 5% peat	**NOEC 32 mg a.s./kg soil dw**	EU	M-289274-01-1
		Statistical Re-analysis	EC_{10}/EC_{20} not determinable	NEW	M-760433-01-1
Folsomia candida	Spiroxamine	28 d Chronic toxicity; 5% peat	NOEC 75 mg a.s./kg soil dw	NEW	M-405276-01-1
		Statistical Re-analysis	EC_{10} 175 mg a.s./kg soil dw EC_{20} 258 mg a.s./kg soil dw	NEW	M-761559-01-1
Folsomia candida	Spiroxamine EC 500	28 d Chronic toxicity; 5% peat	NOEC 25.0 mg/kg soil dw (equivalent to 12.5 mg a.s./kg soil dw) EC_{10} >25.0 mg/kg soil dw (equivalent to >12.5 mg a.s./kg soil dw)	NEW	M-688132-01-1
Folsomia candida	Spiroxamine EC 500	28 d Chronic toxicity; 5% peat	**NOEC 35.0 mg/kg soil dw (equivalent to 17.2 mg a.s./kg soil dw)** EC_{10} 40.8 mg/kg soil dw (equivalent to 20.1 mg a.s./kg soil dw)	NEW	M-761545-01-1
Folsomia candida	KWG 4168-desethyl (M01)	28 d Chronic toxicity; 5% peat	**NOEC 316 mg/kg soil dw**	EU	M-289321-01-1
		Statistical Re-analysis	EC_{10}/EC_{20} not determinable	NEW	M-760431-01-1

Organism	Test item	Test type	Endpoints		Reference
Folsomia candida	KWG 4168-despropyl (M02)	28 d Chronic toxicity; 5% peat	NOEC 316 mg/kg soil dw	EU	M-288905-01-1
		Statistical Re-analysis	**EC_{10} 308 mg/kg soil dw** EC_{20} 402 mg/kg soil dw	NEW	M-760410-01-1
Folsomia candida	KWG 4168-N-oxide (M03)	28 d Chronic toxicity; 5% peat	**NOEC 100 mg/kg soil dw** EC_{10} >100 mg/kg soil dw	NEW	M-687854-01-1
Folsomia candida	KWG 4168-acid (M06)	28 d Chronic toxicity; 5% peat	**NOEC 1000 mg/kg soil dw** EC_{10} >1000 mg/kg soil dw	NEW	M-727026-01-1
Hypoaspis aculeifer	Spiroxamine EC 500	14 d Chronic toxicity; 5% peat	**NOEC 200 mg/kg soil dw (equivalent to 100 mg a.s./kg soil dw)** EC_{10} >200 mg/kg soil dw, (equivalent to >100 mg a.s./kg soil dw)	NEW	M-688129-01-1
Hypoaspis aculeifer	Spiroxamine EC 500	14 d Chronic toxicity; 5% peat	NOEC 1000 mg/kg soil dw (equivalent to 505 mg a.s./kg soil dw)	NEW	M-443019-01-1
Hypoaspis aculeifer	KWG 4168-desethyl (M01)	14 d Chronic toxicity; 5% peat	**NOEC 50 mg/kg soil dw** EC_{10} 94.1 mg/kg soil dw	NEW	M-680684-01-1
Hypoaspis aculeifer	KWG 4168-despropyl (M02)	14 d Chronic toxicity; 5% peat	**NOEC 100 mg/kg soil dw** EC_{10} >100 mg/kg soil dw	NEW	M-680694-01-1
Hypoaspis aculeifer	KWG 4168-N-oxide (M03)	14 d Chronic toxicity; 5% peat	**NOEC 100 mg/kg soil dw** EC_{10} >100 mg/kg soil dw	NEW	M-680687-01-1
Hypoaspis aculeifer	KWG 4168-acid (M06)	14 d Chronic toxicity; 5% peat	**NOEC 1000 mg/kg soil dw** EC_{10} >1000 mg/kg soil dw	NEW	M-727128-02-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
NEW: new study or data generated since the previous EU review or previously not submitted
Values in **bold** have been used in the risk assessment

Toxicity endpoints

In accordance with the Terrestrial Guidance Document (SANCO/10329/2002), to account for the high organic matter content in the test soils, the effect concentrations would be corrected by a factor of 2 for lipophilic substances with log P_{ow} >2. The Log P_{ow} of spiroxamine is 2.79 and 2.98 at pH 7 for diastomers A and B, respectively and at pH 9 these value are 4.88 and 5.08, respectively. Thus, correction of the endpoint would be necessary where artificial soil with a 10% peat content has been used. The Log P_{ow} of spiroxamine-desethyl (M01) is 2.41, 1.97 and >3.64 at pH 4, 7 and 9 respectively. The Log P_{ow} of spiroxamine-despropyl (M02) is 1.95, 1.41 and >3.44 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-N-oxide (M03) is 2.82, 2.59 and 2.63 at pH 4, 7 and 9, respectively. The Log P_{ow} of spiroxamine-carboxylic acid (M06) is 0.45, -0.25 and 0.10 at pH 4, 7 and 9 respectively. Thus, correction of the endpoint for the studies using M01, M02 and M03 would also be necessary but only where artificial soil with a 10% peat content has been used. It is noted that all of the available studies using spiroxamine or its metabolites with *Folsomia* and *Hypoaspis* have used artificial soil with a reduced (5%) peat content therefore correction of the endpoint to account for lipophilicity of the substance is not considered to be necessary.

Four reproduction studies with *Folsomia candida* are available, two with spiroxamine technical and two using Spiroxamine EC 500. For the technical material the lowest endpoint derived out of the two studies was a NOEC of 32 mg a.s./kg soil dw therefore this value has been used in the risk assessment of spiroxamine. For Spiroxamine EC 500, the first study tested up to a maximum concentration of 25 mg product/kg soil dw, at which there were no significant effects relative to the control. A second study was therefore conducted at higher concentrations in order to better define the NOEC. A NOEC of 35 mg product/kg soil dw was determined in the second study and was established based on a LOEC of 55 mg product/kg soil dw. It is therefore appropriate to use the defined NOEC of 35 mg product/kg soil dw in the risk assessment of Spiroxamine EC 500.

For *Hypoaspis aculeifer*, reproduction data for spiroxamine technical are not available. However, the available formulation data are considered to be more relevant for the risk assessment as this has been generated using the representative formulation itself and also represents the toxicity to spiroxamine. Two studies using Spiroxamine EC 500 are available, from which the lowest endpoint was a NOEC of 200 mg product/kg soil dw. This value has therefore been used in the risk assessment below.

Exposure

Full details of the PEC_{soil} calculations have been provided in Document M-CP Section 9 Environmental Fate. The maximum initial and accumulation PEC_{soil} values for spiroxamine and its metabolites, as calculated using FOCUS equations, are given in the table below for the highest application rate of 300 g a.s./ha.

Table CP 10.4.2-2 PEC_{soil} for spiroxamine and its metabolites

Substance	1 x 300 g a.s./ha		2 x 300 g a.s./ha	
	Max PEC_{soil} (mg/kg)	$PEC_{soil\ accumulation}$ (mg/kg)	Max PEC_{soil} (mg/kg)	$PEC_{soil\ accumulation}$ (mg/kg)
Vines				
Spiroxamine	0.200	0.277	0.372	**0.555**
M01	0.022	0.032	0.044	**0.064**
M02	0.016	0.020	0.032	**0.040**
M03	0.017	0.018	0.033	**0.037**
M06	0.012	0.052	0.023	**0.104**

PEC_{soil} values used in the risk assessment are highlighted in **bold**

For the risk assessment below the risk envelope approach has been applied in which the PEC_{soil} values for the proposed use with the highest application rate has been used. Thus, the risk assessment has been conducted for the use on grapes at 2 x 300 g a.s./ha. Furthermore, the $PEC_{soil\ accumulation}$ values were greater than the maximum initial PEC_{soil} values therefore the risk assessment has been conducted using the worst case $PEC_{soil\ accumulation}$ values.

For Spiroxamine EC 500 the formulation PEC_{soil} was determined to be 0.401 mg/kg soil for the maximum application rate of 300 g a.s./ha. Please refer to Document M-CP Section 9 Environmental Fate for further details.

Isomers

For parent spiroxamine the environmental fate soil degradation data currently suggest that there is no significant selective degradation of isomers over time. As a result, the toxicity data generated using the mixture of the isomers (*i.e.* spiroxamine or Spiroxamine EC 500) are considered to represent the toxicity of the isomers in the ratios that would occur in the soil following application. In accordance with the isomer Guidance Document[21] it is therefore not necessary to apply any additional Uncertainty Factor (UF) to the risk assessment (*i.e.* a UF of 1.0 is used).

For the metabolites of spiroxamine there are no chiral data available to be able to make this assessment therefore there is a possibility that selective degradation of isomers could occur in the soil over time. In order to account for any possible increased toxicity to soil organisms as a result of an increase in the ratio of a single isomer, an UF has been applied to the risk assessment of M01, M02, M03 and M06. The UF have been calculated following the recommendations of the isomer Guidance Document and have been presented in the table below.

Table CP 10.4.2-3 Uncertainty Factors determined for the soil meso- and macro-fauna toxicity data with the metabolites of spiroxamine

Test item	Study reference	Test material batch number	Isomer ratio	UF [1]
Folsomia candida				
Spiroxamine	-	-	-	1.0 [2]
M01	M-289321-01-1	921103ELB02	A:B 56:42	4.76
M02	M-288905-01-1	921103ELB03	A:B 55:42	4.76
M03	M-687854-01-1	M26999	D1:D2:D3:D4 22:21:26:31	9.52
M06	M-727126-01-1	AE 1344313-01-03	A:B 47:53	4.26
Hypoaspis aculeifer				
Spiroxamine	-	-	-	1.0 [2]
M01	M-680684-01-1	AE 1344302-PU-01	A:B 52:48	4.17
M02	M-680694-01-1	AE 1344303-PU-01	A:B 83.1:16.0	12.5
M03	M-680687-01-1	M26999	D1:D2:D3:D4 22:21:26:31	9.52
M06	M-727128-01-1	AE1344313-01-03	A:B 47:53	4.26

[21] 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

[1] Changes in stereoisomeric excess are unknown therefore Uncertainty Factor = 100/content of lowest stereoisomer (%) used for ecotox endpoint [as indicted in Table B.1, p.30 of isomer GD] and assumes that the toxicological effects of the mixture can be attributed to a single isomer. This assumes that all enantiomer ratios can be safely assumed to be 50:50. For example A:B ratio of 83.1:16 would be 100/(16/2) = UF of 12.5

[2] No additional UF required for parent as no significant change in isomeric ratios has been demonstrated.

Risk assessment

The risk assessment has been conducted in accordance with the Terrestrial Guidance Document (SANCO/10329/2002).

The effect concentrations for spiroxamine, Spiroxamine EC 500 and for the metabolites are compared to the PEC_{soil} values in the following table.

Table CP 10.4.2-4 Soil meso- and macro-fauna (other than earthworms) risk assessment for spiroxamine, Spiroxamine EC 500 and relevant metabolites following application of Spiroxamine EC 500 to vines

Intended use	**Vines 2 x 300 g a.s./ha**			
Chronic effects on soil meso- and macro-fauna (other than earthworms)				
Test item	**$NOEC/EC_{10}$**	**PEC_{soil}**	**UF[1]**	**TER_{LT}[2] (criterion TER ≥ 5)**
Folsomia candida				
Spiroxamine	32 mg a.s./kg soil dw	0.555 mg a.s./kg soil	1.0	57.7
Spiroxamine EC 500	35 mg product/kg (17.2 mg a.s./kg soil dw)	0.401 mg product/kg soil	1.0	87.3
M01	316 mg/kg soil dw	0.064 mg/kg soil	4.76	1037
M02	308 mg/kg soil dw	0.040 mg/kg soil	4.76	1618
M03	100 mg/kg soil dw	0.037 mg/kg soil	9.52	284
M06	1000 mg/kg soil dw	0.104 mg/kg soil	4.26	2257
Hypoaspis aculeifer				
Spiroxamine EC 500	200 mg product/kg	0.401 mg product/kg soil	1.0	499
	(100 mg a.s./kg soil dw)	0.555 mg a.s./kg soil	1.0	180
M01	50 mg/kg soil dw	0.064 mg/kg soil	4.17	187
M02	100 mg/kg soil dw	0.040 mg/kg soil	12.5	200
M03	100 mg/kg soil dw	0.037 mg/kg soil	9.52	284
M06	1000 mg/kg soil dw	0.104 mg/kg soil	4.26	2257

[1] Uncertainty Factor applied to account for the unknown effect of a possible change in isomer ratios over time

[2] TER calculated as follows: Toxicity endpoint/(PEC_{soil} × UF)

The TER_{LT} values for spiroxamine, Spiroxamine EC 500 and the metabolites all exceed the trigger value of 5 therefore acceptable risks to soil meso- and macro-fauna (other than earthworms), following the proposed uses of Spiroxamine EC 500, can be concluded.

Biodiversity

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on soil meso- and macrofauna (other than earthworms). Therefore, it is considered that the potential impact of the active substance on biodiversity

and the ecosystem, including potential indirect effects *via* alteration of the food web, are covered by the risk assessment for soil meso- and macrofauna (other than earthworms) in this section.

With respect to the risk assessment for non-target soil meso- and macrofauna, which demonstrated an acceptable outcome with large margins of safety and without the need for risk mitigation, the applicant concludes that the use of the representative lead formulation (Spiroxamine EC 500) has a low potential to cause unacceptable effects on biodiversity and the ecosystem *via* trophic interactions. To the best of our knowledge and with the presented safety profile of the spiroxamine a.s., metabolites and the representative lead formulation, the applicant does not foresee any unacceptable effects on biodiversity and the ecosystem with spiroxamine.

CP 10.4.2.1 Species level testing

Data Point:	KCP 10.4.2.1/01
Report Author:	[redacted]
Report Year:	2020
Report Title:	1st final report amendment - Spiroxamine EC 500: Effects on reproduction of the collembola Folsomia candida in artificial soil
Report No:	143091016
Document No:	M-688132-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 (2009) OECD-Guideline for testing chemicals No. 232 "Collembolan Reproduction Test in Soil" (adopted July 25, 2016) ISO-11267 Soil Quality – Inhibition of reproduction of Collembola (Folsomia candida) by soil contaminants, 2014
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

Collembola (*Folsomia candida*) aged 10 to 12 days were exposed to Spiroxamine EC 500 incorporated into artificial soil in a 4 week study in order to assess the effects on mortality and reproduction.

Folsomia candida were exposed to test concentrations of 1.56, 3.13, 6.25, 12.5 and 25.0 mg test item/kg dry weight soil.

There were no statistically significant effects observed on the mortality and reproduction of *Folsomia candida* up to and including the test concentration of 25.0 mg test item/kg dry weight soil.

The NOEC and LOEC values for reproduction were determined to be ≥25.0 and >25.0 mg test item/kg dry weight soil, respectively.

I. Materials and Methods

Materials

Test Material Spiroxamine EC 500

Lot/Batch #: EM4L018425

Purity: Spiroxamine EC 500 50.0% w/w, corresponding to 501.6 g/L

Description:	Yellow liquid
Reanalysis/Expiry date:	09 May 2020
Density:	1.004 g/mL
Treatments	
Test rates:	1.56, 3.13, 6.25, 12.5 and 25.0 mg test item/kg dry weight soil
Test organisms	
Species:	*Folsomia candida,* Collembola, Isotomidae, age 10 – 12 days
Source:	Ibacon GmbH, 64380 Rossdorf, Germany
Feeding:	2 mg of granulated dry yeast at test initiation and after 14 days
Test design	
Test vessel:	Glass containers (volume: 100 mL; diameter: 5 cm) sealed with lids
Test medium:	Artificial soil according to OECD 232 (2016). 5% peat content
Replication:	8 replicates for the control, 4 replicates per test concentration and 1 additional container per treatment to test the pH and water content of the soil at test termination
No. animals/vessel:	10 per test vessel
Duration of test:	4 weeks
Environmental test conditions	
Temperature:	18 - 22°C
pH:	6.0 – 6.2
Photoperiod:	16 hours light: 8 hours dark (400 – 800 lux)

Study Design

This study was conducted in order to assess the effects of Spiroxamine EC 500 on the mortality and reproduction of Collembola (*Folsomia candida*) over 4 weeks.

The Collembola were 10 to 12 days old at test initiation. Ten juvenile Collembola were introduced to the test vessels and placed onto the surface of the artificial soil. The test soil was composed of 74.8% fine quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.2% calcium carbonate.

The artificial soil was kept at 18 to 22°C and the test vessels were exposed to 400 – 800 lux in the controlled environment chamber.

Test concentrations of 1.56, 3.13, 6.25, 12.5 and 25.0 mg test item/kg dry weight soil were applied to the artificial soil. Eight replicates were exposed to the control treatment and four replicates were exposed to the 1.56, 3.13, 6.25, 12.5 and 25.0 mg test item/kg dry weight soil treatments.

At test initiation and after 14 days, the Collembola were fed with approximately 2 mg of granulated dry yeast.

A reference test with the toxic standard, Boric acid, was performed at least once a year to ensure that the laboratory test conditions are adequate and to verify that the response of the test organisms does not change significantly over time.

Water content was checked 14 days after application by reweighing the additional test vessels. If the water loss exceeded 2% of the initial water content, the missing amount of water was added to all vessels of the treatment group.

Reproduction data were observed at test termination. Juveniles were counted twice under binocular microscopes. Statistical analysis was performed on the reproduction data using Shapiro-Wilk's test and Levene's test ($\alpha = 0.01$) to test for normal distribution and homogeneity. Since the reproduction data were normally distributed and homogeneous but did not follow a monotonicity trend (contrast trend) the Dunnett's t-test was used to compare treatment and control values (multiple comparison, $\alpha = 0.05$, one-sided smaller).

Mortality data were observed at test termination. Missing adult Collembola were assumed dead and degraded. Statistical analysis was performed on the mortality data using Fisher's Exact Binomial Test (multiple comparison, with Bonferroni Correction, $\alpha = 0.05$, one-sided greater).

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

Behavioural abnormalities were also recorded at test termination.

II. Results and Discussion

Validity criteria according to the OECD 232 guideline (2016) were met:

- Mean adult mortality should not exceed 20% at the end of the test (actual: 6.3%)
- The mean number of juveniles per vessel should be at least 100 at the end of the test (actual: 390 – 483)
- The coefficient of variation calculated for the number of juveniles should be less than 30% at the end of the definitive test (actual: 7.3%)

There was slight mortality observed at all test concentrations, however the mortality was not statistically significant at any test concentration (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater). The highest and lowest mortalities were observed at concentrations of 6.25 and 12.5 mg test item/kg dry weight soil, respectively. The NOEC and LOEC values for mortality were determined to be ≥25.0 and >25.0 mg test item/kg dry weight soil, respectively.

Table CP 10.4.2.1/01-1 Mortality data observed after 28 days exposure

Treatment group (mg test item/kg artificial soil dry weight soil)	Mean mortality (%)	Standard deviation	Significance[1]
Control	6.3	± 7%	-
1.56	5.0	± 10%	n.s.
3.13	5.0	± 10%	n.s.
6.25	10.0	± 8%	n.s.
12.5	7.5	± 5%	n.s.
25.0	5.0	± 10%	n.s.

[1]Fisher's Exact Test, one-sided greater, $\alpha = 0.05$
- not applicable
n.s. not significantly different compared to the control

There were no statistically significant effects on reproduction of *Folsomia candida* up to and including the highest concentration of 25.0 mg test item/kg soil (Dunnett's t-test, $\alpha = 0.05$, one-sided smaller). The NOEC and LOEC values for reproduction were determined to be ≥25.0 and >25.0 mg test item/kg dry weight soil, respectively.

Table CP 10.4.2.1/01-2 Reproduction data observed after 28 days exposure

Treatment group (mg test item/kg artificial soil dry weight soil)	Mean number of juveniles	Standard deviation	% of control	Significance[1]
Control	449	± 33	-	-
1.56	417	± 19	93	n.s.
3.13	459	± 53	102	n.s.
6.25	468	± 52	104	n.s.
12.5	452	± 19	101	n.s.
25.0	456	± 45	102	n.s.

[1]Dunnett's t-test, α = 0.05, one-sided smaller
- not applicable
n.s. not significantly different compared to the control

To verify the sensitivity of the test system, the reference item (boric acid) was tested at concentrations of 30.5, 48.8, 78.1, 125 and 200 mg/kg soil dry weight in a separate study. In the most recent GLP study, an EC_{50} of 104.6 mg/kg soil dry weight was determined. The effects on the reduction of reproduction showed that the test system was sensitive and reflected the expected toxicity as given in the OECD 232 test guideline (50% reduction in reproduction at about 100 mg/kg soil dry weight).

III. Conclusion

Spiroxamine EC 500 caused no statistically significant effects on mortality and reproduction of Folsomia candida up to and including the highest test concentration of 25.0 mg test item/kg dry weight soil.

The NOEC and LOEC values for mortality were determined to be ≥25.0 and >25.0 mg test item/kg dry weight soil, respectively. The LC_{50} was estimated to be >25.0 mg test item/kg dry weight soil.

The NOEC and LOEC values for reproduction were determined to be ≥25.0 and >25.0 mg test item/kg dry weight soil, respectively (equivalent to ≥12.5 and >12.5 mg a.s./kg soil dry weight, respectively). Due to the lack of a concentration-response relationship, no reliable EC_x-calculation was possible. Therefore, no EC_{10}/EC_{20}-value can be reported. However, the EC_{50} was estimated to be >25.0 mg test item/kg dry weight soil.

Assessment and conclusion by applicant:

This is a new study that has not been previously submitted or evaluated.

Validity criteria according to the OECD 232 guideline (2016) were met.

- Mean adult mortality should not exceed 20% at the end of the test (actual: 6.3%)
- The mean number of juveniles per vessel should be at least 100 at the end of the test (actual: 390 - 483)
- The coefficient of variation calculated for the number of juveniles should be less than 30% at the end of the definitive test (actual: 7.3%)

The reference item also demonstrated sufficient sensitivity of the test organisms. The study is therefore considered acceptable.

The NOEC value for reproduction was determined to be 25.0 mg test item/kg dry weight soil (equivalent to 12.5 mg a.s./kg soil dry weight).

Data Point:	KCP 10.4.2.1/02
Report Author:	[redacted]
Report Year:	2021
Report Title:	Spiroxamine EC 500: Effects on reproduction of the Collembola Folsomia candida in artificial soil
Report No:	156131016
Document No:	M-761545-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 (2009) OECD-Guideline for testing chemicals No. 232 "Collembolan Reproduction Test in Soil" (adopted July 29, 2016) ISO 11267 Soil Quality – Inhibition of reproduction of Collembola (Folsomia candida) by soil contaminants, 2014
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

Collembola (*Folsomia candida*) aged 10 to 12 days were exposed to Spiroxamine EC 500 incorporated into artificial soil in a 4 week study in order to assess the effects on mortality and reproduction.

Folsomia candida were exposed to test concentrations of 35, 55, 75, 95, 115, 135, 180 and 250 mg test item/kg dry weight soil.

There were no statistically significant effects observed on the mortality of *Folsomia candida* up to and including the test concentration of 55 mg test item/kg dry weight soil and no statistically significant effects observed on reproduction at the test concentration of 35 mg test item/kg dry weight soil.

The NOEC and LOEC values for mortality were determined to be 55 and 35 mg test item/kg dry weight soil, respectively. The NOEC and LOEC values for reproduction were determined to be 35 and 55 mg test item/kg dry weight soil, respectively. The EC_{10}, EC_{20} and EC_{50} were determined to be 40.8, 54.6 and 95.3 mg test item/kg dry weight, respectively.

I. Materials and Methods

Materials

Test Material Spiroxamine EC 500

Lot/Batch #: EM4L027093

Purity: Spiroxamine EC 500 49.2% w/w, corresponding to 494.1 g/L

Description: Yellow liquid

Reanalysis/Expiry date: 22 January 2024

Density: 1.004 g/mL

Treatments

Test rates: 35, 55, 75, 95, 115, 135, 180 and 250 mg test item/kg dry weight soil

Test organisms	
Species:	*Folsomia candida,* Collembola, Isotomidae, age 10 – 12 days
Source:	Ibacon GmbH, 64380 Rossdorf, Germany
Feeding:	2 mg of granulated dry yeast at test initiation and after 14 days
Test design	
Test vessel:	Glass containers (volume: 100 mL; diameter: 5 cm) sealed with lids
Test medium:	Artificial soil according to OECD 232 (2016). 5% peat content
Replication:	8 replicates for the control, 4 replicates per test concentration and 1 additional container per treatment to test the pH and water content of the soil at test termination.
No. animals/vessel:	10 per test vessel
Duration of test:	4 weeks
Environmental test conditions	
Temperature:	18 - 22°C
pH:	5.6 - 5.9
Photoperiod:	16 hours light: 8 hours dark (400 – 800 lux)

Study Design

This study was conducted in order to assess the effects of Spiroxamine EC 500 on the mortality and reproduction of Collembola (*Folsomia candida)* over 4 weeks.

The Collembola were 10 to 12 days old at test initiation. Ten juvenile Collembola were introduced to the test vessels and placed onto the surface of the artificial soil. The test soil was composed of 74.8% fine quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.2% calcium carbonate.

The artificial soil was kept at 18 to 22°C and the test vessels were exposed to 400 - 800 lux in the controlled environment chamber.

Test concentrations of 35, 55, 75, 95, 115, 135, 180 and 250 mg test item/kg dry weight soil (equivalent to 17.2, 27.1, 36.9, 46.7, 56.6, 66.4, 88.6 and 123 mg a.s./kg soil) were applied to the artificial soil. Eight replicates were exposed to the control treatment and four replicates were exposed to the 35, 55, 75, 95, 115, 135, 180 and 250 mg test item/kg dry weight soil treatments.

At test initiation and after 14 days, the Collembola were fed with approximately 2 mg of granulated dry yeast.

A reference test with the toxic standard, boric acid, was performed at least once a year to ensure that the laboratory test conditions are adequate and to verify that the response of the test organisms does not change significantly over time.

Water content was checked 14 days after application by reweighing the additional test vessels. If the water loss exceeded 2% of the initial water content, the missing amount of water was added to all vessels of the treatment group.

At the end of the test the content of the test containers was suspended in water, the suspension was tinted with dark ink and stirred with a fine brush. The Collembola drifted to the surface. Adult animals were counted once visually, juvenile animals were counted using FolsomiaCounter, a photo based evaluation software, which automatically determines the number of juvenile animals from a digital photograph.

Statistical analysis was performed on the reproduction data using Shapiro-Wilk's test and Levene's test ($\alpha = 0.01$) to test for normal distribution and homogeneity. Since the reproduction data were normally distributed and homogeneous but did not follow a monotonicity trend (contrast trend) the William's t-test was used to compare treatment and control values (multiple comparison, $\alpha = 0.05$, one-sided smaller).

Mortality data were observed at test termination. Missing adult Collembola were assumed dead and degraded. Statistical analysis was performed on the mortality data using Step-down Cochran-Armitage Test ($\alpha = 0.05$, one-sided greater). An LC_{50} value was calculated by applying Weibull Analysis, values were compensated for control mortality using Abbot's formula. The ECx values for reproduction were calculated by Probit Analysis.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

Behavioural abnormalities were also recorded at test termination.

II. Results and Discussion

Validity criteria according to the OECD 232 guideline (2016) were met.

- Mean adult mortality should not exceed 20% at the end of the test (actual: 4%)
- The mean number of juveniles per vessel should be at least 100 at the end of the test (actual: 1150 to 1484)
- The coefficient of variation calculated for the number of juveniles should be less than 30% at the end of the definitive test (actual: 8.3%)

There was significant mortality observed at test concentrations of 75 mg test item/kg dry weight soil and above (Step-down Cochran-Armitage Test ($\alpha = 0.05$, one-sided greater). The NOEC and LOEC values for mortality were determined to be 55 and 75 mg test item/kg dry weight soil, respectively. The LC_{50} of Spiroxamine EC 500 for *Folsomia candida* in artificial soil was determined to be 153.9 mg test item/kg soil (95% confidence limits of 130.3 to 182.9 mg test item/kg soil). No abnormal behaviour was observed with the surviving Collembola.

Table CP 10.4.2.1/02-1 Mortality data observed after 28 days exposure

Treatment group (mg test item/kg artificial soil dry weight)	Mean mortality (%)	Standard deviation	Significance[1]
Control	4	± 7%	-
35	5	± 15%	n.s.
55	3	± 5%	n.s.
75	23	± 10%	*
95	33	± 15%	*
115	38	± 17%	*
135	40	± 14%	*
180	50	± 12%	*
250	93	± 15%	*

[1] Step-down Cochran-Armitage Test, one-sided greater, $\alpha = 0.05$- not applicable
n.s. Not significantly different compared to the control
* Significantly different compared to the control

There were no statistically significant effects on reproduction of *Folsomia candida* at the test concentration of 35 mg test item/kg soil (Williams t-test, α = 0.05, one-sided smaller). At the concentration of 55 mg test item/kg soil and above reproduction was statistically significantly reduced compared to the control.

Therefore, the NOEC for reproduction was determined to be 35 mg test item/kg soil and the LOEC for reproduction was determined to be 55 mg test item/kg soil. The EC_{10} for *Folsomia candida* in artificial soil was determined to be 40.8 mg test item/kg soil (95% confidence limits of 26.3 to 51.8 mg test item/kg soil). The EC_{20} was determined to be 54.6 mg test item/kg soil (95% confidence limits of 39.8 to 65.4 mg test item/kg soil). The EC_{50} was determined to be 95.3 mg test item/kg soil (95% confidence limits of 83.3 to 107.9 mg test item/kg soil, Probit Analysis).

Table CP 10.4.2.1/02-2 Reproduction data observed after 28 days exposure

Treatment group (mg test item/kg artificial soil dry weight)	Mean number of juveniles	Standard deviation	% of control	Significance[1]
Control	1349	± 412	-	-
35	1242	± 86	92	n.s.
55	1153	± 84	85	*
75	831	± 176	62	*
95	701	± 169	52	*
115	412	± 169	31	*
135	397	± 126	29	*
180	374	± 126	28	*
250	40	± 71	3	*

[1] Williams t-test, α = 0.05, one-sided smaller
n.s. Not significantly different compared to the control
* Significantly different compared to the control

To verify the sensitivity of the test system, the reference item (boric acid) was tested at concentrations of 30.5, 48.8, 78.1, 125 and 200 mg/kg soil dry weight in a separate study. In the most recent GLP study, an EC_{50} of 104.6 mg/kg soil dry weight was determined. The effects on the reduction of reproduction showed that the test system was sensitive and reflected the expected toxicity as given in the OECD 232 test guideline (50% reduction in reproduction at about 100 mg/kg soil dry weight).

III. Conclusion

Spiroxamine EC 500 caused no statistically significant effects on mortality and reproduction of *Folsomia candida* up to and including the concentration of 35 mg test item/kg dry weight soil.

The NOEC and LOEC values for mortality were determined to be 55 and 75 mg test item/kg dry weight soil, respectively. The LC_{50} was estimated to be 153.9 mg test item/kg dry weight soil.

The NOEC and LOEC values for reproduction were determined to be 35 and 55 mg test item/kg dry weight soil, respectively. The EC_{10}, EC_{20} and EC_{50} were determined to be 40.8, 54.6 and 95.3 mg test item/kg dry weight, respectively.

Assessment and conclusion by applicant:

This is a new study that has not been previously submitted or evaluated.

Validity criteria according to the OECD 232 guideline (2016) were met.

- Mean adult mortality should not exceed 20% at the end of the test (actual: 4%)
- The mean number of juveniles per vessel should be at least 100 at the end of the test (actual: 1150 to 1484)
- The coefficient of variation calculated for the number of juveniles should be less than 30% at the end of the definitive test (actual: 8.3%)

The reference item also demonstrated sufficient sensitivity of the test organisms. The study is therefore considered acceptable.

The NOEC value for reproduction was determined to be 35 mg test item/kg dry weight soil (equivalent to 17.2 mg a.s./kg soil dry weight).

Data Point:	KCP 10.4.2.1/03
Report Author:	[redacted]
Report Year:	2020
Report Title:	1st final report amendment - Spiroxamine EC 500: Effects on reproduction of the predatory mite Hypoaspis aculeifer in artificial soil
Report No:	143091089
Document No:	M-688129-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 (2009) OECD 226: Guidelines for the testing of chemicals – Predatory Mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil, adopted July 29, 2016
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

Adult *Hypoaspis aculeifer* were exposed to Spiroxamine EC 500 in a 14-day study to assess the effect on mortality and reproduction.

Hypoaspis aculeifer were exposed in artificial soil to a control and to test concentrations of 25, 50, 100, 200 and 400 mg test item/kg dry weight soil, according to guidelines set out in OECD 226 (2016). Dimethoate was used as a toxic standard.

The NOEC and LOEC values for mortality were determined to be ≥400 and >400 mg test item/kg dry weight soil, respectively.

The NOEC and LOEC values for reproduction were determined to be 200 and 400 mg test item/kg dry weight soil, respectively.

The EC$_{50}$ value was estimated to be >400 mg test item/kg dry weight soil.

I. Materials and Methods

Materials

Test Material Spiroxamine EC 500

Lot/Batch #: EM4L018425

Purity:	Spiroxamine EC 500: 50.0% w/w, corresponding to 501.6 g/L
Description:	Yellow liquid
Reanalysis/Expiry date:	09 May 2020
Density:	1.004 g/mL
Treatments	
Test rates:	25, 50, 100, 200 and 400 mg test item/kg dry weight soil
Test organisms	
Species:	*Hypoaspis aculeifer*, predatory mite, Laelapidae
Source:	Ibacon GmbH, 64380 Rossdorf, Germany
Feeding:	One spatula of cheese mites (*Tyrophagus putrescentiae*) at test initiation and on test days 2, 5, 7, 9 and 12
Test design	
Test vessel:	Glass containers (volume: 100 mL, diameter: 5 cm) with tight screw top lids
Test medium:	Artificial soil, 5% peat content
Replication:	8 replicates for the control, 4 replicates per treatment group and 1 additional container per treatment to test the pH and water content of the test substrate at test termination
No. animals/vessel:	10 per test vessel
Duration of test:	14 days
Environmental test conditions	
Temperature:	18 - 22°C
pH:	6.0 - 6.2
Photoperiod:	16 hours light: 8 hours dark (at 400 – 800 lux)

Study Design

This study was conducted in order to assess the effects on reproduction of Spiroxamine EC 500 on *Hypoaspis aculeifer* over 14 days.

Ten adult female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatments. Concentrations of 25, 50, 100, 200 and 400 mg test item/kg dry weight soil were mixed into the artificial soil. The test soil was composed of 74.8% fine quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.2% calcium carbonate. The soil was prepared according to the guideline OECD 226 (2016).

During the test, *Hypoaspis aculeifer* were fed with cheese mites (*Tyrophagus putrescentiae*) and kept in ventilated glass vessels. Temperatures of 18 - 22°C and a light regime of 400 – 800 Lux, 16 hour light: 8 hour dark were maintained throughout the test in a controlled environment chamber.

A reference test with the toxic standard, BAS 152 11 I (a.s. dimethoate), was performed at least once a year to ensure that the laboratory test conditions are adequate and to verify that the response of the test organisms does not change significantly over time.

Water content was checked 7 days after application by reweighing the additional test vessels. If the water loss exceeded 2% of the initial water content, the missing amount of water was added to all vessels of the treatment group.

Reproduction data were observed at test termination. Juveniles were counted twice under binocular microscopes. Statistical analysis was performed on the reproduction data using Shapiro-Wilk's test and Levene's test (α = 0.01) to test for normal distribution and homogeneity. Since the reproduction data were normally distributed and homogeneous, further statistical analysis was performed using Williams t-test (multiple comparison, α = 0.05, one-sided smaller).

Mortality data were observed at test termination. Missing adult mites were assumed dead and degraded. Statistical analysis was performed on the mortality data using Fisher's Exact Binomial Test (multiple comparison, with Bonferroni Correction, α = 0.05, one-sided greater).

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

II. Results and Discussion

Validity criteria according to the OECD 226 guideline (2016), to which the study was conducted, were met.

- Mean adult female mortality should not exceed 20% at the end of the test (actual: 0%)
- The mean number of juveniles per replicate (with 10 adult females introduced) should be at least 50 at the end of the test (actual: 196 to 237)
- The coefficient of variation calculated for the number of juvenile mites per replicate should not be higher than 30% at the end of the definitive test (actual: 5.9%)

Mortality of *Hypoaspis aculeifer* in the test item treated groups ranged from 0% to 3%. The values were not statistically significantly different compared to the control (Fisher's Exact Test, α = 0.05, one-sided greater). The NOEC and LOEC values were determined to be ≥400 and >400 mg test item/kg dry weight soil, respectively.

Table CP 10.4.2.1/03-1 Mortality data observed after 14 days exposure

Treatment group (mg test item/kg artificial soil dry weight soil)	Mean mortality (%)	Standard deviation	Significance[1]
Control	0	± 0	-
25	3	± 5	n.s.
50	3	± 5	n.s.
100	3	± 5	n.s.
200	3	± 5	n.s.
400	0	± 0	n.s.

[1]Fisher's Exact Test, one-sided greater, α = 0.05
- not applicable
n.s. not significantly different compared to the control

There were no statistically significant effects on reproduction of *Hypoaspis aculeifer* up to and including the test concentration of 200 mg test item/kg dry weight soil (Williams t-test, α = 0.05, one-sided smaller). At the concentration of 400 mg test item/kg dry weight soil a statistically significant decrease of reproduction was observed. The NOEC and LOEC values were determined to be 200 and 400 mg test item/kg dry weight soil, respectively.

Table CP 10.4.2.1/03-2 Reproduction data observed after 14 days exposure

Treatment group (mg test item/kg artificial soil dry weight soil)	Mean	Standard deviation	% of control	Significance[1]
Control	220	± 13	-	-
25	210	± 12	95	n.s.
50	187	± 7	85	n.s.
100	216	± 12	99	n.s.
200	213	± 19	97	n.s.
400	200	± 10	91	*

[1]Williams t-test, α=0.05, one-sided smaller
- not applicable
n.s. not significantly different compared to the control
* significantly different compared to the control

The reference item dimethoate showed statistically significant treatment related effects on reproduction at a concentration of 2.23 mg dimethoate/kg soil and above. The EC_{50} for reproduction was 2.47 mg dimethoate/kg soil. The EC_{50} determined in the reference test is slightly below the recommended range given in the test guideline (3.0 - 7.0 mg a.s./kg soil), however, the results are considered to confirm that the test organisms at this test facility are sensitive to the effects of the reference substance and therefore the results achieved in this study are considered to be valid. The range of the past seven reference tests was between 2.47 to 4.12 mg a.s./kg soil.

III. Conclusion

Spiroxamine EC 500 caused no statistically significant effects on mortality of *Hypoaspis aculeifer* up to and including the test concentration of 400 mg test item/kg dry weight soil. Therefore, the NOEC and LOEC values for mortality were determined to be ≥400 and >400 mg test item/kg dry weight soil, respectively.

The NOEC and LOEC values for reproduction were determined to be 200 and 400 mg test item/kg dry weight soil, respectively (equivalent to 100 and 200 mg a.s./kg dry weight soil, respectively).

EC_x values could not be determined by statistical analysis since there was no adequate concentration response, therefore no EC_{10}/EC_{20} value can be reported. However, the EC_{50} was estimated to be >400 mg test item/kg dry weight soil.

Assessment and conclusion by applicant:

Validity criteria according to the most recent OECD 226 guideline (2016), to which the study was conducted, were met.

- Mean adult female mortality should not exceed 20% at the end of the test (actual: 0%)
- The mean number of juveniles per replicate (with 10 adult females introduced) should be at least 50 at the end of the test (actual: 196 to 237)
- The coefficient of variation calculated for the number of juvenile mites per replicate should not be higher than 30% at the end of the definitive test (actual: 5.9%)

The reference substance was also considered to demonstrate sufficient sensitivity of the test organisms.

The study is therefore considered acceptable.

The NOEC value for reproduction was determined to be 200 mg test item/kg dry weight soil (equivalent to 100 mg a.s./kg dry weight soil).

Data Point:	KCP 10.4.2.1/04
Report Author:	[redacted]
Report Year:	2012
Report Title:	Spiroxamine EC 500E G: Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	KRA-HR-69/12
Document No:	M-443019-01-1
Guideline(s) followed in study:	OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil US EPA OCSPP: None
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 purpose of the study was to assess the effects of Spiroxamine EC 500E G on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 control replicates and 4 replicates for each test item concentration) were exposed to control and treatments. Concentrations of 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil were tested.

The No Observed Effect Concentration (NOEC) was calculated to be ≥ 1000 mg test item/kg dry weight artificial soil. The Lowest Observed Effect Concentration (LOEC) >1000 mg test item/kg dry weight artificial soil.

I. Materials and Methods

Materials

Test Material Spiroxamine EC 500E G

Lot/Batch #: EDFL013642

Purity: Analysed content(s) of a.s.: 508.1 g/L corresponding to 50.5 % w/w

Description: Liquid, yellow-brown

Stability of test compound: Sufficient based on expiration date

Reanalysis/Expiry date: 08 August 2014

Density: 1.006 g/mL

Treatments

Test rates:	100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil
Solvent/vehicle:	Deionised water
Analysis of test concentrations:	No

Test design

Test species:	*Hypoaspis aculeifer*
Test vessel:	Reusable glass vessels (Weck Mini-Sturzglas, volume 140 mL, diameter 5 cm at the bottom, height 7 cm)
Test substrate:	5% Sphagnum-peat, 20% Kaolin clay, 74.7% fine quartz-sand, 0.3% Calcium carbonate
Replication:	Eight control replicates and four replicates for each test item concentration
No. of animals/vessel:	Ten
Duration of test:	14 days (plus two days for extraction)

Environmental test conditions

Temperature:	20 ± 2°C
pH:	Test start: 6.19 to 6.38 Test end: 6.19 to 6.88
Photoperiod:	16 h light : 8 h dark (400 – 800 lux)
Water content:	47.43% to 52.7% of WHC_{max}

Study Design

The purpose of the study was to assess the effects of Spiroxamine EC 500E G on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.

Nominal test concentrations were 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil.

Ten adult female mites were added to each of the four replicate test vessels (eight for the control). Test vessels were reusable glass vessels (Weck Mini-Sturzglas, volume 140 mL, diameter 5 cm at the bottom, height 7 cm), filled with approximately 20 g dry weight artificial soil dry weight.

Directly after the addition of the *Hypoaspis aculeifer*, they were fed with cheese mites (*Tyrophagus putrescentiae*). Cheese mites were bred on brewers yeast in the laboratory. During the continuation of the test the soil mites were fed 3, 7 and 10 days after test start with the cheese mites.

The vessels were kept in a temperature-controlled room at 20 ± 2 °C under a 16-hour light to 8-hour darkness photo period. The light intensity at light period was between 400 - 800 Lux.

The surviving adults and living juveniles were counted as described under bioassay procedure. Missing adults (compared to the number of initially placed test organisms) were considered to be dead, since dead mites cannot be extracted.

The transfer of the test animals was finished within two hours after the application of the test item. After a period of 14 days, the surviving adults and the living juveniles per test vessel were extracted, applying a temperature gradient. The content of each test vessel was carefully transferred to sieve vessels (mesh

size approximately 0.8 mm). Each sieve vessel was put onto another vessel containing a fixing liquid. The vessels were positioned in MCFADYEN-Extractor. The temperature was increased from approximately 25 to 40 °C within two days. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). The extracted mites in the fixing solution were stored in a refrigerator until the start of the counting of surviving adults and juveniles. All *Hypoaspis aculeifer* (adult females and juveniles) were counted under a binocular.

The surviving adults and living juveniles were counted as described under bioassay procedure. Missing adults (compared to the number of initially placed test organisms) were considered to be dead, since dead mites cannot be extracted.

Endpoints of the test were mortality of the adult, female *Hypoaspis aculeifer* in comparison to the initially placed test organisms expressed in % and the number of offspring hatched from the eggs and surviving until the end of the test period per test vessel (reproduction).

For the determination of normal distribution and homogeneity of variance Kolmogorroff-Smirnov Test and Cochran-Test ($\alpha = 0.05$), respectively were used. Data of reproduction were normally distributed and homogeneity of variances was given. Therefore William´s t-test for homogeneous variances (one-sided smaller, $\alpha = 0.05$) was used to determine NOEC and LOEC values. The software used to perform the statistical analysis was ToxRat Pro 2.10.

II. Results and Discussion

Validity criteria according to the guideline to which the study was conducted were met:

- Mean adult female mortality in the controls must not exceed 20% (actual: 5.0%)
- The mean number of juvenile mites per replicate to be at least 50 (actual: 226.8)
- The coefficient of variation for reproduction to be ≤30% (actual: 15.1%)

In the control group 5.0 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤20 % mortality. The LC_{50} could not be calculated and is considered to be >1000 mg test item/kg dry weight artificial soil.

Table CP 10.4.2.1/04-1 Survival of adult, female *Hypoaspis aculeifer* after 14 days

Adults/vessel	Control	Treatment (mg test item/kg dry weight artificial soil)				
		100	178	316	562	1000
Replicate 1	6	9	10	8	10	10
Replicate 2	10	10	10	8	10	10
Replicate 3	10	10	10	9	9	9
Replicate 4	10	10	10	10	10	9
Replicate 5	10	-	-	-	-	-
Replicate 6	10	-	-	-	-	-
Replicate 7	10	-	-	-	-	-
Replicate 8	10	-	-	-	-	-
Mean	**9.5**	**9.8**	**10.0**	**8.8**	**9.8**	**9.5**
Standard deviation	**1.4**	**0.5**	**0.0**	**1.0**	**0.5**	**0.6**
Coefficient of variation	**14.9**	**5.1**	**0.0**	**10.9**	**5.1**	**6.1**
% Mortality	**5.0**	**2.5**	**0.0**	**12.5**	**2.5**	**5.0**

Adults/vessel	Control	Treatment (mg test item/kg dry weight artificial soil)				
		100	178	316	562	1000

Concerning the number of juveniles statistical analysis (William´s t-test, one-sided smaller, α = 0.05) revealed no significant differences between control and any concentration tested.

Therefore, the No-Observed-Effect-Concentration (NOEC) for reproduction was ≥1000 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction was >1000 mg test item/kg dry weight artificial soil. An EC_{50} for reproduction could not be calculated and is considered to be >1000 mg test item/kg dry weight artificial soil.

Table CP 10.4.2.1/04-2 Reproduction of *Hypoaspis aculeifer* after 14 days after test start (Juveniles/replicate)

Adults/vessel	Control	Treatment (mg test item/kg dry weight artificial soil)				
		100	178	316	562	1000
Replicate 1	218	299	370	256	311	363
Replicate 2	309	378	372	308	325	386
Replicate 3	316	359	310	331	346	357
Replicate 4	350	320	330	363	376	340
Replicate 5	374	-	-	-	-	-
Replicate 6	349	-	-	-	-	-
Replicate 7	332	-	-	-	-	-
Replicate 8	366	-	-	-	-	-
Mean	**326.8**	**339.0**	**345.5**	**314.5**	**339.5**	**361.5**
Standard deviation	**49.4**	**36.0**	**30.6**	**45.1**	**28.3**	**19.0**
Coefficient of variation	**15.1**	**10.6**	**8.8**	**14.3**	**8.3**	**5.3**
% Mortality	**100**	**103.7**	**105.7**	**96.3**	**103.9**	**110.6**

The reference item dimethoate produced an LC_{50} of 3.894 mg a.s./kg for mortality and an EC_{50} of 6.62 mg a.s./kg for reproduction. The EC_{50} determined in the reference test is within the recommended range given in the test guideline (3.0 - 7.0 mg a.s./kg soil) therefore the results are considered to demonstrate sufficient sensitivity of the test organism.

III. Conclusion

The No Observed Effect Concentration (NOEC) was calculated to be ≥ 1000 mg test item/kg dry weight artificial soil (equivalent to 505 mg a.s./kg soil). The Lowest Observed Effect Concentration (LOEC) >1000 mg test item/kg dry weight artificial soil.

Assessment and conclusion by applicant:

Validity criteria according to the most recent version of the OECD 226 guideline (2016) were met.

- Mean adult female mortality in the controls must not exceed 20% (actual: 5.0%)
- The mean number of juvenile mites per replicate to be at least 50 (actual: 326.8)

- The coefficient of variation for reproduction to be ≤30% (actual: 15.1%)

The reference substance was also considered to demonstrate sufficient sensitivity of the test organisms.

The study is therefore, considered acceptable.

The NOEC was determined to be 1000 mg test item/kg dry weight artificial soil (equivalent to 505 mg a.s./kg soil).

EC_{10} and EC_{20} values have not been determined as part of this study. However, it is very clear from the results that there was no treatment-related effect whatsoever. In fact, the number of juveniles produced was slightly greater in the majority of treatment groups when compared to the control. For this reason it is considered that no EC_{10} or EC_{20} value would be determinable and the data have not been subject to statistical re-evaluation.

CP 10.4.2.2 Higher tier testing

No data are available. Field data with Spiroxamine EC 500 are not considered necessary as an acceptable risk following the proposed uses has been demonstrated using the available laboratory data.

CP 10.5 Effects on soil nitrogen transformation

The available soil nitrogen transformation data for Spiroxamine EC 500 and the metabolites of spiroxamine are summarized in the table below.

Table CP 10.5-1 Summary of nitrogen transformation studies with spiroxamine and metabolites

Test item	Test type	Endpoints		Reference
Spiroxamine EC 500	Nitrogen transformation	**<25% effect after 42 days at 10.0 mg/kg soil (5.0 mg a.s./kg soil)**	NEW	M-680763-01-1
KWG 4168-desethyl (M01)	Nitrogen transformation	**<25% effect after 28 days at 4.53 mg/kg soil**	EU	M-282056-01-1
KWG 4168-despropyl (M02)	Nitrogen transformation	**<25% effect after 70 days at 5.0 mg/kg soil**	NEW	M-680757-01-1
KWG 4168-N-oxide (M03)	Nitrogen transformation	**<25% effect after 56 days at 6.9 mg/kg soil**	NEW	M-680759-01-1
KWG 4168-acid (M06)	Nitrogen transformation	**<25% effect after 28 days at 5.0 mg/kg soil**	NEW	M-688317-01-1

EU: previously evaluated as part of the original EU review and listed in EFSA conclusion and DAR
NEW: new study or data generated since the previous EU review or previously not submitted
Values in **bold** have been used in the risk assessment

Toxicity endpoints

Nitrogen transformation data for spiroxamine technical are not available. However, the available formulation data are considered to be more relevant for the risk assessment as this has been generated using the representative formulation itself and also represents the toxicity to spiroxamine.

Exposure

Full details of the PEC_{soil} calculations have been provided in Document M-CP Section 9 Environmental Fate. The maximum initial and accumulation PEC_{soil} values for spiroxamine and its metabolites, as calculated using FOCUS equations, are given in the table below for the highest application rate of 300 g a.s./ha.

Table CP 10.5-2 PEC_{soil} for spiroxamine and its metabolites

Substance	1 x 300 g a.s./ha		2 x 300 g a.s./ha	
	Max PEC_{soil} (mg/kg)	$PEC_{soil\ accumulation}$ (mg/kg)	Max PEC_{soil} (mg/kg)	$PEC_{soil\ accumulation}$ (mg/kg)
Vines				
Spiroxamine	0.200	0.277	0.372	**0.555**
M01	0.022	0.052	0.044	**0.064**
M02	0.016	0.020	0.032	**0.040**
M03	0.017	0.018	0.033	**0.037**
M06	0.012	0.052	0.023	**0.104**

PEC_{soil} values used in the risk assessment are highlighted in **bold**

For the risk assessment below the risk envelope approach has been applied in which the PEC_{soil} values for the proposed use with the highest application rate has been used. Thus, the risk assessment has been conducted for the use on grapes at 2 x 300 g a.s./ha. Furthermore, the $PEC_{soil\ accumulation}$ values were greater than the maximum initial PEC_{soil} values therefore the risk assessment has been conducted using the worst case $PEC_{soil\ accumulation}$ values.

For Spiroxamine EC 500 the formulation PEC_{soil} was determined to be 0.401 mg/kg soil for the maximum application rate of 300 g a.s./ha. Please refer to Document M-CP Section 9 Environmental Fate for further details.

Isomers

For parent spiroxamine the environmental fate soil degradation data currently suggest that there is no significant selective degradation of isomers over time. As a result, the toxicity data generated using the mixture of the isomers (*i.e.* Spiroxamine EC 500) are considered to represent the toxicity of the isomers in the ratios that would occur in the soil following application. In accordance with the isomer Guidance Document[22] it is therefore not necessary to apply any additional Uncertainty Factor (UF) to the risk assessment (*i.e.* a UF of 1.0 is used).

For the metabolites of spiroxamine there are no chiral data available to be able to make this assessment therefore there is a possibility that selective degradation of isomers could occur in the soil over time. In order to account for any possible increased toxicity to soil organisms as a result of an increase in the ratio of a single isomer, an UF has been applied to the risk assessment of M01, M02, M03 and M06. The UF have been calculated following the recommendations of the isomer Guidance Document and have been presented in the table below.

[22] 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

Table CP 10.5-3 Uncertainty Factors determined for the nitrogen transformation data with the metabolites of spiroxamine

Test item	Study reference	Test material batch number	Isomer ratio	UF [1]
Spiroxamine	-	-	-	1.0 [2]
M01	M-282056-01-1	921103ELB02	A:B 56:42	4.76
M02	M-680757-01-1	AE 1344303-PU-01	A:B 83.1:16.0	12.5
M03	M-680759-01-1	M26999	D1:D2:D3:D4 22:21:26:31	9.52
M06	M-688317-01-1	AE 1344313-01-03	A:B 47:53	4.26

[1] Changes in stereoisomeric excess are unknown therefore Uncertainty Factor = 100/content of lowest stereoisomer (%) used for ecotox endpoint [as indicted in Table B.1, p 30 of isomer GD] and assumes that the toxicological effects of the mixture can be attributed to a single isomer. This assumes that all enantiomer ratios can be safely assumed to be 50:50. For example A:B ratio of 83.1:16 would be 100/(16/2) = UF of 12.5

[2] No additional UF required for parent as no significant change in isomeric ratios has been demonstrated

Risk assessment

The effect concentrations for Spiroxamine EC 500 and for the metabolites are compared to the PEC_{soil} values in the following table.

Table CP 10.5-4 Soil micro-organism risk assessment for spiroxamine and relevant metabolites following application of Spiroxamine EC 500 to vines

Intended use	Vines 2 x 300 g a.s./ha			
Test item	**Endpoint**	**PEC_{soil}**	**UF [1]**	**Risk acceptable [2]**
Spiroxamine EC 500	<25% effect after 42 days at 10.0 mg/kg soil	0.401 mg product/kg soil	1.0	Yes
	(5.0 mg a.s./kg soil)	0.555 mg a.s./kg soil		
M01	<25% effect after 28 days at 4.53 mg/kg soil	0.064 mg/kg soil	4.76	Yes
M02	<25% effect after 70 days at 5.0 mg/kg soil	0.040 mg/kg soil	12.5	Yes
M03	<25% effect after 56 days at 6.9 mg/kg soil	0.037 mg/kg soil	9.52	Yes
M06	<25% effect after 28 days at 5.0 mg/kg soil	0.104 mg/kg soil	4.26	Yes

[1] Uncertainty Factor applied to account for the unknown effect of a possible change in isomer ratios over time

[2] Risk assessment has compared the NOEC against the PEC_{soil} × UF

Formulated spiroxamine had no significant effect on soil micro-organisms at concentrations up to 5.0 mg a.s./kg soil. This is higher than the maximum PEC_{soil} of 0.555 mg a.s./kg following the worst-case application to grapes. Thus, the margins of safety in the risk assessments are a factor of 9.0 for spiroxamine. This supports the conclusion that under field conditions, the proposed uses of Spiroxamine EC 500 pose no unacceptable risk to non-target soil micro-organisms.

In addition, no significant effects (>25%) were shown in the studies with M01, M02, M03 and M06 at concentrations greatly exceeding the predicted soil concentrations. Acceptable risks to non-target soil micro-organisms from exposure to the metabolites, following application of Spiroxamine EC 500, have therefore also been demonstrated.

Biodiversity

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on soil micro-organisms. Therefore, it is considered that the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects *via* alteration of the food web, are covered by the risk assessment for soil micro-organisms in this section.

With respect to the risk assessments for soil micro-organisms, which demonstrated an acceptable outcome with large margins of safety and without the need for risk mitigation, the applicant concludes that the use of the representative lead formulation (Spiroxamine EC 500) has a low potential to cause unacceptable effects on biodiversity and the ecosystem *via* trophic interactions. To the best of our knowledge and with the presented safety profile of the spiroxamine a.s. metabolites and the representative lead formulation, the applicant does not foresee any unacceptable effects on biodiversity and the ecosystem with spiroxamine.

Summaries of the available soil micro-organism studies have been presented below.

Data Point:	KCP 10.5.04
Report Author:	[redacted]
Report Year:	2020
Report Title:	Spiroxamine EC 500: Effects on the activity of the soil microflora in the laboratory (nitrogen transformation)
Report No:	143091080
Document No:	M-680763-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Storage temperature of soil extracts (no effect)
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 purpose of this study was to assess the effects of the test item on the activity (nitrogen transformation) of soil microflora in the laboratory.

Spiroxamine EC 500 was tested at concentrations of 2.0 and 10 mg test item/kg soil dry weight.

The test item Spiroxamine EC 500 had no impact on nitrogen transformation (nitrate content, mineral nitrogen content and nitrate formation rates) of soil microorganisms when applied at 2.0 mg and 10 mg test item/kg soil dry weight treatment.

I. Materials and Methods

Materials

Test Material Spiroxamine EC 500

Lot/Batch #: EM4L018425

Active ingredients: Spiroxamine (KWG 4168): 50.0% w/w, corresponding to 501.6 g/L

Description: Yellow liquid

Stability of test compound:	Not reported
Reanalysis/Expiry date:	09 May 2020
Density:	1.004 g/mL
Treatments	
Test rates:	1 and 5 mg a.s./kg (corresponding to 2.0 and 10 mg SPX EC 500/kg)
Solvent/vehicle:	Ultrapure water
Analysis of test concentrations:	None
Test design	
Test vessel:	500 mL plastic boxes containing 300 g dw soil
Test soil:	A loamy sand
Source:	In der Speyerer Hohl, No. 977
Replication:	Three per control and test group
Duration of test:	42 days
Environmental test conditions	
Temperature:	20 ± 2 °C
pH:	7.2 to 7.5
Moisture:	48 to 50% of maximum water holding capacity
Photoperiod:	Constant darkness

Study Design

The purpose of this study was to assess the effects of the test item on the activity (nitrogen transformation) of soil microflora in the laboratory.

Triplicate samples of each soil (containing 300 g dry weight (dw)) were tested.

The soil batch used in this study was according to the guideline and was taken from fallow grassland, where no pesticides or organic or mineral fertiliser had been used on the soil for at least four years prior to test initiation. The soil was collected from Rhineland Palatinate district authority, Mechtersheim Germany municipality and the location was “In der Speyerer Hohl “, No. 977”. The soil was a loamy sand.

The water content of one replicate of each treatment group was determined at each sampling date. Water losses were compensated by adding ultrapure water. Throughout the study, the water content ranged from 48% to 50% WHC. The pH was determined at test start and on day 28 in one replicate of each treatment group. Over the course of the study, the pH value was between 7.2 and 7.5.

All solvents or chemicals used were of analytical grade or higher purity. The lucerne meal used was fine powdered lucerne green grass meal; the analysed carbon and nitrogen content was 40.9% and 2.7%, respectively. The ratio of carbon to nitrogen was 15 / 1.

The test item was soluble in water; therefore a stock solution in ultrapure water was prepared by dissolving 44.9 mg Spiroxamine EC 500 in 50 mL ultrapure water and mixed into the soil by means of

a laboratory mixer. Throughout the application the soil was ventilated and the soil water content was adjusted to 48% of WHC.

To the control, acetone treated quartz sand (evaporated) and additionally 0.5% lucerne meal (based on soil dry weight) was mixed into the soil. The soil water content was adjusted to 49% of WHC. The soil water content was determined in one replicate of each treatment group at each sampling.

For the determination of nitrogen content, soil samples were taken within 6 hours after application and afterwards on each sampling date (7, 14, 28 and 42 days). The nitrogen content was determined in each sample of treated and control soils.

For extraction, 24 g to 25 g soil were suspended in 100 mL 0.1 M KCl-solution and agitated for one hour. The suspension was centrifuged (Multifuge 3S+, 4350 rpm) and the extracts were stored deep frozen.

Amounts of 70.8 mg, 74.0 mg, and 72.2 mg ammonium sulfate, sodium nitrite and potassium nitrate, respectively, were diluted in 1000 mL (ammonium sulfate, sodium nitrite) and 100 ml (potassium nitrate) 0.1 M KCl to prepare the standard stock solutions for ammonium-N, nitrite-N and nitrate-N determination. Appropriate aliquots of the stock solutions were automatically diluted by the dilution unity with 0.1 M KCl to prepare 6 standard solutions at a range of 0.5 mg/L to 3.0 mg/L for ammonium-N and nitrite-N and 7 standard solutions at a range of 1.0 mg/L to 12.0 mg/L for nitrate-N determination. Before photometric determination, frozen soil extracts were thawed. For nitrite-N, nitrate-N and ammonium-N determination undiluted extracts were used. For determination undiluted extracts (days 0 to 28) and 1:2 in 0.1 M KCl diluted extracts (day 42) were used.

II. Results and Discussion

Validity criteria according to the OECD 216 (2000) guideline, to which the study was conducted, were met as the control variation between control replicates was less than ± 15% (maximum variation: 3.05%).

No adverse effects of the test item on nitrate content in soil were observed at day 28. At day 28, differences to the control were -1.35% and -12.62% in the 2 mg and 10 mg test item/kg soil dry weight treatment, respectively.

No adverse effects of the test item on nitrate content in soil were observed at test end at day 42. At day 42, differences to the control were -1.88% and -10.14% in the 2 mg and 10 mg test item/kg soil dry weight treatment, respectively.

At day 28 and 42, the difference was statistically significant compared to the control for the high test rate (Student t-test, $\alpha = 0.05$).

Very low nitrite and ammonium contents below 0.8 mg/kg dry weight were measured at day 28 and 42 in control and the test item treatments.

The mineral nitrogen contents in soil were within the trigger range of ± 25% at day 28. At day 28, differences to the control were -2.21% and -12.75% in the 2 mg and 10 mg test item/kg soil dry weight treatment, respectively.

The mineral nitrogen contents in soil were within the trigger range of ± 25% at day 42. At day 42, differences to the control were -1.88% and -10.01% in the 2 mg and 10 mg test item/kg soil dry weight treatment, respectively.

At day 28 and 42, the difference was statistically significant compared to the control for the high test rate (Student t-test, $\alpha = 0.05$), but within the trigger range.

Table CP 10.5/04-1 Nitrogen transformation test, effects of the test item on ammonium (mean values)

Days after treatment	Control		2 mg/kg soil dw		10 mg/kg soil dw	
	Ammonium	CV	Ammonium	Dev. %	Ammonium	Dev. %
0	6.771	1.70	6.484	-4.24	6.488	-4.25
7	1.354	4.95	1.277	-5.69	1.186	-12.41*
14	0.805	4.10	0.710	-11.80	0.984	22.24
28	0.774	59.17	0.457	-40.96	0.594	-23.26
42	0.741	0.81	0.723	-2.43	0.704	-4.99

* Significantly different to the control (t-test at $p \leq 0.05$)

Table CP 10.5/04-2 Nitrogen transformation test, effects of the test item on nitrite (mean values)

Days after treatment	Control		2 mg/kg soil dw		10 mg/kg soil dw	
	Nitrite	CV	Nitrite	Dev. %	Nitrite	Dev. %
0	0.303	5.94	0.262	-13.53*	0.267	-11.88*
7	0.258	0.00	0.258	0.00	0.258	0.00
14	0.258	0.00	0.258	0.00	0.258	0.00
28	0.258	0.00	0.258	0.00	0.258	0.00
42	0.258	0.00	0.258	0.00	0.258	0.00

* Significantly different to the control (t-test at $p \leq 0.05$)

Table CP 10.5/04-3 Nitrogen transformation test, effects of the test item on nitrate (mean values)

Days after treatment	Control		2 mg/kg soil dw		10 mg/kg soil dw	
	Nitrate	CV	Nitrate	Dev. %	Nitrite	Dev. %
0	26.643	0.28	27.075	1.62*	27.017	1.40*
7	23.368	2.94	23.713	1.48	23.366	-0.01
14	26.363	3.05	26.293	-0.27	23.901	-9.34*
28	38.926	0.86	38.400	-1.35	34.013	-12.62*
42	49.628	0.98	48.694	-1.88	44.598	-10.14*

* Significantly different to the control (t-test at $p \leq 0.05$)

Table CP 10.5/04-4 Nitrogen transformation test, effects of the test item on N_{min} (mean values)

Days after treatment	Control		2 mg/kg soil dw		10 mg/kg soil dw	
	N_{min}	CV	N_{min}	Dev. %	N_{min}	Dev. %
0	33.717	0.58	33.821	0.31	33.767	0.15
7	24.980	2.50	25.248	1.07	24.810	-0.68
14	27.426	2.85	27.261	-0.60	25.143	-8.32*

Days after treatment	Control		2 mg/kg soil dw		10 mg/kg soil dw	
	N_{min}	CV	N_{min}	Dev. %	N_{min}	Dev. %
28	39.958	1.43	39.116	-2.11	34.865	-12.75*
42	50.627	0.96	49.674	-1.88	45.560	-10.01*

* Significantly different to the control (t-test at $p \leq 0.05$)

Table CP 10.5/04-5 Nitrogen Transformation Test: Effects of the test item on Nitrate Formation Rates (Mean Values)

Interval[1]	Control		2 mg/kg soil dw			10 mg/kg soil dw		
	Mean mg NO_3-N/kg soil dry weight per day[2]							
Sampling days	mg/day	CV %	mg/day	Dev. %[4]	sig.[5]	mg/day	Dev. %[4]	sig.[5]
0 - 7	-0.468	-21.37	-0.480	2.56	n.s.	-0.522	11.54	n.s.
0 - 14	-0.020	-300.00	-0.056	180.00	n.s.	-0.223	1015.00	*
0 - 28	0.439	3.19	0.405	-7.74	n.s.	0.250	-43.05	*
0 - 42	0.547	2.01	0.515	-5.85	n.s.	0.419	-23.40	*
Interval[1]	Mean mg NO_3-N/kg soil dry weight per day[3]							
Sampling days	mg/day	CV %	mg/day	Dev. %[4]	sig.[5]	mg/day	Dev. %[4]	sig.[5]
0 - 7	-0.468	-21.37	-0.480	2.56	n.s.	-0.522	11.54	n.s.
7 - 14	0.428	12.62	0.368	-14.02	n.s.	0.076	-82.24	*
14 - 28	0.898	4.01	0.865	-3.67	n.s.	0.722	-19.60	*
28 - 42	0.764	6.28	0.735	-3.80	n.s.	0.756	-1.05	n.s.

[1]: Time interval
[2]: Calculated from the mean values of NO_3-N content between the sampling date and day 0
[3]: Calculated from the mean values of NO_3-N content between each sampling date
[4]: Deviation from control
[5]: sig.: Significance according Student-t-test, two sided, $\alpha = 0.05$ (* = significant; n. s.: not significant)
CV: Coefficient of variation (calculated as SD / mean value *100)

The reference item sodium chloride was tested in a GLP study. Sodium chloride was tested at 16 g/kg soil dry weight. The variation of replicate control samples was less than 15%. The reference item had a retarding effect of more than ± 25% compared to the control at days 28 and 96 after application. The results of the study proved sensitivity of the test system and provided assurance that the laboratory test conditions are adequate.

III. Conclusion

After 42 days, the test item Spiroxamine EC 500 had no impact on nitrogen transformation (nitrate content, mineral nitrogen content and nitrate formation rates) of soil microorganisms when applied at 2.0 mg and 10 mg test item/kg soil dry weight treatment (equivalent to 1.0 and 5.0 mg a.s./kg soil dry weight, respectively).

Assessment and conclusion by applicant:

Validity criteria according to the OECD 216 (2000) guideline were met as the control variation between control replicates was less than ± 15% (maximum variation: 3.05%).

The reference item demonstrated sufficient sensitivity of the test system.

The study is therefore considered acceptable.

After 42 days, the test item had no impact on nitrogen transformation of soil microorganisms when applied at rates up to 10 mg test item/kg soil dry weight (equivalent to 5.0 mg a.s./kg soil dry weight, respectively).

Data Point:	KCP 10.5/01
Report Author:	[redacted]
Report Year:	1993
Report Title:	Influence of KWG 4168 EC 500 on microbial mineralization of nitrogen in soil
Report No:	AJO/113193
Document No:	M-008754-01-1
Guideline(s) followed in study:	Guidelines for the Official Testing of Plant Protectants, Part VI, 1-1 "Influence on the Activity of the Soil Microflora", BBA Braunschweig, Germany, March 1990 (2nd ed.)
Deviations from current test guideline:	Yes (refer below) Only a single replicate was tested
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

The effect of exposure to KWG 4168 EC 500 on two lucerne meal amended soils was investigated over 56 days.

It was found that a rate of 1.5 L KWG 4168 EC 500/ha (equivalent to 2 µL product/kg soil dw) had no meaningful influence on the turnover of nitrogen in either a silty sand (1.0 % org. C, pH (KCl) 5.9) or a silt (1.7 % org. C, pH (KCl) 7.0). The 10-fold overdose of 15 L KWG 4168 EC 500 (equivalent to 20 µL product/kg soil dw) caused a temporary reduction of nitrogen mineralisation rates. However, at the end of the experiments, the amounts of nitrate found in the 10-fold overdosed samples of the silty sand and silt were *ca.* 13% and 8% less than in the untreated controls, respectively.

I. Materials and Methods

Materials

Test Material KWG EC 500

Lot/Batch #: 30 0122913

Purity: Not reported

Description: 494.0 g/L

Stability of test compound: Not reported

Reanalysis/Expiry date: 17 March 1994

Density:	Not reported
Treatments	
Test rates:	2 and 20 µL/kg soil dw (1.5 and 15 L test item/ha)
Solvent/vehicle:	Quartz sand
Analysis of test concentrations:	None
Test design	
Test vessel:	500 mL glass bottles containing 250 g dw soil
Test soil:	A silty sand and a sand
Source:	Experimental farms Laacherhof and Höfchen
Replication:	None
Duration of test:	56 days
Environmental test conditions	
Temperature:	20 ± 2°C
pH:	Soil 1 Test start: 6.0 Test end: 6.1 – 6.2
	Soil 2 Test start: 7.0 Test end: 7.1
Photoperiod:	Constant darkness

Study Design

This study was conducted in order to evaluate the effect of exposure to KWG EC 500 on nitrogen mineralisation in two agricultural soils.

Triplicate samples of each soil (equivalent to 10 g dry weight (dw)) were extracted and analysed

Soil 1 was a silty sand from a field that had not received any plant protection products for over 10 years. It consisted of 1.0% organic carbon, and had a microbial biomass of 221 mg microbial carbon/kg soil dw.

Soil 2 was a silt from a field that had not received any plant protection products for five years. This soil consisted of 1.7% organic carbon.

Application rates of equivalent 1.5 and 15 L test item/ha were applied to the soils, corresponding to 2 and 20 µL test item/kg soil dw, respectively.

Sieved soil was supplemented with either 10 g ground quartz sand/kg soil dw (control) or a mixture of quartz sand and the test item, and were mixed with 5000 mg/kg soil dw pulverised lucerne meal. After mixing, 250 g dry weight samples were poured into 500 mL grown glass bottles and closed with parafilm.

Soils were held in the dark at 20 ± 2°C and at about 38.1 and 42.9% water holding capacity for soils 1 and 2, respectively.

Samples were extracted and analysed for ammonium-N, nitrite-N and nitrate-N plus nitrite-N immediately after treatment and after 14, 28, 42 and 56 days.

II. Results and Discussion

Validity criteria were not assessed as part of the study report.

Nitrite was not found in any of the soil samples.

During the 56-day experiments, in soils amended with lucerne meal, it was found that 1.5 L KWG 4168 EC 500/ha (equivalent to 2 µL product/kg soil dw) had no meaningful influence on the turnover of nitrogen in either a silty sand or a silt. The 10-fold overdose of the compound caused a temporary reduction of nitrogen mineralisation rates in a silty sand (max. 24% on day 28).

At the end of the experiments, environmentally relevant differences (>25%) between the treated and control samples were no longer evident.

Table CP 10.5/01-1 Nitrogen mineralisation in soil 1, a silty sand, after exposure to KWG 4168 EC 500

Days after treatment	mg nitrogen/kg soil dw[1] by exposure (µL KWG 4168 EC 500/kg soil dw)					
	0 µL		2 µL		20 µL	
	Ammonium	Nitrate	Ammonium	Nitrate	Ammonium	Nitrate
0	3.36 ± 0.06	20.33 ± 0.49	3.37 ± 0.02	20.13 ± 0.06	3.46 ± 0.12	20.14 ± 0.17
14	1.43 ± 0.06	9.50 ± 0.75	1.34 ± 0.09	8.98 ± 0.99	1.15 ± 0.11*	7.86 ± 0.30
28	1.00 ± 0.06	28.59 ± 0.32	0.93 ± 0.00	26.26 ± 1.35	0.88 ± 0.06	21.54 ±0.45*
42	0.83 ± 0.02	36.65 ± 0.34	0.92 ± 0.04	36.03 ± 0.75	0.86 ± 0.04	30.41 ±0.62*
56	1.17 ± 0.00	45.95 ± 0.62	1.17 ± 0.00	45.09 ± 1.20	1.04 ± 0.45	40.00 ±1.04*

* Significantly different to the control (t-test at $p \leq 0.05$)

Table CP 10.5/01-2 Nitrogen mineralisation in soil 2, a silt, after exposure to KWG 4168 EC 500

Days after treatment	mg nitrogen/kg soil dw[1] by exposure (µL KWG 4168 EC 500/kg soil dw)					
	0 µL		2 µL		20 µL	
	Ammonium	Nitrate	Ammonium	Nitrate	Ammonium	Nitrate
0	4.08 ± 0.14	32.32 ± 0.60	4.32 ± 0.10	32.92 ± 0.27	4.25 ± 0.08	32.83 ± 0.10
14	1.17 ± 0.08	26.91 ± 0.70	1.21 ± 0.21	26.80 ± 0.23	1.06 ± 0.04	24.28 ±0.62*
28	1.01 ± 0.08	48.83 ± 0.35	1.00 ± 0.13	48.74 ± 0.45	0.96 ± 0.06	43.52 ±0.71*
42	1.56 ± 0.00	60.68 ± 0.71	1.56 ± 0.00	60.72 ± 0.73	1.56 ± 0.00	56.01 ±1.54*
56	1.17 ± 0.00	79.70 ± 0.58	1.17 ± 0.00	80.15 ± 1.07	1.17 ± 0.00	73.15 ±0.73*

* Significantly different to the control (t-test at $p \leq 0.05$)

III. Conclusion

At the end of the experiments, the amounts of nitrate found in the 10-fold overdosed samples (15 L product/ha) of the silty sand and silt were *ca.* 13% and 8% less than in the untreated controls, respectively.

When applied as recommended, KWG 4168 EC 500 should not negatively influence the turnover of nitrogen in soils.

Assessment and conclusion by applicant:

The study was conducted to a BBA test guideline and not OECD 216 although the methodology used is consistent.

Validity criteria according to the current OECD 216 (2000) test guideline could not be assessed as it would appear that only a single replicate vessel was tested for each treatment.

The study supports the risk assessment by demonstrating <25% effects at concentrations up to 15 L product/ha but as the report contains only limited details of the test method and because the validity criteria cannot be assessed the study has been submitted as supporting information only.

Data Point:	KCP 10.5/02
Report Author:	[redacted]
Report Year:	1993
Report Title:	Influence of KWG 4168 on glucose stimulated respiration in soil
Report No:	AJO/113093
Document No:	M-008747-01-1
Guideline(s) followed in study:	Guidelines for the Official Testing of Plant Protectants, Part VI, 1-1 "Influence on the Activity of the Soil Microflora", BBA Braunschweig, Germany, March 1990 (2nd ed.).
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

Two agricultural soils were exposed to concentrations of 2 µL and 20 µL KWG 4168/kg dry weight soil to determine the effect on glucose stimulated respiration over 28 days.

It was determined that 1.5 L KWG 4168/ha (equivalent to 2 µL product/kg dry weight soil) and 15 L KWG 4168/ha (equivalent to 20 µL product/kg dry weight soil) had no influence on soil respiration after addition of glucose to a silty sand.

When applied as recommended KWG 4168 should have no influence on degradation of organic carbon in soils.

I. Materials and Methods

Materials

Test Material KWG 4168 EC 500

Lot/Batch #: 30.0122915

Purity: Not reported

Description: Not reported

Stability of test compound: Not reported

Reanalysis/Expiry date: 17 March 1994

Density: Not reported

Treatments

Test rates:	Nominal: Measured:
Solvent/vehicle:	Quartz sand
Analysis of test concentrations:	No

Test design

Test vessel:	1 litre clear glass jars covered with clear glass lids
Replication:	3
Duration of test:	28 days

Environmental test conditions

Temperature:	20 ± 2°C
Photoperiod:	24 hour darkness

Study Design

This study was conducted in order to assess the effects of KWG 4168 EC 500 on soil respiration over 28 days. Concentrations of glucose added were selected based on the results of a preliminary range-finding test.

Soil 1 was a silty sand from a field that plant protection chemicals had not been used on for 12 years. Soil 2 was a silt from a field that plant protection chemicals had not been used on for 5 years.

The application concentrations of the test item were 1.5 L and 15 L KWG 4168/ha which corresponded to 2 µL and 20 µL KWG 4168/kg dry weight soil.

Sieved soil was added with either 10 g ground quartz sand/kg dry weight soil (control) or a mixture of quartz sand and KWG 4168 at test concentrations. The samples were mixed in 4 litre aluminium containers by rolling on a gyrowheel mixer for 15 min at 50 rpm.

After mixing, soil samples equivalent to 750 g dry weight were poured into 1-litre clear glass jars and these were covered with clear glass lids. The lids were loose enough to allow air exchange, but tight enough to slow evaporation of moisture.

Soil 1 required 3000 mg glucose/kg dry weight and soil 2 required 4000 mg/kg to induce maximum respiration rates, this was added, the samples were mixed and then poured into plastic cylinders (3 cm diameter, 23 cm long). The cylinders were connected to a gas analyser and the quantities of carbon dioxide released per hour per kg dry weight soil were measured.

Soils were held in the dark at 20 ± 2°C and about 38.1% (Soil 1) and 42.9% (Soil 2) water capacity.

To determine the influence of the product on glucose stimulated soil respiration, triplicate, moist samples (equivalent to 25 g dry weight) were taken from each treatment on day 0 (within 3 hours after treatment), and after 14 and 28 days of incubation.

II. Results and Discussion

Validity criteria were not assessed as part of the study report.

The test item had no meaningful impact on the respiration of the soil. The CO_2 released from the treated soil was ± 5% of that released from the control.

Table CP 10.5/02-1 CO_2 released from treated soil (mg CO_2 /hour/kg dry weight soil) as compared to untreated control

		% of control		
		0 days after treatment	14 days after treatment	28 days after treatment
Soil 1	2 µL	96.6	96.7	98.8
	20 µL	103.0	98.7	105.0
Soil 2	2 µL	94.9	97.6	97.2
	20 µL	104.8	99.4	103.8

Exposure to the test item did not cause a change in soil pH.

Table CP 10.5/02-2 pH of soils treated with KWG 4168 for 28 days

		0 days after treatment	28 days after treatment
Soil 1	Control	5.9	6.0
	2 µL	5.9	6.1
	20 µL	5.9	6.1
Soil 2	Control	6.9	7.0
	2 µL	6.9	7.0
	20 µL	6.9	7.0

III. Conclusion

During 28-day experiments, 1.5 L KWG 4168/ha (equivalent to 2 uL product/kg dry wt soil) and 15 L KWG 4168/ha (equivalent to 20 uL product/kg dry wt soil) had no influence on soil respiration. The study concluded that when used as recommended, KWG 4168 should have no negative influence on the turnover of organic carbon in soils.

Assessment and conclusion by applicant:

The study was conducted to a BBA test guideline and not OECD 217 although the methodology used is consistent.

Validity criteria according to the current OECD 217 (2000) test guideline could not be assessed as it would appear that only a single replicate vessel was tested for each treatment.

The study supports the risk assessment by demonstrating <25% effects at concentrations up to 15 L product/ha but as the report contains only limited details of the test method and because the validity criteria cannot be assessed the study has been submitted as supporting information only. It is further noted that data on the effects of respiration are not a core data requirement and these results are not required for the risk assessment.

Litter bag study

The following litter bag study is available using Spiroxamine EC 500. Although this study type is no longer considered suitable for use in risk assessment, the study has been submitted for completeness.

Data Point:	KCP 10.5/03
Report Author:	[redacted]
Report Year:	2004
Report Title:	Spiroxamine EC 500: Effects on soil litter degradation
Report No:	LKC-SLD 20/03
Document No:	M-109748-02-1
Guideline(s) followed in study:	Minutes of a meeting on the requirement of data according to EU directive 91/414/EEC, Annex III, point 10.6.2 from Kula and Guske, BBA, Germany March 2001
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

This study was conducted in order to investigate the effects of exposure to KWG 4168 EC 500 on soil degradation.

Six 81 m^2 plots each were used, to which was applied 28.8 g a.s./ha (57.54 g test item/ha). Litter bags containing 4 g wheat straw were buried, and degradation was assessed for the time periods 0 to 29, 0 to 92, and 0 to 173 days through weighing.

Litter degradation in treated test plots was comparable to control plots over the course of the study, at 104.6, 97.8, and 99.5% for the time periods 0 to 29, 0 to 92, and 0 to 173 days, respectively.

Residues of KWG 4168 EC 500 were therefore determined to have no influence on organic matter breakdown after 1, 3, and 6 months.

I. Materials and Methods

Materials

Test Material KWG 4168 EC 500

Lot/Batch #:	PF9004061I
Purity:	501 g/L
Description:	Clear brown liquid
Stability of test compound:	Not reported
Reanalysis/Expiry date:	08 January 2004
Density:	1.001 g/mL

Treatments

Test rates:	28.8 g a.s./ha (57.54 g test item/ha), equivalent to a plateau concentration of 19.2 µg a.s./kg soil
Solvent/vehicle:	Water
Analysis of test concentrations:	None

Test design

Test plots:	9 x 9 m (81 m^2) plots untreated by any formulation including spiroxamine for at least three years
Test soil:	Silt (USDA)
Location:	Bayer experimental farm Höfchen, Burscheid, Germany
Replication:	Six plots per treatment and control
Duration of test:	173 days

Study Design

This study was conducted in order to investigate the effects of exposure to KWG 4168 EC 500 on soil degradation.

Test plots were 9 x 9 m (81 m^2) plots untreated by any formulation including spiroxamine for at least three years. There were six replicates in the treatment and in the control.

Spiroxamine was applied at a rate of 28.8 g a.s./ha (57.54 g test item/ha), equivalent to a plateau concentration of 19.2 µg a.s./kg soil.

Untreated seeds of summer barley, variety "Scarlett", were sown onto all plots at a rate of 166.0 kg/ha. Directly after sowing 48 litter bags (12 x 22 cm, mesh size 8 mm) filled with 4 g of dry wheat straw each were buried per plot. On the same day the calculated annual application rate of 450 g a.s./ha (899.1 g test item/ha), was applied in a volume of 300 L water/ha to the treatment plots.

Degradation was assessed for the time periods 0 to 29, 0 to 92, and 0 to 173 days through weighing.

II. Results and Discussion

The application of the estimated plateau concentration of spiroxamine resulted in soil residues of 21.1 µg a.s./kg dry soil, which is 110.0% of the nominal amount of 19.2 µg/kg. The application of the annual rate of KWG 4168 EC 500 resulted in soil residues of 233 µg a.s./kg dry soil four days after the spray application, corresponding to 73.0% of nominal.

Litter degradation in treated test plots was comparable to control plots over the course of the study, at 104.6, 97.8, and 99.5% for the time periods 0 to 29, 0 to 92, and 0 to 173 days, respectively.

The results of this study show that litter degradation in soil was not inhibited by exposure to the test item. At no sampling time (29, 92, and 173 days after introduction of litter-bags into the soil), could a statistically significant difference in proportion of wheat straw degradation be observed between untreated control plots and the plots treated with the test item.

Table CP 10.5/03-1 Effects of exposure to KWG 4168 EC 500 on litter degradation

	Control	KWG 4168 EC 500	% of control
0 – 29 d			
Wheat straw degraded (g)	0.65	0.68	104.6
Wheat straw degraded (%)	16.25	17.00	
0 – 92 d			
Wheat straw degraded (g)	2.20	2.15	97.8
Wheat straw degraded (%)	55.02	53.82	
0 – 173 d			
Wheat straw degraded (g)	3.55	3.53	99.5

	Control	KWG 4168 EC 500	% of control
Wheat straw degraded (%)	88.67	88.26	

Data are means of four plots

III. Conclusion

Litter degradation in treated test plots was comparable to control plots over the course of the study, at 104.6, 97.8, and 99.5% for the time periods 0 to 29, 0 to 92, and 0 to 173 days, respectively.

Residues of KWG 4168 EC 500 were therefore determined to have no influence on organic matter breakdown after 1, 3, and 6 months.

Assessment and conclusion by applicant:

The study is considered valid in its own right but as litter bag studies are not a core data requirement and are no longer accepted as a refinement study, the study is therefore deemed to be supporting information only.

CP 10.6 Effects on terrestrial non-target higher plants

The available data for spiroxamine with non-target terrestrial plants are presented in the table below.

Table CP 10.6-1 Summary of non-target terrestrial plant studies with Spiroxamine EC 500

Organism	Test item	Test type	Endpoints		Reference
Avena sativa $_m$ *Allium cepa* $_m$ *Beta vulgaris* $_d$ *Brassica rapa* $_d$ *Daucus carota* $_d$ *Glycine max* $_d$	Spiroxamine EC 500	21-day Seedling emergence	NOER 2,400 g a.s./ha ER_{50} >2,400 g a.s./ha	EU	M-136408-02-1
Abutilon theophrasti $_d$ *Amaranthus retroflexus* $_d$	Spiroxamine EC 500	28-day Seedling emergence	NOER <25 g a.s./ha ER_{50} 400 g a.s./ha	EU	M-302061-01-1
Avena sativa $_m$ *Allium cepa* $_m$ *Beta vulgaris* $_d$ *Brassica rapa* $_d$ *Daucus carota* $_d$ *Glycine max* $_d$	Spiroxamine EC 500	21-day Vegetative vigour	NOER 150 g a.s./ha ER_{50} 960 g a.s./ha	EU	M-051682-01-1
Abutilon theophrasti $_d$ *Amaranthus retroflexus* $_d$	Spiroxamine EC 500	21-day Vegetative vigour	NOER 100 g a.s./ha ER_{50} >400 g a.s./ha	EU	M-302060-01-1

d: dicotyledonous; m: monocotyledonous

The available data to assess the toxicity of spiroxamine to non-target terrestrial plants have been generated using the representative formulation, Spiroxamine EC 500, and are therefore considered suitable for use in the risk assessment. The lowest ER_{50} values were determined in the seedling emergence and vegetative vigour studies using *Abutilon theophrasti* and *Amaranthus retroflexus*. It should be noted that there were less than 50% effects in these studies at the maximum rate tested of 400

g a.s./ha therefore the ER_{50} has been set as >400 g a.s./ha and is considered to represent a conservative estimate of the toxicity of Spiroxamine EC 500. This value has been used in the risk assessment below.

Exposure

Effects on non-target terrestrial plants are of concern in the off-field environment, where plants may be exposed to spray drift. The amount of spray drift reaching off-crop habitats is calculated using the appropriate percentile estimates, which depends on the number of applications, and is derived from the BBA (2000[23]) values from the spray-drift predictions of Ganzelmeier & Rautmann (2000[24]).

The worst case representative use of Spiroxamine EC 500 is for two applications to grapes (late) at a maximum rate of 300 g a.s./ha. This use has been considered in the risk assessment below and covers all other representative uses of Spiroxamine EC 500.

The drift rate (predicted environmental rate, $PER_{off-field}$) associated with grapes has been calculated based on spray drift predictions for one application using 90th percentile drift values and for two applications using 82nd percentile drift values. This gives drift rates of 8.02% at 3 m for grapes (late) and 7.23% at 3 m for grapes (late) for one and two applications, respectively. These equate to drift factors of 0.0802 and 0.0723 for one and two applications, respectively.

The calculated drift rates in g a.s./ha for the use on grapes are presented in the following table.

Table CP 10.6-2 Off-field drift rates following application of Spiroxamine EC 500

Crop	Maximum application rate (g a.s./ha)	Number of applications	Drift distance (m)	Drift factor	MAF	$PER_{off-field}$ (g a.s./ha)
Grapes (late)	300	1	3	0.0802	1.0	24.1
		2		0.0723	1.9*	41.2

* Worst case MAF for two applications to soil substrate

The highest $PER_{off-field}$ value has been determined to be 41.2 g a.s./ha and has therefore been used in the risk assessment below.

Isomers

In terms of organism exposure, the critical highest predicted concentrations in the environment will occur immediately or very shortly after application therefore the effects of potential changes in the isomer ratios over time in the environment are not considered to be relevant to the non-target terrestrial plant risk assessment. However, even if exposure to residues over a prolonged period of time were to occur, according to the current residues data set for spiroxamine there are no indications of a significant change in isomer ratios therefore no additional factor need be applied to the risk assessments below (*i.e.* an UF of 1.0 has been used).

Risk assessment for Terrestrial Non-Target Higher Plants

The risk to non-target terrestrial plants in the off-crop environment from spray drift following application of Spiroxamine EC 500 has been assessed by comparing the ER_{50} values from seedling

[23] BBA (2000) Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Public domain.

[24] Ganzelmeier H., Rautmann D. (2000) Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application. Public domain.

emergence and vegetative vigour effects with the highest $PER_{off\text{-}field}$ in order to calculate TER values according to the following equation.

$$TER = \frac{ER_{50}\ (g\ a.s./ha)}{PER_{off\text{-}field}\ (g\ a.s./ha)}$$

The TER value has been evaluated against the trigger value of 5 and are presented in the table below.

Table CP 10.6-3 Spiroxamine EC 500 TER values for non-target terrestrial plants

Crop	Effect	ER_{50} (g a.s./ha)	Application rate g a.s/ha	Off-field exposure			Trigger value
				Distance (m)	PER (g a.s./ha)	TER	
Grapes (late)	Seedling emergence & Vegetative vigour	400	2 x 300	3	41.2	>9.71	5

Based on seedling emergence and vegetative vigour data, an acceptable risk to non-target plants has been demonstrated following the proposed uses of Spiroxamine EC 500, with TER values in excess of the trigger value of 5. No further risk assessment is considered to be necessary.

Biodiversity

No relevant scientifically peer-reviewed open literature could be found on spiroxamine or its major metabolites, from an ecotoxicological perspective, on non-target terrestrial plants. Therefore, it is considered that the potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects *via* alteration of the food web, are covered by the risk assessment for non-target terrestrial plants in this section.

With respect to the NTTP off-field risk assessment, which demonstrated acceptable off-field risks without the need for risk mitigation, the applicant concludes that the use of the representative lead formulation (Spiroxamine EC 500) has a low potential to cause unacceptable effects on biodiversity and the ecosystem *via* trophic interactions. To the best of our knowledge and with the presented safety profile of the spiroxamine a.s. and the representative lead formulation, the applicant does not foresee any unacceptable effects on biodiversity and the ecosystem with spiroxamine.

CP 10.6.1 Summary of screening data

Data Point:	KCP 10.6.1/01
Report Author:	[redacted]; [redacted]
Report Year:	1999
Report Title:	Herbicidal screening data for KWG 4168 EC 500
Report No:	DOM 99123
Document No:	M-027298-01-1
Guideline(s) followed in study:	OECD
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

Screening data were generated in 1999 to assess potential herbicidal effects of KWG 4168 EC 500. The test substance was applied to the soil surface in which plants were subsequently grown and to the foliage of the emerged plants at application rates of 250, 500, 750, 1000, 1500, 2000 and 2250 g a.s./ha. These application rates were up to three times higher than the proposed use rate (at the time) of 750 g a.s./ha.

Test species were monocot maize (*Zea mays*), wild oat (*Avena fatua*), cockspur (*Echinochloa crus-galli*), black twitch (*Alopecurus myosuroides*) and green bristle grass (*Setaria viridis*) and dicot white mustard (*Sinapis alba*), sugar beet (*Beta vulgaris*), cleavers (*Galium aparine*), indian mallow (*Abutilon theophrasti*), common amaranth (*Amaranthus retroflexus*) and ivyleaf morning glory (*Ipomoea hederacea*).

When KWG 4168 EC 500 was applied to the soil (pre-emergence), no effect was observed on maize, wild oat, cockspur or white mustard. Slight phytotoxic effects (<50%) were observed on sugar beet, black twitch and cleavers at the triple proposed application rate. Phytotoxic effects (50-95%) were observed on green bristle grass, indian mallow, common amaranth and ivyleaf morning glory at the triple proposed maximum application rate. Relevant phytotoxic effects (≥50%) were observed on indian mallow and common amaranth at the single proposed maximum application rate.

When KWG 4168 EC 500 was applied to the foliage (post-emergence), slight phytotoxic effects (<50%) were observed on maize, black twitch and wild oat. Phytotoxic effects (≥50%) were observed on sugar beet, cockspur, green bristle grass, indian mallow, common amaranth, cleavers, ivyleaf morning glory and white mustard at the triple proposed maximum application rate.

I. Materials and Methods

Materials

Test Material: KWG 4168 EC 500

Lot/Batch #: 04023/0627/0778

Purity: 50.6%

Description: Not reported

Reanalysis/Expiry date: Not reported

Treatments

Test rates: Nominal: 250, 500, 750, 1000, 1500, 2000 and 2250 g a.s./ha

Test organisms

Species:	Monocot: maize (*Zea mays*), wild oat (*Avena fatua*), cockspur (*Echinochloa crus-galli*), black twitch (*Alopecurus myosuroides*), and green bristle grass (*Setaria virdis*) Dicot: white mustard (*Sinapis alba*), sugar beet (*Beta vulgaris*), cleavers (*Galium aparine*), indian mallow (*Abutilon theophrasti*), common amaranth (*Amaranthus retroflexus*) and ivyleaf morning glory (*Ipomoea hederacea*)

Test design

Test vessel:	Greenhouse pots of 420 cm^2
Test medium:	Soil (texture: sandy loam, organic matter 2.5-3%)
Replication:	Ten seeds per species
Duration of test:	Pre-emergence: final evaluation was done after 21 days; post-emergence: final evaluation was done after 17 days

Environmental test conditions

Temperature:	22/15°C in a day/night cycle
Relative humidity:	50%
Photoperiod:	14 hours light, 10 hours dark (illuminated at 800 lux)

Study Design

The purpose of this report is to summarise screening data generated in November 1999 for non-herbicidal crop protection products

KWG 4168 EC 500 was applied as an EC 500 formulation in 1000 L water/ha at monocot species (maize, wild oat, cockspur, black twitch and green bristle grass) and dicot species (white mustard, sugar beet, cleavers, indian mallow, common amaranth and iveleaf morning glory) at test concentrations of 250, 500, 750, 1000, 1500, 2000 and 2250 g a.s./ha.

Spray treatments were applied in single applications in an automatic spray chamber at a height of 45 cm. Applications were made separately in the pre-emergence and foliar tests.

Typically ten seeds of each species were placed. For the foliar test, the plants were grown in the greenhouse for approximately 14 days prior to application and the final evaluation was completed 17 days after treatment. For the pre-emergence test, the plants were placed within 24 hours prior to application and the final evaluation was completed 21 days after treatment.

Visual phytotoxicity was observed using a rating scale of 0 to 100%, where 100% was compete destruction of above ground parts and 0% was normal growth (no visual damage).

II. Results and Discussion

No validity criteria were assessed as part of the study report.

Table CP 10.6.1/01-1 Values damage observed at the completion of the pre-emergence test

Species	Results (% effect) at different application rates						
	250 g a.s./ha	500 g a.s./ha	750 g a.s./ha	1000 g a.s./ha	1500 g a.s./ha	2000 g a.s./ha	2250 g a.s./ha

Species	Results (% effect) at different application rates						
Maize	0	0	0	0	0	0	0
Sugar beet	20	30	30	30	30	40	40
Black twitch	0	0	0	0	0	0	20
Wild oat	0	0	0	0	0	0	0
Cockspur	0	0	0	0	0	0	0
Green bristle grass	20	30	30	40	40	95	80
Indian mallow	50	50	60	70	70	80	80
Common amaranth	50	50	70	70	80	80	95
Cleavers	40	40	20	40	30	50	40
Ivyleaf morning glory	0	0	0	0	20	20	50
White mustrard	0	0	0	0	0	0	0

Table CP 10.6.1/01-2 Values damage observed at the completion of the foliar applied test

Species	Results (% effect) at different application rates						
	250 g a.s./ha	500 g a.s./ha	750 g a.s./ha	1000 g a.s./ha	1500 g a.s./ha	2000 g a.s./ha	2250 g a.s./ha
Maize	0	10	20	20	30	40	40
Sugar beet	40	50	70	70	80	80	80
Black twitch	20	20	0	30	30	30	30
Wild oat	0	0	0	30	0	40	30
Cockspur	0	20	50	40	50	60	60
Green bristle grass	30	30	50	40	50	50	50
Indian mallow	70	80	90	90	90	95	100
Common amaranth	50	70	80	80	90	90	90
Cleavers	0	30	50	30	60	60	60
Ivyleaf morning glory	20	30	30	50	50	70	80
White mustrard	20	30	50	70	60	80	70

III. Conclusion

In the pre-emergence test, 18% of all species tested showed relevant phytotoxic effects of ≥50% at the single proposed maximum application rate.

In the post-emergence test (foliage), 64 % of all species tested showed relevant phytotoxic effects of ≥50% at the single proposed maximum application rate of 750 g a.s./ha.

Assessment and conclusion by applicant:

The study is considered valid in its own right. As screening data it is accepted that this was a non-GLP study. Validity criteria cannot be assessed as the data generated are not suitable for an assessment against OECD 227 or 208 and this is not considered to be appropriate as the study was simply a screening test.

The results are considered suitable to support the risk assessment but the study has been submitted as supporting information only. It is noted that several Tier I GLP plant studies are available with this formulation and the results of these will be used for the risk assessment.

CP 10.6.2 Testing on non-target plants

Data Point:	KCP 10.6.2/01
Report Author:	[redacted]
Report Year:	2001
Report Title:	Spiroxamine EC 500 - Terrestrial plants toxicity, seedling emergence, Tier II
Report No:	TMK71793
Document No:	M-136008-02-1
Guideline(s) followed in study:	OECD 208 (Draft, 2000)
Deviations from current test guideline:	Yes (refer below) For soybean, a loamy sand with a carbon content of 2.28 ± 0.16% was used because the compression of available soils with a carbon content <1.5% was too high
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The effect of Spiroxamine EC 500 on the seedling emergence of monocot oat (*Avena sativa*), onion (*Allium cepa*) and dicot sugar beet (*Beta vulgaris*), turnip (*Brassica rapa*), carrot (*Daucus carota*) and soybean (*Glycine max*) crops was studied at nominal concentrations of 150, 300, 600, 1200 and 2400 g a.s./ha.

The NOEC and EC_{50} values for shoot height, fresh weight and seedling emergence of all species were 2400 and >2400 g a.s./ha, respectively.

There were no compound related phytotoxic effects.

I. Materials and Methods

Materials

Test Material	Spiroxamine EC 500
Lot/Batch #:	04023/0778(0627)
Purity:	488.9 g/L
Description:	Brown liquid
Reanalysis/Expiry date:	06 March 2001
Relative Density:	1.006 g/cm^3
Treatments	
Test rates:	Nominal: 150, 300, 600, 1200, 2400 g a.s./ha
Solvent/vehicle:	Demineralised water
Test organisms	
Species:	Monocotyledons: oat (*Avena sativa*), onion (*Allium cepa*); Dicotyledons: sugar beet (*Beta vulgaris*), turnip (*Brassica rapa*), carrot (*Daucus carota*) soybean (*Glycine max*)
Source:	Heine & Garvens, D-31157 Sarstedt; Lochow-Petkus GmbH, D-29296 Bergen; Südwestsaat Gbr, D-76437 Rastatt; KWS Kleinwanzlebener Saatzucht AG, D-37555 Einbeck
Test design	
Test vessel:	Plastic flower pots with a diameter of 12 cm
Test medium:	Oat, onion, sugar beet, turnip and carrot: natural soil (texture: sandy loam, grain size: ≤2 mm, carbon content: 1.32 ± 0.10%, pH: 6.5 ± 0.1) Soybean: natural soil (texture: loamy sand, grain size: ≤2 mm, carbon content: 2.28 ± 0.16%, pH: 5.8 ± 0.3)
Replication:	Six replicates per dosage and control per species
No. plants/vessel:	Five plants per test vessel
Duration of test:	21 days
Environmental test conditions	
Temperature:	15 - 26ºC
Relative humidity:	62 – 90%
Photoperiod:	16 hours light, 8 hours dark at 4200 ± 480 lux

Study Design

This study was conducted in order to assess the toxicity of Spiroxamine EC 500 on two monocotyledonae and four dicotyledonae plant species.

Monocotyledonae test species were oat (*Avena sativa*) and onion (*Allium cepa*); dicotyledonae test species were sugar beet (*Beta vulgaris*), turnip (*Brassica rapa*), carrot (*Daucus carota*) and soybean (*Glycine max*).

Plants were grown in 12 cm plastic flowerpots in a climatic hall at 20 ± 10 ºC under a 16 h light 8 h dark photoperiod. Each species consisted of six pots of five plants in each.

The test soil for oat, onion, sugar beet, turnip and carrot was a sandy loam sieved to 2 mm with an organic carbon content of 1.32 ± 0.10% and a pH of 6.5 ± 0.1. The test soil for soybean was a loamy sand sieved to 2 mm with an organic carbon content of 2.28 ± 0.16% and a pH of 5.8 ± 0.3.

At test initiation, serial dilutions of Spiroxamine EC 500 were sprayed on the containers of test medium and sown seeds using a field sprayer at a rate of 200 L/ha. Nominal test concentrations were 150, 300, 600, 1200 and 2400 g a.s./ha of the test item along with a water control.

The test containers were bottom watered and fertilised with nutrient solution throughout the test as needed.

Temperature and humidity were recorded continuously throughout the test using a thermohygrograph.

Visual observations of phytotoxicity and plant mortality were made on days 7, 14 and 21. Phytotoxic symptoms included chlorosis, necrosis and wilting. At test termination, shoot height, fresh weight and phytotoxicity rates were determined.

The NOEC values for inhibition of shoot height, fresh weight and emergence rate were determined by one way analysis of variance followed by Dunnett's test if statistically significant differences compared to control replicates were found.

II. Results and Discussion

Validity criteria according to the study report were met in that the controls seedlings exhibited normal growth throughout the test.

Due to technical reasons, for all plant species the biomass was determined as fresh weight instead of dry weight. This deviation was considered to have no impact on the quality and integrity of the study.

There were no statistically significant effects on the shoot height of any species following exposure to Spiroxamine EC 500 at any treatment.

There were no statistically significant effects on the fresh weight of any species following exposure to Spiroxamine EC 500 at any treatment.

There were no statistically significant effects on the seedling emergence of any species following exposure to Spiroxamine EC 500 at any treatment.

Table CP 10.6.2/01-1 Inhibition of shoot height

Treatment (g a.s./ha)	Shoot height (cm)											
	Oat		Onion		Sugar beet		Turnip		Carrot		Soybean	
		% inhib.		% inhib.		% inhib.		% inhib.		% inhib.		% inhib.
Control	37.3	-	11.2	-	7.8	-	11.5	-	9.4	-	19.9	-
150	35.8	4	11.5	-2	8.9	-14	11.2	3	9.2	3	17.0	15
300	39.0	-5	9.5	16	8.1	-4	11.6	-1	9.9	-6	19.0	4
600	38.4	-3	10.9	3	8.5	-9	11.9	-3	8.7	8	18.8	5
1200	35.2	6	9.9	11	8.0	-3	11.0	4	8.3	12	18.3	8
2400	34.6	7	9.7	13	7.2	7	11.3	1	9.0	5	15.7	21

Table CP 10.6.2/01-2 Inhibition of fresh weight

Treatment (g a.s./ha)	Fresh weight (mg)											
	Oat		Onion		Sugar beet		Turnip		Carrot		Soybean	
		% inhib.		% inhib.		% inhib.		% inhib.		% inhib.		% inhib.
Control	933	-	114	-	705	-	1005	-	90	-	1430	-
150	859	8	131	-15	841	-19	1011	-1	92	-2	1317	3
300	1066	-14	87	24	691	2	1162	-16	98	-9	1392	3
600	1007	-8	122	-7	896	-27	1050	-4	93	-3	1684	-18
1200	775	17	99	13	754	-7	848	16	72	20	1466	-3
2400	803	14	107	6	572	19	934	7	74	18	1140	20

Table CP 10.6.2/01-3 Rate of emergence

Treatment (g a.s./ha)	Rate of emergence											
	Oat		Onion		Sugar beet		Turnip		Carrot		Soybean	
		% inhib.		% inhib.		% inhib.		% inhib.		% inhib.		% inhib.
Control	93	-	77	-	80	-	87	-	87	-	68	-
150	97	-4	70	9	70	13	83	5	70	20	57	16
300	93	0	73	5	63	21	80	8	97	-11	77	-13
600	93	0	77	0	77	4	93	-7	80	8	67	1
1200	93	0	83	-8	83	-4	90	-3	80	8	70	-3
2400	93	0	77	0	73	9	67	23	80	8	67	1

Table CP 10.6.2/01-4 Shoot height after exposure to Spiroxamine EC 500

Species	NOEC* (g a.s./ha)	EC_{25} (g a.s./ha)	p = 95%	EC_{50} (g a.s./ha)	p = 95%
Oat	2400	>2400	-	>2400	-
Onion	2400	>2400	-	>2400	-
Sugar beet	2400	>2400	-	>2400	-
Turnip	2400	>2400	-	>2400	-
Carrot	2400	>2400	-	>2400	-
Soybean	2400	>2400	-	>2400	-

- not determinable
* the highest tested concentration of the test item at which no statistically significant effect is observed

Table CP 10.6.2/01-5 Fresh weight after exposure to Spiroxamine EC 500

Species	NOEC* (g a.s./ha)	EC_{25} (g a.s./ha)	p = 95%	EC_{50} (g a.s./ha)	p = 95%
Oat	2400	>2400	-	>2400	-
Onion	2400	>2400	-	>2400	-
Sugar beet	2400	>2400	-	>2400	-
Turnip	2400	>2400	-	>2400	-
Carrot	2400	>2400	-	>2400	-
Soybean	2400	>2400	-	>2400	-

- not determinable
* the highest tested concentration of the test item at which no statistically significant effect is observed

Table CP 10.6.2/01-6 Seedling emergence after exposure to Spiroxamine EC 500

Species	NOEC* (g a.s./ha)	EC_{25} (g a.s./ha)	p = 95%	EC_{50} (g a.s./ha)	p = 95%
Oat	2400	>2400	-	>2400	-
Onion	2400	>2400	-	>2400	-
Sugar beet	2400	>2400	-	>2400	-
Turnip	2400	>2400	-	>2400	-
Carrot	2400	>2400	-	>2400	-
Soybean	2400	>2400	-	>2400	-

- not determinable
* the highest tested concentration of the test item at which no statistically significant effect is observed

The test item did not cause phytotoxic effects to all tested plant species in the tested concentration range of 150 - 2400 g a.s./ha.

III. Conclusion

Exposure to Spiroxamine EC 500 had no statistically significant effects on any of the species tested in terms of phytotoxicity at any treatment level up to 2400 g a.s./ha. Spiroxamine EC 500 did not cause any inhibitory effects in shoot height, fresh weight or rate of seedling emergence to any of the species tested at any treatment. The EC_{25} and EC_{50} values for shoot height, fresh weight and seedling emergence were all >2400 g a.s./ha.

Assessment and conclusion by applicant:

The study was conducted to a draft version of OECD 208 but did not use the validity criteria that are listed in the current version of OECD 208. The study has therefore been assessed against these criteria:

- Seedling emergence ≥ 70 % (≥70% emergence for oat, onion, sugar beet, turnip and carrot. Emergence was 68% for soybean);
- No visible phytotoxic effects in seedlings (actual: none);
- Mean survival of emerged seedlings ≥ 90 % (actual: achieved);
- Environmental conditions to be identical (actual: achieved with the exception that the soil used for soybean had a higher OC content)

The study was conducted in accordance with a draft version of OECD 208 but the test methods and procedures used are consistent with the current version. Thus, the study is therefore considered to be valid but it is noted that the emergence of control soybean plants was 68% which is just below the criterion of ≥70 %.

The ER_{50} values for shoot height, fresh weight and seedling emergence were all >2400 g a.s./ha.

Data Point:	KCP 10.6.2/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	Spiroxamine EC 500 G: Effect on the seedling growth of two non crop species of non-target terrestrial plants (Tier 2)
Report No:	SE 08/001
Document No:	M-302061-01-1
Guideline(s) followed in study:	OECD 208 (July 2006, adopted): Terrestrial (Non-Target) Plant Test: Seedling emergence and seedling growth test (Tier 2)
Deviations from current test guideline:	Yes (refer below) It was anticipated that the 2 non-crop species would not meet the validity criteria for emergence. Therefore, this criteria was not imposed for this study, although emergence is reported. The seeds were used even if they did not reach the performance criteria recommended in the Guidelines. To provide sufficient seedlings for a valid statistical analysis, 10 seeds were sown per pot (replicate) and in pots where 5 seedlings emerged, additional seedlings were removed. A further modification is that the duration of the study was 28 days after application.
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The effect of Spiroxamine EC 500 G on the seedling emergence of two dicotyledonous (velvetleaf, *Abutilon theophrasti*, redroot pigweed, *Amaranthus retroflexus*) plant species was studied at nominal concentrations of 25, 50, 100, 200 and 400 g a.s./ha.

The growth medium used in the test was sterilised soil (pH: 7.3; organic carbon: 0.81%).

All seeds were planted prior to test item application and the exposure time was 28 days after application. Spray treatments were once applied, at test initiation, at the nominal spray volume of 200 L/ha.

No statistical analysis was done for seedling emergence for either species tested.

For velvetleaf, there were no statistically significant impacts on observed mortality or growth stage development in any treatment groups as compared to the control. Biomass was significantly reduced at all application rates tested as compared to the control. There were slight phytotoxic effects observed including stunting and necrosis.

For redroot pigweed, there were no statistically significant impacts on observed mortality or growth stage development in any treatment groups as compared to the control. Biomass was not significantly reduced in any application rate tested as compared to the control.

The ER_{50} values for both survival and biomass exceeded the maximum rate tested of 400 g a.s./ha.

I. Materials and Methods

Materials

Test Material	Spiroxamine EC 500 G
Lot/Batch #:	PF90087683
Purity:	501 g/L
Description:	Clear brown liquid
Reanalysis/Expiry date:	31 January 2010
Density:	1.006 g/mL
Treatments	
Test rates:	25, 50, 100, 200 and 400 g a.s./ha.
Solvent/vehicle:	None
Analysis of test concentrations:	Analysis of the highest application rate had a recovery of 95.3%
Test organisms	
Species:	Velvetleaf, ABUTH, *Abutilon theophrasti*; redroot pigweed, AMARE, *Amaranthus retroflexus*
Source:	Seeds supplied from commercial sources via Bayer CropScience AG, 65926 Frankfurt am Main
Test design	
Test vessel:	Commercial plastic flower pots (10.5 cm diameter)
Test medium:	Sterilised soil at pH 7.3; C_{org} 0.81%
Replication:	8 replicates
No. plants/vessel:	10 seeds per replicate
Duration of test:	28 days
Environmental test conditions	
Temperature:	23 ± 8 ºC daytime, 18 ± 8 ºC night time
pH:	7.3
Photoperiod:	16 hours light: 8 hours dark (light intensity: <15000 lux)

Study Design

This study was conducted in order to evaluate the effect of Spiroxamine EC 500 G on the seedling emergence and growth of two dicotyledonous plant species.

Test species were two dicotyledonous plants (velvetleaf and redroot pigweed) from two different families.

Plants were grown in commercial plastic flower pots in a glasshouse at 23 ± 8 ºC during the day and 18 ± 8 ºC at night under a 16 hour light 8 hour dark photoperiod. Eight replicates with ten seeds per species were tested.

Sterilised sandy-silt loam was used as the test medium.

At test initiation, spray solution made up of the test item dissolved in deionised water to a volume of 200/L was applied to the soil surface using a spray chamber with an overhead nozzle (set at 30 cm above the sprayed surface).

Observations of phytotoxicity were made on days 7, 14, 21 and 28 according to the EPPO Standard 135. Germination was assessed daily and the first 5 seedlings were considered for the evaluation. Assessments of mortality were made on days 7, 14, 21 and 28. Biomass was determined at final assessment and growth stages at the final assessment were reported according to the phenological growth stages and BBCH identification keys of weed species from the Compendium of Growth Stage Identification Keys for Mono- and Dicotylenous plants.

More than 5 velvetleaf seedlings emerged in all control pots and in total 26 seedlings were removed to leave 5 seedlings per pot for survial and biomass assessments. In no control pot did more than 5 redroot pigweed seedlings emerge.

Statistical analysis was carried out on the mortality and biomass data using the ToxRat software for statistical analysis. No statistical analysis was carried out for emergence data.

Analytical method

Samples of water were analysed using the validated analytical method M-302060-01-1, report reference M-302060-01-1 **(see Doc MCP Section 5)**.

II. Results and Discussion

Validity criteria according to the study report were considered to have been met on the basis that >90% survival of untreated controls was achieved (100% for velvetleaf and 100% for redroot pigweed).

Analysis of the highest application rate revealed the rate to be 95.3% of nominal.

Mortality for both species tested at all test concentrations was 0%, therefore Spiroxamine EC 500 G did not have a statistically significant impact on the survival of either species. For velvetleaf, biomass was significantly reduced at all application rates tested. For redroot pigweed, biomass was not considered as significantly reduced by the statistical software at all application rates tested due to the very high variability within the replicates. There were no effects on growth stage development of treated plants in comparison to the untreated controls at all application rates tested for either species tested.

Table CP 10.6.2/02-1. Summary of the effects of Spiroxamine EC 500 G on tested species

Species	Treatment group (g.a.s.ha)	Survival (%)	Biomass (g)					BBCH (day 28)
			Mean	S.D.	% CV	% Red	Sign.	
Velvetleaf	Control	100	0.235	0.0767	32.6	-	-	12-14
	25	100	0.150	0.0223	14.9	36.4	+	12-14
	50	100	0.147	0.0487	33.0	37.4	+	12-14
	100	100	0.158	0.0411	26.0	32.9	+	12-14
	200	100	0.141	0.0228	16.2	40.3	+	12-14
	400	100	0.125	0.0508	40.5	46.8	+	12-14
Redroot pigweed	Control	100	0.063	0.0341	53.8	-	-	12-16
	25	100	0.035	0.0324	91.9	44.4	+*	12-16
	50	100	0.044	0.0326	73.8	30.5	+*	12-16
	100	100	0.064	0.0203	32.0	-0.2	-	12-16

Species	Treatment group (g a.s.ha)	Survival (%)	Biomass (g)					BBCH (day 28)
			Mean	S.D.	% CV	% Red	Sign.	
	200	100	0.035	0.0227	64.9	45.0	+*	12-16
	400	100	0.048	0.0581	121.8	24.8	+*	12-18

S.D. standard deviation
CV coefficient of variation
* Corrected values

Table CP 10.6.2/02-2 Summary of endpoints

Species	Survival			Biomass		
	NOER (g a.s./ha)	ER_{25} (g a.s./ha)	ER_{50} (g a.s./ha)	NOER (g a.s./ha)	ER_{25} (g a.s./ha)	ER_{50} (g a.s./ha)
Velvetleaf	400#	>400#	>400#	<25#	<25#	>400#
Redroot pigweed	400#	>400#	>400#	<25*	<25*	>400#

Extrapolated values, calculated values were not determined or outside the range tested
* Corrected values

III. Conclusion

Based on the results of this seedling growth study in which the effect of Spiroxamine EC 500 G on two non-crop plant species (velvetleaf and redroot pigweed) was tested under glasshouse conditions, the ER_{50} values for both survival and biomass exceeded the maximum rate tested of 400 g a.s./ha.

Assessment and conclusion by applicant:

Validity criteria according to the current OECD 208 guideline (2006) have been assessed.

- The mean survival of emerged control seedlings is at least 90% for the duration of the study (actual: 100% for both species)
- The seedlings do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and the plants exhibit only normal variation in growth and morphology for that particular species (actual: achieved)
- Environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source (actual: achieved)
- The emergence of velvetleaf in the control was 82.5% of seeds sown (66 seedling emerged from the 81 seeds sown). The emergence of redroot pigweed in the controls was 36.3% of seeds sown (29 seedling emerged from the 80 seeds sown). Thus, the validity criterion of ≥70% emergence was not met for redroot pigweed but this was expected and therefore this criterion is not though to apply here. Additional seedlings were planted so that 5 viable seedlings per pot could be achieved

It is noted that the emergence of redroot pigweed was low but this was fully anticipated prior to the start of the study. The results of the study are considered to be valid and suitable for use in the risk assessment to demonstrate that effects are <50% at a rate of 400 g a.s./ha. The study is therefore considered acceptable.

Data Point:	KCP 10.6.2/03
Report Author:	[redacted]
Report Year:	2001
Report Title:	Spiroxamine EC 500 - Terrestrial plants toxicity, vegetative vigor, tier II
Report No:	TNW71791
Document No:	M-051682-01-1
Guideline(s) followed in study:	OECD 208
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Terrestial non-target plant phytotoxicity was assessed in order to evaluate the toxicity on 6 plant species over a period of 21 days after treatment (determination of EC_{25}, EC_{50} and NOEC values).

The test item was sprayed onto the 2-4 leaf stage plant foliage at various concentrations. During the test the plants were observed for visual phytotoxicity symptoms and total number of dead plants. At the end of the test the shoot height and the dry weight of the shoots were measured.

The most sensitive endpoint was inhibitory effects to soybean dry weight with a NOEC of <150 g a.s./ha. The lowest ER_{50} value determined was 960 g a.s./ha for turnip (dry weight).

ER_{50} values, based on dry weight, for oat, onion, sugar beet, turnip, carrot and soybean were >2400, >2400, 2070, 960, >2400 and 1136 g a.s./ha, respectively. ER_{50} values, based on shoot height, for oat, onion, sugar beet, turnip, carrot and soybean were all >2400 g a.s./ha, respectively.

I. Materials and Methods

Materials

Test Material Spiroxamine EC 500

Lot/Batch #: 05233-00

Purity: Spiroxamine / 485.9 g/L

Description: Brown liquid

Stability of test compound: Not reported

Reanalysis/Expiry date: 17 Aug 2000

Density: Not reported

Treatments

Test rates: 150, 300, 600, 1200 and 2400 g a.s./ha

Solvent/vehicle: None

Analysis of test concentrations: No

Test organisms

Species:	**Monocotyledonae:** *Avena sativa* (oat), *Allium cepa* (onion) **Dicotyledonae:** *Beta vulgaris* (Sugar beet), *Brassica rapa* (turnip), *Daucus carota* (carrot) and *Glycine max* (soybean)
Source:	Heine & Garvens, Wenderter Str. 19, D-31157 Sarstedt, Lochow-Petkus GmbH, Postfach 11 97, D-29296 Bergen, SOLWESTSAAT Gbr. Im Rheinfeld 1-13, D-76437 Rastatt, KWS KLEINWANZLEBENER SAATZUCH AG, D-37555 Einbeck

Test design

Test vessel:	12 cm diameter plastic pots
Test soil:	Certified LUFA soil No. 2.3 (batch-no. Sp2.32300)
Replication:	Six pots
No. animals/vessel:	Five plants per pot
Duration of test:	21 days

Environmental test conditions

Temperature:	15 – 26°C
Relative humidity:	55 – 100%
Photoperiod:	16 h light, 8 h dark. 4680 ± 278

Study Design

Terrestial non-target plant phytotoxicity was assessed in order to evaluate the toxicity on 6 plant species over a period of 21 days after treatment (determination of EC_{25}, EC_{50} and NOEC values).

The Monocotyledonae test species were *Avena sativa* (oat), *Allium cepa* (onion) and the Dicotyledonae test species were *Beta vulgaris* (Sugar beet), *Brassica rapa* (turnip), *Daucus carota* (carrot) and *Glycine max* (soybean). The test item was applied on the plant foliage after the plants had reached a 2-4 leaf stage. The plants were bottom watered and fertilized throughout the test with nutrient solution as needed.

Plants were grown in 12 cm plastic pots at 15 - 26°C under a 16 h light 8 h dark photoperiod at 4680 ± 278 Lux. Each species consisted of six pots of five plants in each.

Test soil was a sandy loam number 2.3. The soil has been stored at the test facility at room temperature (20 ± 5 °C) until use.

Nominal treatment rates were 150, 300, 600, 1200 and 2400 g a.s./ha on the plant foliage along with a water control.

Observations of phytotoxicity were made on days 7, 14 and 21 by visual observations of the plants. Survival and dry weight were determined at test end on day 21 and were checked for statistically significant differences.

H. Results and Discussion

Validity criteria were not specifically assessed as part of the study report but the study was deemed to be valid.

Visual phytotoxicity

The plants were observed on day 7, 14 and 21 for visual phytotoxicity rates.

For oat no test item related phytotoxic effects were observed after 7, 14 and 21 days.

For onion no test item related phytotoxic effects were observed after 7, 14 and 21 days. After 14 days at the concentrations 150 and 600 g a.s./ha, respectively one plant was dead. After 21 days at the concentration 150 g a.s./ha two and at the concentration 600 g a.s./ha one additional plant was dead.

Table CP 10.6.2/03-1 Sugar beet: Phytotoxicity rates after 7, 14 and 21 days

Concentration (g a.s./ha)	Effect	Phytotoxicity rate (%)		
		7 day	14 day	21 day
Control	-	0	0	0
150	-	0	0	0
300	-	0	0	0
600	Chlorosis Necrosis	12 0	<10 0	<10 <10
1200	Necrosis	25	21	21
2400	Necrosis Dead plants	66 0	60 <10	50 <10

Table CP 10.6.2/03-2 Turnip: Phytotoxicity rates after 7, 14 and 21 days

Concentration (g a.s./ha)	Effect	Phytotoxicity rate (%)		
		7 day	14 day	21 day
Control	-	0	0	0
150	-	0	0	0
300	Necrosis	<10	<10	<10
600	Necrosis	27	21	14
1200	Necrosis	54	50	36
2400	Necrosis	71	66	50

Table CP 10.6.2/03-3 Carrot: Phytotoxicity rates after 7, 14 and 21 days

Concentration (g a.s./ha)	Effect	Phytotoxicity rate (%)		
		7 day	14 day	21 day
Control	-	0	0	0
150	-	0	0	0
300	-	0	0	0
600	Necrosis	<10	<10	<10
1200	Necrosis	24	20	15
2400	Necrosis	49	41	33

Table CP 10.6.2/03-4 Soybean: Phytotoxicity rates after 7, 14 and 21 days

Concentration (g a.s./ha)	Effect	Phytotoxicity rate (%)		
		7 day	14 day	21 day
Control	-	0	0	0
150	Necrosis	<10	<10	12
300	Necrosis	<15	<15	13
600	Necrosis	32	26	21
1200	Necrosis	48	41	41
2400	Necrosis	69	67	56

Shoot height

No statistically significant differences of the shoot heights were found for oat and carrot. Statistically significant differences of the shoot heights were found for onion and soybean at concentrations >1200 g a.s./ha, for sugar beet at the concentration 2400 g a.s./ha and for turnip at concentrations >600 g a.s./ha.

Biomass (dry weight)

The biomass was determined as dry weight on day 21.

No statistically significant differences of the dry weight were found for oat and onion at all tested concentrations. Statistically significant differences of the dry weight were found at the concentration 2400 g a.s./ha for sugar beet and carrot, at concentrations ≥300 g a.s./ha for turnip and at all concentrations for soybean.

Inhibition of shoot height and dry weight

Table CP 10.6.2/03-5 Inhibition of shoot height

Concentration (g a.s./ha)	Shoot height (cm)											
	Oat	Inhib. (%)	Onion	Inhib. (%)	Sugar beat	Inhib. (%)	Turnip	Inhib. (%)	Carrot	Inhib. (%)	Soy-bean	Inhib. (%)
Control	43.8	-	21.8	-	13.7	-	16.2	-	27.3	-	30.7	-
150	43.6	0	19.1	12	13.6	0	15.7	3	25.7	6	29.9	2
300	43.7	0	19.8	9	14.3	-4	16.4	-1	26.9	1	29.7	3
600	43.9	0	18.5	15	14.0	-2	13.4	17	25.2	8	28.7	6
1200	46.0	-5	16.6	24	13.1	4	12.0	26	25.1	8	21.4	30
2400	43.5	1	17.3	20	9.2	32	9.8	40	24.6	10	18.3	40

Table CP 10.6.2/03-6 Inhibition of dry weight

Concentration (g a.s./ha)	Dry weight (mg)											
	Oat	Inhib. (%)	Onion	Inhib. (%)	Sugar beat	Inhib. (%)	Turnip	Inhib. (%)	Carrot	Inhib. (%)	Soy-bean	Inhib. (%)
Control	238.5	-	15.5	-	254.1	-	219.3	-	321.8	-	528.3	-
150	198.4	17	13.9	10	256.5	-1	188.5	14	265.6	17	422.7	20

Concentration (g a.s./ha)	Dry weight (mg)											
	Oat	Inhib. (%)	Onion	Inhib. (%)	Sugar beat	Inhib. (%)	Turnip	Inhib. (%)	Carrot	Inhib. (%)	Soy-bean	Inhib. (%)
300	212.7	11	13.8	11	288.2	-13	160.7	27	307.2	5	421.9	20
600	214.5	10	11.8	24	246.5	3	124.3	43	242.7	25	389.7	26
1200	228.4	4	9.8	37	220.4	13	95.0	57	226.2	30	228.3	57
2400	177.3	26	11.0	29	96.2	62	73.9	66	207.6	35	162.9	69

Table CP 10.6.2/03-7 Shoot height: EC_{25} and EC_{50} values with confidence range

Species	EC_{25} (g a.s./ha)	P = 95% (g a.s./ha)	EC_{50} (g a.s./ha)	P = 95% (g a.s./ha)
Oat	>2400	-	>2400	-
Onion	>2400		>2400	-
Sugar beat	2146	1769 – >2400	>2400	-
Turnip	1071	601 – 1908	>2400	-
Carrot	>2400	-	>2400	-
Soybean	1339	981 – 1828	>2400	-

Table CP 10.6.2/03-8 Dry weight: EC_{25} and EC_{50} values with confidence range

Species	EC_{25} (g a.s./ha)	P = 95% (g a.s./ha)	EC_{50} (g a.s./ha)	P = 95% (g a.s./ha)
Oat	2354	1881 – 2944	>2400	-
Onion	926	445 – 1925	>2400	-
Sugar beat	1493	1256 – 1776	2070	1741 – 2461
Turnip	278	184 – 422	960	633 – 1455
Carrot	1073	687 – 1676	>2400	-
Soybean	432	300 – 620	1136	790 – 1632

No effect levels (NOEC)

Table CP 10.6.2/03-9 Shoot height: No effect level concentrations (inhibitory effects)

Species	No observed effect concentration (NOEC) (g a.s./ha)
Oat	2400
Onion	600
Sugar beat	1200
Turnip	300

Species	No observed effect concentration (NOEC) (g a.s./ha)
Carrot	2400
Soybean	600

Table CP 10.6.2/03-10 Dry weight: No effect level concentrations (inhibitory effects)

Species	No observed effect concentration (NOEC) (g a.s./ha)
Oat	1609[#]
Onion	159[#]
Sugar beat	1200
Turnip	150
Carrot	367[#]
Soybean	<150

[#] EC_{10} calculated as NOEC not determinable

III. Conclusion

The test item did not cause phytotoxic effects to oat and onion. Phytotoxic effects were caused at concentrations >600 g a.s./ha for sugar beet and carrot, at concentrations >300 g a.s./ha for turnip and at concentrations >150 g a.s./ha for soybean.

The test item did not cause inhibitory effects in shoot height to oat and carrot in the tested concentration range of 150 - 2400 g a.s./ha.

Effects in shoot height were caused for the plant species sugar beet at the concentrations >1200 g a.s./ha, for the plant species onion and soybean at the concentration >600 g a.s./ha and for the plant species turnip at the concentration >300 g a.s./ha.

Effects in dry weight were caused at concentrations >1609 g a.s./ha for oat, at concentrations >159 g a.s./ha for onion, at concentrations >1200 g a.s./ha for sugar beet, at concentrations >150 g a.s./ha for turnip, at concentrations >367 g a.s./ha for carrot and at all tested concentrations for soybean.

ER_{50} values, based on dry weight, for oat, onion, sugar beet, turnip, carrot and soybean were >2400, >2400, 2070, 960, >2400 and 1136 g a.s./ha, respectively. ER_{50} values, based on shoot height, for oat, onion, sugar beet, turnip, carrot and soybean were all >2400 g a.s./ha, respectively.

Assessment and conclusion by applicant:

The study was conducted to the draft OECD 208 guideline which included vegetative vigour. Validity criteria according to the current OECD 227 (2006) guideline have therefore been assessed were met:

- The seedling emergence is at least 70% (this cannot be confirmed from the study report)
- The control seedlings do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and the plants exhibit only normal variation in growth and morphology for that particular species (actual: no phtotoxicity in controls)
- The mean survival of emerged control seedlings is at least 90% for the duration of the study (achieved)

- Environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source (achieved)

The study is considered to be acceptable. It is noted that the criterion to confirm that the seedlings used to grow the plants for use in the test were viable of ≥70% cannot be confirmed. However, as the necessary number of plants were treated at the start of the test and the survival criteria were met, it is considered that the plants were viable and there is no detrimental impact on the results by not being able to confirm that this criterion was met.

ER_{50} values, based on dry weight, for oat, onion, sugar beet, turnip, carrot and soybean were >2400, >2400, 2070, 960, >2400 and 1136 g a.s./ha, respectively.

Data Point:	KCP 10.6.2/04
Report Author:	[redacted]
Report Year:	2008
Report Title:	Spiroxamine EC 500 G: Effect on the vegetative vigour of two non crop species of non-target terrestrial plants (Tier 2)
Report No:	VV08/002
Document No:	M-302060-01-1
Guideline(s) followed in study:	OECD 227 (July 2006, adopted) modified for a non crop species study
Deviations from current test guideline:	Yes (refer below) It was anticipated that the two species would not meet the criteria for emergence.
Previous evaluation:	yes, evaluated and accepted. RAR (2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The effects of a spray treatment with Spiroxamine EC 500 G on two non-crop non-target plants (velvetleaf and redroot pigweed) were examined over 21 days. Plants at the 2 to 4 leaf stage were sprayed at 200 L/ha, delivering nominally 25, 50, 100, 200 and 400 g a.s./ha to the plants.

Both species treated with Spiroxamine EC 500 G showed slight phytotoxic symptoms of necrosis and stunting after exposure.

The results of this study indicate that exposure to Spiroxamine EC 500 G on two non-crop plant species yielded ER_{50} values for both survival and biomass in excess of 400 g a.s./ha. The most sensitive endpoint was biomass reduction, with a $NOER_{10}$ of 200 and 100 g a.s./ha for velvetleaf and redroot pigweed, respectively.

I. Materials and Methods

Materials

Test Material Spiroxamine EC 500 G

Lot/Batch #: PF90087683

Purity: 49.8% w/w (501 g/L)

Description: Clear brown liquid

Stability of test compound:	Not reported
Reanalysis/Expiry date:	31 January 2010
Density:	1.006 g/mL at 20°C
Treatments	
Test rates:	25, 50, 100, 200 and 400 g a.s./ha
Solvent/vehicle:	None
Analysis of test concentrations:	Analysis of the highest application rate had a recovery of 95.3%
Test organisms	
Species:	Velvetleaf (*Abutilon theophrasti*) and redroot pigweed (*Amaranthus retroflexus*)
Source:	Obtained as untreated seeds from commercial sources *via* Bayer CropScience AG
Test design	
Test vessel:	13 cm diameter plastic pots
Test soil:	Silt loam sieved to 2 mm
Replication:	Eight pots
No. animals/vessel:	Four plants per pot
Duration of test:	21 days
Environmental test conditions	
Temperature:	23 ± 8 °C during the day, 18 ± 8 °C during the night
Relative humidity:	27 – 91%
Photoperiod:	16 h light, 8 h dark. Lighting was natural daylight supplemented by artificial lighting. <15000 lux lamps turn on, >50000 lux shading closes.

Study Design

This study was conducted in order to evaluate the effect of Spiroxamine EC 500 G on the vegetative vigour of two wild dicotyledonous plant species.

Test species were velvetleaf (*Abutilon theophrasti*) and redroot pigweed (*Amaranthus retroflexus*), two non-crop species of different families.

Plants were grown in 13 cm plastic pots in a glasshouse at 23 ± 8 °C during the day and 18 ± 8 °C at night under a 16 h light 8 h dark photoperiod. Each species consisted of eight pots of four plants in each.

Test soil was a silt loam sieved to 2 mm, sterilised with 120°C vapour for 30 minutes and fertilised with 2.4 g Blaukorn per litre. Soil was composed of 59.1% silt, 24.2% clay and 16.7% sand. It had an organic carbon content of 1.30% and a pH of 7.31.

At the 2 to 4 leaf stage, serial dilutions of Spiroxamine EC 500 were sprayed on the plants using a laboratory track sprayer at a rate of 200 L/ha. Nominal test concentrations were 25, 50, 100, 200 and 400 g a.s./ha on the plant foliage along with a water control.

Observations of phytotoxicty were made on days 7, 14 and 21 by visual observations of the plants. Survival and dry weight were determined at test end on day 21.

Analytical method

Samples of water were analysed using the validated analytical method M-302060-01-1, report reference M-302060-01-1 (**see Doc MCP Section 5**).

II. Results and Discussion

The study was deemed to be valid in the study report as the criterion of 90% survival of the control plants was met (100% survival for both species).

The spray chamber was calibrated by weighing the amount of water applied to a known surface area, which gave a mean application volume 92% of nominal. Analysis of the highest application rate revealed the rate to be 95.3% of nominal.

Foliar application of Spiroxamine EC 500 G had no significant impact on the survival of treated velvetleaf plants at any application rates tested in this study. The NOER for survival was therefore 400 g a.s./ha and the ER_{50} value for survival was >400 g a.s./ha.

Shoot dry weight (biomass) was significantly reduced at the highest application rate of 400 g a.s./ha. The NOER with respect to biomass was 200 g a.s./ha and the ER_{50} value for biomass was >400 g a.s./ha.

Slight phytotoxic symptoms visualised as necrosis and stunting were observed at test end at all application rates tested.

There were no effects on growth stage development of treated plants in comparison to the untreated controls at all application rates tested.

Table CP 10.6.2/04-1 Effects of exposure to Spiroxamine EC 500 on velvetleaf

Nominal concentration (g a.s./ha)	Survival (%)	Biomass (g)			
		Mean	SD	% CV	Reduction from control (%)
Control	100	2.268	0.2732	12.0	-
25	100	2.162	0.4046	18.7	4.7
50	100	2.055	0.2810	13.7	9.4
100	100	2.181	0.3269	15.0	3.9
200	100	1.999	0.1952	9.8	11.9
400	100	1.466	0.2962	20.8	35.4*

* Significantly different to the control

Foliar application of Spiroxamine EC 500 G had no significant impact on the survival of treated redroot pigweed plants at any application rates tested in this study. The NOER for survival was therefore 400 g a.s./ha and the ER_{50} value for survival was >400 g a.s./ha.

Shoot dry weight (biomass) was considered as not significantly reduced at all application rates tested. However, the NOER for this endpoint was set at 100 g a.s./ha because of the 32.4% shoot dry weight reduction at the application rate of 200 g a.s./ha which was considered to be biologically relevant. The ER_{50} value for shoot dry weight was >400 g a.s./ha.

Slight to moderate phytotoxic symptoms visualised as necrosis, leaf deformation and stunting were observed at all application rates tested.

There were marginal effects on growth stage development of treated plants in comparison to the untreated controls at all application rates tested.

Table CP 10.6.2/04-2 Effects of exposure to Spiroxamine EC 500 on redroot pigweed

Nominal concentration (g a.s./ha)	Survival (%)	Biomass (g)			
		Mean	SD	% CV	Reduction from control (%)
Control	100	1.456	0.2723	18.7	-
25	100	1.741	0.5969	34.3	-19.6
50	100	1.484	0.4805	32.4	-1.9
100	100	1.351	0.5034	37.2	7.2
200	100	0.984	0.3328	33.8	32.4*
400	100	1.243	0.3277	26.4	14.6*

* Significantly different to the control

A summary of the relevant endpoints is presented in the table below:

Table CP 10.6.2/04-3 Summary of endpoints after exposure to Spiroxamine EC 500 G

Species	Survival (g a.s./L)			Biomass (g a.s./L)		
	NOER	ER_{25}	ER_{50}	NOER	ER_{25}	ER_{50}
Velvetleaf	≥400	>400	>400	200	302.8	>400
Redroot pigweed	≥400	>400	>400	100*	>100	>400

* Corrected value

III. Conclusion

Both species treated with Spiroxamine EC 500 G showed slight phytotoxic symptoms of necrosis and stunting.

The results of this study indicate that exposure to Spiroxamine EC 500 G on two non-crop plant species yielded ER_{50} values for both survival and biomass in excess of 400 g a.s./ha. The most sensitive endpoint was biomass reduction, with a NOER of 200 and 100 g a.s./ha for velvetleaf and redroot pigweed, respectively.

Assessment and conclusion by applicant:

This study was previously evaluated in the DAR (2017), and deemed acceptable.

The study was conducted to the OECD Guideline 227 (2006) "Terrestrial plant test: Vegetative vigour test". Validity criteria according to the OECD 227 (2006) guideline were met:

- Control plant survival ≥90% (actual: 100% both species)
- Control plants to not exhibit visible phytotoxic effects (actual: achieved)

The validity criterion of at least 70% seedling emergence of plants used in the study was anticipated to not be met, and therefore seeds used in the study were not assessed for emergence.

The study is therefore considered to be acceptable and the results suitable for use in the risk assessment.

ER_{50} values for both survival and biomass were determined to be in excess of 400 g a.s./ha.

CP 10.6.3 Extended laboratory studies on non-target plants

No data for extended laboratory studies with non-target terrestrial plants are available. These data are not necessary as an acceptable risk has been demonstrated for the proposed uses of Spiroxamine EC 500 using the available Tier I laboratory data.

CP 10.6.4 Semi-field and field tests on non-target plants

No data for semi-field or field studies with non-target terrestrial plants are available. These data are not necessary as an acceptable risk has been demonstrated for the proposed uses of Spiroxamine EC 500 using the available Tier I laboratory data.

CP 10.7 Effects on other terrestrial organisms (flora and fauna)

All required and available data have been submitted and evaluated in the presented risk assessments. No further data are available or thought to be necessary with other terrestrial organisms.

CP 10.8 Monitoring data

Monitoring of exposure of non-target flora and fauna to spiroxamine has not been conducted. The risk assessments presented in this document demonstrate that there are no unacceptable risks to the environment and non-target organisms when spiroxamine is applied in accordance with the proposed uses of Spiroxamine EC 500.