A14325E

Cyprodinil 300 g/L EC

NOTIFICATION OF AN ACTIVE SUBSTANCE UNDER COMMISSION REGULATION (EU) 844/2012

DOCUMENT M-CP, Section 10

ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
20 May 2016	New data included in response to questions from RMS: Earthworm-eating vertebrate secondary poisoning risk assessment updated using soil accumulation 21-day time-weighted average concentrations Aquatic risk assessment updated using re-modelled surface water concentrations. New RAC values used from the mesocosm study for higher tier refinement of the long-term risk to aquatic invertebrates Some algae statistics updated in order to attempt to derive E_rC_{50} values. Also updated some algae endpoints based on mean measured concentrations. Statistical re-analysis conducted on LR_{50} derived from *Aphidius rhopalosiphi* Tier 1 study. Statistical re-analysis conducted on earthworm reproduction study with A14325E. (All changes highlighted in yellow)	A14325E_10048 9 October 2015 updated 20/5/16
3 February 2017	New data (non-target arthropod (NTA) aged-residue tests) included as recommended by the RMS. NTA risk assessment has been updated (All changes highlighted in green)	A14325E_10048 9 October 2015 updated 20/5/16, 3/2/17

[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 PLANT PROTECTION PRODUCTS

This document supports the application for renewal of the regulatory approval of cyprodinil under Commission Implementing Regulation (EU) 844/2012 of 18 September 2012. This document reviews the ectoxicological studies for the product A14325E containing:

- 300 g/L cyprodinil which was included into Annex I of Council Directive 91/414/EEC (Commission Directive 2006/64/CE of 18 July 2006). This active substance is an approved active substance under Regulation (EC) 1107/2009 (repealing Commission Directive 91/414/EEC) as specified in Commission Implementing Regulation (EU) No. 540/2011 of 25 May 2011.

A14325E is an emulsifiable concentrate (EC) containing 300 g/L cyprodinil for use as a fungicide on barley. A14325E was not a representative formulation in the original EU review of cyprodinil. Representative formulations in the original EU review were UNIX 75 WG (A8779A) and CHORUS 50 WG (A14325E).

In accordance with Commission Implementing Regulation (EU) 844/2012, this document summarises new information which are relevant for the renewal of the approval of cyprodinil under Regulation (EC) 1107/2009. Where appropriate this document refers to the Commission Implementing Regulation (EU) No. 540/2011 for cyprodinil and to the Review Report for cyprodinil (SANCO/4343/2000 final (revised) 28 September 2006), and in particular the endpoints provided in Appendices I and II thereof.

This document covers data and risk assessments which were not part of the original dossier and which are necessary to reflect changes:

- In requirements under Commission Regulation (EU) No 284/2013, and the associated Annex, which repeals Commission Regulation (EU) No 545/2011 which, under Regulation (EC) 1107/2009, replaced the requirements of Annex III to Directive 91/414/EEC
- In scientific and technical knowledge since the approval or last renewal of the approval
- To representative uses

The proposed representative use pattern is included in Document D1.

Each section of this document provides the agreed EU endpoints and if relevant proposals for amended endpoints.

Where new guidance documents have been introduced since the EU review of cyprodinil, an updated evaluation of cyprodinil and A14325E has been included. To adequately assess cyprodinil to the new guidance documents, it may have been necessary to provide new data, if so these are also included.

Information on the detailed composition of A14325E can be found in the confidential dossier of this submission (**Document J**).

Details of all relevant data from the scientific peer reviewed open literature on the active substance, metabolites and breakdown or reaction products and plant protection products containing the active substance have been provided in the **Document M-CA Section 9** and are discussed within the relevant data point of the associated dossier for the active substance, cyprodinil. If the published literature is also relevant to A14325E, it has been discussed within the relevant data point in this document.

Introduction

This section of the submission summarises the ecotoxicological effects of the formulation and evaluates the potential risk to various representatives of terrestrial and aquatic organisms.

Table 10-1: Use pattern of A14325E

Crop	Application method	Spray volume (L/ha)	Maximum individual application rate (g a.s./ha)	Number of applications	Minimum application interval (days)	Application timing
Barley	Spray	150 - 400	450	1-2	14	BBCH 30-61

All Toxicity Exposure Ratios (TERs) and Hazard Quotients (HQs) in the following document are given to 2 significant figures.

Consideration of metabolites

The metabolites that require ecotoxicological assessment according to the EFSA Guidance Documents are given below.

The occurrence and risk from metabolites of cyprodinil have been considered and are discussed in **M-CP Section 9**.

Table 10-2: Metabolites of cyprodinil considered for ecotoxicological risk assessment

Compartment	Metabolites considered for risk assessment
Soil	CGA249287, CGA275535, CGA321915
Surface water	CGA249287, CGA275535, CGA321915, CGA048109 (guanidine), CGA263208 (phenyl guanidine), CA1139A (phenyl guanidine), R008591 (succinic acid), U2, U4,
Sediment	CGA249287

Further information on these metabolites can be found in **M-CA Section 7** for cyprodinil.

The crop metabolism of cyprodinil has been investigated in three crop groups; fruit crops (apple, peach and tomato), root crops (potato) and cereals (wheat), following foliar applications (see MCA Section 6.2.1). It has been concluded that the metabolism pathway is similar in all crops with the parent compound remaining the dominant residue except in potato tubers where the metabolic profile results from the translocation of degradation products through the plant from the soil metabolism of cyprodinil. Where there is a direct contact of cyprodinil with the edible part of the crop, metabolism proceeds mainly via hydroxylation of the phenyl and pyrimidine rings forming metabolites, which then undergo sugar conjugation. Lower levels of other hydroxylated metabolites are also detected. These metabolites are encountered in the rat metabolism and considered covered by the toxicological profile of parent cyprodinil.

Where the edible part of the crop is not exposed to the fungicide spray, metabolism results mainly from the cleavage of the pyrimdine ring with other hydroxylated metabolites identified in both their free and conjugated forms. These potato specific metabolites were not found in the rat metabolism study, but due to the low absolute levels at which they were found in the potato metabolism study, they are not of toxicological relevance.

The metabolism of cyprodinil was investigated in four confined rotational crops studies elucidating the nature of residues following different plant-back intervals. In these studies, cyprodinil radiolabelled in the phenyl or pyrimidinyl ring~~s~~ was applied to bare soil or crops. When radiolabelled cyprodinil was applied on a primary crop at an application rate of 1.25 kg a.s./ha, significant cyprodinil residues were not found in any of the edible parts of the succeeding crops. When cyprodinil was applied to bare soil, the studies identified four major cyprodinil metabolites in the succeeding crops sown at any of the replant intervals. It is concluded that the metabolism of cyprodinil in rotational crops is sufficiently elucidated. Studies on the magnitude of residues in rotational crops confirmed the presence of two plant metabolites which were found at measurable levels at the earliest replanting interval of 30 DAT, whilst parent cyprodinil occurred rarely. However, these metabolites were found to be of no toxicological concern.

The nature of cyprodinil residues in commodities of animal origin was investigated in three metabolism studies, one study in lactating goats and two studies in laying hens, using radiolabelled cyprodinil. The metabolism studies in both ruminants and poultry show that cyprodinil is extensively metabolised and proceeds predominantly via hydroxylation of the phenyl and pyrimidine rings and conjugation with sulphate or glucuronic acid. The majority of the radioactivity was eliminated in the urine and faeces. The four metabolites identified in the studies were all found in the rat metabolism study.

CP 10.1 Effects on Birds and Other Terrestrial Vertebrates

CP 10.1.1 Effects on birds

Toxicity

Summary of endpoints relevant for the risk assessment are presented below:

Table 10.1.1-1: Table of endpoints to assess risk from use of A14325E

Organism	Test type	Endpoint		Endpoints used for the risk assessment	Reference (author, date, Syngenta File No.)
Mallard duck	Acute oral	EU	14 d LD_{50} >500 mg/kg bw	-	***Hakin & Rogers (1992) CGA219417/0062***
Bobwhite quail			14 d LD_{50} > 2000 mg/kg bw	14 d LD_{50} 3776 mg/kg bw [a]	***Hakin & Rogers (1992) CGA219417/0067***
Canary		New	5 d LD_{50} > 5620 mg/kg bw	-	***Hubbard (2015) CGA219417_50779***
Mallard duck	Sub-chronic and reproductive	EU	22 week NOEC = 600 mg/kg diet; NOEL = 102 mg/kg bw	-	***Rogers (1995) CGA219417/0477***
Bobwhite quail		EU	22 week NOEC = 600 mg/kg diet; NOEL = 64 mg/kg bw	64 mg/kg bw	***Rogers (1995) CGA219417/0478***

Note on acute oral studies.

Acute toxicity studies were performed with bobwhite quail and mallard duck. In all cases no mortalities occurred and no toxic symptoms were seen. Regurgitation occurred in the mallard duck study at the two highest dose levels of 1000 and 2000 mg a.s./kg, and the endpoint in the LoEP was set at >500 mg a.s./kg.

The EFSA guidance document states the following:

According to Annex II of Directive 91/414/EEC, the acute oral toxicity of an active substance to a quail species (Japanese quail, Coturnix coturnix japonica or bobwhite quail, Colinus virginianus) or to *mallard duck (Anas platyrhynchos) must be determined. The highest dose used in tests need not normally exceed 2000 mg/kg body weight. Due to issues of regurgitation it is recommended not to use the mallard duck (EFSA, 2007). Where regurgitation or emesis occurs at doses used for risk assessment, additional information is essential to complete the risk assessment. The amount of regurgitated material should be assessed for determination of the ingested dose. In the absence of this information, the lowest overall no observed effect level (NOEL) must be used for risk assessment purposes. Where more than one study has been submitted, the study/studies where no regurgitation has occurred should be used. If, however, mortalities appear in the study in which regurgitation has occurred (at dose levels at or around the LD50 value for the non-regurgitation study), then it is proposed to use the NOEL (for regurgitation or mortality, whichever is lower) from the study where regurgitation has occurred.*

Since no other signs of toxicity other than vomiting were seen in the studies with the mallard, and no effects were seen in the study with the bobwhite quail, it is proposed to use the LD_{50} of >2000 mg a.s./kg.

Cyprodinil metabolites

Since metabolites are formed at <10% of parent level in edible crop parts and mammalian testing indicates that they are less toxic than the parent, it can be concluded that the risk to birds will be low and no further risk assessment is conducted (**cyprodinil; EFSA Scientific Report 51, 2005**).

Exposure

Exposure of birds will be predominantly dietary, through the consumption of residues on food items. Direct exposure of birds to A14325E applications is considered unlikely, since at the time of application and for a short period thereafter, most birds will leave the immediate vicinity of spray operations in response to the human disturbance.

Exposure is calculated according to the **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)**.

Screening step

The Screening step crop groupings and critical use patterns relevant to the uses of A14325E are given in the table below.

Table 10.1.1-2: Screening step crop groupings and critical use patterns relevant to the use of A14325E

Crop group	GAP crop species	Indicator species	Critical use pattern		
			Rate (kg a.s./ha)	No. of apps	App. Interval (days)
Cereals	Barley	Small omnivorous bird	0.450	2	14

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.

$$DDD_{\text{multiple applications}} = \text{application rate (kg a.s./ha) x SV x } MAF_{90}$$

Daily dietary doses for acute exposure to cyprodinil following use of A14325E according to the proposed uses are given in the table below.

Table 10.1.1-3: Screening step – estimates of acute exposure to cyprodinil

Compound	Crop group	Indicator species	Shortcut value (mg/kg bw/day)	App. rate (kg/ha)	No. of apps	App. Interval (days)	MAF	DDD (mg/kg bw/ day)
Cyprodinil	Cereals	Small omnivorous bird	158.8	0.450	2	14	1.2	85.8

The long-term 'daily dietary dose' (DDD) is calculated by multiplying the Shortcut value (SV) based on the mean residues by the application rate in kg a.s./ha.

$DDD_{multiple\ applications}$ = application rate (kg a.s./ha) x SV x f_{twa} x MAF_m

The f_{twa} based upon a default DT_{50} of 10 days is 0.53, as given in the EFSA Guidance Document.

The generic focal species that are relevant for the proposed uses are considered with worst case application rates to calculate long-term DDD values as shown in table below.

Table 10.1.1-4: Screening step - estimates of long-term exposure to cyprodinil

Compound	Crop group	Indicator species	Shortcut value (mg/kg bw/day)	App. rate (kg/ha)	No. of apps	App. Interval (days)	MAF	f_{twa}	DDD (mg/kg bw/day)
Cyprodinil	Cereals	Small omnivorous bird	64.8	0.450	2	14	1.4	0.53	21.6

Tier 1 risk assessment

The Tier 1 assessment initially requires identification of the appropriate crop groupings and generic focal bird species from Annex I of the **EFSA Guidance Document on Risk Assessment for Bird and Mammals**.

The Tier 1 crop groupings and critical use patterns relevant to the uses of A14325E are given in the table below.

Table 10.1.1-5: Tier 1 crop groupings relevant to the use of A14325E

Crop group	GAP crop species	GAP growth stage window (BBCH)	Critical use pattern		
			Rate (kg a.s./ha)	No. of apps	App. Interval (days
Cereals	Barley	BBCH 30-61	0.450	2	14

The generic focal species that are relevant for the proposed uses are considered with worst case application rates to calculate long-term DDD values as shown in table below.

Table 10.1.1-6: Tier 1 – Long-term DDD values for focal species relevant to the use of A14325E

Compound	Crop grouping / growth stage	Generic focal species	Shortcut value (mg a.s/kg bw/day)	App. rate (kg a.s./ha)	No. of apps	MAF	f_{twa}	DDD (mg a.s/kg bw/day)
Cyprodinil	Cereals BBCH 30-39	Small omnivorous bird "lark" Woodlark (*Lullula arborea*)	5.4	0.450	2	1.4	0.53	1.80
	Cereals BBCH ≥40		3.3					1.10

Risks for birds through drinking water

There are two scenarios provided in the EFSA Guidance Document for assessing the risk from drinking water.

The 'Leaf scenario' is relevant for birds taking water that is collected in leaf whorls after application and applies to leafy vegetables forming heads or with a morphology that facilitates collection of rain/irrigation water sufficiently to attract birds.

Since none of the proposed crop uses falls into these categories, the leaf scenario does not apply to the use of A14325E.

Puddle scenario

This scenario is relevant for birds taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This scenario is relevant for all uses of A14325E and should therefore be assessed. The EFSA Guidance Document (ref. 5.5, Step 2b) states the following:

"Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary since the ratio of effective application rate (in g/ha) to acute and long-term endpoint (in mg/kg bw/d) does not exceed 3000 in the case of *more sorptive substances* (Koc > 500 L/kg) as specified in EFSA Guidance Document (ref. 5.5, Step 2b)".

When multiple spray applications are considered, a MAF_m based on the DT_{50} in soil (single first order kinetics, geometric mean as used for PEC_{gw} and PEC_{sw}) may be applied to calculate the effective application rate $AR_{eff.}$

$$AR_{eff} = AR \times MAF_m = AR \times \frac{1 - e^{-nki}}{1 - e^{-ki}}$$

Where:

AR = application rate [g/ha]
k = ln(2)/DT_{50} (rate constant)
n = number of applications
i = application interval (d)

Table 10.1.1-7: Acute risk to birds from drinking water – puddle scenario

Crop group	Compound	Soil DT_{50} (days)	K_{oc} [L/kg]	AR [g a.s./ha]	MAF_m	AR_{eff} [a] [mg a.s./m^2]	LD_{50} [mg a.s./kg bw]	Ratio (AR_{eff} / LD_{50})	No concern ratio
Cereals	Cyprodinil	35.5 ~~33.6~~	1706	450	1.76 ~~1.75~~	79.2 ~~78.8~~	3776	0.021	≤3000
		114.2 ~~118.9~~ [b]			1.92	86.4		<0.043	

[a] The application rate is divided by 10 to convert from g/ha to mg/m^2
[b] this represents the DT_{50} in acidic soils

Table 10.1.1–8: Long-term risk to birds from drinking water – puddle scenario

Compound	Compound	Soil DT_{50} (days)	K_{oc} [L/kg]	AR [g a.s./ha]	MAF_m	AR_{eff} [a] [mg a.s./m^2]	NOEL [mg a.s./kg bw]	Ratio (AR_{eff} / NOEL)	No concern ratio
Cereals	Cyprodinil	35.5 ~~33.6~~	1706	450	1.76 ~~1.75~~	79.2 ~~78.8~~	64	1.24 ~~1.23~~	≤3000
		114.2 ~~118.9~~ [b]			1.92	86.4		1.19	

[a] The application rate is divided by 10 to convert from g/ha to mg/m^2
[b] this represents the DT_{50} in acidic soils

The ratios of the application rates to the toxicity endpoints are clearly less than 3000 indicating low concern for acute and long-term exposure to birds in drinking water from puddles, and no need to carry out further calculations of exposure in puddle water.

Risk assessment for birds

CP 10.1.1.1 Acute oral toxicity

Acute risk is assessed by comparing the relevant DDD from Table 10.1-3 with the appropriate LD_{50} endpoint (summarised in Tables 10.1-1) to give an acute Toxicity: Exposure Ratio (TER_A):

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

The resulting TER_A values for each crop grouping are given in the table below.

Table 10.1.1-9: Screening step - Acute risk (TER_A) to birds from cyprodinil

Compound	Crop group	Indicator species	LD_{50} (mg/kg bw)	DDD (mg a.s./kg bw/day)	TER_A
Cyprodinil	Cereals	Small omnivorous bird	3776	85.8	44

The TER_A value is greater than the Commission Regulation (EU) No. 546/2011 trigger of 10, indicating that acute risk to birds is acceptable following use of A14325E according to this use pattern.

Acute risk assessment to birds through drinking water

Cyprodinil has negligible potential for acute exposure of birds to drinking water (see Table 10.1.1-7).

Long-term toxicity exposure ratio (TER_{LT})

Derivation of the short-term toxicity exposure ratio is no longer a requirement according to **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)** so no short-term risk assessment is presented.

Long-term risk is assessed by comparing the long-term DDD with the worst case NOEC from the reproduction studies, expressed as daily dietary dose, to give a Long-term Toxicity:Exposure Ratio (TER_{LT}):

$$TER_{LT} = \frac{LD_{50}\ (mg/kgbw/day)}{DDD(mg/kgbw/day)}$$

The EFSA Guidance Document indicates that the acute LD_{50}/10 should be used as an endpoint in long-term risk assessment where it is lower than the long-term endpoint.

The endpoint of 64 mg/kg bw/day from a reproduction study has been used in calculations of the TER values since this is lower than the LD_{50}/10 value for the acute LD_{50} endpoint used in the acute risk assessment.

Screening step risk assessment

The TER value calculated for the crop grouping relevant for the use of A14325E is given below:

Table 10.1.1-10: Screening step – long-term (TER_{LT}) risk to birds from cyprodinil

Compound	Crop group	Indicator species	NOEL (mg a.s./kg bw/day)	DDD (mg a.s./kg bw/day)	TER_{LT}
Cyprodinil	Cereals	Small omnivorous bird	64	21.6	**3.0**

The TER_{LT} value is lower than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating a potential long-term risk to birds following use of A14325E according to the proposed use pattern. A tier I risk assessment is presented below.

Table 10.1.1-11: Tier 1 – long-term risk (TER_A) to birds from cyprodinil

Compound	Crop grouping / growth stage	Generic focal species	LD_{50} (mg/kg bw)	DDD (mg a.s/kg bw/day)	TER_{LT}
Cyprodinil	Cereals BBCH 30-39	Small omnivorous bird “lark” Woodlark (*Lullula arborea*)	64	1.80	36
	Cereals BBCH ≥40			1.10	58

The tier I TER_{LT} values are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to birds is acceptable following use of A14325E according to this use pattern.

Long-term risk assessment to birds through drinking water

Cyprodinil has negligible potential for long-term exposure of birds to drinking water (see Table 10.1.1-8).

Effects of secondary poisoning

According to **EFSA Guidance Document on Risk Assessment for Birds and Mammals, 2009**, substances with a log P_{OW} greater than 3 have potential for bioaccumulation. Cyprodinil has a log P_{OW} of 4.0 indicating a potential risk of secondary poisoning therefore a risk assessment is provided.

Risk to earthworm-eating birds

A risk assessment of the risk of secondary poisoning to earthworm-eating birds is conducted using the following equation:

$$TER = \frac{NOEL\,(mg/kg\,bw/day)}{PEC_{worm}\,(mg/kg) \times 1.05}$$

Where:

PEC_{worm} = 21 d time-weighted average $PEC_{soil} \times BCF$
$BCF = C_{worm}/C_{soil} = (0.84 + 0.012\,K_{ow}) / f_{oc} \times K_{oc}$
K_{ow} = Octanol water partition coefficient
K_{oc} = Organic carbon adsorption coefficient
f_{oc} = Organic carbon content of soil (0.02 taken as a default value)

1.05 is a constant used to convert the PEC_{worm} to a daily dose and is based on a 100 g bird eating 104.6 g of worms per day (**Smit 2005** in EFSA Guidance).

The 21-day time-weighted average soil accumulation PEC was used. For details of soil PEC calculations, see the supporting **Document M-CP Section 9**.

The resulting TER value is given in the table below:

Table 10.1.1-12: Long-term risk from secondary poisoning to earthworm-eating birds

Compound	21-day twa $PEC_{s, accum}$ (mg/kg)	K_{ow}	f_{oc}	Koc	BCF	PEC_{worm} (mg/kg)	DDD (mg/kg bw/d)	NOEL (mg/kg bw/d)	TER_{worm}
Cyprodinil	0.272 ~~0.230~~	10000	0.02	1706	3.54	0.96 ~~0.81~~	1.01 ~~0.86~~	64	63 ~~75~~

The TER value exceeds the long-term trigger value of 5, indicating that A14325E poses an acceptable risk to earthworm eating birds.

The main soil metabolites of cyprodinil (CGA249287, CGA275535 and CGA321915) are of low acute oral toxicity to mammals (rat acute oral LD_{50} >2000 mg/kg; refer to **Cyprodinil; EFSA Scientific Report 51, 2005**). Highest log P_{OW} values for CGA249287 and CGA321915 are 1.5 and -0.10, respectively indicating low potential for bioaccumulation. CGA275535 has a log P_{OW} value above 3 (log P_{OW} 3.3 at pH 7.0). Given that the metabolites will be found at lower concentrations than the parent active substance, the risk assessment for the parent is considered to cover the metabolites.

Risk to fish eating birds

A risk assessment of the risk from secondary poisoning to fish-eating birds is conducted using the following equation:

TER = long-term NOEC as daily dietary dose / (PEC_{fish} x 0.159)

Where: $PEC_{fish} = PEC_{water\ (highest\ 3\ wk\ twa)} * BCF_{(whole\ body)}$

The factor of 0.159 is based on a 1000 g bird eating 159 g per day (**Smit, 2005** in EFSA Guidance), and converts the PEC_{fish} to a daily dose.

The worst case Step 3 21-day time-weighted average surface water PECs following use of A14325E after 2 applications in winter wheat were used. For details of surface water PEC calculations, see the supporting **Document M-CP Section 9**.

The resulting TER value is given in the table below:

Table 10.1.1-13: Long-term risk from secondary poisoning to fish-eating birds

Substance	PEC_{water} 21 day TWA (mg/L)	BCF	PEC_{fish} (mg/kg)	DDD (mg/kg/bw/ day)	NOEL (mg/kg bw/day)	TER_{fish}
Cyprodinil	0.0185	400	7.40	1.18	64	54

The TER value exceeds the long-term trigger value of 5, indicating that A14325E poses an acceptable risk to fish eating birds.

Conclusion

The risk assessment indicates that A14325E poses an acceptable risk to birds from secondary poisoning following the proposed use.

Biomagnification in terrestrial food chains

The results from adsorption, distribution, metabolism and excretion (ADME) studies did not indicate a potential for cyprodinil accumulation, as the tissue residues 7 days after application were always <1% of applied dose (refer to the respective **EFSA Scientific Reports for cyprodinil and fludioxonil**).

Also, fish bioaccumulation studies showed rapid depuration of residues of both the parent active substances and major metabolites formed (see Annex Point IIIA 10.2.4).

CP 10.1.1.2 Higher tier data on birds

No other higher tier data on birds are required as the risk assessment presented above indicates an acceptable risk from the supported uses of A14325E.

Relevant Literature on Birds

No scientifically peer-reviewed open literature could be found on A14325E. Details of the literature search undertaken can be found in **M-CA Section 9**.

CP 10.1.2 Effects on terrestrial vertebrates other than birds

Toxicity

Summary of endpoints relevant for risk assessment:

Table 10.1.2-1: Table of endpoints to assess risk from use of A14325E

Test type	Test item	Organism	EU endpoint	Proposed endpoint for risk assessment	Reference (author, date, Syngenta File No.)
Acute	A14325E	Rat	LD_{50} >2000 mg/kg bw	LD_{50} >2000 mg/kg bw	***Straube (2005) CGA219417/1325***
Acute	Cyprodinil	Rat	LD_{50} >2000 mg/kg bw	LD_{50} >2000 mg/kg bw	***Hartmann (1992) CGA219417/0020***
Sub-chronic and reproductive	Cyprodinil	Rat	NOAEL = 72.7 mg/kg bw/day [a]	NOAEL = 72.7 mg/kg bw/day	***Khalil (1993) CGA219417/0162***

[a] The lowest overall mean value was calculated from all of the mean weekly consumption values for the individual sexes (72.7 mg/kg bw/day for males and 96.6 mg/kg bw/day for females)

Cyprodinil metabolites

Exposure of mammals will be predominantly dietary, through the consumption of residues on food items. Direct exposure of mammals to A14325E applications is considered unlikely, since at the time of application and for a short period thereafter, most mammals will leave the immediate vicinity of spray operations in response to the human disturbance.

Exposure is calculated according to the **EFSA Guidance Document on Risk Assessment for Birds and Mammals, 2009**.

Screening step

The Screening step crop groupings and critical use patterns relevant to the uses of A14325E are given in the table below.

Table 10.1.2-2: Screening step crop groupings and critical use patterns relevant to the use of A14325E

Crop group	GAP crop species	Indicator species	Critical use pattern		
			Rate (kg a.s./ha)	No. of apps	App. Interval (days)
Cereals	Barley	Small herbivorous mammal	0.450	2	14

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

$$DDD_{\text{multiple applications}} = \text{application rate (kg as/ha) x SV x } MAF_{90}$$

Daily dietary doses for acute exposure to cyprodinil following use of A14325E according to the various crop groups are given in the table below.

Table 10.1.2-3: Screening step – estimates of acute exposure to cyprodinil

Compound	Crop group	Indicator species	Shortcut value (mg/kg bw/day)	App. rate (kg/ha)	No. of apps	App. Interval (days)	MAF	DDD (mg/kg bw/ day)
A14325E	Cereals	Small herbivorous mammal	118.4	1.52 [a]	2	14	1.2	216
Cyprodinil				0.450				63.9

[a] Based on 0.450 kg a.s./ha (1.5 L formulation/ha). A14325E is a 300 g/L formulation with a density of 1.012 g/mL

Tier 1 risk assessment

For the acute risk assessment, the TER_A value for A14325E at the screening step is less than the relevant trigger value and so a Tier 1 assessment is required.

The Tier 1 assessment initially requires identification of the appropriate crop groupings and generic focal mammal species in Annex I of the EFSA Guidance Document on Bird and Mammal risk assessment.

The Tier 1 crop groupings and critical use patterns relevant to the uses of A14325E are given in the table below.

Table 10.1.2-4: Tier 1 crop groupings relevant to the use of A14325E

Crop group	GAP crop species	GAP growth stage window (BBCH)	Critical use pattern		
			Rate (kg a.s./ha)	No. of apps	App. Interval (days
Cereals	Barley	BBCH 30-61	0.450	2	14

The generic focal species that are relevant for the proposed uses are considered with worst case application rates to calculate long-term DDD values as shown in table below.

Table 10.1.2-5: Tier 1 – Acute DDD values for focal species relevant to the use of A14325E

Compound	Crop grouping / growth stage	Generic focal species	Shortcut value (mg/kg bw/day)	App. rate (kg/ha)	No. of apps	App. Interval (days)	MAF	DDD (mg/kg bw/day)
A14325E	Cereals BBCH ≥20	Small insectivorous mammal "shrew" (*Sorex araneus*)	5.4	1.52	2	14	1.2	9.85
	Cereals BBCH ≥40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	40.9					74.6
	Cereals BBCH 30-39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	8.6					15.7
	Cereals BBCH ≥40		5.2					9.48

The long-term 'daily dietary dose' (DDD) is calculated by multiplying the Shortcut value (SV) based on the mean residues by the application rate in kg a.s./ha.

$DDD_{multiple\ applications}$ = application rate (kg a.s./ha) x SV x f_{twa} x MAF_m

The f_{twa} based upon a default DT_{50} of 10 days is 0.53, as given in the EFSA Guidance Document.

The generic focal species that are relevant for the proposed uses are considered with worst case application rates to calculate long-term DDD values as shown in table below.

Table 10.1.2-6: Screening step – estimates of long-term exposure to cyprodinil

Compound	Crop group	Indicator species	Shortcut value (mg/kg bw/day)	App. rate (kg/ha)	No. of apps	App. Interval (days)	MAF	f_{twa}	DDD (mg/kg bw/day)
Cyprodinil	Cereals	Small herbivorous mammal	48.3	0.450	2	14	1.4	0.53	16.1

Tier 1 risk assessment

For the long-term risk assessment, the TER_{LT} value for cyprodinil at the screening step is less than the relevant trigger values and so a Tier 1 assessment is required.

The Tier 1 assessment initially requires identification of the appropriate crop groupings and generic focal mammal species in Annex I of the EFSA Guidance Document on Bird and Mammal risk assessment.

The Tier 1 crop groupings and critical use patterns relevant to the uses of A14325E are given in the table below.

Table 10.1.2-7: Tier 1 crop groupings relevant to the use of A14325E

Crop group	GAP crop species	GAP growth stage window (BBCH)	Critical use pattern		
			Rate (kg a.s./ha)	No. of apps	App. Interval (days
Cereals	Barley	BBCH 30-61	0.450	2	14

The generic focal species that are relevant for the proposed uses are considered with worst case application rates to calculate long-term DDD values as shown in table below.

Table 10.1.2-8: Tier 1 – Long-term DDD values for focal species relevant to the use of A14325E

Compound	Crop grouping / growth stage	Generic focal species	Shortcut value (mg/kg bw/day)	App. rate (kg/ha)	No. of apps	App. Interval (days)	MAF	f_{twa}	DDD (mg/kg bw/day)
Cyprodinil	Cereals BBCH ≥20	Small insectivorous mammal "shrew" (*Sorex araneus*)	1.9	0.45	2	14	1.4	0.53	0.634
	Cereals BBCH ≥40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)	21.7						7.25
	Cereals BBCH 30-39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)	3.9						1.30
	Cereals BBCH ≥40		2.3						0.768

Exposure to mammals through drinking water

Only the puddle scenario is relevant for risk assessment for mammals through drinking water.

Puddle scenario

The EFSA Guidance Document states:

"Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary since the ratio of effective application rate (in g/ha) to acute and long-term endpoint (in mg/kg bw/d) does not exceed 3000 in the case of *more sorptive substances* (Koc > 500 L/kg) as specified in EFSA Guidance Document (ref. 5.5, Step 2b)".

When multiple spray applications are considered, a MAF_m based on the DT_{50} in soil (single first order kinetics, geometric mean as used for PEC_{gw} and PEC_{sw}) may be applied to calculate the effective application rate $AR_{eff.}$

$$AR_{eff} = AR \times MAF_m = AR \times \frac{1-e^{-nki}}{1-e^{-ki}}$$

Where:

AR = application rate [g/ha]
k = ln(2)/DT_{50} (rate constant)
n = number of applications
i = application interval (d)

Table 10.1.2-9: Acute risk to mammals from drinking water – puddle scenario

Crop group	Compound	Soil DT_{50} (days)	K_{oc} [L/kg]	AR [g a.s./ha]	MAF_m	AR_{eff} [a] [mg a.s./m^2]	LD_{50} [mg a.s./kg bw]	Ratio (AR_{eff} / LD_{50})	No concern ratio
Cereals	Cyprodinil	35.5 ~~33.6~~	1706	450	1.76 ~~1.75~~	79.2 ~~78.8~~	>2000	<0.040 ~~<0.039~~	≤3000
		114.2 ~~118.9~~ [b]			1.92	86.4		<0.043	

[a] The application rate is divided by 10 to convert from g/ha to mg/m^2
[b] this represents the DT_{50} in acidic soils

Table 10.1.2-10: Long-term risk to mammals from drinking water – puddle scenario

Compound	Compound	Soil DT_{50} (days)	K_{oc} [L/kg]	AR [g a.s./ha]	MAF_m	AR_{eff} [a] [mg a.s./m^2]	NOEL [mg a.s./kg bw]	Ratio (AR_{eff} / NOEL)	No concern ratio
Cereals	Cyprodinil	35.5 ~~33.6~~	1706	450	1.76 ~~1.75~~	79.2 ~~78.8~~	72.7	1.09 ~~1.08~~	≤3000
		114.2 ~~118.9~~ [b]			1.92	86.4		1.19	

[a] The application rate is divided by 10 to convert from g/ha to mg/m^2
[b] this represents the DT_{50} in acidic soils

The ratios of the application rates to the toxicity endpoints are below 3000 indicating low concern for acute and long-term exposure to birds in drinking water from puddles, and no need to carry out further calculations of exposure in puddle water.

Risk assessment for other terrestrial vertebrates

Acute toxicity exposure ratio (TER_A)

The acute risk to mammals was assessed by calculation of toxicity exposure ratios (TER_A) according to the following equation:

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

Acute risk was calculated using the lowest acute LD_{50} values for the active substances. According to the Commission Regulation (EU) No. 546/2011, a TER_A value below 10 indicates a potential acute risk to mammals. The results are presented below.

Table 10.1.2-11: Screening step - Acute risk (TER_A) to mammals from A14325E and cyprodinil

Compound	Crop group	Indicator species	LD_{50} (mg/kg bw)	DDD (mg a.s./kg bw/day)	TER_A
A14325E	Cereals	Small herbivorous mammal	>2000	216	**>9.3**
Cyprodinil			>2000	63.9	>31

For cyprodinil the TER_A value is greater than the Commission Regulation (EU) No. 546/2011 trigger of 10, indicating that acute risk to mammals is acceptable following use of A14325E according to the proposed use pattern. For A14325E, however, this was not the case, indicating that a Tier 1 risk assessment is required.

Tier 1 risk assessment

The Tier 1 TER values calculated for cyprodinil are given in the table below.

Table 10.1.2-12: Tier 1 – Acute TER values for focal species relevant to the use of A14325E

Compound	Crop grouping / growth stage	Generic focal species	LD_{50} (mg/kg bw)	DDD (mg a.s./kg bw/day)	TER_A
A14325E	Cereals BBCH ≥20	Small insectivorous mammal "shrew" (*Sorex araneus*)	>2000	9.85	>200
	Cereals BBCH ≥40	Small herbivorous mammal "vole" Common vole (*Microtus arvalis*)		74.6	>27
	Cereals BBCH 30-39	Small omnivorous mammal "mouse" Wood mouse (*Apodemus sylvaticus*)		15.7	>130
	Cereals BBCH ≥40			9.48	>210

The TER_A values are greater than the Regulation (EU) 546/2011 trigger of 10, indicating that acute risk to mammals is acceptable following use of A14325E according to the proposed use pattern.

Acute risk assessment to birds through drinking water

Cyprodinil has negligible potential for acute exposure of mammals to drinking water (see Table 10.1.2-9).

Long-term toxicity exposure ratio (TER_{LT})

According to the **EFSA Guidance Document on Risk Assessment for Birds and Mammals 2009**, short-term risk to mammals is not presented as it is covered by the long-term risk assessment.

The long-term risk to mammals was assessed from long-term TER values, calculated according to the following equation:

$$TER_{LT} = \frac{NOEC(mg/kg\,bw/day)}{Long\text{-}term\,DDD(mg/kg\,bw/day)}$$

The lowest NOEL values for cyprodinil were used to calculate the TER values in order to provide a worst-case scenario. The resulting TER_{LT} values are given below.

Table 10.1.2-13: Screening step - long-term risk (TER_{LT}) to mammals

Compound	Crop group	Indicator species	NOEL (mg a.s./kg bw/day)	DDD (mg a.s./kg bw/day)	TER_{LT}
Cyprodinil	Cereals	Small herbivorous mammal	72.7	16.1	**4.5**

The TER_{LT} value is lower than the Commission Regulation (EU) No. 546/2011 trigger value of 5, indicating that a Tier 1 risk assessment is required.

Tier 1 risk assessment

The Tier 1 TER values calculated for cyprodinil are given in the table below.

Table 10.1.2-14: Tier 1 - long-term TER values for focal species relevant to the use of A14325E

Compound	Crop grouping / growth stage	Generic focal species	NOEL (mg a.s./kg bw/day	DDD (mg/kg bw/day)	TER_{LT}
Cyprodinil	Cereals BBCH ≥20	Small insectivorous mammal “shrew” (*Sorex araneus*)	72.7	0.634	110
	Cereals BBCH ≥40	Small herbivorous mammal “vole” Common vole (*Microtus arvalis*)		7.25	10
	Cereals BBCH 30-39	Small omnivorous mammal “mouse” Wood mouse (*Apodemus sylvaticus*)		1.30	56
	Cereals BBCH ≥40			0.768	95

The TER_{LT} values are greater than the Regulation (EU) 546/2011 trigger of 5, indicating that long-term risk to mammals is acceptable following use of A14325E according to the proposed use pattern.

Long-term risk assessment to mammals through drinking water

Cyprodinil has negligible potential for long-term exposure of mammals to drinking water (see Table 10.1.2-10).

Effects on secondary poisoning

According to **EFSA Guidance Document on Risk Assessment for Birds and Mammals, 2009**, substances with a log P_{OW} greater than 3 have potential for bioaccumulation. Cyprodinil has a log Pow of 4.0, indicating a potential risk of secondary poisoning therefore a risk assessment is provided.

Risk to earthworm eating mammals

A risk assessment of the risk of secondary poisoning to earthworm-eating mammals is conducted using the following equation:

$$TER = \frac{\text{NOEL (mg/kg)}}{PEC_{worm}\text{ (mg/kg)} \ \ x\,1.28}$$

Where:

PEC_{worm} = 21 d time-weighted average $PEC_{soil} \times BCF$
$BCF = C_{worm}/C_{soil} = (0.84 + 0.012\ K_{ow}) / f_{oc} \times K_{oc}$
K_{ow} = Octanol water partition coefficient
K_{oc} = Organic carbon adsorption coefficient
f_{oc} = Organic carbon content of soil (0.02 taken as a default value)

1.28 is a constant used to convert the PEC_{worm} to a daily dose and is based on a 10 g mammal eating 12.8 g of worms per day (**Smit 2005** in EFSA Guidance).

The 21-day time-weighted average soil accumulation PEC was used. For details of soil PEC calculations, see the supporting **Document M-CP Section 9**.

The resulting TER value is given in the table below:

Table 10.1.2-15: Long-term risk from secondary poisoning to earthworm-eating mammals

Compound	PEC s, accum (mg/kg)	K_{ow}	f_{oc}	Koc	BCF	PEC_{worm} (mg/kg)	DDD (mg/kg bw/d)	NOEL (mg/kg bw/d)	TER_{worm}
Cyprodinil	0.272 ~~0.230~~	10000	0.02	1706	3.54	0.96 ~~0.81~~	1.23 ~~1.04~~	72.7	59 ~~70~~

The TER value exceeds the long-term trigger value of 5, indicating that A14325E poses an acceptable risk to earthworm eating mammals.

The main soil metabolites of cyprodinil (CGA249287, CGA275535 and CGA321915) are of low acute oral toxicity to mammals (rat acute oral LD_{50} >2000 mg/kg; refer to **Cyprodinil; EFSA Scientific Report 51, 2005**). Highest log P_{OW} values for CGA249287 and CGA321915 are 1.5 and -0.10, respectively indicating low potential for bioaccumulation. CGA275535 has a log P_{OW} value above 3 (log P_{OW} 3.3 at pH 7.0). Given that the metabolites will be found at lower concentrations than the parent active substance, the risk assessment for the parent is considered to cover the metabolites.

Risk to fish eating mammals

A risk assessment of the risk from secondary poisoning to fish-eating mammals is conducted using the following equation:

TER = long-term NOEC as daily dietary dose / (PEC_{fish} x 0.142)

Where: $PEC_{fish} = PEC_{water\ (highest\ 3\ wk\ twa)} * BCF_{(whole\ body)}$

The factor of 0.142 is based on a 3000 g mammal eating 425 g fish per day (**Smit, 2005** in EFSA Guidance), and converts the PEC_{fish} to a daily dose.

The worst case Step 3 21-day time-weighted average surface water PECs following use of A14325E after 2 applications in winter wheat were used. For details of surface water PEC calculations, see the supporting **Document M-CP Section 9**.

The resulting TER value is given in the table below:

Table 10.1.2-16: Long-term risk from secondary poisoning to fish-eating mammals

Substance	PEC_{water} 21 day TWA (mg/L)	BCF	PEC_{fish} (mg/kg)	ETE (mg/kg/bw/day)	Long-term NOEL (mg/kg bw/day)	TER_{fish}
Cyprodinil	0.0185	400	7.40	1.05	72.7	69

The TER value exceeds the long-term trigger value of 5, indicating that A14325E poses an acceptable risk to fish eating mammals.

Conclusion

The risk assessment indicates that A14325E poses an acceptable risk to mammals from secondary poisoning following the proposed use.

Biomagnification in terrestrial food chains

The results from adsorption, distribution, metabolism and excretion (ADME) studies did not indicate a potential for cyprodinil accumulation, as the tissue residues 7 days after application were always <1% of applied dose (refer to the respective **EFSA Scientific Reports for cyprodinil**).

Also, fish bioaccumulation studies showed rapid depuration of residues of cyprodinil and major metabolites formed (see Annex Point IIIA 10.2.4).

CP 10.1.2.1 Acute oral toxicity to mammals

A mammalian toxicity study, performed on A14325E has been conducted. The title and reference to this study is presented in the report box below. The endpoints are summarised in Table 10.1.2-1 above and discussed in **M-CP, Section 7**.

Report:	K-CP 10.1.2.1/01 Straube E. (2005) CGA219417 300 g/l EC formulation (A14325E): Acute Oral Toxicity Study in the Rat (Up and Down Procedure). RCC Ltd., Toxicology, Wölferstrasse 4, CH-4414 Füllinsdorf, Switzerland. Laboratory Report No. 859442, 23 June 2005. Unpublished. (Syngenta File No. CGA219417/1325)

The acute oral LD_{50} of CGA219417 300 g/l EC formulation (A14325E) to female rats is in excess of 2000 mg/kg bw.

CP 10.1.2.2 Higher tier data on mammals

No other higher tier data on mammals are required as the risk assessment presented above indicates an acceptable risk from the supported uses of A14325E.

Relevant Literature on Wild Mammals

No scientifically peer-reviewed open literature could be found on A14325E. Details of the literature search undertaken can be found in **M-CA Section 9**.

CP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

Toxicity

Summary of endpoints relevant for risk assessment:

Table 10.1.3-1: Table of endpoints to assess risk from use of A14325E

Organism	Test item	Test type	Endpoint	Reference (author, date, Syngenta File No.)
Xenopus laevis	Cyprodinil	Acute	LC_{50} = 12.3 mg/L	***Zhao (2009) CGA219417_11635***

Risk assessment

Guidance on the risk assessment for other terrestrial vertebrate wildlife has yet to be developed. An endpoint is available for effects of cyprodinil on the aquatic phase of *Xenopus laevis* (i.e. tadpoles) so it is appropriate to use surface water concentrations and to derive a Toxicity Exposure Ratio (TER). The

TER_A value has been derived using the worst case FOCUS Step 2 PEC_{SW} value and the result is presented below.

Table 10.1.3-2: Amphibian acute TER value for cyprodinil

Test organism	Test substance	LC_{50} (µg/L)	PEC_{SW} (µg/L)	TER_A	Trigger value
Xenopus laevis	Cyprodinil	12 300	20	620	100

The TER_A value is greater than the trigger indicating that A14325E would pose an acceptable acute risk to amphibian larvae when applied according to proposed use patterns. In addition, there is currently no guidance addressing terrestrial life stages of amphibians and reptiles in PPP risk assessments. Therefore, the risk assessment provided above for birds and mammals is considered to be protective of terrestrial amphibian and reptile species.

Relevant literature on other terrestrial vertebrate wildlife (reptiles and amphibians)

No scientifically peer-reviewed open literature could be found on A14325E. Details of the literature search undertaken can be found in **M-CA Section 9**.

CP 10.2 Effects on Aquatic Organisms

Risk assessment for aquatic organisms

Table 10.2-1: Aquatic vertebrate toxicity data for A14325E and cyprodinil

Organism	Test item	Endpoint (mg/L)		Proposed endpoint for risk assessment (mg/L)	Reference (author, date, Syngenta File No.)
Acute					
Rainbow trout (*Oncorhynchus mykiss*)	A14325E	New	96 h LC_{50} = $6.8_{(nom)}$	96 h LC_{50} = 6.8	***Volz (2005) CGA219417/1354***
Sheepshead minnow *(Cyprinodon variegatus)*	Cyprodinil		96 h LC_{50} = $1.25_{(mm)}$	96 h LC_{50} = 1.25	***Ward et al. (1995) CGA219417/0652***
Xenopus laevis tadpoles			96 h LC_{50} = 12.3	96 h LC_{50} = 12.3	***Zhao (2009) CGA219417_11635***
Chronic					
Fathead minnow *(Pimephales promelas)*	Cyprodinil	EU	NOEC = $0.231_{(mm)}$	NOEC = $0.231_{(mm)}$	***Ward et al. (1995) CGA219417/0653***
Sheepshead minnow		New	NOEC (growth) = $0.0406_{(mm)}$	NOEC (growth) = 0.0406	***Minderhout et al. (2014) CGA219417_50676***

nom = Endpoint derived using nominal concentration
mm = Endpoint derived using mean measured concentration
'New' refers to an endpoint from a study conducted since the previous submission of cyprodinil or a study which was not previously submitted

Table 10.2-2: Aquatic invertebrate data for A14325E and cyprodinil

Organism	Test item	Endpoints (mg/L)		Proposed endpoint for risk assessment (mg/L)	Reference (author, date, Syngenta File No.)
Acute					
Daphnia magna	A14325E	New	48 h EC_{50} = $0.37_{(nom)}$	48 h EC_{50} = 0.37	***Volz (2005) CGA219417/1357***
	Cyprodinil	EU	48 h EC_{50} = $0.033_{(mm)}$	48 h EC_{50} = $0.033_{(mm)}$	***Boeri et al. (1995) CGA219417/0461***
Daphnia longispina			48 h EC_{50} = $0.22_{(mm)}$	48 h EC_{50} = $0.22_{(mm)}$	***Peither (2000) CGA219417/0993***
Daphniopsis sp.			24 h EC_{50} = $0.21_{(mm)}$	24 h EC_{50} = $0.21_{(mm)}$	***Peither (2000) CGA219417/0990***
Simocephalus vetulus			48 h EC_{50} = $0.15_{(mm)}$	48 h EC_{50} = $0.15_{(mm)}$	***Peither (2000) CGA219417/0994***
Gammarus sp.			48 h EC_{50} = $1.8_{(mm)}$	48 h EC_{50} = $1.8_{(mm)}$	***Peither (2000) CGA219417/0998***
Thamnocephalus platyurus			24 h EC_{50} = $0.12_{(mm)}$	24 h EC_{50} = $0.12_{(mm)}$	***Peither (2000) CGA219417/0991***
Ostracoda sp.			48 h EC_{50} = $1.1_{(mm)}$	48 h EC_{50} = $1.1_{(mm)}$	***Peither (2000) CGA 249417/0995***
Brachionus calyciflorus			24 h EC_{50} >$9.5_{(mm)}$	24 h EC_{50} >$9.5_{(mm)}$	***Peither (2000) CGA219417/0992***
Cloeon sp.			48 h EC_{50} = $3.5_{(mm)}$	48 h EC_{50} = $3.5_{(mm)}$	***Peither (2000) CGA219417/0996***
Chaoborus sp.			48 h EC_{50} = $4.0_{(mm)}$	48 h EC_{50} = $4.0_{(mm)}$	***Peither (2000) CGA219417/0999***
Bay shrimp (*Mysidopsis bahia*)		New	96 h LC_{50} = $0.00805_{(mm)}$	96 h LC_{50} = $0.00805_{(mm)}$ [a]	***Ward et al. (1995) CGA219417/0649***
Lymnea stagnalis		EU	48 h EC_{50} = $2.9_{(mm)}$	48 h EC_{50} = $2.9_{(mm)}$	***Peither (2000) CGA219417/0997***
Crassostrea virginica		New	48 h EC_{50} = $0.36_{(mm)}$	-	***Ward et al. (1995) CGA219417/0650***
~~*Asellus aquaticus* (adults)~~			~~96 h EC_{50} = $1.96_{(nom)}$~~	~~96 h EC_{50} = $1.96_{(nom)}$~~	~~***Maynard (2011) CGA219417_11454***~~
Asellus aquaticus (nymphs)			96 h EC_{50} = $2.64_{(nom)}$	96 h EC_{50} = $2.64_{(nom)}$)	***Maynard (2011) CGA219417_11453***
Grandidierella japonica			10 day LC_{50} = 0.42 mg a.s./kg dry weight sediment (mm)	10 day LC_{50} = 0.42 mg a.s./kg dry weight sediment (mm)	***Kreuger & Sutherland (1998) CGA219417/0893***
Hyalella azteca			10 day LC_{50} = 0.73 mg a.s./kg dry weight sediment (mm)	-	***Sutherland & Krueger (1998) CGA219417/0892***
~~*Gammarus pulex*~~		~~LIT~~	~~96 h LC_{50} = 0.69~~	~~96 h LC_{50} = 0.69~~	~~***Beketov & Liess (2008)***~~

Organism	Test item	Endpoints (mg/L)		Proposed endpoint for risk assessment (mg/L)	Reference (author, date, Syngenta File No.)
Chronic					
Mysidopsis bahia	Cyprodinil	New	30 day NOEC = 0.0019$_{(mm)}$	EC_{10} = 0.00197	***Drottar & Kreuger (1999) CGA219417/0926***
Chironomus riparius		EU	28 d NOEC = 25.6 mg/kg sediment$_{(nom)}$ (static test)	28 d NOEC = 25.6 mg/kg sediment$_{(nom)}$	***Grade (2001) CGA249217/0024***
Higher tier studies (micro-mesocosm)					
Aquatic invertebrates	Cyprodinil [a]	New	NOAEAC = 14.6max; 10nom NOEC = 1.8max; 1.5nom ~~NOEC (ETO-RAC, class 2) = 0.0146;~~	NOAEAC = 14.6max; 10nom NOEC = 1.8max; 1.5nom ~~NOEC (ETO-RAC) = 0.0146~~	***Ashwell et al. (2007) CGA219417/1683***

[a] Applied as A14325E
mm = Endpoint derived using mean measured concentration
nom = Endpoint derived using nominal concentration
'New' refers to an endpoint from a study conducted since the previous submission of cyprodinil or a study which was not previously submitted

Comment from RMS: *Concerning the 96h-LC50 of 1.96 mg/L determined for adult Asellus aquaticus (K-CA 8.2.4 .2/02; Maynard 2011a), this endpoint should not be used in the risk assessment given that 10% mortality in control are already reached at 24 hours. This validity criterion is also exceeded at 72 and 96 hours in the similar study conducted with juvenile Asellus aquaticus (K-CA 8.2.4.2/01; Maynard 2011). The study design without sediment is questionable given the type of organism. However, it is the opinion of RMS that the 48h-LC 50 of 2.35 mg/L determined with juvenile Aselfus aquaticus (K-CA 8.2.4.2/01; Maynard 2011) can be used given that only 5% mortality in the control was observed at this time.*

Response from Syngenta: The validity criterion selected by the study director for mortality of the adult *Asellus aquaticus* was on the basis of the adult mortality criterion used for the *Daphnia magna* reproduction test given that the test organisms were confined individually and in the absence of a test guideline. However, the endpoint has been removed from Table 10.2-2 and has not been used in the SSD analysis.

Comment from RMS: *Concerning the LC50 of 0.69 mg/L determined for Gammarus pulex in the publication of Beketov and Liess (2008), can you please provide further details concerning the mortalities in control and test item concentrations? Otherwise, this endpoint could not be used in the risk assessment.*

Response from Syngenta: Control mortality data were not reported in this research article. As requested the endpoint has been removed form Table 10.2-2 and the SSD has been re-run having omitted it. Syngenta originally included this endpoint for transprarency.

Comment from RMS: *Concerning the microcosm study of Ashwell et al. (2007), RMS does not agree with the NOEC of 10 µg/L proposed by applicant. The results clearly demonstrate that Asellus is the critical taxa for defining the study endpoint, due to transient effects observed at low concentration (5 µg/L; class 3a effects) and due to pronounced effects without recovery observed at high concentrations (20 and 50 µg/L). The effects of the class 3a at the test concentration of 5 µg/L in the sample obtained by sweep nets is based on significant reduction of Asellus population compared to the control in at least 4 sampling dates (day 44, day 86, day 100 and day 114) as shown in Table 85 and Figure 91 of the volume 1 (p.187). No clear recovery occurred at the test concentration of 5 µg/L until the end of the test. The significance of the effects observed at 5 µg/L for the sweep net sampling method is supported by the* *abundance of Asellus population measured in the samples, obtained using the ESAS method (See Table 68 and Figure 72). In Table 68, abundance of Asellus in the 5 µg/L and 10 µg/L at sampling day 57 is statistically reduced when compared to the abundance of control and 1.5 µg/L concentration. Even if it is not statistically significant, abundance is also reduced at sampling days 71, 99 and 113 in the 5 µg/L and 10 µg/L when compared to the control. Moreover, when considering the MDD calculations presented in Tables 4 and 6 for Asellus aquaticus in the statistical reanalysis report (Taylor and Dark, 2015), the overall NOEC for ESAS and Sweep nets sampling is stated to be at 1.5 µg/L. Therefore, the NOEC to be used for the ETO-RAC determination has to be 1.5 µg/L. The similarity of the transient effects observed at 5 and 10 µg/L suggests setting the NOEAEC at 10 µg/L for the ERO-RAC determination . The NOEC and NOEAEC from this study should be expressed in nominal concentrations.*

Response from Syngenta: Syngenta consider any effects seen on *Asellus aquaticus* to be transient and not concentration related. The lowest NOEC value reported for *Asellus aquaticus* was 1.5 µg/L in the MDD re-analysis report (Table 6, Taylor & Dark, 2016). However, it should be noted that this reported NOEC value occurred on Day 44 and is bracketed by NOECs of 20 and 50 µg/L on Days 30 and 58 respectively.

No significant effects on *Asellus* abundance was observed at 5 or 10 µg/L in the leaf litterbag samplers at any timepoint within the study, supporting the use of 10 µg/L as the ETO concentration.

In the request for additional information letter ANSES have commented that the NOEC and NOEAEC should be expressed in nominal concentrations. However, according to the aquatic guidance document the maximum measured concentration can be used to derive the mesocosm endpoint. In Section 9.3.5.2 it is stated:

"To evaluate chronic risks (triggered by the tier 1 chronic core data) either the peak concentration or a TWA concentration of the PPP in the relevant matrix (water, sediment) may be used as estimate of $RAC_{sw;ch}$ and PEC estimate"

The higher tier risk assessment for the long-term risk to aquatic invertebrates has been updated using RAC values based on nominal and maximum measured concentrations derived for the NOEC and NOEAEC.

Table 10.2-3: Algae and aquatic macrophyte data for A14325E and cyprodinil

Organism	Test item	Endpoint (mg/L)		Proposed endpoint for risk assessment (mg/L)	Reference (author, date, Syngenta File No.)
Algae					
Pseudokirchneriella subcapitata	A14325E	New	72h E_bC_{50} = 5.3$_{(nom)}$	72h E_bC_{50} = 5.3	***Volz (2005) CGA219417/1358***
Pseudokirchneriella subcapitata ~~*Skeletonema costatum*~~	Cyprodinil		72 h E_rC_{50} = 3.28$_{(mm)}$ [a] ~~96 h E_bC_{50} = 1.75$_{(im)}$~~	72 h E_rC_{50} = 3.28 96 h E_bC_{50} = 1.75	***Ward et al. (1995) CGA219417/0644***
Macrophytes					
Lemna gibba	Cyprodinil	EU	72 h E_yC_{50} = 7.42$_{(im)}$ [a] 7 d EC_{50} = 7.71$_{(im)}$	72 h E_yC_{50} = 7.42$_{(im)}$ 7 d EC_{50} = 7.71	***Ward et al. (1995) CGA219417/0645***

nom = Endpoint derived using nominal concentration
im = Endpoint derived using initial measured concentration
'New' refers to an endpoint from a study conducted since the previous submission of cyprodinil or a study which was not previously submitted
[a] Endpoints modified following re-analysis of the data

Metabolites of cyprodinil

Aquatic organisms may be exposed to metabolites of cyprodinil. Tests have been conducted with CGA249287, CGA275535, CGA321915, CGA263208 and CA1139A.

The results from toxicity tests with representative freshwater species conducted with metabolites are summarised in the tables below.

Table 10.2-4: Toxicity to aquatic organisms to cyprodinil metabolites

Test species	Metabolite	Endpoint	Value (mg/L)	Reference (author, date, Syngenta File No.)
Fish				
Oncorhynchus mykiss	CGA249287	96-h acute LC_{50}	55	***Maetzler (1999) CGA249287/0007***
	CGA275535		2.1	***Pfeifle (2001) CGA275535/0017***
	CGA263208 (phenyl guanidine)		2.1	***Vial (1991) CA1059/0009***
	CA1139A (carbonate salt of phenyl guanidine)		>100	***Grade (1992) CA1139/0008***
Aquatic invertebrates				
Daphnia magna	CGA249287	48-h acute EC_{50}	>100	***Maetzler (1999) CGA249287/0008***
	CGA275535		6.8	***Maetzler (2001) CGA275535/0016***
	CGA321915		>98	***Eckenstein (2015) CGA321915_10005***
	CGA263208 (phenyl guanidine)		20.6	***Vial (1991) CA1059/0010***
	CA1139A (carbonate salt of phenyl guanidine)		15.7	***Grade (1992) CA1139/0009***
Chironomus riparius [a]	CGA321915		>97	***Tobler (2015) CGA321915_10009***
Algae				
Pseudokirchneriella subcapitataa	CGA249287	72-h E_rC_{50}	>100	***Maetzler (1999) CGA249287/0006***
	CGA275535		18	***Maetzler (2001) CGA275535/0015***
	CGA321915	72-h E_bC_{50}	>99	***Eckenstein (2015) CGA321915_10004***
Desmodesmus subspicatus	CGA263208 (phenyl guanidine)		1.86	***Vial (1991) CA1059/0012***
	CA1139A (carbonate salt of phenyl guanidine)		3.80	***Rufli (1992) CA1139/0010***
Sediment dwellers				
Chironomus riparius	CGA249287	28 d NOEC	25.6 mg/kg	***Grade (2001) CGA249217/0024***

[a] although *Chironomus riparius* is a sediment dweller, this data is presented in this section because the exposure regime was acute and young larvae were exposed in water only, no sediment being present

An aqueous photolysis study carried out in 2015 has yielded several new metabolites including guanidine (CGA048109), phenyl guanidine (CGA263208), succinic acid (R008591), U2 and U4. Studies have previously been conducted with phenyl guanidine on carbonic acid (CGA263208) and carbonate (CA1139A) salts. These endpoints are presented in Table 10.2-4. As U2 and U4 have yet to be identified at the time of writing this document no further discussion on these metabolites is presented here.

Guanidine and succinic acid are ubiquitous compounds in the terrestrial and aquatic environments. Also, according to **Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater**[1] ……. *if a substance is an organic compound of aliphatic structure, with a chain length of 4 or less, which consists only of C, H, N or O atoms and which has no "alerting structures" such as epoxide, nitrosamine, nitrile or other functional groups of known toxicological concern."* Also succinic acid is designated "Generally Recognised as Safe" or GRAS by USFDA therefore can be added to food without testing.

Exposure

Aquatic organisms may be exposed to A14325E, cyprodinil and its major metabolites through spray drift, run-off and drainage from the application site into adjacent water bodies. Exposure of aquatic organisms from these routes was estimated by calculating Predicted Environmental Concentrations in surface water (PEC_{SW}) (see **M-CP Section 9** for details of calculations).

A14325E

Due to the differences in environmental fate and behaviour of the constituents of A14325E in aquatic systems, the only PEC_{SW} for risk assessment is the maximum instantaneous PEC_{SW} from entry through spray-drift immediately after a single application. This PEC_{SW} was calculated using the following equation:

$$PEC_{SW}\ [\mu g/L] = \frac{\%\ \text{drift (90th percentile)} \times \text{application rate [g/ha]}}{\text{water depth (30 cm)} \times 10}$$

The PEC_{SW} values following a single application of A14325E to barley are presented below.

Table 10.2-5: A14325E: Predicted Environmental Concentrations (PEC) in surface water

Application rate [g A14325E /ha]	Crop	Drift buffer [m]	Drift rate [%]	Initial PEC_{SW} [µg A14325E/L]
1 application (90th percentile drift)				
1653[a]	Cereals	1 m	2.77	15.3
		5 m	0.57	3.14

[a] The rate of formulation was based on a specific density of 1.102 g/mL with a maximum application of 1.50 L/ha (based on an application rate of 450 g a.s/ha).

Cyprodinil and its metabolites

PEC_{SW} values for cyprodinil and its relevant metabolites were calculated using the FOCUS surface water models following one and two applications of A14325E. FOCUS Step 1 and 2 PEC_{SW} and PEC_{SED} values were calculated using an extreme worst-case exposure scenario. For full details of the assumptions used in the exposure calculations, see **M-CP Section 9**.

The resulting worst-case FOCUS Step 1 and 2 PEC_{SW} and PEC_{SED} values for cyprodinil and its metabolites are presented below. For FOCUS Step 2, concentrations were estimated for Northern and Southern Europe.

[1] Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated Under Council Directive 91/414/EEC. (SANCO/221/2000-rev.10; 25 February 2003).

Table 10.2-6: FOCUS Step 1 and 2 PEC_{SW} values for cyprodinil following application of A14325E to cereals

Use pattern	Step	Region	1 x 450 g a.s./ha		2 x 450 g a.s./ha	
			Max PEC_{SW} [µg/L]	Max PEC_{SED} [µg/kg]	Max PEC_{SW} [µg/L]	Max PEC_{SED} [µg/kg]
Winter and spring cereals BBCH 30-61	Step 1	-	50.1	798	100	1600
	Step 2	North Europe	~~6.12~~ 8.80	~~97.0~~ 142	~~11.4~~ 16.6	~~182~~ 269
		South Europe	~~10.6~~ 16.0	~~173~~ 264	~~20.0~~ 30.3	~~328~~ 502

Table 10.2-7: FOCUS Step 1 and 2 PEC_{SW} values for CGA249287, CGA275535, CGA321915 and CGA263208 following application of A14325E to cereals

Step	No of apps	Region	CGA275535	CGA321915	CGA263208	CGA249287	
			Max PEC_{SW} [µg/L]				Max PEC_{SED} [µg/kg]
1	1	-	10.0	~~5.14~~ 5.17	-	~~12.1~~ 23.5	~~20.8~~ 93.0
	2		20.1	10.3	6.12	~~24.2~~ 47.1	~~41.5~~ 186
2	1	North Europe	~~0.081~~ 0.129	~~0.493~~ 0.808	-	~~1.64~~ 4.04	~~2.79~~ 15.9
	2		~~0.081~~ 0.129	~~0.921~~ 1.55	~~0.698~~ 1.01	~~3.13~~ 7.79	~~5.31~~ 30.7
	1	South Europe	~~0.161~~ 0.258	~~0.986~~ 1.62	-	~~2.79~~ 7.66	~~4.76~~ 30.3
	2		~~0.161~~ 0.258	~~1.84~~ 3.10	~~1.22~~ 1.86	~~5.38~~ 14.8	~~9.20~~ 58.8

Table 10.2-8: Maximum PEC$_{SW}$ values for cyprodinil following applications to winter cereals at FOCUS Step 3

Application scenario	Scenario	Waterbody	Option 1	Water DT$_{50}$ = 158.8 d	Sediment DT$_{50}$ = 1000 d	Option 2	Water DT$_{50}$ = 1000 days	Sediment DT$_{50}$ = 158.8 days
			PEC$_{SW}$ (µg/L)	PEC$_{SED}$ (µg/kg)	Dominant Route of Entry	PEC$_{SW}$ (µg/L)	PEC$_{SED}$ (µg/kg)	Dominant Route of Entry
Winter cereals 1 x 450 g a.s/ha BBCH 30	D1	ditch	3.25	26.8	Drift	3.25	23.2	Drift
	D1	stream	2.52	13.1	Drift	2.52	11.3	Drift
	D2	ditch	3.28	21.9	Drift	3.28	18.6	Drift
	D2	stream	2.77	12.7	Drift	2.77	12.3	Drift
	D3	ditch	2.84	1.88	Drift	2.84	1.88	Drift
	D4	pond	0.098	1.19	Drift	0.098	1.14	Drift
	D4	stream	2.37	0.264	Drift	2.37	0.255	Drift
	D5	pond	0.100	0.985	Drift	0.100	0.947	Drift
	D5	stream	2.51	0.191	Drift	2.51	0.190	Drift
	D6	ditch	2.84	1.98	Drift	2.84	1.98	Drift
	R1	pond	0.180	2.96	Runoff	0.186	2.77	Runoff
	R1	stream	1.87	3.25	Drift	1.87	3.12	Drift
	R3	stream	2.65	4.36	Drift	2.65	4.35	Drift
	R4	stream	1.88	6.16	Drift	1.88	6.14	Drift
Winter cereals 2 x 450 g a.s/ha BBCH 30	D1	ditch	4.29	47.1	Drift	4.29	39.4	Drift
	D1	stream	2.19	23.1	Drift	2.18	20.0	Drift
	D2	ditch	6.43	53.2	Drainage	6.43	45.4	Drainage
	D2	stream	4.02	30.5	Drainage	4.02	26.0	Drainage
	D3	ditch	2.49	2.39	Drift	2.49	2.36	Drift
	D4	pond	0.145	2.16	Drainage	0.149	2.09	Drainage
	D4	stream	2.10	0.587	Drift	2.10	0.567	Drift
	D5	pond	0.137	1.59	Drift	0.139	1.53	Drift
	D5	stream	2.29	0.711	Drift	2.29	0.708	Drift
	D6	ditch	2.50	4.07	Drift	2.50	4.03	Drift
	R1	pond	0.466	6.94	Runoff	0.481	6.58	Runoff
	R1	stream	2.99	8.34	Runoff	2.99	8.01	Runoff
	R3	stream	2.33	5.51	Runoff	2.33	5.24	Runoff
	R4	stream	1.85	9.13	Runoff	1.85	7.91	Runoff

Table 10.2-9: Maximum PEC_{SW} values for cyprodinil following applications to spring cereals at FOCUS Step 3

Application scenario	Scenario	Waterbody	Option 1	Water DT_{50} = 158.8 d	Sediment DT_{50} = 1000 d	Option 2	Water DT_{50} = 1000 days	Sediment DT_{50} = 158.8 days
			PEC_{SW} (µg/L)	PEC_{SED} (µg/kg)	Dominant Route of Entry	PEC_{SW} (µg/L)	PEC_{SED} (µg/kg)	Dominant Route of Entry
Spring cereals 1 x 450 g a.s/ha BBCH 30	D1	ditch	3.46	30.5	Drift	3.45	25.7	Drift
	D1	stream	2.53	15.7	Drift	2.53	13.6	Drift
	D3	ditch	2.85	2.03	Drift	2.85	2.03	Drift
	D4	pond	0.098	1.23	Drift	0.098	1.18	Drift
	D4	stream	2.33	0.296	Drift	2.33	0.289	Drift
	D5	pond	0.100	0.965	Drift	0.100	0.928	Drift
	D5	stream	2.47	0.156	Drift	2.47	0.156	Drift
	R4	stream	1.94	6.43	Runoff	1.94	6.41	Runoff
Spring cereals 2 x 450 g a.s/ha BBCH 30	D1	ditch	4.78	62.3	Drift	4.79	52.6	Drift
	D1	stream	2.21	32.8	Drift	2.20	28.3	Drift
	D3	ditch	2.49	2.62	Drift	2.49	2.60	Drift
	D4	pond	0.175	2.33	Drainage	0.179	2.26	Drainage
	D4	stream	2.12	0.722	Drift	2.12	0.702	Drift
	D5	pond	0.137	1.57	Drift	0.138	1.51	Drift
	D5	stream	2.15	0.202	Drift	2.15	0.200	Drift
	R4	stream	2.01	9.57	Runoff	2.01	8.12	Runoff

Crop / surrogate crop[b]	Scenario	Water body	Single application			Multiple application		
			PEC_{SW} [µg/L]	PEC_{SED} [µg/kg]	Main route of entry to water body for max. PEC_{SW}	PEC_{SW} [µg/L]	PEC_{SED} [µg/kg]	Main route of entry to water body for max. PEC_{SW}
Winter barley / winter cereals	D1	Ditch	3.28	28.5	Drift	4.33	50.1	Drift
	D1	Stream	2.52	14.0	Drift	2.19	24.8	Drift
	D2	Ditch	3.33	23.8	Drift	6.82	57.1	Drainage
	D2	Stream	2.80	13.6	Drift	4.27	32.8	Drainage
	D3	Ditch	2.84	1.89	Drift	2.49	2.39	Drift
	D4	Pond	0.098	1.23	Drift	0.159	2.24	Drainage
	D4	Stream	2.37	0.285	Drift	2.10	0.632	Drift
	D5	Pond	0.100	0.989	Drift	0.139	1.60	Drift
	D5	Stream	2.51	0.191	Drift	2.29	0.712	Drift
	D6	Ditch	2.84	1.99	Drift	2.50	4.08	Drift
	R1	Pond	0.188	3.00	Runoff	0.484	7.02	Runoff
	R1	Stream	1.87	3.25	Drift	3.04	8.34	Runoff
	R3	Stream	2.65	4.33	Drift	2.41	5.51	Runoff
	R4	Stream	1.88	6.12	Drift	1.88	9.27	Runoff
Spring barley / spring cereals	D1	Ditch	3.51	32.7	Drift	4.87	66.6	Drift
	D1	Stream	2.53	17.0	Drift	2.21	35.2	Drift
	D3	Ditch	2.85	2.03	Drift	2.49	2.63	Drift
	D4	Pond	0.098	1.27	Drift	0.190	2.42	Drainage
	D4	Stream	2.33	0.319	Drift	2.12	0.770	Drift
	D5	Pond	0.100	0.969	Drift	0.139	1.58	Drift
	D5	Stream	2.47	0.157	Drift	2.15	0.203	Drift
	R4	Stream	1.97	6.39	Runoff	2.04	9.72	Runoff

Table 10.2-10: Time weighted average PEC_{SW} of cyprodinil at Step 3

Crop / surrogate crop[b]	Scenario	Water body	Single application TWA PEC_{SW} [µg/L] [a]			Multiple application TWA PEC_{SW} [µg/L] [a]		
			7 day	21 day	28 day	7 day	21 day	28 day
Winter barley / winter cereals	D1	Ditch	2.57	2.04	1.86	3.59	2.92	2.72
	D1	Stream	0.690	0.665	0.660	1.30	1.22	1.21
	D2	Ditch	2.60	1.34	1.14	3.13	2.59	2.35
	D2	Stream	2.13	0.939	0.776	2.36	1.37	1.20
	D3	Ditch	0.417	0.142	0.107	0.403	0.138	0.193
	D4	Pond	0.088	0.077	0.073	0.137	0.120	0.114
	D4	Stream	0.054	0.019	0.014	0.123	0.044	0.033
	D5	Pond	0.090	0.079	0.076	0.130	0.118	0.114
	D5	Stream	0.035	0.012	0.009	0.125	0.051	0.039
	D6	Ditch	0.432	0.150	0.115	1.01	0.408	0.315
	R1	Pond	0.175	0.159	0.154	0.452	0.411	0.396
	R1	Stream	0.140	0.084	0.067	0.390	0.232	0.188
	R3	Stream	0.190	0.107	0.085	0.332	0.201	0.160
	R4	Stream	0.563	0.266	0.208	0.578	0.282	0.223
Spring barley / spring cereals	D1	Ditch	2.77	2.20	2.01	4.05	3.32	3.24
	D1	Stream	0.665	0.642	0.640	1.58	1.54	1.52
	D3	Ditch	0.457	0.155	0.117	0.415	0.273	0.207
	D4	Pond	0.088	0.076	0.073	0.165	0.144	0.135
	D4	Stream	0.063	0.022	0.017	0.155	0.055	0.041
	D5	Pond	0.090	0.079	0.075	0.128	0.116	0.112
	D5	Stream	0.028	0.009	0.007	0.026	0.017	0.013
	R4	Stream	0.615	0.288	0.225	0.636	0.306	0.242

[a] based on simulation option 2 as the default DT_{50} in surface water results in higher TWA values

Crop / surrogate crop[b]	Scenario	Water body	Single application TWA PEC_{SW} [µg/L]			Multiple application TWA PEC_{SW} [µg/L]		
			7 day	21 day	28 day	7 day	21 day	28 day
Winter barley / winter cereals	D1	Ditch	2.60	2.06	1.88	3.62	2.95	2.76
	D1	Stream	0.735	0.706	0.700	1.38	1.30	1.28
	D2	Ditch	2.65	1.40	1.19	3.24	2.71	2.46
	D2	Stream	2.16	0.973	0.810	2.43	1.44	1.31
	D3	Ditch	0.420	0.143	0.107	0.405	0.139	0.194
	D4	Pond	0.088	0.077	0.073	0.147	0.129	0.122
	D4	Stream	0.059	0.021	0.016	0.135	0.049	0.037
	D5	Pond	0.090	0.080	0.076	0.130	0.118	0.114
	D5	Stream	0.036	0.012	0.009	0.127	0.053	0.040
	D6	Ditch	0.434	0.151	0.117	1.01	0.408	0.314
	R1	Pond	0.177	0.160	0.155	0.455	0.413	0.399
	R1	Stream	0.139	0.084	0.067	0.388	0.231	0.187
	R3	Stream	0.181	0.104	0.083	0.335	0.202	0.159
	R4	Stream	0.553	0.263	0.206	0.568	0.279	0.222
Spring barley / spring cereals	D1	Ditch	2.82	2.25	2.06	4.12	3.39	3.33
	D1	Stream	0.724	0.697	0.696	1.71	1.67	1.65
	D3	Ditch	0.459	0.156	0.118	0.417	0.275	0.208
	D4	Pond	0.088	0.076	0.073	0.175	0.153	0.144
	D4	Stream	0.069	0.024	0.018	0.168	0.060	0.045
	D5	Pond	0.090	0.079	0.075	0.129	0.116	0.112
	D5	Stream	0.030	0.010	0.007	0.027	0.018	0.013
	R4	Stream	0.605	0.284	0.222	0.627	0.303	0.240

[a] based on simulation option B (see Table 9.2.5-1) as the default DT_{50} in surface water results in higher TWA values
[b] according to FOCUS guidance

Table 10.2-11: FOCUS Step 4 Global Maximum PEC_{SW} for cyprodinil following single and multiple applications to winter cereals (Option 1[1])

			Mitigation options							
Vegetative strip (m)			-		-		10 - 12		18 – 20	
No spray buffer (m)			5		10		10		20	
Nozzle reduction (%)			-		-		-		-	
Crop	**Scenario**	**Water body**	**PEC_{SW} (µg/L)**	**Dominant entry route**	**PEC_{SW} (µg/L)**	**Dominant entry route**	**PEC_{SW} (µg/L)**	**Dominant entry route**	**PEC_{SW} (µg/L)**	**Dominant entry route**
Winter cereals 1 x 450 g a.s/ha BBCH 30	D1	ditch	1.28	Drainage	1.10	Drainage				
	D1	stream	0.927	Drift	0.722	Drainage				
	D2	ditch	2.13	Drainage	2.13	Drainage				
	D2	stream	1.34	Drainage	1.34	Drainage				
	D3	ditch	0.788	Drift	0.437	Drift				
	D4	pond	0.115	Drainage	0.084	Drainage				
	D4	stream	0.907	Drift	0.492	Drift				
	D5	pond	0.117	Drainage	0.086	Drainage				
	D5	stream	0.950	Drift	0.512	Drift				
	D6	ditch	0.792	Drift	0.602	Drainage				
	R1	pond	0.188	Runoff	0.176	Runoff	0.091	Runoff	0.054	Runoff
	R1	stream	1.08	Runoff	1.08	Runoff	0.490	Runoff	0.257	Runoff
	R3	stream	1.37	Runoff	1.37	Runoff	0.625	Runoff	0.328	Runoff
	R4	stream	1.80	Runoff	1.80	Runoff	0.821	Runoff	0.430	Runoff
Winter cereals 2 x 450 g a.s/ha BBCH 30	D1	ditch	2.04	Drainage	2.04	Drainage				
	D1	stream	1.36	Drainage	1.36	Drainage				
	D2	ditch	6.42	Drainage	6.42	Drainage				
	D2	stream	4.02	Drainage	4.02	Drainage				
	D3	ditch	0.677	Drift	0.381	Drift				
	D4	pond	0.171	Drainage	0.144	Drainage				
	D4	stream	0.782	Drift	0.491	Drainage				
	D5	pond	0.171	Drainage	0.125	Drainage				
	D5	stream	0.834	Drift	0.447	Drift				
	D6	ditch	1.11	Drainage	1.11	Drainage				
	R1	pond	0.486	Runoff	0.462	Runoff	0.224	Runoff	0.120	Runoff
	R1	stream	2.99	Runoff	2.99	Runoff	1.36	Runoff	0.710	Runoff
	R3	stream	2.33	Runoff	2.33	Runoff	1.05	Runoff	0.546	Runoff
	R4	stream	1.85	Runoff	1.85	Runoff	0.841	Runoff	0.440	Runoff

[1] $DT_{50,WATER}$ = 158.8 days, $DT_{50,SEDIMENT}$ = 1000 days

Table 10.2-12: FOCUS Step 4 Global Maximum PEC_{SW} for cyprodinil following single and multiple applications to winter cereals (Option 2[1])

			Mitigation options							
Vegetative strip (m)			-		-		10 - 12		18 – 20	
No spray buffer (m)			5		10		10		20	
Nozzle reduction (%)			-		-		-		-	
Crop	**Scenario**	**Water body**	**PEC_{SW} (µg/L)**	**Domi-nant entry route**	**PEC_{SW} (µg/L)**	**Domi-nant entry route**	**PEC_{SW} (µg/L)**	**Domi-nant entry route**	**PEC_{SW} (µg/L)**	**Domi-nant entry route**
Winter cereals 1 x 450 g a.s/ha BBCH 30	D1	ditch	1.28	Drainage	1.10	Drainage				
	D1	stream	0.926	Drift	0.722	Drainage				
	D2	ditch	2.13	Drainage	2.13	Drainage				
	D2	stream	1.34	Drainage	1.34	Drainage				
	D3	ditch	0.788	Drift	0.437	Drift				
	D4	pond	0.115	Drainage	0.084	Drainage				
	D4	stream	0.907	Drift	0.492	Drift				
	D5	pond	0.117	Drainage	0.086	Drainage				
	D5	stream	0.950	Drift	0.512	Drift				
	D6	ditch	0.792	Drift	0.602	Drainage				
	R1	pond	0.195	Runoff	0.182	Runoff	0.095	Runoff	0.054	Runoff
	R1	stream	1.08	Runoff	1.08	Runoff	0.490	Runoff	0.257	Runoff
	R3	stream	1.37	Runoff	1.37	Runoff	0.625	Runoff	0.328	Runoff
	R4	stream	1.81	Runoff	1.81	Runoff	0.821	Runoff	0.430	Runoff
Winter cereals 2 x 450 g a.s/ha BBCH 30	D1	ditch	2.04	Drainage	2.04	Drainage				
	D1	stream	1.36	Drainage	1.36	Drainage				
	D2	ditch	6.42	Drainage	6.42	Drainage				
	D2	stream	4.02	Drainage	4.02	Drainage				
	D3	ditch	0.677	Drift	0.381	Drift				
	D4	pond	0.173	Drainage	0.147	Drainage				
	D4	stream	0.782	Drift	0.491	Drainage				
	D5	pond	0.174	Drainage	0.127	Drainage				
	D5	stream	0.834	Drift	0.447	Drift				
	D6	ditch	1.11	Drainage	1.11	Drainage				
	R1	pond	0.502	Runoff	0.476	Runoff	0.232	Runoff	0.124	Runoff
	R1	stream	2.99	Runoff	2.99	Runoff	1.36	Runoff	0.710	Runoff
	R3	stream	2.33	Runoff	2.33	Runoff	1.05	Runoff	0.546	Runoff
	R4	stream	1.85	Runoff	1.85	Runoff	0.841	Runoff	0.440	Runoff

[1] $DT_{50,WATER}$ = 1000 days, $DT_{50,SEDIMENT}$ = 158.8 days

Table 10.2-13: FOCUS Step 4 Global Maximum PEC_{SW} for cyprodinil following single and multiple applications to spring cereals (Option 1[1])

			Mitigation options							
Vegetative strip (m)			-		-		10 - 12		18 – 20	
No spray buffer (m)			5		10		10		20	
Nozzle reduction (%)			-		-		-		-	
Crop	**Scenario**	**Water body**	**PEC_{SW} (µg/L)**	**Dominant entry route**	**PEC_{SW} (µg/L)**	**Dominant entry route**	**PEC_{SW} (µg/L)**	**Dominant entry route**	**PEC_{SW} (µg/L)**	**Dominant entry route**
Spring cereals 1 x 450 g a.s/ha BBCH 30	D1	ditch	1.48	Drainage	1.24	Drainage				
	D1	stream	0.935	Drift	0.774	Drainage				
	D3	ditch	0.792	Drift	0.441	Drift				
	D4	pond	0.115	Drainage	0.084	Drainage				
	D4	stream	0.883	Drift	0.476	Drift				
	D5	pond	0.116	Drainage	0.086	Drainage				
	D5	stream	0.939	Drift	0.506	Drift				
	R4	stream	1.94	Runoff	1.94	Runoff	0.884	Runoff	0.463	Runoff
Spring cereals 2 x 450 g a.s/ha BBCH 30	D1	ditch	2.77	Drainage	2.77	Drainage				
	D1	stream	1.74	Drainage	1.74	Drainage				
	D3	ditch	0.679	Drift	0.383	Drift				
	D4	pond	0.183	Drainage	0.174	Drainage				
	D4	stream	0.779	Drift	0.616	Drainage				
	D5	pond	0.170	Drainage	0.125	Drainage				
	D5	stream	0.793	Drift	0.421	Drift				
	R4	stream	2.01	Runoff	2.01	Runoff	0.913	Runoff	0.478	Runoff

[1] $DT_{50,WATER}$ = 158.8 days, $DT_{50,SEDIMENT}$ = 1000 days

Table 10.2-14: FOCUS Step 4 Global Maximum PEC_{SW} for cyprodinil following single and multiple applications to spring cereals (Option 2[1])

			Mitigation options							
Vegetative strip (m)			-		-		10 - 12		18 – 20	
No spray buffer (m)			5		10		10		20	
Nozzle reduction (%)			-		-		-		-	
Crop	**Scenario**	**Water body**	**PEC_{SW} (µg/L)**	**Domi-nant entry route**	**PEC_{SW} (µg/L)**	**Domi-nant entry route**	**PEC_{SW} (µg/L)**	**Domi-nant entry route**	**PEC_{SW} (µg/L)**	**Domi-nant entry route**
Spring	D1	ditch	1.48	Drainage	1.24	Drainage				
cereals	D1	stream	0.934	Drift	0.774	Drainage				
1 x	D3	ditch	0.793	Drift	0.441	Drift				
450 g	D4	pond	0.115	Drainage	0.084	Drainage				
a.s/ha	D4	stream	0.883	Drift	0.476	Drift				
BBCH	D5	pond	0.117	Drainage	0.086	Drainage				
30	D5	stream	0.939	Drift	0.506	Drift				
	R4	stream	1.94	Runoff	1.94	Runoff	0.884	Runoff	0.463	Runoff
Spring	D1	ditch	2.77	Drainage	2.77	Drainage				
cereals	D1	stream	1.74	Drainage	1.74	Drainage				
2 x	D3	ditch	0.679	Drift	0.383	Drift				
450 g	D4	pond	0.187	Drainage	0.177	Drainage				
a.s/ha	D4	stream	0.779	Drift	0.616	Drainage				
BBCH	D5	pond	0.173	Drainage	0.126	Drainage				
30	D5	stream	0.793	Drift	0.421	Drift				
	R4	stream	2.01	Runoff	2.01	Runoff	0.913	Runoff	0.478	Runoff

[1] $DT_{50,WATER}$ = 1000 days, $DT_{50,SEDIMENT}$ = 158.8 days

Risk assessment for aquatic organisms

The A14325E and cyprodinil risk assessments were carried out following application according to the proposed use.

The risk assessments followed the recently noted EFSA (2013) Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. The assessment is a tiered procedure which derives Regulatory Acceptable Concentrations (RACs) from the effects data by applying assessment factors appropriate to the taxon and tier assessed. The RAC is compared to the appropriate PEC_{SW} value. If the RAC is > PEC, then the risk is acceptable, otherwise the assessment should be refined with higher tiers.

Table 10.2-1~~0~~5: Derivation of RAC values for use in the Tier I risk assessment – A14325E

Species	Substance	Exposure System	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
Oncorhynchus mykiss	A14325E	96 h, s	LC_{50} = 6 800	100	68
Daphnia magna		48 h, s	EC_{50} = 370	100	3.7
Pseudiokirchneriella subcapitata		72 h, s	E_rC_{50} = 5 300	10	530

s = static system

Table 10.2-1~~1~~6: Derivation of RAC values for use in the Tier I risk assessment – cyprodinil and metabolites

Test organism	Substance	Exposure system	Endpoints (µg/L)	AF	Tier 1-RAC (µg/L)
Fish					
Cyprinodon variegatus	Cyprodinil	96 h, f	LC_{50} = 1 250	100	12.5
Oncorhynchus mykiss	CGA249287	96 h, s	LC_{50} = 55 000		550
	CGA275535	96 h, s	LC_{50} = 2 100		21
	CGA263208 [a]	96 h, s	LC_{50} = 2 100		21
Cyprinodon variegatus	Cyprodinil	34 d, f	NOEC = 40.6	10	4.06
Aquatic invertebrates					
Mysidopsis bahia	Cyprodinil	96 h, f	LC_{50} = 8.05	100	**0.0805**
Daphnia magna	CGA249287	48 h, s	EC_{50} >100 000		>1 000
	CGA275535	48 h, s	EC_{50} = 6 800		68
	CGA321915	48 h, s	EC_{50} >98 000		>980
	CGA263208 [a]	48 h, s	EC_{50} = 20 600		206
Mysidopsis bahia	Cyprodinil	30 d, f	EC_{10} = 1.97	10	**0.197**
Aquatic insect					
Chironomus riparius	CGA321915	48 h, s	EC_{50} >97 000	100	970
Sediment dwellers					
Chironomus riparius	Cyprodinil	27 d, spiked sediment	NOEC = 80 000 (µg/kg)	10	8 000 µg/kg
Grandidierella japonica		10 d, spiked sediment	LC_{50} = 420	100	4.2
Chironomus riparius	CGA249287	28 d, spiked sediment	NOEC = 25 600 (µg/kg)	10	2 560 µg/kg
Algae					
Psudokirchneriella subcapitata [c] ~~*Skeletonema costatum*~~	Cyprodinil	72 h, s	E_rC_{50} = 3 200 ~~EC_{50} = 1 750~~	10	~~175~~ 320
Psudokirchneriella subcapitata	CGA249287		$E_{rb}C_{50}$ >100 000		>10 000
	CGA275535		E_rC_{50} = 18 000		1 800
	CGA321915		$E_{rb}C_{50}$ >99 000		>9 900
	CGA263208		E_bC_{50} = 1 860		186
Macrophytes					
Lemna gibba	Cyprodinil	7 d, s	EC_{50} = 7 710	10	771
Mesocosm					
Invertebrates	Cyprodinil [b]		NOAEAC = 14.6_{max}; 10_{nom}	3	4.86, 3.33
			NOEC = 1.8_{max}; 1.5_{nom}	2	0.90, 0.75

s = static system
f = flow-through system
max = maximum measured concentration
nom = nominal concentration
[a] result was derived from a study conducted with CA1139A, a carbonate salt of phenyl guanidine
[b] tested as A14325E
[c] Represents worst case endpoint based on E_rC_{50} values derived for all algal species

Risk assessment for A14325E

Following the EFSA Guidance Document on Aquatic Risk Assessment (July 2013)[2], the RACs are compared to the exposure values using the PEC/RAC ratio. The risk assessment is presented in the table below.

Table 10.2-17 ~~2~~: Tier 1 risk assessment for A14325E based on spray drift following applications to cereals

1 x 1.5 L A14325E/ha				
Group		**Fish - acute**	**Invertebrate - acute**	**Algae**
Tier 1 RAC (µg/L)		**68**	**3.7**	**530**
Spray drift distances	**PEC (µg/L)**	**PEC/RAC**		
1 m	15.3	0.23	**4.1**	0.03
5 m	3.14	-	0.85	-

Values in bold are above the trigger value of 1 and hence further consideration is needed for these taxa/scenario combinations

The PEC/RAC ratios for fish and algae are below 1 for a 1 m spray drift buffer indicating acceptable risk for these groups. For the acute risk to aquatic invertebrates, however, a 5 m drift buffer was required to achieve acceptable risk.

It is clear from the list of endpoints table (Table 10.2.1 to 10.2-3) that the toxicity of cyprodinil to fish, *Daphnia* and algae is not significantly enhanced by formulating it as A14325E. The toxicity of A14325E is therefore considered to be driven by the active substance and the acute risk to fish and aquatic invertebrates will be refined by consideration of the toxicity of cyprodinil.

Risk assessment for cyprodinil

From table 10.2-16 ~~11~~ it is clear that the lowest tier 1 $RAC_{sw;ac}$ is 0.0805 µg/L, based on the toxicity to the aquatic invertebrate species *Mysidopsis bahia* (mysid).

The lowest tier 1 $RAC_{sw;ch}$ is 0.197 µg/L, based on aquatic invertebrates, the mysid.

Following the EFSA Guidance Document on Aquatic Risk Assessment (July 2013), the tier 1 RACs are compared to the exposure values derived for FOCUS Steps 1 to 3. These are presented in Tables 10.2-18 ~~13~~ to 10.2-21 ~~16~~.

[2] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 186 pp. doi:10.2903/j.efsa.2013.3290.

Table 10.2-18: Tier 1 risk assessment for cyprodinil based on FOCUS Step 1, 2 and 3 PECs for 1 x 450 g a.s./ha application to winter cereals

Group			Fish - acute	Fish - chronic	Invertebrate - acute	Invertebrate - chronic	Algae	Macrophyte	Group	Sediment dweller - acute	Sediment dweller - chronic
Tier 1 RAC (µg/L)			**12.5**	**4.06**	**0.0805**	**0.197**	**320**	**742**	**Tier 1 RAC (µg/kg)**	**4.2**	**8000**
FOCUS Scenario		**PEC_{sw} (µg/L)**	**PEC/RAC ratio (pelagic species)**						**PEC_{sed} (µg/kg) [b]**	**PEC/RAC (benthic species)**	
Step 1		50.1	**4.0**	**12**	**622**	**254**	0.16	0.068	798	**190**	0.0998
Step 2	N EU	8.8	0.70	**2.2**	**109**	**45**	0.028	0.012	142	**34**	-
	S EU	16	**1.28**	**3.9**	**199**	**81**	0.050	0.022	264	**63**	-
Step 3 [a]	D1 ditch	3.25	0.26	0.80	**40**	**16**	-	-	26.8	**6.4**	-
	D1 stream	2.52	0.20	0.62	**31**	**13**	-	-	13.1	**3.1**	-
	D2 ditch	3.28	0.26	0.81	**41**	**17**	-	-	21.9	**5.2**	-
	D2 stream	2.77	0.22	0.68	**34**	**14**	-	-	12.7	**3.0**	-
	D3 ditch	2.84	0.23	0.70	**35**	**14**	-	-	1.88	0.45	-
	D4 pond	0.098	0.01	0.024	**1.2**	0.50	-	-	1.19	0.28	-
	D4 stream	2.37	0.19	0.58	**29**	**12**	-	-	0.264	0.06	-
	D5 pond	0.10	0.01	0.025	**1.2**	0.51	-	-	0.985	0.23	-
	D5 stream	2.51	0.20	0.62	**31**	**13**	-	-	0.191	0.05	-
	D6 ditch	2.84	0.23	0.70	**35**	**14**	-	-	1.98	0.47	-
	R1 pond	0.186	0.01	0.046	**2.3**	0.94	-	-	2.96	0.70	-
	R1 stream	1.87	0.15	0.46	**23**	**9.5**	-	-	3.25	0.77	-
	R3 stream	2.65	0.21	0.65	**33**	**13**	-	-	4.36	**1.0**	-
	R4 stream	1.88	0.15	0.46	**23**	**9.5**	-	-	6.16	**1.5**	-

[a] Highest PEC_{SW} was used
Values in bold are above the trigger value of 1 and hence further consideration is needed for these taxa/scenario combinations

Table 10.2-13: Tier 1 risk assessment for cyprodinil based on FOCUS Step 1, 2 and 3 PECs for 1 x 450 g a.s./ha application to winter cereals

Group			**Fish - acute**	**Fish - chronic**	**Invertebrate - acute**	**Invertebrate - chronic**	**Algae**	**Macrophyte**	**Group**	**Sediment dweller - acute**	**Sediment dweller - chronic**
Tier 1 RAC (µg/L)			**12.5**	**4.06**	**0.0805**	**0.197**	**175**	**771**	**Tier 1 RAC (µg/kg)**	**4.2**	**8000**
FOCUS Scenario		**PEC_{sw} (µg/L)**	**PEC/RAC ratio (pelagic species)**						**PEC_{sed} (µg/kg)**	**PEC/RAC (benthic species)**	
Step 1		50.1	**4.0**	**12**	**622**	**254**	**0.29**	**0.065**	798	**190**	
Step 2	N EU	6.12	0.49	**1.5**	**76**	**31**	0.035	0.0079	97	**23**	-
	S EU	10.6	0.85	**2.6**	**132**	**54**	0.061	0.014	173	**41**	-
Step 3	D1 ditch	3.28	-	0.81	**41**	**17**	-	-	28.5	**6.8**	-
	D1 stream	2.52	-	0.62	**31**	**13**	-	-	14.0	**3.3**	-
	D2 ditch	3.33	-	0.82	**41**	**17**	-	-	23.8	**5.7**	-
	D2 stream	2.80	-	0.69	**35**	**14**	-	-	13.6	**3.2**	-
	D3 ditch	2.84	-	0.70	**35**	**14**	-	-	1.89	0.45	-
	D4 pond	0.098	-	0.024	**1.2**	0.50	-	-	1.23	0.29	-
	D4 stream	2.37	-	0.58	**29**	**12**	-	-	0.285	0.07	-
	D5 pond	0.100	-	0.025	**1.2**	0.51	-	-	0.989	0.24	-
	D5 stream	2.51	-	0.62	**31**	**13**	-	-	0.191	0.05	-
	D6 ditch	2.84	-	0.70	**35**	**14**	-	-	1.99	0.47	-
	R1 pond	0.188	-	0.046	**2.3**	0.95	-	-	3.00	0.71	-
	R1 stream	1.87	-	0.46	**23**	**9.5**	-	-	3.25	0.77	-
	R3 stream	2.65	-	0.65	**33**	**13**	-	-	4.33	**1.0**	-
	R4 stream	1.88	-	0.46	**23**	**9.5**	-	-	6.12	**1.5**	-

Values in bold are above the trigger value of 1 and hence further consideration is needed for these taxa/scenario combinations

Table 10.2-19: Tier 1 risk assessment for cyprodinil based on FOCUS Step 1, 2 and 3 PECs for 2 x 450 g a.s./ha applications to winter cereals

Group			**Fish - acute**	**Fish - chronic**	**Invertebrate - acute**	**Invertebrate - chronic**	**Algae**	**Macrophyte**	**Group**	**Sediment dweller - acute**	**Sediment dweller - chronic**
Tier 1 RAC (µg/L)			**12.5**	**4.06**	**0.0805**	**0.197**	**320**	**742**	**Tier 1 RAC (µg/kg)**	**4.2**	**8000**
FOCUS Scenario		**PEC$_{sw}$ (µg/L)**	**PEC/RAC ratio (pelagic species)**						**PEC$_{sed}$ (µg/kg)** [b]	**PEC/RAC (benthic species)**	
Step 1		100	**8.0**	**25**	**1242**	**508**	0.31	0.13	1600	**381**	**0.20**
Step 2	N EU	16.6	**1.33**	**4.1**	**206**	**84**	0.052	0.022	269	**64**	-
	S EU	30.3	**2.42**	**7.5**	**376**	**154**	0.09	0.041	502	**120**	-
Step 3 [a]	D1 ditch	4.29 [a]	0.34	**1.1**	**53**	**22**	-	-	47.1	**11**	-
	D1 stream	2.19 [b]	0.18	0.54	**27**	**11**	-	-	23.1	**5.5**	-
	D2 ditch	6.43 [a]	0.51	**1.6**	**80**	**33**	-	-	53.2	**13**	-
	D2 stream	4.02 [a]	0.32	**1.0**	**50**	**20**	-	-	30.5	**7.3**	-
	D3 ditch	2.49 [a]	0.20	0.61	**31**	**13**	-	-	2.39	**0.57**	-
	D4 pond	0.149 [c]	0.01	0.037	**1.9**	**0.76**	-	-	2.16	**0.51**	-
	D4 stream	2.1 [a]	0.17	0.52	**26**	**11**	-	-	0.587	**0.14**	-
	D5 pond	0.139 [c]	0.011	0.034	**1.7**	**0.71**	-	-	1.59	**0.38**	-
	D5 stream	2.29 [a]	0.18	0.56	**28**	**12**	-	-	0.711	**0.17**	-
	D6 ditch	2.5 [a]	0.20	0.62	**31**	**13**	-	-	4.07	**0.97**	-
	R1 pond	0.481 [c]	0.038	0.12	**6.0**	**2.4**	-	-	6.94	**1.7**	-
	R1 stream	2.99 [a]	0.24	0.74	**37**	**15**	-	-	8.34	**2.0**	-
	R3 stream	2.33 [a]	0.19	0.57	**29**	**12**	-	-	5.51	**1.3**	-
	R4 stream	1.85 [a]	0.15	0.46	**23**	**9**	-	-	9.13	**2.2**	-

[a] Highest PEC$_{SW}$ was used
Values in bold are above the trigger value of 1 and hence further consideration is needed for these taxa/scenario combinations

Table 10.2-14: Tier 1 risk assessment for cyprodinil based on FOCUS Step 1, 2 and 3 PECs for 2 x 450 g a.s./ha applications to winter cereals

Group			Fish – acute	Fish – chronic	Invertebrate – acute	Invertebrate – chronic	Algae	Macrophyte	Group	Sediment dweller – acute	Sediment dweller – chronic
Tier 1 RAC (µg/L)			12.5	4.06	0.0805	0.197	175	771	Tier 1 RAC (µg/kg)	4.2	8000
FOCUS Scenario		PEC_{sw} (µg/L)	PEC/RAC ratio (pelagic species)						PEC_{sed} (µg/kg)	PEC/RAC (benthic species)	
Step 1		100	**8.0**	**25**	**1200**	**510**	0.57	0.13	1600	**380**	0.20
Step 2	N EU	11.4	0.91	**2.8**	**140**	**58**	0.065	0.015	182	**43**	-
	S EU	20.0	**1.60**	**4.9**	**250**	**100**	0.11	0.026	328	**78**	-
Step 3	D1 ditch	4.33	0.35	**1.1**	**54**	**22**	-	-	50.1	**12**	-
	D1 stream	2.19	0.18	0.54	**27**	**11**	-	-	24.8	**5.9**	-
	D2 ditch	6.82	0.55	**1.7**	**85**	**35**	-	-	57.1	**14**	-
	D2 stream	4.27	0.34	**1.1**	**53**	**22**	-	-	32.8	**7.8**	-
	D3 ditch	2.49	0.20	0.61	**31**	**13**	-	-	2.39	0.57	-
	D4 pond	0.159	0.01	0.039	**2.0**	**0.81**	-	-	2.24	0.53	-
	D4 stream	2.10	0.17	0.52	**26**	**11**	-	-	0.632	0.15	-
	D5 pond	0.139	0.011	0.034	**1.7**	0.71	-	-	1.60	0.38	-
	D5 stream	2.29	0.18	0.56	**28**	**12**	-	-	0.712	0.17	-
	D6 ditch	2.50	0.20	0.62	**31**	**13**	-	-	4.08	0.97	-
	R1 pond	0.484	0.039	0.12	**6.0**	**2.5**	-	-	7.02	**1.7**	-
	R1 stream	3.04	0.24	0.75	**38**	**15**	-	-	8.34	**2.0**	-
	R3 stream	2.41	0.19	0.59	**30**	**12**	-	-	5.51	**1.3**	-
	R4 stream	1.88	0.15	0.46	**23**	**10**	-	-	9.27	**2.2**	-

Values in bold are above the trigger value of 1 and hence further consideration is needed for these taxa/scenario combinations

Table 10.2-20: Tier 1 risk assessment for cyprodinil based on FOCUS Step 1, 2 and 3 PECs for 1 x 450 g a.s./ha application to spring cereals

Group			Fish - acute	Fish - chronic	Invertebrate - acute	Invertebrate - chronic	Algae	Macrophyte	Group	Sediment dweller - acute	Sediment dweller - chronic
Tier 1 RAC (µg/L)			12.5	4.06	0.0805	0.197	320	742	Tier 1 RAC (µg/kg)	4.2	8000
FOCUS Scenario		PEC_{sw} (µg/L)	PEC/RAC ratio (pelagic species)						PEC_{sed} (µg/kg) [b]	PEC/RAC (benthic species)	
Step 1		50.1	**4.0**	**12**	**622**	**254**	0.16	0.068	798	**190**	0.10
Step 2	N EU	8.8	0.70	**2.2**	**109**	**45**	0.028	0.0119	142	**34**	0.02
	S EU	16	**1.28**	**3.9**	**199**	**81**	0.050	0.022	264	**63**	0.03
Step 3 [a]	D1 ditch	3.46 [b]	0.28	0.85	**43**	**18**	-	-	30.5	**30.5**	-
	D1 stream	2.53 [a]	0.20	0.62	**31**	**13**	-	-	15.7	**15.7**	-
	D3 ditch	2.85 [a]	0.23	0.70	**35**	**14**	-	-	2.03	**2.03**	-
	D4 pond	0.098 [a]	0.01	0.024	**1.2**	0.50	-	-	1.23	**1.23**	-
	D4 stream	2.33 [a]	0.19	0.57	**29**	**12**	-	-	0.296	0.30	-
	D5 pond	0.1 [a]	0.01	0.025	**1.2**	0.51	-	-	0.965	0.97	-
	D5 stream	2.47 [a]	0.20	0.61	**31**	**13**	-	-	0.156	0.16	-
	R4 stream	1.94 [a]	0.16	0.48	**24**	**10**	-	-	6.43	**6.4**	-

[a] Highest PEC_{SW} was used
Values in bold are above the trigger value of 1 and hence further consideration is needed for these taxa/scenario combinations

Table 10.2-15: Tier 1 risk assessment for cyprodinil based on FOCUS Step 1, 2 and 3 PECs for 1 x 450 g a.s./ha application to spring cereals

Group			Fish - acute	Fish - chronic	Invertebrate - acute	Invertebrate - chronic	Algae	Macrophyte	Group	Sediment dweller - acute	Sediment dweller - chronic
Tier 1 RAC (µg/L)			12.5	4.06	0.0805	0.197	175	771	Tier 1 RAC (µg/kg)	4.2	8000
FOCUS Scenario		PEC_{sw} (µg/L)	PEC/RAC ratio (pelagic species)						PEC_{sed} (µg/kg)	PEC/RAC (benthic species)	
Step 1		50.1	**4.0**	**12**	**620**	**250**	0.29	0.06	798	**190**	0.10
Step 2	N EU	6.12	0.49	**1.5**	**76**	**31**	0.035	0.0079	97	**23**	0.01
	S EU	10.6	0.85	**2.6**	**130**	**54**	0.061	0.014	173	**41**	0.02
Step 3	D1 ditch	3.51	–	0.86	**44**	**18**	–	-	32.7	**7.8**	-
	D1 stream	2.53	–	0.62	**31**	**13**	–	–	17	**4.0**	-
	D3 ditch	2.85	–	0.70	**35**	**14**	–	–	2.03	0.48	-
	D4 pond	0.098	–	0.024	**1.2**	0.50	–	–	1.27	0.30	-
	D4 stream	2.33	–	0.57	**29**	**12**	–	–	2.12	0.50	-
	D5 pond	0.10	–	0.025	**1.2**	0.51	–	–	0.969	0.23	-
	D5 stream	2.47	–	0.61	**31**	**13**	–	–	0.157	0.04	-
	R4 stream	1.97	–	0.49	**24**	**10**	–	–	6.39	**1.5**	-

Values in bold are above the trigger value of 1 and hence further consideration is needed for these taxa/scenario combinations

Table 10.2-21: Tier 1 risk assessment for cyprodinil based on FOCUS Step 1, 2 and 3 PECs for 2 x 450 g a.s./ha applications to spring cereals

Group			**Fish - acute**	**Fish - chronic**	**Invertebrate - acute**	**Invertebrate - chronic**	**Algae**	**Macrophyte**	**Group**	**Sediment dweller - acute**	**Sediment dweller - chronic**
Tier 1 RAC (µg/L)			**12.5**	**4.06**	**0.0805**	**0.197**	**320**	**742**	**Tier 1 RAC (µg/kg)**	**4.2**	**8000**
FOCUS Scenario		**PEC_{sw} (µg/L)**	**PEC/RAC ratio (pelagic species)**						**PEC_{sed} (µg/kg)** [b]	**PEC/RAC ratio (benthic species)**	
Step 1		100	**8.0**	**25**	**1242**	**508**	0.31	0.13	1600	**381**	0.20
Step 2	N EU	16.6	**1.33**	**4.1**	**206**	**84**	0.052	0.022	269	**64**	-
	S EU	30.3	**2.42**	**7.5**	**376**	**154**	0.09	0.041	502	**120**	-
Step 3 [a]	D1 ditch	4.79 [c]	0.38	**1.2**	**60**	**24**	-	-	62.3	**15**	-
	D1 stream	2.21 [b]	0.18	0.5	**27**	**11**	-	-	32.8	**8**	-
	D3 ditch	2.49 [a]	0.20	0.54	**27**	**11**	-	-	2.62	0.62	-
	D4 pond	0.179 [c]	0.01	0.044	**2.2**	0.91	-	-	2.33	0.55	-
	D4 stream	2.12 [a]	0.17	0.52	**26**	**11**	-	-	0.722	0.17	-
	D5 pond	0.138 [c]	0.01	0.034	**1.7**	0.70	-	-	1.57	0.37	-
	D5 stream	2.15 [a]	0.17	0.53	**27**	**11**	-	-	0.202	0.05	-
	R4 stream	2.01 [a]	0.16	0.50	**25**	**10**	-	-	9.57	**2.3**	-

[a] Highest PEC_{SW} was used
Values in bold are above the trigger value of 1 and hence further consideration is needed for these taxa/scenario combinations

~~Table 10.2-16: Tier 1 risk assessment for cyprodinil based on FOCUS Step 1, 2 and 3 PECs for 2 x 450 g a.s./ha applications to spring cereals~~

Group			Fish - acute	Fish - chronic	Invertebrate - acute	Invertebrate - chronic	Algae	Macrophyte	Group	Sediment dweller - acute	Sediment dweller - chronic
Tier 1 RAC (µg/L)			12.5	4.06	0.0805	0.197	175	771	Tier 1 RAC (µg/kg)	4.2	8000
FOCUS Scenario		PEC$_{sw}$ (µg/L)	PEC/RAC ratio (pelagic species)						PEC$_{sed}$ (µg/kg)	PEC/RAC ratio (benthic species)	
Step 1		100	**8.0**	**25**	**1200**	**510**	0.57	0.13	1600	**380**	0.20
Step 2	N EU	11.4	0.91	**2.8**	**140**	**58**	0.065	0.015	182	**43**	-
	S EU	20	1.60	**4.9**	**250**	**100**	0.11	0.026	328	**78**	-
Step 3	D1 ditch	4.87	–	**1.2**	**60**	**25**	–	–	66.6	**16**	-
	D1 stream	2.21	–	0.54	**27**	**11**	–	–	35.2	**8.4**	-
	D3 ditch	2.49	–	0.61	**31**	**13**	–	–	2.63	0.63	-
	D4 pond	0.19	–	0.047	**2.4**	0.96	–	–	2.42	0.58	-
	D4 stream	2.12	–	0.52	**26**	**11**	–	–	0.77	0.18	-
	D5 pond	0.139	–	0.034	**1.7**	0.71	–	–	1.58	0.38	-
	D5 stream	2.15	–	0.53	**27**	**11**	–	–	0.203	0.05	-
	R4 stream	2.04	–	0.50	**25**	**10**	–	–	9.72	**2.3**	-

~~Values in bold are above the trigger value of 1 and hence further consideration is needed for these taxa/scenario combinations~~

For taxa/scenario combinations where the PEC/RAC ratio is above the trigger value of 1, with the exception of the acute risk to sediment dwellers, the risk assessment has been refined using FOCUS Step 3 PEC$_{SW}$ values. These refinements are presented below:

Refinement of the acute risk assessment to aquatic invertebrates

Given that the RAC for aquatic invertebrates represents the lowest endpoint for the acute risk assessment, refinement of the risk to this group will be protective of acute toxicity to other groups.

The acute invertebrate risk assessment for cyprodinil is based on a 96-hour LC_{50} of 8.05 µg a.s./L for *Mysidopsis bahia*. This value is the lowest endpoint generated from tests with 13 other species, where EC_{50} values range between 0.033 and >9.5 mg a.s./L.

Given the number of endpoints that are available, one refinement option is to construct a species sensitivity distribution using the program ETX 2.0[3]. For convenience the list of endpoints for acute invertebrates is presented in the table below.

Table 10.2-22 ~~17~~: Acute cyprodinil toxicity endpoints for aquatic invertebrates, for probabilistic risk assessment

Test organism	Taxonomy		EC/LC_{50} (mg a.s./L)	Reference
	Subphylum	Order		
Mysidopsis bahia	Crustacean	Mysida	0.00805	***Ward (1995)***
Daphnia magna	Crustacean	Cladocera	0.033	***Boeri et al (1995)***
Thamnocephalus platyurus	Crustacean	Anostraca	0.12	***Peither (2000)***
Simocephalus vetulus	Crustacean	Anomopoda	0.15	***Peither (2000)***
Daphniopsis sp.	Crustacean	Cladocera	0.21	***Peither (2000)***
Daphnia longispina	Crustacean	Cladocera	0.22	***Peither (2000)***
Ostracoda	Crustacean	Podocopa	1.1	***Peither (2000)***
Gammarus sp.	Crustacean	Amphipoda	1.8	***Peither (2000)***
Lymnea stagnalis	Mollusca (phylum)	-	2.9	***Peither (2000)***
Cloeon sp.	Arthropoda (phylum)	-	3.5	***Peither (2000)***
Chaoborus sp.	Hexapoda	-	4.0	***Peither (2000)***
Brachionus calyciflorus	Rotifera	Ploima	> 9.5	***Peither (2000)***
Asellus aquaticus (nymphs)	Crustacean	Isopoda	2.35	***Maynard (2011)***
~~*Asellus aquaticus* (adults)~~	~~Crustacean~~	~~Isopoda~~	~~1.96~~	~~***Maynard (2011)***~~
~~*Gammarus pulex*~~	~~Crustacean~~	~~Amphipoda~~	~~0.69~~	~~***Beketov & Liess (2008)***~~

Clearly the most sensitive taxa are the crustaceans. As discussed in the aquatic guidance document when considering the quality of acute toxicity data used to construct the SSD:

'If the toxicity data comprise several different genera/families/orders of the potentially sensitive taxonomic group (see section 8.4.3 for further guidance), including Ephemeroptera/Plecoptera/Trichoptera taxa (EPT) for insecticides, a lower AF in the proposed range may be selected. However, if another valid SSD can be constructed with a more limited dataset containing the most sensitive species, and the HC_5 derived from this SSD curve is lower than that of the SSD curve using toxicity data for a wider array of taxa, a higher AF in the proposed range may be selected to be applied to the SSD from the wider set.'

Given that the crustaceans are the most sensitive group an SSD has been constructed based on endpoints derived for them. The SSD distribution is presented in Figure 10.2-1.

[3] Vlaardingen PLA van, Traas TP, Wintersen AM, Aldenberg T. ETX 2.0. A Program to Calculate Hazardous Concentrations and Fraction Affected, Based on Normally Distributed Toxicity Data. RIVM The Netherlands.

The resulting median HC_5 value is 13.6 14.14 µg a.s./L (95% CI 1.71 – 50.39 1.79 – 44.4 µg a.s./L).

According to the aquatic guidance document an assessment factor (AF) of 3 – 6 is recommended for this type of data. Several aspects need to be considered when selecting an appropriate AF from an SSD distribution. For ease of reference these are directly quoted below.

1. *The quality of the acute toxicity data used to construct the SSD*. If the toxicity data comprise several different genera/families/orders of the potentially sensitive taxonomic group (see section 8.4.3 for further guidance), including Ephemeroptera/Plecoptera/Trichoptera taxa (EPT) for insecticides, a lower AF in the proposed range may be selected. However, if another valid SSD can be constructed with a more limited dataset containing the most sensitive species, and the HC_5 derived from this SSD curve is lower than that of the SSD curve using toxicity data for a wider array of taxa, a higher AF in the proposed range may be selected to be applied to the SSD from the wider set.

2. *The lower limit value of the HC_5*. If the lower limit HC_5 derived from the curve is less than 1/3 of the median HC_5, a higher AF in the proposed range may be warranted.

3. *The lower tier RACs on the basis of standard toxicity data (tier 1), standard and additional toxicity data (Geomean approach) and tier 3 data*. The size of the AF should ideally not result in an SSD-$RAC_{sw;ac}$ higher than the tier 3 RAC derived from effect class 1 and 2 of micro- mesocosm studies, nor should it result in an SSD-$RAC_{sw;ac}$ lower than the tier 1 $RAC_{sw;ac}$ on the basis of standard test species and/or the Geomean- $RAC_{sw;ac}$ and/or method 3 to 5 (EFSA, 2006a) on the basis of the same toxicity data that were used to construct the SSD. Note that according to EFSA (2006a), the Geomean approach aims to achieve the same average level of protection as in the tier 1 effect assessment but can be predicted more accurately because of the availability of additional toxicity data for the relevant taxonomic groups.

4. *The position of the toxicity data in the lower tail of the SSD (around the HC_5)*. If in the lower tail the toxicity data, overall, are positioned on the right side of the SSD curve, the derived HC_5 estimate may be considered relatively "conservative" for the most sensitive species. This may be a reason to adopt a lower AF from the proposed range. In contrast, if in the lower tail the toxicity data are, overall, positioned on the left side of the SSD curve, this may be a reason to adopt a higher AF from the proposed range.

5. *The steepness of the SSD curve*. In the case of a relatively steep SSD curve (e.g. less than a factor of 100 between lowest and highest $L(E)C_{50}$ value used to construct the SSD curve), a higher AF from the proposed range is recommended since exposure concentrations that exceed the $RAC_{sw;ac}$ may have ecotoxicological consequences for a larger number of taxa.

6. *Considering information on chronic effects*. If acute to chronic ration (acute EC_{50}/chronic EC_{10}) is larger than 10, then an AF in the higher range may be warranted.

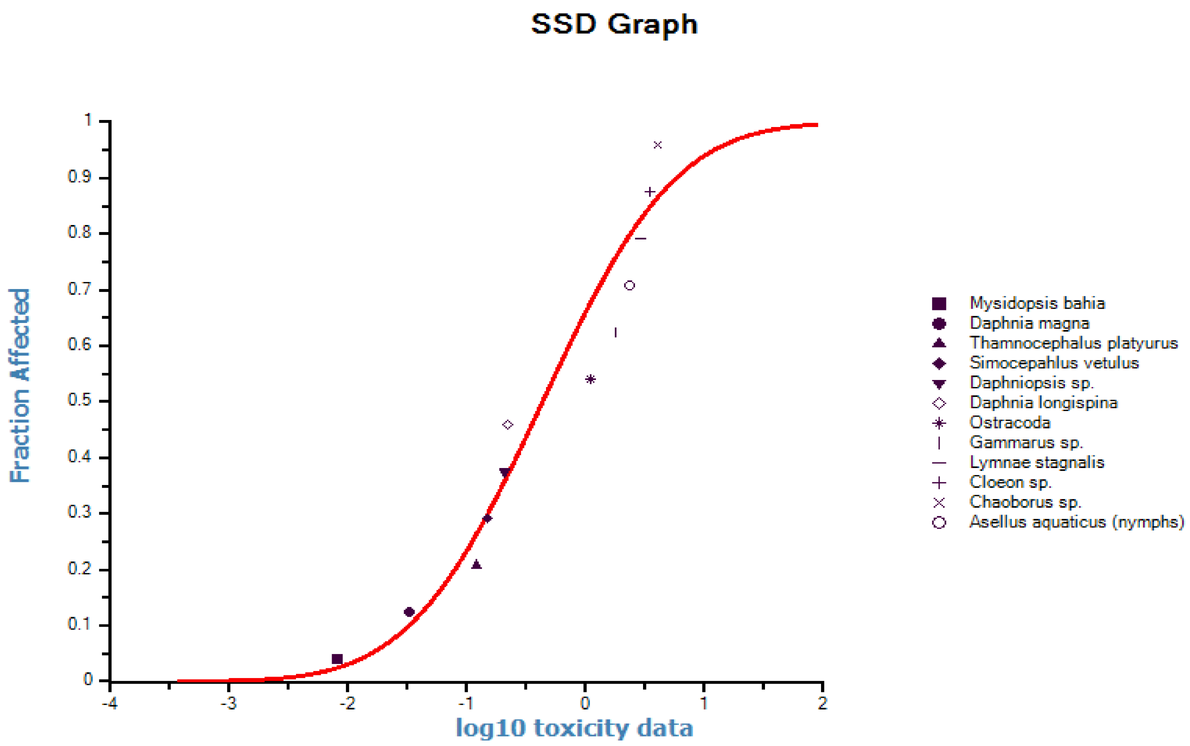

Figure 10.2-1: Species sensitivity distribution (SSD) for acute exposure of crustaceans to cyprodinil

It is proposed that an AF of 3 is applied to the HC_5 of 14.14 ~~13.6~~ µg a.s./L giving an $SSD\text{-}RAC_{sw;ac}$ of 4.71 ~~4.53~~ µg a.s./L. Justification is provided below by considering the data set presented in Table 10.2-20 against the above aspects:

1. The most sensitive taxa have been used to construct the SSD and several different orders are represented – **therefore a lower assessment factor can be justified here.**

2. The lower limit of the HC_5 is less than 1/3 of the median HC_5

3. The size of the AF should ideally not result in an $SSD\text{-}RAC_{sw;ac}$ higher than the tier 3 RAC derived from effect class 1 and 2 of micro- mesocosm studies, nor should it result in an $SSD\text{-}RAC_{sw;ac}$ lower than the tier 1 $RAC_{sw;ac}$ on the basis of standard test species – **therefore a lower assessment factor can be justified here.**

4. In the lower tail the toxicity data, overall, are positioned on the right side of the SSD curve - **therefore a lower assessment factor can be justified here.**

5. The SSD curve is relatively shallow in that there is greater than a factor of 100 between lowest and highest $L(E)C_{50}$ - **therefore a lower assessment factor can be justified here.**

6. **The acute to chronic ratio for** *Mysidopsis bahia* is 4 - **therefore a lower assessment factor can be justified here.**

In addition to these points, the test for normality was acceptable for all three tests (Anderson-Darling, Kolmogorov-Smirnov and Cramer von Mises) for all significance levels.

The FOCUS Step 3 PEC_{SW} values for all application scenarios have been compared with the SSD-$RAC_{sw;ac}$ RAC of 4.53 µg a.s./L. These are shown in the table below.

Table 10.2-23: Higher-tier acute risk assessment using a refined SSD-RAC of 4.71 µg a.s./L for aquatic invertebrates for cyprodinil – FOCUS Step 3 for cereals

Crop	Scenario	Application scenarios for A14325E in cereals			
		1 x 450 g a.s./ha		2 x 450 g a.s./ha	
		PEC (µg/L)	PEC/RAC ratio	PEC (µg/L)	PEC/RAC ratio
Winter cereals	D1 ditch	3.25	0.69	4.29	0.91
	D1 stream	2.52	0.54	2.19	0.46
	D2 ditch	3.28	0.70	6.43	**1.4**
	D2 stream	2.77	0.59	4.02	0.85
	D3 ditch	2.84	0.60	2.49	0.53
	D4 pond	0.098	0.021	0.149	0.032
	D4 stream	2.37	0.50	2.1	0.45
	D5 pond	0.1	0.021	0.139	0.030
	D5 stream	2.51	0.53	2.29	0.49
	D6 ditch	2.84	0.60	2.5	0.53
	R1 pond	0.186	0.039	0.481	0.10
	R2 stream	1.87	0.40	2.99	0.63
	R3 stream	2.65	0.56	2.33	0.49
	R4 stream	1.88	0.40	1.85	0.39
Spring cereals	D1 ditch	3.46	0.73	4.79	**1.0**
	D1 stream	2.53	0.54	2.21	0.47
	D3 ditch	2.85	0.61	2.49	0.53
	D4 pond	0.098	0.021	0.179	0.038
	D4 stream	2.33	0.49	2.12	0.45
	D5 pond	0.1	0.02	0.138	0.029
	D5 stream	2.47	0.52	2.15	0.46
	R4 stream	1.94	0.41	2.01	0.43

Values in bold are above the trigger value of 1 and hence further consideration is needed for these taxa/scenario combinations

Table 10.2-18: Higher-tier acute risk assessment using a refined SSD-RAC of 4.53 µg a.s./L for aquatic invertebrates for cyprodinil – FOCUS Step 3 for cereals

Crop	Scenario	Application scenarios for A14325E in cereals			
		1 x 450 g a.s./ha		2 x 450 g a.s./ha	
		PEC (µg/L)	PEC/RAC ratio	PEC (µg/L)	PEC/RAC ratio
Winter cereals	D1 ditch	3.28	0.72	4.33	0.96
	D1 stream	2.52	0.56	2.19	0.48
	D2 ditch	3.33	0.74	6.82	**1.5**
	D2 stream	2.80	0.62	4.27	0.94
	D3 ditch	2.84	0.63	2.49	0.55
	D4 pond	0.098	0.022	0.159	0.035
	D4 stream	2.37	0.52	2.10	0.46
	D5 pond	0.100	0.022	0.139	0.031
	D5 stream	2.51	0.55	2.29	0.51
	R1 pond	2.84	0.63	2.50	0.55
	R1 stream	0.188	0.042	0.484	0.11
	R2 stream	1.87	0.41	3.04	0.67
	R3 stream	2.65	0.58	2.41	0.53
	R4 stream	1.88	0.42	1.88	0.42
Spring cereals	D1 ditch	3.51	0.77	4.87	**1.1**
	D1 stream	2.53	0.56	2.21	0.49
	D3 ditch	2.85	0.63	2.49	0.55
	D4 pond	0.098	0.022	0.190	0.042
	D4 stream	2.33	0.51	2.12	0.47
	D5 pond	0.100	0.02	0.139	0.031
	D5 stream	2.47	0.55	2.15	0.47
	R4 stream	1.97	0.43	2.04	0.45

Values in bold are above the trigger value of 1 and hence further consideration is needed for these taxa/scenario combinations

The vast majority of the PEC/SSD-$RAC_{sw;ac}$ values are less than 1 indicating acceptable acute risk to aquatic invertebrates following use of A14325E according to the proposed use pattern. However, for winter cereals for the D2 ditch scenario and spring cereals for the D1 ditch scenario the PEC/SSD-$RAC_{sw;ac}$ ratios are greater than 1, indicating the need for further consideration of the risk to aquatic invertebrates.

Refinement is presented below in which the PEC/RAC values have been calculated using FOCUS Step 4 values.

Table 10.2-24: Refinement of acute risk to aquatic invertebrates using FOCUS Step 4 PECsw

$FOCUS_{sw}$ step 4 – PEC/RAC ratio – Cyprodinil on cereals

Organisms: Aquatic invertebrates

Toxicity endpoint: RAC – 4.71 µg a.s./L

Crop	Mitigation options	Vegetative strip (m)	5 m non-spray buffer zone (corresponding to ≤ 95 % drift reduction)		Trigger
			PEC_{SW} (µg/L)	PEC/RAC ratio	
Winter cereals	FOCUS Step 4*				

2 x 450 g a.s./ha	D2 / ditch	None	2.13	0.45	1
Spring cereals	**FOCUS Step 4***				
2 x 450 g a.s./ha	D1 / ditch	None	1.48	0.31	1

The refined PEC/RAC$_{sw;ac}$ values are less than 1 indicating acceptable acute risk to aquatic invertebrates following use of A14325E according to the proposed use pattern when consideration is given to a 5m non-spray buffer zone.

Refinement of acute risk to aquatic invertebrates using the Ashwell *et al.* (2007) mesocosm ETO-RAC

A mesocosm study was conducted using a 300 EC formulation A14325E (***Ashwell et al, 2007***) (details are provided in M-CA Section 8, CA 8.2-8) to a community typical for a lentic freshwater community, containing phyto- and zooplankton and macroinvertebrates. Intended initial concentrations were 0 – 1.5 – 5 – 10 – 20 – 50 µg a.s./L. Immediately after each of the three applications, the test compound was mixed in the water layer of the microcosms. Measurements in dosing solutions and water indicated that the test systems received the intended doses. Shortly after the applications, 75-80%, 119-154% and 118-156% of the target amount was measured in the water of the test systems.

MDD analysis of the available data for zooplankton demonstrated that typically small to large effects could be determined throughout the study for five parameters. As these evaluations included sensitive taxa (*Daphnia* sp.) and organisms from the three main zooplankton groups (cladocera, copepoda and rotifera), the data generated are considered robust and reliable for ETO-RAC derivation and a NOEC (class 1) of 14.6 µg a.s./L (based on measured concentrations) is recommended for zooplankton. If an NOEAEC (class 3A) is required for ERO-RAC, it can be considered to be 67.5 µg a.s./L (based on measured concentrations).

The NOEC (Effect class 1) from this study has been determined as 14.6 µg a.s./L (based on mean measured concentrations) . The appropriate assessment factor recommended by the EFSA aquatic guidance is 2, resulting in an ETO-RAC of 7.3 µg a.s./L.

In the table below, the PEC/RAC values based on the ETO- RAC of 7.3 µg a.s./L have been presented for the two scenarios that previously failed the risk assessment.

Table 10.2-19: Higher-tier acute risk assessment for aquatic invertebrates using a refined ETO-RAC of 7.3 µg a.s./L

Crop	Scenario	Application scenarios for A14325E in cereals	
		2 x 450 g a.s./ha	
		PEC (µg/L)	PEC/RAC ratio
Winter cereals	D2 ditch	6.82	0.93
Spring cereals	D1 ditch	4.87	0.67

The refined PEC/ETO-RAC$_{sw;ac}$ values are less than 1 indicating acceptable acute risk to aquatic invertebrates following use of A14325E according to the proposed use pattern.

Acute risk to fish

For the acute risk assessment for fish, the PEC/RAC ratios were greater than 1 for several FOCUS Step 3 scenarios (please refer to Tables 10.2.18 ~~13~~ to 10.2-21 ~~16~~). Given that the acute RAC for fish is 12.5 µg a.s./L and is therefore higher than the ~~mesocosm~~ ~~ETO-~~ SSD RAC of ~~7.3~~ 4.71 µg a.s./L, the acute invertebrate risk assessment would cover also cover the acute risk to fish.

Refinement of the acute risk to sediment dwellers

For the acute risk assessment to sediment dwellers, some of the PEC/RAC ratios presented in Tables 10.2.18 ~~13~~ to 10.2-21 ~~16~~ are above 1 indicating the need for further refinement.

In the mesocosm study conducted by Ashwell *et al.* (2007) the effects of cyprodinil, applied as A14325E, on Chironomidae were evaluated. %MDD values for Chironomidae ranged from 17 to 29 between day -27 and day 29, meaning small effects could be reliably determined for this sampling period, which included all three applications of the test item. From day 43 to day 71, %MDD values were >100, meaning no effects could be reliably determined. From day 85 and for the remainder of the study, %MDD values ranged between 62 and 88, meaning medium to large effects could be reliably determined.

As a result, the data for this taxon are considered reliable (category one) and suitable for use in ETO-RAC derivation. In addition, as no clear treatment related effects were seen at the maximum tested concentration (50 µg a.s./L), the endpoint for Chironomidae are also suitable for ERO-RAC derivation. Therefore, the mitigation proposed to address the acute risk to invertebrates will also address the acute risk to sediment-dwellers.

Overall conclusion

When applied in accordance with the uses supported in this submission A14325E poses an acceptable acute risk to aquatic organisms.

Long-term risk to aquatic invertebrates

The lowest tier 1 $RAC_{sw;ch}$ is 0.197 µg a.s./L, based on data for aquatic invertebrates, the mysid shrimp. As shown in Tables 10.2.18 ~~13~~ to 10.2-21 ~~16~~, acceptable risk was not achieved when this $RAC_{sw;ch}$ was compared to FOCUS Step 3 surface water concentrations.

Based on EFSA Aquatic Guidance, the chronic risk can be refined using a default 7-d twa. However, it should not be used if the following apply:

- If the RAC is from studies where exposure is not maintained – *exposure was maintained throughout the mysid study.*
- When the effect is based on a developmental endpoint during a specific lifestage that may last a short time only – *the endpoint is based on survival of the F1 generation.*
- When the effect is based on mortality early in the test or the acute:chronic ratio both based on mortality is <10 – *mortality did not occur early in the test.*
- If latency has been demonstrated or might be expected – *there is no evidence for latency of effects*.

There is no reason not to use the 7-d twa in the chronic risk assessment. PEC/RAC values for FOCUS Step 3 7 d TWA concentrations are presented in the table below.

Table 10.2-25: Higher-tier long-term risk assessment for aquatic invertebrates using FOCUS Step 3 TWA PEC$_{SW}$ concentrations (RACsw;ch = 0.197 µg a.s./L) for winter cereals

Crop	Scenario	Application scenarios for A14325E in cereals			
		1 x 450 g a.s./ha		2 x 450 g a.s./ha	
		PEC (µg/L)	PEC/RAC ratio	PEC (µg/L)	PEC/RAC ratio
Winter cereals	D1 ditch	2.57	**13**	3.59	**18**
	D1 stream	0.69	**3.5**	1.3	**6.6**
	D2 ditch	2.6	**13**	3.13	**16**
	D2 stream	2.13	**11**	2.36	**12**
	D3 ditch	0.417	**2.1**	0.403	**2.0**
	D4 pond	0.088	0.45	0.137	0.70
	D4 stream	0.054	0.27	0.123	0.62
	D5 pond	0.09	0.46	0.13	0.66
	D5 stream	0.035	0.18	0.125	0.63
	D6 ditch	0.432	**2.2**	1.01	**5.1**
	R1 pond	0.175	0.89	0.452	**2.3**
	R1 stream	0.14	0.71	0.39	**2.0**
	R3 stream	0.19	0.96	0.332	**1.7**
	R4 stream	0.563	**2.9**	0.578	**2.9**
Spring cereals	D1 ditch	2.77	**14**	4.05	**21**
	D1 stream	0.665	**3.4**	1.58	**8.0**
	D3 ditch	0.457	**2.3**	0.415	**2.1**
	D4 pond	0.088	0.45	0.165	0.84
	D4 stream	0.063	0.32	0.155	0.79
	D5 pond	0.09	0.46	0.128	0.65
	D5 stream	0.028	0.14	0.026	0.13
	R4 stream	0.615	**3.1**	0.636	**3.2**

Values in bold are above the trigger value of 1 and hence further consideration is needed for these taxa/scenario combinations

~~**Table 10.2-20: Higher-tier long-term risk assessment for aquatic invertebrates using FOCUS Step 3 TWA PEC$_{SW}$ concentrations (RACsw;ch = 0.197 µg a.s./L) for winter cereals**~~

~~Crop~~	~~Scenario~~	~~Application scenarios for A14325E in cereals~~			
		~~1 x 450 g a.s./ha~~		~~2 x 450 g a.s./ha~~	
		~~PEC (µg/L)~~	~~PEC/RAC ratio~~	~~PEC (µg/L)~~	~~PEC/RAC ratio~~
~~Winter cereals~~	~~D1 ditch~~	~~2.60~~	~~**13**~~	~~3.62~~	~~**18**~~
	~~D1 stream~~	~~0.735~~	~~**3.7**~~	~~1.38~~	~~**7.0**~~
	~~D2 ditch~~	~~2.65~~	~~**13**~~	~~3.24~~	~~**16**~~
	~~D2 stream~~	~~2.16~~	~~**11**~~	~~2.43~~	~~**12**~~
	~~D3 ditch~~	~~0.420~~	~~**2.1**~~	~~0.405~~	~~**2.1**~~
	~~D4 pond~~	~~0.088~~	~~0.45~~	~~0.147~~	~~0.75~~
	~~D4 stream~~	~~0.059~~	~~0.30~~	~~0.135~~	~~0.69~~
	~~D5 pond~~	~~0.090~~	~~0.46~~	~~0.130~~	~~0.66~~
	~~D5 stream~~	~~0.036~~	~~0.18~~	~~0.127~~	~~0.64~~
	~~R1 pond~~	~~0.434~~	~~**2.2**~~	~~1.01~~	~~**5.1**~~
	~~R1 stream~~	~~0.177~~	~~0.90~~	~~0.455~~	~~**2.3**~~

	R2 stream	0.139	0.71	0.388	**2.0**
	R3 stream	0.181	0.92	0.335	**1.7**
	R4 stream	0.553	**2.8**	0.568	**2.9**
Spring cereals	D1 ditch	2.82	**14**	4.12	**21**
	D1 stream	0.724	**3.7**	1.71	**8.7**
	D3 ditch	0.459	**2.3**	0.417	**2.1**
	D4 pond	0.088	0.45	0.175	0.89
	D4 stream	0.069	0.35	0.168	0.85
	D5 pond	0.090	0.46	0.129	0.65
	D5 stream	0.030	0.15	0.027	0.14
	R4 stream	0.605	**3.1**	0.627	**3.2**

Values in bold are above the trigger value of 1 and hence further consideration is needed for these taxa/scenario combinations

Some of the PEC/RAC ratios are greater than 1 and therefore further refinement is required. This is presented below.

Refinement of the long-term risk to aquatic invertebrates (RACsw;ch)

A mesocosm study was conducted using a 300 EC formulation A14325E (***Ashwell et al, 2007***) (details are provided in M-CA Section 8, CA 8.2-8) to a community typical for a lentic freshwater community, containing phyto- and zooplankton and macroinvertebrates. Intended initial concentrations were 0 – 1.5 – 5 – 10 – 20 – 50 µg a.s./L. Immediately after each of the three applications, the test compound was mixed in the water layer of the microcosms. Measurements in dosing solutions and water indicated that the test systems received the intended doses. Shortly after the applications, 75-80%, 119-154% and 118-156% of the target amount was measured in the water of the test systems.

The long-term risk to aquatic invertebrates will be refined using the ETO–RACs of 7.3 0.75 and 0.90 µg a.s./L and ERO-RACs of 3.33 and 4.86 µg a.s./L, derived following re-evaluation of the data from the study.

In the table below, the PEC/RAC values based on the ETO–RAC of 7.3 µg a.s./L have been presented for the two scenarios that previously failed the risk assessment.

Refinement of the chronic risk to aquatic invertebrates using a ETO-RAC of 0.75 µg a.s./L derived from the mesocosm study of Ashwell (2007)

Table 10.2-26: Higher-tier long-term risk assessment using an ETO-RAC of 0.75 µg a.s./L derived from the Ashwell *et al.* mesocosm study – FOCUS Step 3 for cereals

Crop	Scenario	Application scenarios for A14325E in cereals			
		1 x 450 g a.s./ha		2 x 450 g a.s./ha	
		PEC (µg/L)	PEC/RAC ratio	PEC (µg/L)	PEC/RAC ratio
Winter cereals	D1 ditch	3.25	**4.3**	4.29	**5.7**
	D1 stream	2.52	**3.4**	2.19	**2.9**
	D2 ditch	3.28	**4.4**	6.43	**8.6**
	D2 stream	2.77	**3.7**	4.02	**5.4**
	D3 ditch	2.84	**3.8**	2.49	**3.3**
	D6 ditch	2.84	**3.8**	2.5	**3.3**
	R1 pond	-	-	0.481	0.64
	R1 stream	-	-	2.99	**4.0**
	R3 stream	-	-	2.33	**3.1**
	R4 stream	1.88	**2.5**	1.85	**2.5**
Spring cereals	D1 ditch	3.46	**4.6**	4.79	**6.4**
	D1 stream	2.53	**3.4**	2.21	**3.0**
	D3 ditch	2.85	**3.8**	2.49	**3.3**
	R4 stream	1.94	**2.6**	2.01	**2.7**

Values in bold are above the trigger value of 1 and hence further consideration is needed for these taxa/scenario combinations

The majority of the PEC/RAC ratios are higher than the trigger value. Refined risk assessments have been presented in the tables below for the scenarios that failed.

Table 10.2-27: Refinement of long term risk to aquatic invertebrates using FOCUS Step 4 PECsw – PEC/ETO-RAC (0.75 µg a.s./L) ratio – cyprodinil on winter cereals 1 x 450 g a.s./ha

Scenario	Mitigation options	Non-spray buffer zone (corresponding to ≤ 95 % drift reduction)						Trigger
	Vegetative strip (m)	5 m		10 m		20 m		
		PEC_{SW} (µg/L)	PEC/RAC ratio	PEC_{SW} (µg/L)	PEC/RAC ratio	PEC_{SW} (µg/L)	PEC/RAC ratio	
D1 / ditch	None	1.28	**1.7**	1.1	**1.5**	-	-	1
D1 / stream	None	0.927	**1.2**	0.722	1.0	-	-	1
D2 / ditch	None	2.13	**2.8**	2.13	**2.8**	-	-	1
D2 / stream	None	1.34	**1.8**	1.34	**1.8**	-	-	1
D3 / ditch	None	0.788	**1.1**	0.437	0.58	-	-	1
D6 / ditch	None	0.792	**1.1**	0.602	0.8	-	-	1
R4 / stream	None	1.81	**2.4**	1.81	**2.4**	-	-	1
	10 – 12	-	-	0.821	**1.1**	-	-	
	18 - 20	-	-	-	-	0.43	0.57	

Table 10.2-28: Refinement of long term risk to aquatic invertebrates using FOCUS Step 4 PECsw – PEC/ETO-RAC (0.75 µg a.s./L) ratio – cyprodinil on winter cereals 2 x 450 g a.s./ha

Scenario	Mitigation options	Non-spray buffer zone (corresponding to ≤ 95 % drift reduction)						Trigger
	Vegetative strip (m)	5 m		10 m		20 m		
		PEC_{SW} (µg/L)	PEC/RAC ratio	PEC_{SW} (µg/L)	PEC/RAC ratio	PEC_{SW} (µg/L)	PEC/RAC ratio	
D1 / ditch	None	2.04	**2.7**	2.04	**2.7**	-	-	1
D1 / stream	None	1.36	**1.8**	1.36	**1.8**	-	-	1
D2 / ditch	None	6.42	**8.6**	6.42	**8.6**	-	-	1
D2 / stream	None	4.02	**5.4**	4.02	**5.4**	-	-	1
D3 / ditch	None	0.677	0.90	-	-	-	-	1
D6 / ditch	None	1.11	**1.5**	1.11	**1.5**	-	-	1
R1 / stream	None	2.99	**4.0**	2.99	**4.0**	-	-	1
	10 – 12	-	-	1.36	**1.8**	-	-	
	18 - 20	-	-	-	-	0.71	0.95	
R3 / stream	None	2.33	**3.1**	2.33	**3.1**	-	-	1
	10 – 12	-	-	1.05	**1.4**	-	-	
	18 - 20	-	-	-	-	0.546	0.73	
R4 / stream	None	1.85	**2.5**	1.85	**2.5**	-	-	1
	10 – 12	-	-	0.841	**1.1**	-	-	
	18 - 20	-	-	-	-	0.44	0.59	

Table 10.2-29: Refinement of long term risk to aquatic invertebrates using FOCUS Step 4 PECsw – PEC/ETO-RAC (0.75 µg a.s./L) ratio – cyprodinil on spring cereals 1 x 450 g a.s./ha

Scenario	Mitigation options	Non-spray buffer zone (corresponding to ≤ 95 % drift reduction)						Trigger
	Vegetative strip (m)	5 m		10 m		20 m		
		PEC_{SW} (µg/L)	PEC/RAC ratio	PEC_{SW} (µg/L)	PEC/RAC ratio	PEC_{SW} (µg/L)	PEC/RAC ratio	
D1 / ditch	None	1.48	**2.0**	1.24	**1.7**	-	-	1
D1 / stream	None	0.935	**1.2**	0.774	**1.0**	-	-	1
D3 / ditch	None	0.793	**1.1**	0.441	0.59	-	-	1
R4 / stream	None	1.94	**2.6**	1.94	**2.6**	-	-	1
	10 – 12	-	-	0.884	**1.2**	-	-	
	18 - 20	-	-	-	-	0.463	0.6	

Table 10.2-30: Refinement of long term risk to aquatic invertebrates using FOCUS Step 4 PECsw – PEC/ETO-RAC (0.75 µg a.s./L) ratio – cyprodinil on spring cereals 2 x 450 g a.s./ha

Scenario	Mitigation options	Non-spray buffer zone (corresponding to ≤ 95 % drift reduction)						Trigger
	Vegetative strip (m)	5 m		10 m		20 m		
		PEC_{SW} (µg/L)	PEC/RAC ratio	PEC_{SW} (µg/L)	PEC/RAC ratio	PEC_{SW} (µg/L)	PEC/RAC ratio	
D1 / ditch	None	2.77	**3.7**	2.77	**3.7**	-	-	1
D1 / stream	None	1.74	**2.3**	1.74	**2.3**	-	-	1
D3 / ditch	None	0.679	0.91	-	-	-	-	1
R4 / stream	None	2.01	**2.7**	2.01	**2.7**	-	-	1
	10 – 12	-	-	0.913	**1.2**	-	-	
	18 - 20	-	-	-	-	0.478	0.64	

The table below summarises mitigation required for the various scenarios when an ETO-RAC of 0.75 is used to refine the risk assessment

Table 10.2-31: Mitigation measures required to resolve the long-term risk assessment for aquatic invertebrates when using an ETO-RAC of 0.75 µg a.s./L

Scenario	Winter cereals 1 x 450 g a.s./ha	Winter cereals 2 x 450 g a.s./ha	Spring cereals 1 x 450 g a.s./ha	Spring cereals 2 x 450 g a.s./ha
	Mitigation required			
D1 ditch	NR	NR	NR	NR
D1 stream	10 m DB	NR	NR	NR
D2 ditch	NR	NR		
D2 stream	NR	NR		
D3 ditch	10 m DB	5 m DB	10 m DB	5 m DB
D6 ditch	10 m DB	NR		
R1 stream	-	20 m DB + 18 – 20 m VS		
R3 stream	-	20 m DB + 18 – 20 m VS		
R4 stream	20 m DB + 18 – 20 m VS	20 m DB + 18 – 20 m VS	20 m DB + 18 – 20 m VS	20 m DB + 18 – 20 m VS

DB = Drift buffer
VS = vegetated filter strip
NR = risk assessment could not be resolved using this ETO – RAC
- Acceptable risk was achieved for these scenarios using a lower tier risk assessment
Shaded boxes represent scenarios that are not relevant to spring cereal cultivation

Refinement of the chronic risk to aquatic invertebrates using an ETO-RAC of 0.90 derived from the mesocosm study of Ashwell (2007)

Table 10.2-32: Higher-tier long-term risk assessment using an ETO-RAC of 0.90 µg a.s./L derived from the Ashwell *et al.* mesocosm study – FOCUS Step 3 for cereals

Crop	Scenario	Application scenarios for A14325E in cereals			
		1 x 450 g a.s./ha		2 x 450 g a.s./ha	
		PEC (µg/L)	PEC/RAC ratio	PEC (µg/L)	PEC/RAC ratio
Winter cereals	D1 ditch	3.25	**3.61**	4.29	**4.77**
	D1 stream	2.52	**2.80**	2.19	**2.43**
	D2 ditch	3.28	**3.64**	6.43	**7.14**
	D2 stream	2.77	**3.08**	4.02	**4.47**
	D3 ditch	2.84	**3.16**	2.49	**2.77**
	D6 ditch	2.84	**3.16**	2.5	**2.78**
	R1 pond	-	-	0.481	0.53
	R1 stream	-	-	2.99	**3.32**
	R3 stream	-	-	2.33	**2.59**
	R4 stream	1.88	**2.09**	1.85	**2.06**
Spring cereals	D1 ditch	3.46	**3.84**	4.79	**5.32**
	D1 stream	2.53	**2.81**	2.21	**2.46**
	D3 ditch	2.85	**3.17**	2.49	**2.77**
	R4 stream	1.94	**2.16**	2.01	**2.23**

Values in bold are above the trigger value of 1 and hence further consideration is needed for these taxa/scenario combinations

The majority of the PEC/RAC ratios are higher than the trigger value. Refined risk assessments have been presented in the tables below for the scenarios that failed.

Table 10.2-33: Refinement of long term risk to aquatic invertebrates using FOCUS Step 4 PECsw – PEC/ETO-RAC (0.90 µg a.s./L) ratio – cyprodinil on winter cereals 1 x 450 g a.s./ha

Scenario	Mitigation options	Non-spray buffer zone (corresponding to ≤ 95 % drift reduction)				Trigger
	Vegetative strip (m)	5 m		10 m		
		PEC_{SW} (µg/L)	PEC/RAC ratio	PEC_{SW} (µg/L)	PEC/RAC ratio	
D1 / ditch	None	1.28	**1.4**	1.1	**1.2**	1
D1 / stream	None	0.927	**1.0**	0.722	0.8	1
D2 / ditch	None	2.13	**2.4**	2.13	**2.4**	1
D2 / stream	None	1.34	**1.5**	1.34	**1.5**	1
D3 / ditch	None	0.788	0.88	0.437	0.49	1
D6 / ditch	None	0.792	0.88	0.602	0.67	1
R4 / stream	None	1.81	**2.0**	1.81	**2.0**	1
	10 – 12	-	-	0.821	0.91	

Table 10.2-34: Refinement of long term risk to aquatic invertebrates using FOCUS Step 4 PECsw – PEC/ETO-RAC (0.90 µg a.s./L) ratio – cyprodinil on winter cereals 2 x 450 g a.s./ha

Scenario	Mitigation options	Non-spray buffer zone (corresponding to ≤ 95 % drift reduction)						Trigger
	Vegetative strip (m)	5 m		10 m		20 m		
		PEC_{SW} (µg/L)	PEC/RAC ratio	PEC_{SW} (µg/L)	PEC/RAC ratio	PEC_{SW} (µg/L)	PEC/RAC ratio	
D1 / ditch	None	2.04	**2.3**	2.04	**2.3**	-	-	1
D1 / stream	None	1.36	**1.5**	1.36	**1.5**	-	-	1
D2 / ditch	None	6.42	**7.1**	6.42	**7.1**	-	-	1
D2 / stream	None	4.02	**4.5**	4.02	**4.5**	-	-	1
D3 / ditch	None	0.677	0.75	0.381	0.42	-	-	1
D6 / ditch	None	1.11	**1.2**	1.11	**1.2**	-	-	1
R1 / stream	None	2.99	**3.3**	2.99	**3.3**	-	-	1
	10 – 12	-	-	1.36	**1.5**	-	-	
	18 - 20	-	-	-	-	0.71	0.79	
R3 / stream	None	2.33	**2.6**	2.33	**2.6**	-	-	1
	10 – 12	-	-	1.05	**1.2**	-	-	
	18 - 20	-	-	-	-	0.546	0.61	
R4 / stream	None	1.85	**2.1**	1.85	**2.1**	-	-	1
	10 – 12	-	-	0.841	0.93	-	-	

Table 10.2-35: Refinement of long term risk to aquatic invertebrates using FOCUS Step 4 PECsw – PEC/ETO-RAC (0.90 µg a.s./L) ratio – cyprodinil on spring cereals 1 x 450 g a.s./ha

Scenario	Mitigation options	Non-spray buffer zone (corresponding to ≤ 95 % drift reduction)				Trigger
	Vegetative strip (m)	5 m		10 m		
		PEC_{SW} (µg/L)	PEC/RAC ratio	PEC_{SW} (µg/L)	PEC/RAC ratio	
D1 / ditch	None	1.48	**1.6**	1.24	**1.4**	1
D1 / stream	None	0.935	**1.0**	0.774	0.86	1
D3 / ditch	None	0.793	0.88	0.441	0.49	1
R4 / stream	None	1.94	**2.2**	1.94	**2.2**	1
	10 – 12	-	-	0.884	1.0	

Table 10.2-36: Refinement of long term risk to aquatic invertebrates using FOCUS Step 4 PECsw – PEC/ETO-RAC (0.90 µg a.s./L) ratio – cyprodinil on spring cereals 2 x 450 g a.s./ha

Scenario	Mitigation options	Non-spray buffer zone (corresponding to ≤ 95 % drift reduction)						Trigger
	Vegetative strip (m)	5 m		10 m		20 m		
		PEC_{SW} (µg/L)	PEC/RAC ratio	PEC_{SW} (µg/L)	PEC/RAC ratio	PEC_{SW} (µg/L)	PEC/RAC ratio	
D1 / ditch	None	2.77	**3.1**	2.77	**3.1**	-	-	1
D1 / stream	None	1.74	**1.9**	1.74	**1.9**	-	-	1
D3 / ditch	None	0.679	0.75	0.383	0.43	-	-	1
R4 / stream	None	2.01	**2.2**	2.01	**2.2**	-	-	1
	10 – 12	-	-	0.913	**1.0**	-	-	
	18 - 20	-	-	-	-	0.478	0.53	

The table below summarises mitigation required for the various scenarios when an ETO-RAC of 0.90 µg a.s./L is used to refine the risk assessment

Table 10.2-37: Mitigation measures required to resolve the long-term risk assessment for aquatic invertebrates when using an ETO-RAC of 0.90 µg a.s./L

Scenario	Winter cereals 1 x 450 g a.s./ha	Winter cereals 2 x 450 g a.s./ha	Spring cereals 1 x 450 g a.s./ha	Spring cereals 2 x 450 g a.s./ha
	Mitigation required			
D1 ditch	NR	NR	NR	NR
D1 stream	10 m DB	NR	NR	NR
D2 ditch	NR	NR		
D2 stream	NR	NR		
D3 ditch	5 m DB	5 m DB	5 m DB	10 m DB
D6 ditch	5 m DB	NR		
R1 stream	-	20 m DB + 18 – 20 m VS		
R3 stream	-	20 m DB + 18 – 20 m VS		
R4 stream	10 m DB + 10 – 12 m VS	10 m DB + 10 – 12 m VS	10 m DB + 10 – 12 m VS	20 m DB + 18 – 20 m VS

DB = Drift buffer
VS = vegetated filter strip
NR = risk assessment could not be resolved using this ETO – RAC
- Acceptable risk was achieved for these scenarios using a lower tier risk assessment
Shaded boxes represent scenarios that are not relevant to spring cereal cultivation

Refinement of the chronic risk to aquatic invertebrates using an ERO-RAC of 3.33 µg a.s./L derived from the mesocosm study of Ashwell (2007)

Table 10.2-38: Higher-tier long-term risk assessment using an ERO-RAC of 3.33 µg a.s./L derived from the Ashwell *et al.* mesocosm study – FOCUS Step 3 for cereals

Crop	Scenario	Application scenarios for A14325E in cereals			
		1 x 450 g a.s./ha		2 x 450 g a.s./ha	
		PEC (µg/L)	PEC/RAC ratio	PEC (µg/L)	PEC/RAC ratio
Winter cereals	D1 ditch	3.25	0.98	4.29	**1.29**
	D1 stream	2.52	0.76	2.19	0.66
	D2 ditch	3.28	0.98	6.43	**1.93**
	D2 stream	2.77	0.83	4.02	**1.21**
	D3 ditch	2.84	0.85	2.49	0.75
	D6 ditch	2.84	0.85	2.5	0.75
	R1 pond			0.481	0.14
	R1 stream			2.99	0.90
	R3 stream			2.33	0.70
	R4 stream	1.88	0.56	1.85	0.56
Spring cereals	D1 ditch	3.46	**1.04**	4.79	**1.44**
	D1 stream	2.53	0.76	2.21	0.66
	D3 ditch	2.85	0.86	2.49	0.75
	R4 stream	1.94	0.58	2.01	0.60

Values in bold are above the trigger value of 1 and hence further consideration is needed for these taxa/scenario combinations

Refined risk assessments have been presented in the tables below for the scenarios that failed.

Table 10.2-39: Refinement of long term risk to aquatic invertebrates using FOCUS Step 4 PECsw – PEC/ERO-RAC (3.33 µg a.s./L) ratio – cyprodinil on winter cereals 2 x 450 g a.s./ha

Scenario	Mitigation options	Non-spray buffer zone (corresponding to ≤ 95 % drift reduction)				Trigger
	Vegetative strip (m)	5 m		10 m		
		PEC_{SW} (µg/L)	PEC/RAC ratio	PEC_{SW} (µg/L)	PEC/RAC ratio	
D1 / ditch	None	2.04	0.61	-	-	1
D2 / ditch	None	6.42	**1.9**	6.42	**1.9**	1
D2 / stream	None	4.02	**1.2**	4.02	**1.2**	1

Table 10.2-40: Refinement of long term risk to aquatic invertebrates using FOCUS Step 4 PECsw – PEC/ERO-RAC (3.33 µg a.s./L) ratio – cyprodinil on spring cereals 1 x 450 g a.s./ha

Scenario	Mitigation options	Non-spray buffer zone (corresponding to ≤ 95 % drift reduction)		Trigger
		5 m		
	Vegetative strip (m)	PEC_{SW} (µg/L)	PEC/RAC ratio	
D1 / ditch	None	1.48	0.44	1

Table 10.2-41: Refinement of long term risk to aquatic invertebrates using FOCUS Step 4 PECsw – PEC/ERO-RAC (3.33 µg a.s./L) ratio – cyprodinil on spring cereals 2 x 450 g a.s./ha

Scenario	Mitigation options	Non-spray buffer zone (corresponding to ≤ 95 % drift reduction) 5 m		Trigger
	Vegetative strip (m)	PEC_{SW} (µg/L)	PEC/RAC ratio	
D1 / ditch	None	2.77	0.8c	1

The table below summarises mitigation required for the various scenarios when an ERO-RAC of 3.33 µg a.s./L is used to refine the risk assessment

Table 10.2-42: Mitigation measures required to resolve the long-term risk assessment for aquatic invertebrates when using an ERO-RAC of 3.33 µg a.s./L

Scenario	Winter cereals 1 x 450 g a.s./ha	Winter cereals 2 x 450 g a.s./ha	Spring cereals 1 x 450 g a.s./ha	Spring cereals 2 x 450 g a.s./ha
	Mitigation required			
D1 ditch	-	5 m DB	5 m DB	5 m DB
D2 ditch	-	NR		
D2 stream	-	NR		

DB = Drift buffer
VS = vegetated filter strip
NR = risk assessment could not be resolved using this ETO – RAC
- Mitigation is not required

Refinement of the chronic risk to aquatic invertebrates using an ERO-RAC of 4.86 µg a.s./L derived from the mesocosm study of Ashwell (2007)

Table 10.2-43: Higher-tier long-term risk assessment using an ERO-RAC of 4.86 µg a.s./L derived from the Ashwell *et al.* mesocosm study – FOCUS Step 3 for cereals

Crop	Scenario	Application scenarios for A14325E in cereals			
		1 x 450 g a.s./ha		2 x 450 g a.s./ha	
		PEC (µg/L)	PEC/RAC ratio	PEC (µg/L)	PEC/RAC ratio
Winter cereals	D1 ditch	3.25	0.67	4.29	0.88
	D1 stream	2.52	0.52	2.19	0.45
	D2 ditch	3.28	0.67	6.43	**1.32**
	D2 stream	2.77	0.57	4.02	0.83
	D3 ditch	2.84	0.58	2.49	0.51
	D6 ditch	2.84	0.58	2.5	0.51
	R1 pond	-	-	0.481	0.10
	R1 stream	-	-	2.99	0.62
	R3 stream	-	-	2.33	0.48
	R4 stream	1.88	0.39	1.85	0.38
Spring cereals	D1 ditch	3.46	0.71	4.79	0.99
	D1 stream	2.53	0.52	2.21	0.45
	D3 ditch	2.85	0.59	2.49	0.51
	R4 stream	1.94	0.40	2.01	0.41

Values in bold are above the trigger value of 1 and hence further consideration is needed for these taxa/scenario combinations

Refined risk assessments have been presented in the tables below for the scenarios that failed.

Table 10.2-44: Refinement of long term risk to aquatic invertebrates using FOCUS Step 4 PECsw – PEC/ERO-RAC (4.86 µg a.s./L) ratio – cyprodinil on winter cereals 2 x 450 g a.s./ha

Scenario	Mitigation options	Non-spray buffer zone (corresponding to ≤ 95 % drift reduction)				Trigger
	Vegetative strip (m)	5 m		10 m		
		PEC_{SW} (µg/L)	PEC/RAC ratio	PEC_{SW} (µg/L)	PEC/RAC ratio	
D2 / ditch	None	6.42	**1.3**	6.42	**1.3**	1

~~**Table 10.2-21: Higher-tier long-term risk assessment using an ETO-RAC of 7.3 µg a.s./L derived from the Ashwell *et al.* mesocosm study – FOCUS Step 3 for cereals**~~

~~Crop~~	~~Scenario~~	~~Application scenarios for A14325E in cereals~~			
		~~1 x 450 g a.s./ha~~		~~2 x 450 g a.s./ha~~	
		~~PEC (µg/L)~~	~~PEC/RAC ratio~~	~~PEC (µg/L)~~	~~PEC/RAC ratio~~
~~Winter cereals~~	~~D1 ditch~~	~~3.28~~	~~0.45~~	~~4.33~~	~~0.59~~
	~~D1 stream~~	~~2.52~~	~~0.35~~	~~2.19~~	~~0.30~~
	~~D2 ditch~~	~~3.33~~	~~0.46~~	~~6.82~~	~~0.93~~
	~~D2 stream~~	~~2.80~~	~~0.38~~	~~4.27~~	~~0.58~~
	~~D3 ditch~~	~~2.84~~	~~0.39~~	~~2.49~~	~~0.34~~
	~~D4 pond~~	~~0.098~~	~~0.013~~	~~0.159~~	~~0.022~~
	~~D4 stream~~	~~2.37~~	~~0.32~~	~~2.10~~	~~0.29~~
	~~D5 pond~~	~~0.100~~	~~0.014~~	~~0.139~~	~~0.019~~
	~~D5 stream~~	~~2.51~~	~~0.34~~	~~2.29~~	~~0.31~~
	~~R1 pond~~	~~2.84~~	~~0.39~~	~~2.50~~	~~0.34~~
	~~R1 stream~~	~~0.188~~	~~0.026~~	~~0.484~~	~~0.066~~
	~~R2 stream~~	~~1.87~~	~~0.26~~	~~3.04~~	~~0.42~~
	~~R3 stream~~	~~2.65~~	~~0.36~~	~~2.41~~	~~0.33~~
	~~R4 stream~~	~~1.88~~	~~0.26~~	~~1.88~~	~~0.26~~
~~Spring cereals~~	~~D1 ditch~~	~~3.51~~	~~0.48~~	~~4.87~~	~~0.67~~
	~~D1 stream~~	~~2.53~~	~~0.35~~	~~2.21~~	~~0.30~~
	~~D3 ditch~~	~~2.85~~	~~0.39~~	~~2.49~~	~~0.34~~
	~~D4 pond~~	~~0.098~~	~~0.013~~	~~0.190~~	~~0.026~~
	~~D4 stream~~	~~2.33~~	~~0.32~~	~~2.12~~	~~0.29~~
	~~D5 pond~~	~~0.100~~	~~0.014~~	~~0.139~~	~~0.019~~
	~~D5 stream~~	~~2.47~~	~~0.34~~	~~2.15~~	~~0.29~~
	~~R4 stream~~	~~1.97~~	~~0.27~~	~~2.04~~	~~0.28~~

Long-term risk to other groups

For the risk assessment for long-term risk to fish the PEC/RAC ratios were greater than 1 for several FOCUS Step 3 scenarios (please refer to Tables 10.2.18 ~~13~~ to 10.2-21 ~~16~~). For the sake of completeness the PEC/RAC ratios have been refined as described below.

Refinement of the long-term risk to fish

Two fish early life stage studies have been conducted with cyprodinil, one with *Cyprinodon variegates* and the other with *Pimephales promelas*. Since the endpoints for both studies are based on growth parameters it is acceptable to derive a geometric mean from the two endpoints of 40.6 and 231 µg a.s./L, respectively. Refinement has been presented in the table below.

Table 10.2-45: Higher-tier long-term risk assessment for fish using an RAC of 9.68 µg a.s./L (geometric mean of 40.6 µg a.s./L [*Cyprinodon variegates*] and 231 µg a.s./L [*Pimephales promelas*]) – FOCUS Step 3 PEC_{SW}

Crop	Scenario	Application scenarios for A14325E in cereals			
		1 x 450 g a.s./ha		2 x 450 g a.s./ha	
		PEC (µg/L)	PEC/RAC ratio	PEC (µg/L)	PEC/RAC ratio
Winter cereals	D1 ditch	3.25	0.34	4.29	0.44
	D2 ditch	3.28	0.34	6.43	0.66
	D2 stream	2.77	0.29	4.02	0.42
Spring cereals	D1 ditch	3.46	0.36	4.79	0.49

~~Table 10.2-22: Higher-tier long-term risk assessment for fish using an RAC of 9.68 µg a.s./L (geometric mean of 40.6 µg a.s./L [*Cyprinodon variegates*] and 231 µg a.s./L [*Pimephales promelas*]) – FOCUS Step 3 PEC~~$_{SW}$

~~Crop~~	~~Scenario~~	~~Application scenarios for A14325E in cereals~~			
		~~1 x 450 g a.s./ha~~		~~2 x 450 g a.s./ha~~	
		~~PEC (µg/L)~~	~~PEC/RAC ratio~~	~~PEC (µg/L)~~	~~PEC/RAC ratio~~
~~Winter cereals~~	~~D1 ditch~~	~~3.28~~	~~0.34~~	~~4.33~~	~~0.45~~
	~~D1 stream~~	~~2.52~~	~~0.26~~	~~2.19~~	~~0.23~~
	~~D2 ditch~~	~~3.33~~	~~0.34~~	~~6.82~~	~~0.70~~
	~~D2 stream~~	~~2.80~~	~~0.29~~	~~4.27~~	~~0.44~~
	~~D3 ditch~~	~~2.84~~	~~0.29~~	~~2.49~~	~~0.26~~
	~~D4 pond~~	~~0.098~~	~~0.010~~	~~0.159~~	~~0.016~~
	~~D4 stream~~	~~2.37~~	~~0.24~~	~~2.10~~	~~0.22~~
	~~D5 pond~~	~~0.100~~	~~0.010~~	~~0.139~~	~~0.014~~
	~~D5 stream~~	~~2.51~~	~~0.26~~	~~2.29~~	~~0.24~~
	~~R1 pond~~	~~2.84~~	~~0.29~~	~~2.50~~	~~0.26~~
	~~R1 stream~~	~~0.188~~	~~0.019~~	~~0.484~~	~~0.050~~
	~~R2 stream~~	~~1.87~~	~~0.19~~	~~3.04~~	~~0.31~~
	~~R3 stream~~	~~2.65~~	~~0.27~~	~~2.41~~	~~0.25~~
	~~R4 stream~~	~~1.88~~	~~0.19~~	~~1.88~~	~~0.19~~
~~Spring cereals~~	~~D1 ditch~~	~~3.51~~	~~0.36~~	~~4.87~~	~~0.50~~
	~~D1 stream~~	~~2.53~~	~~0.26~~	~~2.21~~	~~0.23~~
	~~D3 ditch~~	~~2.85~~	~~0.29~~	~~2.49~~	~~0.26~~
	~~D4 pond~~	~~0.098~~	~~0.010~~	~~0.190~~	~~0.020~~
	~~D4 stream~~	~~2.33~~	~~0.24~~	~~2.12~~	~~0.22~~
	~~D5 pond~~	~~0.100~~	~~0.010~~	~~0.139~~	~~0.014~~
	~~D5 stream~~	~~2.47~~	~~0.26~~	~~2.15~~	~~0.22~~
	~~R4 stream~~	~~1.97~~	~~0.20~~	~~2.04~~	~~0.21~~

All of the PEC/RAC values are below the trigger of 1 indicating acceptable long-term risk to fish following application of A14325E according to the proposed use pattern.

Long-term risk assessment for cyprodinil for sediment dwelling organisms using the plateau concentration

The accumulation of cyprodinil in sediment needs to be considered in the risk assessment. The worst-case plateau concentration derived using FOCUS Step 3 modelling was estimated to be 279 ~~298~~ µg a.s./kg. **Comparing this with the Tier 1 RAC of 8 000 µg/kg gives a PEC/RAC ratio of 0.035 ~~0.037~~, indicating acceptable risk for sediment accumulation of cyprodinil following application of A14325E according to the proposed use pattern.**

Cyprodinil metabolites

The risk to aquatic organisms from the cyprodinil metabolites is presented in the table below.

Table 10.2-2346: Risk to aquatic organisms from cyprodinil metabolites (FOCUS Step 2)

Test organism	Substance	Tier 1-RAC (µg/L)	Max PEC_{SW} [µg/L]	PEC/RAC ratio
Oncorhynchus mykiss	CGA249287	550	5.38	0.0098
	CGA275535	21	0.161	0.0077
	CGA263208	21	1.22	0.058
Daphnia magna	CGA249287	>1 000	5.38	<0.0054
	CGA275535	68	0.161	0.0024
	CGA321915	>980	1.84	<0.0019
	CGA263208	206	1.22	0.0059
Chironomus riparius	CGA321915	970	1.84	0.0019
Chironomus riparius	CGA249287	2 560 µg/kg	5.38 µg/kg	0.0021
Psudokirchneriella subcapitata	CGA249287	>10 000	5.38	<0.00054
	CGA275535	1 800	0.161	0.000089
	CGA321915	>9 900	1.84	<0.00019
	CGA263208	186	1.22	0.0066

All of the PEC/RAC values are below the trigger of 1 indicating acceptable risk to aquatic organisms for metabolites of cyprodinil following application of A14325E according to the proposed use pattern.

Risk assessment for CGA249287 for sediment dwelling organisms using the plateau concentration

The accumulation of CGA249287 in sediment needs to be considered in the risk assessment. The worst-case plateau concentration derived using FOCUS Step 2 modelling was estimated to be 263 ~~41.2~~ µg a.s./kg. **Comparing this with the Tier 1 RAC of 2 560 µg/kg gives a PEC/RAC ratio of 0.10 ~~0.016~~, indicating acceptable risk from this metabolite for sediment accumulation following application of A14325E according to the proposed use pattern.**

CP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Report:	K-CP 10.2.1/01 Volz E. (2005) Cyprodinil 300 EC Formulation (A14325E): Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) in a 96-hour static test. RCC Ltd, Itingen, Switzerland. Report Number 859284. (Syngenta File No. CGA219417/1354)

Guidelines

OECD Guideline for Testing of Chemicals, No. 203, Fish, Acute Toxicity Test, 1992; EU Commission Directive 92/69/EEC, C.1: Acute Toxicity for Fish, 1992; United States Environmental Protection Agency: Ecological Effects Test Guidelines OPPTS 850.1075, Fish Acute Toxicity Test, Freshwater and Marine; Public Draft, April 1996.

GLP: Yes

Executive Summary

The acute toxicity of Cyprodinil 300 EC Formulation (A14325E) to rainbow trout (*Oncorhynchus mykiss*) was determined. This study was run with nominal concentrations of 1.0, 2.2, 4.6, 10 and 22 mg

A14325E/L together with a dilution water control. The 96 h LC_{50} based on nominal concentrations was estimated to be 6.8 mg A14325E/L.

Materials

Test Material:	A14325E
Description:	Yellow liquid
Lot/Batch #:	SMU5BP002
Actual content of ai:	Cyprodinil (CGA219417): 303 g/L corresponding to 29.9% w/w
Specific density:	1.012 g/cm^3
Test concentrations:	Dilution water control and nominal formulation concentrations of 1.0, 2.2, 4.6, 10 and 22 mg A14325E/L
Vehicle and/or positive control:	None
Analysis of test concentrations:	Yes (based on measurement of Cyprodinil (CGA219417))
Test organism	
Species:	Rainbow trout (*Oncorhynchus mykiss*)
Source:	P. Hohler, trout breeding station Zeiningen, Switzerland
Acclimatisation period:	7 days
Treatment for disease:	None
Length of fish	5.2 ± 0.3 cm (mean ± SD)
Weight of fish	1.20 ± 0.26 g (mean ± SD)
Feeding:	None during test
Environmental conditions	
Test temperature:	13–14 °C
pH:	8.5 to 8.6
Dissolved oxygen:	9.2–9.7 mg/L
Total hardness of dilution water:	191 ($CaCO_3$)
Lighting:	16 hours daily photoperiod (fluorescent light) with 30-minute dawn and dusk transition periods
Length of test:	96 hours

Study Design and Methods

Experimental dates: 25th April 2005 to 9th May 2005

Test procedure and apparatus

One glass aquarium with 15 litres test medium was used for each test concentration and the control.

Seven fish were randomly allocated to each prepared test vessel. The test was conducted under static conditions with gentle aeration of the test media.

Preparation of test solutions

A concentrated stock solution of nominal 1.00 g/L was freshly prepared by completely dissolving 999.9 mg of the test item in 1000 mL of test water using stirring for 30 minutes at room temperature. This stock solution was used in a series of dilutions to prepare the test media of all test item concentrations.

Analytical method

The concentrations of cyprodinil (CGA219417) in the test solutions were measured at 0 and 96 hours using high performance liquid chromatography.

Observations for mortality and symptoms of toxicity

Observations were made at 3, 24, 48, 72 and 96 hours. The LC_{50} values, and their respective 95% confidence intervals, were calculated at the various time intervals by Moving Average Interpolation.

Physical and chemical parameters

Daily measurements of pH, temperature and dissolved oxygen concentration in the test solutions were made throughout the 96-hour period.

Statistical analysis

The 96 h LC_{50} was estimated using the moving average linear interpolation model.

Results and Discussion

In the analysed test media of nominal 4.6, 10 and 22 mg/L the measured test item concentrations (based on the determination of cyprodinil) at the start of the test ranged from 101 to 103% of the nominal values. During the test period of 96 hours there was a decrease of the cyprodinil concentration in the test media. At the end of the test 43 to 97% of the nominal values were found. The reported biological results are based on the nominal concentrations of the test item since a formulation was tested.

Table 10.2.1-1: A14325E: Effects on the survival of rainbow trout (*Oncorhynchus mykiss*) following exposure for 96 hours in a static test

Nominal concentration of formulation	**Cumulative percentage mortality observed**[a]				
(mg A14325E/L)	**3 h**	**24 h**	**48 h**	**72 h**	**96 h**
Dilution water control	0	0	0	0	0
1.0	0	0	0	0	0
2.2	0	0	0	0	0
4.6	0	0	0	0	0
10	0 [b]	0 [b]	43	100	--
22	0 [b]	100	--	--	--
LC_{50} (mg A14325E/L)		15	11	6.8	6.8
95% confidence interval (mg A14325E/L)		n.c.	n.c.	n.c.	n.c.
Calculation method		Moving Average Interpolation	Moving Average Interpolation	Moving Average Interpolation	Moving Average Interpolation

[a] Seven fish were exposed in each test vessel, one replicate per treatment.
[b] Symptoms of toxicity observed, including apathy, swimming at the bottom of the aquarium, convulsions, lying on side or back on the bottom and tumbling during swimming.
--: All fish dead.
n.c.: could not be calculated.

The 96-hour NOEC (no observed effect concentration) is defined as the highest tested concentration at which no mortalities or significant sublethal effects occurred within the test period: 4.6 mg A14325E/L.

Conclusion

The 96-hour LC_{50} for A14325E to rainbow trout (*Oncorhynchus mykiss)* was found to be 6.8 mg/L, based on nominal concentrations of formulation.

(Volz E, 2005)

Report:	K-CP 10.2.1/02 Volz E. (2005a) Cyprodinil 300 EC Formulation (A14325E): Acute toxicity to *Daphnia magna* in a 48-hour immobilization test. RCC Ltd, Itingen, Switzerland. Report Number 859286. (Syngenta File No. CGA219417/1357)

Guidelines

OECD Guideline for Testing of Chemicals, No. 202, *Daphnia sp.*, Acute Immobilisation Test, 2004; EU Commission Directive 92/69/EEC, C.2, Acute Toxicity for Daphnia, 1992; US EPA 1996, Ecological Effects Test Guidelines, OPPTS 850.1010, Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids, US EPA Prevention, Pesticides and Toxic Substances (7101), EPA 712-C-96-114 April 1996, "Public Draft".

GLP: Yes

Executive Summary

The acute toxicity of Cyprodinil 300 EC Formulation (A14325E) to *Daphnia magna* was determined. This study was run with nominal concentrations of 0.022, 0.046, 0.10, 0.22, 0.46 and 1.0 mg A14325E/L together with a medium control. The 48 h EC_{50} based on nominal concentrations was estimated to be 0.37 mg A14325E/L.

Materials

Test Material:	A14325E
Description:	Yellow liquid
Lot/Batch #:	SMU5BP002
Actual content of ai:	Cyprodinil (CGA219417): 303 g/L corresponding to 29.9% w/w
Density:	1.012 g/cm^3
Test concentrations:	Culture medium control and nominal formulation concentrations of 0.022, 0.046, 0.10, 0.22, 0.46 and 1.0 mg A14325E/L
Vehicle and/or positive control:	None
Analysis of test concentrations:	Yes (based on measurement of Cyprodinil (CGA219417))
Test organism	
Species:	*Daphnia magna*
Source:	Continuous laboratory cultures originally obtained from the University of Sheffield, UK defined as Clone 5.
Treatment for disease:	Non
Feeding:	None during test
Environmental conditions	
Test temperature:	20 °C
pH:	7.8 to 7.9
Dissolved oxygen:	8.5 to 8.9 mg/L
Total hardness of dilution water:	250 mg/L $CaCO_3$

Lighting:	A 16-hour daily photoperiod (with a 30 minute transition period). Light intensity during the light period was between approximately 570 and 740 Lux.
Length of test:	48 hours

Study Design and Methods

Experimental dates: 25th April 2005 to 6th May 2005

Test procedure and apparatus

The test was performed in 100 mL glass beakers, covered with lids, filled with 50 mL of test medium. At each test concentration and for the control 20 daphnids were used, divided between four replicates with five daphnids each. The daphnids were randomly distributed among the test vessels at initiation of the test. The test was conducted under static conditions with no aeration.

Preparation of test solutions

A stock solution with a nominal concentration of 1.00 g/L was prepared by dissolving 300.3 mg of the test item completely in 300 mL of test water using intense stirring for 15 minutes at room temperature. Adequate volumes of the intensively mixed stock solution were added to test water to prepare the test media with the nominal test concentrations as stated above. The test media were prepared just before introduction of the daphnids (*i.e.* start of the test). The control consisted of reconstituted test water only.

Analytical method

The concentrations of cyprodinil (CGA219417) in the test solutions were measured at 0 and 48 hours using a high performance liquid chromatography method.

Observations of effects

The immobility of the daphnids was determined by visual observations after 24 and 48 hours of exposure. Those organisms not able to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile.

The median effect concentration (EC_{50}) was defined as the concentration resulting in 50% immobilization of the *Daphnia* in the time period specified.

The appearance of the test media was visually recorded at the start of the test and after 24 and 48 hours.

Physical and chemical parameters

At the start and at the end of the test, the pH values, the dissolved oxygen concentrations, and the water temperature were determined in each test concentration and the control.

Results and Discussion

In the analysed test media of nominal 0.10, 0.22, 0.46 and 1.0 mg/L the measured test item concentrations (based on the determination of cyprodinil) from the start and the end of the test ranged from 98 to 104% of the nominal values. The reported biological results are based on the nominal concentrations of the test item.

Table 10.2.1-2: Effects of A14325E on Daphnia magna in a 48 hour static test

Nominal test item concentration (mg A14325E/L)	No. of daphnids tested	Immobilized daphnids after 24 hours		Immobilized daphnids after 48 hours	
		No.	%	No.	%
Control	20	0	0	0	0
0.022	20	0	0	0	0
0.046	20	0	0	0	0
0.10	20	0	0	0	0
0.22	20	0	0	2	10
0.46	20	6	30	14	70
1.0	20	16	80	20	100
EC_{50} (mg A14325E/L)		0.64		0.37	
95% confidence interval (mg A14325E/L)		0.49 – 0.86		0.33 – 0.41	
Calculation method		Probit Analysis		Probit Analysis	

The NOEC (no observed effect concentration) is defined as the highest tested concentration in which there was no observed effect on the *Daphnia* within the period of the test. Therefore, 48-hour NOEC = 0.10 mg A14325E/L

Conclusion

The 48-hour EC_{50} for A14325E to *Daphnia magna* was found to be 0.37 mg A14325E/L, based on the nominal concentration of formulation.

(Volz E, 2005a)

Report:	K-CP 10.2.1/03 Volz E. (2005b) Cyprodinil 300 EC Formulation (A14325E): A 96-hour algal growth inhibition test with *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*). RCC Ltd, Itingen, Switzerland. Report Number 859282. (Syngenta File No. CGA219417/1358)

Guidelines

OECD Guideline for Testing of Chemicals, No. 201: Alga, Growth Inhibition Test, 1984; EU Commission Directive 92/69/EEC, C.3: Algal Inhibition Test, 1992; US EPA 1996, Ecological Effects Test Guidelines, OPPTS Test Guideline 850.5400, Algal Toxicity, Tiers I and II, US EPA Prevention, Pesticides and Toxic Substances (7101), EPA 712-C-96-164, April 1996, "Public Draft"

GLP: Yes

Executive Summary

Toxicity of Cyprodinil 300 EC Formulation (A14325E) to the green alga *Pseudokirchneriella subcapitata* was determined. This study was run with a culture medium control together with nominal formulation concentrations of 0.29, 0.64, 1.4, 3.1, 6.8 and 15 mg A14325E/L. The 72 and 96 h E_rC_{50} based on nominal concentrations were both estimated to be 12 mg A14325E/L. The 72 and 96 h E_bC_{50} based on nominal concentrations were estimated to be 5.3 and 5.9 mg A14325E/L, respectively.

Materials

Test Material:	A14325E
Description:	Yellow liquid
Lot/Batch #:	SMU5BP002
Actual content of ai:	Cyprodinil (CGA219417): 303 g/L corresponding to 29.9% w/w
Specific density:	1.012 g/cm^3
Test concentrations:	Culture medium control and nominal formulation concentrations of 0.29, 0.64, 1.4, 3.1, 6.8 and 15 mg A14325E/L
Vehicle and/or positive control:	None
Analysis of test concentrations:	Yes (based on measurement of cyprodinil (CGA219417))
Test organism	
Species:	*Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*), Strain No. 61.81 SAG
Source:	Collection of Algal Cultures (SAG, Institute for Plant Physiology, University of Göttingen, Germany). The algae are cultivated in the RCC laboratories under standardized conditions according to the test guidelines.
Environmental conditions	
Test temperature:	22–23 °C
pH:	test start: 7.9 to 8.1 test end: 8.0 to 8.5
Lighting:	Continuous at 6480 to 6970 Lux (mean 6700 Lux)
Length of test:	96 hours

Study Design and Methods

Experimental dates: 22nd April to 11th May 2005

Test procedure and apparatus

The test design included three replicates per test concentration and six replicates of the control. Volumes of 15 mL algal suspension for each replicate were continuously stirred by magnetic stirrers in 50 mL Erlenmeyer flasks. The flasks were covered with glass dishes. They were incubated in a temperature controlled water bath and continuously illuminated.

Small volumes of all test concentrations and the control (0.4–1.0 mL) were taken from all test flasks after 24, 48, 72 and 96 hours of exposure. The algal cell densities in the samples were determined by counting with an electronic particle counter.

In addition, after 96 hours exposure, a sample was taken from the control and from a test concentration with reduced algal growth (nominal 6.8 mg/L). The shape of the algal cells was examined microscopically in these samples.

Preparation of test solutions

A stock solution of nominal 1.00 g/L was prepared by dissolving 300.25 mg of the test item completely in 300 mL of test water using ultrasonic treatment (10 minutes) and intense stirring (10 minutes at room temperature). This intensively mixed stock solution was used in a series of dilutions to prepare the test media of all test item concentrations. The control consisted of synthetic test water only.

The test was started (0 hours) by inoculation of each test concentration and the control with 10,000 algal cells per mL of test medium.

Analytical method

The concentrations of cyprodinil (CGA219417) in the test solutions were measured at 0 and 96 hours using a high performance liquid chromatography method.

Physical and chemical parameters

The pH was measured at the start and at the end of the test. The water temperature was measured daily in a flask incubated under the same conditions as the test flasks. The appearance of the test media was also recorded daily.

The appearance of the test media was visually recorded at the start of the test and after 0, 24, 48, 72 and 96 hours.

Analysis of biological data

The algal cell densities were measured at each time period and the means of these values were calculated. The areas under the growth curve and the growth rates were calculated for each replicate culture, according to the formulae given in the OECD Guideline 201.

The 72- and 96-hour E_bC_{50} and E_rC_{50} values (the respective concentrations of the test item corresponding to 50% inhibition of algal biomass (b) and growth rate (r) compared to the control), and the corresponding EC_{10} value and EC_{90} values and their 95%-confidence limits were calculated by Probit Analysis.

For the determination of the 72- and 96-hour LOEC and NOEC, the calculated mean biomass and the mean growth rate at the test concentrations were tested for significant differences when compared to the control values by a Dunnett-test.

Results and Discussion

In the analysed test media of nominal 1.4, 3.1, 6.8 and 15 mg/L the measured concentrations of the active ingredient cyprodinil at the start of the test ranged from 96 to 101% of the nominal values. At the end of the test 73 to 103% of the nominal values were found. The reported biological results are based on the nominal concentrations of the test item since a formulation was tested.

Table 10.2.1-3: A14325E - mean values for the area under the growth curve (AUC) and the growth rate (r) for effects on *Pseudokirchneriella subcapitata*

Nominal test item concentration (mg A14325E/L)	Timescale							
	0-24 h		0-48 h		0-72 h		0-96 h	
	Areas under the growth curves (AUC) and % inhibition of AUC							
	AUC [a]	I_{AUC} (%)	AUC [a]	I_{AUC} (%)	AUC [a]	I_{AUC} (%)	AUC [a]	I_{AUC} (%)
Control	53	0.0	407	0.0	1900	0.0	5746	0.0
0.29	47	11.4	386	5.2	1936	-1.9	6115	-6.4
0.64	49	7.6	374	8.1	1885	0.8	6247	-8.7
1.4	45	14.4	318	21.7	1700	10.5	5753	-0.1
3.1	39	26.9	290	28.8	1395	26.6	4507	21.6
6.8	31	41.7	208	48.8	930	51.0	2834	50.7
15	19	64.8	58	85.7	124	93.5	239	95.8

	Growth rate (r) and % inhibition of r							
	r (1/day)	I_r (%)	r (1/day)	I_r (%)	r (1/day)	I_r (%)	r (1/day)	I_r (%)
Control	1.68	0.0	1.63	0.0	1.54	0.0	1.35	0.0
0.29	1.58	5.9	1.62	0.9	1.55	-1.2	1.37	-1.7
0.64	1.62	3.5	1.59	2.8	1.54	-0.6	1.39	-3.0
1.4	1.56	7.2	1.50	8.2	1.52	0.7	1.37	-1.6
3.1	1.44	14.4	1.46	10.2	1.44	6.2	1.31	3.4
6.8	1.27	24.4	1.28	21.2	1.29	15.7	1.18	12.7
15	0.94	44.3	0.50	69.0	0.52	66.4	0.48	64.3

[a] AUC x 10,000
-% inhibition: increase in growth relative to that of control

Table 10.2.1-4: A14325E - Statistical analysis of effects on *Pseudokirchneriella subcapitata*

Endpoint		Timescale	
		72 h	96 h
Biomass	E_bC_{50} (mg A14325E/L)	5.3	5.9
	95% Confidence limits (mg A14325E/L)	4.0–7.2	4.7–7.5
	NOEC	1.4	1.4
	LOEC	3.1	3.1
Growth rate	E_rC_{50} (mg A14325E/L)	12	12
	95% Confidence limits (mg A14325E/L)	9.3–17	Not determinable
	NOEC	1.4	1.4
	LOEC	3.1	3.1

Conclusion

The 96-hour E_bC_{50} for effects of A14325E on the green alga *Pseudokirchneriella subcapitata* was found to be 5.9 mg A14325E/L and the 96-hour E_rC_{50} 12 mg A14325E/L, based on nominal concentrations of formulation.

(Volz E, 2005b)

CP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

Additional long-term or chronic studies with A14325E are not required as acute studies indicate the formulated product is no more toxic than expected on the basis of the active substance toxicity and hence risk can be adequately assessed using the chronic toxicity data for the active substance.

CP 10.2.3 Further testing on aquatic organisms

A mesocosm study was conducted using a 300 EC formulation A14325E (***Ashwell et al, 2007***) (details are provided in M-CA Section 8, CA 8.2-8) to a community typical for a lentic freshwater community, containing phyto- and zooplankton and macroinvertebrates. Intended initial concentrations were 0 – 1.5 – 5 – 10 – 20 – 50 µg a.s./L. Immediately after each of the three applications the test compound was mixed in the water layer of the microcosms. Measurements in dosing solutions and water indicated that the test systems received the intended doses. Shortly after the applications 75-80%, 119-154% and 118-156% of the target amount was measured in the water of the test systems.

MDD analysis of the available data for zooplankton demonstrated that typically small to large effects could be determined throughout the study for five parameters. As these evaluations included sensitive taxa (*Daphnia* sp.) and organisms from the three main zooplankton groups (cladocera, copepoda and rotifera), the data generated are considered robust and reliable for ETO-RAC derivation and a NOEC (class 1) of 10 µg a.s./L (based on nominal concentrations) and 14.6 µg a.s./L (based on mean measured concentrations) is recommended for zooplankton. If an NOEAEC (class 3A) is required for ERO-RAC it can be considered to be 50 µg a.s./L.

CP 10.3 Effects on Arthropods

CP 10.3.1 Effects on bees

Toxicity

Summary of endpoints relevant for the risk assessment:

Table 10.3.1-1: Table of endpoints to assess risk from use of A14325E

Organism	Test item	Test type	Endpoint		Reference (author, date, Syngenta File No.)
Honey bee	Cyprodinil	Acute contact	EU	LD_{50} >784 µg a.s./bee	***Boeri et al. (1995d) CGA219417/0532***
	Cyprodinil [a]	Acute Oral		Oral 72h LD_{50} >125 µg a.s./bee	***Candolfi (1995) CGA219417/0375***
	A14325E	Acute Oral	New study	Oral 72h LD_{50} >408 µg/bee (>121 µg a.s./bee)	***Bocksch (2005) CGA219417/1317***
		Acute Contact		Contact 72h LD_{50} >675 µg/bee (>200 µg a.s./bee)	
		Adult chronic	New Study	10 day LD_{50} 69.7 µg consumed a.s./bee/day 10 day NOED 44.2 µg consumed a.s./bee/day 10 day NOEC 1.284 g a.s./kg food	***Ruhland (2014) A14325E_10065***
		Chronic larval	New Study	8 day NOEC 0.110 g a.s./kg diet 8 day NOED 17.3 µg a.s./larva 8 day LD_{50} 45.7 µg a.s./larva	***Kleebaum (2014) A14325E_10067***

[a] tested as A8637C

Exposure

Applications of pesticides can potentially result in exposure of bees either through direct over-spray, or by contact with residues on plants whilst bees are foraging for food. For cyprodinil, it is possible that bees will be exposed to significant residues, as A14325E may occur in honeydew secreted by aphids during periods of high infestation. Therefore the in-field scenario represents a worst-case, short-term source of exposure.

Exposure through contact from drift to bees foraging in the off-field environment is a relevant exposure route; however, the level of exposure is clearly lower than in-field, and as such is covered by the in-field contact risk assessment.

In order to consider an extreme worst-case scenario and provide a conservative assessment, the maximum application rate of 450 g a.s./ha when 1.5 L A14325E is applied at the maximum proposed rate has been used in the risk assessment below.

Risk assessment for bees

The risk to bees has been assessed following the EPPO 2010 scheme[4] as proposed in the list of guidance documents relevant to the implementation of Regulation 1107/2009, published in the official EU Journal 2013/C 95/01 and 95/02.

Acute risk assessment

The potential acute risk from use of A14325E was assessed using the maximum single application rates and the LD_{50} values to calculate hazard quotients in accordance with the current Terrestrial Guidance Document[5] and EPPO 2010.

$$\text{Hazard Quotient} = \frac{\text{Maximum application rate (g formulation/ ha)}}{\text{Acute } LD_{50} \text{ (µg/bee)}}$$

Table 10.3.1-2: Risk to bees from oral exposure to A14325E

Test substance	Application rate (g/ha)	Oral LD_{50} (µg/bee)	Hazard quotient
A14325E	1653 [a]	>408	<4.1
Cyprodinil	450	>112.5	<3.6

[a] The application rate is based on a specific density of 1.102 g/mL with a maximum application of 1.50 L/ha (based on an application rate of 450 g a.s/ha).

Both of the hazard quotients for cyprodinil and A14325E are less than 50, indicating that the risk to bees is acceptable following use of A14325E according to the proposed use pattern.

Table 10.3.1-3: Risk to bees from contact exposure to A14325E

Test substance	Application rate (g/ha)	Contact LD_{50} (µg/bee)	Hazard quotient
A14325E	1653 [a]	675	<2.4
Cyprodinil	450	>784	<0.57

[a] The application rate is based on a specific density of 1.102 g/mL with a maximum application of 1.50 L/ha (based on an application rate of 450 g a.s/ha).

Both of the hazard quotients for cyprodinil and A14325E are less than 50, indicating that the risk to bees is acceptable following use of A14325E according to the proposed use pattern.

Chronic Risk Assessment

Chronic adult and larval bee studies have been conducted according to the data requirements under 1007/2009. The endpoints from these studies have been assessed by adapting the EPPO 2010 scheme.

[4] EPPO/OEPP (2010) Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees (PP 3/10(3)). Bulletin OEPP/EPPO Bulletin 40: 323-331.
[5] Anonymous (2002b). Guidance Document on terrestrial ecotoxicology under Council Directive 91/414/EEC. SANCO/10329/2002. 17 October 2002.

Larval assessment:

Following the EPPO scheme for assessing potential risks to larvae (point 4 on the scheme), the scheme suggests that effects on growth or development can be excluded when considering cyprodinil, since it is not an IGR, and shows no effects on juvenile stages in other organisms as demonstrated by the risk assessments for non-target arthropods, and soil organisms (Collembola and *Hypoaspis*). Thus cyprodinil can be categorised as posing a low risk to bees.

However a chronic larval study is available and this potential low risk can be further demonstrated by carrying out a worst-case risk assessment through the calculation of a TER value as set out in the EPPO 2010 scheme (point 5 on the scheme).

A worst-case of potential exposure via residues in pollen / nectar can be estimated based on the default worst-case residue of 1 mg a.s./kg proposed in the EPPO 2010 scheme (see Note 6), based on a database of measured values from aerial plant parts as a surrogate for nectar and pollen.

The default residues can then be combined with a measure of consumption in order to estimate the exposure. Worst case data from **Rortais *et al.*, 2005**[6] as proposed in the EPPO scheme have been used to estimate the consumption by bee larvae:

Worst case: drone larvae consuming 98.2 mg sugar in 6.5 days (= 15.1 mg sugar /day).

Thus considering residues of 1 mg a.s./kg sugar x consumption of 15.1 mg sugar/bee/day

Total exposure ETE = 0.0151 µg a.s./bee/day

This can be compared to the cyprodinil larval NOEC of 17.3 µg a.s./bee/developmental period, which is = 2.16 µg a.s./bee/day (based on an 8 day study duration).

- TER = NOEL (µg a.s./bee/day)/ ETE (µg a.s./bee/day)

  = 2.16/0.0151= 140

The EPPO 2010 scheme proposes a trigger of 1 for assessment of the risk to honey bees. It is clear that with a TER value of 140 there is a wide safety margin, indicating that the proposed uses of cyprodinil pose an acceptable risk to bee larval development.

Adult chronic assessment:

The EPPO 2010 scheme does not recommend a chronic assessment for adults for foliar spray applications. However, as an approach is proposed as an assessment refinement for seed coatings/soil treatments (point 7 on the scheme), this approach can be adapted to provide a worst-case assessment for foliar sprays.

A worst-case of potential exposure via residues in pollen / nectar can be estimated as before based on the default worst-case value of 1 mg a.s./kg proposed in the EPPO 2010 scheme (see Note 6), based on a database of measured values from aerial plant parts as a surrogate for nectar and pollen.

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[6] Agnès RORTAIS, Gérard ARNOLD, Marie-Pierre HALM, Frédérique TOUFFET-BRIENS (2005) Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. Apidologie 36 (2005) 71–83

The default residues can then be combined with a measure of consumption in order to estimate the exposure. Worst case data from Rortais *et al*., 2005 as proposed in the EPPO 2010 scheme have been used to estimate the consumption by bee foragers:

Worst case: forager consuming 128 mg nectar/day.

Thus considering residues of 1 mg a.s./kg sugar x consumption of 28 mg nectar/bee/day

Total exposure ETE = 0.128 µg a.s./bee/day

This can be compared to the cyprodinil adult NOEL of 44.2 µg a.s./bee/day.

- TER = NOEL (µg a.s./bee/day)/ ETE (µg a.s./bee/day)

  = (44.2/0.128) = 350

The EPPO 2010 scheme proposes a trigger of 1 for assessment of the risk to honey bees when a NOEL is used in this assessment. It is clear that with a TER value of 350 there is a wide safety margin, indicating that the proposed uses of cyprodinil pose an acceptable chronic risk to adult bees.

Tests on chronic toxicity and larval and brood development have been carried out in accordance with the **Annexes to Regulation 283/2013 and 284/2013**. **The results of these tests indicate that the use of cyprodinil in A14325E poses an acceptable risk to bees.**

CP 10.3.1.1 Acute toxicity to bees

CP 10.3.1.1.1 Acute oral toxicity to bees

Report:	K-CP 10.3.1.1.1/01 Bocksch S (2005). Cyprodinil (CGA219417) 300 g/L EC Formulation (A14325E): Acute Toxicity to the Honeybee, *Apis mellifera* L. in the Laboratory. GAB Biotechnologie GmbH, Niefern-Öschelbronn, Germany. Report Number 20051088/01-BLEU. (Syngenta File No. CGA219417/1317)

Guidelines

OECD (1998): Guideline for the testing of chemicals; Honey bees; acute oral toxicity test; 213.

OECD (1998): Guideline for the testing of chemicals; Honey bees; acute contact toxicity test; 214.

GLP: Yes

Executive Summary

The oral and contact toxicity of A14325E to the honeybee (*Apis mellifera* L.) was determined in an oral limit test and a contact dose response test.

In the oral test bees were fed with the nominal dose of 100 µg a.s./bee. The actual consumption by bees in the oral test was 121 µg a.s. /bee. In the contact test bees were treated with the doses of 12.5, 25.0, 50.0, 100 and 200 µg a.s./bee by topical application.

The 48-hour oral LD_{50} for A14325E was >121 µg a.s./bee (nominally equivalent to 408 µg A14325E/bee) and the 48-hour contact LD_{50} was >200 µg a.s./bee (nominally equivalent to 675 µg A14325E/bee).

No behavioural differences between the surviving bees in the test item treatment and the control bees were observed at the end of the test period.

Materials

Test Material:	A14325E
Description:	Yellow liquid; 300 g/L cyprodinil (CGA219417 EC (300))
Lot/Batch #:	SMU5BP002
Purity:	303 g/L (29.9 % (w/w)) cyprodinil (CGA219417 EC (300))
Stability of test compound:	Sufficient for test purpose
Test doses:	Oral test: nominal 100 µg a.s./bee, actual consumption 121 µg a.s./bee. Contact test: 12.5, 25.0, 50.0, 100 and 200 µg a.s./bee.
Vehicle and/or positive control:	Tap water vehicle and control; positive control Dimethoate 40 EC
Test animals	
Species:	*Apis mellifera carnica*
Source:	Beekeeper Mr. Berthold Nengel, Brückenstraße 12, 56348 Dahlheim, Germany
Food:	50 % aqueous sucrose solution
Environmental conditions	
Temperature:	25.0 - 27 °C (oral test); 24.0 – 27.5 °C (> 27.0 °C for 90 minutes) (contact test)
Humidity:	54 % to 65 % RH (oral test); 40 % to 64 % RH (contact test)
Photoperiod:	24 hour darkness

Study Design and Methods

Experimental dates: 19th April to 12th May 2005.

The test was carried out with a single concentration of A14325E in the oral test and five concentrations in the contact test, four concentrations of the reference item and a control. Bees were exposed to the test item by feeding and topical application.

For the oral toxicity test, A14325E was dissolved in tap water to make a stock solution. The final dose was prepared by mixing an appropriate amount of the stock solution with an appropriate amount of 50 % aqueous sucrose solution, such that 20 µL contained the required amount of test item per bee even though 25 µL was provided. Before bees were permitted to feed, they starved for 2 hours. A quantity of 250 µL of test item and reference item solution was offered for 5 hours and 15 minutes to each cage of 10 bees to ensure sufficient consumption of test or reference item. Bees within a cage shared the test solution and therefore are assumed to have received a similar dose. The amount of test solution consumed by each replicate (consisting of 10 bees) was determined by weighing the feeders (eppendorf cups) before and after feeding. After the test solutions were consumed, the bees were supplied *ad libitum* with untreated 50 % aqueous sucrose solution.

For the contact toxicity test A14325E was dissolved in tap water. After the bees had been anaesthetised with carbon dioxide they were treated individually by topical application with a micro-applicator. 2 µL of test item solution, control or reference item solution were applied dorsally to the thorax of each bee, respectively. After application the bees were returned to the test cages and fed with a 50 % aqueous sucrose solution *ad libitum*. Between every application, the outside of the micro-applicator needle was cleaned with a mixture of water and a water-wetting agent. This reduced the surface tension of the applied solution and ensured that the drop of the test item solution spread out immediately after application.

The number of dead bees in the individual test cages was recorded after 4 h, 24 h and 48 h. In case of symptoms of poisoning the behavioural differences between the bees of the control group and those of the test item treatment were noted at each observation interval.

Results and Discussion

Oral test

Table 10.3.1.1.1-1: Mean mortality and total consumption in the acute oral toxicity test with A14325E

Treatment	Nominal Dose (µg a.s./bee)	Measured consumed dose (µg a.s./bee)	Mortality (%)	
			24 h	48 h
Control (sugar solution)	0	-	0.0	0.0
A14325E	100	121	10.0	12.0
Reference item: dimethoate	0.08	0.10	8.0	16.0
	0.10	0.11	14.0	28.0
	0.14	0.17	78.0	84.0
	0.22	0.27	100.0	100.0

The oral LD_{50} values for A14325E at 24 and 48 hours were both > 121 µg a.s./bee.

The oral LD_{50} values for the reference item dimethoate at 24 and 48 hours were 0.14 (95 confidence limits 0.13 to 0.15) and 0.13 (95% confidence limits 0.12 to 0.14) µg a.s./bee, respectively.

No behavioural abnormalities of the bees that could be attributed to the exposure to the test item were observed during the test.

Contact test

Table 10.3.1.1.1-2: Mean mortality in the acute contact toxicity test with A14325E

Treatment	Dose (µg a.s./bee)	Mortality (%)		Corrected mortality (%)	
		24 h	48 h	24 h	48 h
Control (tap water)	0	4.0	4.0	-	-
A14325E	12.5	4.0	6.0	0.0	2.1
	25.0	4.0	10.0	0.0	6.3
	50.0	4.0	4.0	0.0	0.0
	100	6.0	6.0	2.1	2.1
	200	36.0	38.0	33.3	35.4
Reference item: dimethoate	0.10	12.0	30.0	8.3	27.1
	0.15	38.0	44.0	35.4	41.7
	0.26	84.0	88.0	83.3	87.5
	0.36	86.0	86.0	85.4	85.4

The contact LD_{50} values for A14325E at 24 and 48 hours were both > 200 µg a.s./bee.
The contact LD_{50} values for the reference item dimethoate at 24 and 48 hours were 0.18 (95 confidence limits 0.16 to 0.21) and 0.13 (95% confidence limits 0.13 to 0.18) µg a.s./bee, respectively.

No behavioural differences between the surviving bees in the test item treatment and the control bees were observed at the end of the test period.

Conclusions

The 48-hour oral LD_{50} for A14325E was found to be >121 µg a.s./bee (nominally equivalent to 408 µg A14325E/bee) and the 48-hour contact LD_{50} was > 200 µg a.s./bee (nominally equivalent to 675 µg A14325E/bee).

(Bocksch S, 2005)

CP 10.3.1.1.2 Acute contact toxicity to bees

See Point CP 10.3.1.1.1 above.

CP 10.3.1.2 Chronic toxicity to bees

Chronic toxicity data for bees is a new data requirement under the **Annexes to Regulation 283/2013 and 284/2013**, applicable where there is a possibility that bees may be exposed. In order to minimise testing, and as the formulated product is considered to be indicative of the effects of the active substance for bees, tests have only been carried out with the formulated substance and these are summarised in M-CA Section 8, CA 8.5.1.2. The results are summarised in Table 10.3.1-1.

CP 10.3.1.3 Effects on honey bee development and other honey bee life stages

Larval and brood development data for bees is a new data requirement under the **Annexes to Regulation 283/2013 and 284/2013**, applicable where there is a possibility that bees may be exposed. In order to minimise testing, and as the formulated product is considered to be indicative of the effects of the active substance for bees, tests have only been carried out with the formulated substance and these are summarised in M-CA Section 8 Point 8.5.1.3. The results are summarised in Table 10.3.1-1.

CP 10.3.1.4 Sub-lethal effects

As the risk to bees is acceptable following use of A14325E according to the proposed use pattern, further tests are not necessary.

CP 10.3.1.5 Cage and tunnel tests

As the risk to bees is acceptable following use of A14325E according to the proposed use pattern, further tests are not necessary.

CP 10.3.1.6 Field tests with honeybees

As the risk to bees is acceptable following use of A14325E according to the proposed use pattern, further tests are not necessary.

CP 10.3.2 Effects on non-target arthropods other than bees

The toxicity of A14325E to non-target arthropods has been investigated. The testing and risk assessment strategy used here follows the approach recommended in the ESCORT 2 guidance document (**Candolfi et al. 2001**)[7] as proposed by **EC Guidance Document on Terrestrial Ecotoxicology** [8].

Toxicity

The toxicity of A14325E to non-target arthropods has been investigated by carrying out Tier I and II tests with the product on *Aphidius rhopalosiphi* and *Typhlodromus pyri*. These two species are tested, in accordance with ESCORT 2, as representative non-target arthropods since they have been found to be particularly sensitive species, and therefore can be considered as indicators of potential effects to the most sensitive non-target arthropods in the field. Additionally, Tier II tests have been carried out with *Chrysoperla carnea* and *Coccinella septempunctata.* The results of these studies are summarised below.

[7] Candolfi MP, Barrett KL, Campbell PJ, Forster R, Grandy N, Huet M-C, Lewis G, Oomen PA, Schmuck R, Vogt H (2000) 'Guidance Document on regulatory testing procedures for plant protection products with non-target arthropods' From the workshop, European Standard Characteristics of Non-target Arthropod Regulatory Testing (ESCORT 2) 21-23 March 2000.

[8] EC Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO/10329, 17 October 2002.

Table 10.3.2-1: Table of endpoints to assess risk from use of A14325E

Species	Test type[a]	Treatment rate (mL/ha)	Result	Reference
Typhlodromus pyri	T1 Laboratory, glass plate	Dose response: 93.67, 375, 750, 1950, 3000	LR_{50} = 1943 mL A14325E/ha Reproduction: no effect >50% up to 750 mL/ha (the highest rate tested for fecundity)	***Vaughan (2005) CGA219417/1298***
Aphidius rhopalosiphi	T1 Laboratory, glass plate	Dose response: 23.33, 93.67, 375, 750, 1950, 3000	LR_{50} = 156 [a] ~~241~~ mL A14325E/ha Reproduction: no effect >50% at 23.33 mL/ha (the only rate tested)	***Vinall (2005) CGA219417/1297*** ***Statistical reanalysis: Paul (2016) A14325E_10096***
Typhlodromus pyri	2-D extended laboratory, plant substrate	Dose response: 23.33, 93.67, 375, 750, 1950, 3000	LR_{50} >3000 mL A14325E/ha. Reproduction: no effect >50% at up to 1950 mL/ha; >50% effect at 3000 mL/ha	***Vaughan (2005) CGA219417/1332***
Aphidius rhopalosiphi	3-D extended laboratory, on whole plant	Dose response: 23.33, 93.67, 375, 750, 1950, 3000	LR_{50} >3000 mL A14325E/ha. Reproduction: no effect >50% at up to 3000 mL/ha	***Vinall (2005) CGA219417/1332***
Chrysoperla carnea	2-D extended laboratory, plant substrate	Dose response: 23.33, 93.67, 375, 750, 1950, 3000	LR_{50} = 2393 mL A14325E/ha. Reproduction: no unacceptable effect up to 750 mL/ha (the highest tested for reproduction)	***Spincer (2005) CGA219417/1375***
Coccinella septempunctata	2-D extended laboratory, plant substrate	Dose response: 23.33, 93.67, 375, 750, 1950, 3000	LR_{50} >3000 mL A14325E/ha. Reproduction: no unacceptable effect up to 3000 mL/ha	***Spincer (2005) CGA219417/1392***
Typhlodromus pyri	Extended laboratory aged-residue test	2 x 1500 mL/ha at a 14-d interval	No effects >50% on mortality of reproduction	***Fallowfield (2017) A14325E_10107***
Chrysoperla carnea	Extended laboratory aged-residue test	2 x 1500 mL/ha at a 14-d interval	No effects >50% on mortality of reproduction	***Vaughan (2016) A14325E_10106***

T1 = Tier 1
[a] re-estimated at the request of the RMS

Risk assessment for other non-target arthropods

The risk to non-target arthropods is assessed using the approach recommended in the published ESCORT 2 document (**Candolfi et al. 2001**)[9] and the **EC Guidance Document on Terrestrial Ecotoxicology** [10].

In-field

Exposure

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[9] Candolfi MP, Barrett KL, Campbell PJ, Forster R, Grandy N, Huet M-C, Lewis G, Oomen PA, Schmuck R, Vogt H (2000) 'Guidance Document on regulatory testing procedures for plant protection products with non-target arthropods' From the workshop, European Standard Characteristics of Non-target Arthropod Regulatory Testing (ESCORT 2) 21-23 March 2000.

[10] EC Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO/10329, 17 October 2002.

Non-target arthropods living in the crop can be exposed to residues from A14325E by direct contact either as a result of overspray or through contact with residues on plants and soil or in food items. A14325E is applied at a maximum rate of 1.5 L formulation/ha. The maximum in-field exposure (Predicted Environmental Rate, PER) to foliar-dwelling or soil-dwelling organisms is therefore 1.5 L formulation/ha, assuming the worst-case (contradiction) of 100% crop interception for foliar exposure and 80% crop interception for soil exposure, respectively.

The in-field exposure (predicted environmental residue, PER) is calculated according to ESCORT 2 using the following equation:

$$PER_{in-field} = Application \text{ rate (g a.s./ha)}$$

The maximum predicted environmental residues (PER) occurring within the field after application of A14325E at the maximum application rate are presented below.

Table 10.3.2-2: In-field PER values for application of A14325E

Crop	Application rate (mL/ha)	Foliar exposure		Soil exposure		
		MAF	PER (foliar) mL product/ha	MAF	Crop interception (%)	PER (soil) mL product/ha
Cereals	1500	1.7	2550	1.9	80	570

Risk Assessment

The in-field risk to non-target arthropods was assessed by calculating Hazard Quotients (HQs) for the two sensitive indicator species, *T. pyri* and *A. rhopalosiphi*, using the following equation:

$$\text{In - field HQ} = \frac{\text{PER}_{\text{in-field}} \text{ (mL/ha)}}{\text{LR}_{50} \text{ (mL/ha)}}$$

The resulting HQ values are presented, to 2 significant figures, in the table below. When using Tier I data the risk is considered to be acceptable if the HQ is less than 2.

Table 10.3.2-3: In-field HQs for non-target arthropods

Crop	Species	LR_{50} (g/ha)	In-field foliar exposure		In-field soil exposure		Trigger value
			PER (mL/ha)	HQ	PER (mL/ha)	HQ	
Cereals	*A. rhopalosiphi*	156 [a] ~~241~~	2550	**16** ~~**10.6**~~	570	**3.7** ~~**2.4**~~	2
	T. pyri	1943		1.3		0.29	

[a] re-estimated at the request of the RMS

The in-field soil and foliar HQ values for *Typhlodromus pyri* are <2 for both foliar and soil exposure crops, indicating an acceptable risk. However, the in-field foliar HQ values for *A. rhopalosiphi* are above the trigger of 2 for both crops, indicating the need for further refinement. A higher tier risk assessment has therefore been conducted and is presented below.

Refined in-field risk assessment

Higher tier tests have been conducted according to the requirements of ESCORT 2 and summarised in Table 10.3.2-1. According to ESCORT 2, in higher tier tests, lethal and sub-lethal effects <50% compared to the control, at test rates equivalent to the relevant PER are considered acceptable.

The in-field assessment is presented in the table below.

Table 10.3.2-4: In-field risk assessment for non-target arthropods

Test species	Endpoints (mL A14325E/ha)		Toxicity endpoint <50% at ≥PER			
			Foliage		Soil	
			PER (mL/ha)	Acceptable risk	PER (mL/ha)	Acceptable risk
T. pyri	LR_{50}	>3000	2550	Yes	570	Yes
	NOER (reproduction)	1950		No		
A. rhopalosiphi	LR_{50}	>3000	2550	Yes	570	Yes
	NOER (reproduction)	3000				
C. carnea	LR_{50}	2393	2550	Yes	570	Yes
	NOER (reproduction)	750		No		
C. septempunctata	LR_{50}	>3000	2550	Yes	570	Yes
	NOER (reproduction)	3000				

The risk assessment based on data for *A. rhopalosiphi* and *C. septempunctata* is acceptable. The risk assessment based on data for *T. pyri* is acceptable with respect to mortality but unacceptable with respect to risk of effects on fecundity from foliar residues, as the PER foliar lies between the rate showing no unacceptable effects and the rate showing unacceptable effects. The risk assessment based upon *C. carnea* data is acceptable with respect to mortality from foliar residues, and is unacceptable with respect to fecundity. Based on this assessment, there is still some potential risk to some non-target arthropods, requiring further consideration which is given below.

According to ESCORT 2, any initial in-field effects are considered acceptable provided that the potential for recovery within one year can be demonstrated. In order to demonstrate potential for recovery, the degradation of foliar residues of A14325E have been modelled using first order degradation kinetics [11], to determine the time after last application when residue levels will fall below the no-unacceptable effect rate, which in this case is 750 mL product/ha. This is conservative, as it is the highest rate tested for reproduction in *C. carnea*, and no unacceptable effects were seen at this rate. The foliar DT_{50} value for cyprodinil is 4.5 days. The time taken for foliar residues to fall below the acceptable toxicity threshold of 750 mL/ha is shown in the table below.

Table 10.3.2-5: A14325E effects on non-target arthropods: Time taken for residues to fall to an acceptable level

Use pattern	Exposure surface	Acceptable residue level (mL/ha)	DT_{50} (days)	PER after last foliar application (g/ha)	Time after last application at which residues fall to an acceptable level (day)
Cereals	Foliar	750	4.5	1673	6

Even when considering this most sensitive endpoint and worst-case degradation, effects in-field demonstrate an acceptable potential for re-colonisation of any affected populations within the one year

[11] $PER_{(t)} = PER_{initial}(e^{-kt})$
Where: t = time elapsed (days) ; $k = \ln(2) / DT_{50}$ in days

recovery period stipulated by ESCORT 2. Therefore, even based on this conservative assessment, and using laboratory test data, the potential for recovery is clearly acceptable according to ESCORT 2 guidelines.

In addition, no unacceptable effects are shown off-field (see below), allowing recovery from any initial effects by immigration from source off-field areas.

Extended laboratory aged residue tests have subsequently been conducted. *T. pyri* protonymphs and *C. carnea* larvae were exposed to fresh residues of A14325E after two applications of 1500 mL/ha with a 14-day spray interval. For both species effects <50% were observed for both morality and reproduction for bioassays initiated with fresh residues (0 DAT) and residues aged under rain protection (7 DAT for *C. carnea* and 14 DAT for *T.pyri*). This demonstrates an acceptable risk to these species following application of A14325E according to the proposed use pattern.

Conclusion: A14325E poses an acceptable in-field risk to non-target arthropods, according to the proposed use patterns.

Off-field

Exposure

Risk assessment of areas immediately surrounding the crop is considered important since these areas represent a natural reservoir for immigration, emigration and reproduction of arthropod populations and provide increased species diversity. Exposure of non-target arthropods living in off-field areas to A14325E will mainly be due to spray drift from field applications. Off-field areas are assumed to be densely vegetated and thus spray drift is unlikely to reach bare ground. Therefore, evaluation of exposure *via* soil residues in off-field areas was not considered. Off-field foliar PER values were calculated from in-field foliar PERs in conjunction with drift values published by the *BBA (2000)*[12] as shown in the following equation:

$$\text{Off-field foliar PER} = \frac{\text{Maximum in-field foliar PER x (\% drift/100)}}{\text{vegetation distribution factor}}$$

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.

The drift value at 1 m distance is 2.77% of the application rate (90th percentile drift). The drift factor (% drift/100) is therefore 2.77/100 = 0.0277.

The resulting $PER_{off\text{-}field}$ values are shown below.

[12] 90th percentile drift according to 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

Table 10.3.2-6: Off-field foliar Predicted Environmental Rates (PER)

Study type	Maximum in-field foliar PER [a] (mL product/ha)	drift factor (% drift/100)	Vegetation distribution factor	Off-field foliar PER (mL product/ha)
2-D	2550	0.0277	10	7.06
3-D			0	70.6

[a] See Table CP 10.3.2-2

Risk Assessment

The off-field risk to non-target arthropods was assessed by calculating Hazard Quotients (HQs) for the two sensitive indicator species, *T. pyri* and *A. rhopalosiphi*, using the following equation:

$$\text{Off-field HQ} = \frac{\text{PER (g/ha)}}{\text{LR}_{50}\text{(g/ha)}}$$

The resulting HQ values are presented, to 2 significant figures, in the table below. When using Tier I data the risk is considered to be acceptable if the HQ is less than 2.

Table 10.3.2-7: Off-field risk assessment for non-target arthropods

Crop	Species	LR_{50} (g/ha)	PER (g/ha)	HQ	Trigger value
Cereals	*A. rhopalosiphi*	156 [a] ~~241~~	7.06	0.045 ~~0.029~~	2
	T. pyri	1943		0.0036	

[a] re-estimated at the request of the RMS

The off-field HQ values are below the trigger value of 2, indicating that A14235E poses an acceptable off-field risk to non-target arthropods.

In addition, although not strictly required by ESCORT 2, sub-lethal effects were tested in the Tier I studies. The results were as follows:

- *A. rhopalosiphi* showed no unacceptable reduction in fecundity (i.e. reduction was not >50%) at 23.33 mL formulation/ha (the only rate tested), compared to the control.
- *T. pyri* showed no unacceptable reduction in fecundity (i.e. reduction was not >50%) at up to 750 mL formulation/ha (the highest fecundity rate tested)

Conclusion: A14325E poses an acceptable off-field risk to non-target arthropods, according to the proposed use patterns.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

Report:	K-CP 10.3.2.1/01 Vaughan R. (2005) Cyprodinil (CGA219417) 300 g L^{-1} EC formulation (A14325E): A laboratory bioassay of the effects of fresh residues on the predatory mite, *Typhlodromus pyri* (Acari: Phytoseiidae). Mambo-Tox Ltd, Southampton, UK. Report Number SYN-05-5. (Syngenta file No. CGA219417/1298)

Guidelines

Blümel *et al*. (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products.

GLP: Yes.

Executive Summary

The aim of the study was to determine the effects of fresh dry residues of A14325E on the predatory mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae), under worst-case laboratory test conditions on glass.

A14325E was evaluated in a bioassay at five rates, equivalent to 3000, 1950, 750, 375 and 93.67 mL product/ha. Also included in the definitive test were a control treatment of purified water and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate) applied at a rate of 15 mL product/ha (nominally 6 g ai/ha). All treatments were applied to glass plates at a volume rate equivalent to 200 L spray solution/ha. Mortality was evaluated over 7 days exposure, and fecundity over a further 7 days.

Under worst-case laboratory test conditions, the 7-day LR_{50} (median lethal rate) for *Typhlodromus pyri* was equivalent to 1943 mL A14325E/ha. A14325E had no effects >50% on fecundity at rates up to 750 mL/ha, the highest tested for fecundity.

Materials

Test Material:	A14325E
Description:	Clear amber-coloured fluid, nominally containing 300 g/L CGA219417 (cyprodinil)
Lot/Batch #:	SMU5BP002
Purity:	303 g/L
Stability of test compound:	The test item is assumed to be stable for the period of use in the study, pending concurrent batch analysis.
Test rates:	3000, 1950, 750, 375 and 93.67 mL A14325E/ha
Vehicle and control:	Purified water
Toxic reference:	Perfekthion EC (400 g dimethoate/L) in deionised water (15 mL product/ha)
Spray volume rate:	200 L spray solution/L
Application method:	Potter Laboratory Spray Tower, calibrated for each treatment preparation.
Test organisms	
Species:	*Typhlodromus pyri* (Acari: Phytoseiidae).
Source:	Culture maintained at Test Facility.
Food:	1:1 v/v mixture of walnut (*Juglans regia* L.) and apple (*Malus* sp. var. Winter Banana)
Test substrate:	Glass plates formed from two microscope slide cover slips, each 22 mm x 40 mm in area and thickness No.1 (i.e. 0.13-0.16 mm).
Environmental test conditions	
Temperature:	24 to 26°C
Humidity:	49 to 81% relative humidity (fluctuations below 60% RH were for short periods, not more than 2 h).
Photoperiod:	16 h daily photoperiod (600-1500 lux)

Study Design and Methods

Experimental dates: 22nd March to 5th April 2005.

The test substrate comprised glass plates formed from two microscope slide cover slips, each 22 mm x 40 mm in area. Following treatment, the glass plates were left to dry and then placed onto damp tissue paper, with their treated surface uppermost. The bioassay was initiated approximately 1 h after treatments were applied, *i.e.* once residues on the treated glass plates had dried. An oblong-ring of 'Non-Drying Insect Glue' was drawn on each plate to make an arena in which to confine the mites. Twenty protonymphal *T. pyri* were placed at the centre of each replicate arena, with four replicates (80 mites in total) prepared per treatment. The mites were fed regularly with untreated pollen for food. Their survival was assessed over a 7-day period, by which time they were adult. The sex of the adult mites was determined and they were then left *in situ* so that their reproduction could be assessed over a further 7 days. These further assessments were carried out for the control and for treatment rates of the test item that had resulted in < 50% corrected mortality. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated.

Results and Discussion

Table 10.3.2.1-1: Effects of residues of A14325E on glass on mortality and fecundity of the mite, *Typhlodromus pyri*, under laboratory test conditions

Treatment	Rate (mL/ha)	Mean % mortality 7 DAT	Corrected % mortality 7 DAT	Mean number of eggs per female [a]	Effects on reproduction [b] (%)
Control	-	19	-	8.2	-
A14325E	3000	70	63	n.a.	-
	1950	61	52	n.a.	-
	750	38	23	4.5 *	45
	375	29	12	5.9	28
	93.67	21	3	9.2	-12
Perfekthion	15	99	98	-	-

[a] Treatments compared by one-way ANOVA and Dunnett's Test ($\alpha = 0.05$). Asterisks indicate test item treatments that differed significantly from the control (* $P < 0.05$).
[b] Change in numbers of eggs per female, relative to control (after Blümel *et al*., 2000). A positive value indicates a decrease and a negative value an increase.
n.a. not tested at this rate.

A Probit regression analysis indicated that the 7-day LR_{50} (median lethal rate) was 1943 mL product/ha (95% confidence limits = 1437 and 2924 mL product/ha).

Conclusion

The effects of A14325E on the predatory mite, *Typhlodromus pyri*, were evaluated under worst-case laboratory test conditions on glass. The 7-day LR_{50} (median lethal rate) was determined as being 1943 mL product/ha. A14325E had no statistically significant effect on the reproduction of mites at rates of up to and including 375 mL product/ha, and no effects >50% up to 750 mL/ha (the highest rate tested for fecundity).

(Vaughan R, 2005)

Report:	K-CP 10.3.2.1/02 Vinall S. (2005) Cyprodinil (CGA219417) 300 g L^{-1} EC formulation (A14325E): A laboratory bioassay of the effects of fresh residues on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). Mambo-Tox Ltd, Southampton, UK. Report Number SYN-05-4. (Syngenta file No. CGA219417/1297)

Guidelines

Mead-Briggs *et al*. (2000). A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera, Braconidae).

GLP: Yes.

Executive Summary

The aim of the study was to determine the effects of fresh dry residues of A14325E on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera, Braconidae), under worst-case laboratory test conditions on glass.

A14325E was evaluated at six application rates, equivalent to 3000, 1950, 750, 375, 93.67 and 23.33 mL product/ha. Also included in the definitive test were a water-treated control and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate), applied at a rate of 0.20 mL product/ha (0.08 g ai/ha). All treatments were applied to glass plates at a volume rate of 200 L/ha. Assessments of treatment effects were made over 48 h. To assess any sub-lethal effects, reproduction assessments were then carried out for insects from the control and from all treatment rates of the test item that had resulted in < 50% corrected mortality. Fifteen female wasps were confined individually over untreated aphid-infested barley plants for 24 h, before being removed. The plants were left for a further 10 days before the number of aphid mummies that developed was recorded.

The median lethal rate (LR_{50}) was estimated to be 241.1 mL product/ha. A14325E had no effect >50% on the fecundity of the wasps at 23.33 mL product/ha, the only rate tested for fecundity.

Materials

Test Material:	A14325E
Description:	Clear amber-coloured fluid, nominally containing 300 g/L CGA219417 (cyprodinil)
Lot/Batch #:	SMU5BP002
Purity:	303 g/L
Stability of test compound:	The test item is assumed to be stable for the period of use in the study, pending concurrent batch analysis.
Test rates:	3000, 1950, 750, 375, 93.67 and 23.33 mL A14325E/ha
Vehicle and control:	Purified water
Toxic reference:	Perfekthion EC (400 g dimethoate/L) in deionised water (0.2 mL/ product ha)
Spray volume rate:	200 L spray solution/ha
Application method:	Potter Laboratory Spray Tower, calibrated for each treatment preparation.
Test organisms	
Species:	*Aphidius rhopalosiphi* De Stefani-Perez. (Hymenoptera: Braconidae)
Source:	Culture maintained at Test Facility on cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*).
Food:	1:3 v/v solution of honey in water or 10% w/v solution of fructose in water.
Test substrate:	Glass plates
Environmental test conditions	
Temperature:	Mortality assessment phase: 20 to 21°C Fecundity assessment phase: 19 to 21°C

Humidity: Mortality assessment phase: 65 to 84% relative humidity

Photoperiod: Mortality assessment phase: 16 h daily photoperiod (1100-1650 lux)
Fecundity assessment phase: 16 h daily photoperiod (4100-4500 lux)

Study Design and Methods

Experimental dates: 29th March to 11th April 2005.

Treatments were applied to glass plates which were then used to form the floor and ceiling of shallow arenas. Ten adult wasps (including a minimum of five females) were placed in each replicate arena (n = 4 per treatment rate). Assessments of treatment effects were made at 2, 24 and 48 h. To assess any sub-lethal effects, fecundity assessments were then carried out for insects from the control and from all treatment rates of the test item that had resulted in < 50% corrected mortality. Fifteen female wasps were confined individually over untreated aphid-infested barley plants for 24 h, before being removed. The plants were left for a further 10 days before the number of aphid mummies that developed was recorded.

Results and Discussion

Table 10.3.2.1-2: Effects of fresh residues of A14325E on glass on mortality and fecundity of *Aphidius rhopalosiphi* under laboratory test conditions

Treatment	Rate (mL product/ha)	% mortality at 48 h [a]	Corrected % mortality at 48 h	Mean number mummies per surviving female [a]	Effect on reproduction [b] (%)
Control	0	0	-	40.4	-
A14325E	3000	80	~~100~~ 80	n.a.	-
	1950	83	~~55~~ 83	n.a.	-
	750	65	~~0~~ 65	n.a.	-
	375	65	~~0~~ 65	n.a.	-
	93.67	55	~~0~~ 55	n.a.	-
	23.33	0	0	30.8	24
Perfekthion	0.2	100	~~95~~ 100	n.a.	-

[a] The results for the test item treatment and control were compared by t-test for unmatched pairs but they did not differ significantly (P > 0.05).
[b] A positive value indicates a decrease in reproduction, relative to the control.
n.a. not assessed in this treatment.

A Probit analysis on these data indicated that the 48-h LR_{50} was 241.1 mL A14325E/ha (95% confidence limits = 123.6 and 410.7mL product/ha).

Conclusion

The effects of A14325E on the parasitic wasp, *Aphidius rhopalosiphi*, were evaluated under worst-case laboratory test conditions on glass. The median lethal rate (LR_{50}) for the test item was estimated to be 241.1 mL product/ha. A14325E had no statistically significant or >50% effect on the fecundity of the wasps at 23.33 mL product/ha, the only rate tested for fecundity.

(Vinall S, 2005)

***Comment from RMS:** Tier 1 laboratory study with A14325E on Aphidius rhopalosiphi (K-CP 10.3.2.1/02; Vinall, 2005): the LR50 (241.1 mL A14325E/ha) should be recalculated with another method than Probit given that mortality exceeds 50% at 93.67 ml/ha and at higher rates.*

Response from Syngenta: The LR_{50} has been re-estimated as requested and a summary of the re-analysis is presented below. The Tier 1 risk-assessment has been updated accordingly.

Report:	Submitted at the request of the RMS K-CP 10.3.2.1/03, Paul KB (2016). Estimation of the LR_{50} for the following report**:** Cyprodinil (CGA219417) 300 g/L^{-1} EC formulation (A14325E): A laboratory bioassay of the effects of fresh residues on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae), statistical re-analysis. (Syngenta File No: A14325E_10096).

Summary

Mambo-tox report number SYN-05-4 (Vinall S, 2005; Syngenta file number CGA219417/1297), for the effect of fresh residues of A14325E to the parasitic wasp, *Aphidius rhopalosiphi*, did not provide appropriate estimates of the LR_{50} and a request was issued by a regulatory body to update the study endpoint using more appropriate statistical methods. Consequently, the data generated in the *Aphidius rhopalosiphi* study were re-analysed in an attempt to fulfil this objective.

The tested application rates of A14325E were 23.33, 93.67, 375, 750, 1950 and 3000 mL product/ha, tested with an untreated control. With 4 replicates per treatment of 10 parasitic wasps.

Statistical analysis in the original report estimated the LR50 to be 241.1 mL product/ha (95 % C.I. 123.6 to 410.7 mL product/ha) using the Probit model, and the mean response from each group.

The models employed during the re-analysis were Probit, Probit (with arcsine transformation), Weibull and the trimmed Spearman-Karber.

Only the trimmed Spearman-Karber was considered to give an appropriate estimate due to the nature of the dataset and estimated values.

The LR_{50} was estimated to be 156 mL product/ha (C.I. - 97.6 to 249 mL product/ha)

Methods

A series of statistical methods were employed to attempt to recalculated the *Aphidius rhopalosiphi* study endpoint.

Initially Probit analysis was performed, however in order to improve accuracy and appropriateness, the Probit model was constructed using all observed results. Further, arcsine transformation of the dataset was also conducted to improve data distribution followed by Probit analysis.

Subsequently, the data was fitted to the Weibull model due to its ability to account for skewed data.

Finally, the non-parametric trimmed Spearman-Karber model which is appropriate for deviations from monotonicity and normality.

Statistical analysis was performed using SPSS statistics package (v. 24), Excel (v 15.211), R (v. 3.2.4) and USEPA TSK.

Results

For the Probit model; transformation and use of all data did not improve data normality, or correct for a slight skew within the dataset, and model assumptions were not met. Further, the data showed non-monotonicity at the higher levels of application, which can effect a reliable interpolation. The derived values and poor fit, made the interpolations unreliable.

The Weibull model again was inappropriate considering the dataset and interpolated value, despite its ability to better cope with skew of the dataset, it is unable to account for non-monotonicity seen within the higher application rates of the dataset.

Only the trimmed Spearman-Karber was considered to give an appropriate estimate due to the nature of the dataset and estimated values. The model is distribution free, is able to cope with non-monotonic trends, and focuses on those values and application rates which contribute to the critical value of interest by trimming the upper and lower portions of the curve.

The LR_{50} was estimated to be 156 mL product/ha (C.I. - 97.6 to 249 mL product/ha)

Table 10.3.2.1-3: Summary results of statistical analysis using various methods

Method	Software	LR_{50} Estimate (mL product/ha)	95% Confidence Interval		Comment
			Lower Bound	Upper Bound	
Probit (log_{10})	SPSS	241	124	411	Poor fit for data. Over weights higher concentrations. Unable to account for non-monotonicity.
Arcsin transformation Probit (log_{10})	SPSS	311	160	547	Poor fit for data. Over weights higher concentrations. Unable to account for non-monotonicity.
Weibull	R	204	38.9 (as S.E.)		Unable to account for non-monotonicity.
Trimmed Spearman-Karber	USEPA TSK	156	97.6	249	Valid non-parametric / distribution free, robust test, appropriate to the data and conservative endpoint

Conclusion

The results of these analysis indicate that the LR_{50} estimated from the TSK model is the most appropriate and conservative. The model can be considered biologically (accounts for all relevant populations) and statistically reasonable and robust. The model does not overweight the interpolation based on the dataset extremes or, distribution of data which do not meet the model assumptions.

It is concluded that the LR_{50} is estimated to be 156 mL product/ha (C.I. - 97.6 to 249 mL product/ha).

(Paul K., 2016)

CP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

Report:	K-CP 10.3.2.2/01 Vaughan R. (2005a) Cyprodinil (CGA219417) 300 g L^{-1} EC formulation (A14325E): An extended laboratory bioassay of the effects of fresh residues on the predatory mite, *Typhlodromus pyri* (Acari: Phytoseiidae). Mambo-Tox Ltd, Southampton, UK. Report Number SYN-05-9. (Syngenta File No. CGA219417/1332)

Guidelines

Blümel *et al*. (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products.

GLP: Yes.

Executive Summary

The aim of the study was to determine the effects of fresh dry residues of A14325E on the predatory mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae), under extended laboratory test conditions (2-dimensional).

A14325E was evaluated in a bioassay at six rates, equivalent to 3000, 1950, 750, 375, 93.67 and 23.33 mL product/ha (nominally equivalent to 900, 585, 225, 112.5, 28.1 and 7 g ai/ha). Also included in the definitive test were a control treatment of purified water and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate) applied at a rate of 30 mL product/ha (nominally 6 g ai/ha). All treatments were applied to leaf discs taken from French bean plants (*Phaseolus vulgaris* L.), at a volume rate equivalent to 200 L spray solution/ha. The leaf discs were left to dry and then placed onto wet cotton wool, with their treated surface uppermost. A ring of a sticky non-drying gel was drawn on each disc to create the arenas in which mites were then confined. Twenty protonymphal *T. pyri* were placed on each replicate arena, with four replicates (80 mites in total) prepared per treatment. The mites were fed regularly with untreated pollen for food. Their survival was assessed over a 7-day period, by which time they were adult, and fecundity was evaluated for a further 7 days.

The 7-day LR_{50} (median lethal rate) for A14325E to *Typhlodromus pyri* was determined as being greater than the maximum treatment rate of 3000 mL product/ha. A14325E had no statistically significant or >50% effect on the reproduction of mites at rates of up to and including 1950 mL product/ha.

Materials

Test Material:	A14325E
Description:	Amber liquid, nominally containing 300 g/L CGA219417 (cyprodinil).
Lot/Batch #:	SMU5BP002
Purity:	303 g/L cyprodinil
Stability of test compound:	The test item is assumed to be stable for the period of use in the study, pending concurrent batch analysis.
Test rates:	3000, 1950, 750, 375, 93.67 and 23.33 mL A14325E/ha (nominally equivalent to 900, 585, 225, 112.5, 28.1 and 7 g ai/ha)
Vehicle and control:	Purified water
Toxic reference:	Perfekthion EC (nominally 400 g dimethoate/L) in deionised water (30 mL product/ha)

Spray volume rate: 200 L spray solution/ha
Application method: Potter Laboratory Spray Tower, calibrated for each treatment preparation.

Test organisms
Species: *Typhlodromus pyri* (Acari: Phytoseiidae).
Source: Culture maintained at Test Facility.
Food: 1:1 v/v mixture of walnut (*Juglans regia* L.) and apple (*Malus* sp. var. Winter Banana)

`est substrate: Leaf discs taken from first true leaves of dwarf French beans (*Phaseolus vulgaris* L., var. The prince).

Environmental test conditions
Temperature: 25-27°C
Humidity: 72% to 93% relative humidity
Photoperiod: 16 h daily photoperiod (900-1400 lux)

Study Design and Methods

Experimental dates: 14th to 28th June 2005.

The test substrate comprised leaf discs taken from dwarf French bean plants, *Phaseolus vulgaris.* The bioassay was initiated approximately 1 h after treatments were applied, *i.e.* once residues on the leaf discs had dried. The leaf discs were placed onto damp cotton wool and a ring of a sticky non-drying gel drawn around the edge of each to create circular arenas in which mites were confined. Twenty protonymphal *T. pyri* were placed at the centre of each replicate arena, with four replicates (80 mites in total) prepared per treatment. The mites were fed regularly with untreated pollen for food. Their survival was assessed over a 7-day period, by which time they were adult. The sex of the adult mites was determined and they were then left *in situ* so that their reproduction could be assessed over a further 7 days. These further assessments were carried out for the control and for treatment rates of the test item that had resulted in < 50% corrected mortality. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated.

Results and Discussion

Table 10.3.2.2-1: Effects of foliar residues of A14325E on mortality and fecundity of the mite, *Typhlodromus pyri*, under extended laboratory test conditions

Treatment	Rate (mL/ha)	Mean % mortality (at 7 DAT) [a]	Corrected % mortality (at 7 DAT)	Mean number of eggs per female [b]	Effects on reproduction [c] (%)
Control	-	19	-	6.0	-
A14325E	3000	25	8	1.9 *	68
	1950	28	11	3.6	40
	750	31	15	3.4	43
	375	33	17	3.9	35
	93.67	25	8	4.7	22
	23.33	24	6	3.3	45
Perfekthion	30	61 ***	52	-	-

[a] The results of mortality assessments were compared using Fisher's Exact Test. Asterisks indicate treatment means that differed significantly from the control (*** $P < 0.001$).

[b] Treatments compared by one-way ANOVA and Dunnett's Test. Asterisks indicate treatment means that differed significantly from the control (*P < 0.05).
[c] Change in numbers of eggs per female, relative to control (after Blümel *et al*., 2000). A positive value indicates a decrease.

Conclusion

The effects of A14325E on the predatory mite, *Typhlodromus pyri*, were evaluated under extended laboratory test conditions (2-dimensional). The 7-day LR_{50} (median lethal rate) was determined as being greater than the maximum treatment rate of 3000 mL product/ha. A significant reduction in the reproductive capacity of the mites was seen at a treatment rate of 3000 mL product/ha. A14325E had no statistically significant or >50% effect on the fecundity of mites at rates of up to and including 1950 mL product/ha.

(Vaughan R, 2005a)

Report:	K-CP 10.3.2.2/02 Vinall S. (2005a) Cyprodinil (CGA219417) 300 g L^{-1} EC formulation (A14325E): An extended laboratory bioassay of the effects of fresh residues on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). Mambo-Tox Ltd, Southampton, UK. Report Number SYN-05-10. (Syngenta File No. CGA219417/1390)

Guidelines

Mead-Briggs *et al.* (in preparation). An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera, Braconidae).

GLP: Yes.

Executive Summary

The aim of this study was to determine the effects of A14325E on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera, Braconidae) under extended laboratory test conditions (3-dimensional).

A14325E was evaluated at six application rates, equivalent to 3000, 1950, 750, 375, 93.67 and 23.33 mL product/ha. Also included in the test were a water-treated control and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate), applied at a rate of 7.5 mL product/ha (nominally 3 g ai/ha). Treatments were applied at a volume rate equivalent to 400 L spray solution/ha to pots of seedling barley. Once dry, the barley plants were enclosed within cylindrical, ventilated collars. Five wasps were confined in each arena, with six replicates (*i.e.* a total of 30 wasps) prepared for each treatment. Assessments of mortality effects were made over 48 h. To assess any significant sub-lethal effects, reproduction assessments were then carried out for the control and from all treatment rates of the test item that had resulted in < 50% corrected mortality. Fifteen female wasps were confined individually for 24 h over untreated barley plants previously infested with cereal aphids. The wasps were then removed and the plants left for a further 9 days before the number of 'mummies' that had developed was recorded.

The 48-h LR_{50} (median lethal rate) was greater than 3000 mL A14325E/ha and the reproductive performance of surviving wasps was not affected >50% at treatment rates up to and including 3000 mL A14325E/ha.

Materials

Test Material: A14325E

Description:	Liquid, nominally containing 300 g/L CGA219417 (cyprodinil).
Lot/Batch #:	SMU5BP002
Purity:	303 g/L cyprodinil
Stability of test compound:	The test item is assumed to be stable for the period of use in the study, pending concurrent batch analysis.
Test rates:	3000, 1950, 750, 375, 93.67 and 23.33 mL A14325E/ha
Vehicle and control:	Deionised water
Toxic reference:	Perfekthion EC (nominally 400 g dimethoate/L) in deionised water, applied at a rate of 7.5 mL product/ha)
Spray volume rate:	400 L spray solution/ha
Application method:	Potter Laboratory Spray Tower, calibrated for each treatment preparation.
Test organisms	
Species:	*Aphidius rhopalosiphi* De Stefani-Perez. (Hymenoptera: Braconidae)
Source:	Culture maintained at Test Facility on cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*).
Food:	1:3 v/v solution of honey in water or 10% w/v solution of fructose in water.
Test substrate:	Barley (*Hordeum vulgare* var. Chime) seedlings. Groups of 10 seedlings per replicate pot, with two expanded leaves and cut to be approximately 10 cm tall.
Environmental test conditions	
Temperature:	Mortality assessment phase: 19 to 22°C Fecundity assessment phase: 19 to 21°C
Humidity:	Mortality assessment phase: 66 to 72% relative humidity
Photoperiod:	Mortality assessment phase: 16 h daily photoperiod (2600 lux) Fecundity assessment phase: 16 h daily photoperiod (4500-7200 lux)

Study Design and Methods

Experimental dates: 15th to 27th June 2005.

Pots of barley seedlings (6 replicates per treatment) were treated and left for up to 1 h to dry. Once dry, the pots of plants were enclosed within cylindrical, ventilated collars. Five wasps were confined in each arena, with six replicates (*i.e.* a total of 30 wasps) prepared for each treatment. For the definitive bioassay there were three variant treatment rates for the test item, a control and a toxic reference treatment. Assessments of treatment effects were made over 48 h. The behaviour of the wasps was assessed during the first 3 h after treatment, to determine whether there was any apparent repellence from the treated plants, and wasp survival was assessed over a period of 48 h. To assess any sub-lethal effects, reproduction assessments were then carried out for the control and for both treatment rates of the test item. Fifteen female wasps from each treatment were confined individually for 24 h over untreated barley plants previously infested with cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*). The wasps were then removed and the plants left for a further 9 days before the number of ‘mummies’ (parasitised aphids containing wasp pupae) that had developed was recorded.

Results and Discussion

Table 10.3.2.2-2: Effects of fresh foliar residues of A14325E on mortality and fecundity of *Aphidius rhopalosiphi* under extended laboratory test conditions

Treatment	Rate (mL/ha)	% mortality at 48 h	Corrected % mortality at 48 h	Mean number mummies per surviving female [a]	Effect on reproduction [b] (%)
Control	-	0	-	45.1	-
A14325E	3000	7	7	24.4 ***	46
	1950	0	0	44.7	1
	750	0	0	42.3	6
	375	0	0	43.4	4
	93.67	0	0	51.8	-15
	23.33	0	0	52.7	-17
Perfekthion	7.5	100	100	-	-

[a] The results for the test items treatments were compared to the control by one-way ANOVA but did not differ significantly.
[b] A positive value indicates and decrease and a negative value an increase in reproduction relative to the control.

Conclusion

The 48-h LR_{50} (median lethal rate) for effects of A14325E on *A. rhopalosiphi* under extended laboratory conditions (3-Dimensional) was greater than the highest test rate, *i.e.* > 3000 mL A14325E/ha. The fecundity of surviving wasps was significantly affected at the 3000 mL/ha treatment rates but there were no effects >50% at all rates, including 3000 mL/ha.

(Vinall S, 2005a)

Report:	K-CP 10.3.2.2/03 Spincer D. (2005) Cyprodinil (CGA219417) 300 g L^{-1} EC formulation (A14325E): An extended laboratory test to evaluate the effects of fresh residues on the green lacewing, *Chrysoperla carnea* (Neuroptera, Chrysopidae). Mambo-Tox Ltd, Southampton, UK. Report Number SYN-05-12. (Syngenta File No. CGA219417/1375)

Guidelines

Vogt H, Bigler H, *et al*. (2000). Laboratory method to test effects of plant protection products on larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae). In: *Guidelines to evaluate side-effects of plant protection products to non-target arthropods; IOBC, BART and EPPO Joint Initiative*. Eds. Candolfi MP, Blümel S, *et al.*. IOBC Publication. ISBN 92-9067-129-7.

GLP: Yes.

Executive Summary

The aim of this study was to determine the LR_{50} (median lethal rate) of A14325E on larvae of the lacewing, *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae), under extended laboratory test conditions (2-dimensional). The reproductive potential of the resultant adult lacewings was also checked.

A14325E was evaluated at six application rates, equivalent to 3000, 1950, 750, 375, 93.67 and 23.33 mL product/ha. These were compared to a water-treated control and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate), applied at a rate of 200 mL/ha (nominally 80 g ai/ha). Treatments were applied to leaves of the dwarf French bean (*Phaseolus vulgaris* L.) and once residues had dried the leaves were used to line the floor of test arenas (n = 40 per treatment) into which individual larvae of *C. carnea* (2-3 days old) were introduced. The larvae were fed with untreated eggs of the Angoumois grain moth, *Sitotroga cerealella* (Oliver) and any pre-imaginal mortality of the lacewings was recorded. A check was then made for sub-lethal effects on the reproductive performance of the adults surviving in the control and in treatment groups of the test item where corrected mortality was < 50%. For this, the egg-laying activity of grouped females was monitored for two 24-h periods and the viability of the eggs was determined.

The mean numbers of eggs produced in all the treatments evaluated was ≥ 15 eggs/female/day and the mean egg viability was ≥ 70%. These two thresholds are viewed as being indicative of no harmful treatment effects.

The LR_{50} for A14325E to the green lacewing, *Chrysoperla carnea*, was 2393 mL product/ha (nominally 717.9 g ai/ha). There were no unacceptable effects on reproduction at treatment rates up to and including 750 mL A14325E/ha, the highest rate tested for reproduction.

Materials

Test Material:	A14325E
Description:	liquid, nominally containing 160 g/L SAN619 and 300 g/L cyprodinil
Lot/Batch #:	SMU5BP002
Purity:	303 g/L cyprodinil
Stability of test compound:	The test item is assumed to be stable for the period of use in the study, pending concurrent batch analysis.
Treatment rates:	3000, 1950, 750, 375, 93.67 and 23.33 mL A14325E/ha
Vehicle and control:	Purified water
Toxic reference:	Perfekthion EC (400 g dimethoate/L) in deionised water (200 mL product/ ha)
Spray volume rate:	400 L spray solution/ha
Application method:	Hand-held sprayer powered by compressed air (Azo Ltd., Ede, The Netherlands). Spray boom fitted with 80° flat-fan nozzles (XR Teejet 8002 VS) and spray pressure set at 2.5 bar.
Test organisms	
Species:	*Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae).
Source:	Culture maintained at Test Facility.
Food:	UV-killed eggs of the Angoumois grain moth, *Sitotroga cerealella* (Oliver) (Lepidoptera, Gelechiidae)
Test substrate:	First true leaves of dwarf French beans (*Phaseolus vulgaris* L., var. The prince).
Environmental test conditions	
Temperature:	23-26°C
Humidity:	52-75% relative humidity
Photoperiod:	16 h daily photoperiod (2600-2650 lux)

Study Design and Methods

Experimental dates: 23rd June to 28th July 2005.

Excised French bean leaves (40 replicates per treatment) were treated on their upper (adaxial) surface and left for up to 1 h to dry. Arenas were then assembled and 2- to 3-day-old lacewing larvae individually confined on the upper treated surface. The larvae were provided with untreated moth eggs for food and pre-imaginal mortality was assessed. The adults were then grouped together, with treatments kept in separate boxes. A check was made for sub-lethal effects on the reproductive performance of the surviving adults in the control and in the test item treatments with <50% mortality. For this the egg-laying activity of all surviving females was monitored for two 24-h periods in one week and the viability of the eggs produced was then determined.

Results and Discussion

Table 10.3.2.2-3: Effects of fresh foliar residues of A14325E on mortality and fecundity of the lacewing, *Chrysoperla carnea,* exposed under extended laboratory test conditions

Treatment	Rate (mL product/ha)	% pre-imaginal mortality [a]	Corrected % pre-imaginal mortality [b]	Mean number eggs/female/day [c]	Mean percentage egg viability [d]	Effects on reproduction [e] (%)
Control	-	15.0	-	30.44	86.47	-
A14325E	3000	70.0 ***	64.7	n.a.	n.a.	-
	1950	57.5 ***	50.0	n.a.	n.a.	-
	750	22.5	8.8	22.58	85.41	27
	375	12.5	0	25.91	86.58	15
	93.67	23.1	9.5	20.59	90.14	29
	23.33	12.8	0	24.58	83.52	22
Perfekthion	200	100.0 ***	100.0	n.a.	n.a.	-

[a] Pre-imaginal mortality in individual treatments was compared to the control by Fisher's Exact Test. Asterisks indicate where differences were significant (*** $P < 0.001$).
[b] The corrected pre-imaginal mortality was calculated using Abbott's formula.
[c] Based on two 24-h long assessments made for each oviposition box in each treatment.
[d] Based on all eggs laid on the fibrous tissue sheet lining the lid of each oviposition box.
[e] Percentage change in mean number of viable eggs per female, relative to control. A positive value indicates a decrease.
n.a.: not assessed in theis treatment.

The LR_{50} for A14325E was estimated by Probit analysis to be 2393 mL product/ha (nominally 717.9 g ai/ha).

The reproductive performance of insects in the control and all the four A14325E treatment rates evaluated reached the threshold of ≥ 15 eggs/female/day and ≥ 70% egg viability, currently viewed as being indicative of no harmful treatment effects (Vogt *et al*. 2000).

Conclusion

The effects of A14325E on the green lacewing, *Chrysoperla carnea*, were evaluated under extended laboratory test conditions (2-dimensional). In terms of pre-imaginal mortality, the LR_{50} for A14325E was calculated to be 2393 mL product/ha (nominally 717.9 g ai/ha). There were no unacceptable effects on reproduction at treatment rates of up to and including 750 mL A14325E/ha, the highest rate tested for reproduction.

(Spincer D, 2005)

Report:	K-CP 10.3.2.2/04 Spincer D. (2005a) Cyprodinil (CGA219417) 300 g L^{-1} EC formulation (A14325E): An extended laboratory test to evaluate the effects of fresh residues on the ladybird beetle, *Coccinella septempunctata*. Mambo-Tox Ltd, Southampton, UK. Report Number SYN-05-11. (Syngenta file No. CGA219417/1392)

Guidelines

Schmuck R, Candolfi MP *et al.* (2000). A laboratory test system for assessing effects of plant protection products on the plant-dwelling insect *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). In: *Guidelines to evaluate side-effects of plant protection products to non-target arthropods; IOBC, BART and EPPO Joint Initiative*. Eds. Candolfi MP, Blümel S, *et al.*. IOBC Publication. ISBN 92-9067-129-7.

GLP: Yes.

Executive Summary

The aim of this study was to determine the LR_{50} (median lethal rate) of A14325E on larvae of the seven-spotted ladybird, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), under extended laboratory test conditions (2-dimensional). The reproductive potential of the resultant adult beetles was also checked.

A14325E was evaluated at six application rates, equivalent to 3000, 1950, 750, 375, 93.67 and 23.33 mL product/ha. These were compared to a water-treated control and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate), applied at a rate of 200 mL/ha (nominally 80 g ai/ha). Treatments were applied to leaves of the dwarf French bean (*Phaseolus vulgaris* L.) and once residues had dried the leaves were used to line the floor of test arenas (n = 40 per treatment) into which individual 3- to 5-day-old larvae of *C. septempunctata* were then confined. The larvae were fed with pea aphids and any pre-imaginal mortality of the ladybirds was recorded. A check was then made for sub-lethal effects on the reproductive performance of the adults surviving in the control and in the three highest treatment groups of the test item where corrected mortality was < 50%. This was done by individually confining females with males and counting the number of eggs produced over a 14-day period. The viability of these eggs was assessed by counting the number of larvae hatching over a 5-day period.

In terms of pre-imaginal mortality following the exposure of larvae to freshly-dried residues, the median lethal rate (LR_{50}) for A14325E to *Coccinella septempunctata* was greater than the maximum test rate, *i.e.* > 3000 mL product/ha. No detrimental effects on the reproduction of surviving insects were observed at application rates up to and including 3000 mL A14325E/ha.

Materials

Test Material:	A14325E
Description:	liquid, nominally containing 160 g/L SAN619 and 300 g/L cyprodinil
Lot/Batch #:	SMU5BP002
Purity:	303 g/L cyprodinil
Stability of test compound:	The test item is assumed to be stable for the period of use in the study, pending concurrent batch analysis.
Test rates:	3000, 1950, 750, 375, 93.67 and 23.33 mL A14325E/ha
Vehicle and control:	Purified water
Toxic reference:	Perfekthion EC (400 g dimethoate/L) in deionised water (200 mL product/ha)
Spray volume rate:	400 L spray solution/ha
Application method:	Hand-held sprayer powered by compressed air (Azo Ltd., Ede, The Netherlands). Sray boom fitted with 80° flat-fan nozzles (XR Teejet 8002 VS) and spray pressure set at 2.5 bar.
Test organisms	
Species:	*Coccinella septempunctata* L. (Coleoptera: Coccinellidae).

Source: Culture maintained at Test Facility.
Food: Pea aphids (*Acyrthosiphon pisum* Harris)

Test substrate: First true leaves of dwarf French beans (*Phaseolus vulgaris* L., var. The prince).

Environmental test conditions
Temperature: 23-27°C
Humidity: 60-90% relative humidity
Photoperiod: 16 h daily photoperiod (2600-2650 lux)

Study Design and Methods

Experimental dates: 14th July to 14th September 2005.

Excised French bean leaves (40 replicates per treatment) were treated on their upper (adaxial) surface and left for up to 1 h to dry. Arenas were then assembled and 4- to 5-day-old ladybird larvae individually confined on the upper treated surface. The larvae were provided with untreated pea aphids for food and pre-imaginal mortality was assessed. Once sexually mature, the adult females were confined individually with a male beetles and a check was made for sub-lethal effects on their reproductive performance, for the control and the three highest treatment rates of the test item with <50% mortality. For this, the egg-laying activity of all surviving females was monitored for two weeks and the viability of all of the eggs produced was determined.

Results and Discussion

Table 10.3.2.2-4: Effects of fresh foliar residues of A14325E on mortality and reproduction of the ladybird *Coccinella septempunctata,* exposed under extended laboratory test conditions

Treatment	Rate (mL product/ha)	% pre-imaginal mortality [a]	Corrected % pre-imaginal mortality [b]	Mean eggs/♀/day	Mean % viability	Mean viable eggs/♀/day
Control	-	12.5	-	4.2	88.9	3.7
A14325E	3000	34.3 *	24.9	4.8	89.9	4.3
	1950	35.0 *	25.7	5.4	71.6	3.9
	750	32.5	22.9	9.8	88.0	8.6
	375	15.0	2.9	n.a.	n.a.	-
	93.67	17.5	5.7	n.a.	n.a.	-
	23.33	12.5	0.0	n.a.	n.a.	-
Perfekthion	200	100.0 ***	100.0	n.a.	n.a.	-

[a] Pre-imaginal mortality in individual treatments was compared to the control by Fisher's Exact Test. Asterisks indicate where differences were significant (* $P < 0.05$, *** $P < 0.001$).
[b] The corrected pre-imaginal mortality was calculated using Abbott's formula.
n.a.: not assessed for this treatment.

The fecundity in the control and all test item treatment rates exceeded the minimum requirement of 2.0 viable eggs/female/day cited in the test guideline (Schmuck *et al*. 2000).

Conclusion

The effects of A14325E on the ladybird *Coccinella septempunctata* were evaluated under extended laboratory test conditions (2-dimensional). Following exposure of larvae to freshly-dried residues and assessment of pre-imaginal mortality, the median lethal rate (LR_{50}) for A14325E was shown to be greater than the maximum test rate (i.e. > 3000 mL product/ha). No detrimental effects on the reproduction of surviving insects were observed at application rates up to and including 3000 mL A14325E/ha.

(Spincer D, 2005a)

Report:	K-CP 10.3.2.2/05 Fallowfield L. (2017), Cyprodinil (A14325E) – Aged-residue extended laboratory tests to determine effects on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae). Report Number SYN-16-42. Mambo-Tox Ltd. 2 Venture Road, University Science Park, Southampton SO16 7NP, United Kingdom (Syngenta file No. A14325E_10107).

Guideline

IOBC, Blümel *et al*. (2000)

GLP: Yes.

Executive Summary

The effects of both fresh and aged foliar residues of A14325E on the predatory mite *Typhlodromus pyri* were evaluated under extended laboratory test conditions. When sweet pepper plants were treated twice at a rate of 1500 mL product/ha, with a 14-day interval in-between applications, both 0-day-old and 14-day-old residues of A14325E had no unacceptable effects on either the survival or the subsequent reproductive capacity of the mites.

Materials

Test Material	A14325E Cyprodinil EC (300)
Lot/Batch #:	SMO3A100
Actual content of active ingredient:	29.1% w/w (295 g/L)
Description:	Light yellow liquid
Stability of test compound:	Stable under standard conditions.
Recertification date:	31 January 2017
Measured density:	1.014 g/mL

Treatments	
Test rate:	1500 mL product/ha
Control:	Purified water
Toxic standard:	BAS 152 11 I (nominally 400 g dimethoate/L) in purified water, applied at a rate of 60 mL product/ha in 400 L water/ha
Spray volume rate:	400 L spray solution/ha
Application method:	Hand-held hydraulic boom sprayer
Number of applications:	2 (times T1 and T2) for A14325E and the control; 1 for the toxic reference
Spray interval:	14 days

Test organisms	
Species:	*Typhlodromus pyri* Scheuten (Acari: Phytoseiidae)
Age:	Less than 24 h old newly emerged protonymphs
Source:	Culture maintained at Test Facility (originally: Katz Biotech AG, Baruth,

	Germany)
Feeding:	Almond (*Prunus* sp. var. Butte) and apple (*Malus* sp. var. Red Delicious) pollen
Test design	
Arenas:	Treated sweet pepper leaf discs placed on water saturated cotton wool in a 9 cm diameter Petri dish base. The edge of each disc was gently pushed down to ensure contact with the cotton wool. One end of a 5-mm-wide strip of was laid onto the leaf disc and the other end was extended onto the cotton wool, so as to provide the mites with a source of water. A line of sticky gel was drawn around the edge of the leaf disc, as a barrier to prevent the dispersal of the mites. This sticky barrier enclosed an area of approximately 12.5 cm^2. Following the placement of the mites a 5-cm-high clear plastic collar was placed around each arena.
Replication:	5
Number of mites/arena:	20
Duration of test:	14 days for each bioassay
Environmental test conditions	
Temperature:	0 DAT bioassay: 25-26ºC 14 DAT bioassay: 25-26ºC
Humidity:	0 DAT bioassay: 60-78% 14 DAT bioassay: 71-79%
Photoperiod:	0 DAT bioassay: 16 h photoperiod (900-1200 lux). 14 DAT bioassay: 16 h photoperiod (900-1200 lux).

Study Design and Methods

Experimental dates: 8th August to 19th September 2016

Treatments were applied to sweet pepper plants (*var. Bellboy*) using a hand-held sprayer with 2-m-wide boom, at a volume rate equivalent to 400 L spray solution/ha. Both between treatment applications and afterwards, the treated pepper plants were maintained outdoors, with their foliage protected from rainfall by suspending a sheet of polythene permeable to UV light above them.

The initial (0 DAT) bioassay was set up within 1 h of treatments being applied, i.e. once residues on the treated plants had dried. For the 14 DAT bioassay thereafter, leaves were removed from the treated plants, for the preparation of the arenas. Using a fine brush, twenty 1- to 2-day-old mites (protonymphs) were placed at the centre of each arena. Untreated pollen (ca. 1 mg) was sprinkled in the centre of each arena and this was replenished every day. The cotton wool supporting the leaf discs was also rewetted every day.

The condition of the mites was assessed with the aid of a binocular microscope at 1 and 7 days after initiation of the bioassays. To assess any sub-lethal effects, reproduction assessments were then carried out for each bioassay. For this the numbers of male and female mites in each replicate were recorded at 7 DAI to confirm that there was a male to female ratio of at least one male per five females. Any eggs that were produced prior to 7 DAI were discarded. For 7 days, the total egg production (numbers of eggs plus live and dead juvenile stages) was recorded for each unit. Three assessments of oviposition activities were carried out between 7 and 14 DAI. Any eggs and nymphs present were recorded and then removed. In addition, the numbers and condition of the adult female and male mites in each arena was recorded on each date.

The percentage mortality was derived after 7 days. The numbers of any *stuck*, *drowned* or *missing* mites were added to the number of dead mites found in each treatment to derive the overall 'mortality'. The corrected percentage mortality (taking into account any control treatment losses) was derived using

Abbott's formula. Where there was treatment mortality at 7 d, this was compared to the control using Fisher's Exact Test (α = 0.05). For the fecundity assessments, the datasets for both bioassays were first checked for normality using a Shapiro-Wilk test (α = 0.05) and then subjected to t-test for independent samples (α = 0.05.

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table 10.3.2.2-5: Effects of fresh and aged-residues of A14325E on mortality and reproduction of *Typhlodromus pyri*, when exposed under extended laboratory test conditions.

Treatment	Mean % mortality at 7 d [a]	Corrected mean % mortality at 48 h [b]	Mean number of eggs per female	% Effect on reproduction compared to control (R-value) [d]
0 DAT bioassay			-	
Control	12	-	11.9	-
A14325E, 2 x 1500 mL/ha	19	8.0	10.0 *	16.1
Toxic reference	100 *	100	-	-
14 DAT bioassay				
Control	12	-	11.3	
A14325E, 2 x 1500 mL/ha	21	10	9.9 *	12.2

a Mortality at 7 days after test initiation (DAI). For each bioassay, results for the test-item treatment were compared to the control using Fisher's Exact Test (α = 0.05). Treatments marked with an asterisk (*) differed significantly from the control.
b Corrected mortalities were calculated using Abbott's formula.
c Reproduction assessed 7-14 days DAI. ~ indicates where treatments were not evaluated. For each bioassay, the results were compared by t-test for independent samples. Treatments that differed significantly from the control are indicated with an asterisk (*).
d Change in numbers of eggs per female, relative to control (after Blümel *et al.*, 2000). A positive value indicates a decrease.

Validity criteria

The validity criteria for the test were met since:

- Mortality within the control treatment over the initial 7 days was 12% for both (should not exceed 20%.
- Mortality within the toxic-reference treatment in the 0 DAT bioassay was 100 % (should exceed 50%).
- The mean cumulative number of eggs produced in the control treatment between 7 and 14 days was 11.9 for the 0 DAT bioassay and 11.3 for the 1§4 DAT bioassay (should be ≥ 4.0 per female).

Conclusions

The effects of both fresh and aged foliar residues of A14325E on the predatory mite *Typhlodromus pyri* were evaluated under extended laboratory test conditions. When sweet pepper plants were treated twice at a rate of 1500 mL product/ha, with a 14-day interval in-between applications, both 0-day-old and 14-day-old residues of A14325E had no unacceptable effects on either the survival or the subsequent reproductive capacity of the mites.

(Fallowfield, 2017)

Report:	K-CP 10.3.2.2/06 Vaughan R. (2016), Cyprodinil EC (A14325E) – Aged-residue extended laboratory tests to determine effects on the green lacewing *Chrysoperla carnea* (Neuroptera, Chrysopidae).Report Number SYN-16-41. Mambo-Tox Ltd. 2 Venture Road, University Science Park, Southampton SO16 7NP, United Kingdom (Syngenta File No. A14325E_10106)

Guidelines

Vogt *et al*. Laboratory method to test effects of plant protection products on larvae of *Chrysoperla carnea* (2000)

GLP: Yes.

Executive Summary

The effect of two sequential applications of A14325E on the green lacewing, *Chyrsoperla carnea*, was evaluated under extended laboratory test conditions. On each occasion, the test item was applied to French bean plants at a rate of 1.5 L product/ha (nominally 450 g a.s./ha), with a 14-day interval between applications. Neither fresh nor 7-day field-aged residues had harmful effects on either the survival or reproductive capacity of the lacewings.

Materials

Test Material	A14325E Cyprodinil EC (300)
Lot/Batch #:	SMO3A100
Actual content of active ingredient:	29.1% w/w (295 g/L)
Description:	Light yellow liquid
Stability of test compound:	Stable under standard conditions.
Recertification date:	31 January 2017
Measured density:	1.014 g/mL

Treatments	
Test rate:	1500 mL product/ha
Control:	Purified water
Toxic standard:	Perfekthion EC (nominally 400 g dimethoate/L) in purified water, applied at a rate of 200 mL product/ha.
Spray volume rate:	200 L spray solution/ha
Application method:	Laboratory tray sprayer (3 bar pressure, 80° flat-fan nozzle)
Number of applications:	2 (times T1 and T2) for A14325E and the control; 1 for the toxic reference
Spray interval:	14 days

Test organisms	
Species:	*Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae).
Age:	< 24 hours
Source:	Culture maintained at Test Facility (Originally: Biological Crop Protection, Ashford, Kent)
Feeding:	Larvae: UV light-killed eggs of *S. cerealella* every 1 – 3 days Adults: Artificial diet, water and 1:2-1:3 honey/water solution

Test design - Mortality phase

Arenas:	French bean leaves, which were used to line the floor of simple test arenas. Each arena comprised a square glass plate (7.5 cm x 7.5 cm), a Perspex supporting plate of similar size, with a 5-cm-diameter hole cut through it, and an acrylic cylinder (44 mm internal diameter, ca. 2.5 cm tall).
Replication:	40
No. of larvae/arena :	1

Test design – Fecundity phase

Arenas:	Polystyrene boxes (15 cm x 27 cm x 10 cm) with close-fitting lids.
Replication:	1
Duration of test:	14 days

Environmental test conditions

Temperature:	0 DAT bioassay: 24.5-26.3ºC 7 DAT bioassay: 24.3-26.3ºC
Humidity:	0 DAT bioassay: 59-80% RH 7 DAT bioassay: 59-79% RH
Photoperiod:	0 DAT bioassay: 16 h photoperiod (3300-4400 lux) 7 DAT bioassay: 16 h photoperiod (2800-4400 lux)

Study Design and Methods

Experimental dates: 10th August to 5th October 2016

Treatments were applied to potted French bean plants (*Phaseolus vulgaris* L.). Both between and after the two spray applications, prior to foliage being collected for extended laboratory bioassays, the treated plants were maintained outdoors. They were stored under a suspended sheet of polythene, permeable to UV light, to protect them from any rainfall.

An initial bioassay commenced within approximately 60 minutes of the plants being sprayed at time T2, hereafter referred to as 0 days after treatment (DAT). Further bioassays were initiated at 7 and 14 DAT, although the toxic reference treatment was not included in these.

For each bioassay, treated leaves were collected from the plants and used to line the floor of individual test arenas (n = 40 per treatment). Into each was placed a single 2- or 3-day old larva of *C. carnea*. The larvae were fed with untreated eggs of the Angoumois grain moth, *Sitotroga cerealella* (Oliver), and any subsequent pre-imaginal mortality of the lacewings was recorded.

A check was then made for sub-lethal effects on the reproductive performance of the adult lacewings for the test item and the control. For this, the egg-laying activity of grouped females was monitored for two 24-h periods and the subsequent viability of the eggs was determined. These assessments took place between 26 and 35 days after the initiation of each respective bioassay.

The pre-imaginal mortality in each treatment was compared to that in the control by Fisher's Exact Test ($\alpha = 0.05$).

Effects on lacewing reproduction in the individual test item treatments are normally assessed on the basis of "triggers", as specified in the guideline of Vogt et al. (2000). Namely, if treatment effects are to be deemed harmless, there should be a mean of ≥ 15 eggs produced per female per day and the mean egg-hatching rate should be ≥ 70%.

The percentage effect of the individual treatments on lacewing fecundity, relative to the control, was also calculated for each treatment.

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table 10.3.2.2-6: Effects of fresh and aged-residues of A14325E on mortality and reproduction of *Chrysoperla carnea*, when exposed under extended laboratory test conditions

Treatment	Mean % pre-imaginal mortality [a]	Corrected % pre-imaginal mortality [b]	Mean number eggs/female/day	Mean % hatching rate	Mean viable eggs/female/day	% Effect on reproduction compared to control (R-value) [d]
0 DAT bioassay						
Control	15.0	-	31.8	89.7	28.5	-
A14325E, 2 x 1500 mL/ha	27.5	14.7	34.8	88.2	30.7	-7.7
Toxic reference	87.5 *	85.3	-		-	-
7 DAT bioassay						
Control	15.0	-	35.5	89.3	31.7	-
A14325E, 2 x 1500 mL/ha	20.0	5.9	35.0	90.7	31.7	0.0

a For each bioassay, individual treatments were compared by Fisher's Exact Test (α = 0.05). Treatment means marked with an asterisk (*) differed significantly from the control.
b Mortality corrected for any control treatment deaths using Abbott's formula.
c Based on two 24-h assessments made for each oviposition box in each treatment.
d Percentage change in mean number of viable eggs per female, relative to control. A negative indicates an increase.

Validity criteria

The validity criteria were met since:

- The pre-imaginal mortality in the control was 15.0% for both bioassays (must be ≤ 20%)
- Mean egg production in the control was 31.8 and 35.5 eggs per female per day for the 0 and 7 DAT bioassays respectively (must be ≥ 15 eggs)
- Mean viability of the eggs was 89.7 and 89.3% for the 0 and 7 DAT bioassays respectively (must be ≥ 70 %.)
- Mortality in the toxic reference treatment was 87.5 % (must be ≥ 50%)

Conclusions

The effect of two sequential applications of A14325E on the green lacewing, *Chyrsoperla carnea*, was evaluated under extended laboratory test conditions. On each occasion, the test item was applied to French bean plants at a rate of 1.5 L product/ha (nominally 450 g a.s./ha), with a 14-day interval between applications. Neither fresh nor 7-day field-aged residues had harmful effects on either the survival or reproductive capacity of the lacewings.

(Vaughan, 2016)

CP 10.3.2.3 Semi-field studies with non-target arthropods

As risk assessments based on endpoints from laboratory tests indicate that A14325E poses an acceptable risk to non-target arthropods, semi-field tests were not considered necessary.

CP 10.3.2.4 Field studies with non-target arthropods

As risk assessments based on endpoints from laboratory tests indicate that A14325E poses an acceptable risk to non-target arthropods, semi-field tests were not considered necessary.

CP 10.3.2.5 Other routes of exposure for non-target arthropods

No other routes of exposure are considered relevant for non-target arthropods after use of A14325E as recommended.

Relevant Literature on non-target arthropods other than bees

No relevant scientifically peer-reviewed open literature could be found on A14325E. Details of the literature search undertaken can be found in **M-CA Section 9**.

CP 10.4 Effects on Non-Target Soil Meso- and Macrofauna

Toxicity

The endpoints relevant for the risk assessment are given below.

Table 10.4-1: Table of endpoints for earthworms

Organism	Test item	Test type	Endpoint		Endpoint used for the risk assessment	Reference (author, date, Syngenta File No.)
Earthworm	A14325E	Chronic and reproductive	New	NOEC = 41.27 mg A14325E/kg	NOEC = 41.27 mg A14325E/kg	***Schmidt 2005 CGA219417/1320***
			EC_{10}/EC_{20} [a]	Not possible to estimate due to lack of a significant concentration response		***EC_{10}/EC_{20} estimate Taylor & Allen (2016) A14325E_10095***
	Cyprodinil (A8779A)		EU	NOEC = 3.75 kg a.s./ha (≅ 5 mg a.s./kg)	20 mg a.s./kg [b]	***Nienstedt (2001) CGA219417/1029***
			EC_{10}/EC_{20} [a]	Not possible to estimate due to lack of a significant concentration response		***EC_{10}/EC_{20} estimate Taylor & Joyce (2015) CGA8779A_10235***
	Cyprodinil (A8779A)		EU	NOEC = 15 kg a.s./ha (≅ 20 mg a.s./kg)		***Ehlers (2001) CGA219417/1028***
			EC_{10}/EC_{20} [a]	Not possible to estimate due to lack of a significant concentration response		***EC_{10}/EC_{20} estimate Taylor & Pickering (2015) CGA8779A_10237***
	CGA249287		EU	NOEC = 1.13 mg/kg soil d.w.	NOEC = 1.13 mg/kg	***Pfeifle (2001) CGA249287/0020***
			EC_{10}/EC_{20} [a]	Not possible to estimate due to lack of a significant concentration response		***Taylor & Pickering (2015) CGA249287_10008***
	CGA275535		New	NOEC (reproduction) = 556 mg/kg soil d.w.; EC_{10} = 385 mg/kg; EC_{20} = 638 mg/kg	NOEC = 556 mg/kg	***Lührs (2014) CGA275535_10002***
	CGA321915			NOEC/EC_{10}/EC_{20} (reproduction) = 1000 mg/kg soil d.w.	NOEC/EC_{10} = 1000 mg/kg	***Lührs (2015) CGA321915_10012***

[a] Values estimated in accordance with **Commission Regulation (EU) No 283/2013**
[b] For the tests conducted by Neinstedt and Ehlers the NOEC values represent the highest concentrations tested. Therefore the endpoint of 20 mg a.s./kg derived by Ehlers 2001 will be used for the risk assessment

Comment from the RMS: *Effects on reproduction of A14325E on Eisenia fetida (K-CP 10.4.1.1/0 1; Schmidt, 2005): given the high variability of the effects on reproduction, please calculate an EC10 with confidence intervals to check the robustness of the value. This value may be more relevant for the chronic risk assessment of A14325E to earthworms.*

Response from Syngenta: An attempt was made to re-analyse the data. It was not possible to determine EC_{10} or EC_{20} values. A summary of the statistical analysis is presented in Section 10.4.1.1.

Table 10.4-2: Table of endpoints for non-target soil meso- and macro-fauna

Organism	Test item	Test type	Endpoint		Endpoint used for the risk assessment	Reference (author, date, Syngenta File No.)
Folsomia candida	Cyprodinil [a]	28 day chronic	New	NOEC = 29.4 mg A14325E/kg (8.67 mg a.s./kg); EC_{10} = 53.2 mg A14325E//kg (15.7 mg a.s./kg); EC_{20} = 67.7 mg A14325E (20 mg a.s./kg)[c]	NOEC = 29.4 mg a.s./kg	***Lührs (2014) A14325E_10061***
	Cyprodinil [b]			NOEC/EC_{10}/EC_{20} = 105 mg A8637C/kg (52.5 mg a.s./kg) [d, e]		***Lührs (2014) A8637C_10314***
	CGA249287			NOEC = 31 mg/kg soil; EC_{10} = 7.9 mg/kg; EC_{20} = 22.7 mg/kg	NOEC = 31 mg/kg	***Vinall (2012) CGA249287/10003***
	CGA275535			NOEC = 171.5 mg/kg soil [f]	NOEC = 171.5 mg/kg	***Lührs (2014) CGA275535_10004***
	CGA321915			NOEC/EC_{10}/EC_{20} = 1000 mg/kg soil d.w. [e]	NOEC/EC_{10} = 1000 mg/kg	***Lührs (2015) CGA321915_10010***
Hypoaspis aculeifer	Cyprodinil [a]	14 day chronic		NOEC/EC_{10}/EC_{20} = 1000 mg A14325E/kg soil (295 mg a.s./kg) [c, e]	NOEC/EC_{10} = 277.8 mg/kg	***Lührs (2014) A14325E_10062***
	Cyprodinil [b]			NOEC/EC_{10}/EC_{20} = 555.6 mg A8637C/kg (277.8 mg a.s./kg) [d, f]		***Lührs (2014) A8637C_10312***
	CGA249287			NOEC = 74 mg/kg soil; EC_{10} = 70.5 mg/kg; EC_{20} = 321.3 mg/kg	NOEC = 74 mg/kg	***Schultz (2014) CGA249287_10005***
	CGA275535			NOEC = 171.5; EC_{10} = 104.6 mg/kg; EC_{20} = 272.5 mg/kg	NOEC = 171.5 mg/kg	***Lührs (2014) CGA275535_10000***
	CGA321915			NOEC/EC_{10}/EC_{20} = 1000 mg/kg soil	NOEC/EC_{10} = 1000 mg/kg	***Lührs (2015) CGA321915_10011***

[a] Tested as A14325E
[b] Tested as A8637C
[c] Concentrations converted to active substance content based on nominal formulation composition of 295 g cyprodinil/L
[d] Concentrations converted to active substance content based on nominal formulation composition of 500 g cyprodinil/kg
[e] It was not possible to estimate EC_{10} or EC_{20} values as the NOEC was derived for the highest concentration tested
[f] It was not possible to estimate EC_{10} or EC_{20} values as a significant concentration response could not be derived

The exposure to soil organisms was estimated by calculating the maximum instantaneous predicted environmental concentrations in soil (PEC_S) (see **M-CP, Section 9**). For multiple applications, the worst-case maximum PEC_S will be immediately after the final application.

Since A14325E is rapidly broken down into its constituent parts on contact with soil and/or crop material, it was appropriate to calculate the PEC_S for A14325E following a single application only.

The PEC_S was calculated as described in the **M-CP Section 9**. The resulting PEC_S values are presented below.

Table 10.4-3: Maximum peak PEC_S values for A14325E, cyprodinil and soil metabolites following application of A14325E at 1500 mL product/ha to cereals

Formulation/ compound	$PEC_{S, initial}$ [mg/kg]	$PEC_{S, plateau}$ [mg/kg]	$PEC_{S, peak\ accum}$ [mg/kg]
A14325E	**0.441**	-	-
Cyprodinil	0.236	0.043	**0.279**
CGA249287	0.006	0.005	**0.011**
CGA275535	**0.054**	-	-
CGA321915	0.009	0.008	**0.017**

Numbers in bold are used for the risk assessment

CP 10.4.1 Earthworms

Risk assessment for earthworms

An acute risk assessment is no longer required in accordance with the guidance in **Annexes to Regulation 284/2013**.

The potential long-term risk of cyprodinil and relevant soil metabolites was assessed by calculating long-term TER (TER_{LT}) values by comparing the NOEC or the adjusted NOEC, if appropriate, and the PEC_S using the following equation:

$$TER_{LT} = \frac{NOEC\,(mg/kg)}{PEC_S\ (mg/kg)}$$

For substances with log P_{OW} values greater than 2, there was a need to reduce the NOEC by a factor of 2 in order to account for the relatively high organic matter content of the artificial test soil (10%) compared to agricultural soils in accordance with the EPPO guidelines (**EPPO, 2002**). Since the log P_{OW} values of the cyprodinil metabolites CGA249287 and CGA321915 are less than 2 (1.5 and -0.10 respectively) there was no need to reduce the NOEC by a factor of 2. The log P_{OW} values of cyprodinil and its metabolite CGA275535 are greater than 2 (4.0 and 3.3 respectively), therefore the NOECs have been reduced by a factor of 2.

The resulting TER_{LT} values are presented below:

Table 10.4.1-1: Long-term TER values for earthworms

Formulation/ compound	Endpoint [mg/kg]	$NOEC_{adjusted}$ [mg/kg]	Maximum PEC_S [mg/kg	TER_{LT}	Trigger
A14325E	NOEC = 41.27	20.6	0.441	47	5
Cyprodinil [a]	NOEC = 20	10	0.279	36	
CGA249287	NOEC = 1.13	-	0.011	100	
CGA275535	NOEC = 556	278	0.054	5 100	
CGA321915	NOEC = 1000	-	0.017	59 000	

[a] Tested as A8779A (a 750 mg/kg WG formulation)

The long-term TER values for the tested metabolites and cyprodinil all exceed the Commission Regulation (EU) No. 546/2011 long-term trigger value of 5, indicating that the long-term risk to earthworms is acceptable following use of A14325E according to the proposed use pattern.

CP 10.4.1.1 Earthworms – sub-lethal effects

Report:	K-CP 10.4.1.1/01 Schmidt T. (2005) Cyprodinil (CGA219417) EC 300 (A14325E): Effects of a 300 g/L EC formulation on survival, growth, and reproduction of the earthworm *Eisenia fetida.* RCC Ltd, Itingen, Switzerland. Report No. 859246. (Syngenta File No. CGA219417/1320)

Guidelines

International Standards ISO 11268-2: 1998(E)

BBA VI, 2-2 (1994)

OECD Guideline No. 222 (2004)

GLP: Yes

Executive Summary

In a 56 day subchronic toxicity study, earthworms of the species *Eisenia fetida* were exposed to the test item A14325E in artificial soil. The test item was mixed into the soil at concentrations of 10.32, 17.18, 20.63, 30.95 and 41.27 mg A14325E/kg dry soil. The control substrate was prepared analogously to the test substrates but without test item. The reference item Derosal® (4.2 mg/kg dry soil) was tested in parallel.

Mortality and body weight change of the adult worms were assessed after an exposure of 28 days. The cocoons and juvenile earthworms remained in the vessels for additional 28 days. The reproduction rate was determined by counting the number of offspring hatched from the cocoons after this additional test period.

The highest concentration without toxic effects (NOEC) of A14325E on *Eisenia fetida* after the test period was 41.27 mg/kg dry soil (corresponding to 12.23 mg cyprodinil/kg dry soil), the highest concentration tested.

Materials

Test Material:	A14325E
Description:	Liquid yellow emulsifiable concentrate
Lot/Batch #:	SMU5BP002
Actual content of ai (measured):	Cyprodinil (CGA219417): 303 g/L
Test concentrations:	10.32, 17.18, 20.63, 30.95 and 41.27 mg A14325E/kg dry soil (corresponding to 3.06, 5.09, 6.12, 9.17 and 12.23 mg cyprodinil/kg dry soil and to 10.20, 16.98, 20.39, 30.58 and 40.78 µL A14325E/kg dry soil)
Vehicle:	Tap water
Test organism	
Species:	*Eisenia fetida*
Source:	In-house culture
Food:	Air-dried and finely ground horse manure during test
Environmental conditions	
Temperature:	18 - 21 °C

Photoperiod:	16-hour daily photoperiod (light intensity within 570-640 Lux)
Artificial soil:	10 % Sphagnum peat (shredded, sieved through 2°mm)
	20% Kaolinite clay (content of Al_2O_3: 36.4%)
	70% Sand (Sihelco 36)
	0.5% Calcium carbonate ($CaCO_3$)
Soil moisture content:	35%
Length of test:	56 days (exposure of 28 days for adults, exposure of 28 days for juveniles)

Study Design and Methods

In life dates: 5th April to 3rd June, 2005.

Adult earthworms (*Eisenia fetida*), approximately four months old with a clitellum, were exposed to A14325E, mixed into artificial soil of the test units. The test item was dissolved in tap water and mixed into the artificial soil. The artificial soil of the control test containers were treated analogously to the test substrates but without test item. Test containers treated with the reference item Derosal® ran in parallel (4.2 mg Derosal®/kg dry soil, corresponding to 2.5 mg Carbendazim/dry soil). 600 g dry weight (i.e. approx. 750 g wet weight) per vessel was used. The worms were introduced into the test containers immediately after mixing the artificial soil with the test item. Four replicates with 10 worms per replicate were used for the test item concentrations and the reference item treatment, eight replicates for the control. The worms were acclimatized to the test conditions two days before test start.

Air-dried and finely ground horse manure was used as food source during the study. The adult earthworms were fed once per week with approximately 4-6 g food per vessel. The offspring was fed only once at the start of the second four weeks.

After four weeks of exposure of the adults, the content of each test vessel was emptied and the living adult worms were counted and checked for any abnormal behaviour or other adverse effects. At the beginning (prior to exposure) and at the end of the first four weeks of the test, the adult test organisms of each test vessel were weighed (at the start each individually, at the end all together from each test vessel). The adults were removed from the test vessels and the cocoons and juvenile earthworms remained in the treated soils for additional four weeks.

At the test termination after 56 days, the number of live juveniles per test vessel was determined. The test vessels were warmed up in a water bath at 60 °C for approximately 15-20 minutes to encourage the juvenile earthworms to rise to the soil surface. The juvenile earthworms at the soil surface were counted and removed. The test vessels were warmed up for a second time for about 10 minutes and the surface was searched for juveniles. Afterwards, each test vessel was emptied and intensely searched for additional juveniles that may have remained in the soil.

Results and Discussion

Table 10.4.1.1-1: Chronic effects on *Eisenia fetida* exposed in artificial soil – adult mortality and weight change

Nominal concentration of A14325E (mg/kg dry soil)	Replicates, N =	Mean % adult mortality after 28 days	Bodyweight per worm at test start (mg)		Decrease of bodyweight per worm at test end (mg) [a]		Mean % decrease in body weight
			Mean	SD	Mean	SD	
Control	8	0	426	25	-13	15	-3
10.32	4	0	427	14	3	11	1
17.18	4	0	426	17	-9	5	-2
20.63	4	0	430	22	-3	8	-1
30.95	4	0	424	7	8	10	2
41.27	4		426	21	3	27	1
Toxic reference: Derosal 4.2 mg/kg	4	0	415	14	1	4	0

[a] Tested for statistically significant differences in mean body fresh weight loss of the treatments versus the control at test termination: results of a Dunnett-test, one-sided (smaller), α = 0.05; except reference item with 2-sided t-test, α = 0.05. No significant differences found.

Table 10.4.1.1-2: Chronic effects on *Eisenia fetida* exposed in artificial soil – reproduction

Nominal test item concentration (mg/kg)	Juvenile worms per test vessel		Reproduction rate (per surviving adult)				Statistical evaluation [a]
	Mean	SD	Mean	SD	CV (%)	% of control	
control	61.5	10.4	6.2	1.0	16.9	---	—
10.32	52.8	21.2	5.3	2.1	40.3	85.8	Not significant
17.18	53.8	9.4	5.4	0.9	17.5	87.4	Not significant
20.63	36.5	5.9	3.7	0.6	16.2	59.3	Significant [b]
30.95	46.8	19.6	4.7	2.0	41.9	76.0	Not significant
41.27	43.0	13.5	4.3	1.3	31.4	69.9	Not significant
Reference item: Derosal 4.2 mg/kg	14.0	5.4	1.4	0.5	38.2	22.8	Significant [c]

[a] Statistical comparison of the mean reproduction rate (per surviving adult), results of a Dunnett-test, one-sided smaller, α = 0.05.
[b] This statistical significance is not considered as a treatment-related effect since the reproduction rates of the two higher test concentrations were not statistically significantly different from the control.
[c] t-test, two-sided, α = 0.05.

In the control, an average of 62 juvenile worms per test vessel were found and the coefficient of variance of the reproduction rate in the control was 17%. Thus, the validity criteria of the test guidelines (at least 30 juveniles per test vessel, coefficient of variance of reproduction ≤30%) were met.

Conclusion

The NOEC for chronic effects of A14325E on *Eisenia fetida* over 56 days was 41.27 mg/kg dry soil (corresponding to 12.23 mg cyprodinil/kg dry soil), the highest concentration tested.

(Schmidt T, 2005)

An attempt was made to estimate EC_{10} and EC_{20} values for mortality, reproduction and biomass. A summary is presented below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CP 10.4.1.1/02 Taylor S., & Allen, M. (2016) Cyprodinil (CGA219417) EC 300 (A14325E) – Effects of a 300 g/L EC Formulation on Survival, Growth and Reproduction of the Earthworm *Eisenia fetida* - Statistical Re-analysis. Report Number CEA.1713. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: A14325E_10095)

Summary

The report from RCC Ltd, study number 859246 (Schmidt, 2005), for the growth and reproductive toxicity test of cyprodinil with the earthworm (*Eisenia fetida*), did not provide estimates of the EC_{10} or EC_{20} for the response variables of biomass change, mortality or reproduction. Consequently the data generated in this study were intended to be re-analysed in an attempt to provide these values.

Statistical analyses revealed that no ECx values could reliably be determined for any of the re-analysed parameters.

Statistical Analysis

No mortality was observed after 28 days of exposure for any test concentration. In addition, there were no statistically significant differences in the feeding activity or biomass change between each of the treatment concentrations and the control in the original report. As a result, these parameters were not statistically analysed and no ECx values could reliably be determined.

For reproduction, Probit analysis with linear maximum likelihood regression was used in an attempt to determine the concentration response function for reproduction. Chi^2 was used as a goodness of fit measure. The proportion of variance explained by the dose/ response function was determined and is presented as the coefficient of determination, r2 ($0 \le r2 \le 1$).

All computations were carried out in the Statistical program: ToxRat Professional 2.10.05 (ToxRat Solutions GmbH, 2001-2010).

Results

For the reproduction, there was no significant dose response ($p(F) = 0.490$) and therefore EC_{10} and EC_{20} values could not be reliably determined.

Conclusion

No EC_{10} or EC_{20} values for reproduction could be reliably calculated due to a lack of significant dose response.

(Taylor S. & Allen M. 2016)

CP 10.4.1.2 Earthworms – field studies

Not required as the risk assessment conducted using laboratory data indicates acceptable risk for earthworms following application of A14325E according to the proposed use pattern.

Relevant Literature on Earthworms

No relevant scientifically peer-reviewed open literature could be found on A14325E. Details of the literature search undertaken can be found in **M-CA Section 9**.

CP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

Risk assessment for other non-target soil meso- and macrofauna (other than earthworms)

The potential long-term risk of cyprodinil and relevant soil metabolites to other non-target soil meso- and macro-fauna was assessed by calculating long-term TER (TER_{LT}) values by comparing the NOEC values and the maximum instantaneous PEC_S using the following equation:

$$TER_{LT} = \frac{\text{NOEC (mg/kg)}}{PEC_S \text{ (mg/kg)}}$$

For substances with log P_{OW} values greater than 2, there was a need to reduce the NOEC by a factor of 2 in order to account for the relatively high organic matter content of the artificial test soil (10%) compared to agricultural soils in accordance with the EPPO guidelines (**EPPO, 2002**). Since the log P_{OW} values of the cyprodinil metabolites CGA249287 and CGA321915 are less than 2 (1.5 and -0.10 respectively) there was no need to reduce the NOEC by a factor of 2. The log P_{OW} values of cyprodinil and its metabolite CGA275535 are greater than 2 (4.0 and 3.3 respectively), however, all tests were conducted in artificial soil containing 5% peat so therefore there was no need to reduce the endpoint.

The resulting TER_{LT} values are presented below:

Table 10.4.2-1: Long-term TER values for other soil meso- and macro-fauna

Organism	**Test substance**	**Endpoint (mg/kg soil)**	**PEC_S (mg a.s./kg soil)**	**TER_{LT}**	**Trigger value**
Folsomia candida	A14325E	NOEC = 53.2	0.441	120	5
	Cyprodinil [a]	NOEC = 29.4	0.279	110	
	CGA249287	NOEC = 31	0.011	2 800	
	CGA275535	NOEC =171.5	0.054	3 200	
	CGA321915	NOEC = 1000	0.017	59 000	
Hypoaspis acuelifer	A14325E	NOEC = 1000	0.441	2 300	
	Cyprodinil	NOEC = 277.8	0.279	1 000	
	CGA249287	NOEC = 74	0.011	6 700	
	CGA275535	NOEC = 171.5	0.054	3 200	
	CGA321915	NOEC = 1000	0.017	59 000	

[a] Endpoint derived for a test conducted with A14325E

The long-term TER values all exceed the Commission Regulation (EU) No. 546/2011 long-term trigger value of 5, indicating that the long-term risk to *Folsomia candida* and *Hypoaspis aculeifer* is acceptable following use of A14325E according to the proposed use pattern.

CP 10.4.2.1 Species level testing

New studies have been carried out for A14325E with *Folsomia candida* and *Hypoaspis aculeifer* to fulfil current data requirements for in Regulation 283/2013 and 284/2013. Endpoints from these studies are considered to cover effects for the active substance. The endpoints are summarised in Table 10.4-1 above. Summaries of these studies are presented in M-CA Section 8.

CP 10.4.2.2 Higher tier testing

Higher tier tests were not conducted as the risk assessment above indicates acceptable risk to soil macro- and meso-organisms other than earthworms.

Relevant literature on non-target soil meso- and macrofauna (other than earthworms)

No relevant scientifically peer-reviewed open literature could be found on A14325E. Details of the literature search undertaken can be found in **M-CA Section 9**.

CP 10.5 Effects on Soil Nitrogen Transformation

The toxicity of A14325E, cyprodinil and metabolites to soil microbial activity in terms of nitrogen transformation is summarised below.

Table 10.5-1: Table of endpoints to assess risk from use of A14325E

Test type	Test item	Endpoint (mg/kg)		Reference (author, date, Syngenta File No.)
N-transformation	A14325E	New	NOEC = 20.33	***Hammesfahr (2015) A14325E_10057***
	Cyprodinil	EU	NOEC = 26.7	***Wütrich (1993) CGA219417/0209***
	CGA249287		NOEC = 3.33	***Grade (2000) CGA249287/010***
	CGA275535		NOEC = 1.15	***Seyfried (2001) CGA275535/020***
	CGA321915	New	NOEC = 5.10	***Hammesfahr (2015) CGA321915_10008***

Exposure

The exposure to soil organisms was estimated by calculating the maximum instantaneous predicted environmental concentrations in soil (PEC_S) as presented under CP 10.4, above (see **M-CP, Section 9**). The PEC_s are repeated below for convenience.

Table 10.5-2: Maximum peak PEC_S values for A14325E, cyprodinil and soil metabolites following application of A14325E at 1500 mL product/ha to cereals

Formulation/ compound	$PEC_{S,\ initial}$ [mg/kg]	$PEC_{S,\ plateau}$ [mg/kg]	$PEC_{S,\ peak\ accum}$ [mg/kg]
A14325E	**0.441**	-	-
Cyprodinil	0.236	0.043	**0.279**
CGA249287	0.006	0.005	**0.011**
CGA275535	**0.054**	-	-
CGA321915	0.009	0.008	**0.017**

Numbers in bold are used for the risk assessment

Risk assessment for Soil Nitrogen Transformation

As a worst case approach the peak accumulation PECs have been compared with the NOECs derived for nitrogen transformation by soil micro-organisms. This comparison, presented as 'Ratio of NOEC:PECs' is presented in the table below.

Table 10.5-3: Risk assessment for effects on soil micro-organisms

Test substance	NOEC (mg/kg)	PEC_S (mg a.s./kg)	Ratio of NOEC:PEC_S
A14325E	20.33	0.441	46
Cyprodinil	26.7	0.279	96
CGA249287	3.33	0.011	300
CGA275535	1.15	0.054	21
CGA321915	5.10	0.017	300

[1] Initial PECs
[2] Peak accumulation PEC_S

A14325E had no significant effect on soil micro-organisms at 20.33 mg A14325E/kg. This is approximately 46 times higher than the maximum PEC_S of 0.441 mg A14325E/kg following the worst-case application. This indicates that the risk to non-target soil micro-organisms is acceptable following use of A14325E according to the proposed use pattern.

Furthermore, the NOEC for cyprodinil and all metabolites range from 21 to 300 times higher than the maximum soil concentrations.

Laboratory testing

A summary of a study conducted with the representative formulation is presented below.

Report:	K-CP 10.5/01 Hammesfahr U. (2014) Cyprodinil EC (A14325E) - Effects on Activity of Soil Microflora (Carbon and Nitrogen) in the Laboratory, Report Number 92781080, Institut für Biologische Analytik und Consulting, IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany (Syngenta file No. A14325E_10057).

Guidelines

OECD guidelines 216, Soil Microorganisms: Nitrogen Transformation Test (2000)

OECD guidelines 217, Soil Microorganisms: Carbon Transformation Test (2000)

GLP: Yes

Executive Summary

A14325E was applied to the soil at concentrations of 4.07 mg /kg dry soil and 20.33 mg /kg dry soil. The test item caused no adverse effects on soil nitrogen transformation (measured as NO_3-N-production) and on soil carbon transformation (measured as O_2-consumption) at the end of the 28-day incubation period.

Materials

Test Material	A14325E Cyprodinil EC
Lot/Batch #:	SMO3A100
Actual content of active ingredients:	29.1 % w/w, corresponding to 295 g/L
Description:	Light yellow liquid
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	End of January 2017
Density:	1014 kg/m3
Treatments	
Test rates:	4.07 and 20.33 mg /kg dry soil
Control:	Deionised water
Toxic standard:	Sodium chloride (Potassium nitrate, Sodium nitrite and Ammonium sulfate used as reference items in continuous flow analysis).
Test design	
Soil type:	Loamy sand
Test units:	Disposable plastic boxes; each box contained different amounts of soil for the two tests: Carbon transformation test: 750 g to 1000 g soil d.w box size 1 L, filled up to 6 cm nitrogen transformation test: 250 g to 500 g soil d.w, box size approximately 0.5 L, filled up to 6 cm
Replication:	3
Sampling intervals :	
Duration of test:	28 days
Environmental test conditions	
Temperature:	20°C ± 2°C
pH of soil:	6.8 – 7.0
Soil moisture content:	48% to 52% WHC
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 12th September to 14th October 2014

Soil samples were treated with the A14325E at two doses, 4.07 and 20.33 mg /kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

The test item was mixed with deionised water and the test solution was subsequently mixed with the soil in the laboratory mixer. Water was added to the soil to achieve a water content of approximately 45 % of WHC. The water content of the soil in each test vessel was determined at test start (after application) and adjusted once a week to the required range of 40 - 50 % of WHC.

Three replicate soil samples were prepared for each treatment rate and the control for the nitrogen transformation test and carbon transformation test.

Mean nitrogen content (mg NO_3/kg soil d.w.), standard deviation and coefficient of variation as well as the mean nitrogen content/day (mg NO_3/kg soil d.w./day) were calculated for each treatment group and sampling date.

For the evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated (based on the mean nitrogen content/day) for each sampling date.

The amount of oxygen consumed by soil microorganisms was calculated based on the pressure decrease in the reaction vessel. The oxygen consumption was calculated by regression analysis of the linear part of the respiration curve over 12 hours.

Data for short-term respiration and soil nitrogen contents were tested for normality and homogeneity of variance using the R/S-Test (a = 0.05) and Levene´s test (a = 0.05), respectively. The Student t-test (pair wise comparison, two-sided, a = 0.05) was used for comparison of treated and control values.

The software used to conduct the statistical analysis was ToxRat Professional, Version 2.10.05.

Results and Discussion

Results from the nitrogen transformation test and the carbon transformation test are summarised in the tables below.

Table 10.5-4: Effects on nitrogen transformation in soil after treatment with A14325E

Time Interval (days)	Control	4.07 mg test item/kg soil dry weight		20.33 mg test item/kg soil dry weight	
	NO_3-N [mg/kg soil d.w./day]	NO_3-N [mg/kg soil d.w./day]	Deviation from control [%][1)]	NO_3-N [mg/kg soil d.w./day]	Deviation from control [%]
0 - 7	-1.26	-1.27	-0.79	-0.79	37.30
0 - 14	2.53	2.58	-1.98	2.59	-2.37
0 - 28	1.87	1.88	-0.53	1.88	-0.53

The calculations were performed with non-rounded values
Negative values indicate an increase relative to the control
No statistically significant differences between the control and the test item treatments were calculated

Table 10.5-5: Effects on carbon transformation in soil after treatment with A14325E

Days after application	Control		4.07 mg test item/kg soil dry weight		20.33 mg test item/kg soil dry weight	
	O_2-consumption [mg/kg soil d.w./h]	CV [%]	O_2-consumption [mg/kg soil d.w./h]	Deviation from control [%][1)]	O_2-consumption [mg/kg soil d.w./h]	Deviation from control [%
0	11.171	0.95	12.548	-12.33	12.593	-12.73
7	8.967	8.84	9.612	-7.19	9.873	-10.10
14	10.698	2.00	11.133	-4.07	11.293	-5.56
28	9.614	3.23	9.622	-0.08	9.044	5.93

Based on O_2-consumption; - = inhibition; + = stimulation
Negative values indicate an increase relative to the control
* Statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, p ≤ 0.05)

Validity criteria

The validity criteria are listed below:

- The coefficient of variation in the carbon transformation tests was 0.95 – 3.23% (must be ≤ 15 %).
- The reference item must have a retarding or stimulating effect of more than ± 25% compared to the control at day 28 after application.

Conclusions

The test item had no impact on carbon transformation and nitrogen transformation (nitrate content, mineral nitrogen content and nitrate formation rate) of soil microorganisms when applied at 4.07 mg and 20.33 mg test item/kg soil dry weight treatment.

(Hammesfahr U. 2014)

CP 10.6 Effects on Terrestrial Non-Target Higher Plants

Toxicity

The effect of A14325E on seedling emergence and vegetative vigour in 6 plant species was evaluated in a glasshouse study (***Büche, 2005***). Pre- and post-emergence applications of A14325E at rates up to and including 450 g cyprodinil /ha did not have an adverse effect on seedling emergence or subsequent shoot growth. Further details of the study are provided under CP 10.6.1 below.

Exposure

Effects on non-target plants are of concern in the off-crop environment, where they may be exposed to spray drift. The amount of spray drift reaching off-crop habitats is calculated using the 90th percentile estimates derived by the **BBA (2000)**[13] from the spray-drift predictions of ***Ganzelmeier & Rautmann***

[13] 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.

(2000)[14]. Only a single application is considered as factors such as plant growth will reduce residues per unit area between multiple applications. For a single application of A14325E, as a worst case 2.77% of the in-field application rate is assumed to reach areas at a minimum distance of 1 m from the edge of the orchard.

The single application rate of A14325E is 450 g a.s./ha, giving a maximum off-crop predicted environmental rate ($PER_{off\text{-}crop}$) of 12.5 g cyprodinil/ha.

Risk assessment for Terrestrial Non-Target Higher Plants

A14325E is a fungicide and is therefore not expected to have any significant herbicidal activity.

The potential risk of cyprodinil, formulated as A14325E, to non-target plants is evaluated by comparing toxicity with the maximum predicted residue concentration. The off-field PER of 12.5 g a.s./ha is below 400 g a.s./ha, i.e. the rate which showed no ecologically relevant effects on six plant species. It is therefore considered that the proposed use of A14325E is highly unlikely to affect non-target higher plants in the off-field environment.

Conclusion

When applied in accordance with the uses supported in this submission A14325E does not pose an unacceptable risk to non-target plants.

CP 10.6.1 Summary of screening data

Report:	K-CP 10.6.1/01 Büche C. (2005) Herbicide Profiling Test to evaluate the phytotoxicity of Cyprodinil (CGA219417) EC 300 (A14325E) to terrestrial non-target higher plants. RCC, Itingen, CH. Report Number 859247. (Syngenta File No. CGA219417/1324)

Guidelines

None (screening test)

GLP: No.

Executive summary

The effects of A14325E on emergence (pre-emergence treatment) and vegetative vigour (post-emergence treatment) of non-target terrestrial plants was tested under glasshouse conditions at rates of up to 450 g a.s./ha. Test species were *Allium cepa, Avena fatua, Glycine max, Beta vulgaris, Brassica napus* and *Cucumis sativus*. Evaluation of phytotoxicity was by visual observation and rating using a descriptive scale. There were no effects observed on emergence and growth, or vegetative vigour, in any test species.

It is therefore concluded that A14325E had no unacceptable effects on the test species at rates 450 g a.s./ha.

[14] Ganzelmeier H., Rautmann D. (2000) Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application. Public domain.

Materials

Test Material:	A14325E
Description:	Light yellow to brown liquid
Lot/Batch #:	SMU5BP002
Purity:	Cyprodinil nominal: 300 g/L.
Stability of test compound:	Not stated
Test rates:	14.07, 28.13, 56.25, 112.5, 225, 450 g cyprodinil/ha
Vehicle and control:	Purified water
Toxic reference:	None
Application volume:	500 L/ha
Test organisms	
Species:	*Allium cepa, Avena fatua, Glycine max, Beta vulgaris, Brassica napus*, *Cucumis sativus*
Source:	Sativa Rheinau GmbH, Reinau, Switzerland; and Landi Oberbaselbeit AG, Gelterkinden, Switzerland
Food:	Fertilizer: Universol® Orange
Environmental test conditions	
Temperature:	Monitored glasshouse conditions; limits not stated
Photoperiod:	>14 hours light / <10 hours dark dailly.
Irrigation:	Bottom irrigation every 1-3 days, as required.
Soil:	Clay loam of local origin, with a layer of LUFA Speyer 2.3 sieved soil on top.

Study Design and Methods

In life dates: 30th March to 7th June 2005.

The effects of A14325E on emergence and vegetative vigour was assessed on 6 species of higher terrestrial plant. For the emergence test, the test item was sprayed onto the soil over the seeds (pre-emergence). For the vegetative vigour test, the test item was applied onto the plant surface, post-emergence. Application was by laboratory track sprayer with a flat jet Teejet 8003EVS nozzle at 2.3 bar pressure. Two replicates per treatment were used, with four to approximately twenty seeds each, depending on the seed size of the species. The seedling emergence test was assessed over 28 days. The vegetative vigour test was assessed over 21 days after treatment. Visible phytotoxic effects were used as the endpoint, using a defined rating scale from 0 (no effects) to 10 (100% inhibition or mortality).

Results and Discussion

Table 10.6.1-1: A14325E - effects [a] on seedling emergence of 6 species of higher terrestrial plants

Application rate (g/ha a.s.):	450	225	112.5	56.25	28.13	14.07
Brassica napus	0	0	0	0	0	0
Avena fatua	0	0	0	0	0	0
Beta vulgaris	0	0	0	0	0	0
Cucumis sativus	0	0	0	0	0	0
Glycine max	0	0	0	0	0	0
Allium cepa	0	0	0	0	0	0

[a] Scale from 0 to 10: 0 = no observable effects on germination and growth, emergence identical to that of the untreated control; 10 = 100% inhibition of germination or complete destruction of above ground parts; 5 = estimated 50% injury or germination inhibition. Data given are the average of two replicates.

Table 10.6.1-2: A14325E - effects [a] on vegetative vigour of 6 species of higher terrestrial plants

Application rate (g/ha a.s.):	450	225	112.5	56.25	28.13	14.07
Brassica napus	0	0	0	0	0	0
Avena fatua	0	0	0	0	0	0
Beta vulgaris	0	0	0	0	0	0
Cucumis sativus	0	0	0	0	0	0
Glycine max	0	0	0	0	0	0
Allium cepa	0	0	0	0	0	0

[a] Scale from 0 to 10: 0 = vigorous healthy plants, indistinguishable from the untreated control; 10 = 100% plant injury, complete destruction of plant parts above ground; 5 = estimated 50% injury or inhibition of growth. Data given are the average of two replicates.

Conclusion

At rates up to and including 450 g a.s./ha of A14325E, no effects were seen on any of the plant species tested in either the seedling emergence or vegetative vigour test.

(Büche C, 2005)

CP 10.6.2 Testing on non-target plants

Further testing is not required since A14325E does not exhibit herbicidal activity.

CP 10.6.3 Extended laboratory studies on non-target plants

Extended laboratory tests were not conducted as the risk assessment above indicates acceptable risk to non-target plants.

CP 10.6.4 Semi-field and field tests on non-target plants

Extended laboratory tests were not conducted as the risk assessment above indicates acceptable risk to non-target plants.

Relevant Literature on Non-Target Plants

No relevant scientifically peer-reviewed open literature could be found on A14325E. Details of the literature search undertaken can be found in **M-CA Section 9**.

CP 10.7 Effects on Other Terrestrial Organisms (Flora and Fauna)

No further data on other terrestrial organisms is required.

Risk assessment for Other Terrestrial Organisms (Flora and Fauna)

No further risk assessments on other terrestrial organisms are required.

CP 10.8 Monitoring Data

There are no records of reported incidents related to use of A14325E or cyprodinil from monitoring data. No monitoring studies are needed for cyprodinil for ecotoxicological purposes as an acceptable risk has been identified for its proposed uses.