Draft Assessment Report

Evaluation of Active Substances

Plant Protection Products

Prepared according to **assimilated Regulation No 1107/2009** as it applies in Great Britain

Inpyrfluxam

Volume 3 – B.9 (S-2399 60 g/L EC)

Ecotoxicology

Great Britain

March 2026

Version History

When	What
November 2025	Initial DAR
March 2026	Updates made after ECP

Contents

B.9 Ecotoxicology Data and Assessment of Risks for Non-target Species

Background information

'S-2399 60 g/L EC' is the representative formulation for the approval of the new fungicidal active substance 'inpyrfluxam'. 'S-2399 60 g/L EC' is an EC (emulsifiable concentrate) formulation, containing 90 g inpyrfluxam/L and is intended for professional field use on cereals.

Enantiomeric ratios of inpyrfluxam and batches tested

All batches tested in the ecotoxicology studies in terms of impurities were considered comparable to the reference specification based on the technical equivalence assessment (see section C.1.4.2 of Volume 4 for full details).

Inpyrfluxam is a resolved isomer and not a mixture of isomers. Chiral analysis of selected samples was undertaken in plant, animal and environmental metabolism studies and in no case was any isomerisation observed and therefore no further consideration of the risk to non-target organisms from enantiomers of Inpyrfluxam is required. The metabolites 1'-CH_2OH-S-2840 and 1'-COOH-S-2840 are composed of two diastereomers pairs due to having a chiral carbon.

For each non-target organism group the change in isomeric ratio has been considered in Volume 1, section 2.11.7. No toxicity data was provided with non-target organisms with the individual enantiomers of the relevant metabolites. No significant shift was identified in the isomeric ratio of either metabolite by HSE residues and HSE environmental fate and behaviour and no further consideration was required by HSE toxicology and therefore no further consideration of the risk to non-target organisms is required.

Environmentally significant metabolites

The following table provides a summary of the environmentally significant metabolites as identified in Section B.8 of Volume 3:

Table B.9.0-1 Environmentally significant metabolites for inpyrfluxam

Environmental compartment	Metabolite(s)
Soil	3'-OH-S-2840, 1'-COOH-S-2840

Environmental compartment	Metabolite(s)
Groundwater	1'-COOH-S-2840
Surface water	3'-OH-S-2840, 1'-COOH-S-2840
Sediment	No environmentally significant metabolites
Air	No environmentally significant metabolites

Uses

The following table outlines the intended uses of inpyrfluxam.

Use-No.	Crop and/or situation (crop destination / purpose of crop)	F, G, or I	Application rate					
			Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season	g as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max
1	Wheat winter (TRZAW), Wheat spring (TRZAS), Durum wheat (TRZDU)	F	Foliar spray	BBCH 30-71 Spring	a)1 b)1	a)1.5 b)1.5	a)90 b)90	75-300
2	Barley winter (HORVW), Barley spring (HORVS)	F	Foliar spray	BBCH 30-71 Spring	a)1 b)1	a)1.5 b)1.5	a)90 b)90	75-300

B.9.1 Effects on birds and other terrestrial vertebrates

B.9.1.1 Effect on birds

No studies with birds were submitted with the formulation.

Summary of endpoints

The below table contains a summary of the available data with birds and the active substance.

The active substance studies are evaluated in Volume 3CA B9.

Toxicity data

Table B.9.1.1-1: Summary of 'inpyrfluxam' toxicity endpoints relevant for the risk assessment for birds

Species	Substance	Exposure system	Endpoint	Reference (Author, date)
Acute toxicity				
Northern bobwhite (*C. virginianus*)*	**Inpyrfluxam**	14 day acute oral study	LD_{50}[a] = >2250 mg a.s/kg bw	CA 8.1.1.1/01 [redacted] and [redacted] 2014
Mallard duck (*A. platyrhynchos*)*	**Inpyrfluxam**	14 day acute oral study	LD_{50}[b] = > 1350 mg a.s./kg bw	CA 8.1.1.1/02 [redacted] and [redacted] 2016
Short-term dietary toxicity				
Northern bobwhite (*C. virginianus*)*	**Inpyrfluxam**	Short-term, dietary (5 days)	LD_{50} = >5620 ppm (>1348 mg a.s./kg bw/d)	CA 8.1.1.3/01 [redacted] and 2015a
Zebra finch (*T. guttata*)	**Inpyrfluxam**	Short-term, dietary (5 days)	**$LD0^{c}$ =** 253 ppm **(38 mg a.s/kg bw/d)** **NOEL** = 80ppm **(19 mg a.s/kg bw/d)**	CA 8.1.1.2/03 [redacted] and 2017
Mallard duck (*A. platyrhynchos*)*	**Inpyrfluxam**	Short-term, dietary (5 days)	LD_{50} = > 5620 ppm (>2136 mg a.s./kg bw/d)	CA 8.1.1.2/02 [redacted] and [redacted] 2014b
Reproductive toxicity				
Northern bobwhite (*C. virginianus*)*	**Inpyrfluxam**	Reproduction, dietary (21 weeks)	NOED[d] = 44.3 mg a.s./kg bw/day	CA 8.1.1.3/01 [redacted] and 2015a

Species	Substance	Exposure system	Endpoint	Reference (Author, date)
Mallard duck (*A. platyrhynchos*)*	**Inpyrfluxam**	Reproduction, dietary (20 weeks)	NOEC[d] = 1000 ppm (130 mg a.s./kg bw/day)	CA 8.1.1.3/02 [redacted] and 2015b

*These studies are not relied upon in the risk assessment.
[a] Calculation of $LD_{10/20}$ not possible due to study design (limit test)
[b] $LD_{10/20}$ values could not be obtained with the mortality and regurgitation pattern observed during the test
[c] A definitive LD_{50} value could not be calculated due to a lack of clear dose response. As a worst case the lower value of a range (38-50 mg a.s./kg bw/day) taken from the raw data was used for risk assessment purposes. Additionally, there was no mortality at the lower value (38 mg a.s./kg bw/day) which was LD_0
[d] $EC_{10/20}$ values could not be calculated as no effects on mortality or reproduction were seen in the study
Endpoints highlighted in **bold** used in the risk assessment

Choice of acute avian endpoint for use in the risk assessment

Active substance

Two acute oral and three short-term dietary studies with the active substance have been provided for the acute avian risk assessment. The acute oral toxicity study with northern bobwhite quail produced the highest endpoint of the acute studies (>2250 mg a.s/kg bw/d).The lowest acute endpoint was for the mallard duck (1350 mg a.s/kg bw/d). Ordinarily, this would be the endpoint considered for risk assessment; however, three short-term dietary studies were submitted and the endpoint from the zebra finch short-term dietary study (38 mg a.s/kg bw/d) is the lowest endpoint. Therefore, this endpoint will be used in the acute risk assessment. Conversion of the LD_{50} (432 ppm) to mg/kg bw/d is not considered to be appropriate due to the demonstrated concentration-related food avoidance (SANCO/4145/2000 – final)[1], therefore, the highest concentration with no effect on adults (LC_0) has been selected for use in the acute risk assessment.

There is noted uncertainty with the mallard short term dietary study as, *"OECD 205 (1984) recommends an age of 10 – 17 days for mallard duck on study initiation. The study conductor initiated the study when ducklings were 5 days old. The age of ducklings at study initiation likely impacts their sensitivity to pesticides. The degree and directionality of the difference in pesticide sensitivity between 5 days old and 10 - 17 days old individuals is unclear". Anas platyrhychos* displayed low sensitivity to inpyrfluxam with no mortality and a 10.8% reduction in bodyweight at 2335 mg a.s./kg bw/day (5620 ppm) between Days 0 – 5, which was no longer observed on Day 8. Furthermore, in the *Anas platyrhychos* acute oral toxicity study no mortality was observed at 1350 mg a.s./kg bw and 38 % mortality at 2250 mg a.s./kg bw for individuals that consumed the full dose. For comparison,

[1] European Commission, 2002c. Guidance Document on Risk Assessment for Birds and Mammals Under CouncilDirective 91/414/EEC. SANCO/4145/2000

Taeniopygia guttata displayed an LC_0 = 38 mg a.s/kg bw in the short-term dietary study. In terms of concentration, the sensitivity difference between 5-day and 10 – 17-day old ducklings would have to be > 10 to impact the risk assessment, where the *Taeniopygia guttata* short-term dietary endpoint is selected. This is highly unlikely. Therefore, HSE considers the selection of 5-day old ducklings a minor deviation with no impact on the subsequent risk assessment.

No justification has been provided by the applicant for why so many additional studies have been submitted for birds.

An LD_0 of 38 mg a.s./kg b.w. will be used in the acute avian risk assessment.

Formulation

No avian studies with the formulation have been submitted.

Choice of chronic avian endpoints for use in the risk assessment

Active substance

Two dietary reproductive toxicity studies were submitted, one with the northern bobwhite and one with the mallard duck. The lowest endpoint was northern bobwhite with a NOEL of 44.3 mg a.s/kg bw/d. However, the short-term dietary study on zebra finch had a NOEL of 19 mg a.s/kg bw/d. In this study, at the 142 ppm test concentration, three birds were noted with clinical signs and one bird was noted with a ruffled appearance on Day 1. Two birds were recorded with loss of coordination on Day 4 and one of those birds showed a slight ruffled appearance on Day 5. As the endpoint for adults is to reflect the possibility that mating and nest building behaviour might be impacted by the birds being unwell, these are considered to be relevant effects. As this is lower than the endpoints obtained from the dietary reproductive studies, this endpoint is considered to be the critical endpoint for use in risk assessment.

An NOEL of 19 mg a.s./kg b.w./day will be used in the chronic avian risk assessment.

B.9.1.2 Effect on terrestrial vertebrates other than birds

Summary of endpoints

The tables below provide acute and reproductive mammalian toxicity endpoint from studies evaluated in Volume 3 B6.

Table B 9.1.2 -1: Acute toxicity for the risk assessment of mammals from 'Inpyrfluxam'

Species[1]	Substance	Exposure System	Results	Reference
Acute active substance				
Wistar rats	Inpyrfluxam	Toxic class method	50 < LD_{50} < 300 mg/kg bw	Vol. 3 CA B.6.2.1 [redacted] (2015a)
Wistar rats	Inpyrfluxam	Up and down procedure	**LD_{50} = 180 mg/kg bw** (95% confidence interval: 30.08 mg/kg bw to 735 mg/kg bw)	Vol. 3 CA B.6.2.1 [redacted] (2017a)
Wister rats	3'-OH-S-2840 (99.6% purity)	Toxic class method	LD_{50}[a)] >2000 mg/kg bw	Vol. 3 CA B.6.8.1 [redacted] (2017b)
Wister rats	1'-COOH-S-2840 (99.8% purity)	Toxic class method	LD_{50}[a)] >2000 mg/kg bw	Vol. 3 CA B.6.8.1 [redacted] (2017c)

Endpoints in **bold** suitable for use in risk assessment.
a) For the acute toxic class (OECD 423) method where only a range is provided, an 'LD_{50} cut-off' value can be obtained from the charts in Annex 2 of the Test Guideline.

Table B 9.1.2 -2: Reproductive toxicity endpoints for the risk assessment of mammals from S-2399 60 g/l EC

Endpoint	NOAEL (mg a.s./kg bw/d)	Reference	Studies to check
Body weight change[1],	31.7 mg/kg bw/day (500 ppm in diet)	90 day study in rats Vol. 3 CA B.6.3.2 [redacted] (2016)	Repeated dose 28-day oral toxicity study in rodents (OECD 407)

Endpoint	NOAEL (mg a.s./kg bw/d)	Reference	Studies to check
	27.8 mg/kg bw/day (500 ppm in the diet) Body weight effects on adults and offspring	Two-generation reproductive toxicity study in rat Vol. 3 CA B.6.6.1 [redacted] (2017)	Sub-chronic oral toxicity study-rodent 90 day study (OECD 408) Multi-generation study (OECD 416) Developmental studies (OECD 414)
	25 mg/kg bw/day Body weights in dams and foetuses	Developmental toxicity study in rat Vol. 3 CA B.6.6.2 [redacted] (2017a)	
	60 mg/kg bw/day Body weights in does	Developmental toxicity study in rabbits Vol. 3 CA B.6.6.2 [redacted] (2017c)	
Behavioural effects and systemic toxicity[2]	30 mg/kg bw reduced body temperature and motor activity	Acute neurotoxicity study in rats Vol. 3 CA B.6.7.1 [redacted] (2016b)	Repeated dose 28-day oral toxicity study in rodents (OECD 407) Sub-chronic oral toxicity study-rodent 90-day study (OECD 408) Multi-generation study (OECD 416) Developmental studies (OECD 414)
Indices of gestation, litter size, pup and litter weight[3]	27.8 mg/kg bw/day (500 ppm in diet) litter weight	Two-generation reproductive toxicity study in rat Vol. 3 CA B.6.6.1 [redacted] (2017)	Multi-generation study (OECD 416) Developmental studies (OECD 414)
	25 mg/kg bw/day Foetus weight	Developmental toxicity study in rat Vol. 3 CA B.6.6.2 [redacted] (2017a)	

Endpoint	NOAEL (mg a.s./kg bw/d)	Reference	Studies to check
Indices of viability, pre- and post-implantation loss	No effects 86 mg/kg bw/day (top dose)	Two-generation reproductive toxicity study in rat Vol. 3 CA B.6.6.1 [redacted] (2017)	Multi-generation study (OECD 416) Developmental studies (OECD 414)
	No effects 80 mg/kg bw/day (top dose)	Developmental toxicity study in rat Vol. 3 CA B.6.6.2 [redacted] (2017a)	
Embryo/foetal toxicity including teratological effects	No effects		Multi-generation study (OECD 416) Developmental studies (OECD 414)
Number aborting and number delivering early	No effects 86 mg/kg bw/day (top dose)	Two-generation reproductive toxicity study in rat Vol. 3 CA B.6.6.1 [redacted] (2017)	Multi-generation study (OECD 416) Developmental studies (OECD 414)
	60 mg/kg bw/day Abortions	Developmental toxicity study in rabbits Vol. 3 CA B.6.6.2 [redacted] (2017c)	
Systemic toxicity and effects on adult body weight	See above		Multi-generation study (OECD 416) Developmental studies (OECD 414)
Indices of post-natal growth[4], indices of lactation and data on physical landmarks	No effects		Multi-generation study (OECD 416) Developmental studies (OECD 414)
Survival and general toxicity up to sexual maturity	No effects		Multi-generation study (OECD 416) Developmental studies (OECD 414)

[1] Included as an indicator for parental effects which may disrupt reproduction.

[2] Effects derived from absorption of the substance that causes modification of an organ or an apparatus (biochemical, physiological and/or morphological). Examples include behavioural or physiological impairment (e.g. reduced locomotive activity, altered reflexes).
[3]Any effects in foetal body weight should be evaluated in the context of all pertinent data including other developmental effects as well as maternal toxicity.
[4] For example body weight gain, ear and eye opening, tooth eruption, hair growth and effects on sexual maturation such as age and body-weight at vaginal opening or balano-preputial separation.
Endpoints highlighted in **bold** are for use in risk assessment.

Choice of acute mammalian endpoints for use in the risk assessment

Active substance

Two acute oral toxicity studies with rats was conducted with the active substances and have been provided for use in the acute mammal risk assessment. The toxic class method produced a range between 50 < LD_{50} < 300 mg/kg bw. This was refined in the second study using the up and down procedure allowed refinement to give an was an LD_{50} of 180 mg a.s./kg b.w. ([redacted] 2017a).

An LD_{50} of 180 mg a.s./kg b.w. will be used in the acute mammal risk assessment.

Formulation

No mammalian studies submitted using the formulation.

Choice of chronic mammalian endpoint for use in the risk assessment

Active substance

Mammalian toxicity studies have been evaluated by the toxicology specialist (see Volume 3 B.6. for full evaluations) and have been considered below in order to derive the chronic mammalian endpoints (see Table B.9.1.2-2 for a summary).

The applicant proposed that the developmental toxicity study in the rat derived from [redacted] (2017a) was the appropriate study to use to set the endpoint for the reproductive mammalian risk assessment. This study derived a NOAEL of 25 mg a.s./kg bw/d based on the effects on body weight, body weight gain and food consumption for maternal toxicity. It is agreed that body weight and body weight gain are ecotoxicologically relevant effects that have the potential to impact fitness, competition, and consequently reproduction. In terms of maternal toxicity, at the high dose (80 mg/kg bw/d), decreased mean body weights (statistically significant on gestation days 12, 18 and 20) and body weight gains (statistically significant from gestation days 6-9) were noted throughout the treatment period. These are considered treatment related and adverse.

At 80 mg/kg bw/d, there was a significant decrease in food consumption from gestation days 6-9 to termination. This is considered treatment related and adverse.

In terms of systemic toxicity, the study by [redacted] (2016b), a NOAEL of 30 mg a.s/kg bw/d was proposed. No mortality was observed in any of the groups (0, 30, 100 or 200 mg/kg bw). At 200 mg/kg bw, decreased muscle tone was noted in two females on the first day, which disappeared on day two. This is considered adverse. At 100 and 200 mg/kg bw, there were significant decreases in body temperature and motor activity in females. These changes are considered to be related with systemic toxicity, but not neurotoxicity. No treatment related changes were noted in body weight at necropsy or histopathology in either sex.

A 2-generational study on rats by [redacted] (2017) produced a NOAEL of 86 mg/kg bw/d as dietary administration caused no effects on fertility and reproductive performance. In [redacted] (2017), no effects on viability/reproductive performance were noted at the highest concentration of 80 mg/kg bw/d; therefore a NOAEL of 80 mg/kg bw/d was determined for reproductive toxicity.

In terms of developmental toxicity, statistically significant decreases in mean foetal weight were observed at 80 mg/kg bw/d. This is considered treatment related and adverse. Numerous malformations noted in the treated groups, but the incidences were similar to those in controls. Among the malformations, the unusual occurrence of cyclopia was also noted in one foetus at the high dose. Although cyclopia is observed spontaneously, the incidence of cyclopia is quite low and this malformation had not appeared in the HCD of the testing facility. Therefore, relation to treatment could not be excluded and an additional developmental toxicity study in rats was conducted to determine whether the malformation was likely to be treatment related. In terms of developmental toxicity, a NOAEL of 25 mg a.s/kg bw/d based on development toxicity can be established based on effects on foetal weight and one occurrence of cyclopia at 80 mg/kg bw/d.

An additional study ([redacted] 2017b) administered doses of 0 or 90 mg/kg bw/d. In the treated dams, significant decreases in mean body weight and body weight gain were noted from gestation day 9. This is considered treatment related and adverse. Significant decrease in mean foetal weight was also observed and was considered treatment related and adverse. No cyclopia was observed in any foetus in this study, and the single occurrence of cyclopia in [redacted] (2017a) was concluded to not be related in inpyrfluxam treatment.

In terms of aborting and delivering early, the study by [redacted] (2017c), produced a NOAEL of 60 mg/kg bw for maternal toxicity in rabbits. Adverse effects were noted in the maternal animals at 200 mg/kg bw/d from gestation day 6 to 27. This included effects on body weight gain, food consumption and clinical signs of toxicity (red discharge and abortions). There were no adverse effects noted in the foetuses. A NOAEL of 200 mg/kg bw/d (highest dose tested) can be established for developmental toxicity as there were no adverse effects.

Body weight is one of the parameters listed in the guidance as relevant for the reproductive assessment of wild mammals. The guidance does not differentiate between males and females. In this case the lowest NOAEL is for females and foetuses. As a result this endpoint will be used to set the overall endpoint for the wild mammal risk assessment.

The lowest chronic mammalian endpoints for the active substance are for rats based on body weights change in dams and foetuses (2017a) and indices of gestation, litter size, pup and litter weight on foetus weight (2017a), which both had endpoints of 25 mg a.s/kg bw/d.

The chronic endpoint proposed for use in the risk assessment is 25 mg a.s./kg b.w./day, based on foetus weight.

B.9.2 Risk assessment for birds and other terrestrial vertebrates

B.9.2.1 Risk assessment for birds

Acute toxicity to birds

Inpyrfluxam is a broad-spectrum fungicide, belonging to the succinate dehydrogenase inhibitor (SDHI) family of fungicides. It is proposed to be applied as a foliar spray on winter and spring cereals (BBCH 30-71).

Table 9.2.1 -1 Screening assessment of the acute risk for birds due to S-2399 use in cereals using the worst-case application rate

Intended use	Cereals				
Active substance	Inpyrfluxam				
Application rate (kg/ha)	0.09				
Acute toxicity (mg/kg bw)	38				
TER criterion	10				
Crop scenario	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER**
Cereals	Small omnivorous bird	158.8	1	14.29	**2.66**

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

The acute TER value for the intended use is below the trigger value of 10 and requires further consideration at Tier 1. A first tier risk assessment is shown in the table below.

Table 9.2.1 – 2: First tier assessment of the acute risk for birds due to 'inpyrfluxam' use in cereals using the worst-case application

Intended use	Cereals
Active substance	Inpyrfluxam
Application rate (kg/ha)	0.09
Acute toxicity (mg/kg bw)	38
TER criterion	1

Crop scenario	**Generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD (mg/kg bw/d)**	**TER**
Cereal BBCH 30 -39	Small omnivorous bird "lark"	12.0	1	1.08	35.19
Cereals BBCH ≥ 40	Small omnivorous bird "lark"	7.2	1	0.65	58.64
Cereals late post-emergence (May-June) BBCH 71-89	Small insectivorous bird "passerine"	57.6	1	5.18	7.33
Cereals late season – seed heads	Small granivorous/insectivorous bird "bunting"	4.0	1	0.36	105.56

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

Geometric mean refinement

A geomean approach has been proposed by the applicant to refine the endpoint derived from the short-term dietary studies. The geometric mean LD_{50} was calculated to be > 478.29 mg a.s./kg bw/day, which is more than a factor of 10 greater than the worst-case lowest endpoint for zebra finch. According to EFSA guidance (2009), when the most sensitive species (zebra finch) is more than a factor of 10 below the geometric mean of all the tested species, the most sensitive species should be used in the risk assessment with an assessment factor of 1. It should be noted that this endpoint is also conservative as it is based on an LD_0 as opposed to an LD_{50}. This is because the LD_{50} in the study could not be converted from ppm, the unit the endpoint is in, to mg a.s./kg bw due to food avoidance in the study at this concentration. Therefore, the highest concentration with no mortality and no food avoidance was selected for use in the study and converted into mg a.s./kg bw. This gives a suitable acute endpoint of 38 mg a.s/kg.

In this instance acceptable risk can be concluded for all crop scenarios above using the geomean refinement as the TER value is greater than the trigger value of 1. No further

consideration is required. Acceptable acute risk to birds from the proposed GAP has been demonstrated.

A first tier risk assessment using the proposed assessment factor of 1 for late post-emergence BBCH 71 – 89 has been presented below.

Long-term/reproductive toxicity to birds

Table 9.2.1 – 3: Screening assessment of the reproductive risk to birds due to 'inpyrfluxam' use in cereals using the worst-case application rate

Intended use	Cereals				
Active substance	Inpyrfluxam				
Application rate (kg/ha)	0.09				
Reproductive toxicity (mg/kg bw)	19				
TER criterion	5				
Crop scenario	**Indicator species**	**SV_m**	**MAF x TWA**	**DDD_{90} (mg/kg bw/d)**	**TER**
Cereals	Small omnivorous bird	64.8	1.0 x 0.53	3.09	6.15

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

An acceptable reproductive risk to birds was demonstrated at screening stage with the TER being greater than the trigger value of 5. No further consideration is required for the reproductive risk.

Effects of secondary poisoning on birds

The log P_{ow} of 'inpyrfluxam' amounts to 3.65 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

Table 9.2.1 - 4: Assessment of the risk for earthworm-eating birds due to exposure to 'inpyrfluxam' *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Inpyrfluxam	Comments
PEC_{soil} (mg/kg soil)	0.069	PEC_{soil} (twa = 21 d) + $PEC_{soil\ plateau}$ (i.e. 0.023 + 0.003) (Section B.9.1.3)
P_{ow}	4467	log Pow = 3.65
Koc	647	
foc	0.02	Default
BCF_{worm}	4.207	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.2903	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.3048	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	19	
TER_{lt}	62.33	Trigger = 5

TER values shown in **bold** fall below the relevant trigger

As the above assessment demonstrates the TER to be above the trigger value 5, the risk to earthworm-eating birds from the proposed use of S-2399 60 G/L EC in cereals is considered to be acceptable.

Risk assessment to fish-eating birds via secondary poisoning

Table 9.2.1 – 5: Assessment of the risk for fish-eating birds due to exposure to 'inpyrfluxam' *via* bioaccumulation in fish (secondary poisoning)

Parameter	Inpyrfluxam	Comments
PEC_{sw} (mg/L)	0.000831	Worst-case from drift entry
BCF_{fish}	215.4*	Range 29-30
PEC_{fish}	0.179	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.02846	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	19	
TER_{lt}	667.59	Trigger = 5

TER values shown in **bold** fall below the relevant trigger

*The worst-case BCF value has been used. As the risk assessment demonstrated a large margin of safety, this has not been considered further.

As the above assessment demonstrates the TER to be above the trigger value 5, the risk to fish-eating birds from the proposed use of S-2399 60 g/L EC in cereals is considered to be acceptable.

Risk assessment for birds through drinking water

Two drinking water risk assessment scenarios for birds are available according to EFSA/2009/1438: the leaf scenario, and the puddle scenario. The leaf scenario is not relevant for use on cereals as they are not leafy vegetables and do not produce leaf whorls. The risk to birds via drinking water from the application of 'S-2399' via the leaf scenario does therefore not require consideration.

Puddle scenario

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 when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

With a K(f)oc value of 647 ml/g, 'inpyrfluxam' belongs to the group of more sorptive substances. Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the critical value of 3000 for at least one scenario, a quantitative risk assessment (calculation of TER values) is not necessary and an acceptable risk to birds from exposure via contaminated drinking water following the proposed uses of S-2399 60 g/L EC is concluded.

Table 9.2.1 –6: Drinking water assessment for birds

Effective application rate (g/ha)	=	90		
Acute toxicity (mg/kg bw)	=	38	quotient	= 2.37
Reprod. toxicity (mg/kg bw/d)	=	19	quotient	= 4.7

Metabolite assessment

In accordance with the guidance of EFSA (2009) it must be identified if any metabolites are likely to be formed in avian food items which may then be consumed by relevant focal species.

Metabolism in rotational crops was investigated using pyrazole and phenyl labelled inpyrfluxam. From the available plant metabolism data, metabolites were considered relevant where they were formed at ≥ 10% total reactive residues (TRR) OR where the TRR was < 10%, but the metabolite was present at ≥ 0.05 mg/kg. For apple, soybean, oilseed rape, maize, sorghum, rice, and potatoes (primary crops), eight plant metabolites, 3'-OH-S-2840, 1'-CH_2OH-S-2840, *N*-des-Me-2840, *N*-des-Me-DFPA (conjugate), Glc-NDM-S-2399A, Gly-1'-CH2OH-S-2840, DFPA-$CONH_2$, and 1'-COOH-S-2840 conjugate were considered relevant. For rotational crops, two further metabolites, DFPA and N-des-

Me-1'-CH_2OH-S-2840 were considered relevant in lettuce, sorghum and radish (for full details on studies see Volume 3CA B7, sections 7.2.1 and 7.6.1).

The measured concentrations are provided in Table B.9.2.1-7 below. It should be noted that this data does not provide a specific indication of plant metabolites present at the time birds would be in the field and therefore potentially exposed. It does, however, provide an indication that these metabolites are formed at significant levels in plant material as part of the plant metabolism of the active substance. Therefore, there is the potential for them to be present in food items consumed by birds and their further consideration is required with respect to the bird dietary risk assessment.

Please note that Table B.9.2.1-7 only contains data for metabolites that occurred at ≥ 10% TRR or were < 10% TRR but detected at ≥ 0.05 mg/kg in those parts of the plant considered to be consumed by birds.

Table B.9.2.1-7: Maximum metabolite percentage formation of total reactive residues (TRR) in plant metabolism studies

Primary crops			
Crop: Apple (Fruit); applied at 3 x 649 g a.s./ha			
Matrix	Apple (whole fruit) (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
3'-OH-S-2840	0.035	11.5	Not reported
Matrix	Apple (whole fruit) (phenyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
3'-OH-S-2840	0.014	11	Not reported
Crop: Soybean (Pulses/oilseeds); applied at 2 x 108 g a.s./ha			
Matrix	Soybean forage (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
3'-OH-S-2840	0.308	22.1	BBCH 60
1'-CH_2OH-S-2840	0.050	3.6	
Matrix	Soybean Hay (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
3'-OH-S-2840	0.349	14.7	BBCH 60
1'-CH_2OH-S-2840	0.087	3.7	
N-des-Me-S-2840	0.054	2.3	
Crop: Soybean (pulses/oilseeds); applied at 2 x 113 g a.s/ha			
Matrix	Soybean mature seeds (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
N-des-Me-DFPA (conjugate)	0.038	17.5	BBCH 75
Matrix	Soybean immature pods (pyrazolyl label)		**BBCH at sampling/harvest**

Metabolite	**mg eq./kg**	**% TRR**	BBCH 75
3'-OH-S-2840	0.065	9.2	
Crop: Soybean (pulses/oilseeds); applied at 2 x 107 g a.s/ha			
Matrix	Soybean forage (phenyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
3'-OH-S-2840	0.238	15.3	BBCH 60
1'-CH_2OH-S-2840	0.058	3.7	
Matrix	Soybean hay (phenyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
3'-OH-S-2840	0.321	14.3	BBCH 60
N-des-Me-S-2840	0.053	2.4	
Glc-NDM-S-2399A	0.085	3.8	
Crop: Soybean (pulses/oilseeds); applied at 2 x 111 g a.s/ha			
Matrix	Soybean mature pods (phenyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
3'-OH-S-2840	0.094	12.7	BBCH 75
Matrix	Soybean immature pods (phenyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
3'-OH-S-2840	0.057	9.0	BBCH 75
Crop: Rice (cereals/grass crops); applied at 1 x 95 g a.s/ha			
Matrix	Rice straw (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
3'-OH-S-2840	0.102	12.0	28 days before BBCH 77
Matrix	Rice hulls (pyrazole label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
1'-CH_2OH-S-2840	0.517	33.9	28 days before BBCH 77
3'-OH-S-2840	0.088	5.8	
Gly-1'-CH2OH-S-2840	0.110	7.2	
Matrix	Rice grain (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
Gly-1'-CH_2OH-S-2840	0.101	16.0	28 days before BBCH 77
Crop: Rice (cereals/grass crops); applied at 1 x 108.1 g a.s/ha			
Matrix	Rice straw (phenyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
3'-OH-S-2840	0.055	6.0	28 days before BBCH 77
Matrix	Rice hulls (phenyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
1'-CH_2OH-S-2840	0.277	18.0	28 days before BBCH 77
3'-OH-S-2840	0.087	5.6	
Gly-1'-CH2OH-S-2840	0.118	7.1	

Crop: Rice (cereals/grass crops); applied at 1 x 391 g a.s/ha			
Matrix	Rice forage (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
1'-CH_2OH-S-2840	0.279	7.2	BBCH 13 - 14
3'OH-S-2840	0.142	3.6	
Gly-1'-CH_2OH-S-2840	1.010	26.0	
Matrix	Rice straw (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
1'-CH_2OH-S-2840	0.365	23.2	BBCH 13-14
Gly-1'-CH_2OH-S-2840	0.498	31.7	
Matrix	Rice hulls (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
1'-CH_2OH-S-2840	0.070	40.1	BBCH 13 -14
DFPA-$CONH_2$	0.031	17.5	
Matrix	Rice grain (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
N-des-Me-DFPA	0.002	23.1	BBCH 13 - 14
Crop: Rice (cereals/grass crops); applied at 1 x 357 g a.s/ha			
Matrix	Rice forage (phenyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
Gly-1'-CH_2OH-S-2840	0.315	16.7	BBCH 13- 14
1'-CH_2OH-S-2840	0.118	6.3	
Matrix	Rice straw (phenyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
1'-CH2OH-S-2840	0.276	25.7	BBCH 13 - 14
Gly-1'-CH2OH-S-2840	0.407	38.2	
Matrix	Rice hulls (phenyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
1'-CH_2OH-S-2840	0.084	53.2	BBCH 13 - 14
Crop: Potato (root crop) applied at 1 x 4.99 g a.s/100 kg tubers			
Matrix	Potato tubers (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
1'-COOH-S-2840 conjugate	0.008	18.5	BBCH 00
N-des-Me-DFPA	0.004	10.1	
Rotational crops**			
Crop: Lettuce; applied at 233 – 236 g a.s/ha			
Matrix	Lettuce immature leaves (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
1'-CH_2OH-S-2840 conjugate	0.010	14.0	BBCH 44 - 49
DFPA (free) (2^{nd} rotation)	0.022	22.4	

N-des-Me-DFPA (free) (3rd rotation)	0.077	18.5	
Matrix	Lettuce mature leaves (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
1'-CH_2OH-S-2840 (conjugate)	0.010	13.5	BBCH 44 - 49
DFPA (free) (1st rotation)	0.010	13.0	
DFPA (conjugate) (2nd rotation)	0.014	16.6	
DFPA (free) (3rd rotation)	0.004	15.6	
N-des-Me-DFPA (free) (3rd rotation)	0.006	28.0	
Matrix	Lettuce immature leaves (phenyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
1'-CH_2OH-S-2840 (conjugate)	0.007	15.7	BBCH 44 - 49
3'-OH-S-2840 (free) (2nd rotation)	0.06	10.9	
Matrix	Lettuce mature leaves (phenyl label)		
Metabolite	**mg eq./kg**	**%TRR**	
1'-CH_2OH-S-2840 (conjugate)*	0.021	21.2	
3'-OH-S-2840 (free) (2nd rotation)	0.008	11.7	
Crop: Sorghum; applied at 233 – 236 g a.s/ha			
Matrix	Sorghum forage (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
DFPA (conjugate)	0.021	11.1	BBCH 85 - 89
Matrix	Sorghum grain (pyrazolyl label)		
Metabolite	**mg eq./kg**	**% TRR**	
DFPA (free)	0.011	22.6	
Matrix	Sorghum straw (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
1'-CH_2OH-S-2840 (conjugate) (1st rotation)	0.078	10.3	BBCH 85 - 89
1'-CH_2OH-S-2840 (conjugate) (2nd rotation)	0.080	7.2	
DFPA (conjugate) (2nd rotation)	0.129	11.6	
N-des-Me-DFPA (conjugate)	0.059	4.5	

Matrix	Sorghum forage (phenyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
1'-CH_2OH-S-2840 (conjugate) (1st rotation)	0.013	13.8	BBCH 85 - 89
1'-CH_2OH-S-2840 (conjugate) (2nd rotation)	0.015	13.0	
N-des-Me-1'-CH_2OH-S-2840 (conjugate) (2nd rotation)	0.015	12.3	
Matrix	Sorghum straw (phenyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
1'-CH_2OH-S-2840 (conjugate) (1st rotation)	0.092	13.5	BBCH 85 - 89
1'-CH_2OH-S-2840 (conjugate) (2nd rotation)	0.167	13.3	
3'-OH-S-2840 (conjugate)	0.098	7.8	
N-des-Me-1'-CH_2OH-S-2840 (conjugate) (2nd rotation)	0.111	8.9	
Crop: Radish; applied at 233 – 236 g a.s/ha			
Matrix	Radish immature roots (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
DFPA (free) (2nd rotation)	0.007	11.2	BBCH 44 - 49
DFPA (free)	0.005	23.6	
1'-COOH-S-2840 (free)	0.003	14.3	
Matrix	Radish mature roots (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
DFPA (free)	0.003	13.7	Not reported
Matrix	Radish immature tops (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolite	**mg eq./kg**	**% TRR**	
DFPA (conjugate)	0.014	10.2	BBCH 44 - 49
DFPA-$CONH_2$ (free) (1st rotation)	0.025	18.5	
N-des-Me-S-2840 (free) (2nd rotation)	0.027	12.6	
N-des-Me-1'-CH_2OH-S-2840 (conjugate) (2nd rotation)	0.026	12.3	

DFPA-$CONH_2$ (free) (2nd rotation)	0.037	17.1	
1'-COOH-S-2840 (conjugate) (3rd rotation)	0.005	12.0	
Matrix	Radish mature tops (pyrazolyl label)		**BBCH at sampling/harvest**
Metabolites	**mg.eq/kg**	**% TRR**	
N-des-Me-1'-CH_2OH-S-2840 (conjugate) (1st rotation)	0.029	13.0	BBCH 44 - 49
DFPA-$CONH_2$ (free)	0.023	10.3	
N-des-Me-S-2840 (free) (2nd rotation)	0.038	10.2	
N-des-Me-S-2840 (free) (3rd rotation)	0.009	12.8	
Matrix	Radish immature roots (phenyl label)		**BBCH at sampling/harvest**
Metabolites	**mg.eq/kg**	**% TRR**	
3'-OH-S-2840 (free) (2nd rotation)	0.003	11.9	BBCH 44 - 49
1'-COOH-S-2840 (free) (3rd rotation)	0.003	25.7	
Matrix	Radish mature roots (phenyl label)		**BBCH at sampling/harvest**
Metabolites	**mg.eq/kg**	**% TRR**	
3'-OH-S-2840 (free)	0.003	11.1	BBCH 44 - 49
1'-COOH-S-2840 (free) (3rd rotation)	0.005	19.1	
Matrix	Immature tops (phenyl label)		**BBCH at sampling/harvest**
Metabolites	**mg.eq/kg**	**%TRR**	
N-des-Me-S-2840 (free)	0.016	14.3	BBCH 44 - 49
N-des-Me-1'-CH_2OH-S-2840 (conjugate)	0.015	13.0	
1'-COOH-S-2840 (conjugate) (3rd rotation)	0.021	25.6	
Matrix	Radish mature tops (phenyl label)		**BBCH at sampling/harvest**
Metabolites	**mg.eq/kg**	**% TRR**	
3'-OH-S-2840 (conjugate)	0.013	10.2	BBCH 44 - 49
N-des-Me-S-2840 (free) (1st rotation)	0.014	10.7	
N-des-Me-1'-CH_2OH-S-2840 (conjugate)	0.012	11.5	
N-des-Me-S-2840 (free) (3rd rotation)	0.012	13.6	

1'-COOH-S-2840 (conjugate) (3rd rotation)	0.019	22.1	

**values displayed are from the 1st crop rotation (unless stated otherwise) and are protective of any other observations in other rotations
* highest (worst-case) value has been included

On the basis of the above table, it is clear that several metabolites occur in plant material at a level that requires further consideration:

- 3'-OH-S-2840
- 1'-CH_2OH-S-2840
- *N*-des-Me-S-2840
- *N*-des-Me-DFPA
- Glc-NDM-S-2399A
- Gly-1'-CH_2OH-S2840
- DFPA-$CONH_2$
- 1'-COOH-S-2840
- DFPA
- *N*-des-Me-1'-CH_2OH-S-2840

In order to determine whether a dietary risk assessment is necessary for these metabolites for birds, metabolism studies conducted on poultry have been checked, since a risk assessment will not be required if the metabolites in question are formed at sufficient levels in birds as the risk will be considered covered by the risk assessment for the active substance.

There is one available hen metabolism study in Volume 3CA B7, section B7.2.2:

- ([redacted] 2017) Metabolism of [14C]S-2399 (2 radiolabels) in Laying Hens 21-SEP-2016 (Final Report) 26-APR-2017 (Amended Final Report)

The results of the hen metabolism study in the context of the above relevant metabolites have been considered below:

Table B.9.2.1-8: Summary of the hen metabolism studies

Metabolite	Present in the poultry study?
3'-OH-S-2840	Pyrazolyl label: 1.92 % TRR in eggs, 2.66 % in abdominal fat, 2.18 % in subcutaneous fat Phenyl label: 2.52% TRR in eggs, 1.45% TRR in abdominal fat,

Metabolite	Present in the poultry study?
1'-CH_2OH-S-2840*	Pyrazolyl label: **31.59% TRR in eggs**, **11.08% TRR in thigh**, 5.59% TRR in breast, 2.66% TRR in abdominal fat, 2.57% TRR in subcutaneous fat, 2.05% TRR in excreta (day 4) Phenyl label: **29.79% TRR in egg**, **10.8% TRR in thigh**, 3.38% TRR in breast, 2.32% TRR in abdominal fat
N-des-Me-S-2840	Pyrazolyl label: 4.62 % TRR in liver, 5.02 % TRR in eggs, 3.25% TRR in abdominal fat, 3.17% TRR in subcutaneous fat Phenyl label: 9.50% TRR in liver, 5.58% TRR in eggs, 2.52% TRR in abdominal fat
N-des-Me-DFPA	N/A
Glc-NDM-S-2399A	N/A
Gly-1'-CH_2OH-S2840	N/A
DFPA-$CONH_2$	Pyrazolyl label: 5.02 % TRR in eggs, **14.49 % TRR in thighs, 11.83 % TRR in breast**
DFPA	N/A
1'-COOH-S-2840*	Pyrazolyl label: 6.51% TRR in liver, 9.79% TRR in thigh, **11% TRR in breast**, 1.18% TRR in abdominal fat, 29.23% TRR in excreta (day 4) Phenyl label: **10.97%** TRR in liver, 4.68% TRR in egg, **16.4% TRR in thigh, 10.59% TRR in breast**, 3.19% TRR in abdominal fat, **35.21% TRR in excreta (day 4)**
N-des-Me-1'-CH2OH-S-2840	N/A

N/A – not applicable (not detected in study)
*Values are the sum of the combination of A and B isomers
Values in **bold** are ≥ 10% TRR.

The applicant has stated that for bird metabolites:

"The significant plant metabolites identified in MCA 6 (Residues) are 3'-OH-S-2840, 1'-CH_2OH-S-2840 and 1'-COOH-S-2840. No avian studies are available with these

metabolites, however information on their toxicity can be taken from the mammalian studies within MCA 5 (Toxicology)".

HSE consider this to be suitable justification for the metabolites 1'-CH_2OH-S-2840 and 1'-COOH-S-2840 as they are present in the hen metabolism study at >10%. However, this cannot be considered for the metabolite 3'-OH-S-2840. This will be considered further below.

Based on the above, the following metabolites can be considered to be covered by the parent as they are present at > 10% TRR in the hen metabolism study.

- 1'-COOH-S-2840
- 1'CH_2OH-S-2840
- DFPA-CONH2

The metabolite Gly-1'-CH_2OH-S2840 can also be excluded on the basis that this is a sugar conjugate of 1'-CH_2OH-S-2840 and that the sugar would be cleaved in the GI tract. This is proposed by HSE Toxicology (B.6.1.1); but this can be considered to be the case for the birds based on vertebrate metabolism. As the 1'-CH_2OH-S-2840 metabolite is a major bird metabolite and has been excluded, no further consideration is required.

The following metabolites are not considered to be covered by the toxicity studies on the parent and require further consideration:

- 3'-OH-S-2840
- *N*-des-Me-S-2840
- *N*-des-Me-DFPA
- Glc-NDM-S-2399A
- DFPA
- *N*-des-Me-1'-CH_2OH-S-2840

For Glc-NDM-S-2399A, HSE Toxicology have said that "*as the conjugated form of the metabolite N-des-Me-S-2840, the toxicity profile of this metabolite is expected to be comparable / less severe compared to its aglycon form N-des-Me-S-2840".* This is supported by QSAR information. This provides some evidence that this metabolite could be excluded based on a weight of evidence approach. However, a risk assessment has been conducted for completeness.

As these metabolites cannot be excluded, a worst-case risk assessment has been conducted for both acute and reproductive risk.

The below risk assessment assumes a worst-case 10 times greater toxicity than the parent in the first instance.

Table B.9.2.1-9: Acute tier 1 risk assessment for birds from metabolites in food items

Intended use	Cereals				
Active substance	Inpyrfluxam				
Application rate (kg/ha)	0.09[a]				
Acute toxicity (mg/kg bw)	3.8[b]				
TER criterion	10				
Crop scenario	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD (mg/kg bw/d)**	**TER**
Cereal BBCH 30-39	Small omnivorous bird 'lark'	12	1	1.08	**3.52**
Cereal BBCH ≥ 40	Small omnivorous bird 'lark'	7.2	1	0.65	**5.86**
Cereals late post-emergence (May-June) 71-89	Small insectivorous bird "passerine"	57.6	1	5.18	**0.73**
Late season – seed heads	Small granivorous/insectivorous bird "bunting"	4.0	1	0.36	10.56

[a] As a worst-case initial consideration, the maximum application rate of the parent has been considered

[b] As no toxicity data is available for the metabolites, they are assumed to be 10 times more toxic than the active substance

The TER values in the worst-case acute tier 1 bird risk assessment are below the trigger value of 10 for all indicator species, with the exception of small granivorous/insectivorous bird "bunting"; therefore, further consideration is required. To assess the risk from the remaining metabolites, HSE has considered the metabolite with the highest %TRR present in the hen metabolism studies. This is for the metabolite N-des-Me-DFPA present at a maximum of 28% TRR. This has been considered in the risk assessment below.

Table B.9.2.1-10: Acute risk assessment for birds from metabolites in food items

Intended use	Cereals
Active substance	Inpyrfluxam
Application rate (kg/ha)	0.09[a]

Acute toxicity (mg/kg bw)	3.8[b]				
TER criterion	10				
Crop scenario	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD (mg/kg bw/d)**	**TER**
Cereal BBCH 30-39	Small omnivorous bird 'lark'	12	1	0.30[c)]	12.57
Cereal BBCH ≥ 40	Small omnivorous bird 'lark'	7.2	1	0.18[c)]	20.94
Cereals late post-emergence (May-June) BBCH 71-89	Small insectivorous bird "passerine"	57.6	1	1.45[c)]	**2.62**

a) As a worst-case initial consideration, the maximum application rate of the parent has been considered
b) As no toxicity data is available for the metabolites, they are assumed to be 10 times more toxic than the active substance
c) DDD amended in line with highest metabolite %TRR (N-des-Me-DFPA at 28% TRR)

An acceptable acute risk to birds was able to be demonstrated for small omnivorous bird 'lark' at BBCH ≥ 40 and BBCH 30-39. No further consideration is required for these scenarios. However, an unacceptable risk has been demonstrated for the scenario for cereals late post-emergence (May-June) BBCH 71-89 based on a trigger value of 10. Further consideration is required.

As considered above, a geomean refinement was considered appropriate whereby a trigger value of 1 can be used. In this instance, acceptable acute risk to birds from the metabolites of inpyrfluxam can be demonstrated for small insectivorous bird "passerine" as the derived TER value of 2.62 is greater than the geometric mean refinement trigger value of 1. Acceptable acute risk to birds from the proposed use has been demonstrated and no further consideration is required.

The reproductive risk to birds from metabolites has been considered using 10 times greater toxicity than the parent as a worst-case scenario in the first instance.

Table B.9.2.1-11: Reproductive screening risk assessment for birds from metabolites in food items

Intended use	Cereals				
Active substance	Inpyrfluxam				
Application rate (kg/ha)	0.09 a)				
Reproductive toxicity (mg/kg bw)	1.9 b)				
TER criterion	5				
Crop scenario	**Indicator species**	**SV_m**	**MAF x TWA**	**DDD_{90} (mg/kg bw/d)**	**TER**
Cereals	Small omnivorous bird	64.8	1.0 c)	3.09	**0.33**

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

a) As a worst-case initial consideration, the maximum application rate of the parent has been considered

b) As no toxicity data is available for the metabolites, they are assumed to be 10 times more toxic than the active substance

c) *An fTWA of 1 was used for the metabolite as the pattern of decline is not as straightforward as the active substance since residues initially increase before decreasing. Therefore, a worst-case as-sumption of a 100% conversion from parent at start and maintenance of this over the whole averaging period was used at first tier*

As the TER for the worst-case screening assessment is below the trigger value of 5, unacceptable reproductive risk from the worst-case screening assessment has been demonstrated. This has been considered further at Tier 1 below.

Table B.9.2.1-12: Reproductive tier I risk assessment for birds from metabolites in food items

Intended use	Cereals
Active substance	Inpyrfluxam
Application rate (kg/ha)	0.09 a)
Reproductive toxicity (mg/kg bw)	1.9 b)
TER criterion	5

Crop scenario	Indicator species	SV_m	MAF x TWA c)	DDD_{90} (mg/kg bw/d)	TER
BBCH 30 - 39	Small omnivorous bird 'lark'	5.4	1	0.486	**3.90**
BBCH ≥ 40	Small omnivorous bird 'lark'	3.3	1	0.297	6.39
Cereals late post-emergence (May-June) BBCH 71-89	Small insectivorous bird "passerine"	22.4	1	2.02	**0.94**
Late season – seed heads	Small granivorous/insectivorous bird "bunting"	4.7	1	0.42	**4.49**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose
a) As a worst-case initial consideration, the maximum application rate of the parent has been considered
b) As no toxicity data is available for the metabolites, they are assumed to be 10 times more toxic than the active substance
c) An fTWA value of 1 was used for the metabolites as the pattern of decline is not as straightforward as for active substances, since residues initially increase before decreasing. Therefore, a worst-case assumption of an initial 100% conversion from the parent at the start and maintenance of this over the whole averaging period has been used at first tier.

The TER value for "small omnivorous bird 'lark'" in the worst-case long-term/reproductive tier 1 bird risk assessment is below the trigger value of 5 at BBCH 30-39, as small insectivorous bird "passerine" for cereals late post-emergence (May-June) BBCH 71-89 and small granivorous/insectivorous bird "bunting" for and late season – seed heads; therefore, further consideration is required. To assess the risk from the remaining metabolites, HSE has considered the metabolite with the highest %TRR present in the hen metabolism studies. This is the metabolite N-des-Me-DFPA present at 28%.

Table B.9.2.1-13: Reproductive risk assessment for birds from metabolites in food items

Intended use	Cereals					
Active substance	Inpyrfluxam					
Application rate (kg/ha)	0.09 a)					
Reproductive toxicity (mg/kg bw)	1.9 b)					
TER criterion	5					
Crop scenario	**Indicator species**	**SV_m**	**MAF x TWA** c)	**DDD_{90} (mg/kg bw/d)**	**DDD_{90} (mg/kg bw/d) corr.** d)	**TER**
BBCH 30 - 39	Small omnivorous bird 'lark'	5.4	1	0.486	0.13	13.96
Cereals late post-emergence (May-June) BBCH 71-89	Small insectivorous bird "passerine"	22.4	1	2.02	0.56	**3.39**
Late season – seed heads	Small granivorous/insectivorous bird "bunting"	4.7	1	0.42	0.12	15.8

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose
a) As a worst-case initial consideration, the maximum application rate of the parent has been considered
b) As no toxicity data is available for the metabolites, they are assumed to be 10 times more toxic than the active substance
c) An fTWA value of 1 was used for the metabolites as the pattern of decline is not as straightforward as for active substances, since residues initially increase before decreasing. Therefore, a worst-case assumption of an initial 100% conversion from the parent at the start and maintenance of this over the whole averaging period has been used at first tier.
D) DDD amended based on highest percentage TRR for metabolites in plant residues (28% N-des-Me-DFPA)

Based on the worst-case tier 1 risk assessment, an acceptable reproductive risk to birds from the metabolites present in food items at late post-emergence growth stages has been demonstrated, with the exception of the BBCH 71-89 scenario. Further consideration is required.

Risk assessments have been carried out below to consider the risk from each individual metabolite that could not be excluded based on hen metabolism data for the crop scenario BBCH 71-89 (small insectivorous bird "passerine"). The above assessment considered the worst case across all metabolites; therefore, the risk assessments presented below have been carried out to determine exactly which of the six metabolites require further consideration.

Table B.9.2.1-14: Reproductive risk to birds from individual metabolites present in food items

Intended use	Cereals					
Active substance	Inpyrfluxam					
Application rate (kg/ha)	0.09 a)					
Reproductive toxicity (mg/kg bw)	1.9 b)					
TER criterion	5					
Crop scenario	**~~Indicator~~ Generic focal species**	**SV_m**	**MAF x TWA** c)	**Metabolite (max %TRR)**	**DDD_{90} (mg.kg bw/d) corr** d)	**TER**
Cereals late post-emergence (May-June) BBCH 71-89	Small insectivorous bird “passerine”	22.4	1	3’OH’S-2840 (22.1%)	0.45	**4.26**
Cereals late post-emergence (May-June) BBCH 71-89	Small insectivorous bird “passerine”	22.4	1	DFPA (23.6%)	0.47	**4.0**
Cereals late post-emergence (May-June) BBCH 71-89	Small insectivorous bird “passerine”	22.4	1	N-des-Me-S-2840 (14.3%)	0.28	6.59
Cereals late post-emergence (May-June) BBCH 71-89	Small insectivorous bird “passerine”	22.4	1	Glc-NDM-S-2399A (3.8%)	0.077	24.8

Cereals late post-emergence (May-June) BBCH 71-89	Small insectivorous bird “passerine”	22.4	1	N-des-Me-1’CH_2OH-S-2840 (13%)	0.26	7.25

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose
a) As a worst-case initial consideration, the maximum application rate of the parent has been considered
b) As no toxicity data is available for the metabolites, they are assumed to be 10 times more toxic than the active substance
c) An fTWA value of 1 was used for the metabolites as the pattern of decline is not as straightforward as for active substances, since residues initially increase before decreasing. Therefore, a worst-case assumption of an initial 100% conversion from the parent at the start and maintenance of this over the whole averaging period has been used at first tier.
d)DDD amended based on highest percentage TRR for metabolites in plant residues

Based on the above risk assessments, an acceptable reproductive risk is demonstrated for the metabolites: N-des-Me-1’-CH2OH-S-2840, Glc-NDM-S-2399A and N-des-Me-S-2840. However, an unacceptable risk is demonstrated for the metabolites: N-des-Me-DFPA, 3’OH’-S-2840 and DFPA. Further consideration is required.

As the reproductive risk to small insectivorous bird “passerine” at BBCH 71-89 is below the trigger value of 5 for three metabolites (N-des-Me-DFPA, 3’OH’-S-2840 and DFPA), the risk has been considered further by HSE ecotoxicology using a weight of evidence approach.

As the tier 1 risk assessment for birds based on the toxicity endpoint of the parent divided by 10 shows high risk, further consideration is required. The assumption of metabolites being 10 times more toxic than the parent is a worst-case assumption in the absence of toxicity data for metabolites. As no data has been provided considering the effects of individual metabolites on birds, HSE have used the available mammalian toxicology data for the relevant metabolites which fail at tier 1 to consider the reproductive risk to birds. There is some uncertainty extrapolating toxicity data between mammals and birds, but as they are both vertebrates and as the reproductive active substance (parent) NOAELs are broadly similar (19 mg a.s/kg bw/d in birds compared to 25 mg a.s/kg bw/d in mammals), this provides some indication that extrapolation for metabolites is suitable in this instance to assess the reproductive risk to birds in the cereals late post-emergence (May-June) BBCH 71-89 scenario based on mammalian data.

The risk assessment for mammals for the parent demonstrates low risk with a moderate margin of safety (TER = 10.9 compared to a trigger value of 5), with the reproductive endpoint for mammals (25 mg/kg bw/d) showing broadly similar toxicity to the reproductive endpoint for birds (19 mg/kg bw/d).

In terms of metabolites, the mammalian toxicity endpoint for 3’OH’-S-2840 is 37.9 mg/kg/d, which shows similar toxicity to the parent.

For the metabolite N-des-Me-DFPA, the lowest mammalian toxicity endpoint is 300 mg/kg/d based on pre-natal developmental toxicity in rabbit. This demonstrates more than 10 times less toxicity than the parent.

Likewise, DFPA toxicity to mammals produced an endpoint of 250 mg/kg bw/d for pre-natal developmental toxicity in rabbit, demonstrating that the metabolite is 10 times less toxic than the parent.

Based on similar levels of toxicity between the active and the metabolites, as demonstrated from extrapolation of mammalian toxicology data, the TER values for N-des-Me-DFPA, 3'OH'-S-2840 and DFPA would be 33.9, 42.6 and 40.0, respectively, based on 1:1 toxicity. These TER values are greater than the standard reproductive trigger value of 5 and demonstrate an acceptable risk.

HSE Ecotoxicology have also considered the dosing of the experiments to see if this provides more confidence relating to the NOAELs as these have the potential to be influenced by the doses chosen in the experiment. The doses used in the zebra finch study are higher, and have greater intervals, than the rat metabolism study. The doses used in the rat study were; 0, 10, 25 and 80 mg/kg bw/d, and the doses used in the dietary zebra finch study were; 0, 80, 142, 253, 450 and 800 ppm a.s. The determined NOAEL in the rat study was the second-lowest dose (based on foetus weight), and the lowest dose in the bird study (based on clinical signs). There is some uncertainty as the doses are not directly comparable, with doses in the rat study in mg/kg bw/d and food concentrations in the finch study in ppm, which can be converted to mg/kg bw/d, and the endpoints being based on different toxic effects. However, as the NOAELs derived from the studies are broadly comparable (rat NOAEL = 25 mg/kg bw/d, bird NOAEL = 19 mg a.s. bw/d, or 80ppm), and as these are the towards the lower range of doses tested in both studies, this provides some confidence that the NOAELs are not notably influenced by the doses used in the experiments.

To add to the weight of evidence, the avian and mammalian toxicity of two other active substances (Bixafen and Pydiflumetofen) with similar modes of action to inpyrfluxam (SDHI) have been considered. The reproductive bird endpoint for bixafen is 30 mg a.s./kg bw/d and the reproductive mammalian endpoint is 33.3 mg a.s./kg bw/d. These are very similar endpoints and provide additional evidence that extrapolation between avian and mammalian toxicity is suitable for SDHI active substances. For Pydiflumetofen, the reproductive bird endpoint is 90.1 mg a.s/kg bw/d, and the reproductive mammalian endpoint is 36 mg a.s./kg bw/d. Whilst these are not as similar, higher toxicity is demonstrated for mammals compared to birds. This provides further evidence that the extrapolation of mammalian toxicity to avian toxicity does not underestimate the potential risk. Additionally, it is noted that both bixafen and inpyrfluxam are pyrazole-carboxamides, whereas pydiflumetofen is an N-methoxy-(phenyl-ethyl)-pyrazole-carboxamide. HSE Ecotoxicology have further considered the avian and mammalian NOECs available for the

five other authorised pyrazole-carboxamides, all of which have very similar long-term avian and mammalian NOECs, with penthiopyrad having the greatest difference, with the bird endpoint being 3.8 times greater than the mammalian endpoint. Ultimately, this information supports the evidence that it is appropriate to extrapolate avian and mammalian toxicity for inpyrfluxam to use the parent toxicity endpoints for birds for the metabolites.

Ultimately, it is unlikely that the risk to birds from the metabolites will be higher than the parent due to similar or reduced toxicity of the metabolites to mammals. Therefore, the above weight of evidence approach can be used to address the risk to small insectivorous bird “passerine” at BBCH 71-89.

Additionally, it should also be considered that the metabolites were identified from studies on the residues formed in plants. The risk assessment assumes that birds will be exposed from consumption of these residues on plant material. However, the focal species of concern has a diet of 100% insects according to EFSA 2009. It is not clear if these metabolites would form on insects and, if so, if they would be formed at similar levels as for plants.

HSE Ecotoxicology have considered the plant harvest studies in plant matter that are available in the B7 dossier in order to assess whether the TWA value of 1 in the above risk assessment is overly conservative . As these trials are not suitable for quantitative use from an ecotoxicology perspective, these studies have been used to qualitatively assess whether the metabolites 3’OH’S-2840, DFPA and N-des-Me-DFPA show a decline over time, and whether the TWA of 1 can be considered overly conservative. The metabolite N-des-Me-DFPA demonstrates the worst-case TER value in the above risk assessments (3.39), but based on the residue data available, was present at the lowest amounts (most values unbound at <0.01 mg/kg) which generally stays constant, with no increase or decrease shown. Based on the low levels of this metabolite, this provides some evidence that the risk assessment for N-des-Me-DFPA can be considered conservative. This case cannot be made for 3’OH’S-2840 and DFPA as it is not clear that the maximum formation was reached for 3’OH’S-2840 as residues were still increasing, and inconsistent results were shown between studies for DFPA. However, as the above TER values for these metabolites are closer to the trigger value of 5 (4.0 and 4.26), the risk can be considered to be sufficiently addressed based on the weight of evidence from toxicity data above. It is noted that whilst the residue data can be considered qualitatively, there are uncertainties with these studies; including: uncertainty of extrapolating residue data in plants to insects based on the relevant generic focal species of ‘small insectivorous bird – passerine’, and the uncertainty of whether the time period where the maximum % TRR for each of the metabolites from plant data is sufficiently captured in the residue data. HSE Ecotoxicology consider the reproductive risk to birds from the proposed use of inpyrfluxam to be acceptable based on the weight of evidence presented.

HSE Ecotoxicology also note that the reproductive risk assessment for birds demonstrates acceptable risk when considering risk up to BBCH 69. It is noted that application later than BBCH 69 was unlikely; therefore, the likelihood of this specific risk assessment scenario occurring is considered to be low. HSE Efficacy confirmed that the final fungicide application to wheat and durum wheat is usually made at BBCH 59 or BBCH 63 – 65. HSE Efficacy also confirmed that barley is even less likely to have applications after BBCH 69. Approval for the proposed use of inpyrfluxam remains up to BBCH 71; however, for the reasons stated above, this risk to birds from metabolites is considered to be acceptable.

HSE considers the results of the reproductive risk to birds from metabolites do not demonstrate acceptable risk based on TER values; however, following the EFSA Bird and Mammal guidance document (2009), a weight of evidence approach based on available mammalian toxicology data from the metabolites, consideration of the focal species diet and TER values based on 1:1 toxicity between the parent and metabolites has been used to consider acceptable reproductive risk to birds from metabolites. The initial conclusion from HSE is that reproductive risk to birds from metabolites of the parent found in food items is acceptable.

HSE note that in order to definitively remove the assumption of the metabolites being ten times more toxic than the parent, new toxicity studies would be required and this would require three new chronic vertebrate studies. For welfare reasons, HSE consider these are unnecessary.

Ultimately, based on the weight of evidence presented above, acceptable reproductive risk to birds from the metabolites N-des-Me-DFPA, 3'OH'-S-2840 and DFPA can be concluded and no further consideration is required.

Risk to birds via secondary poisoning

The log Pow of the metabolite 3'-OH-S-2840 is 2.53 and is therefore below the trigger value of 3. However, no log Pow values are available for the metabolites N-des-Me-S-2840, N-des-Me-DFPA, Glc-NDM-S-2399A, DFPA, or N-des-Me-1'-CH_2OH-S-2840 and so it is not known whether these metabolites would exceed the trigger value for consideration. None of these metabolites were identified as environmentally significant in any environmental compartment by HSE Environmental Fate and Behaviour, however, and therefore no further consideration is required.

B.9.2.2 Risk assessment for terrestrial vertebrates other than birds

Acute toxicity to mammals

'Inpyrfluxam' is a broad-spectrum fungicide, belonging to the succinate dehydrogenase inhibitor (SDHI) family of fungicides. It is applied as a foliar spray on winter and spring cereals (BBCH 30-71).

Table 9.2.2 -1: Screening assessment for the acute risk of mammals for the proposed worst-case application rate

Intended use	Cereals				
Active substance	Inpyrfluxam				
Application rate (kg/ha)	0.09				
Acute toxicity (mg/kg bw)	180				
TER criterion	10				
Crop scenario	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER**
Cereals	Small herbivorous mammal	118.4	1	10.65	16.9

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

An acceptable acute risk to mammals for the proposed worst-case use of Inpyrfluxam has been demonstrated at the screening stage, with a TER above the trigger value of 10. No further consideration is required.

Long-term/reproductive toxicity to mammals

Table 9.2.2 – 2: Screening assessment for the reproductive risk to mammals for the proposed worst-case application rate

Intended use	**Cereals**					
Active substance	Inpyrfluxam					
Application rate (kg/ha)	0.09					
Reproductive toxicity (mg/kg bw/d)	25					
TER criterion	5					
Crop scenario	**Indicator species**	**SVm**	**MAF_{90}**	**MAF x TWA**	**DDD_{90} (mg/kg bw/d)**	**TER**
Cereals	Small herbivorous mammal	48.3	1	1.0 x 0.53	2.3	10.9

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

An acceptable reproductive risk to mammals from the proposed worst-case use of Inpyrfluxam has been demonstrated at screening stage with a TER above the trigger value of 5. Therefore, no further consideration is required.

Effects of secondary poisoning on mammals

The log P_{ow} of 'inpyrfluxam' amounts to 3.65 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating mammal*s via secondary poisoning*

Table 10.1.2-3: Assessment of the risk for earthworm-eating mammals due to exposure to 'inpyrfluxam' *via* bioaccumulation in earthworms (secondary poisoning)

Parameter	Inpyrfluxam	Comments
PEC_{soil} (mg/kg soil)	0.069	PEC_{soil} (twa = 21 d) + $PEC_{soil\ plateau}$ (i.e. 0.023 + 0.003) (Section B.9.1.3)
P_{ow}	4467	log Pow = 3.65
Koc	647	
foc	0.02	Default
BCF_{worm}	4.21	$BCF_{worm/soil}$ = ($PEC_{worm,ww}/PEC_{soil,dw}$) = (0.84 + 0.12 × P_{ow}) / foc × Koc
PEC_{worm}	0.29	PEC_{worm} = PEC_{soil} × $BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.37	DDD = PEC_{worm} × 1.28
NOEL (mg/kg bw/d)	25	
TER_{lt}	67.28	Trigger = 5

TER values shown in **bold** fall below the relevant trigger

As the above assessment demonstrates the TER to be above the trigger value 5, the risk to earthworm-eating mammals from the proposed use of S-2399 60 g/L EC in cereals is considered acceptable.

Risk assessment to fish-eating mammals via secondary poisoning
According to EFSA/2009/1438, the risk for piscivorous mammals is assessed for a small mammal of 3000 g body weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

Table 9.2.2 - 4: Assessment of the risk for fish-eating mammals due to exposure to 'inpyrfluxam' *via* bioaccumulation in fish (secondary poisoning)

Parameter	Inpyrfluxam	Comments
PEC_{sw} (maximum initial) (mg/L)	0.000831	Worst-case from drift entry (Section B.9.2.5)
BCF_{fish}	215.4*	Range 29-30
PEC_{fish}	0.179	PEC_{fish} = PEC_{water} × BCF_{fish}

Daily dietary dose (mg/kg bw/d)	0.02542	DDD = PEC_{fish} × 0.142
NOEL (mg/kg bw/d)	25	
TER_{lt}	983.57	Trigger = 5

TER values shown in **bold** fall below the relevant trigger
*The worst-case BCF value has been used. As the risk assessment demonstrated a large margin of safety, this has not been considered further.

As the above assessment demonstrates the TER to be above the trigger value 5, the risk to fish-eating mammals from the proposed use of S-2399 60 g/L EC in cereals is considered to be acceptable.

Risk assessment for mammals through drinking water

Two drinking water risk assessment scenarios for birds are available according to EFSA/2009/1438: the leaf scenario, and the puddle scenario. The leaf scenario is not relevant for mammals and so only the puddle scenario will be considered.

Puddle scenario

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 when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

With a K(f)oc value of 651 ml/g, 'inpyrfluxam' belongs to the group of more sorptive substances. Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the critical value of 3000 for at least one scenario, a quantitative risk assessment (calculation of TER values) is not necessary and an acceptable risk to mammals from exposure *via* contaminated drinking water following the proposed uses of S-2399 60 g/L EC is concluded.

Table 9.2.2 –5: Acute and reproductive drinking water exposure assessment for mammals

Effective application rate (g/ha)	=	90		
Acute toxicity (mg/kg bw)	=	180	quotient	= 0.5
Reprod. toxicity (mg/kg bw/d)	=	25	quotient	= 3.6

An acceptable acute and reproductive risk to mammals via drinking water exposure has been demonstrated for the proposed use of inpyrfluxam.

Metabolite assessment

In accordance with the guidance of EFSA (2009), it must be identified if any metabolites are likely to be formed in mammalian food items which may then be consumed by relevant focal species. The available plant metabolism data has already been considered in section B.9.2.1 and the conclusions are repeated below for clarity.

Metabolism in rotational crops was investigated using pyrazole and phenyl labelled inpyrfluxam. From the available plant metabolism data, metabolites were considered relevant where they were formed at ≥ 10% total reactive residues (TRR) or where the TRR was < 10%, but the metabolite was present at ≥ 0.05 mg/kg. For apple, soybean, oilseed rape, maize, sorghum, rice, and potatoes (primary crops), eight plant metabolites, 3'-OH-S-2840, 1'-CH_2OH-S-2840, *N*-des-Me-2840, *N*-des-Me-DFPA (conjugate), Glc-NDM-S-2399A, Gly-1'-CH2OH-S-2840, DFPA-$CONH_2$, and 1'-COOH-S-2840 conjugate were considered relevant. For rotational crops, two further metabolites, DFPA and N-des-Me-1'-CH_2OH-S-2840 were considered relevant in lettuce, sorghum and radish (for full details on studies see Volume 3CA B7, sections 7.2.1 and 7.6.1).

The measured concentrations are provided in Table B.9.2.1-3 below. It should be noted that this data does not provide a specific indication of plant metabolites present at the time mammals would be in the field and therefore potentially exposed. It does, however, provide an indication that these metabolites are formed at significant levels in plant material as part of the plant metabolism of the active substance. Therefore, there is the potential for them to be present in food items consumed by mammals and their further consideration is required with respect to the mammal dietary risk assessment.

Please note that Table B.9.2.1-3 only contains data for metabolites that occurred at ≥ 10% TRR or were < 10% TRR but detected at ≥ 0.05 mg/kg in those parts of the plant considered to be consumed by mammals.

On the basis of the data summarised in section B.9.2.1, it is clear that several metabolites occur in plant material at levels that require further consideration:

- 3'-OH-S-2840
- 1'-CH_2OH-S-2840
- *N*-des-Me-S-2840
- *N*-des-Me-DFPA
- Glc-NDM-S-2399A
- Gly-1'-CH_2OH-S2840
- DFPA-$CONH_2$
- 1'-COOH-S-2840
- DFPA
- *N*-des-Me-1'-CH_2OH-S-2840

In order to determine whether a dietary risk assessment is necessary for these metabolites for mammals, metabolism studies conducted on rats have been checked, since a risk assessment will not be required if the metabolites in question are formed at sufficient levels in mammals as the risk will be considered covered by the risk assessment for the active substance.

There are two available rat metabolism studies in Volume 3CA B6, section B.6.1.1. The studies are as follows:

- Study 1 [redacted] (2016a) Metabolism of S-2399 in rats
- Study 2 [redacted] (2016b) Metabolism of inpyrfluxam in rats – repeated exposure

The results of these rat metabolism studies in the context of the above relevant metabolites have been considered below:

Table B.9.2.1-4: Summary of the rat metabolism studies

Metabolite	Present in the rat study?
3'-OH-S-2840	N/A
1'-CH_2OH-S-2840	4.1% administered dose in faeces (males, single exposure, pyrazolyl), 1.0% administered dose in faeces (female, single exposure, pyrazolyl), 1.1% administered dose in bile (male, single exposure, pyrazolyl), 0.5% administered dose in bile (female, single exposure, pyrazolyl), 4.4% of TRR in faeces in males (repeated exposure)
N-des-Me-S-2840	1.0% administered dose in faeces (males, single exposure, pyrazolyl), 2.2% administered dose in faeces (females, single exposure, pyrazolyl), 3% TRR in faeces in females (repeated exposure)
N-des-Me-DFPA	N/A
Glc-NDM-S-2399A	N/A
Gly-1'-CH_2OH-S2840	N/A
DFPA-$CONH_2$	N/A
1-COOH-S-2840	**15% of administered dose in urine (males, single exposure, pyrazolyl)**, 2.4% of administered dose in faeces (male, single

	exposure, pyrazolyl), 12.3% administered dose in urine (females, single exposure, phenyl), 2.4% administered dose in faeces (female, single exposure, phenyl), 3.2% administered dose in bile (males, single exposure, pyrazoyl), 0.6% administered dose in bile (female, single exposure, pyrazolyl), 10.4% TRR in urine in males (repeated exposure, pyrazolyl), 3.9% TRR in faeces in males (repeated exposure),
DFPA	N/A
N-des-Me-1'-CH_2OH-S-2840	3.4% of administered dose in urine (males, single exposure, pyrazolyl), 7.8% of administered dose in faeces (males, single exposure, pyrazolyl), 4.1% administered dose in urine (females, single exposure, phenyl), 6.3% administered dose in faeces (females, single exposure, pyrazolyl), 8.6% administered dose in faeces (females, single exposure, phenyl), 0.8% administered dose in bile (females, single exposure, pyrazolyl), 3.0% TRR in urine in male and female (repeated exposure), **10% of TRR in faeces in females (repeated exposure)**, 9.1% in male faeces (repeated exposure) 0.8% administered dose in bile (female, single exposure, pyrazolyl)

N/A – not applicable (not found in studies)
Values in **bold** are present at > 10% TRR and can be excluded.

Based on the above information, the following metabolites can be excluded as they are present at >10% TRR

- 1-COOH-S-2840
- *N*-des-Me-1'-CH_2OH-S-2840

Therefore, the following metabolites require further consideration:

- 3'-OH-S-2840
- 1'-CH_2OH-S-2840
- *N*-des-Me-S-2840
- *N*-des-Me-DFPA
- Glc-NDM-S-2399A
- Gly-1'-CH_2OH-S2840
- DFPA-$CONH_2$
- DFPA

The applicant has stated the following regarding metabolites present in the rat metabolism studies:

"*The toxicity of 1'-CH_2OH-S-2840 is considered to be covered by the toxicity of parent as the ADME data show that it represents >10 % of the urinary and biliary excretion. Studies with 1'-COOH-S-2840 and 3'-OH-S-2840 show them to have comparable or lower toxicity to the parent compound, and therefore the toxicological endpoints for S-2399 are appropriate for these metabolites also".*

HSE agree with the above reasoning and can exclude these metabolites with no further consideration required.

HSE Toxicology has provided further information on the remaining metabolites either not present in the rat metabolism studies or present at <10% TRR. These details can be found in Part B6, section B.6.8.1.2.

Metabolite data is available that can exclude the following metabolites from requiring further consideration as they are not more toxic than the parent: *N*-des-Me-DFPA, DFPA and DFPA-$CONH_2$. Based on this, these metabolites can be excluded.

Further consideration for the remaining metabolites has also been provided by HSE Toxicology. For Gly-1'-CH_2OH-S2840, the following is stated: *"As the sugar conjugate of 1'-CH2OH-S-2840, a predicted major rat metabolite, covered by parent – the sugar will be cleaved in the GI tract releasing the predicted major rat metabolite".* Therefore, HSE Ecotoxicology agree that the risk from this metabolite is covered by 1'CH_2OH-S-2840, which is a major rat metabolite; therefore does not need consideration.

HSE Toxicology consideration for N-des-Me-S-2840 provided the following: *"No additional relevant alert for general toxicity was identified in the comparative QSAR analysis compared to inpyrfluxam".* Based on this, HSE Ecotoxicology can exclude this metabolite and no further consideration is required as the risk is covered by the parent.

In relation to Glc-NDM-S-2399A, HSE Toxicology state the following *"As the conjugated form of the metabolite N-des-Me-S-2840, the toxicity profile of this metabolite is expected to be comparable / less severe compared to its aglycon form N-des-Me-S-2840; no concern for genotoxicity was identified in the comparative QSAR analysis of N-des-Me-S-2840 compared to inpyrfluxam".* Again, HSE agree with the above consideration and can exclude the above metabolite as the risk is covered by the parent.

From the above consideration provided by the applicant, HSE Toxicology, and the rat metabolism studies, all metabolites can be excluded and no further acute or reproductive risk assessment nor consideration of the risk via secondary poisoning is required to consider the risk to mammals.

B.9.3 Effect on aquatic organisms

B.9.3.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Reference:	KCP 10.2.1/01
Report Title:	S-2399 6 EC: Fish, Acute Toxicity Test
Author(s) & year:	[redacted] (2020a)
Document No, Authority registration No:	[redacted] Study No. 3202418 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0120
Substance used:	S-2399 6 EC, V16-7L1901, 6.544 ± 0.024% w/w
Method of analysis:	LC-TOF/MS
Guideline(s):	OECD 203 (2019)
Deviations:	Yes, see HSE Comments
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

MATERIALS AND METHODS

MATERIALS

Test material S-2399 6 EC

Batch code: V16-7L1901

Active Substance Content: 6.544 ± 0.024% w/w or 65.44 ± 0.24 g/kg or 60.68 ± 0.23 g/L (verified by certificate of analysis)

Description: Amber liquid

Stability of test compound: Not stated

Retest date: 17 May 2021

Storage on Receipt: Room Temperature (15 – 30°C)

TREATMENTS

Test concentrations: Control (dilution water), nominal formulation concentrations of 0.0625, 0.125, 0.25, 0.50 and 1.0 mg/L, nominal active substance concentrations of 0.00409, 0.00818, 0.0164, 0.0327, 0.0654 mg a.s./L, geometric mean measured active substance concentrations of 0.00365, 0.00772, 0.0136, 0.0357, 0.0709 mg a.s./L

Solvent: None

Toxic reference None

Analysis of test concentrations:	Yes, at 0 (new media), 48 (old and new media) and 96 hours (old media) (all treatment levels and control) based on analysis of S-2399 using liquid chromatography-time of flight mass spectrometry (LC-TOF/MS) system. The limit of quantification of the analytical method is 0.0005 mg S-2399/L

TEST ORGANISMS

Species:	Rainbow trout (*Oncorhynchus mykiss*)
Source:	[redacted]
Acclimatisation period:	≥ 9 days
Treatment for disease:	None reported
Weight and length of a representative sample of fish (n = 10):	Mean wet weight: 1.6 g (range: 1.4 to 1.7 g) Mean length: 57 mm (range: 52 to 60 mm)
Loading rate:	0.54 g fish/L
Feeding:	Proprietary fish food, added to holding tanks over two feeding intervals in quantities dictated by fish size. The fish were not fed throughout test or 24 hours before.

TEST DESIGN

Test vessels:	Single 20 L constructed glass aquaria, each fitted with an appropriate lid, containing 20 L of media.
Test medium:	Treated mains water
Replication:	One replicate
No of fish per tank:	7
Exposure regime:	Semi-static
Duration:	96 hours

TEST CONDITIONS

Test temperature:	12.6 – 13.0 °C
pH:	7.01 – 8.30
Salinity	Not applicable
Dissolved oxygen:	94.2 – 105.1 % Air saturation value (ASV)
Hardness of dilution water:	68-131 mg $CaCO_3$/L
Lighting:	photoperiod of 16 hours light and 8 hours darkness with *ca* 30-minute dawn/dusk phases

STUDY DESIGN AND METHODS

Experimental dates: the 96-hour definitive exposure was conducted from 14 to 18 October 2019.

Test organism and acclimatisation

The Rainbow trout (*Oncorhynchus mykiss*), juvenile), commonly used in acute freshwater toxicity tests, was selected as the test species. Prior to testing, fish were acclimatised for ≥ 9 days under species appropriate conditions. During acclimatisation the fish were fed commercially prepared over two feeding intervals during the day. Uneaten food and debris was siphoned or cleaned from the tanks as required. The mortality rate of the stock batch of fish was 0% in the 7 days prior to the definitive test.

Test water

The dilution water (referred to hereafter as treated mains water (TMW)) used for conducting the tests was treated mains water which was pumped to the laboratory through an activated carbon filter, particulate filter and a UV steriliser. Representative samples of the dilution water source were analysed periodically for the presence of pesticides, PCBs and toxic metals. None of the compounds listed in Annex 3 of OECD 203 (2019) were above the Limit Of Detection (LOD). Chlorine content was 0.06 and 0.00 mg Cl_2/L at exposure initiation and termination respectively.

Definitive test and dose preparation

A semi-static test system was employed for the definitive test, due to the low stability of the test substance, with test media preparation at 0 and 48 hours over a test duration of 96 hours. One replicate of seven fish per test concentration and control were tested. A dilution water control was tested in parallel. At test start, seven randomly selected fish from a holding stock were added to test and control vessels.

At test start and media renewal at 48 hours, *ca.* 50 mg of test item was dispersed in 1000 mL of treated mains water to give a 50 mg/L stock. Aliquots (i.e. 400, 200, 100, 50 and 25 mL) of the 50 mg/L stock were each separately dispersed in a volume of 20 L of treated mains water to give the 1.0, 0.50, 0.25, 0.125 and 0.0625 mg product/L test concentrations, respectively (corresponding to 0.00409, 0.00818, 0.0164, 0.0327 and 0.0654 mg a.s./L). The control was prepared by adding treated mains water only to the test vessel. The test was conducted in a temperature-controlled laboratory (12 ± 2ºC) with a 16-hour light: 8-hours dark lighting cycle with ca 30-minute dawn/dusk phases.

Measurements and observations

All fish were observed for mortality and toxic symptoms or modified behaviour at ca. 2, 5, 24, 48, 72 and 96 hours. Additional observations were made at 24-hour + ca. 6-hour intervals and findings were recorded. Fish exhibiting toxic symptoms at or above the severe severity limit were humanely killed and classed as mortalities.

At the start of the test (0 hours) and media renewal at 48 hours samples of freshly prepared test media, and at 48 and 96 hours samples of old test media, were taken from the control and the test media preparation vessels for chemical analysis. Samples were analysed by injection onto a LC-TOF/MS system. Geometric mean measured concentrations were calculated.

At the start of the test and at approximately 24-hour intervals during the test, the media pH, concentration of dissolved oxygen (% air saturation value and mg/L) and the individual test media temperatures were recorded in the freshly prepared and old test media as appropriate. Continuous temperatures were also documented with the use of a max/min thermometer. Hardness and chlorine content of the control media were determined at the start of the test. In error, the hardness and chlorine content were not determined at media renewal at 48 hours as required by the protocol.

Statistical analysis
The LC50 values at 24 and 48 hours were calculated using untrimmed Spearman-Kärber whilst the 72 and 96-hour LC50 values were calculated using Binomial/Graphical method. The NOEC values were estimated empirically.

Statistical analysis was performed using the CETIS program v 1.8.6.8.

RESULTS AND DISCUSSION
The test preparations were observed to be colourless solutions throughout the duration of the test.

Mortality
Effects on mortality are summarised in Table 9.3-1 and endpoints are presented in Table 9.3-2.

Table 9.3-1: Cumulative mortality in rainbow trout

Nominal concentration [mg product/L]	Geometric mean measured concentration [mg a.s./L]	No. of fish exposed	Cumulative mortalities recorded [no.] / [%]			
			24 h	48 h	72 h	96 h
Control		7	0 / 0	0 / 0	0 / 0	0 / 0
0.0625	0.00365	7	0 / 0	0 / 0	0 / 0	0 / 0
0.125	0.00772	7	0 / 0	0 / 0	0 / 0	0 / 0
0.25	0.0136	7	0 / 0	0 / 0	0 / 0	0 / 0

Nominal concentration [mg product/L]	Geometric mean measured concentration [mg a.s./L]	No. of fish exposed	Cumulative mortalities recorded [no.] / [%]			
			24 h	48 h	72 h	96 h
0.50	0.0357	7	3 / 43	5 / 71	7 / 100	7 / 100
1.0	0.0709	7	7 / 100	7 / 100	7 / 100	7 / 100

Table 9.3-2: Acute toxicity of S-2399 60 G/L EC to rainbow trout (*Oncorhynchus mykiss*)

Endpoint	Value (95% confidence limits)	
	Nominal concentration [mg product/L]	Mean measured concentration [mg a.s./L]
24-hour LC_{50}	0.53 (0.41 – 0.68)	0.0353 (0.0259 – 0.0481)
48-hour LC_{50}	0.43 (0.34 – 0.55)	0.0279 (0.0210 – 0.0370)
72-hour LC_{50}	0.35 (0.27 – 0.46)	0.0220 (0.0153 – 0.0317)
96-hour LC_{50}	0.35 (0.27 – 0.46)	0.0220 (0.0153 – 0.0317)
24 to 96-hour NOEC	0.25	0.0136

Abnormal effects and other observations

There were no toxic symptoms in fish observed within the study duration of 96 hours at nominal test concentrations up to and including 0.25 mg product/L (corresponding to 0.0136 mg a.s./L). In the highest treatment group of nominal 1.0 mg product/L (corresponding to 0.0709 mg a.s./L), all fish died within 2 hours. Moderate (e.g. fish swimming abnormally or lying on bottom of tank) and severe (fish classed as dead) toxic effects were observed at the nominal concentration of 0.50 mg product/L (corresponding to 0.0357 mg a.s./L) starting in all fish at 2 hours (6× moderate, 1× severe). All fish were dead after ca. 54 hours in this treatment group.

Analytical results

Geometric mean measured concentrations were 0.00365, 0.00772, 0.0136, 0.0357 and 0.0709 mg a.s./L corresponding to 89, 94, 82, 109 and 108% of nominal. Results are presented based on nominal (product) and mean measured (active substance) concentrations. A summary of the analytical results is presented in Table 9.3-3.

Table 9.3-3: Summary of analytical results

Nominal concentration [mg product/L]	Nominal concentration [mg a.s./L]*	Measured concentration [mg a.s./L] / [%] of nominal				Geometric mean measured concentration [mg a.s./L]	[%] of nominal (active substance)
		0 hours (fresh)	48 hours (old)	48 hours (fresh)	96 hours (old)		
Control		< LOQ	< LOQ	< LOQ	< LOQ	n.a.	n.a.
0.0625	0.00409	0.00482 / 118	0.00289 / 71	0.00434 / 106	0.00293 / 72	0.00365	89
0.125	0.00818	0.00978 / 120	0.00617 / 75	0.00911 / 111	0.00645 / 79	0.00772	94
0.25	0.0164	0.0151 / 92	0.0106 / 65	0.0170 / 104	0.0124 / 76	0.0136	83
0.50	0.0327	0.0390 / 119	0.0299 / 91	0.0373 / 114	0.0375 / 115	0.0357	109
1.0	0.0654	0.0643 / 98	0.0707 / 108	0.0744 / 114	0.0747 / 114	0.0709	108

n.a. not applicable

* Based on an active substance purity of 65.44 g/kg

Validity criteria

The validity criteria for the study were met according to OECD 203 (2019) (Table 9.3-4).

Table 9.3-4: Compliance with OECD 203 validity criteria

Validity criterion	Required	Obtained
Mortality in the control(s)	≤ 10 %	0 %
Test conditions	Constant conditions	A semi-static design was selected. Constant conditions were maintained.
Dissolved oxygen concentration	At least 60 % of the air saturation value throughout the test	Dissolved oxygen concentration > 60 % of the air saturation throughout the test.
Concentration of substance	Analytical measurement of test concentrations is compulsory. At least 80 % of the nominal concentration throughout the test. If the deviation from the nominal concentration is greater than 20 % results should be based on the measured concentration.	Analytical measurements were performed. The semi-static design resulted in the geometric mean measured concentrations staying within 80% of the nominal concentrations. Individual timepoints did, however, diverge from this range (recoveries ranged from 92 - 120 % in fresh solutions and 65 -115% in old solutions). Therefore, endpoints were derived using the geometric mean measured concentrations.

CONCLUSIONS

The acute 96-hour LC50 for rainbow trout (Oncorhynchus mykiss) exposed to S-2399 6 EC was determined to be 0.35 mg product/L based on nominal concentrations and 0.0220 mg a.s./L based on geometric mean measured concentrations. The corresponding NOEC was determined to be 0.25 mg test item/L (nominal) or 0.0136 mg a.s./L (geometric mean measured).

HSE COMMENTS

The study was carried out according to and evaluated against the OECD 203 guideline (2019). All validity criteria outlined in OECD 203 (2019) were satisfactorily met for the duration of the study. There were no significant deviations to the guideline.

The following deviations were noted:
OECD 203 (2019) paragraph (§) 9 states that the study report method include a description of the apparatus used to carry out the test and appropriate documentation to validate equipment functionality. The equipment used throughout the study was not reported. Appropriate documentation to validate equipment functionality was also omitted. These omissions are unlikely to affect study outcome considering the up-to-date GLP certification of the study laboratory. HSE consider this a minor deviation.

OECD 203 (2019) § 10 states, *"test vessels should be randomly positioned in the test area and shielded from unwanted disturbance (excessive noise, vibration, light)"*. This was not detailed in the study report. HSE consider this a minor deviation.

OECD 203 (2019) § 14 outlines the criteria for test fish population mortality and health during the acclimatisation period. No detailed information was provided detailing signs of disease, stress, malformations or treatments against disease or parasites within 14 days prior to testing. The study conductors reported no mortality for the seven days prior to exposure initiation, as well as no mortality or sub-lethal effects during the exposure period in the test dilution water controls, indicating the health of the test fish population was acceptable. HSE consider this a minor deviation which is not expected to impact the study outcome.

OECD 203 (2019) § 15 presents the chemical characteristic requirements of water used during the study. It states, *"any water which conforms to the chemical characteristics of acceptable dilution water as listed in Annex 3 is suitable as a test water".* The study report attached a representative analytical report detailing the levels of toxic metals and other elements within test water. The (analytical sensitivity) for many elements (Cu, Fe, Pb, Zn, Al and Cd) were not sensitive enough to determine whether the water conformed to the chemical characteristics detailed in Annex III of OECD 203 (2019). The lack of mortality or sub-lethal effects within the dilution water control suggests the water selected is not expected to impact the study outcome. HSE considers this a minor deviation.

OECD 203 (2019) § 16 details required chemical testing of dilution water. Within this paragraph it states, "*analyses of nitrate and chlorine should be performed on each batch of dilution water to demonstrate that the limits specified in Annex 3 are not exceeded*". The study reported chlorine levels in the test water was 60 µg/L at exposure initiation. Annex III defines a maximum concentration of 10 µg/L. Again, the lack of mortality or sub-lethal effects in the dilution water control suggests residual chlorine levels did not impact the health of test fish. Furthermore, the chlorine and water hardness were not measured at 48 hours. HSE consider this a minor deviation which is not expected to impact the study outcome.

OECD 203 (2019) § 19 states, *"Light: should be within the photoperiod ranges specified for the test species (Annex 2) and with an intensity of 10-20 µE/m2 /s, 540-1000 lux, or 50-100 ft-c (ambient laboratory levels)"*. A specific light intensity range was not reported. Since the

validity criteria have been met HSE consider this a minor deviation which is not expected to impact the study outcome.

OECD 203 (2019) § 21 concerns integrating existing sources of information into test concentration selection if such information is available. The study provided no evidence of this approach being attempted. HSE note the omission of this point is not expected to impact the study outcome. HSE consider this a minor deviation.

OECD 203 (2019) § 25 covers the requirements for analytical determinations. It states, *"TOC…should be measured…at the beginning of the exposure in the dilution water".* This was not reported. However, the lack of mortality or sub-lethal effects in the control demonstrates the suitability of the test water. HSE consider this a minor deviation.

OECD 203 (2019) § 33 outlines the requirements for the test report, including the reporting of test substance physico-chemical properties. The study report did not provide the required physico-chemical properties or a structural formula. Analytical measurement at exposure initiation and completion confirmed the presence of the test substance in the test solution indicating the omission of the physico-chemical properties did not impact test integrity. Furthermore, geometric mean measured concentrations were within 80 % - 120 % of the nominal concentration. HSE consider this a minor deviation which is not expected to impact the study outcome.

Although not a deviation from OECD 203 (2019), a 96-hour concentration response graph was not provided since the slope cannot be meaningfully represented for experiments with less than two partial mortalities. In this scenario, a graph is not a requirement of OECD 203 (2019).

Finally, although mean measured concentrations, expressed as mg a.s/L, were provided, they were not also reported expressed as mg product/L. As the measured concentration of active substance did fall outside the 80 – 120 % range for some timepoints, this should have reported. This, however, can be easily calculated using the active substance purity of 65.44 g/kg product.

The method of analysis used in the study was evaluated by HSE Chemistry. The conclusions of their evaluation are reproduced below. Please see Volume 3 CA, section B5 for more details.

"*The analytical method is not fully validated according to SANTE/2020/12830 rev. 1 for the determination of the active substance inpyrfluxam in treated mains water as the matrix effects and stock solution stability have not been determined. However, the study was generated prior to the implementation of SANTE 2020/12830 rev.1 and SANCO 3029 rev.4 did not require matrix effects and stock solution stability to be addressed. As all other*

validation requirements have been met (including the minimum validation requirements outlined in SANTE 2020/12830 rev.1), the method is considered to be fit for purpose."

The above study was conducted to GLP and considered valid.

The agreed endpoint suitable for use in the risk assessment is: 96-hour LC_{50} = 0.0220 mg S-2399/L, which equates to 0.336 mg product/L.

Reference:	KCP 10.2.1/02
Report Title:	S-2399 6 EC: Acute Toxicity to *Daphnia magna*
Author(s) & year:	[redacted] (2020b)
Document No, Authority registration No:	Smithers ERS Limited Study No. 3202417 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0116
Substance used:	S-2399 6 EC, V16-7L1901, 6.544 ± 0.024% w/w
Method of analysis:	LC-TOF/MS
Guideline(s):	OECD 202 (2004)
Deviations:	Yes, see HSE Comments
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

MATERIALS

Test Material	S-2399 60 G/L EC
Lot/Batch #:	V16-7L1901
Active substance content:	Nominal: 60 g S-2399/L Analysed: 6.544 ± 0.024 % (w/w) or 65.44 ± 0.24 g/kg or 60.68 ± 0.23 g/L (verified by the certificate of analysis)
Description:	Amber liquid
Retest date:	17 May 2021
Treatments	
Test concentrations:	Elendt M4 medium control and nominal concentrations of 0.625, 1.25, 2.5, 5.0 and 10 mg product/L (0.0409, 0.0818, 0.164, 0.327 and 0.654 mg a.s./L)
Negative control:	Elendt M4 medium
Positive control:	Potassium dichromate
Analysis of test concentrations:	Yes, analysis at 0 and 48 hours using liquid chromatography-time of flight mass spectrometry (LC-

	TOF/MS) analysis. The LOQ of the procedure is 0.001 µg/mL.
Test organisms	
Species:	*Daphnia magna* Straus
Source:	MicroBio Tests Inc., Belgium
Feeding:	None during test
Culture medium:	Elendt M4 medium
Test design	
Test vessels:	Glass tall form beakers (100 mL) containing approximately 50 mL media
Test medium:	Elendt M4 medium
Replication:	4 replicates of 5 juvenile daphnids
Exposure regime:	Static
Duration:	48 hours
Environmental conditions	
Test temperature:	18.7 to 20 °C (initiation and termination measurements) 18.8 to 21 °C (continuous measurement)
pH range:	7.26 to 7.87
Dissolved oxygen:	97.1 to 101.4 % air saturation value (ASV)
Lighting:	16-hour light, 8-hour dark photoperiod

STUDY DESIGN AND METHODS

Experimental dates: 2 to 4 October 2019

Test organism

Daphnia magna, commonly used in freshwater invertebrate toxicity testing, were selected for this study. *D.magna* were cultured in Elendt M4 medium and fed daily with a concentrated suspension of *Chlorella vulgaris*. Regular testing using the reference toxicant potassium dichromate was performed to assess culture sensitivity. Juveniles (< 24 hours old, second brood onwards) were removed from healthy cultures and actively swimming individuals were selected for the definitive test.

Test media and dose preparation

The dilution solution used in the study was Elendt M4 medium, which is recommended for the long-term culture of *D.magna* in OECD 211 (2012)[2]. At the start of the test, 10 mg (actual: 10.01 mg) of test substance was dissolved in a final volume of 1000 mL of Elendt M4 medium, with the aid of vigorous stirring for 30 minutes, to give the 10 mg/L test concentration. Serial dilutions were prepared in Elendt M4 from the 10 mg/L test concentration to give the remaining test concentrations of 5.0, 2.5, 1.25 and 0.625 mg

[2] OECD (2012), Test No. 211: *Daphnia magna* Reproduction Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264185203-en

product/L. The control was prepared by adding Elendt M4 only to the vessels.

Definitive test
Five juvenile *Daphnia magna*, less than 24 hours old, were added to each test and control vessel, using a wide bore glass pipette/tube to avoid damaging the animals during transfer. The *Daphnia magna* were not fed during the test.

In parallel, abiotic vessels were also prepared for the control and each test concentration which contained no test organisms and were incubated alongside the test vessels. These vessels were used for analytical sampling at 48 hours.

Measurements and observations
After 24 and 48 hours, the *Daphnia magna* in each test vessel were observed for evidence of immobility. The observations differentiated between mobile and immobile daphnids. An individual was considered immobile if, when the contents of the test vessel were briefly agitated, it did not swim during a 15-second period of observation. In addition, *Daphnia magna* submerged in the body of the test media and those that were held at the surface of the test media were also recorded.

The pH, dissolved oxygen concentration (% air saturation value (ASV) and mg/L) and temperatures were determined in freshly prepared test media at the start of the test and in the old media at 48 hours. Continuous temperatures were measured using a digital (min/max) thermometer in an additional vessel maintained in the test area. The test vessels were not aerated during the test.

At the start of the test (0 hours), 10 mL samples of freshly prepared test media were taken from the control and the test media preparation vessels for chemical analysis. At 48 hours, 10 mL samples were also taken for chemical analysis from the pooled old test media from the test and control groups and abiotic vessels. Samples were analysed by injection onto a LC-TOF/MS system.

Statistical analysis
Toxicity results were expressed in terms of the concentration that immobilised 50% of the *Daphnia magna* after a 24 and 48 hours exposure (24 and 48-hour EC_{50}).

The highest nominal concentration where no significant immobilisation was observed was defined as the no observed effect concentration (NOEC). The concentration immediately above the NOEC where significant effects were observed was defined as the lowest observed effect concentration (LOEC).

Statistical analysis was performed using the CETIS program v 1.8.6.8. The EC_{50} values were calculated using linear interpolation. The NOEC and LOEC were estimated

empirically. The toxicity results are expressed in terms of the nominal formulation concentrations and mean measured active substance (a.s.) concentrations.

RESULTS AND DISCUSSION

Immobilisation

Immobilisation data for the definitive test are summarised in Table 9.3-5 and endpoints are presented in Table 9.3-6.

Table 9.3-5: Immobilisation of *Daphnia magna*

Nominal concentration [mg product/L]	Mean measured concentration [mg a.s./L]	No. of Daphnia exposed	Immobilisation [no.] / [%]	
			24 h	48 h
Control		20	0 / 0	0 / 0
0.625	0.0404	20	0 / 0	0 / 0
1.25	0.0797	20	0 / 0	0 / 0
2.5	0.161	20	0 / 0	0 / 0
5.0	0.311	20	9 / 45	15 / 75
10	0.673	20	19 / 95	20 / 100

Table 9.3-6: Acute toxicity of S-2399 60 G/L EC to *Daphnia magna*

Endpoint	Value (95% confidence limits)	
	Nominal concentration [mg product/L]	Mean measured concentration [mg a.s./L]
24 h EC_{50}	5.4 (4.3 – 6.0)	0.34 (0.27 – 0.40)
48 h EC_{50}	4.0 (3.5 – 4.8)	0.26 (0.23 – 0.30)
24/48 h LOEC	5.0	0.31
24/48 h NOEC	2.5	0.16

All daphnids (mobile and immobile) were observed submerged in the media with no daphnids observed trapped at the surface. Adverse effects (unusual or abnormal behaviour) except for immobility were not observed among daphnids exposed to any of the treatment levels tested or control.

The 48-hour reference test established that the EC_{50} value for *D.magna* and potassium dichromate was 1.3 mg/L (conducted 15 – 17 January 2019). This result was within the

expected range for *D.magna* exposed to potassium dichromate (0.6 – 2.1 mg/L from ISO 6341:2012).

The 48-hour concentration response visualised in Figure 9.3.1-1.

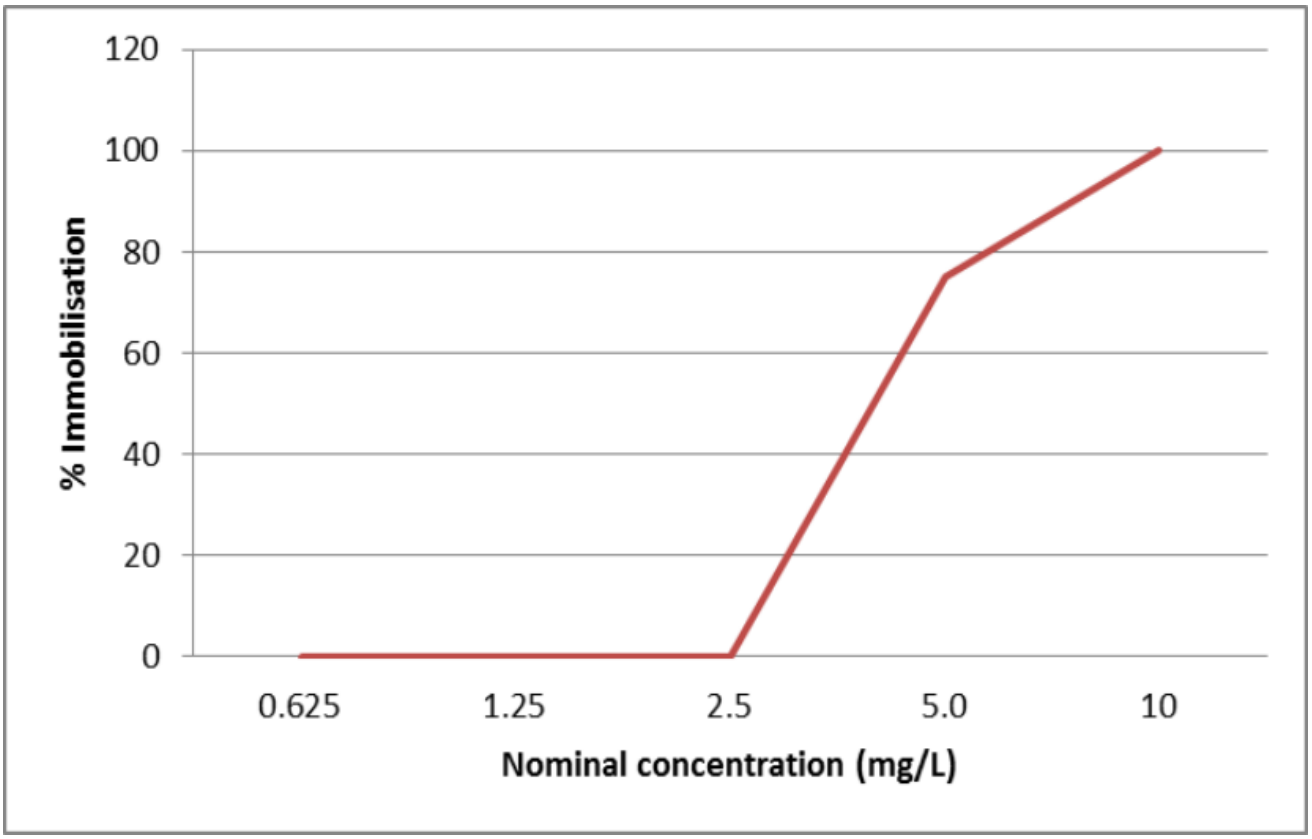

Figure 9.3-1: The 48-hour dose-response curve for S-2399 60 G/L EC and *D.magna*

Chemical analysis

The results of the chemical analysis are present in Table 9.3-7. The percentage of the nominal concentration ranged from 92 - 109 %. The test preparations were colourless and no undissolved test substance was observed in any of the test preparations.

Table 9.3-7: Analytical measurements at exposure initiation and termination

Nominal formulation concentration (mg/L)	Nominal active substance concentration (mg a.s./L)*	Measured concentration (mg/L)				Mean measured active substance concentration (mg a.s./L)	% nominal of active substance
		0 hours (new media)	% nominal of active substance	48 hours (old media)	% nominal of active substance		
Control	Control	<LOQ	-	<LOQ	-	-	-
0.625	0.0409	0.0404	99	0.0404	99	0.0404	99
1.25	0.0818	0.0798	99	0.0795	97	0.0797	97
2.5	0.164	0.163	99	0.158	96	0.161	98
5.0	0.327	0.321	98	0.300	92	0.311	95
10	0.654	0.711	109	0.634	97	0.673	103

LOQ = Limit of quantification of the analytical method (0.001 mg/L)
a.s. = active substance
*based on active substance purity of 65.44 g/kg
- = Not applicable

The results from the abiotic vessels showed measured concentrations to range from 97 to 103% of nominal active substance. Given that the results from the abiotic samples were similar to those obtained from the vessels containing the test organisms, it was considered that the test substance was stable in the test media over a 48-hour period with or without the presence of organisms.

Validity criteria
The validity criteria for the study were met according to OECD 202 (2004) (Table 9.3-8).

Table 9.3-8: Compliance with OECD 203 validity criteria

Validity criterion	Required	Obtained
Immobilisation in the controls	≤ 10 % immobilisation or other signs of disease or stress in the control(s) (dilution water	Dilution media control: 0 %

Validity criterion	Required	Obtained
	control, solvent control)	
Dissolved oxygen concentration	≥ 60 % of the air saturation value (≥ 3 mg/L dissolved oxygen) in all test vessels throughout the exposure	Dissolved oxygen concentration ranged from 97.1 to 101.4 % (8.41 mg/L to 9.57 mg/L).

CONCLUSION

Based on mean measured concentrations, the 48 hour EC_{50} for S-2399 6 EC to *D.magna* was 0.26 mg a.s./L (0.23 – 0.30) or 3.97 mg product/L (3.52 – 4.58). The 48-hour NOEC was 0.16 mg .a.s./L or 2.5 mg product/L.

HSE COMMMENTS

The study was carried out according to GLP and follows OECD 202 (2004). All validity criteria were met.

OECD 202 (2004) § 9 states, "*stock animals must be maintained in culture conditions (light, temperature, medium) similar to those to be used in the test*". The temperature used for laboratory cultures was not reported precluding the comparison of the culture and test temperatures. Considering *D.magna* stocks were described as healthy this reporting omission is not expected to have impacted study integrity. HSE considers this deviation acceptable.

OECD 202 (2004) § 18 states, "*the temperature should be within the range of 18°C and 22°C, and for each single test it should be constant within ±1 °C*". Temperature ranged from 18.7 to 20 °C (initiation and termination measurements) and 18.8 to 21 °C (continuous measurement). This deviation is not expected to have impacted test integrity given that temperatures remained within the 20 ± 2ºC overall range specified in OECD 202 (2004) and no immobilisation was observed in the control group. HSE considers this deviation acceptable.

OECD 202 (2004) § 25 and 26 detail the appropriate statistical methods to be used when calculating EC_{50} values and associated confidence intervals. There was only one concentration with partial mortality, which precludes the use of standard classical maximum likelihood methods for fitting probit or logit methods. OECD 202 (2004) states, "*where the standard methods of calculating the EC50 are not applicable to the data obtained, the highest concentration causing no immobility and the lowest concentration producing 100 per cent immobility should be used as an approximation for the EC50 (this being considered*

the geometric mean of these two concentrations)". This approach was not taken by the study conductor. Instead, they performed linear interpolation. If the approach recommended by the guidance document is performed the 48-hour EC_{50} = 0.329 mg a.s./L (geometric mean of 0.161 and 0.673 mg a.s./L). The provided 48-hour EC_{50} = 0.26 mg a.s./L is more protective and therefore retained for the purposes of risk assessment.

OECD 202 (2004) § 27 outlines the requirements for the test report. Within the test conditions section, it states that light intensity should be included. This was not reported. The only information provided was that cultures were maintained under fluorescent lighting. This reporting omission is not expected to have impacted study integrity as there was no reported immobilisation in the control group. Therefore, HSE considers this a minor deviation.

Finally, OECD 202 (2004) Annex 3 outlines the recommended preparation of Elendt M4 medium. The study conductor added 47.9 mg/L of Na2SeO4 instead of the recommended 43.8 mg/L of Na2SeO3 when preparing the medium. Considering the lack of control immobilisation and healthy *Daphnia* cultures HSE consider this a minor deviation.

The method of analysis used in the study was evaluated by HSE Chemistry. The conclusions of their evaluation are reproduced below. Please see Volume 3 CA, section B5 for more details.

"*The analytical method is not fully validated according to SANTE/2020/12830 rev. 1 for the determination of the active substance inpyrfluxam in Elendt medium as the matrix effects and stock solution stability have not been determined. However, the study was generated prior to the implementation of SANTE 2020/12830 rev.1 and SANCO 3029 rev.4 did not require matrix effects and stock solution stability to be addressed. As all other validation requirements have been met (including the minimum validation requirements outlined in SANTE 2020/12830 rev.1), the method is considered to be fit for purpose.*"

The agreed endpoint suitable for use in the risk assessment is: 48-hour EC_{50} = 0.26 mg a.s./L or 3.97 mg product/L.

Reference:	KCP 10.2.1/03
Report Title:	S-2399 6 EC: Inhibition of Growth to the Alga *Raphidocelis subcapitata* (Formerly known as *Pseudokirchneriella subcapitata*)
Author(s) & year:	[redacted] (2020c)

Document No, Authority registration No:	Smithers ERS Limited Study No. 3202416 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0119
Substance used:	S-2399 6 EC, V16-7L1901, 6.544 ± 0.024% w/w
Method of analysis:	LC-TOF/MS
Guideline(s):	OECD 201 (2011)
Deviations:	Yes, see HSE Comments
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

MATERIALS

Test material S-2399 6 EC

Lot/Batch: V16-7L1901

Active Substance Content: 6.544 ± 0.024% w/w or 65.44 ± 0.24 g/kg or 60.68 ± 0.23 g/L (verified by certificate of analysis)

Description: Amber liquid

Density: 0.9273 g/mL

Receipt date: 29 January 2019

Retest date: 17 May 2021

Storage on Receipt: Room Temperature (15 – 30 °C)

TREATMENTS

Test concentrations: Control (dilution media) and nominal concentrations of 0.32, 1.0, 3.2, 10 and 32 mg product/L (corresponding to 0.0209, 0.0654, 0.209, 0.654 and 2.09 mg a.s./L, respectively), mean measured concentrations were 0.0201, 0.0678, 0.205, 0.729 and 2.15 mg a.s./L

Toxic reference Potassium dichromate, tested in a separate study in January 2019 (test facility study number: 3202327). E_rC_{50} = 1.3 mg/L

Analysis of test concentrations: Yes, at 0 and 72 hours (all treatment levels and control) based on analysis of S-2399 using liquid chromatography-time of flight mass spectrometry (LC-TOF/MS) system. The limit of quantification (LOQ) of the analytical method is 0.001 mg S-2399/L

TEST ORGANISMS

Species: *Raphidocelis subcapitata,* Strain 278/4 (formerly known as *Pseudokirchneriella subcapitata*)

Pre-culture:	Exponentially growing pre-culture, prior to testing duplicate starter cultures were prepared and incubated under test conditions to achieve starting cell density
Inoculation rate:	1×10^4 cells/mL
Source:	Originally obtained from the Culture Collection of Algae and Protozoa (CCAP), Oban, UK

TEST DESIGN

Test vessels:	Sterile glass 250 mL Erlenmeyer (conical) flasks filled with 100 mL of test or control medium and capped with foam bungs
Test medium:	OECD TG 201 medium (dilution media)
Replication:	Six inoculated test vessels were prepared for the control and three replicate test vessels for each test concentration An additional inoculated vessel was prepared for the controls and test substance concentration for initial water quality analysis
Exposure regime:	Static test conditions with no renewal of the test media
Duration:	72 hours

TEST CONDITIONS

Test temperature:	22.5 – 23.2 °C
pH:	Test start: 7.86 – 7.89 (with algae) and 7.84 – 7.90 (without algae) Test end: 7.87 – 7.95 (with algae) Maximum pH variation: 0.08 units
Lighting:	Continuous light at a light intensity of 4780 to 7350 lux (at 0 hours: 6900 – 7350 lux; at 72 hours: 4780 – 5800 lux).
Shaking:	Orbital shaker at 100 revolutions per minute (rpm)

STUDY DESIGN AND METHODS

Experimental dates: The exposure phase of the definitive test was conducted between 15 and 18 October 2019.

Test organism and culturing

The test organism, *Raphidocelis subcapitata*, is a representative species of the freshwater aquatic phytoplankton.

Test media

OECD TG 201 medium was prepared according to Annex 3 of OECD 201 (2011).

Definitive test and dose preparation
Initial media preparation work indicated that the test substance formed a homogenous, hazy dispersion at a concentration of 100 mg/L which appeared to dissolve over a 72-hour period under test conditions. At the start of the test, 31.96 mg of test formulation was dissolved in a final volume of 1000 mL of OECD medium, with the aid of vigorous stirring for 30 minutes, to give the 32 mg/L test concentration. Dilutions were prepared in OECD medium to give the remaining test concentrations of 10, 3.2, 1.0 and 0.32 mg/L. The control was prepared by adding OECD medium only to the vessels.

A media blank (OECD media only) was prepared to establish background counts on each sampling occasion. This vessel was incubated alongside the other test vessels. Background counts were subtracted from the cell counting results for each of the inoculated test vessels. The resulting cell counts were then used to calculate the yield and the corresponding specific growth rates.

Measurements and observations
At approximately 24-hour intervals after the start of the incubation period, pre-determined volumes of test media (1.0 mL at 24 hours and 0.5 mL at 48 and 72 hours) were removed from each incubated test vessel and transferred to individually identified cell counting vials. The contents of each vial were diluted to a 10 mL final volume with an electrolyte solution (Coulter Counter Isoton® II diluent). The cell density of the vial contents was then determined using a particle counter (Z2 Coulter Counter®).

The appearance and colour of the test media was recorded at the start and end of the test.

The light intensity within the incubator was monitored at the start and end of the test at five different positions in the incubator. The test vessels were randomly re-positioned daily to minimise any differences in light intensity across the test area. Temperature was recorded continuously using a digital min/max thermometer. At test initiation, the pH of freshly prepared test media, before and after algal cell inoculation, was determined. The pH in each test vessel was also determined at the end of the test.

At the start of the test (0 hours), 10 mL samples of freshly prepared test media were taken from the control and the test media preparation vessels for chemical analysis. At 72 hours, 10 mL samples were also taken for chemical analysis from the pooled old test media from the test and control groups. In each case duplicate samples were taken, one for chemical analysis and one as a ‘back-up’ should further analysis be required.

Data processing and statistical analysis
The algal cell concentration data were evaluated using three approaches: area under the growth curve (AUC), average specific growth rate and final yield.

The AUC was calculated using the formula:

$$A = \frac{N_1 - N_0}{2} \times (t_1) + \frac{N_1 + N_2 - 2N_0}{2} \times (t_2 - t_1) + \frac{N_{n-1} + N_n - 2N_0}{2} \times (t_n - t_{n-1})$$

The average specific growth rate (μ) was calculated using the formula:

$$\mu = \frac{\ln N_n - \ln N_0}{t_n - t_0}$$

where:
N_0 = initial measured cell concentration at time t_0 (cells/mL)
N_1 = measured cell concentration at time t_1 (cells/mL)
N_2 = measured cell concentration at time t_2 (cells/mL)
N_n = measured cell concentration at time t_n (cells/mL)
t_1 = time of first measurement after the beginning of the test (h)
t_2 = time of second measurement after the beginning of the test (h)
t_n = time of nth measurement after the beginning of the test (h)

The final yield was calculated using the formula:

$$Y = Final\ time\ point\ cell\ concentration\ (cells/mL) - Initial\ (inoculum)\ cell\ concentration\ (cells/mL)$$

The percentage inhibition (I_A %) of cell growth was calculated using the following equation:

$$I_A(\%) = \frac{A_c - A_s}{A_c} \times 100$$

where:
A_c = mean values of Y, A or μ determined for the control treatment
A_s = mean values for Y, A or μ determined for each treatment concentration

Section-by-section percentage inhibition in growth rate and section-by-section growth rates for control vessels are also reported.

Statistical analysis was performed using the CETIS program v 1.8.6.8. The toxicity results are expressed in terms of the nominal formulation concentrations and mean measured active substance concentrations.

The no observed effect concentrations (NOEC) for yield, average specific growth rate and

the 24 and 48-hour area under the growth curve were determined using Dunnett Multiple Comparison Test. The NOEC for the 72-hour area under the growth curve was determined using Williams Multiple Comparison Test.

The EC_{10}, EC_{20} and EC_{50} values for the 72-hour final yield, the 0-24, 0-48, 0-72, 24-48 and 48-72 hour average specific growth rate (μ) time intervals and the 24, 48 and 72- hour area under the growth curve (A) were estimated using linear interpolation. To distinguish between EC_x values, estimated using final yield, AUC and growth rates, the symbols E_yC_x, E_bC_x and E_rC_x were used, respectively.

RESULTS AND DISCUSSION

Chemical analysis

The results of the chemical analysis of test media samples during the definitive test are presented in Table 9.3-9.

Table 9.3-9: Measured concentrations of active substance S-2399 throughout definitive exposure

Nominal concentration [mg product/L]	Nominal concentration [mg a.s./L] *	Measured test concentration [mg a.s./L] / [%] of nominal		Mean measured concentration [mg a.s./L]	[%] of nominal (active substance)
		0 hours (fresh)	72 hours (old)		
Control		< LOQ	< LOQ	n.a.	n.a.
0.32	0.0209	0.0225 / 108	0.0177 / 85	0.0201	96
1.0	0.0654	0.0727 / 111	0.0628 / 96	0.0678	104
3.2	0.209	0.212 / 101	0.198 / 95	0.205	98
10	0.654	0.746 / 114	0.711 / 109	0.729	111
32	2.09	2.25 / 108	2.05 / 98	2.15	103

n.a. not applicable

* Based on an active substance purity of 65.44 g/kg

Given that the test substance is a formulated product, the results were calculated based on nominal formulation concentration and mean measured active substance concentration.

Water quality and environmental conditions

The freshly prepared (new) test media for the control and test concentrations at the start of the test were observed to be colourless solutions.

The appearance of the test media following the 72-hour exposure period are summarised in

Table 9.3-10.

Table 9.3-10: Colour and appearance of test mediums after 72-hour exposure period

Nominal formulation concentration (mg/L)	Colour of Test Medium	Appearance of Test Medium
Control	Slightly green	Homogenous, hazy dispersion of algae cells
0.32		
1.0		
3.2	Colourless	Solution
10		
32		

Biological effects

The results for cell growth, yield and AUC of *Raphidocelis subcapitata* during the definitive test for each replicate are presented in Table 9.3-11.

Table 9.3-11: Cell growth, yield and AUC of *Raphidocelis subcapitata* during the definitive test

Nominal formulation conc. (mg/L)	Mean measured conc. (mg a.s./L)	Cell Concentration (cells/mL)			Mean Cell Concentration (cells x 10^4 / mL)			Mean Final Yield per Treatment (cells x 10^4 / mL) (% inhibition relative to control)	Mean AUC (x 10^6 cell/mL) (% inhibition relative to control)
		24-hour	48-hour	72-hour	24-hour	48-hour	72-hour	72-hour	72-hour
Control	Control	30010	126180	433860	2.97	13.1	45.7	44.7 (n.a.)	8.745 (n.a.)
		27030	122940	426320					
		24620	123380	467600					
		32610	130480	458200					
		32170	153600	519680					
		31580	130760	436040					
0.32	0.0201	25150	139920	507480	3.20	15.3	51.1	50.1 (-12)	9.978 (-

Nominal formulation conc. (mg/L)	Mean measured conc. (mg a.s./L)	Cell Concentration (cells/mL)			Mean Cell Concentration (cells x 10^4 / mL)			Mean Final Yield per Treatment (cells x 10^4 / mL) (% inhibition relative to control)	Mean AUC (x 10^6 cell/mL) (% inhibition relative to control)
		24-hour	48-hour	72-hour	24-hour	48-hour	72-hour	72-hour	72-hour
		29370	136920	447300					14)
		41380	183320	577560					
1.0	0.0678	22490	106320	456660	2.55	11.2	43.1	42.1 (6)	7.874 (10)
		30610	110140	423940					
		23290	119460	413200					
3.2	0.205	18080	39780	155880	1.97	5.04	19.2	18.2 (59)[a]	3.382 (61)[b]
		18950	50900	206360					
		22020	60640	212540					
10	0.729	15560	17140	21760	1.58	1.85	2.66	1.66 (96)[a]	0.5435 (94)[b]
		15690	16440	23680					
		16170	21980	34480					
32	2.15	13170	17840	16220	1.35	1.64	1.71	0.707 (98)[a]	0.3217 (96)[b]
		25230	31620	33660					
		2040	-300	1340					

[a] Statistically significantly different from controls (Dunnett Multiple Comparison Test (a = 0.05)

[b] Missing Williams Multiple Comparison Test for AUC

n.a. not applicable

The calculated average specific growth rates are presented in Table 9.3-12.

Table 9.3-12: Calculated average specific growth rates

Nominal formulation concentration (mg/L)	Average specific growth rate, "μ" (x 10^{-2} $hours^{-1}$)			
	0-24 hours	24-48 hours	48-72 hours	0-72 hours (% inhibition relative to control)
Control	4.51	6.20	5.20	5.31 (n.a)
0.32	4.75	6.59	5.03	5.46 (-3)
1.0	3.85	6.21	5.62	5.23 (1)
3.2	2.81	3.87	5.58	4.09 (23)[a]
10	1.91	0.626	1.46	1.33 (75)[a]
32	-0.540	0.735	-0.045	-0.145 (103)[a]

[a] Statistically significantly different from controls (Dunnett Multiple Comparison Test (a = 0.05)
n.a. not applicable

The 72-hour growth rates for individual control replicates, required to calculate the coefficient of variation (CV), are presented in Table 9.3-13.

Table 9.3-13: 72-hour growth rates for individual control replicates

Vessel number	Section-by-section growth rates
	0 – 72 hours
Control V1	0.052363
Control V2	0.05212
Control V3	0.053403
Control V4	0.053121
Control V5	0.05487
Control V6	0.052433
Mean	0.053052
Standard deviation	0.001016
Coefficient of variation (%)	1.91

Table 9.3-14 presents the section-by-section growth rates for the control replicates and the CV between individual time frames within a replicate, as well as the mean CV (part of the validity criteria below).

Table 9.3-14: Section-by-section growth rate of *Raphidocelis subcapitata* in the control

Vessel Number	Section-by-section growth rates			Mean	Standard deviation	Coefficient of variation (%)
	0-24 hours	24-48 hours	48-72 hours			
Control V1	0.045789	0.059841	0.051459	0.052363	0.007069	14
Control V2	0.041432	0.063115	0.051812	0.052120	0.010845	21
Control V3	0.037541	0.067155	0.055514	0.053403	0.014919	28
Control V4	0.049251	0.057775	0.052337	0.053121	0.004316	8
Control V5	0.048685	0.065138	0.050786	0.054870	0.008954	16
Control V6	0.047914	0.059202	0.050182	0.052433	0.005971	11
Mean coefficient of variation (%)						16

Growth curves for *Raphidocelis subcapitata* during the definitive test are visualised in Figure 9.3.1-2. Note that both the x and y-axis are linear.

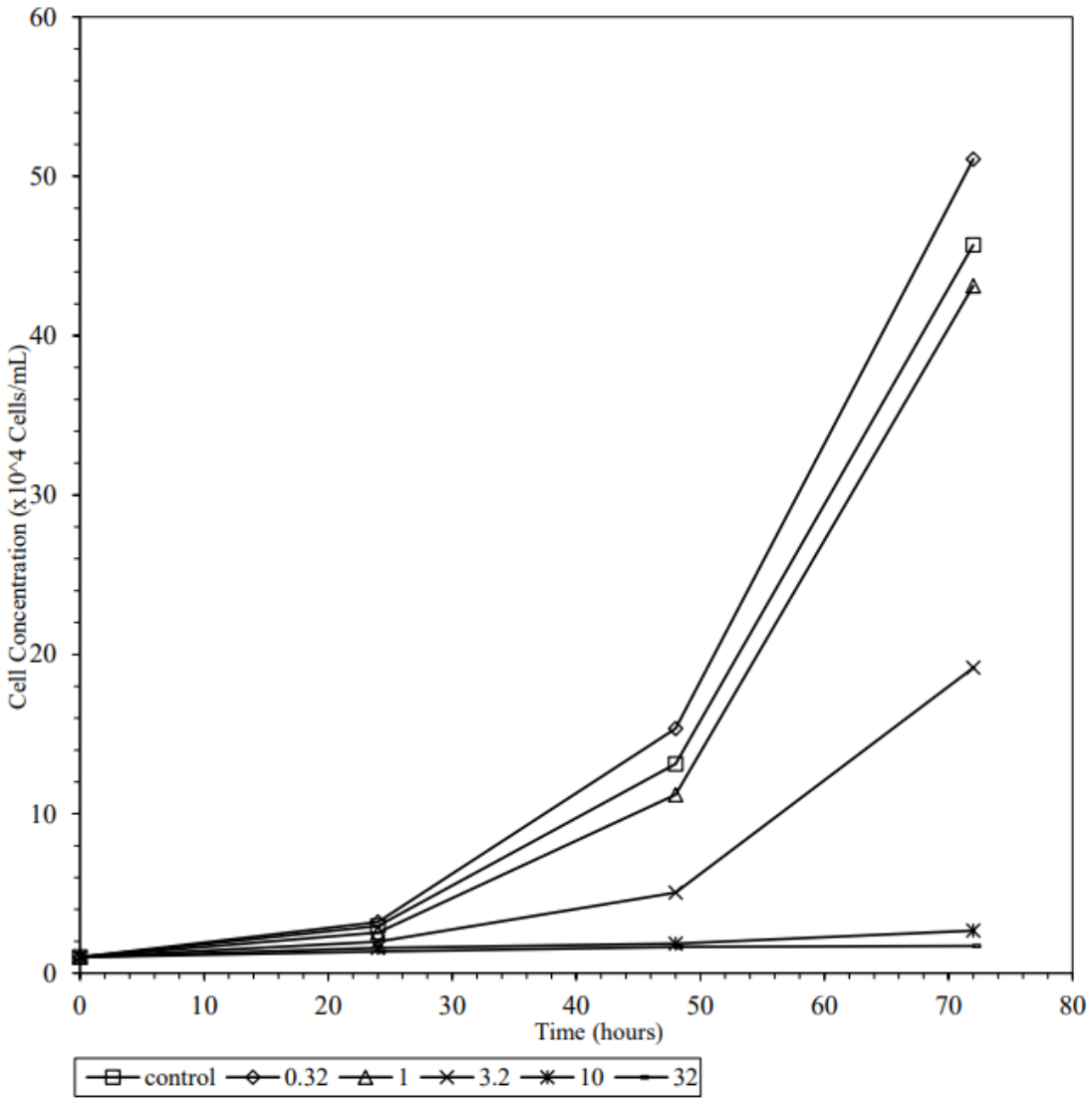

Figure 9.3-2: Growth curves for *Raphidocelis subcapitata* during the definitive test for each treatment level

Endpoints for yield, AUC and growth rate are summarised in Table 9.3-15.

Table 9.3-15: Summary of endpoints

Endpoint	Yield	AUC	Growth rate
	Nominal concentration [mg product/L]		
72-hour EC_{10}	0.92 (0.37 – 1.5)	0.71 (0.42 – 1.4)	1.6 (1.2 – 2.0)
72-hour EC_{20}	1.3 (0.81 – 1.8)	1.1 (0.63 – 1.5)	2.6 (1.8 – 3.6)
72-hour EC_{50}	2.5 (2.0 – 3.1)	2.4 (1.9 – 3.1)	5.8 (4.9 – 6.9)

Endpoint	Yield	AUC	Growth rate
	Nominal concentration [mg product/L]		
72-hour NOEC	1.0	1.0	1.0
72-hour LOEC	3.2	3.2	3.2
	Mean measured concentration [mg a.s./L]		
72-hour EC_{10}	0.0630 (0.0300 – 0.107)	0.0499 (0.0316 – 0.0914)	0.112 (0.0827 – 0.141)
72-hour EC_{20}	0.0908 (0.0575 – 0.130)	0.0789 (0.0449 – 0.115)	0.178 (0.135 – 0.224)
72-hour EC_{50}	0.172 (0.136 – 0.209)	0.164 (0.127 – 0.204)	0.447 (0.367 – 0.528)
72-hour NOEC	0.0678	0.0678	0.0678
72-hour LOEC	0.205	0.205	0.205

Validity criteria

The validity criteria for the study were met according to OECD 201 (2011) (Table 9.3-16).

Table 9.3-16: Compliance with OECD 201 (2011) validity criteria

Validity criterion	Required	Obtained
Mean cell count (cells/mL)) control increase (biomass surrogate)	Increase by a factor of at least 16	45.7*
Coefficient of variation of average specific growth rates at 72h	≤ 7 %	1.91 %
Mean coefficient of variation for section-by-section specific growth rates (individual replicates – 0-24, 24- 48, 48-72 hours)	≤ 35 %	16 %

*Modified by HSE to correctly reflect fold increase

CONCLUSIONS

Based on nominal concentrations, the 72-hour E_yC_{50}, E_bC_{50} and E_rC_{50} values were estimated to be 2.5, 2.4 and 5.8 mg/L, respectively. The corresponding NOEC values for yield, biomass and growth rate were considered to be 1.0 mg/L.

Based on mean measured active substance (a.s.) concentrations, the 72-hour E_yC_{50}, E_bC_{50} and E_rC_{50} values were estimated to be 0.172, 0.164 and 0.447 mg a.s./L, respectively. The corresponding NOEC values for yield, biomass and growth rate were considered to be 0.0678 mg a.s./L.

HSE COMMENTS

The study was carried out according to and evaluated against the OECD 201 (2011) guideline. All validity criteria outlined in OECD 201 (2011) were satisfactorily met for the duration of the study.

The following deviations were noted:

In relation to OECD 201 (2001) paragraph (§) 12 it is noted that the toxic reference result (E_rC_{50} = 1.3 mg potassium dichromate/L) is in line with the mean value quoted in ISO 8692 (2012) (E_rC_{50} = 1.19 mg potassium dichromate/L). Therefore, the test strain selected displays expected toxicant sensitivity and is suitable.

OECD 201 (2001) paragraph (§) 16 details equipment that are required to carry out the study (and (§) 61 outlines associated reporting requirements). Regarding light measurement instruments, it suggests using a spherical (4 π) receptor (which responds to direct and reflected light from all angles above and below the plane of measurement), or a 2 π receptor (which responds to light from all angles above the measurement plane). The study conductor did not report which light measurement instrument was used. HSE consider this a minor reporting omission.

OECD 201 (2001) paragraph (§) 26 specifies the requirements for initiating and maintaining an inoculum culture. It states that once an inoculum culture is set up the study conductor should, "*measure the increase in biomass in the inoculum culture to ensure that growth is within the normal range for the test strain under the culturing conditions*". This check was not outlined in the study report. Within Annex 2 of OECD 201 (2001) it gives a growth rate of 1.5 – 1.7 day^{-1} for *R.subcapitata* (21 °C, 70 $\mu Em^{-2}s^{-1}$). This equates, if 1.6 day^{-1} is used, to an increase by a factor of 121.5 in cell count (cells/mL; surrogate for biomass). This is significantly higher than the reported 45/46 average factor increase (1.27/1.28 day^{-1}) for control vessels, which indicates that the growth rate of the cells used in the study was below the normal range for *R.subcapitata*. If the check outlined in paragraph (§) 26 had been performed this lower-than-expected growth rate would have been detected. Nevertheless, the control factorial increase was above the validity criteria threshold. Also, the low variation

between the growth rates of the control replicates, indicates that the lower-than-expected growth rate was present for all replicates. HSE considers this deviation acceptable.

OECD 201 (2001) paragraph (§) 41 states that, "*microscopic observation should be performed to verify a normal and healthy appearance of the inoculum culture and to observe any abnormal appearance of the algae (as may be caused by the exposure to the test substance) at the end of the test*" (and (§) 61 outlines associated reporting requirements). This was either not performed or not reported by the study conductor. In the case of recording the abnormal appearance of algae at test termination, omitting this may have resulted in morphological perturbations not being detected. If these occurred at concentrations lower than those where effects on average specific growth rate, yield and AUC were detected, this may have resulted in inappropriately high endpoint estimates. HSE notes this uncertainty and will consider it during risk assessment.

OECD 201 (2001) paragraph (§) 44 discusses the best practices for plotting growth curves. It states that, "*logarithmic scales are mandatory and generally give a better presentation of variations in growth pattern during the test period. Note that exponential growth produces a straight line when plotted on a logarithmic scale*". Cell concentration is not plotted on the logarithmic scale in Figure 9.3.1-2. This is a minor reporting error that had no impact on the study results. Therefore, HSE considers this deviation acceptable.

OECD 201 (2001) paragraph (§) 45 outlines the identification of procedural errors. For the 32 mg product/L nominal treatment level there appears to be an inoculation error for one of the replicates. After 24 hours there were 2040 cell/mL when the replicates were seeded with 10000 cells/mL initially. The other two replicates from this treatment level had > 10000 cell/ml after 24 hours, which suggests the low cell count for the other replicate was the result of a procedural error, rather than due to the cytotoxic properties of the test substance at high concentrations. The procedural error is unlikely have impacted the results for specific growth rate and yield as the 32 mg product/L nominal treatment level was left out of the statistical analyses (although this was not drawn attention to in the study report). It was, however, included for the AUC analyses and was not detected as an outlier. As specific growth rate and yield, the two primary endpoints listed in OECD 201 (2011), were analysed without this outlier HSE considers this a minor deviation, which is unlikely to have greatly impacted the reported NOECs and E_xC_{50}s.

OECD 201 (2001) paragraph (§) 48 gives the equation used to calculate the average specific growth rate. This was incorrectly reported by the study conductor and has been corrected in this study summary. The growth rates reported for all treatment levels used the correct equation showing the error was only one of reporting. There was also the issue of the incorrectly reported units in Table D, which should be $hour^{-1}$, which further raises the issue that the guideline stipulates the use of day^{-1}. Despite these reporting issues, the underlying data has been reported, which makes it possible to express the results in units that align

with OECD 201 (2011). This allows for the comparison of control growth rates to the expected growth rate outlined in Annex 2 OECD 201 (2011). Therefore, HSE considers these reporting deviations as acceptable.

OECD 201 (2001) paragraph (§) 49 recommends the comparison of section-by-section specific growth rates. It states. "*a significantly lower specific growth rate on day one than the total average specific growth rate may indicate a lag phase…, a lag phase in exposed cultures may indicate recovery after initial toxic stress*". For the 1 mg product/L nominal treatment level, there is the possibility that the 0 - 24 hour average specific growth rate was lower than for the control. The 24-48 hour and 48-72 hour average specific growth rates recovered to control levels. This suggests a toxicant-induced lag phase may be present between 0 – 24 hours. Therefore, it seems likely from the results that 24 or 48-hour endpoint estimates would be lower than the reported 72-hour endpoint values and a more conservative approach.

OECD 201 (2001) paragraph (§) 57 discusses approaches for estimation of NOECs and LOECs. The study conductor used the recommended Dunnett's (growth rate and yield) and William's Tests (AUC) without justifying why each test was deployed. The William's Test is usually deployed when data is monotonic, which was not the case for AUC. This is a minor deviation from the guidance, which HSE considers acceptable.

OECD 201 (2001) paragraph (§) 61 defines the material that must be included in the test report. There were a number of reporting omissions:

The test report requires "*calculated response variables for each treatment replicate, with mean values and coefficient of variation for replicates*". Growth rates & AUCs were not reported at the level of treatment replicate, only their means for each treatment-level time-period combination. Cell concentration data was provided, however, for each replicate making the calculation of individual replicate growth rates possible. HSE consider this a minor deviation.

The test report requires, "graphical presentation of the concentration/effect relationship". A concentration-effect curve was not provided, although the effect of treatment was visualised in Figure 9.3.1-2. HSE consider this a minor reporting omission.

The test report requires "*if ANOVA has been used, the size of the effect which can be detected (e.g. the least significant difference)*". Dunnett's Test is a post-hoc test following an ANOVA. Therefore, the size of the effect that can be detected should have been reported.

The test report should mention, "*any stimulation of growth found in any treatment*". This was evident from the data but not discussed. Given the statistical model selected was compatible with growth stimulation this oversight did not affect the estimated endpoints. HSE consider

this a minor reporting omission and acceptable.

Lastly, there were two reporting errors/omissions in both the study report:

In the study report no AUC values were reported at all beyond the E_bE_x point estimates These reporting errors did not impact the integrity of the generated data and HSE considers them minor errors.

A William's Test was performed on the AUC endpoint. The results of this test were not reported. As the result of this test can be deduced by the reported LOEC/NOEC values for the AUC endpoint, HSE considers this a minor reporting error.

The method of analysis used in the study was evaluated by HSE Chemistry. The conclusions of their evaluation are reproduced below. Please see Volume 3 CA, section B5 for more details.

"*The analytical method is not fully validated according to SANTE/2020/12830 rev. 1 for the determination of the active substance inpyrfluxam in OECD medium as the matrix effects and stock solution stability have not been determined. However, the study was generated prior to the implementation of SANTE 2020/12830 rev.1 and SANCO 3029 rev.4 did not require matrix effects and stock solution stability to be addressed. As all other validation requirements have been met (including the minimum validation requirements outlined in SANTE 2020/12830 rev.1), the method is considered to be fit for purpose.*"

The above study was conducted to GLP and considered valid.

The agreed endpoints suitable for use in the risk assessment are:

72-hour E_rC_{10} = 0.112 mg a.s./L, which equates to 1.71 mg product/L
72-hour E_rC_{20} = 0.178 mg a.s./L, which equates to 2.72 mg product/L
72-hour E_rC_{50} = 0.447 mg a.s./L, which equates to 6.83 mg product/L

72-hour E_yC_{10} = 0.0630 mg a.s./L, which equates to 0.963 mg product/L
72-hour E_yC_{20} = 0.0908 mg a.s./L, which equates to 1.39 mg product/L
72-hour E_yC_{50} = 0.172 mg a.s./L, which equates to 2.63 mg product/L

72-hour E_bC_{10} = 0.0499 mg a.s./L, which equates to 0.763 mg product/L
72-hour E_bC_{20} = 0.0789 mg a.s./L, which equates to 1.21 mg product/L
72-hour E_bC_{50} = 0.164 mg a.s./L, which equates to 2.51 mg product/L

Therefore, based on specific growth rate, the preferred indicator of acute toxicity according to OECD 201 (2011), the 72-hour E_rC_{50} = 0.447 mg a.s./L, which equates to

6.83 mg product/L

B.9.3.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

No chronic toxicity studies for aquatic organisms were submitted with the representative formulation as the test item.

B.9.3.3 Further testing on aquatic organisms

No further testing on aquatic organisms with the representative formulation was submitted.

B.9.4 Risk assessment for aquatic organisms

The following risk assessment has been conducted according to the EFSA (2013) guidance document[3].

B.9.4.1 Exposure

Exposure estimates have been taken from Volume 3, section B.8 (Environmental fate dossier). Predicted Environmental Concentrations (PECs) used for risk assessment have been established by the Environmental Fate evaluator.

Relevant metabolites for consideration in the risk assessment are outlined below in Table 9.4-1.

Table 9.4-1: Relevant metabolites for consideration during aquatic risk assessment

Metabolite	Relevant environmental compartments
3'-OH-S-2840	surface water
1'-COOH-S-2840	surface water, groundwater

HSE Environmental Fate and Behaviour have confirmed that no consideration of the change in isomeric ratio is required as the enantiomer ratio did not change and a single assessment for the sum of the enantiomers was considered appropriate. Additionally, 1'-COOH-S-2840 is a major metabolite in the environment and this metabolite was investigated and it was

[3] 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, 268 pp. doi:10.2903/j.efsa.2013.3290.

concluded that the enantiomers did not need to be considered separately. Detailed consideration can be found in Volume 1 Section 2.12.15.

B.9.4.2 Toxicity

Active substance (Inpyrfluxam)

The data available to address the toxicity of the active substance, inpyrfluxam, is summarised below (Table 9.4-2). Studies that were not considered suitable for use in risk assessment are indicated in the table.

Table 9.4-2: Endpoints relevant for inpyrfluxam (S-2399)

Test substance	Test organism	Test system	Endpoint		Reference
Acute toxicity to fish					
Inpyrfluxam S-2399	*Oncorhynchus mykiss*	96-hour, static	LC_{50}	**0.031 mg a.s./L (t.w.a)**	KCA 8.2.1/01 [redacted] 2014a
Inpyrfluxam S-2399	*Lepomis macrochirus*	96-hour, static	LC_{50}	0.054 mg a.s./L (t.w.a)	KCA 8.2.1/02 [redacted] 2014b
Inpyrfluxam S-2399	*Pimephales promelas*	96-hour, static	LC_{50}	0.050 mg a.s./L (t.w.a)	KCA 8.2.1/03 [redacted] 2014c
Inpyrfluxam S-2399	*Cyprinus carpio*	96-hour, static	LC_{50}	0.067 mg a.s./L (t.w.a)	KCA 8.2.1/04 [redacted] 2014d
Inpyrfluxam S-2399	*Cyprinodon variegatus*	96-hour, static	LC_{50}	0.15 mg a.s./L (m.m)	KCA 8.2.1/05 [redacted] 2014e
Inpyrfluxam S-2399	*Poecilia reticulata*	96-hour, static	LC_{50}	0.35 mg a.s./L (t.w.a)	KCA 8.2.1/06 [redacted] 2016c

Test substance	Test organism	Test system	Endpoint		Reference
Inpyrfluxam S-2399	*Oryzias latipes*	96-hour, static	LC_{50}	0.79 mg a.s./L (t.w.a)	KCA 8.2.1/07 [redacted] 2016a
Inpyrfluxam S-2399	*Danio rerio*	96-hour, static	LC_{50}	0.30 mg a.s./L (t.w.a)	KCA 8.2.1/08 [redacted] 2016b
Inpyrfluxam S-2399	Species sensitivity distribution (SSD)	96-hour, static	HC_5	**0.018 mg a.s./L** (based on LC_{50} data)	KCA 8.2.1/09 [redacted] and [redacted] 2017
Chronic toxicity to fish					
Inpyrfluxam S-2399	*Pimephales promelas*	32-day, flow-through	LC_{10}	**0.0066 mg a.s./L (m.m)**	KCA 8.2.2.1/01 [redacted] 2014
Inpyrfluxam S-2399	*Cyprinodon variegatus*	34-day, flow-through	NOEC[a]	0.009 mg a.s./L (m.m)	KCA 8.2.2.1/02 [redacted] 2017
Bioconcentration in fish					
Inpyrfluxam S-2399	*Lepomis macrohirus*	31-day, flow-through	Lipid normalised, growth corrected, kinetic bioconcentration factor ($BCF_{kgL,TRR}$)	**215.4 L/kg (Total ^{14}C residue basis)** **38.4 L/kg (S-2399)**	KCA 8.2.2.3/01 [redacted] 2015

Test substance	Test organism	Test system	Endpoint		Reference
			Lipid normalised steady state bioconcentration factor ($BCF_{SSL, S-2399}$)	**38.4 L/kg (S-2399)**	
Acute toxicity to invertebrates					
Inpyrfluxam S-2399	*Daphnia magna*	48-hour, static	EC_{50}	**1.1 mg a.s./L** (t.w.a)	KCA 8.2.4.1/01 [redacted] 2014f
Inpyrfluxam S-2399	*Americamysis bahia*	48-hour, static	LC_{50}	**1.1 mg a.s./L** (m.m)	KCA 8.2.4.2/01 [redacted] 2014g
Long-term toxicity to invertebrates					
Inpyrfluxam S-2399	*Daphnia magna*	21-day, static-renewal	NOEC	0.13 mg a.s./L (t.w.a)	KCA 8.2.5.1/01 [redacted] 2014h
			EC_{10} (reproduction)	**0.21 mg a.s./L** (t.w.a)	
Inpyrfluxam S-2399	*Americamysis bahia*	28-day, flow through	NOEC	Not determined	KCA 8.2.5.2/01 [redacted] 2016 **To be used as supporting information only**
Toxicity to sediment-dwelling organisms					
Inpyrfluxam S-2399	*Chironomus dilutus*	62-day, static renewal	NOEC	Not determined	KCA 8.2.5.4/01 [redacted]

Test substance	Test organism	Test system	Endpoint		Reference
					[redacted] 2015 **Not suitable for use in risk assessment**
Inpyrfluxam S-2399	*Hyalella azteca*	42-day, static spiked sediment (with surface water renewal)	NOEC	Not determined	KCA 8.2.5.4/02 [redacted] 2016 **Not suitable for use in risk assessment**
Inpyrfluxam S-2399	*Leptocheirus plumulosus*	28-day, static renewal	NOEC[a]	**10.26 mg a.s./kg sediment (t.w.a)**	KCA 8.2.5.4/03 [redacted] 2017
Toxicity to algae					
Inpyrfluxam S-2399	*Pseudokirchneriella subcapitata*	96-hour, static	E_rC_{50} (72 h)	>23 mg a.s./L (m.m)	KCA 8.2.6.1/01 [redacted] 2015a
Inpyrfluxam S-2399	*Navicula pelliculosa*	96-hour, static	E_rC_{50} (72 h)	10.1 mg a.s./L (m.m)	KCA 8.2.6.2/01 [redacted] 2015b

Test substance	Test organism	Test system	Endpoint		Reference
Inpyrfluxam S-2399	*Anabaena flos-aquae*	96-hour, static	E_rC_{50} (72 h)	Not determined	KCA 8.2.6.2/02 [redacted] 2015 **Not suitable for use in risk assessment**
Inpyrfluxam S-2399	*Skeletonema costatum*	96-hour, static	E_rC_{50} (96 h)	1.28 mg a.s./L (m.m)	KCA 8.2.6.2/03 [redacted] 2015d
Geomean of two diatom endpoints (*Skeletonema costatum* and *Navicula pelliculosa*)				**3.60 mg a.s./L**	-
Toxicity to aquatic macrophytes					
Inpyrfluxam S-2399	*Lemna gibba*	7-day, semi-static	E_rC_{50}	**> 24 mg a.s./L (m.m)**	KCA 8.2.7/01 [redacted] 2016
Further testing on aquatic organisms					
Inpyrfluxam S-2399	*Crassostrea virginica*	96-hour, -flow through	EC_{50}	>0.99 mg a.s./L (m.m)	KCA 8.2.8/01 [redacted] 2016

nom. = nominal; m.m. = arithmetic mean measured; g.m. = geometric mean measured; t.w.a = time-weighted average; i.m = initial measured.

[a] Due to the lack of model fit robust EC_{10} and EC_{20} values could not be generated.

Endpoints in **bold** were used in risk assessment.

Selection of endpoints for Tier 1 risk assessment for the active substance S-2399

The following endpoints were selected for use in the tier 1 risk assessment for S-2399.

Acute toxicity to fish

Eight acute toxicity studies were conducted with fish using the following species: *Onchyrynchus mykiss, Lepomis macrochirus, Pimephales promelas, Cyprinus carpio, Cyprinodon variegatus, Poecilia reticulata, Oryzias latipes and Danio rerio.* All eight studies were considered valid for use in risk assessment.

For two studies, there were issues that require clarification: 1) for KCA 8.2.1/02 (*Lepomis macrochirus*), there was a high degree of mortality variation between the two 0.058 mg a.s./L replicates, the only concentration that elicited partial mortality, after 96 hours. This resulted in increased uncertainty associated with the reported LC_{50}. HSE considers this uncertainty satisfactorily addressed by the use of seven additional endpoints during the SSD Tier 2 refinement to estimate a HC_5; and 2) for KCA 8.2.1/04 (*Cyprinus carpio*), the dissolved oxygen validity criterion was not met for the duration of the study. There was a malfunction in the aeration pump at the 72-hour interval. This resulted in a replicate A of 0.023 mg/L and replicates B of 0.023, 0.037, 0.059 and 0.094 mg/L (nominal concentration) treatment groups presenting < 60 % DO. No mortality was observed at the 0.023 and 0.037 mg/L treatment levels and 1/20 fish died in the 0.059 mg/L treatment group. For 0.095 mg/L replicate B, 4 % DO was recorded at 72 hours. However, 90 % mortality was already present at the previous timepoint. For comparison, 0.095 mg/L replicate A had 100 % mortality after 48 hours. This demonstrates that a strong toxicant effect was already present and the 100 % mortality in replicate B after 96 hours is very likely treatment-related rather than due to the reduction in DO levels. HSE also notes that this aerator pump malfunction would have resulted in a toxicity overestimate if it impacted the study at all. Therefore, due to its marginal effect on the LC_{50} endpoint and to prevent further invertebrate testing, HSE accepts the use of this endpoint in risk assessment.

Beyond *Onchyrynchus mykiss*, the seven additional studies were conducted for a Tier 2 Species Sensitivity Distribution (SSD) refinement. For Tier 1, the lowest endpoint from the eight species is selected (**LC_{50} = 0.031 mg a.s./L**). This is done to highlight the need for refinement, at which point a SSD will be performed. This is in accordance with Table 26 of EFSA Journal 2013;11(7):3290, which states, "*if more data than indicated in the second column (≥ 5 aquatic vertebrates or ≥ 8 invertebrates) are available, the Geomean approach could still be applied, but it is recommended to preferably apply the SSD approach*".

Long-term toxicity to fish

Two fish early-life stage toxicity tests were conducted using *Pimephales promelas* and *Cyprinodon variegatus.* Both studies were considered valid for use in risk assessment. According to Regulation (EU) No 283/2013, one chronic study is required "*where exposure of surface water is likely and the substance is deemed to be stable in water, that is to say there is less than 90 % loss of the original substance over 24 hours via hydrolysis*". This is the case for S-2399 (see B8 for further information). No justification was provided for why two chronic ELS studies were submitted. For *C. variegatus*, the NOEC was revised down

from 0.045 mg a.s./L to 0.009 mg a.s./L as statistically significant effects at the 0.015 mg a.s./L were deemed treatment related and biologically relevant by HSE. For *P. promelas*, the NOEC = 0.0075 mg/L proposed by the applicant was amended to a **LC_{10} = 0.0066 mg/L**. In accordance with the Corrigendum of the Aquatic guidance document (EFSA, 2016)[4], the lowest available endpoint, **0.0066 mg/L**, is selected for chronic risk assessment.

Acute toxicity to aquatic invertebrates

Two acute aquatic invertebrate toxicity studies were submitted. An acute test with *Daphnia magna* was correctly submitted in accordance with Regulation (EU) No 283/2013. An additional acute aquatic invertebrate toxicity study was submitted. This is only required if the active substance has an insecticidal mode of action or shows insecticidal activity. Inpyrfluxam has a fungicidal mode of action and does not meet the threshold of insecticidal activity according to footnote 29 of EFSA Journal 2013;11(7):3290 (NTA and bee quotients < 2 and 50 respectively). Consequently, the *Americamysis bahia* acute study appears to be surplus to Regulation (EU) No 283/2013. The acute toxicity endpoints for *D. magna* and *A. bahia* are both **1.1 mg a.s/L** and this value will be used in risk assessment for aquatic crustaceans. Both the *D. magna* and *A. bahia* were considered valid for use in risk assessment.

Chronic toxicity to aquatic invertebrates

Two chronic studies were submitted, for *D. magna* and *A. bahia*. The *D. magna* study was considered valid for use in risk assessment whereas the *A. bahia* study was not (see below). According to Regulation (EU) No 283/2013 "*A long-term or chronic toxicity study on aquatic invertebrates shall be provided for all active substances where exposure of surface water is likely and the substance is deemed to be stable in water, that is to say there is less than 90 % loss of the original substance over 24 hours via hydrolysis*". As with chronic fish toxicity, this is the case for S-2399 (see B8 for further information). No justification was provided for why two chronic aquatic invertebrate studies were submitted.

The *A. bahia* study was categorised as supporting information only as no EC_{10} could be reliably estimated and there was uncertainty regarding the reported NOEC. Offspring per female, a key reproductive endpoint, reported inconclusive results with no NOEC determined. Moreover, several other variables outlined in the followed guideline (US EPA OCSPP 850.1350 (1996)) were not fully captured. Despite this, *A. bahia* and *D. magna* acute endpoints are available and equivalent, suggesting that the sensitivity of the two species to inpyrfluxam is comparable. Also, the availability of the *D. magna* study fulfils the data requirement set out by Regulation (EU) No 283/2013. During the subsequent risk assessment, derived chronic RACs will be compared to the concentrations tested in the *A. bahia* study to assess the implications of the inconclusive offspring reproduction results.

[4] Network on Pesticide Steering Consultation for the corrigendum of the Aquatic guidance document (EFSA PPR Panel, 2013) Minutes Held on 14-15 September 2016, Parma (Agreed on 27th September 2016), https://www.efsa.europa.eu/sites/default/files/event/160914b-m.pdf

EFSA Journal 2013;11(7):3290 recommends the use of EC_{10}s in preference to NOECs. Therefore, the *D. magna* **EC_{10} = 0.21 mg a.s./L** is selected for use in risk assessment.

Regulation (EU) No 283/2013 data point 8.2.5.3. also states that a *Chironomus spp.* study, where the active substance is applied to the water overlying sediment, is required for active substances "*that have other effects on insect growth and development*". The two chronic aquatic invertebrate studies both reported effects on growth for individuals exposed to inpyrfluxam. For *A. bahia*, F_0 male total length was affected at 0.36 mg a.s./L. For *D. magna*, both parental body length and dry weight were affected at 0.54 mg a.s./L. For *A. bahia*, the NOEC was based on the growth effect observed on F_0 male total length. It appears that a data gap has been identified: inpyrfluxam does have growth and developmental effects for insects and a water spiked *Chironomus spp.* study, required by Section 8.2.5.3 of Regulation (EU) No 283/2013, has been omitted.

HSE Ecotoxicology submitted a RAI to the applicant asking for a reasoned case to support the omission of a water spiked *Chironomus spp.* study. The applicant provided a reasoned case to support this omission in the RAI response, reproduced in full below.

'In the Regulation (EU) No 283/2013, it is stated that "*If the active substance is an insect growth regulator, an additional study on chronic toxicity shall be carried out using relevant non-crustacean species such as Chironomus spp*." and "*The active substance shall be applied to the water overlying sediment and effects on survival and development of Chironomus riparius, including effects on emergence of adults, shall be measured to provide endpoints for those substances considered to interfere with insect moulting hormones or that have other effects on insect growth and development.".* Inpyrfluxam is not insect growth regulator and has no such mode of action.

Significant effects on length and weight of *D. magna* were reported by [redacted] (2014h, TPW-0007) for the top concentration of 0.54 mg a.s./L only. However, the most sensitive parameter was reproduction setting the NOEC at 0.13 mg a.s./L (LOEC = 0.27 mg a.s./L) or the EC10 was determined at 0.21 mg a.s./L applied for risk assessments. The EC10 estimates for body length and dry weight are 0.49 and 0.34 mg a.s./L, respectively. Accordingly, risk of effects on growth parameters are not expected at concentrations of concern for reproduction, which are considered for the assessment of risk for aquatic invertebrate species based on the NOEC derived.

Furthermore, the effect on body length (-12%) was observed in comparison to the solvent control. Body length in the solvent control was by 3% increased compared to the control. Weight was even increased by 11% in the solvent control as compared to the control. A positive effect of the solvent on growth is not considered likely which to some extend relativises the biological relevance of the effect at the top dose tested.

In the study on *Americamysis* ([redacted] 2016/2020, TPW-0041), only total body length reduction (-6 %) in comparison to controls and only for males was reported setting the NOEC at 0.18 mg a.s./L (LOEC = 0.36 mg a.s./L). Comparing the total body length in males with the solvent control shows a reduction of 4% only. It is considered questionable if these effect levels are of any biological relevance for the test organisms. Besides, significant effects were not observed for dry body weight and in females, where body length was increased by about 2% in comparison to solvent controls.

With reference to the chronic study on *Daphnia magna*, where control test organism growth was increased (+ 3%) in the solvent control as compared to the negative control, in the study on *Americamysis bahia*, the negative control showed stronger growth (+ 2% in males and +3% in females).

Overall, the comparative growth observed in the different controls is contradictory between the two studies and, in combination with the sex-specific findings in mysid shrimp as well as the low magnitude of effects, doubt the relevance of growth effects on aquatic invertebrates.

Most importantly, the NOEC of 0.13 mg a.s./L from the study on *Daphnia magna* is based on effects on reproductive output and this value is lower than the NOEC for growth from either of the tested species. Therefore, the risk for aquatic invertebrates from chronic exposure arguably is not driven by effects on growth.

Furthermore, a sediment dweller test is made available on *Chironomus dilutus* even though not in a spiked water design. Generally, the Applicant is of the opinion, that these data sufficiently cover the development and emergence of insect species. The NOEC of 92 mg a.s./L corresponds to the limit concentration tested and includes emergence as test parameter. The limit concentration (NOEC) is much in excess of the NOEC for chronic toxicity for daphnids which, accordingly, is considered to be protective of any potential risk for insect growth and development.

Finally, overall risk assessments are driven by toxicity to fish, i.e. these assessments are protective of the risk for aquatic invertebrates.'

HSE Ecotoxicology broadly agrees with this response and accepts the justification for omitting the water spiked *Chironomus spp.* study. This is predominantly based on the applicant outlining how the risk from chronic exposure for aquatic invertebrates is driven by reproduction, not growth endpoints, and, overall, how the risk assessment is driven by fish, not aquatic invertebrates. No further consideration of the potential data gap is required.

Toxicity to sediment-dwelling organisms

Three chronic studies for sediment dwelling organism were submitted: two for amphipods (*Leptocheirus plumulosus* and *Hyalella azteca*) and one for midges (*Chironomus dilutus*). Due to difficulties with determining NOECs for key response variables, two studies (*Hyalella azteca* and *Chironomus dilutus*) were concluded to be not reliable and not suitable for use in risk assessment. The *Leptocheirus plumulosus* was considered reliable and suitable for use in risk assessment. According to Regulation (EU) No 283/2013, "*when accumulation of an active substance in aquatic sediment is indicated or predicted by environmental fate studies, the impact on a sediment-dwelling organism shall be assessed. The chronic risk to Chironomus riparius or Lumbriculus spp. shall be determined*". EFSA Journal 2013;11(7):3290 footnote 30 expands on this by identifying the relevant fate study and level of active substance sediment accumulation. It outlines that a sediment dwelling organism study is required when a "*water/sediment study showed > 10 % of applied radioactivity at or after day 14 present in the sediment and chronic daphnia test (or other comparable study with insects) NOEC < 0.1 mg/L*". Regardless of the results from the water/sediment study, no chronic aquatic invertebrate NOEC < 0.1 mg/L. Therefore, no sediment-dwelling organism study is strictly required according to EFSA Journal 2013;11(7):3290. However, for completeness, a sediment dwelling risk assessment was performed. The NOEC reported for *Leptocheirus plumulosus* (**10.26 mg/kg**) was selected for risk assessment as it is lowest reported chronic endpoint with the lowest degree of associated uncertainty.

Toxicity to algae

Four studies were conducted with the active substance selecting *Pseudokirchneriella subcapitata*, *Navicula pelliculosa, Anabaena flos-aquae* and *Skeletonema costatum.* The study with *Anabaena flos-aquae* did not meet the validity criteria of OECD 201 (2011) and was not suitable for use in risk assessment. The three other studies were considered valid for use in risk assessment. According to Regulation (EU) No 283/2013, for active substances that do not exhibit herbicidal activity, like inpyrfluxam, only one algal study is required. No justification was provided for the additional three studies submitted. When additional studies are available a geometric mean approach should be taken according to EFSA Journal 2013;11(7):3290. The Corrigendum of the Aquatic guidance document (EFSA PPR Panel, 2013)[5] clarified that this approach is applicable to chronic algal endpoints. The lowest value between the green algae endpoint (*Pseudokirchneriella subcapitata)* and geomean of the two diatoms endpoints (*Navicula pelliculosa* and *Skeletonema costatum*) is selected. This is the geomean of the two diatom endpoints (E_rC_{50} = **3.60 mg a.s./L**).

Toxicity to aquatic macrophytes

One study was available using *Lemna gibba*. The study was considered valid for use in risk assessment. According to Regulation (EU) No 283/2013, an aquatic macrophyte study is

[5] Network on Pesticide Steering Consultation for the corrigendum of the Aquatic guidance document (EFSA PPR Panel, 2013) Minutes Held on 14-15 September 2016, Parma (Agreed on 27th September 2016), https://www.efsa.europa.eu/sites/default/files/event/160914b-m.pdf

only required for herbicides, plant growth regulators and other active substances displaying herbicidal activity. Inpyrfluxam does not display herbicidal activity, with no phytotoxicity observed at the maximum application rate for six vascular plant species (see KCP 10.6.2/02 study summary in 3CA B9 document). Therefore, no aquatic macrophyte study is required according to Regulation (EU) No 283/2013 and EFSA Journal 2013;11(7):3290. For completeness, an aquatic macrophyte risk assessment was performed using E_rC_{50} = **24 mg a.s./L**. Changes in appearance and morphology were not reported in the submitted study. The impact of this reporting omission was explored further using a margin of safety approach during risk assessment.

Toxicity to additional aquatic organisms

Data for *Crassostrea virginica* are not required by Regulation (EU) No 283/2013. HSE Ecotoxicology notes that the study did not determine an EC_{50} over the concentrations tested (EC_{50} > 0.99 mg/L). When compared to the two other acute aquatic invertebrate endpoints (E/LC_{50} = 1.1 mg/L), the *C. virginica* study does not indicate increased sensitivity to inpyrfluxam. As a consequence, this study will not be considered further.

As per EFSA (2013), the risk from the active substance will be assessed using the appropriate PEC values for the proposed uses of inpyrfluxam. Endpoints are adjusted by an Assessment Factor (AF) and the values used (100 for acute endpoints and 10 for chronic endpoints) in the lower tier are taken from Commission Implementing Regulation 546/2011. RAC values will be compared to the relevant PECs in a tiered process for both acute and chronic risks to aquatic organisms. The RACs to be used in the risk assessment are presented in Table 9.4-3 below.

Table 9.4-3: Regulatory acceptable concentrations (RAC) for inpyrfluxam for each organism group (Tier 1)

Group	**Fish**		**Invertebrates**		**Algae**	**Aquatic macrophyte**	**Group**	**Sed. Dwell. Prolonged**
Test species :	**Acute *O. mykiss***	**Chronic *P. promelas***	**Acute *D. magna* / *A. bahia***	**Chronic *D.magna***	***Geomean of two diatom species***	***Lemna gibba***	**Test species :**	***Leptocheirus plumulosus***
End-point [µg a.s./L]	LC_{50}	NOEC	EC_{50}	EC_{10}	E_rC_{50}	E_rC_{50}	**End-point**	NOEC
	31	6.6	1100	210	3600	24000	**[µg a.s./kg]**	10260
AF	100	10	100	10	10	10	**AF**	10

Group	Fish		Invertebrates		Algae	Aquatic macrophyte	Group	Sed. Dwell. Prolonged
Test species:	Acute *O. mykiss*	Chronic *P. promelas*	Acute *D. magna / A. bahia*	Chronic *D.magna*	*Geomean of two diatom species*	*Lemna gibba*	Test species:	*Leptocheirus plumulosus*
RAC [µg a.s./L]	0.31	0.66	11	21	360	2400	**RAC [µg a.s./kg]**	1026

Previously, HSE noted the inconclusive reproduction results of the chronic *A. bahia* study and stated that the lowest derived chronic RAC would be compared to the concentrations tested in the *A. bahia* study. The lowest concentration tested in the chronic *A. bahia* study was 23 µg a.s./L. The lowest chronic RAC in Table 9.4-3 above is 0.66 µg a.s./L This RAC is 35 times lower than the lowest concentration tested and 273 times lower than the concentration for which effects were statistically supported and followed a concentration-response. This demonstrates that the risk assessment is sufficiently protective against any potential chronic effects on *A. bahia*.

Metabolites of inpyrfluxam

The tier-1 data available to address the toxicity of the active substance inpyrfluxam metabolites are summarised below (Table 9.4-4).

Table 9.4-4: Summary of toxicity data related to the metabolites of inpyrfluxam

Test substance	Test organism	Test system	Endpoint (mg met./L)		Reference
Acute toxicity to fish					
3'-OH-S-2840	*Oncorhynchus mykiss*	96-hours, static	LC_{50}	> 6.2 (m.m.)	CA 8.2.1/10 [redacted] 2016a
1'-COOH-S-2840	*Oncorhynchus mykiss*	96-hours, static	LC_{50}	> 50 (m.m.)	CA 8.2.1/11 [redacted] 2016b

m.m. = mean measured

Metabolites 3'-OH-S-2840 and 1'-COOH-S-2840 are relevant for the water (see B8 document) but not the sediment compartment. This is based on the 1:1 toxicity extrapolation between the a.s. and metabolites (see below). This results in the same conclusion for the metabolites as the a.s. in the above section: that a sediment study and associated risk

assessment is not required. Only an acute fish study was submitted for each metabolite. To estimate RACs for other taxonomic groups, the assessment scheme presented in Section 10.2.4 of EFSA Journal 2013;11(7):3290 was followed. The first key step in the assessment scheme is Step 4, "*Identify the species or taxonomic group determining the lowest tier 1 $RAC_{sw;ac}$ for the a.s. Is the acute metabolite L(E)C_{50} > 10 times the a.s. L(E)C_{50} (on a molar basis)".* The lowest tier 1 RAC for inpyrfluxam is the acute fish LC_{50} = 0.31 µg a.s./L. This endpoint is compared on a molar basis to the acute fish 3'-OH-S-2840 and 1'-COOH-S-2840 endpoints below.

The equation used to compare endpoints on a molar basis:

$$LC_{50\ metabolite} > 10 \times \frac{M_{r\ metabolite}}{M_{r\ active}} \times LC_{50\ active}$$

M_r = Molecular mass

For 3'-OH-S-2840:

$$6200\ \mu g\ p.m./L > 10 \times \frac{349.38}{333.38} \times 31\ \mu g\ a.s./L = 325\ \mu g\ a.s./L$$

For 1'-COOH-S-2840:

$$50000\ \mu g\ p.m./L > 10 \times \frac{363.36}{333.38} \times 31\ \mu g\ a.s./L = 338\ \mu g\ a.s./L$$

Both metabolites are clearly ten times less toxic than inpyrfluxam for fish, the most sensitive taxonomic group. In this situation (Step 6 of the assessment scheme), it should be assumed that "*the acute and chronic toxicity of the metabolite is equal to the toxicity of the a.s. for all first tier taxonomic groups*".

EFSA Journal 2013;11(7):3290 also specifies that a chronic metabolite assessment is required when "*exposure of surface water is likely and the metabolite is deemed to be stable in water, as defined in the data requirements, that is, there is less than 90 % loss of the original substance over 24 hours via hydrolysis under relevant pH conditions (Commission Regulation (EU) No 283/2013 and 284/2013). However, as hydrolysis studies are rarely available for metabolites, the 90 % loss trigger can be applied on data from other abiotic/biotic degradation studies.*". HSE Environmental Fate and Behaviour were consulted on this point and confirmed that chronic assessments are required for 1'-COOH-S-2840 and 3'-OH-S-2840.

For 1'-COOH-S-2840, exposure in water is likely as the metabolite displayed a 10 % maximum occurrence in the water-sediment studies (112 DAT). No clear decline phase was observed in water, indicating stability.

For 3'-OH-S-2840, exposure in water is likely via drainflow due to it being a major soil

metabolite. Also, it forms in water via aqueous photolysis, though not as a major metabolite (slightly below 10 % threshold although still increasing at water-sediment study end). Although the metabolite demonstrates a gradual decline in water, HSE Environmental Fate and Behaviour concluded it satisfies the above exposure and stability criteria, considering its presence and persistence in multiple compartments.

Based on this, the initial RACs for the two metabolites are presented in Table 9.4-5 below.

Table 9.4-5: Regulatory acceptable concentrations (RAC) for metabolites of inpyrfluxam for each organism group

Test species:	**Fish (Acute)**		**Fish (Chronic)**		**Aquatic invertebrates (Acute)**		**Aquatic invertebrates (Chronic)**		**Algae**	
	O. mykiss		***Extrapolated from P. promelas inpyrfluxam study***		***Extrapolated from D. magna inpyrfluxam study***		***Extrapolated from D. magna inpyrfluxam study***		***Extrapolated from P. subcapitata inpyrfluxam study***	
Metabolite:	3'-OH-S-2840	1'-COOH-S-2840	3'-OH-S-2840	1'-COOH-S-2840	3'-OH-S-2840	1'-COOH-S-2840	3'-OH-S-2840	1'-COOH-S-2840	3'-OH-S-2840	1'-COOH-S-2840
Endpoint [µg metabolite/L]	6200	50000	6.6	6.6	1100	1100	210	210	3600	3600
AF	100	100	10	10	100	100	10	10	10	10
RAC [µg metabolite/L]	62	500	0.66	0.66	11	11	21	21	360	360

Formulation (S-2399 60 G/L EC)

Toxicity data, regarding the representative formulation 'S-2399 60 G/L EC', available for use in risk assessment are summarised in Table 9.4-6 below.

Table 9.4-6: Summary of available toxicity endpoints for the formulation S-2399 60 G/L EC

Test substance	Test organism	Test system	Endpoint	Result	Reference
S-2399 60 G/L EC [a]	*Oncorhynchus mykiss*	96-hour, static	LC_{50}	0.022 mg a.s./L (m.m)	CP 10.2.1/01 [redacted] 2020a
S-2399 60 G/L EC [a]	*Daphnia magna*	48-hour, static	EC_{50}	0.26 mg a.s./L (m.m)	CP 10.2.1/02 [redacted] 2020b
S-2399 60 G/L EC [a]	*Pseudokirchneriella subcapitata*	72-hour, static	72 hr E_rC_{50}	0.447 mg a.s./L (m.m)	CP 10.2.1/03 [redacted] 2020c

m.m = mean measured concentration; nom = nominal concentration

[a] Active substance content of the formulation; density 0.9273 g/mL, 60.68 g a.s./L (corresponding to 6.544% w/w)

The three studies conducted with the representative formulation were all considered valid and suitable for use in risk assessment. For KCP 10.2.1/03 (*Pseudokirchneriella subcapitata*), there are suggestions that a toxicant-induced lag phase may have been present between 0 – 24 hours. Therefore, it seems likely from the results that 24-hour endpoint estimates would have been lower than the reported 72-hour endpoint values and a more conservative approach. This uncertainty will be explored using a margin of safety approach in the following risk assessment.

HSE has compared the S-2399 60 G/L EC formulation toxicity to the active substance toxicity for acute fish, acute invertebrate and algal studies (Table 9.4-7). All endpoints are expressed in terms of mg a.s./L to facilitate comparison. In line with the SANCO Technical Equivalence guidance (EC (2012))[6], where the formulation is a factor of ≥ 3 times more toxic than the active substance, a separate risk assessment for formulation spray drift is required.

[6] EC (European Commission), 2012. Guidance Document on the assessment of the equivalence of technical materials of substances regulated under Regulation (EC) No 1107/2009. SANCO/10597/2003 rev. 10.1, 13 July 2012

Table 9.4-7: Comparison of active substance toxicity vs formulation toxicity

Test organism	Endpoint	Active substance (mg a.s./L)	Formulation (mg a.s./L)	Factor difference
Onchyrynchus mykiss	LC_{50}	0.031 (m.m.)	0.022 (m.m.)	1.41
Daphnia magna	EC_{50}	1.1 (m.m.)	0.26 (m.m.)	4.23
Pseudokirchneriella subcapitata	E_rC_{50}	> 23 (m.m.)	0.447 (m.m.)	51.45

For acute invertebrates and algae, S-2399 60 G/L EC > 3 times more toxic than the active substance alone. Therefore, for these taxonomic groups a separate formulation spray drift risk assessment is required. For fish, the toxicity of the formulation is within a factor of 3 of the active substance. Therefore, the risk from the formulation can be considered covered by the risk assessment for the active substance and a formulation specific spray drift risk assessment is not required. However, for completeness, a spray drift risk assessment with the formulation endpoint is performed. For algae, *Pseudokirchneriella subcapitata* (green algae) was not the most sensitive algal taxonomic group tested for the active substance. Diatoms were the most sensitive with an $EC_{50\ geomean}$= 3.60 mg a.s./L. This is considered further in the formulation risk assessment carried out below.

The corresponding RACs for use in the risk assessment are detailed in Table 9.4-8 below.

Table 9.4-8: Regulatory acceptable concentrations (RACs) for 'S-2399 60 G/L EC' for each organism group

Test species:	Fish	Invertebrates	Algae
	O. mykiss	***D. magna***	***P. subcapitata***
Endpoint [µg product/L]	LC_{50}	EC_{50}	E_rC_{50}
	336[1] (22 µg a.s./L)	3973[1] (260 µg a.s./L)	6830[1] (447 µg a.s./L)
AF	100	100	10
RAC [µg formulation/L]	3.36 (0.22 µg a.s./L)	39.73 (2.6 µg a.s./L)	683 (44.7 µg a.s./L)

[1]Back-calculated from mean measured concentrations of active substance.

B.9.4.3 Risk assessment

B.9.4.3.1 Active substance (inpyrfluxam)

Tier 1

Risk assessment for the active substance (inpyrfluxam) is summarised in Table 9.4-9. The PEC values used relate to the intended use in cereals (BBCH 30 – 71) of 90 g a.s./ha.

Table 9.4-9: First-tier risk assessment for exposure to inpyrfluxam after use on cereals at 90 g a.s./L

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophytes	Group	Sed. Dwell. Prolonged
Test species		***Oncorhynchus mykiss***	***P. promelas***	***Daphnia magna***	***Daphnia magna***	Geomean of 2 diatoms	***Lemna gibba***	Test species	*L. plumulosus*
Endpoint (µg/L)		LC_{50}	LC_{10}	EC_{50}	EC_{10}	Geomean E_rC_{50}	E_rC_{50}	Endpoint	NOEC
		31	6.6	1100	210	3600	24000	(µg/kg)	10260
AF		100	10	100	10	10	10	AF	10
RAC (µg/L)		0.31	0.66	11	21	360	2400	RAC (µg/kg)	1026
Entry route	PEC_{sw} (µg/L)	PEC/RAC						$PEC_{sed,\ accumulation}$ (µg/kg)	PEC/RAC
Spray drift (1 m)	0.831	**2.68**	**1.26**	0.076	0.040	0.0023	0.00035	14.516	0.014
Drainflow	0.692	**2.23**	**1.05**	0.063	0.033	0.0019	0.00029	12.093	0.012

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**.

For the proposed use on cereals at 90 g a.s./ha, there is an unacceptable acute and chronic risk to fish via spraydrift and drainflow. Further consideration is required. For the aquatic macrophyte *L. gibba*, changes in appearance and morphology were not reported in the submitted study. Given the large margin of safety in the spray drift and drainflow risk assessments, this reporting omission will not have altered the conclusion of acceptable risk. No further consideration of this point is required. For all other exposure route organism group combinations, an acceptable risk can be concluded for inpyrfluxam use in cereals (90 g a.s./ha) at the first-tier stage.

Refined drift exposure assessment

The acute and chronic risk to fish via spray drift was recalculated after reducing inpyrfluxam exposure to water bodies through the use of a 5 m non-sprayed buffer zone.

Table 9.4-10: Refined spray drift exposure assessment for inpyrfluxam usage in cereals through the use of a 5 m buffer zone

Intended use		Cereals	
Active substance		S-2399	
Application rate [g a.s./ha]		1 × 90	
Nozzle reduction	**No-spray buffer [m]**	**Default distance**	**Rautmann drift**
		1 m	5 m
	Exposure Scenario	**PEC_{sw} [µg/L]**	
None	Drift	0.831	0.171
Tier 1-RAC [µg/L]		Acute fish	
0.31		**PEC/RAC ratio**	
None	Drift	**2.68**	0.55
Tier 1-RAC [µg/L]		Chronic fish	
0.66		**PEC/RAC ratio**	
None	Drift	**1.26**	0.26

PEC: Predicted environmental concentration; RAC: Regulatory Acceptable Concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**.

With the 5 m buffer zone refinement, an acceptable acute and chronic risk via spraydrift for fish can be concluded for the proposed use of inpyfluxam in cereals.

An unacceptable acute and chronic risk via drainflow for fish remains. The acute risk is addressed at the Tier 2B stage. The chronic risk requires a higher tier drainflow (HTDF) consideration.

Tier 2B

To refine the acute fish RAC, a SSD was performed with the eight submitted acute fish studies. This is the recommended approach if ≥ 5 endpoints are available for vertebrates (Regulation (EU) No 283/2013). The software selected to estimate the hazardous concentration to 5 % of species tested (HC_5) was a web-based risk assessment tool called "Webfram". This is no longer available. HSE re-estimated the HC_5 using the softwares "ET_x" and "Mosaic" (https://mosaic.univ-lyon1.fr/), which found the HC_5 = 17.9 µg/L (95 % CI = 3.35 to 42.7 µg/L) and HC_5 = 22.0 µg/L (95 % CI = 8.81 to 76.5 µg/L) respectively.

For the ET_x estimate, the model fit was appraised through the use of an Anderson-Darling Test and a visual appraisal. The Anderson-Darling Test passed with $p < 0.01$. For the visual appraisal (Figure 9.7.1-1), the two lowest LC_{50} values were on the right-hand side of the fitted curve, suggesting that the fitted distribution in the tail of the SSD is relatively worst-case compared with the data points. HSE consdiers the ET_x estimate reliable and notes it is in very close agreement with the HC_5 = 18 µg/L provided by the applicant.

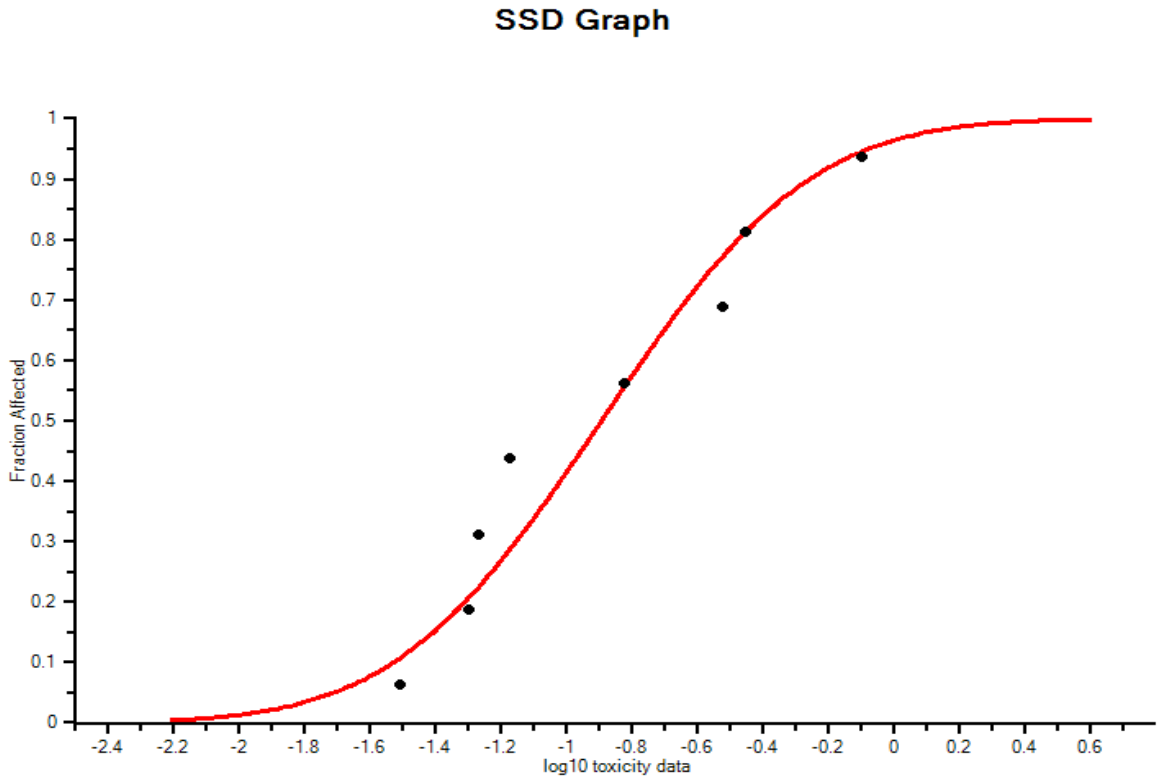

Figure 9.4-1: SSD model fit using the ET_x program. Note the lowest LC_{50} values are to the right of the model prediction.

For the Mosaic estimate, a log-normal distribution was fitted. Fit was appraised visually and determined as acceptable by HSE Ecotoxicology. Similar to the ET_x model, the lowest LC_{50} data point is to the right of the fitted model, likely resulting in a conservative HC_5. HSE Ecotoxicology considers the Mosaic estimate reliable.

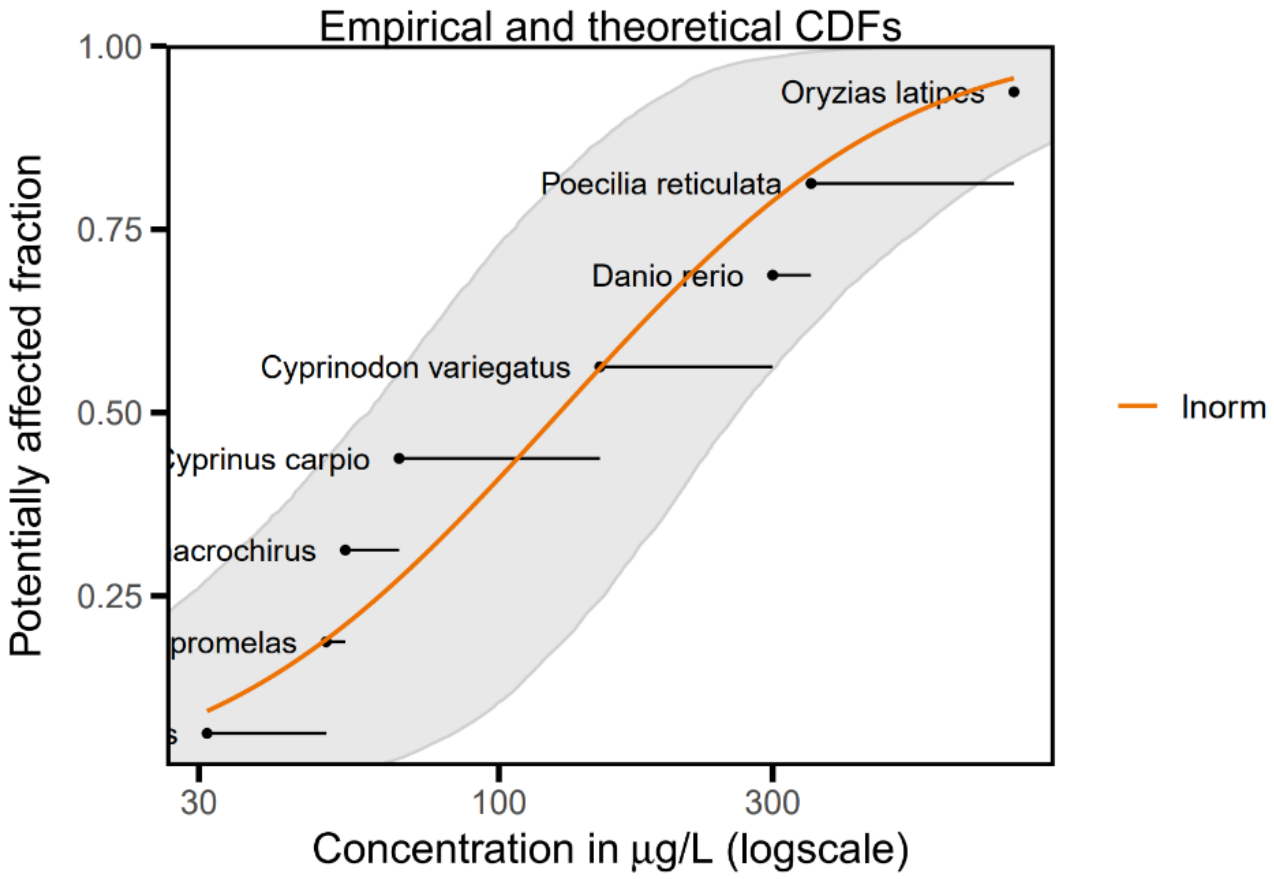

Figure 9.4-2: SSD model fit using the Mosaic program. The black points and lines correspond to the empirical cumulative distribution of the data. Note the lowest LC_{50} value is to the right of the model prediction (if extrapolations had been plotted).

For risk assessment, the lower of the two estimates calculated by HSE Ecotoxicology is selected, which is HC_5 = 17.9 µg a.s./L. The AF of 9 was taken from EFSA Journal 2013;11(7):3290, suitable for a median acute HC_5 based on 96h LC_{50} endpoints for fish.

Table 9.4-11: Refined acute fish drainflow exposure assessment for inpyrfluxam usage in cereals through the use of a RAC derived from a SSD

Group		Fish acute
Test species		**Based on 8 species**
Endpoint		HC_5
(µg/L)		17.9
AF		9
RAC (µg/L)		1.99
Entry route	**PEC_{sw} (µg/L)**	**PEC/RAC**

Drainage	0.692	0.348

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Using the refined RAC an acceptable acute risk to fish via drainage can be concluded.

HTDF

Late (BBCH71) winter cereal application

To resolve the outstanding unacceptable chronic risk to fish via drainage, HSE Environmental Fate and Behaviour conducted HTDF modelling. Please refer to Section B8 (3CP) for the full HTDF report. A summary of the HTDF PECs modelling is presented in tandem with the triggered case-by-case ecotoxicological assessment.

The HTDF modelling performed by HSE Environmental Fate and Behaviour provided PECs associated with an application to winter cereals at BBCH71. HTDF modelling reported > 3 exceedance years for the Denchworth Medium and Denchworth Wet soil climate scenarios (see table below). Please refer to https://www.hse.gov.uk/pesticides/data-requirements-handbook/fate/macro.htm for a full description of the HTDF procedure. The winter cereals application ~~is~~ was initially considered worst-case for the spring cereals application. Please refer to Document 3CA B8 ~~for a justification.~~ and the late (BBCH71) spring cereal application HTDF assessment in the following section for further information.

Throughout this HTDF risk assessment, based on the chemical properties of inpyrfluxam (log P_{ow} = 3.65), it is assumed that inpyrfluxam exposure to fish is predominantly through water, rather than through diet. This is in line with OECD 305 (2012), which provides a threshold of log P_{ow} > 5 for chemicals, above which potential environmental exposure may be largely via diet. As inpyrfluxam is below this threshold, it is assumed that the dominant inpyrfluxam exposure route is comparable between fish life stages and differing energy sources between life stages (yolk sac for larvae vs exogenous feeding for more developed life stages) does not greatly impact inpyrfluxam exposure.

Table 9.4-12 Summary of HTDF modelling. Shows the number of exceedance years out of 30 using a RAC = 0.66 µg a.s./L

Soil scenario	S-2399 exceedance years (out of 30)		
	Dry	Medium	Wet
Denchworth	0	**4**	**6**

Hanslope	0	0	0
Brockhurst	0	0	0
Clifton	0	0	0

As the RAC is set by a chronic fish endpoint (larval survival) there is no defined upper limit threshold for the number of exceedance years. Acceptable risk can be demonstrated through a more detailed case-by-case assessment, which considers the size, frequency and the duration of exceedances. As the RAC is set by effects on larval survival, the Specific Protection Goal (SPG) for the Ecological Threshold Option (ETO) requiring negligible effects on individual survival for fish (vertebrates), with a high degree of certainty, is relevant to the HTDF results.

To determine whether this fish ETO SPG has been met, HSE Ecotoxicology has performed a case-by-case assessment where the magnitude, duration and dependency of each RAC exceedance has been described and then compared to the larval survival effects observed across an equivalent timescale in the *P. promelas* effects study. **Only larval survival was compared to the exceedances; if all other endpoints from the *P. promelas* effects study are used to set the RAC, the drainflow risk assessment passes at the lower tier. For total length, body weight and % of live normal larvae, the agreed NOEC = 7.5 µg/L, resulting in a RAC = 0.75 µg/L. This leads to a PEC/RAC < 1 (0.923).**

The magnitude, duration and dependency of each RAC exceedance is described in the sections below.

Denchworth Medium (2002, 2008, 2009 and 2012)

Out of the 30 modelled years, four years reported exceedances above the RAC for the Denchworth Medium soil/climate scenario. The frequency, duration and magnitude of the exceedances is summarised in table below.

Table 9.4-13 The frequency, duration and magnitude of the exceedances for the Denchworth Medium soil/climate scenario

Year	Exceedances in each year (days)	Longest sequence (days)	$PEC_{SW,max}$ (µg/L)
2002	1	1	0.671
2008	2	2	0.798

2009	3	2	0.705
2012	1	1	0.666

For 2002, 2008 and 2012 the total number of exceedance days matched the longest sequence of exceedance days. These exceedance profiles can be adequately described by the longest sequence of exceedance days and the $PEC_{sw,max}$. This was not the case for 2009, where the exceedance profile constituted two exceedance windows separated by four days. These two exceedance windows are not independent. As a conservative approach, HSE Ecotoxicology has classified this exceedance profile as a seven day exceedance period with a $PEC_{sw,max}$ = 0.705 µg/L. The four yearly exceedance profiles defined above can be considered independent. The closest two defined exceedance profiles were January 2008 and November 2009.

Denchworth Wet

Out of the 30 modelled years, six years reported exceedances above the RAC for the Denchworth Wet soil/climate scenario. The frequency, duration and magnitude of the exceedances is summarised in table below.

Table 9.4-14 The frequency, duration and magnitude of the exceedances for the Denchworth Wet soil/climate scenario

Year	Exceedances in each year (days)	Longest sequence (days)	$PEC_{sw,max}$ (µg/L)
2000	1	1	0.704
2002	1	1	0.738
2007	3	2	0.838
2008	13	8	0.953
2009	15	12	0.966
2012	2	2	0.703

HSE Ecotoxicology has split these exceedance years into two groups, based on their independence and duration.

2000, 2002, and 2012

2000, 2002 and 2012 can all be described as independent exceedance profiles, with their duration and magnitude adequately captured by Table 9.4-14.

2007, 2008 and 2009

A more detailed approach is required for 2007, 2008 and 2009 to adequately describe the exceedance profiles. The PECs against time for these years are displayed in figure below.

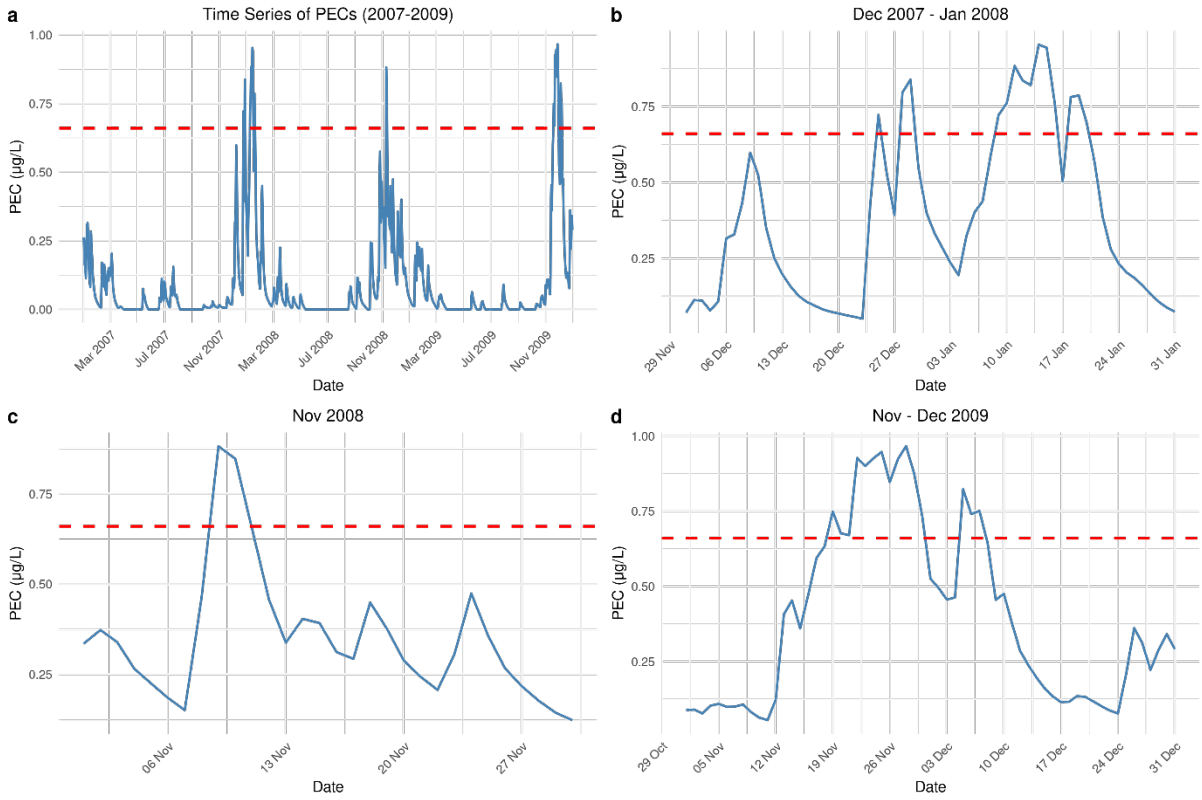

Figure 9.4-3 a) Time series plot of PECs for the years 2007 - 2009 for the Denchworth Wet soil climate scenario. The RAC is shown by the red dashed horizontal line. b), c) and d) show the three independent exceedance profiles during this time.

From 2007 – 2009 there are three independent exceedance profiles, December 2007 to January 2008, November 2008 and November to December 2009. For December 2007 to January 2008, the exceedance profile can be conservatively described as a 27 day exceedance period with a $PEC_{sw,max}$ = 0.953 µg/L. For November 2008, the exceedance profile can be conservatively described as a 2 day exceedance period with a $PEC_{sw,max}$ = 0.882 µg/L. For November to December 2009, the exceedance profile can be conservatively described as a 19 day exceedance period with a $PEC_{sw,max}$ = 0.966 µg/L. The two closest exceedance profiles are 10 months apart.

Next, comparisons are made between the exceedance profiles described above and the larval survival effects observed in the *P. promelas* study over matched time periods.

Comparison of PEC exposure profiles and larval survival effects

Table 9.4-15 reproduces the larval survival data per day for all concentrations tested in the *P. promelas* study.

Table 9.4-15: Day level larval survival results for inpyrfluxam

Day post-hatch	Control	Solvent control	Mean measured concentration (µg a.s./L)				
			1.6	2.7	4.6	7.5	13[a)]
1	80 LF	80 LF	80 LF	80 LF	80 LF	80 LF	40 LF (all lethargic)
2	80 LF	80 LF	80 LF	80 LF	80 LF	80 LF	40 LF
3	80 LF	80 LF	80 LF	80 LF	78 LF (2 DF)	79 LF (1 DF)	39 LF (1 DF)
4	80 LF	80 LF	80 LF	80 LF	78 LF	79 LF	39 LF
5	80 LF	80 LF	80 LF	80 LF	78 LF	79 LF	39 LF
6	80 LF	80 LF	80 LF	80 LF	78 LF	79 LF	39 LF
7	80 LF	80 LF	80 LF	80 LF	78 LF	79 LF	39 LF
8	80 LF	79 LF (1 DF)	80 LF	80 LF	78 LF	79 LF	39 LF
9	80 LF	79 LF	80 LF	80 LF	78 LF	79 LF	39 LF (all small)

10	80 LF	79 LF	80 LF	80 LF	78 LF	79 LF	39 LF (all small)
11	80 LF	79 LF	80 LF	80 LF	78 LF	79 LF	39 LF (all small)
12	80 LF	79 LF	80 LF	80 LF	78 LF	79 LF	39 LF (all small)
13	80 LF	79 LF	80 LF	80 LF	78 LF	79 LF	39 LF (all small)
14	80 LF	79 LF	80 LF	80 LF	78 LF	79 LF	39 LF (all small)
15	80 LF	79 LF	80 LF	80 LF	78 LF	79 LF	39 LF (all small)
16	80 LF	79 LF	80 LF	80 LF	78 LF	79 LF	39 LF (all small)
17	80 LF	79 LF	80 LF	80 LF	78 LF	79 LF	39 LF (all small)
18	80 LF	79 LF	80 LF	80 LF	78 LF	79 LF	39 LF (all small)
19	80 LF	79 LF	80 LF	80 LF	78 LF	79 LF	39 LF (all small)
20	80 LF	79 LF	80 LF	80 LF	78 LF	79 LF	39 LF (all small)
21	80 LF	79 LF	80 LF	80 LF	78 LF	78 LF (1 DF)	39 LF (all small)

22	80 LF	79 LF	80 LF	80 LF	78 LF	78 LF	39 LF (all small)
23	80 LF	79 LF	80 LF	80 LF	78 LF	78 KF	39 LF (all small)
24	80 LF	79 LF	80 LF	80 LF	78 LF	78 LF	37 LF (all small) (2 DF)
25	80 LF	79 LF	80 LF	80 LF (1 pale)	78 LF	78 LF	37 LF (all small)
26	80 LF	79 LF	80 LF	80 LF (1 pale)	78 LF	78 LF	37 LF (all small)
27	80 LF	79 LF	80 LF	80 LF (1 pale)	78 LF	78 LF	37 LF (all small)
28	77 LF	77 LF	75 LF	72 LF	70 LF	74 LF	30 LF

Note: 20 larvae per replicate, four replicates per treatment (total = 80).
LF: Live fry; DF: Dead fry.
[a)] In replicates A and B, fry were inadvertently discarded and replicates were not loaded.

In Table 9.4-16 below, the $PEC_{sw, max}$ for independent exceedance profiles are compared to NOECs derived for equivalent time periods from the *P. promelas* larval survival data. This is performed for all Denchworth Medium exceedance profiles and Denchworth Wet exceedance profiles from 2000, 2002, 2012 and November 2008.

Table 9.4-16 Case-by-case HTDF risk assessment for larval survival

Scenario	Month	Year	Conservative exposure length	$PEC_{sw, max}$ (µg/L)	$NOEC_{survival}$ (µg/L)	RAC (µg/L)	PEC/RAC
Denchworth Medium	November	2002	1	0.671	13	1.3	0.52

Denchworth Medium	January	2008	2	0.798	13	1.3	0.61
Denchworth Medium	November	2009	7	0.705	13	1.3	0.54
Denchworth Medium	December	2012	1	0.666	13	1.3	0.51
Denchworth Wet	November	2000	1	0.704	13	1.3	0.54
Denchworth Wet	November	2002	1	0.738	13	1.3	0.57
Denchworth Wet	November	2008	2	0.882	13	1.3	0.68
Denchworth Wet	December	2012	2	0.703	13	1.3	0.54

After 7 days, 2.5 % larval mortality was observed in the *P. promelas* chronic study at the highest concentration tested. A NOEC = 13 µg/L (or EC_{10} > 13 µg/L) can be set without statistical modelling: even if 13 µg/L was significantly different from the control, such a small larval mortality would not be considered biologically relevant. The same NOEC can be applied to all exposure periods < 7 days. HSE Ecotoxicology reiterates that the reported sub-lethal effects on growth are covered by the lower tier risk assessment using the NOEC = 7.5 µg/L. Using the chronic AF = 10, a RAC = 1.3 µg/L was defined for all exposure periods < 7 days. In the above table, the $PEC_{sw,\ max}$ of exceedance profiles was compared to the corresponding time period adjusted RAC. This adds a level of conservatism as the PEC_{twa} across the relevant exceedance profile would be equal to or lower than the maximum. Even for this conservative approach, comparing the $PEC_{sw,\ max}$ to the time period adjusted RAC indicates an acceptable risk (PEC/RAC < 1) for the above independent exceedance profiles. In fact, all the above exceedance profiles, except Denchworth Medium 2009, represent exceedances of short duration (≤ 2 days). Due to their short duration, a higher degree of certainty can be attributed to the indicated acceptable risk for these exceedance profiles. Without these 6 exceedance years (Denchworth Medium: 2002, 2008 and 2012; Denchworth Wet: 2000, 2002 and 2012) the criterion of no more than three RAC

exceedance years out of 30 is reached for both Denchworth Medium and Wet, in accordance with the 'MACRO higher tier drainflow modelling for pesticide registration in Great Britain and Northern Ireland' guidance.

Two exceedance profiles remain: December 2007 to January 2008 (27-day exceedance period with a $PEC_{sw,max}$ = 0.953 µg/L) and November to December 2009 (19-day exceedance period with a $PEC_{sw,max}$ = 0.966 µg/L). For these exceedance profiles, particularly the 27-day exceedance period with a $PEC_{sw,max}$ = 0.953 µg/L, comparing the $PEC_{sw,max}$ to a corresponding time period adjusted NOEC is less definitive. In the *P. promelas* hazard study, a 28-day EC_{10} = 6.6 µg/L was determined. To what degree this endpoint is driven by 19-day or 27-day exposure is unclear. Expressed differently, it is possible that an exposure profile constituting 27 days of constant exposure, followed by one day in clean, unspiked water, would lead to similar effects as 28 days of constant exposure. Therefore, for the 27-day exceedance period with a $PEC_{sw,max}$ = 0.953 µg/L, it is not possible to conclude negligible effects with a high degree of certainty for fish. A 28-day time-weighted average could be calculated for the exceedance profile, if the day before or after exceedance were included (day before included = 0.605 µg/L; day after included = 0.607 µg/L), and compared to the 0.66 µg/L RAC derived from the 28-day EC_{10}. This comparison would suggest an acceptable risk (PEC/RAC < 1). However, it is possible that comparing time-weighted averages from variable exposure profiles to effects from constant exposure hazard studies is misleading. For example, the 28-day exposure profile with a twa = 0.605 – 0.607 µg/L, could result in greater effects than a 28-day continuous exposure at 0.66 µg/L, due to the existence of pulses up to $PEC_{sw,max}$ = 0.953 µg/L. The same uncertainties affect the 19-day exposure profile with a $PEC_{sw,max}$ = 0.966 µg/L, albeit to a lesser degree.

Taken together, it is not possible to discount non-negligible effects for the 27-day exceedance profile defined between December 2007 and January 2008 with a high degree of certainty. Therefore, across the 30-year simulation window, there is one exceedance profile of concern regarding chronic fish survival, and arguably two of potential concern (including November and December 2009). It must be stressed that this potential risk is restricted to one soil, climate scenario, Denchworth Wet, which represents a small percentage of total cropping area for cereals. This is demonstrated by the results of the weighted level of exceedance approach. The percentage of exceedance years for each soil-climate scenario is multiplied by the extent of the crop grown under each soil-climate condition and summed. The results, presented in the table below, are well above the 90 % threshold.

Table 9.4-17: Weighted levels of exceedance approach

Soil drainage status	Weighted safe/not safe years (%)
not drained	48.84
peat	3.05
drained but 'safe'	47.62
drained and not 'safe'	0.48
Total safe 'years'	99.52

To conclude, based on an assessment that accounts for exceedance duration, the number of exceedance years containing exceedance profiles of concern (one for Denchworth Wet; zero for Denchworth Medium), or potential concern (two for Denchworth Wet; zero for Denchworth Medium), is lower than the threshold of three for both Denchworth Medium and Wet, in accordance with the 'MACRO higher tier drainflow modelling for pesticide registration in Great Britain and Northern Ireland' guidance. The threshold of 3 was set with the aim of providing the equivalent level of protection that a 90th percentile PEC provides in other exposure assessments (for example spray drift) where it is accepted that a reasonable worst case is a more appropriate threshold than an absolute worst case. For Denchworth Wet, one, or potentially two, exceedance profiles exist that may result in non-negligible chronic effects in fish. HSE Ecotoxicology notes that comparable exceedances are possible for risk assessments where the current no more than three exceedance years out of 30 criterion is met. In such instances, no appreciation of exceedance profile magnitude or duration is required.

For the remaining exceedances of ≤ 7 days, in addition to the indicated acceptable risk for larval survival across the relevant exceedance time frames (Table 9.4-16), there is evidence to suggest that larvae will not be present during winter, when all exceedances occur. Salmonoids are the main group of fish in the UK which spawn over winter (November to January), but their eggs do not typically hatch until March or April. Therefore, it is unlikely that larvae will be present when the RAC is exceeded. The risk to more developed fish life stages (juveniles and adults) over these time frames (≤ 7 days) is likely covered by the acute risk assessment, where juvenile fish are exposed over a 4-day period. Of the exceedances outlined in Table 9.4-16, only one has an exceedance period of > 4 days (Denchworth Medium, 2009), the exposure period not covered by the acute risk assessment. If this exceedance profile is classed as one of concern, the threshold of three exceedances is still

not breached for either Denchworth Medium (one) or Wet (two). For the remaining exceedance periods from Table 9.4-16 (≤ 2-day exceedance periods), the acute RAC = 1.99 µg/L can be confidently applied, which indicates an acceptable risk for juvenile and adult fish.

To further strengthen the above conclusion, the ECP suggested exploring the agronomic likelihood of applications occurring post-BBCH 69. HSE Efficacy were consulted and provided the following consideration, "*The final fungicide application to wheat (called 'T3') is usually made at BBCH 59 or BBCH 63-65. Although applications can be made outside these timings the majority of applications occur before the end of flowering (BBCH 69), as this will give the best disease control. The same principles apply to durum wheat. Barley is less likely to have applications post-BBCH 69. Please refer to Fungicide programmes for wheat | AHDB and Fungicide programmes for barley | AHDB*". As the likelihood of post-BBCH 69 applications is low, further HTDF modelling was performed for winter cereals using an application timing of BBCH 69 by HSE Environmental Fate and Behaviour. Fewer exceedances occurred at this application timing compared to BBCH 71, highlighting that the real-world risk of inpyrfluxam is likely lower than those outlined in the risk assessment conducted in line with the GAP. HSE Ecotoxicology stresses, however, that the risk assessment conducted in line with the GAP demonstrated an acceptable risk and this further consideration is provided as supporting information only.

Therefore, HSE Ecotoxicology has concluded that an acceptable risk to fish from chronic exposure via drainflow for all life stages can be demonstrated for an application to winter cereals at BBCH71 for inpyrfluxam. No further consideration is required for winter cereal applications.

Late (BBCH71) spring cereal application

A further HTDF assessment was requested during the ECP meeting. The ECP questioned whether the late (BBCH71) winter cereal application can be considered worst-case for the late (BBCH71) spring cereal application in all instances. For context, HSE Environmental Fate and Behaviour excluded Denchworth soil scenarios from the spring cereal application modelling based on several lines of evidence supporting the low probability of spring cereals being sown on Denchworth soils. The ECP did not agree with this decision, stating that farmers may attempt to sow spring cereals on heavy/wet soils like Denchworth, particularly in response to winter-sown crop failure. Consequently, HSE Ecotoxicology has performed a further HTDF assessment for the late (BBCH 71) spring cereal application, analogous to the assessment initially performed for the late (BBCH 71) winter cereal application.

A summary of the HTDF modelling results for an application to spring cereals at BBCH 71 is presented below.

Table 9.4-18 Summary of HTDF modelling for late (BBCH 71) spring cereal application. Shows the number of exceedance years out of 30 using a RAC = 0.66 µg a.s./L

Soil scenario	S-2399 exceedance years (out of 30)		
	Dry	Medium	Wet
Denchworth	0	**4**	**9**
Hanslope	0	0	1
Brockhurst	0	0	0
Clifton	0	0	0

The magnitude, duration and dependency of each RAC exceedance is described in the sections below.

Denchworth Medium

Out of the 30 modelled years, four years reported exceedances above the RAC for the Denchworth Medium soil/climate scenario. The frequency, duration and magnitude of the exceedances are summarised in table below.

Table 9.4-19 The frequency, duration and magnitude of the exceedances for the Denchworth Medium soil/climate scenario

Year	Exceedances in each year (days)	Longest sequence (days)	$PEC_{sw,max}$ (µg/L)
2002	4	2	0.742
2008	2	2	0.802
2009	9	8	0.982
2012	2	2	0.734

For 2008 and 2012 the total number of exceedance days matched the longest sequence of exceedance days. These exceedance profiles can be adequately described by the longest sequence of exceedance days and the $PEC_{sw,max}$. This was not the case for 2002 or 2009.

For 2002, the exceedance profile constituted three exceedance windows. The exceedance profile is visualised in the following figure.

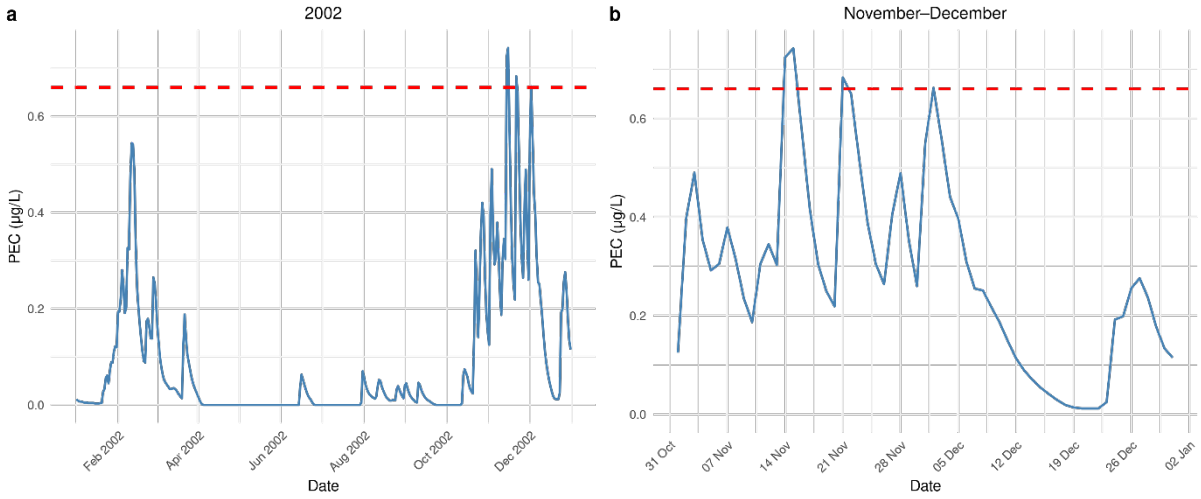

Figure 9.4-4 a) Time series plot of PECs for the year 2002 for the Denchworth Medium soil climate scenario. The RAC is shown by the red dashed horizontal line. b) shows the three non-independent exceedance windows during this time.

As the three exceedance windows are not independent, as a conservative approach, HSE Ecotoxicology has classified this exceedance profile as a 19 day exceedance period with a $PEC_{sw,max}$ = 0.742 µg/L. For 2009, the exceedance profile constituted two exceedance windows separated by 9 days. As the two exceedance windows are not independent, HSE Ecotoxicology has classified this exceedance profile as a 17 day exceedance period with a $PEC_{sw,max}$ = 0.982 µg/L. The four yearly exceedance profiles defined above can be considered independent. The closest two defined exceedance profiles were January 2008 and November 2009.

Denchworth Wet

Out of the 30 modelled years, nine years reported exceedances above the RAC for the Denchworth Wet soil/climate scenario. The frequency, duration and magnitude of the exceedances is summarised in table below.

Table 9.4-20 The frequency, duration and magnitude of the exceedances for the Denchworth Wet soil/climate scenario

Year	Exceedances in each year (days)	Longest sequence (days)	$PEC_{SW,max}$ (µg/L)
1998	1	1	0.683
2000	3	2	0.796

Year	Exceedances in each year (days)	Longest sequence (days)	$PEC_{SW,max}$ (µg/L)
2002	2	2	0.863
2007	3	2	0.852
2008	11	8	1.002
2009	18	14	1.166
2012	5	3	0.788
2015	4	2	0.754
2019	1	1	0.698

HSE Ecotoxicology has split these exceedance years into two groups, based on their independence and duration.

1998, 2000, 2002, 2012, 2015, 2019

1998, 2002 and 2019 can all be described as independent exceedance profiles, with their duration and magnitude adequately captured by Table 9.4 14. For 2000, the exceedance profile comprises of two exceedance windows, 5 days apart. As the two exceedance windows are not independent, HSE Ecotoxicology has classified this exceedance profile as a 7 day exceedance period with a $PEC_{sw,max}$ = 0.796 µg/L. For 2012, the exceedance profile comprises of three exceedance windows. The exceedance profile is visualised in the following figure.

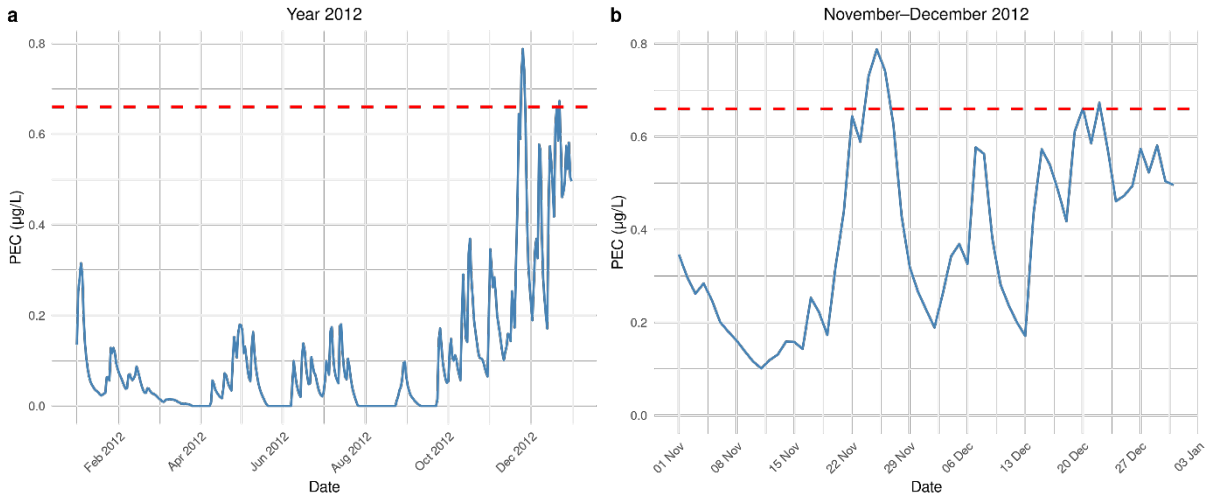

Figure 9.4-5 a) Time series plot of PECs for the year 2012 for the Denchworth Wet soil climate scenario. The RAC is shown by the red dashed horizontal line. b) shows the three non-independent exceedance windows during this time.

As the three exceedance windows are not independent, HSE Ecotoxicology has classified this exceedance profile as a 29 day exceedance period with a $PEC_{sw,max}$ = 0.788 µg/L. For 2015, the exceedance profile comprises of two exceedance windows eight days apart. As the two exceedance windows are not independent, HSE Ecotoxicology has classified this exceedance profile as a 11 day exceedance period with a $PEC_{sw,max}$ = 0.754 µg/L.

2007, 2008, 2009

A more detailed approach is required for 2007, 2008 and 2009 to adequately describe the exceedance profiles. The PECs against time for these years are displayed below in figure below.

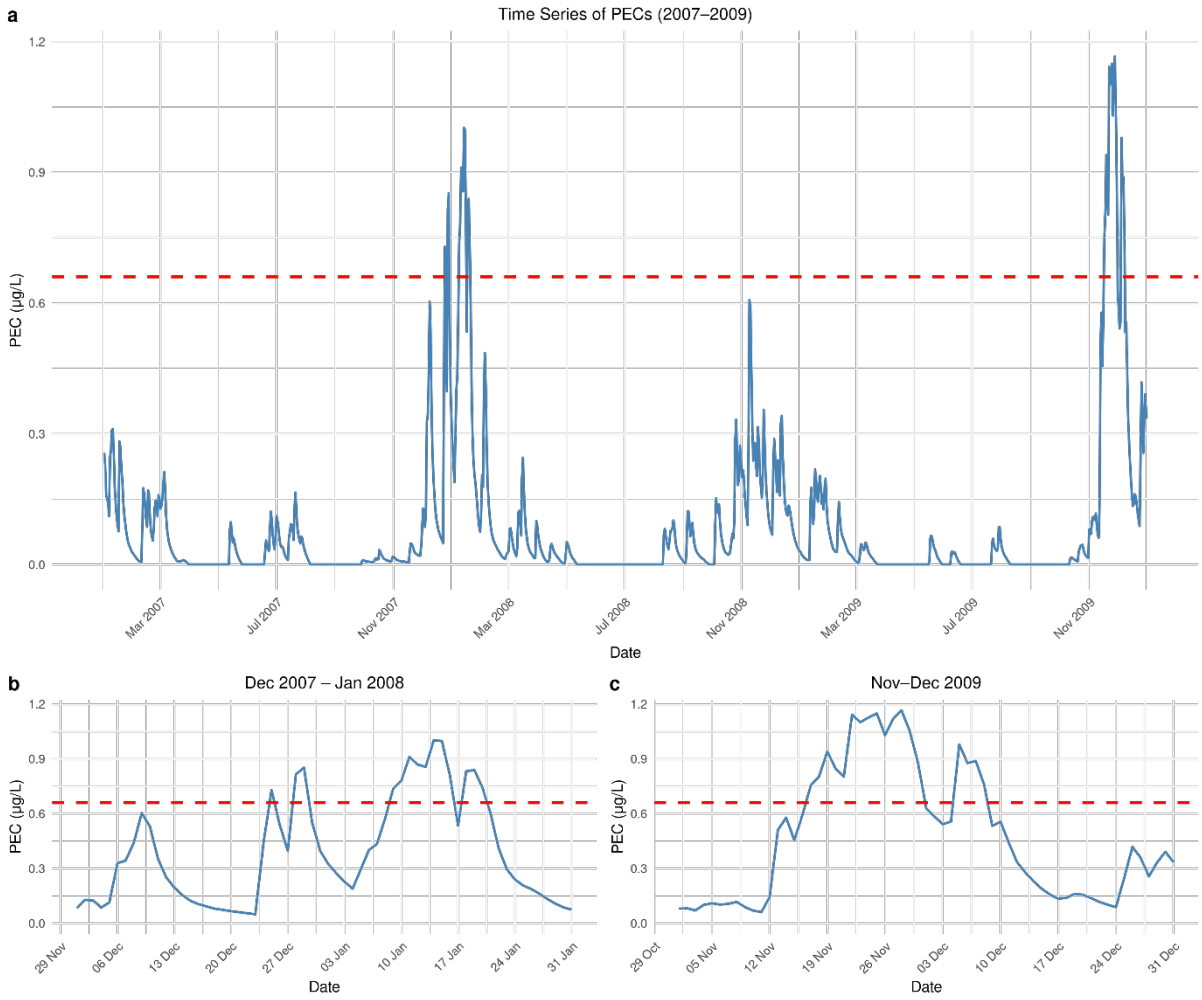

Figure 9.4-6 a) Time series plot of PECs for the years 2007 - 2009 for the Denchworth Wet soil climate scenario. The RAC is shown by the red dashed horizontal line. b) and c) show the two independent exceedance profiles during this time.

From 2007 – 2009 there are two independent exceedance profiles, December 2007 to January 2008 and November to December 2009. For December 2007 to January 2008, the exceedance profile can be conservatively described as a 27 day exceedance period with a $PEC_{sw,max}$ = 1.002 µg/L. For November to December 2009, the exceedance profile can be conservatively described as a 22 day exceedance period with a $PEC_{sw,max}$ = 1.166 µg/L.

Next, comparisons are made between the exceedance profiles described above and the larval survival effects observed in the *P. promelas* study over matched time periods.

Comparison of PEC exposure profiles and larval survival effects

Please refer to **Table 9.4-15** for the larval survival data per day for all concentrations tested in the *P. promelas* study.

In the table below, the $PEC_{sw,max}$ for independent exceedance profiles are compared to NOECs derived for equivalent time periods from the *P. promelas* larval survival data. This

is performed for Denchworth Medium exceedance profiles from 2008 and 2012 and Denchworth Wet exceedance profiles from 1998, 2002 and 2019.

Table 9.4-21 Case-by-case HTDF risk assessment for larval survival (Denchworth Medium: 2008 and 2012, Denchworth Wet: 1998, 2002, and 2019)

Scenario	Month	Year	Conservative exposure length	$PEC_{sw, max}$ (µg/L)	$NOEC_{survival}$ (µg/L)	RAC (µg/L)	PEC/RAC
Denchworth Medium	January	2008	2	0.802	13	1.3	0.617
Denchworth Medium	December	2012	2	0.734	13	1.3	0.565
Denchworth Wet	November	1998	1	0.683	13	1.3	0.525
Denchworth Wet	November	2002	2	0.863	13	1.3	0.664
Denchworth Wet	November	2019	1	0.698	13	1.3	0.537

For the five exceedance profiles above, the maximum exposure period is 2 days. In the *P. promelas* chronic study, over 2 days 0 % larval mortality was observed at the highest concentration tested. A NOEC = 13 µg/L (or EC_{10} > 13 µg/L) can be set without statistical modelling. Therefore, a RAC = 1.3 µg/L can be set for exposure periods ≤ 2 days with a high degree of certainty. This endpoint is most applicable to larvae as it is derived from a chronic larval study. For other life stages (juveniles and adults), the five exceedance profiles are also covered by the acute risk assessment, where juveniles are exposed over a 4-day period. During the acute risk assessment, a RAC = 1.99 µg/L was determined. Using either RAC results in a PEC/RAC < 1, demonstrating acceptable risks to all fish life stages. At this stage, only two Denchworth Medium exceedance profiles of potential concern remain, which is below the threshold of three. Therefore, an acceptable risk to fish can be concluded for the Denchworth Medium soil, climate scenario after a late (BBCH 71) spring cereal application. Further consideration is required for the Denchworth Wet soil, climate scenario, presented below.

Five Denchworth Wet exceedance profiles remain. Exceedance profiles from 2000 and 2015 are compared to NOECs derived for equivalent time periods from the *P. promelas* larval survival data in the table below.

Table 9.4-22 Case-by-case HTDF risk assessment for larval survival (Denchworth Wet: 2000 and 2015)

Scenario	Month	Year	Conservative exposure length	$PEC_{sw,\ max}$ (µg/L)	$NOEC_{survival}$ (µg/L)	Larval mortality (%)	RAC (µg/L)	PEC/RAC
Denchworth Wet	November	2000	7	0.796	13	2.5	1.3	0.612
Denchworth Wet	December	2015	11	0.754	13	2.5	1.3	0.580

For 2000 and 2015, the maximum exposure length was 11 days. After 11 days in the accompanying chronic larval study, one larval mortality out of 40 test individuals had been observed (Day 3) for the highest tested concentration (13 µg/L). No further mortalities were observed until Day 24 (a further two mortalities). It is the view of HSE Ecotoxicology that the low mortality at Day 11, accompanied by a lack of mortalities for a further 13 days, support the absence of latency of effects. Bioaccumulation is one mechanism that can drive latency of effects. A bioconcentration in fish study (KCA 8.2.2.3/01) was submitted for inpyrfluxam and did not report a bioconcentration factor (BCF) of concern ($BCF_{SSL,\ TRR}$ = 211.8 L/kg fish, $BCF_{SSL,\ S\text{-}2399}$ = 38.4 L/kg fish) with a maximum $t_{1/2g}$ = 0.401 days. This provides further support for the absence of latency of effects. Taken together, HSE Ecotoxicology considers a NOEC = 13 µg/L over the 11-day time period appropriate. Comparing the $PEC_{sw,max}$ to the resulting RAC = 1.3 µg/L leads to PEC/RAC < 1 for both exceedance profiles. HSE Ecotoxicology reiterates that defining the exceedance profiles as constant exposure to the $PEC_{sw,max}$ over the defined periods is conservative. To demonstrate this, the PEC_{twa} = 0.493 µg/L for the 2000 exceedance profile and PEC_{twa} = 0.444 µg/L for the 2015 exceedance profile. Taken together, an acceptable risk can be concluded for the 2000 and 2015 exceedance profiles.

HSE Ecotoxicology refers the reader to the late (BBCH71) winter cereal application HTDF case-by-case assessment for additional arguments surrounding larval presence during exceedances, a description of how the 'MACRO higher tier drainflow modelling for pesticide registration in Great Britain and Northern Ireland' guidance is applied, and the lower risk associated with BBCH69 applications, which are in practice more likely than BBCH71.

Three exceedance profiles remain for Denchworth Wet, 2007/2008, 2009 and 2012. As the exceedance profiles for 2007 and 2008 were non-independent, this has been classified as one independent exceedance year. The case-by-case assessment has concluded three exceedance years of concern, which is within the threshold of no more than three exceedance years in 30 years. Consequently, the three outstanding exceedance years have not been considered further. Therefore, an acceptable risk to fish from chronic exposure via drainflow can be concluded for the late (BBCH71) spring cereal application for inpyrfluxam. This provides further support to the case presented by HSE Environmental fate and behaviour relating to the relevance of the Denchworth soil type for spring cereal applications.

B.9.4.3.2 Metabolites of inpyrfluxam

Tier 1

Risk assessments for the metabolites 3'-OH-S-2840 and 1'-COOH-S-2840 are summarised for the intended use (90 g a.s./ha on cereals, BBCH 30 – 71) in Table 9.4-16 and Table 9.4-17 below:

Table 9.4-23: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the 3'-OH-S-2840 metabolite, based on UK national modelling for the use of S2399 in cereals

Group		Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic	Algae
Test species		***O. mykiss***	***extrapolated***	***extrapolated***	***extrapolated***	***extrapolated***
Endpoint		LC_{50}	EC_{10}	EC_{50}	EC_{10}	E_rC_{50}
(µg/L)		>6200	6.6	1100	210	3600
AF		100	10	100	10	10
RAC (µg/L)		62	0.66	11	21	360
Entry route	**PEC_{sw} (µg/L)**	**PEC / RAC**				
Drift (1 m)	0.075	0.0012	0.1136	0.0068	0.0036	0.0002
Drainage	0.210	0.003	0.318	0.019	0.01	0.001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.4-24: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the 1'-COOH-S-2840 metabolites, based on UK national modelling for the use of S2399 in cereals

Group		Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic	Algae
Test species		*O. mykiss*	*extrapolated*	*extrapolated*	*extrapolated*	*extrapolated*
Endpoint		LC_{50}	EC_{10}	EC_{50}	EC_{10}	E_rC_{50}
(µg/L)		>50000	6.6	1100	210	3600
AF		100	10	100	10	10
RAC (µg/L)		500	0.66	11	21	360
Entry route	PEC_{sw} / PEC_{gw} (µg/L)	PEC / RAC				
Drift (1 m)	0.091	0.0002	0.1379	0.0083	0.0043	0.0003
Drainage	0.863	0.002	**1.31**	0.078	0.041	0.002
Ground water	0.18	0.0004	0.273	0.016	0.009	0.0005

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

For the proposed 90 g a.s./ha use on cereals, an acceptable spray drift risk to aquatic organisms can be concluded for all relevant inpyrfluxam metabolites.

For groundwater, no assessment is required for 3'-OH-S-2840 as the highest PEC_{gw} = 0.01 µg/L, which is below the 0.1 µg/L trigger. For 1'-COOH-S-2840, the PEC_{gw} = 1.802 µg/L (spring cereals, late scenario, Hamburg, PEARL, see Section B8 (3CP) for more details) was divided by 10, to represent the dilution factor that occurs when groundwater becomes surface water. This is in accordance with SANCO/3268/2001 (2002). An acceptable risk can be concluded for all organism groups.

However, for drainage exposure, the chronic fish scenario indicates an unacceptable risk for metabolite 1'-COOH-S-2840 when using a RAC of 0.66 µg/L. A direct 1:1 extrapolation from the a.s. chronic fish endpoint for 1'-COOH-S-2840 is likely overly conservative. Given that the a.s. chronic fish endpoint is based on larval survival, it is reasonable to assume that the mortality-based acute toxicity ratio between the a.s. and 1'-COOH-S-2840 reflects their relative mortality-based chronic toxicity. After molecular weight adjustment, the acute fish endpoint for 1'-COOH-S-2840 is 1,480 times higher than that of the a.s., demonstrating significantly lower toxicity. Using this relationship, a chronic fish endpoint for 1'-COOH-S-2840 can be estimated as:

$$\left(\frac{363.36}{333.38} \times 6.6\ \mu g\ a.s./L\right) \times 1480 = 10646\ \mu g\ met./L$$

A RAC would usually be derived by applying an AF = 10 for chronic endpoints. Given the uncertainty in the extrapolation, HSE has applied an AF = 100 instead. This results in a chronic fish RAC = 106.5 µg met./L for 1'-COOH-S-2840. Using this refined RAC leads to a PEC/RAC = 0.0081, which demonstrates a large margin of safety even with an AF = 100. From this, an acceptable risk for the chronic fish scenario can be concluded for 1'-COOH-S-2840 drainflow exposure.

For the remaining endpoints from the chronic fish study with the active substance, a NOEC of 7.5 µg/L and resulting RAC of 0.75 µg/L were determined. Using this sub-lethal chronic RAC and assuming a 1:1 toxicity relationship between the active substance and metabolite, the lower-tier drainflow assessment fails (PEC/RAC = 1.15). For inpyfluxam and 1'-COOH-S-2840, the toxicity relationship for sub-lethal chronic effects is unknown. However, based on the acute mortality toxicity relationship, it can be inferred that the toxophore relevant to fish has been lost in 1'-COOH-S-2840.

The 'Opinion of the Scientific Panel on Plant health, Plant protection products and their Residues on a request from EFSA related to the assessment of the acute and chronic risk to aquatic organisms with regard to the possibility of lowering the uncertainty factor if additional species were tested.' (The EFSA Journal (2005) 301, 1-45) discusses common acute-to-chronic toxicity ratios for substances. The document states: "*A factor of 10 was felt to be supported by most data (especially neutral organics) with some exceptions (e.g. anilines) where larger factors may be appropriate.*"

For 1'-COOH-S-2840, if the active substance's sub-lethal chronic endpoint were representative of the metabolite, the ratio between the acute endpoint (>50,000 µg/L) and chronic endpoint (7.5 µg/L) would exceed 6,667. Considering the historical data reviewed in EFSA Journal (2005) 301, 1-45, such an acute-to-chronic toxicity ratio is highly unlikely. Supporting this is the absence of any toxic symptoms recorded during the acute fish toxicity study for 1'-COOH-S-2840.

Taken together, HSE Ecotoxicology considers it implausible that the sub-lethal chronic effects of 1'-COOH-S-2840 are of a similar magnitude to those of the active substance. Therefore, further toxicity data on the sub-lethal chronic effects of 1'-COOH-S-2840 are not considered necessary to conclude an acceptable chronic risk for this compound, particularly given the objective to reduce vertebrate testing. No further consideration is required and the risk from 1'-COOH-S-2840 is considered sufficiently addressed by the assessment for the active substance.

B.9.4.3.3 Formulation 'S-2399 60 G/L EC'

Tier 1

Risk assessment for the formulation 'S-2399 60 G/L EC' is summarised in Table 9.4-18. The PEC values used relate to the use of 'S-2399 60 G/L EC' on cereals at 90 g a.s./ha. PECs and RACs have been expressed in µg a.s/L. Only the risk via spray drift is considered. Formulations would not expect to remain intact to result in exposure via drainflow.

Table 9.4-25: First-tier risk assessment for exposure to S-2399 60 G/L EC after use on cereals at 90 g a.s./L

Group		**Fish**	**Inverteb. acute**	**Algae**
Test species		***O. mykiss***	***D. magna***	***P. subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg a.s/L)		22	260	447
AF		100	100	10
RAC (µg a.s/L)		0.22	2.6	44.7
Entry route	**PEC_{sw} (µg a.s./L)**	**PEC/RAC**		
Spray drift (1 m)	0.831	**3.78**	0.320	0.0186
Spray drift (5 m)	0.171	0.78	-	-

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**.

An acceptable acute risk for the formulation via spray drift can be concluded for fish and aquatic invertebrates at 5 m and 1 m, respectively.

For algae, *Pseudokirchneriella subcapitata* (green algae) was not the most sensitive algal taxonomic group tested for the active substance. Diatoms were the most sensitive with an $EC_{50\ geomean}$= 3.6 mg a.s./L. For *Pseudokirchneriella subcapitata*, the only algal species with active substance and product data available, the formulation is 51.45 more toxic than the active substance. For the formulation risk assessment, HSE applied this factor to the active substance diatom geomean to account for the different sensitivities between different algal taxonomic groups (estimated formulation diatom $EC_{50geomean}$ = 70.0 µg a.s./L).

Table 9.4-26: Formulation risk assessment for algae correcting for most sensitive algal taxonomic group

Group		**Algae**
Test species		**Adjusted diatom geomean**
Endpoint		Geomean
(µg a.s/L)		70
AF		10
RAC (µg a.s/L)		7
Entry route	**PEC_{sw} (µg/L)**	**PEC/RAC**
Spray drift (1 m)	0.831	0.119

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**.

After using an approximate estimate of the formulation endpoint for the most sensitive algal taxonomic group as an input for the formulation risk assessment, an acceptable risk to algae is still concluded. Although the method for approximating the formulation endpoint is simplistic, the large margin of safety (at 1 m, at 5 m this would be even larger) addresses any potential uncertainties associated with this approach.

There was one potential toxicity underestimation from the *Pseudokirchneriella subcapitata* formulation study (KCP 10.2.1/03) that may have impacted the risk assessment: the suggestion of a toxicant-induced lag phase between 0 – 24 hours and the failure to estimate a 24-hour endpoint that would have reflected this potential toxicity increase. Given the large margin of safety indicated by the PEC/RAC ratios above, this potential toxicity underestimate is highly unlikely to alter the conclusions of the risk assessment for algae. Therefore, HSE deems this margin of safety protective of this potential toxicity underestimate.

B.9.4.3.4 Conclusion

Inpyrfluxam

An acceptable risk to all aquatic organisms, except fish, was concluded at the first tier for inpyrfluxam. An acceptable acute and prolonged risk to fish for exposure via spray drift was demonstrated with the implementation of a 5 m buffer zone. An acceptable acute risk to fish for exposure via drainflow was demonstrated at Tier 2B using a SSD refined RAC. An acceptable prolonged risk to fish for exposure via drainflow was demonstrated through a detailed case-by-case assessment of the HTDF modelling results.

Metabolites

An acceptable risk to all aquatic organisms was demonstrated at the first tier for 3'-OH-S-2840. For 1'-COOH-S-2840, an acceptable risk was demonstrated for all aquatic organisms at the first tier once the chronic fish RAC was revised to reflect the reduced toxicity of 1'-COOH-S-2840 to fish.

Formulation 'S-2399 60 G/L EC '

An acceptable risk to all aquatic organism groups was demonstrated at the first tier for exposure to the formulation via spray drift.

B.9.5 Effects on arthropods

B.9.5.1 Effects on bees

B.9.5.1.1 Acute toxicity to bees

Reference:	KCP 10.3.1.1.1/01
Report Title:	S-2399 60 g/L EC: Acute Oral and Contact Toxicity to the Honey Bee, *Apis mellifera* L., under Laboratory Conditions
Author(s) & year:	[redacted] (2019)
Document No, Authority registration No:	Eurofins Scientific Study No. S19-00421 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0107
Substance used:	S-2399 6 EC, V16-7L1901, 6.544 ± 0.024% w/w
Method of analysis:	n/a
Guideline(s):	OECD 213 (1998) and 214 (1998)
Deviations:	Yes, see HSE Comments
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

MATERIALS

Test material	S-2399 60 g/L EC
Batch number:	V16-7L1901
Active substance content:	60.68 ± 0.23 g/L (verified by certificate of analysis)
Density:	0.9273 (g/mL)
Storage on receipt:	Store in the original container < 30º C, in a dry and well-ventilated place.

Date of receipt	28 Jan 2019

TREATMENTS

Nominal test doses:	Oral toxicity test: 47.92, 95.83, 191.67, 383.34 and 766.67 µg test item/bee, equivalent to 3.14, 6.27, 12.54, 25.08 and 50.17 µg a.s./bee (nominal dose) Oral toxicity test: 43.29, 70.38, 99.38, 202.30 and 320.20 µg test item/bee, equivalent to 2.83, 4.61, 6.50, 13.24 and 20.95 µg a.s./bee (actual ingested dose) Contact toxicity test: 47.92, 95.83, 191.67, 383.34 and 766.67 µg test item/bee, equivalent to 3.14, 6.27, 12.54, 25.08 and 50.17 µg a.s./bee (nominal) .
Control:	Oral toxicity test: 50 % (w/v) aqueous sucrose solution (C) Contact toxicity test: deionised water (C)
Toxic reference:	BAS 152 11 I (Dimethoate, analysed 429 g/L, nominal 40 % w/v EC, verified by certificate of analysis) Oral toxicity test: 0.060, 0.090, 0.140 and 0.210 µg dimethoate/bee (nominal). Contact toxicity test: 0.080, 0.120, 0.180 and 0.270 µg dimethoate/bee (nominal).
Toxic reference batch:	FRE-001578

TEST ORGANISMS

Species:	Honey Bees (*Apis mellifera* L), adult worker bees
Source:	Queen-right, healthy colony from commercial apiary (beehives registered in the Local Government Administration under the official number 176-V-026)
Acclimatisation period:	Bees were randomly collected from the outer combs of the hive and distributed into test cages one day before start of exposure. The collected honey bees were kept under test conditions until test start.
Diet:	50 % (w/v) aqueous sucrose solution was used as food. Feeding was done *ad libitum* during acclimatisation and the test period, except during starvation and feeding of application solution(s) in oral exposure.

TEST DESIGN

Test cages:	Steel lined with filter paper (base: 8.5 cm x 4.5 cm; height: 6.0 cm)
Replication:	Test item and control = 5, reference item = 4
No of bees per tank:	10
Duration:	48 hours

TEST CONDITIONS

Test temperature:	Oral exposure: 24.9 - 25.7 °C Contact exposure: 24.8 – 25.2 °C
Relative humidity:	Oral exposure: 55.4 – 60.5 % Contact exposure: 56.9 – 59.7 %
Lighting:	24 h darkness, except during application and assessments

STUDY DESIGN AND METHODS

Experimental dates: 15 May 2019 to 17 May 2019

Test organism and treatment

The hive used for honey bee collection was adequately fed, healthy and reported as disease free. No chemical substances (such as antibiotics, anti-*Varroa* treatments, pesticides, etc.) were used in the hive for at least one month prior to the test.

Lethal effects of the test substance on the honeybee, *Apis mellifera* L., after oral and contact exposure were assessed at five doses of S-2399 60 g/L EC under laboratory conditions. In addition, one untreated control and a reference item (four doses) were tested. For the oral treatment, the test substance was provided via feeding solution. For the contact treatment, the test substance was applied to the dorsal side of the bee thorax.

In the oral toxicity test, Dimethoate EC 400 was tested at nominal doses of 0.060, 0.090, 0.140 and 0.210 µg dimethoate/bee. The actual ingested doses were 0.051, 0.076, 0.111 and 0.137 µg dimethoate/bee. For the contact toxicity test, Dimethoate EC 400 was tested at 0.080, 0.120, 0.180 and 0.270 µg dimethoate/bee.

Oral toxicity test

A quantity of 200 µL of test or reference substance application solution was offered to each cage of ten bees. The bees in one replicate share through trophallaxis the application solution and thus receive similar doses. The actual amount of test solution consumed by each replicate was determined by weighing the feeders before and after feeding. The bees were starved 2 hours prior to application start. Each test unit was provided with the application solution for up to 6 hours, to ensure a sufficient intake. The feeders were then removed, and the bees were provided *ad libitum* with a 50 % (w/v) aqueous sucrose solution.

Contact toxicity test

After the bees had been anaesthetised with carbon dioxide they were treated individually by

applying 1 µL test item or 2 µL untreated control or reference substance application solution dorsally to the thorax of the bee. Application was performed using a hand operated micro-applicator. After treatment, the bees were returned to the test cages and fed with a 50% (w/v) aqueous sucrose solution *ad libitum.*

Dose preparation
For the oral treatment, a stock solution (equal to the highest test solution) was prepared by dispersing 0.7666 g test item in 20 mL 50 % (w/v) aqueous sucrose solution. The lower test solutions were prepared by dilution of the stock solution (5, 2.5, 1.25 and 0.625 mL added to 10 mL sucrose solution each).

For the contact treatment, a stock solution (equal to the highest test solution) was prepared by dispersing 3.8335 g test item in 10 mL deionised water. The lower test solutions were prepared by dilution of the stock solution (2.5, 1.25, 0.625 and 0.313 mL added to 5 mL deionised water each).

Measurements and observations
Mortality of the bees was assessed at 4, 24 and 48 hours after test start (start of feeding or after contact application). At the same observation times, any abnormal behaviour (affected, apathy, cramps, moribund) in comparison to the control bees was documented. The consumption of application solution per replicate was determined by weighing the feeders at the start and at the end of the feeding application period.

The actual uptake of test/reference item per bee was calculated according to the following formula:

$$D = \frac{T_d \times A_c}{T_c}$$

D = actual uptake of test (reference) item/bee [µg/bee]
T_d= target dose of test (reference) item [µg/bee]
A_c= actual consumption of application solution/replicate [mg/replicate]
T_c= target consumption of 200 µL, corresponding 238 mg of application solution [mg/replicate]
Temperature and humidity were recorded continuously with appropriate, calibrated equipment.

Statistical analysis:
Mortality was determined after 24 and 48 hours after exposure and corrected for the control results following Abbott's formula:

$$M = \frac{M_t - M_c}{100 - M_c} \times 100$$

M = Corrected mortality (%)
M_t = Mortality in the treated group [%]
M_c = Mortality in the control group [%]

The 24-hour and 48-hour LD_{50} oral and contact values with 95 % confidence limits of the reference item were calculated by a Weibull and Probit analysis method using linear max. likelihood regression. The 24-hour and 48-hour LD_{50} oral values with 95 % confidence limits of the test item were calculated by a Probit analysis method using linear max. likelihood regression. The 24-hour and 48-hour NOED oral values were determined by a Step-down Cochran-Armitage Test Procedure. The 24-hour and 48-hour LD_{50} contact values with 95 % confidence limits of the test item were calculated by a Trimmed Spearman-Karber procedure (interpolation method). The 24-hour and 48-hour NOED contact values could not be determined due to response obtained, so were empirically estimated.

Analysis was performed using ToxRat® Professional, Version 3.2.1.

RESULTS AND DISCUSSION

Biological effects

Oral toxicity test

The results from the oral toxicity test are summarised in Table 9.5-1.

Table 9.5-1: Oral toxicity of S-2399 60 g/L EC to honeybees (Apis mellifera L.)

Dose		Mean mortality [%]			Corrected mortality [%] [a]		
Target	Actual uptake						
[µg product/bee]	[µg product/bee]	4 h	24 h	48 h	4 h	24 h	48 h
Control (sucrose)							
-	-	0.0	2.0	4.0	-	-	-
Test item (S-2399 60 g/L EC)							
47.92	43.29	0.0	0.0	2.0	0.0	-2.04	-2.08
95.83	70.38	0.0	4.0	6.0	0.0	2.04	2.08
191.67	99.38	0.0	6.0	8.0	0.0	4.08	4.17
383.34	202.30	0.0	32	36	0.0	30.61	33.33
766.67	320.20	0.0	60	60	0.0	59.18	58.33

Dose		Mean mortality [%]			Corrected mortality [%] [a]		
Target	Actual uptake						
[µg product/bee]	[µg product/bee]	4 h	24 h	48 h	4 h	24 h	48 h
Endpoints oral toxicity							
	µg S-2399 60 g/L EC/bee			**µg a.s./bee**			
24-hour LD_{50} (95% CI)	277.55 (239.84 – 338.39)			18.16 (15.69 – 22.14)			
48-hour LD_{50} (95% CI)	274.95 (237.37 – 335.31)			17.99 (15.53 – 21.94)			
24-hour NOED	99.38			6.50			
48-hour NOED	99.38			6.50			

[a] According to the formula of Schneider-Orelli (1947).
CI: confidence limits.

The mean consumption per treatment in shown in Table 9.5-2.

Table 9.5-2: Mean consumption of S-2399 60 g/L EC per treatment

Target dose	Consumed solution		Consumed dosed
[µg product/bee]	g	%	g product
Control (sucrose)			
0	0.221	93.03	-
Test item (S-2399 60 g/L EC)			
47.92	0.215	90.34	43.29
95.83	0.175	73.45	70.38
191.67	0.123	51.85	99.38
383.34	0.16	52.77	202.30
766.67	0.099	41.76	320.20

Sub-lethal effects were also detected and presented in Table 9.5-3.

Table 9.5-3: Behavioural abnormalities in the oral toxicity test with S-2399 60 g/L EC

Test item: S-2399 60 g/L EC [µg test item (Nominal)/bee]	4-h					24-h					48-h				
	Alive	A	Ap	C	Mb	Alive	A	Ap	C	Mb	Alive	A	Ap	C	Mb
0	50	0	0	0	0	49	0	0	0	0	49	0	0	0	0
47.92	50	0	0	0	0	50	0	0	0	0	49	0	0	0	0
95.83	50	1	0	0	0	48	0	0	0	0	47	0	0	0	0
191.67	50	19	0	0	0	47	7	0	0	0	46	0	0	0	0
383.34	50	31	0	0	0	34	10	0	0	0	32	2	0	0	0
766.67	50	50	0	0	0	20	8	0	0	0	20	1	0	0	0

A = affected
Ap = Apathy
C = Cramps
Mb = Moribund

Abnormal behaviour was pronounced for the 191.67 µg test item (Nominal)/bee treatment level and above after 4 hours. These behavioural effects gradually reduced throughout the experiment from 38 to 100 % to 5 to 6.3 % of individuals affected (range of individuals affected in three highest treatment levels).

The 24- and 48-hour LD_{50} values for the reference item were 0.100 and 0.090 µg dimethoate/bee, respectively.

Contact toxicity test
The results from the contact toxicity test are summarised in Table 9.5-4.

Table 9.5-4: Contact toxicity of S-2399 60 g/L EC to honeybees (Apis mellifera L.)

Target dose	Mean mortality [%]		
[µg product/bee]	4 h	24 h	48 h
Control (water)			
-	0.0	0.0	0.0
Test item (S-2399 60 g/L EC)			
47.92	4.0	10	16

Target dose	Mean mortality [%]		
[µg product/bee]	4 h	24 h	48 h
95.83	10	26	26
191.67	2.0	24	34
383.34	2.0	30	40
766.67	12	84	94
Endpoints contact toxicity			
	µg S-2399 60 g/L EC/bee	**µg a.s./bee**	
24-hour LD_{50} (95% CI)	324.59 (272.10 – 387.20)	21.24 (17.81 – 25.34)	
48-hour LD_{50} (95% CI)	252.91 (211.14 – 302.94)	16.55 (13.82 – 19.82)	
24-hour NOED	< 47.92	< 3.14	
48-hour NOED	< 47.92	< 3.14	

CI: confidence limits.

Table 9.5-5 presents the behavioural abnormalities detected in the contact toxicity test.

Table 9.5-5: Behavioural abnormalities in the contact toxicity test with S-2399 60 g/L EC

Test item: S-2399 60 g/L EC [µg test item (Nominal)/bee]	4 h					24 h					48 h				
	Alive	A	Ap	C	Mb	Alive	A	Ap	C	Mb	Alive	A	Ap	C	Mb
0	50	0	0	0	0	50	0	0	0	0	50	0	0	0	0
47.92	48	31	0	0	2	45	8	0	0	1	42	7	0	0	0
95.83	45	31	0	0	8	37	13	0	0	0	37	13	0	0	0
191.67	49	21	0	0	1	38	5	0	0	1	33	2	0	0	0
383.34	49	16	1	0	1	35	4	0	0	1	30	2	0	0	0
766.67	44	5	0	0	39	8	8	0	0	0	3	3	0	0	0

A = affected
Ap = Apathy
C = Cramps
Mb = Moribund

After 4 hours behavioural abnormalities were detected for all treatment levels after contact exposure. As with oral exposure these effects did reduce over time. The 47.92 and 95.83 µg test item (Nominal)/bee treatment levels, however, displayed a higher proportion of individuals affected after 48 hours for contact exposure, with 16.7 and 35 % of individuals still displaying behavioural abnormalities.

The 24- and 48-hour LD_{50} values for the reference item were 0.194 and 0.178 µg dimethoate/bee, respectively.

Validity criteria
The validity criteria for the study were met according to OECD 213 (1998) and OECD 214 (1998) (Table 9.5-6).

Table 9.5-6: Compliance with OECD 213 and OECD 214 validity criteria

Validity criterion	Required	Obtained
OECD 213		
Control mortality	≤ 10 %	4 %
Dimethoate toxicity (24-hour LD_{50})	0.10 – 0.35 µg/bee	0.1 µg/bee
OECD 214		
Control mortality	≤ 10 %	0 %
Dimethoate toxicity (24-hour LD_{50})	0.10 – 0.30 µg/bee	0.194 µg/bee

CONCLUSIONS
The acute contact and oral toxicity on the honeybee *Apis mellifera* L. were investigated under laboratory conditions over a period of 48 hours. The 48-hour oral LD_{50} of S-2399 60 g/L EC was 274.95 µg product/bee and the 48-hour oral NOED was 99.38 µg product/bee. The 48-hour contact LD_{50} of S-2399 60 g/L EC was 252.91 µg product/bee and the 48-hour contact NOED was < 47.92 µg product/bee. All validity criteria were fulfilled.

HSE COMMENTS
The study was carried out according to and evaluated against the OECD 213 (1998) and OECD 214 (1998) guidelines. All validity criteria outlined in OECD 213 (1998) and OECD 214 (1998) were satisfactorily met for the duration of the study. There were no significant

deviations to the guidelines. Treatment with the toxic reference (dimethoate) indicated the sensitivity of the bees and reliability of the test system was appropriate for both oral and contact toxicity.

The following minor deviations were noted for OECD 213 (1998) and OECD 214 (1998):
OECD 213 (1998) § 10 states that test cages should be randomly placed in the experimental room. This was either not performed or reported by the study conductor.

OECD 213 (1998) § 19 and 20 outline the length of the test and observation schedule. These paragraph state, "*the duration of the test is 48 h after the test solution has been replaced with sucrose solution alone*" and "*mortality is recorded at 4 h after start of the test and thereafter at 24h and 48h (i.e. after given dose)*". The study conductor recorded morality 4, 24 and 48 hours after the start of feeding, which suggests that the study was terminated 48 hours after the provision of spiked feed, not 48 hours after the consumption of the spiked feed (or after maximum 6-hour spiked feed consumption period). The study, therefore, was terminated 6 hours early for the higher treatment levels, where individuals failed to consume the full quantity of spiked feed. For the 766.67 µg test item/bee treatment level there was no increase in mortality between 24 to 48 hours, which suggest this reduction in the observation period is unlikely to have underestimated the effect of the test item. For the 191.67 and 383.34 µg test item/bee treatment levels, however, there were small increases (2 and 4 %) in mortality from 24 to 48 hours, which may have been greater than if the observation period had continued for the additional six hours. This deviation and its impact will be considered at the risk assessment stage by appraising the margin of safety for the formulated product.

OECD 213 (1998) § 25 and OECD 214 (1998) § 23 describe what the test report must include. An approximate age in weeks of the bees used in the study was not provided. Also, the results of preliminary range finding studies were not reported. Bees were collected from the outer combs of the colony. Honey bees exhibit temporal polyethism. Therefore, it is expected that bees collected from the outer combs will be of a middling age and approximately age matched as these individuals are not foraging (older bees) or nursing brood (younger bees). Collecting individuals from the outer comb is a standard approach for approximate age matching and HSE considers this acceptable. The failure to report the results of preliminary range finding tests is not in keeping with the guideline. This reporting omission, however, does not impact the validity of the definitive test. HSE considers this a minor deviation.

OECD 214 (1998) § 17 states, "*a volume of 1 µl of solution containing the test substance at the suitable concentration should be applied with a microapplicator to the dorsal side of the thorax of each bee. Other volumes may be used, if justified*". During this study 2 µl was used for the control and reference item since a higher volume ensures a more reliable dispersion of the application solution. HSE accepts this justification but questions why 2 µl was not used for the test item as well if this volume improves dispersion of the solution.

HSE notes that the acute contact exposure study was repeated due to the validity criteria not being met on the first attempt. The validity criteria was met on the second attempt, the results of which are presented in the study report and this summary.

The above study was conducted to GLP and considered valid.

The agreed endpoints suitable for use in the risk assessment are: 48-hour oral LD_{50} = 274.95 µg test item/bee, which equates to 17.99 µg a.s./bee, and 48-hour contact LD_{50} = 252.91 µg test item/bee, which equates to 16.55 µg a.s./bee.

Reference:	KCP 10.3.1.1.1/02
Report Title:	S-2399 60 g/L EC: Acute oral and contact Toxicity to the Bumblebee *Bombus terrestris* L., under Laboratory Conditions
Author(s) & year:	[redacted] (2020)
Document No, Authority registration No:	Eurofins Scientific Study No. S19-00422 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0121
Substance used:	S-2399 6 EC, V16-7L1901, 6.544 ± 0.024% w/w
Method of analysis:	LC-MS/MS
Guideline(s):	OECD 246 and 247
Deviations:	Yes, see HSE Comments
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	No, not considered in current SANCO/10329/2002 risk assessment

I. MATERIALS AND METHODS

A. MATERIALS

1. Test item: S-2399 60 g/L EC (Emulsifiable Concentrate)
Description: Clear liquid
Lot/Batch: V16-7L1901
Active substance content: Nominal: 60 g S-2399/L
Analysed: 60.68 g S-2399/L
Density: 0.9273 (kg/m3)
Molecular weight: 333.76 g/mol
Purity: 60 / 60.68 (nominal / analytical)
Water solubility: Not stated

Analysis of concentrations: 95.83, 191.67, 383.33, 766.67 and 1533.33µg test item/bumblebee. LOQ = 240 mg test item/L. LOD = 72 mg test item/L
CAS number: 1352994-67-2

2. **Control:**
Oral toxicity test: 50 % (w/v) aqueous sucrose solution
Contact toxicity test: Deionised water containing 0.1 % Triton X solution

3. **Reference item:** Dimethoate 40% w/v EC (BAS 152 11 I)
Description: Not stated
Lot/Batch: FRE-001578
Active substance content: Dimethoate 429.0 g/L analysed
Density: 1.076 g/mL

B. STUDY DESIGN AND METHODS

1. **Test species:** Bumblebee, Bombus terrestris L. (Hymenoptera, Apidae); young adult worker bumblebees
Age: Young adult worker bees
Source: Queen-right, healthy colonies (containing ~ 60 – 80 bumblebee workers) from a commercial supplier (Biomip Biological Quality, SL, Almería, Spain).
Collection date: 06 May 2019
Collection method: Not stated
Replicates: Oral **=** 30. Contact = 35
Number of bees/cage: 1
Duration: 48 hrs (oral). 72 hrs (contact)

Acclimatisation: Bumblebees were collected from 13 hives and distributed into test cages one day before start of exposure.
Diet: Bumblebees were fed via 1 mL syringes deprived of the tip and kept in a position to prevent the syringes from sticking into the Nicot® cage.
50 % (w/v) aqueous sucrose solution was used as food. Feeding was done ad libitum during acclimatisation and the test period, except during starvation and feeding of application solution(s) in oral exposure.

2. **Test units:** In both tests, the bees were kept in Nicot® cages.

3. **Environmental conditions**

Table 9.5-7: Summary of environmental conditions of bumblebees exposed to S-2399 60EC for acute oral and contact toxicity testing

Variable	Required OECD 246 (2017)	Obtained
Temperature (°C)	25 ± 2 °C	24.8 – 25.7 °C
Relative Humidity (%)	60 ± 20 %	55.4 – 60.5 %

Photoperiod	Constant darkness	Throughout the test, bumblebees were kept in constant darkness, except during handling and assessments

Study dates: 07 May 2019 - 10 May 2019

B. STUDY DESIGN AND METHODS

1. Test organism and treatment:

Lethal effects of the test substance on the bumblebee, *Bombus terrestris* L., after oral and contact exposure were assessed at five doses of S-2399 60 G/L EC under laboratory conditions. In addition, an untreated control and a reference item (one dose) were tested. For the oral treatment, the test substance was provided via feeding solution. For the contact treatment, the test substance was applied to the dorsal side of the bee thorax.

In the oral toxicity test, Dimethoate EC 400 was tested at nominally 3.0 µg dimethoate/bumblebee. The actual ingested dose was similar to the target dose. For the contact toxicity test, Dimethoate EC 400 was tested at 10.0 µg dimethoate/bumblebee.

Oral toxicity test

S-2399 60 G/L EC was tested at nominally 95.83, 191.67, 383.33, 766.67 and 1533.33 µg test item/bumblebee (equivalent to 6.25, 12.50, 25.00, 50.00 and 100 µg a.s./bumblebee). The actual ingested doses were 90.61, 176.09, 350.61, 532.69 and 508.69 µg product/bumblebee (equivalent to 5.91, 11.48, 22.87, 34.74 and 33.18 µg a.s./bumblebee). A control group, receiving untreated 50% (w/v) aqueous sucrose solution was tested in parallel. Each treatment group consisted of 35 replicates containing one test organism each. A quantity of 40 µL of test or reference substance application solution was offered to each cage of one bumblebee. The actual amount of test solution consumed by each replicate was determined by weighing the feeders before and after feeding. The bumblebees were starved 3 hours prior to application start. Each test unit was provided with the application solution for up to 4 hours. The feeders were then removed, and the bumblebees were provided *ad libitum* with a 50 % (w/v) aqueous sucrose solution for the reminder of the test. Individuals that did not consume at least 80 % of the mean consumption of the respective treatment group within the 4 hours of exposure were discarded from the test.

Contact toxicity test

S-2399 60 G/L EC was tested at nominally 191.67, 383.33, 766.67, 1533.33 and 3066.67 µg test item/bumblebee (equivalent to 12.50, 25.00, 50.00, 100 and 200 µg a.s./bumblebee). A control group, receiving deionised water containing 0.1 % Triton X solution, was tested in parallel. Each treatment group consisted of 30 replicates containing one test organism each. After the bumblebees had been chilled with dry ice, they were treated individually by applying 4 µL test item, untreated control or reference substance application solution dorsally to the thorax of the bumblebee. Application was performed using a Burkard hand micro-applicator. After treatment, the bumblebees were returned to the test cages and fed with a 50% (w/v) aqueous sucrose solution *ad libitum.*

2. Dose preparation:

For the oral treatment, five test solutions were prepared by dispersing 0.0241, 0.0477, 0.0957, 0.1919 and 0.3835 g test item in 10 mL 50 % (w/v) aqueous sucrose solution.
For the contact treatment, five test solutions were prepared by dispersing 0.4794, 0.9585, 1.9165, 3.8334 and 7.6669 g test item in 10 mL deionised water with 0.1 % Triton X.

3. Measurements and observations:

Mortality of the bees was assessed at 4, 24 and 48 hours after test start (start of feeding or after contact application). Moreover, thereafter 72 hours in the contact toxicity test. At the same observation times, any abnormal behaviour in comparison to the control bumblebees was documented. The consumption of application solution per replicate was determined by weighing the feeders at the start and at the end of the feeding application period.

At the start of the test (0 days), samples of the control group and the lowest and highest test item treated solutions for both, oral and contact tests were taken. Samples were analysed by LC-MS/MS). Method validation data are presented in Document Part B, Section 5.

Temperature and relative air humidity were recorded at intervals of 1 hour with calibrated data loggers.
LOQ = 240 mg test item/L. LOD = 72 mg test item/L.

4. Statistical analysis:

Mortality was determined after 24, 48 and 72 hours after exposure and corrected for the control results following Schneider-Orelli (1947) formula. The LD_{50} oral and contact values with 95 % confidence limits could not be statistically calculated because mortalities did not reach 50 % in any test item group in both oral and contact tests. Therefore, LD_{50} values were empirically estimated. NOED values, for both oral and contact tests, were determined using Chi^2 2x2 table test with Bonferroni correction.

Analysis was performed using ToxRat® Professional, Version 3.2.1 (ToxRat Solutions GmbH) and Microsoft© Office Excel 2010 v.14.0.

C RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

Oral toxicity test

The actual consumed doses of S-2399 60 g/L EC in the treatments of nominal 95.83, 191.67, 383.33, 766.67 and 1533.33µg product/bumblebee were 90.61, 176.09, 350.61, 532.69 and 508.69 µg product/bumblebee and the cumulative mean mortality after 48 hours was 7.69, 4.35, 0.00, 9.09 and 4.17 %, (corrected: 4.98, 1.53, -2.94, 6.42 and 1.35 %) respectively.

The number of non-feeder bumblebees range from any individuals in the control group to 24 individuals in the target dose of 766.67 µg product/bumblebee. A repellency effect towards the test item was observed because the number of non-feeders was clearly higher in all the test item treatments compared to the control group. In addition, the mean amount of food consumed per treatment decreased as the concentration of test item increased.

Moreover, mortality in control and test reference groups was 2.86 and 100 %, respectively at the end of the test. No moribund bumblebees were recorded at any assessment.

Results of the oral toxicity test and relevant endpoints are summarized in Table 9.5-8.

Table 9.5-8: Oral toxicity of S-2399 60 g/L EC to bumblebees (*Bombus terrestris*)

Dose		Mean mortality [%]			Corrected mortality [%] [a]		
Target	Actual uptake						
[µg product/bumblebee]	[µg product/bumblebee]	4 h	24 h	48 h	4 h	24 h	48 h
Control (sucrose)							
-	-	0.0	0.0	2.86	-	-	-
Test item (S-2399 60 g/L EC)							
95.83	90.61	0.0	0.0	7.69	0.0	0.0	4.98
191.67	176.09	0.0	0.0	4.35	0.0	0.0	1.53
383.33	350.61	0.0	0.0	0.0	0.0	0.0	-2.94
766.67	532.69	0.0	9.09	9.09	0.0	9.09	6.42
1533.33	508.69	0.0	4.17	4.17	0.0	4.17	1.35
Endpoints oral toxicity							
	µg S-2399 60 g/L EC/bumblebee			**µg a.s./bumblebee**			
24-hour LD_{50} (95% CI)	> 532.69			> 34.74			
48-hour LD_{50} (95% CI)	> 532.69			> 34.74			
24-hour NOED	532.69			34.74			
48-hour NOED	532.69			34.74			

[a] According to the formula of Schneider-Orelli (1947).
CI: confidence limits.

The mean consumption per treatment is shown in the Table 9.5-9.

Table 9.5-9: Mean consumption of S-2399 60 g/L EC per treatment

Target dose	Consumed solution		Consumed dosed/bumblebee		Number of feeders
[µg product/bumblebee]	mg	%	[µg product/bumblebee]	[µg a.s./bumblebee]*	
Control (sucrose)					
0	45.2	94.9	-	-	35

Target dose	Consumed solution		Consumed dosed/bumblebee		Number of feeders
[µg product/bumblebee]	mg	%	[µg product/bumblebee]	[µg a.s./bumblebee]*	
Test item (S-2399 60 g/L EC)					
95.83	45.0	94.6	90.61	5.91	26
191.67	43.7	91.9	176.09	11.48	23
383.33	43.5	91.5	350.61	22.87	19
766.67	33.1	69.5	532.69	34.74	11
1533.33	15.8	33.2	508.69	33.18	24

* Based on the purity of the active ingredient according to the available certificate of analysis.

Contact toxicity test

In the test item doses of 191.67, 383.33, 766.67, 1533.33 and 3066.67 µg product/bumblebee, the cumulative mean mortality after 72 hours was 0.00, 10.00, 6.67, 10.00 and 33.33%, respectively.

Results of the contact toxicity test and relevant endpoints are summarized in the Table 9.5-10.

Table 9.5-10: Contact toxicity of S-2399 60 g/L EC to bumblebees (*Bombus terrestris*)

Target dose	Mean mortality [%]				Corrected mortality [%] [a]			
[µg product/bee]	4 h	24 h	48 h	72 h	4 h	24 h	48 h	72 h
Control (deionised water containing 0.1 % Triton X solution)								
-	0.00	0.00	6.67	6.67	-	-	-	-
Test item (S-2399 60 g/L EC)								
191.67	0.00	0.00	0.00	0.00	0.00	0.00	-7.14	-7.15
383.33	0.00	6.67	6.67	10.00	0.00	6.67	0.00	3.57
766.67	0.00	3.33	6.67	6.67	0.00	3.33	0.00	0.00
1533.33	0.00	3.33	6.67	10.00	0.00	3.33	0.00	6.25
3066.67	0.00	16.67	30.0	33.33	0.00	16.67	25.00	30.56
Endpoints contact toxicity								
	µg S-2399 60 g/L EC/bumblebee				**µg a.s./bumblebee**			
24-hour LD_{50} (95% CI)	> 3066.67				> 200.00			
48-hour LD_{50} (95% CI)	> 3066.67				> 200.00			
72-hour LD_{50} (95% CI)	> 3066.67				> 200.00			

Target dose	Mean mortality [%]				Corrected mortality [%] [a]			
[µg product/bee]	4 h	24 h	48 h	72 h	4 h	24 h	48 h	72 h
24-hour NOED	3066.67				200.00			
48-hour NOED	3066.67				200.00			
72-hour NOED	3066.67				200.00			

[a] According to the formula of Schneider-Orelli (1947).
CI: confidence limits.

B. ANALYTICAL RESULTS

Mean measured concentrations ranged between 95 – 112 % of nominal. Since the measured concentration in the samples was within 20 % of nominal, the concentrations of the test item were confirmed, and the endpoints are based on nominal concentrations.

Results of the analytical phase are summarized in the Table 9.5-11.

Table 9.5-11: Analytical results

Nominal concentration (mg a.s./mL)	Measured concentration (mg a.s./mL)	% of nominal	Matrix
Oral toxicity test			
Control	< LOD	-	50 % (w/v) aqueous sucrose solution
0.16	0.16	101	
2.50	2.59	104	
Contact toxicity test			
Control	< LOD	-	0.1 % Triton X Solution
3.13	2.96	95	
50.00	56.10	112	

LOD = limit of detection of 72 mg test item/L (30 % of the LOQ) for sucrose solution and water + 0.1% Triton X.

C. VALIDITY CRITERIA

The acute oral study fulfilled the validity criteria outlined in the most recent EU test guideline (OECD 247, 2017) as detailed below:

- Average mortality for the total number of controls must not exceed 10% at the end of the test. Average mortality was 2.86% in the untreated control.
- Average mortality for the reference item group must exceed 50% at the end of the test. Average mortality was 100% in the reference item group.

The acute contact study fulfilled the validity criteria outlined in the most recent EU test guideline (OECD 246, 2017) as detailed below:

- Average mortality for the total number of controls must not exceed 10% at the end of

the test. Average mortality was 6.67% in the untreated control.
- Average mortality for the reference item group must exceed 50% at the end of the test. Average mortality was 93.33% in the reference item group.

I. CONCLUSION

The acute oral and contact toxicity on the bumblebee, *Bombus terrestris* L., were investigated under laboratory conditions over a period of 48 and 72 hours, respectively. The 48-hour oral LD_{50} of S-2399 60 g/L EC was > 532.69 µg product/bumblebee and the 48-hour oral NOED was 532.69 µg product/bumblebee. The 48- and 72-hour contact LD_{50} of S-2399 60 g/L EC were > 3066.67 µg product/bumblebee and the 48- and 72-hour contact NOED were 3066.67 µg product/bumblebee.

HSE COMMENTS
This study was conducted under GLP and OECD 246 (2027) and OECD 247 (2017) guidelines. It has been assessed against these same guidelines.
The concentrations of the test item were maintained between 80-120% of the nominal value throughout the test. Results should be based on nominal concentrations, as has been expressed by the applicant.

In the oral toxicity test, Dimethoate EC 400 was tested at nominally 3.0 µg dimethoate/bumblebee. OECD 247 (2017) guidelines state that 4ug dimethoate/bee has shown to be suitable to achieve 50% mortality. It is stated in the full study report that due to the test facility experience about the high sensitivity of the organisms, a dose of 3.0 µg dimethoate/bumblebee was used in the oral test instead of 4.0 µg dimethoate/bumblebee as is indicated in the OECD guideline No. 247. HSE conclude that this is an acceptable deviation and has no impact on the interpretation of results as the bees were shown to be meeting the sensitivity requirements for the test in terms of mortality at the 25% reduced dose of the reference item.

There was a deviation to protocol in that behavioural abnormalities in the reference item treatment were not recorded. The applicant has stated that this is because the reference item is known to be toxic to honeybees and therefore effects are expected, and that the dose range covers the expected LD_{50} values. However, as the test was conducted on bumblebees rather than honeybees, behavioural abnormalities should have been reported as per OECD 246 (2017) and 247 (2017) guidelines. The reference item group in the oral toxicity study recorded 100.00 % mortality, and the reference item group in the contact toxicity study recorded 93.33 % mortality. As the validity criteria require the average mortality in the reference item groups in oral and contact toxicity studies to exceed 50%, the study is considered valid.

The contact toxicity study was extended to 72 hours as there was a >10% increase in mortality in several test groups between 24 and 48 hours. The mortality continued to increase in several groups between 48 and 72 hours. In OECD 246 (2017) guidelines, studies should be extended to a maximum of 96 hours. However, as there was only a 3% increase in mortality between 48 and 72 hours, it is unlikely that the bees would have reached 50% mortality even if extended for 96 hours. HSE accepts the 72-hour endpoint.

In the oral toxicity study there was a repellency effect towards the test item. The number of non-feeders was higher in all the test item treatments compared to the control group. The actual uptake figures generally show an increase as the test concentration increases, but less food is being consumed as a percentage of the target dose. For dose verification, the amount of application solution consumed was determined by weighing the feeders before and after feeding. Individuals that did not consume at least 80 % of the mean consumption within the four hours of exposure were discarded from the test. It is not stated that non-feeders were replaced (as per OECD 247 guidelines), therefore the number of replicates for oral toxicity is below the minimum requirement of 30 individuals. For the acute oral test, it is noted that for the reference control, 766.67 and 1533.33 µg a.s./bumblebee treatment groups, there were 1, 24 and 11 bees, respectively, that consumed less than 80 % of the mean food uptake per treatment. These bees were considered non-feeders and, therefore, were not included in statistical evaluations (n = 35, 26, 23, 19, 11, 24 in the treatment groups). There were no non-feeders in the control group. According to OECD 247 (2017), an excess of bees should be acclimatised and used per treatment in order to replace non-feeders with feeders. In addition, there appears to be a dose-response relationship in the number of bees that consumed <80 % of the mean dose and, as there did not appear to be an issue with test bees consuming the control sucrose solution, this suggests avoidance of the test item. As the non-feeder data were removed and the statistical evaluations were carried out with only the bees that consumed >80 % of the mean food uptake, this removes the potential distortion that these datapoints could introduce due to incomplete consumption of the test item. However, this results in low numbers of individuals at several test doses.

HSE explored the impact of the reduced sample size using Binomial theory. The highest dose tested = 532.69 µg product/bumblebee with 11 feeders and one mortality after 48 hours. The probability of observing up to one mortality from 11 feeders if 532.69 µg product/bumblebee is the LD_{50} is equal to 0.586 % (cumulative probability: $P(X \leq 1)$ = 0.00586). Therefore, there is at least a 99 % confidence that the LD_{50} is greater than this dose. This demonstrates that the LD_{50} quoted below is robust despite the reduced sample size and is suitable for use in risk assessment.

As all validity criteria were met for this study, it is still considered valid.

The use of statistics is suitable for this study.

The analytical methods of this study were not evaluated by HSE Chemistry.

The endpoints for use in risk assessment are:
Oral LD_{50} > 532.69 µg product/bumblebee
Contact LD_{50} > 3066.67 µg product/bumblebee (72 hours, based on nominal concentrations)

B.9.5.1.2 Chronic toxicity to bees

Reference:	KCP 10.3.1.2/01
Report Title:	S-2399 60 g/L EC: Chronic Oral Toxicity Test (10-Day Feeding) to the Honey Bee (*Apis mellifera* L.) under Laboratory Conditions

Author(s) & year:	[redacted] (2021a)
Document No, Authority registration No:	Eurofins Scientific Study No. S20-00800 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0135
Substance used:	S-2399 6 EC, V16-7L1901, 6.544 ± 0.024% w/w
Method of analysis:	LC-MS/MS
Guideline(s):	OECD 245 (2017)
Deviations:	Yes, see HSE Comments
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

MATERIALS AND METHODS

MATERIALS

Test material S-2399 60 g/L EC

Description: Yellow clear liquid

Batch number: V16-7L1901

Active substance content: 60.68 ± 0.23 g/L (verified by certificate of analysis)
6.544 ± 0.024 % w/w (verified by certificate of analysis)

Density: 0.9273 (g/mL)

Storage on receipt: Stored at ambient temperature in the storeroom for GLP test products; protected from sunlight, heat and humidity. Storage in original container and in dark conditions

Date of receipt 21 Feb 2020

Expiry date: 17 May 2021

TREATMENTS

Nominal test doses: 652.33, 1435.12, 3157.27, 6945.99 and 15281.18 mg test item/kg
feeding solution (equivalent to 42.69, 93.91, 206.61, 454.55 and 1000.00 mg active substance/kg feeding solution)

Control: Untreated feeding solution

Toxic reference: BAS 152 65 I (Dimethoate, analysed 414 g/L, verified by certificate of analysis)

0.90 mg dimethoate/kg feeding solution.

Toxic reference batch: 10248664A

TEST ORGANISMS

Species: Honey bee (*Apis mellifera* L.), young adult worker bees not

	older than 48 hours.
Source:	Commercial beehives from the in-house Test Facility stock, adequately fed, healthy and as far as possible disease-free and queen-right. The hives from which the test organisms were obtained were not previously exposed to any chemical treatments within four weeks of test initiation.
Acclimatisation period:	Two days before the beginning of the test, brood frames containing capped cells from which adults were expected to emerge within 24 hours were taken from the honey bee hives and put in a bioclimatic chamber. One day prior to test start, the bees were randomly collected directly from the frames, introduced into test units and kept under test conditions until the start of the test. Acclimatisation period comprised from bee collection to the start of the test. Dead and moribund bees were rejected and replaced by healthy bees before starting the test.
Diet:	50 % (w/v) aqueous sucrose solution *ad libitum* during acclimatisation period. 0.10 mL/bee/day 50 % (w/v) aqueous sucrose solution, spiked with or without the test item depending on the treatment group, during exposure period.
TEST DESIGN	
Test cages:	Steel lined with filter paper (base: 8.5 cm x 4.5 cm; height: 6.0 cm)
Replication:	Each treatment group consisted of 50 test organisms, divided in 5 parallel replicates (cages), each containing 10 bees. 5 evaporation controls containing feeding solutions but no bees.
No of bees per cage:	10
Duration:	10 days (exposure)
TEST CONDITIONS	
Test temperature:	33.0 – 33.8 °C (Acclimatisation) 32.6 – 33.9 °C (Test)
Relative humidity:	56.5 – 68.2 % (Acclimatisation) 55.2 – 67.6 % (Test)
Lighting:	Constant darkness except during feeding and assessments

STUDY DESIGN AND METHODS

Experimental dates: 12 August 2020 to 22 August 2020

Treatment

A fresh test item stock solution was obtained daily by mixing a defined amount of test item with a defined amount of 50 % (w/v) aqueous sucrose solution. This stock solution was used also as the highest test item concentration feeding solution. The remaining test item feeding solutions were freshly prepared every day by mixing aliquots of the stock solution with 50 % (w/v) aqueous sucrose solution.

Starting on the day of the first application and for the following ten days, each cage was provided daily with fresh feeding solution. Treatments were applied using 5 mL syringes containing 1 mL (0.10 mL/bee) of the corresponding feeding solution, injected with a micropipette into the syringes. Syringes with the feeding solutions were weighted before application and then inserted in one of the holes of the upper surface of the cages. The bees in one replicate share the food and thus receive similar doses (trophallaxis). The feeding solutions remained in the cage up to 24 ± 2 h and then were weighted again to calculate feeding solution consumption. These consumption values were corrected for evaporation using the evaporation controls set up in parallel.

Measurements and observations

Biological

Mortality was recorded on a daily basis starting 24 hours (± 2 hours) after the first application and for the whole duration (10 days) of the test. At each assessment time dead bees were removed for sanitary reasons. Behavioural abnormalities such as symptoms of poisoning were recorded at each observation interval. In the reference group, behavioural abnormalities assessments were not conducted as it was assumed that moribund and affected bees of the reference group would die by the end of the test.

Behavioural abnormalities were recorded according to the following categories:
a = affected (bees still upright and attempting to walk but showing signs of reduced coordination).
ap = apathetic (bees show only low or delayed reactions to stimulation, e.g. light or blowing; bees are sitting motionless in the unit or are able to walk but not correctly).
c = cramps (bees contracting abdomen or entire body).
m = moribund (bees cannot walk and show only very feeble movements of legs and antennae; only weak response to stimulation; e.g. light or blowing; bees may recover but usually die).
v = vomiting.

Analytical

Samples of the control, the highest (T5, used as stock solution as well) and lowest concentration of the test item treatments were taken on D9, directly after preparation. The samples were taken by duplicate, one sample for shipping (A) and one for retention (R), with a volume of 2 mL each. The concentration of S-2399 in the samples was analysed by liquid chromatography with tandem mass spectrometry (LC-MS/MS, LOQ = 65 mg test item/kg, LOD = 19.5 mg test item/kg).

Data processing and statistical analysis:

The cumulative mortality [%] for each treatment group was calculated from the number of bees at the end of the assay (D10) in relation to the number of introduced test organisms per treatment group. Cumulative mortality of each test item and reference item group is expressed as percentage of the control populations after an adjustment according to Abbott's formula:

$$M = \frac{t - c}{100 - c} \times 100$$

M = Corrected mortality (%)
T = Mortality in the treated group [%]
c = Mortality in the control group [%]

Consumption of feeding solution per bee was calculated by dividing the total consumption of the replicate by the number of living bees in that replicate at start of feeding. For each treatment group, the mean consumption of application solution/bee was calculated by averaging the replicate values.

The mean intake of test / reference item per bee was calculated according to the following formula:

$$Mi = MC\,1000 \times C$$

Mi = Mean intake of test / reference item [µg/bee]
MC = Mean consumption of feeding solution [mg/bee]
C = Concentration of test / reference item in feeding solution [mg/kg]

The evaporation out of the feeding solution syringes was determined by daily weighing of the syringes of the respective, additional test cages. Over the whole test period the mean value of evaporation per day was determined and the daily feeding solution consumption of the control group, test and reference item treatment groups was corrected by the corresponding mean value of evaporation on the corresponding day. If these calculations resulted in a negative consumption value, i.e. feeding solution consumption was lower than

the mean daily evaporation, consumption was assumed to be equal to zero for all related calculations.

For statistical calculation of mortality results, the Step-down Cochran-Armitage test was used (α = 0.05, one-sided greater) to evaluate whether there was a significant difference between the mortality data of the test item groups and the control group. Thus, the NOEDD and NOEC values were determined. The LDD_{50} and LC_{50} along with its 95% confidence limits were determined by Weibull analysis. The LDD_{50} value was calculated using the actual consumption of the feeding solutions.

Analysis was performed using ToxRat® Professional, Version 3.3.0 (ToxRat Solutions GmbH) and Microsoft Office Excel 2013® Version 15.0.

RESULTS AND DISCUSSION

Biological effects

Consumption data, used to calculate dose level consumed per bee, and mortality data are summarised in Table 9.5-12.

Table 9.5-12: Mortality of bees in the chronic toxicity feeding test after 10 days

Test concentration	Mean feeding solution consumed ± SD	Dose level consumed		Total number dead	Cumulative mortality [%] ± SE	Corrected mortality [%] [a]
		[µg product/bee]	[µg a.s./bee]			
[mg product/kg feeding solution]	[mg/bee/day]	Daily / Cumulative	Daily / Cumulative			
Control (sucrose)						
0	19.5 ± 8.0	0	0	3	6.00 ± 4.00	-
Test item (S-2399 60 g/L EC)						
652.33	18.6 ± 8.1	12.14 / 121.43	0.79 / 7.95	4	8.00 ± 3.74	2.13
1435.12	15.7 ± 5.9	22.52 / 225.19	1.47 / 14.74	4	8.00 ± 3.74	2.13
3157.27	14.7 ± 6.5	46.56 / 465.62	3.05 / 30.47	8	16.00 ± 4.00	10.64
6945.99	13.0 ± 4.1	90.00 /	5.89 /	11	22.00 [b] ±	17.02

		900.00	58.90		6.63	
15281.18	11.2 ± 4.5	171.56 / 1715.63	11.23 / 112.27	37	74.00 [b] ± 6.78	72.34
Reference item (dimethoate) [c]						
0.9	14.2 ± 12.5	0.01 / 0.11	-	49	98.00 ± 2.00	97.87

Endpoints chronic toxicity					
Concentration [mg/kg feeding solution]			**Dose [µg/bee/day]**		
	S-2399 60 g/L EC	**a.s.: S-2399**		**S-2399 60 g/L EC**	**a.s.: S-2399**
10-day NOEC	3157.27	206.61	10-day NOEDD	46.56	3.05
10-day LC_{50} (95% CI)	11295.68 (9537.19 – 13615.86)	739.19 (624.11 – 891.02)	10-day LDD_{50} (95% CI)	133.83 (116.16 – 156.54)	8.76 (7.60 – 10.24)

[a] According to Abbott's formula (1925) modified by Schneider-Orelli (1947).
[b] Significantly increased compared to the Control group (step-down Cochran-Armitage test procedure, one sided greater, α = 0.05).
[c] For the reference item, the concentration/dose values are expressed in active substance (dimethoate).
CI: confidence limits.
SD: standard deviation.
SE: standard error.

Behavioural abnormalities associated with S-2399 60 g/L EC treatment are presented in Table 9.5-13.

Table 9.5-13: Behavioural abnormalities on treatment with S-2399 60 g/L EC

Treatment group (code)		**Conc.**	**Affected bees [%] [a]**									
		[mg t.i./kg f.s.]	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**
Control	**(C)**	--	0.0	0.0	0.0	0.0	2.1	0.0	0.0	0.0	0.0	0.0
Test item	**(T1)**	652.33	0.0	0.0	0.0	2.1	2.1	2.1	0.0	0.0	0.0	0.0
	(T2)	1435.12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Treatment group (code)		Conc.	Affected bees [%] [a]									
		[mg t.i./kg f.s.]	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
S-2399 60 g/L EC	(T3)	3157.27	0.0	0.0	0.0	2.1	2.1	0.0	0.0	0.0	0.0	4.8
	(T4)	6945.99	0.0	4.1	2.1	4.2	8.5	4.4	4.7	7.1	4.9	5.1
	(T5)	15281.18	6.5	15.9	2.4	5.6	16.1	26.9	20.8	5.3	7.1	15.4

t.i. = test item; f.s. = feeding solution

[a] Percentage of affected individuals in relation to total living individuals

Symptoms of intoxication (mainly affected bees but also some moribund individuals) were observed predominantly in the two highest treatments levels throughout the exposure period (Day 2 – 10 for T4 and Day 1 - 10 for T5).

Analytical results

Analytical results are presented in Table 9.5-14.

Table 9.5-14: Analytical results

Nominal concentration (mg product/kg)	Nominal concentration (mg a.s./kg)	Measured concentration (mg a.s./mL)	% of nominal	Matrix
Control	Control	< LOD	-	50 % (w/v) aqueous sucrose solution
652.33	42.69	38.1	89	
15281.18	1000	928	93	

LOD = limit of detection of 1.28 mg test item/kg.

Mean measured concentrations ranged between 89 – 93 % of nominal. Since the measured concentration in the samples was within 20 % of nominal, the concentrations of the test item were confirmed, and the endpoints are based on nominal concentrations.

Validity criteria

The validity criteria for the study were met according to OECD 245 (2017).

Table 9.5-15: Compliance with OECD 245 (2017) validity criteria

Validity criterion	Required	Obtained
Control mortality	≤ 15 %	6 %
Dimethoate toxicity	≥ 50 %	98 %

CONCLUSIONS

In this chronic toxicity feeding study with S-2399 60 g/L EC on the honey bee *Apis mellifera* L., the 10-day LDD50 was determined at 133.83 µg product/bee/day (corresponding to 8.76 µg a.s./bee/day). The 10-day NOEDD, based on daily consumed doses, was determined to be 46.56 µg product/bee/day (corresponding to 3.05 µg a.s./bee/day).

HSE COMMENTS

The study was carried out according to and evaluated against the OECD 245 (2017) guideline. All validity criteria were satisfactorily met for the duration of the study. There were no significant deviations from the guideline. Treatment with the toxic reference (dimethoate) indicated the sensitivity of the bees and reliability of the test system was appropriate.

The following minor deviations were noted for OECD 245 (2017):
OECD 245 (2017) § 22 states the minimum volume of a syringe feeder should be 2 mL. The study supplied a volume of 1 mL in a 5 mL syringe. No justification is given for the minimum volume requirement of 2 mL. The impacts of this deviation are unclear but probably minor as bee consumption was accurately estimated during the study. HSE considers this an acceptable deviation.

OECD 245 (2017) § 34 outlines requirements for the test report. It requires that biological effects observed, such as anti-feeding effects, are discussed. For S-2399 60 g/L EC there was a clear anti-feeding effect as its concentration in feeding solution increased, captured by mean feeding solution consumed per bee per day. This was not explicitly discussed, although the results were clearly presented. HSE considers this a minor and acceptable deviation.

The method of analysis used in the study was evaluated by HSE Chemistry. The conclusions of their evaluation are reproduced below. Please see Volume 3 CA, section B5 for more details.

"*The analytical method is not fully validated according to SANTE/2020/12830 rev. 1 for the determination of the active substance inpyrfluxam in 50% w/v aqueous sucrose solution as the standard solution stability has not been determined. However, the study was generated prior to the implementation of SANTE 2020/12830 rev.1 and SANCO 3029 rev.4 did not require standard solution stability to be addressed. As all other validation requirements have been met (including the minimum validation requirements outlined in SANTE 2020/12830 rev.1), the method is considered to be fit for purpose.*"

The above study was conducted to GLP and considered valid.

The agreed endpoints suitable for use in the risk assessment are: 10-day LDD_{50} = 133.83 µg product/bee/day which equates to 8.76 µg a.s./bee/day; a NOEC of

3157.27 mg product/kg feeding solution, which equates to 206.61 mg a.s./kg; and a NOEDD of 46.56 µg product/bee/day, which equates to 3.05 a.s./bee/day.

B.9.5.1.3 Effects on honey bee development and other honey bee life stages

Reference:	**KCP 10.3.1.3/01**
Report Title:	S-2399 60 g/L EC: Honey Bee (*Apis mellifera* L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions
Author(s) & year:	[redacted] (2021b)
Document No, Authority registration No:	Eurofins Scientific Study No. S20-00798 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0136
Substance used:	S-2399 6 EC, V16-7L1901, 6.544 ± 0.024% w/w
Method of analysis:	LC-MS/MS
Guideline(s):	OECD 239 (2021)
Deviations:	Yes, see HSE Comments
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

MATERIALS AND METHODS

MATERIALS

Test material S-2399 60 g/L EC

Description: Yellow clear liquid

Batch number: V16-7L1901

Active substance content: 60.68 ± 0.23 g/L (verified by certificate of analysis)
6.544 ± 0.024 % w/w (verified by certificate of analysis)

Density: 0.9273 (g/mL)

Storage on receipt: Stored at ambient temperature in the storeroom for GLP test products; protected from sunlight, heat and humidity. Storage in original container and in dark conditions

Date of receipt 21 Feb 2020

Expiry date: 17 May 2021

TREATMENTS

Nominal test doses: Nominally 15.28, 33.62, 73.96, 162.71 and 357.97 µg product/larva (corresponding to 1.00, 2.20, 4.84, 10.65

	and 23.43 µg a.s./larva) equivalent to a concentration of 99.23, 218.30, 480.26, 1056.57 and 2324.46 mg product/kg diet (corresponding to 6.49, 14.29, 31.43, 69.14 and 152.11 mg a.s./kg diet, respectively).
Control:	Untreated feeding solutions
Toxic reference:	BAS 152 I (Dimethoate, content of a.s. analysed: 98.2 % w/w) 7.39 µg dimethoate/larva corresponding to a concentration of 48 mg dimethoate/kg diet
Toxic reference batch:	COD-002332

TEST ORGANISMS

Species:	Honey bee (*Apis mellifera* L.), synchronized first instar (L1) larvae not older than 30 hours at grafting time.
Source:	Commercial beehives from the in-house test facility stock, adequately fed, healthy and as far as possible disease-free and queen-right. The hives from which the larvae were obtained were not previously exposed to any chemical treatments within four weeks of test initiation.
Acclimatisation period:	At Day -3, the queen from at least three colonies was isolated for one day within a queen excluder placed on a single frame with empty cells in their own hive, to provide known-aged eggs and subsequent larvae. At Day -2, maximum 30 hours after isolation, the queens were released. Frames containing eggs were left in the excluder cages until hatching (Day 1). Three frames from different hives, containing the highest number of synchronized larvae, were selected for grafting in the laboratory.
Diet:	Diet A (D1, volume administered: 20 µL/larva): 50 % weight of royal jelly + 50 % weight of an aqueous solution containing 2 % weight of yeast extract, 12 % weight of glucose and 12 % weight of fructose. Diet B (D3, volume administered: 20 µL/larva): 50 % weight of royal jelly + 50 % weight of an aqueous solution containing 3 % weight of yeast extract, 15 % weight of glucose and 15 % weight of fructose. Diet C (from D4 to D6, volume administered: 30 µL/larva; 40 µL/larva and 50 µL/larva respectively): 50 % weight of royal jelly + 50 % weight of an aqueous solution containing 4 % weight of yeast extract, 18 % weight of

glucose and 18 % weight of fructose.

TEST DESIGN

Test units:	Crystal polystyrene grafting cells (NICOTPLAST, 9mm diameter, sterilised). Each cell was placed into a well of a sterile 48-well cellular culture plate. Plates were placed into hermetically sealed Plexiglas desiccators, containing a dish filled with a saturated potassium sulphate (K_2SO_4) solution in order to keep a water saturated atmosphere from day 1 until day 7. On day 7, the well plates were transferred to another Plexiglas desiccator, containing a dish with a saturated sodium chloride (NaCl) solution to maintain a slightly lower relative humidity until day 15. On day 15, each plate was transferred into an emergence box in an incubator.
Replication:	For each treatment group, 48 larvae from three different hives were tested over 22 days. Each hive equates to one replicate; 16 larvae from each replicate were used.
Duration:	22 days

TEST CONDITIONS

Test temperature:	32.9 – 34.8 °C (Day 1 - 7) 32.0 – 35.2 °C (Day 7 - 15) 33.4 – 34.9 °C (Day 15 - 22)
Relative humidity:	57.9 - 100 % (Day 1 – 7) 45.2 – 84.0 % (Day 7 - 15) 58.4 – 66.4 % (Day 15 - 22)
Lighting:	Constant darkness except during feeding and assessments

STUDY DESIGN AND METHODS

Experimental dates: 25 May 2020 to 15 June 2020

Test organism

On Day 1, the combs were transferred to the laboratory using an insulated container to avoid temperature variation. In the laboratory, the three combs were used for grafting. On Day 1 the test was initiated with larvae in excess. 20 µL of diet A was dropped into each grafting cell of the well plate. Using a grafting tool, one larva was delicately transferred from the comb to each cell on the surface of the diet. Reserve plates were prepared containing larvae of the same replicate hives. Before first application of the test product on Day 3, it was assured that all larvae used were of similar size and alive. Thus, non-suitable larvae per replicate were replaced across all treatment groups by individuals from the reserve plates.

Diet and feeding
The larval diet was freshly prepared prior the applications and stored in a fridge at ≤ 5 ºC (0.1 - 1.9 ºC). Each larva was fed once a day (except on day 2) with a standardized amount of artificial diet until day 6 (20 µL/larva at D3; 30 µL/larva at D4; 40 µL/larva at D5; 50 µL/larva at D6 with a cumulative volume of 140 µL/larva (total)). For feeding, a multi stepper pipette was used. Care was taken to avoid touching and drowning the larvae when feeding them. Food was dropped next to the larva, along the wall of the grafting cell. For the preparation of the larval diet, a commercial royal jelly was used. The absence of antibiotics, pesticides and heavy metals in the royal jelly was confirmed by a non-GLP multi-residues analysis.

Dose preparation
A test item stock solution was prepared on day 3 by mixing 0.2557 g of test item with 10 mL of deionised water and stored in the refrigerator until day 6. This stock solution used also as the highest test item concentration application solution. The remaining test item application solutions were prepared by serial dilutions of the stock solution in deionised water. The final diets were treated with 1 mL of the corresponding application solutions. Control group individuals were fed with untreated diet. The reference item stock solution was prepared by mixing 0.0269 g reference item with 5 mL of deionised water. This stock solution was used as the application solution and 0.05 mL mixed with 0.45 mL of deionised water was mixed in the final diet. The volume of application solution in the diet did not exceed 10 % of the final diet volume.

Measurement and observations
Assessment of larval mortality were conducted before feeding from day 4 to day 8. Larvae were recorded as dead if no respiration (movement of spiracles) was observed using a stereo microscope. Mortality during pupation phase was assessed on day 15 and bee emergence was assessed on day 22. Other observations (larval appearance and size) were recorded to aid in the interpretation of mortality in comparison to the control group. Additionally, the presence of uneaten food was qualitatively recorded on day 8.

Samples of the control group stock solution and all the concentrations of the test item were taken on days 3 – 6 directly after preparation. The concentration of S-2399 in the samples was analysed by liquid chromatography with tandem mass spectrometry (LC-MS/MS, LOQ = 10 mg test item/kg (0.654 mg S-2399/kg) for larval diet and 10 mg test item/L (0.654 mg S-2399/L) for deionised water, LOD = 3 mg test item/kg (0.196 mg S-2399/kg)
for larval diet and 3 mg test item/L (0.196 mg S-2399/L) for deionised water).

Air temperature and relative air humidity were recorded at intervals of 15 minutes with calibrated data loggers placed into each desiccator from day 1 until the end of the test (day 22).

Data evaluation

The calculation of the equivalent doses per larva [µg test item/larva] was based on the given test item concentration [mg test item/L diet], the feeding volume per larva (both for the daily volumes and the cumulative 140 µL per developmental period) and the density of the diet (1.1 g/mL). The following formula was used:

$$TD = \frac{TC}{1000} \times VF \times \rho$$

T_D = test itemdose per larva [µg test item/larva]
T_C = test item concentration [mg test item/L diet]
V_F = feeding volume per larva [µL]
ρ = density of larval diet [g/cm^3]

The cumulative larval mortality [%] for each treatment group was calculated from the number of dead larvae on day 8 (D8) in relation to the total number of larvae per treatment group across all replicates after selection on day 3 (D3).

Mortality during the pupation phase was evaluated on day 15 (D15). The cumulative pupae mortality [%] for each treatment group was calculated from the number of larvae that had not transformed into pupae on D15 in relation to the total number of individuals after selection on D3.

The adult emergence rate [%] for each treatment group was evaluated on day 22 (D22) and was calculated from the number of adult emerged bees on D22 in relation to the total number of larvae per treatment group after selection on D3.

In case control mortality occurred, the cumulative mortalities for each test and reference item groups were expressed as percentage of the control populations after an adjustment according to the formula of Abbott (1925), modified by Schneider-Orelli (1947):

$$M_{corr} = \frac{M_T - M_C}{100 - M_C} \times 100$$

M_{corr} = Corrected mortality [%]
M_C = Mortality in the control group [%]
M_T = Mortality in the test group [%]

Statistical analysis

All individuals who did not consume the entire diet on day 8 were discarded from the statistics since the dose they have consumed is not exactly known.

For statistical calculations, the qualitative trend analysis by contrasts (monotonicity of concentration/response, α = 0.05) revealed a linear trend between concentration and mortality at the different developmental phases (day 3 – day 8, day 3 – day 15 and day 3 – day 22). Due to no signs of extra binomial variance were found in the data (Tarone's procedure, α = 0.01), the Step-down Cochran-Armitage test was used (α = 0.05, one-sided greater) to evaluate whether there was a significant difference between mortality data of the test item groups and the control group. Thus, the NOEC and NOED values were determined. For day 8 and 22, the trimmed Spearman-Karber procedure was used to calculate the EC_{50}/ED_{50}. The $EC_{10,20}$/$ED_{10,20}$ could not be calculated statistically, but were empirically estimated from the results.

Analysis was performed using ToxRat® Professional, Versions 3.2.1 and 3.3.0 (ToxRat Solutions GmbH) and Microsoft Office Excel 2013® Version 15.0.

RESULTS AND DISCUSSION

Biological effects

A summary of the relationship between test concentration, dose and mortality, alongside estimated chronic toxicity endpoints, are presented in Table 9.5-16.

Table 9.5-16: Mortality and emergence of larva after repeated exposure to S-2399 60 g/L EC

Test concentration [mg product/kg diet]	Dose level		Cumulative mortality [%]			Corrected mortality [%] [b]			Day 22 mean adult emergence (%)
	[µg product/larva] [a]	[µg a.s./larva] [a]	Day 8	Day 15	Day 22	Day 8	Day 15	Day 22	
Control (untreated diet)									
-	-	-	6.25	8.33	12.50	-	-	-	87.50
Test item (S-2399 60 g/L EC)									
99.23	15.28	1.00	6.25	10.42	10.42	0.00	2.27	-2.38	89.58
218.30	33.62	2.20	10.42	14.58	14.58	4.44	6.82	2.38	85.42
480.26	73.96	4.84	6.25	8.33	8.33	0.00	0.00	-4.76	91.67
1056.57	162.71	10.65	8.33	10.42	10.42	2.22	2.27	-2.38	89.58
2324.46	357.97	23.43	77.08 [c]	81.25 [c]	83.33 [c]	75.56 [c]	79.55 [c]	80.95 [c]	16.67
Reference item (dimethoate) [d]									
48.0	7.39	-	93.75	93.75	97.92	93.33	95.45	97.62	2.08

Test concentration [mg product/kg diet]	Dose level		Cumulative mortality [%]			Corrected mortality [%] [b]			Day 22 mean adult emergence (%)
	[µg product/larva] [a]	[µg a.s./larva] [a]	Day 8	Day 15	Day 22	Day 8	Day 15	Day 22	
Endpoints chronic toxicity									
Concentration [mg/kg diet]				**Dose [µg/larva]**					
	S-2399 60 g/L EC	**a.s.: S-2399**				**S-2399 60 g/L EC**		**a.s.: S-2399**	
8-day NOEC	1056.57	69.14		8-day NOED		162.71		10.65	
15-day NOEC	1056.57	69.14		15-day NOED		162.71		10.65	
22-day NOEC	1056.57	69.14		22-day NOED		162.71		10.65	
8-day $EC_{10,20}$ (95% CI)	1056.57 – 2324.46 [e]	69.14 – 152.11 [e]		8-day $ED_{10,20}$ (95% CI)		162.71 - 357.97 [e]		10.65 – 23.43	
22-day $EC_{10,20}$ (95% CI)	1056.57 – 2324.46 [e]	69.14 – p152.11 [e]		22-day $ED_{10,20}$ (95% CI)		162.71 - 357.97 [e]		10.65 – 23.43	
8-day EC_{50} (95% CI)	1802.96 (1609.06 – 2020.22)	117.99 (105.30 – 132.20)		8-day ED_{50} (95% CI)		277.66 (247.80 – 311.11)		18.17 (16.22 – 20.36)	
22-day EC_{50} (95% CI)	1787.23 (1623.67 – 1967.27)	116.96 (106.25 – 128.74)		22-day ED_{50} (95% CI)		275.23 (250.05 – 302.96)		18.01 (16.36 – 19.83)	

[a] Based on the cumulative volume of 140 µL/larva (total).
[b] Corrected for control mortality according to Abbott modified by Schneider-Orelli.
[c] Significantly increased compared to the control group (step-down Cochran-Armitage test procedure, one sided greater, α = 0.05).
[d] For the reference item, the concentration/dose values are expressed in active substance (dimethoate).
[e] Values empirically estimated from the results because no statistically significant dose/response was found.
CI: confidence limits.

On day 8 no individuals were observed with uneaten food or other affections. At the end of the test, in the final assessment of the emergence on day 22, no emerged bees were

recorded as being affected (i.e. malformation)

Analytical results
Results of the analytical phase are presented in Table 9.5-17.

Table 9.5-17: Analytical results

Nominal concentration (mg product/kg)	Nominal concentration (mg a.s./kg)	Day	Measured concentration (mg a.s./kg)	% of nominal	Matrix
Control	Control	3	< LOD	-	Larval diet
Control	Control	4	< LOD	-	
Control	Control	5	< LOD	-	
Control	Control	6	< LOD	-	
99.23	6.49	3	6.70	103	
99.23	6.49	4	6.42	99	
99.23	6.49	5	6.37	98	
99.23	6.49	6	5.81	89	
218.3	14.3	3	14.5	102	
218.3	14.3	4	14.5	102	
218.3	14.3	5	13.7	96	
218.3	14.3	6	12.9	90	
480.26	31.4	3	30.9	98	
480.26	31.4	4	31.2	99	
480.26	31.4	5	29.7	95	
480.26	31.4	6	27.6	88	
1056.57	69.1	3	69.9	101	
1056.57	69.1	4	73.2	106	
1056.57	69.1	5	69.9	101	
1056.57	69.1	6	65.6	95	
2324.46	152	3	155	102	
2324.46	152	4	154	101	

Nominal concentration (mg product/kg)	Nominal concentration (mg a.s./kg)	Day	Measured concentration (mg a.s./kg)	% of nominal	Matrix
2324.46	152	5	152	100	
2324.46	152	6	154	101	
Nominal concentration (mg product/L)	**Nominal concentration (mg a.s./L)**	**Day**	**Measured concentration (mg a.s./mL)**	**% of nominal**	**Matrix**
25569.04	1673	3	1965	117	Deionised water
25569.04	1673	4	1835	110	
25569.04	1673	5	1880	112	
25569.04	1673	6	1920	115	

LOD = limit of detection set at 3 mg product/kg (0.196 mg a.s./kg) for larval diet and for deionised water (30 % of the LOQ).

Mean measured concentrations in the larval diet and stock solutions ranged between 88 – 106 and 110 – 117% of nominal, respectively. Since the measured concentration in the samples was within 20 % of nominal, the concentrations of the test item were confirmed, and the endpoints are based on nominal concentrations.

Environmental conditions

The air temperature was recorded out of the established range of 34 – 35 ºC for more than 30 minutes in the following occasions: 31 May 2020 temperature was lower than the minimum stabilised during 1 hour 45 minutes without falling below 32.0 ºC; 01 Jun 2020, the temperature was above the maximum stabilised for 45 minutes, without exceeding 35.1 ºC; and 05 Jun 2020, the temperature was above the maximum stabilised for 1 hour 15 minutes, without exceeding 35.2 ºC.

All deviations in humidity from the prescribed range were short-term (< 30 minutes)

Validity criteria

The validity criteria for the study were met according to OECD 239 (2021).

Table 9.5-18: Compliance with OECD 239 (2021) validity criteria

Validity criterion	Required	Obtained
Control larval mortality (Day 8)	≤ 15 %	6.25 %
Control emergence rate (Day 22)	≥ 70 %	87.5 %
Dimethoate larval toxicity (Day 8)	≥ 50 %	93.75 %

CONCLUSIONS

In this 22-day repeated exposure larval toxicity test with S-2399 60 g/L EC, the 22-day ED_{50} was determined at 275.23 µg product/larva (corresponding to 18.01 µg a.s./larva). The 22-day NOED, was determined to be 162.71 µg product/larva (corresponding to 10.65 µg a.s./larva).

HSE COMMENTS

The study was carried out according to and evaluated against the OECD 239 (2021) guideline. All validity criteria were satisfactorily met for the duration of the study. There were no significant deviations from the guideline. Treatment with the toxic reference (dimethoate) indicated the sensitivity of the bees and reliability of the test system was appropriate.

The following minor deviations were noted for OECD 239 (2021):

OECD 239 (2021) § 9 to 11 define humidity requirements during the larval and pupal stages. The humidity should be reduced from 95 % to 80 % on Day 8 of the experiment. This was performed on Day 7 in the study. As larval mortality and emergence rates met the validity criteria, this deviation is considered acceptable by HSE.

OECD 239 (2021) § 12 recommends conducting a pre-test before the start of the season with a representative number of larvae per potential test colony to inspect bee colonies for signs of variability and test performance. This was either not performed or not reported. Control mortality met the mortality criteria for all replicates and sensitivity was demonstrated for each replicate (colony) with the toxic reference dimethoate. HSE considers this a minor deviation/reporting omission.

OECD 239 (2021) § 18 details the preparation and storage of stock solutions. It states that, "*the stock solution should be prepared freshly at each feeding day unless the stability of the test chemical has been demonstrated in former studies*". The stock solution was prepared

on Day 3 and stored (refrigerated) until Day 6. The analytical results of stock solution samples from Day 3 – 6 demonstrate that no degradation of the test item occurred. HSE consider this a minor deviation with no impact on the study results.

OECD 239 (2021) § 20 states, "*a sample of the solution used to prepare the diet with the highest and lowest concentrations will be stored in a freezer at ≤-20°C in order to be further checked for analytical determination of the actual concentration of the test chemical*". Of the test solutions, only the stock solution was analytically determined. The study, however, performed analytical measurements on the diet solutions for each day of exposure, which comprehensively demonstrated that the larvae were exposed to target concentrations and doses. HSE considers this an acceptable deviation.

OECD 239 (2021) § 23 describes acceptable temperature deviations. It states, "*the temperature in the incubator is kept between 34ºC and 35ºC. Temporary deviations are allowed, however temperature should not drop below 23°C or go above 40°C, and these deviations should not last, as far as possible, more than 30 minutes once every 24 hour*". There were three temperature deviations that lasted for longer than 30 minutes. Given that the control larval mortality and emergence validity criteria were met these extended fluctuations outside the specified 34 to 35 ºC are considered acceptable by HSE.

OECD 239 (2021) § 33 outlines how the mortality of various life stage is calculated. It states, "*the pupal mortality is calculated in percentage by comparing the number pupae failed to emerge, including those bees without emergence on D22 and dead pupae removed during pupa stage – from D8 to D22 – to the number of bees entering pre-pupa stage on D8*". In this study, pupal mortality was, "*evaluated on day 15 (D15). The cumulative pupae mortality [%] for each treatment group was calculated from the number of larvae that had not transformed into pupae on D15 in relation to the total number of individuals after selection on D3*". As the main endpoints of interest are NOEC/NOED and EC_{50}/ED_{50} after 22 days (adult emergence), which was calculated correctly, this mortality data evaluation deviation is considered minor.

OECD 239 (2021) § 38 defines the requirements of the test report. The results should contain a graph of the fitted dose-response model, the slope of the concentration-response curve and its corresponding 95% confidence limits and the criteria for goodness of fit. These were not included in the results section of the study report.

The method of analysis used in the study was evaluated by HSE Chemistry. The conclusions of their evaluation are reproduced below. Please see Volume 3 CA, section B5 for more details.

"*The analytical method is not fully validated according to SANTE/2020/12830 rev. 1 for the determination of the active substance inpyrfluxam in larva diet and deionised water as the*

standard solution stability has not been determined. However, the study was generated prior to the implementation of SANTE 2020/12830 rev.1 and SANCO 3029 rev.4 did not require standard solution stability to be addressed. As all other validation requirements have been met (including the minimum validation requirements outlined in SANTE 2020/12830 rev.1), the method is considered to be fit for purpose."

The above study was conducted to GLP and considered valid.

The agreed endpoints suitable for use in the risk assessment are: 22-day ED_{50} = 275.23 µg product/larva (corresponding to 18.01 µg a.s./larva) and 22-day NOED = 162.71 µg product/larva (corresponding to 10.65 µg a.s./larva)

B.9.5.1.4 Sub-lethal effects

Sub-lethal effects were assessed in all studies in B.9.5.1.1 to 3. Please refer to the individual study summaries and the overall sub-lethal effects summary provided in B.9.6.1.

B.9.5.1.5 Cage and tunnel tests

No studies submitted. No further testing is required, as an acceptable risk was demonstrated based on a first-tier risk assessment.

B.9.5.1.6 Field tests with honeybees

No studies submitted. No further testing is required, as an acceptable risk was demonstrated based on a first-tier risk assessment.

B.9.5.2 Effects on non-target arthropods other than bees

The following table provides a summary of the endpoints generated from the studies conducted with the active substance inpyrfluxam for non-target arthropods. Full study summaries and evaluation can be found in Section B.9.5.2

B.9.5.2 – 1: Toxicity endpoints for the risk assessment of non-target arthropods for the formulation S-2399 6 g/l EC

Species	Test system	Test substance	Endpoint	Results	References
First tier studies					
Aphidius rhopalosiphi	Glass plate (2D)	S-2399 60 g/L EC [a]	LR_{50}	809 mL product/ha **(49.1 g a.s./ha)**	CP 10.3.2.1/01 [redacted] 2019a
			ER_{50}	> 400 mL product/ha (24.3 g a.s./ha)	
			NOER	< 64 mL product/ha	

Species	Test system	Test substance	Endpoint	Results	References
				(3.88 g a.s./ha)	
Typhlodromus pyri	Glass plate (2D)	S-2399 60 g/L EC [a]	LR_{50} ER_{50} NOER	>1000 mL **(60.68 g a.s./ha)** > 1000 mL product/ha (> 60.7 g a.s./ha) 400 mL product/ha (24.3 g a.s./ha)	CP 10.3.2.1/02 [redacted] 2019b

[a] Active substance content of the formulation; density 0.9273 g/mL, 60.68 g a.s./L (corresponding to 6.544% w/w)
Endpoints highlighted in **bold** used in the risk assessment.

Reference:	KCP 10.3.2.1/01
Report Title:	S-2399 60g/L EC: Effects on the Parasitoid *Aphidius rhopalosiphi* in the Laboratory – Dose Response Test
Author(s) & year:	[redacted] (2019a)
Document No, Authority registration No:	Study No. 141471001, TPW-0106
Substance used:	S-2399 6 EC, V16-7L1901, 6.544 ± 0.024% w/w
Method of analysis:	n/a
Guideline(s):	Mead-Briggs *et al.* (2000)
Deviations:	Yes, see HSE Comments
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

I. MATERIALS AND METHODS

A.MATERIALS

1. Test item: S-2399 60 g/L EC (Emulsifiable Concentrate)
Description: Yellow liquid
Lot/Batch: V16-7L1901
Active substance content: Nominal: 60g S-2399/L
Analysed: 60.68 ± 0.23 g S-2399/L or 65.44 ± 0.24 g/kg or 6.544 ± 0.024 %(w/w)
Density: 0.9273 g/ml
Test concentrations: 0.294, 0.736, 1.84, 4.60 and 11.5 g product/L
Spray equipment: Laboratory-spraying equipment. Nozzle - teejet 80015

EVS

2.Control: Deionised water (200 L/ha)

3.Reference item: Perfekthion (BAS 152 11 I)
Description: Blue liquid
Lot/Batch: FRE-0015878
Active substance content: Dimethoate 492.0 g/L analysed
Density: 1.076 g/cm^3

B: STUDY DESIGN AND METHODS

1.Test species: *Aphidius rhopalosiphi* (destefani-Perez)
Age: Adults; ≤ 48 hours (males and females)
Source: Katz Biotech AG, Baruth, Germany
Replicates: 40 (4 x 10 per control, reference and test concentration group)
Acclimation: Approximately 1 – 2 days under test conditions
Duration: Mortality phase = 48 hours. Reproduction = 11 – 12 days.
Diet: *Ad libitum* 10% fructose solution provided during exposure in small test tubes; on cotton wool during acclimatisation
Exposure units: Two glass plates (13 × 13 cm) held apart in an aluminium frame (13 × 1.5 × 1 cm per side) and held together with two clamps. 6 ventilation holes on three sides covered with cloth, and one side with one small hole (approximately 1 cm) of inserting and feeding test animals
Post-exposure units: Untreated pots (13 cm) with barley seedlings (*Hordeum vulgare* ´Sunshine´; 14 – 25 seedlings, 9 days old) infested with 100 to 200 host aphids (*Rhopalosiphum padi*) of all developmental stages, enclosed with a clear polyacrylic cylinder (30 cm high, 10 cm) with two holes (70 × 195 mm) closed with fine gauze for ventilation and another hole (approximately 2 cm) closed with cotton wool for parasitoid introduction. Top closed with fine gauze and soil surface covered with thin layer of quartz sand.

2.Environmental conditions

A summary of the environmental conditions obtained in this study are shown in Table B.9.5.2 - 2 below.

Table B.9.5.2 - 2: Environmental conditions obtained in the study compared to guideline recommendations

Variable	Guidelines	Obtained
Temperature	20 ± 2 °C	19 – 22°C
Relative humidity	60-90 %	Acclimatisation/exposure: 67 – 72% Post-exposure: 79 – 81%

Variable	Guidelines	Obtained
Photoperiod	16h light : 8h dark	16 h light : 8 dark
Light intensity	400- 3000 lux (first 48hrs) 4000-20000 lux (post-parasitisation)	940 – 1550 lux (acclimatisation/exposure/parasitisation period) 9290 – 12380 lux (post-parasitisation period).

Study dates: 26th March 2019 – 28 May 2019

1. **Test organism and treatment:**

A. rhopalosiphi were exposed to glass plates treated with S-2399 60 G/L EC at single target application rates of 64, 160, 400, 1000 and 2500 ml product/ha in 200 L/ha water (corresponding to 3.88, 9.71, 24.3, 60.7 and 152 g a.s./ha, respectively). A deionised water control and the toxic reference item Perfekthion at an application rate of 0.3 ml/ha in 200 L/ha deionised water were tested in parallel. Glass plates were sprayed with test solutions using calibrated (by weighing glass plates prior to and directly after spraying) laboratory spray equipment (Fa. Schachtner; Ludwigsburg, Germany) fitted with a Teejet 80015 EVS nozzle at a rate of 200 L/ha corresponding to 2 mg/cm^2 ± 10% (spraying pressure: 2.5 bar; spraying speed: 2.75 km/h; spraying distance: 30 cm). The exposure was conducted in four replicates per treatment groups with 10 impartially selected individual wasps (three males and seven females) each introduced after drying of exposure units for 35 to 40 minutes after application. Wasps were exposed for approximately 48 hours. For the post-exposure (parasitisation) period, one female per unit (replicate) in 20 replicates per treatment group were impartially selected and transferred to post-exposure units using an aspirator. At the end of the 24-hour parasitisation period, females were removed from the exposure units before a post-parasitisation period of 11 to 12 days (nine units evaluated after 11 days and 9 to 11 units evaluated 12 days after parasitisation).

2. **Dose preparation:**

Based on the results of a range finding test, spray solutions of 0.294, 0.736, 1.84, 4.60 and 11.5 g product/L corresponding to 64, 160, 400, 1000 and 2500 ml product/ha in 200 L/ha water were prepared separately by weighing 0.0736, 0.184, 0.460, 1.15 and 2.88 g test item in 250 ml deionised water each. The reference item solution of 1.5 µl Perfekthion/L corresponding to an application rate of 0.3 ml Perfekthion/ha was prepared by dissolving 0.75 µl Perfekthion in 500 ml deionised water.

3. **Measurements and observations:**

The numbers of parasitoids alive, affected (as behavioural abnormalities), moribund and dead were recorded approximately 2, 24 and 48 hours after test initiation. Reproduction as assessed as the number of aphid mummies counted 11 to 12 days after the end of 24-hour parasitisation period in all replicates where the females were alive after the 24-hour parasitisation period (n = 18 – 20). An assessment of reproduction was performed for those treatments where corrected mortality was ≤ 50%.

Test conditions were recorded with suitable instruments. Short-term deviations (< 2 hours) are partly unavoidable due to handling and are not reported.

4. Statistical analysis:

Mortality (dead plus moribund wasps) was determined 48 hours after exposure and corrected for the control results following Abbott (1925) and improvements by Schneider-Orelli (1947). The number of aphid mummies obtained from the maximum of 20 replicates per treatment group was used to calculate the mean aphid mummies produced per female (± standard deviation) within the 24 hours parasitisation period (post-exposure period). The LR_{50} for mortality was calculated applying Moving Averages. Mortality data were analysed for significance using Fisher Exact Test (a = 0.05). For the analysis of the test item data the Bonferroni correction was applied (Bonferroni-Holm Fisher's Exact Test). Reproduction data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test (α = 0.05) and the Levene's test (α = 0.05), respectively. Because the reproduction data were normally distributed and homogeneous, the Williams t-test (multiple comparison, one-sided, α = 0.05) was used.

Analysis was performed using Toxrat® Professional, Version 3.30 (Toxrat Solutions gmbh).

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

There was no mortality on controls and no treatment-related mortality up to and including 400 ml product/ha. Statistically significant treatment-related mortality of 72.5 and 100.0% was reported for dose levels of 1000 and 2500 ml product/ha, respectively. 100% mortality was observed for the reference item treatment.

Reproduction was statistically significantly affected at all dose rates, but the effect on reproduction was always below the trigger value of 50 %.

The results are summarised in Table B.9.5.2 - 3. A summary of observations are presented in Table B.9.5.2 - 4, and endpoints are summarised in Table B.9.5.2 - 5.

Table B.9.5.2 - 3: Percent mortality and reproduction in *Aphidius rhopalosiphi* after exposure to S-2399 60 g/L EC

Application rate [ml product/ha]	Mortality		Reproduction	
	[%] ± SD	[%] Corrected	Mummies/female (parasitisation rate)	Reduction of parasitisation efficiency [%]
Control	0.0 ± 0.0	-	45.0 ± 18.7	-
64	2.5 ± 5.0	2.5	35.2 ± 17.5 [c]	21.6
160	2.5 ± 5.0	2.5	30.6 ± 15.8 [c]	32.0
400	0.0 ± 0.0	0.0	30.7 ± 13.5 [c]	31.7
1000	72.5 ± 15.0 [a]	72.5	N.a	N.a
2500	100.0 ± 0.0 [a]	100.0	N.a	N.a
Reference Item (0.3 L Perfekthion/ha)	100.0 ± 0.0 [b]	100.0	N.a	N.a

SD = Standard Deviation; n.a. Not applicable/assessed

[A] Statistically significantly different from control (Bonferroni-Holm Fisher Exact Test; α =

0.05)
[B] Statistically significantly different from control (Fisher Exact Test; α = 0.05)
[C] Statistically significantly different from control (Williams t-test; α = 0.05)
A summary of the sub-lethal effects and mortality are presented in Table B.9.5.2 - 3.

Table B.9.5.2 - 4: Summary of mortalities and sub-lethal observations of *A. Rhopalosiphi* exposed to S-2399 60 g/L EC over 48 hours

Application rate (ml product/ha)	2 hours	24 hours	48 hours
Control	-	-	-
64	-	-	1 dead
160	-	-	1 dead
400	-	-	-
1000	8 affected 10 moribund 1 dead	10 affected 4 moribund 16 dead	8 affected 29 dead
2500	1 moribund 32 dead	All dead	-

Table B.9.5.2 - 5: Table of endpoints

Endpoint	[mL product/ha] (95% confidence limit)	[g a.s./ha] (95% confidence limit)
Mortality LR_{50}	809 (671 – 976)	49.1 (40.7 – 59.2)
Mortality NOER	400	24.3
Mortality LOER	1000	60.7
Reproduction ER_{50}	> 400	> 24.3
Reproduction NOER	< 64	< 3.88
Reproduction LOER	64	3.88

B. VALIDITY CRITERIA

The study fulfilled the validity criteria outlined in the test guidelines (Mead-Briggs *et al*. 2000), as detailed below:

- The mortality in the controls should be ≤ 13%. Actual control mortality was 0%.
- The corrected mortality in the toxic reference should be ≥ 50%. Actual corrected toxic reference mortality was 100.0%.
- Reproduction rate in controls should be ≥ 5 mummies per female. Actual reproduction rate was 45.0 mummies per female (mean).
- There should not be more than two parasitoids producing zero values in the control. There were no parasitoids producing zero values in the control.

III. CONCLUSION

The 48-hour LR_{50} for *Aphidius rhopalosiphi* S-2399 60G/L EC in a standard laboratory glass plate test was 809 ml product/ha (i.e. 49.1 mg a.s./ha) in 200 L water/ha. An NOER based

on mortality was set at 400 ml product/ha. The NOER for reproduction is < 64 ml product/ha (i.e. < 3.88 g a.s./ha). The ER_{50} for reproduction is estimated to be > 400 ml product/ha (i.e. > 24.3 g a.s./ha).

HSE COMMENTS
This study was conducted to Mead-Briggs *et al.* (2000) and Mead-Briggs *et al.* (2010) guidelines and has been assessed against these same guidelines.

It is noted that the dimethoate content in the reference item (492.0g/L) was higher than the guideline recommendation (400 g/L) which adds uncertainty. However, the validity criteria have been met, so this does not invalidate the study.

The 400ml product/ha group did not record any mortalities or sub-lethal effects. This is perhaps unexpected when the 64 and 160 ml product/ha groups recorded 2.5% mortality after 48 hours, and the 1000ml product/ha group recorded 75.5% mortality and does not fit with the trend of the overall data.

The parasitisation of the 64, 160 and 400 ml product/ha groups all recorded rates with overlapping confidence limits to each other, but these results were all still significantly different from the control group. All validity criteria were met, so the study is considered valid.

The use of statistics is suitable for this study, although probit regression analysis is generally recommended in the guidelines to determine the robustness of the LR_{50} result. This study used a moving average regression model. This is not considered to invalidate the study or interpretation of results.

The agreed endpoints to use in risk assessment are:

- **48-hour LR_{50} = 809 mL product/ha (49.1 g a.s/ha)**

Reference:	**KCP 10.3.2.1/02**
Report Title:	S-2399 60 g/L EC: Effects on the Predatory Mite *Typhlodromus pyri* in the Laboratory – Dose Response Test
Author(s) & year:	[redacted] (2019b)
Document No, Authority registration No:	Ibacon GmbH Study No. 141471063 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0105
Substance used:	S-2399 6 EC, V16-7L1901, 6.544 ± 0.024% w/w
Method of analysis:	n/a
Guideline(s):	Blümel et al. (2000)
Deviations:	Yes, see HSE Comments
GLP or GEP:	Yes

Acceptability:	Yes
Study relied upon:	Yes

I.MATERIALS AND METHODS

A.MATERIALS

1.Test material	S-2399 60 g/L EC
Description:	Yellow clear liquid
Batch number:	V16-7L1901
Active substance content:	60.68 ± 0.23 g/L (verified by certificate of analysis) 6.544 ± 0.024 % w/w (verified by certificate of analysis)
Density:	0.9273 (g/mL)
Storage on receipt:	At 20 ± 5 °C, in the dark
Expiry date:	17 May 2021
TREATMENTS	
Nominal test doses:	64 mL product/ha or 3.88 g a.s./ha in 200 L deionised water/ha (corresponding to 0.294 g product/L) 160 mL product/ha or 9.71 g a.s./ha in 200 L deionised water/ha (corresponding to 0.736 g product/L) 400 mL product/ha or 24.3 g a.s./ha in 200 L deionised water/ha (corresponding to 1.84 g product/L) 1000 mL product/ha or 60.7 g a.s./ha in 200 L deionised water/ha (corresponding to 4.60 g product/L) 2500 mL product/ha or 152 g a.s./ha n 200 L deionised water/ha (corresponding to 11.5 g product/L)
Control:	Deionised water (200 L/ha)
Toxic reference:	Perfekthion (BAS 152 11 I, Dimethoate, content of a.s. analysed: 429 g/L) 8 mL Perfekthion in 200 L deionised water/ha (corresponding to 40 µL Perfekthion/L)
Toxic reference batch:	FRE-001578
Expiry date:	November 17, 2019
Application rate:	2 mg/cm² ± 10 % (corresponding to 200 L spray liquid/ha)
Spraying equipment:	Laboratory-spraying equipment (Fa. Schachtner, D-71640 Ludwigsburg)
Spray nozzle type:	TeeJet 80015 EVS (distance to glass plate: 30 cm, spraying pressure: 2.5 bar, spraying speed: 2.75 km/h)

B.TEST ORGANISMS

Species:	Predatory mite (*Typhlodromus pyri* Scheuten), Protonymphs, not older than 24 hours.
Acclimatisation:	Under test conditions
Source:	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837

	Baruth (Eggs on delivery)
Diet:	A mixture of pine (*Pinus sp.*) and birch (*Betula sp.*) pollen (3:1) *ad libitum* on the day of the test start and on each assessment day except for the last one (at least every four days). Water provided between cover slides (via capillary action)
C.TEST DESIGN	
Test container:	Plastic trays (11 cm x 11 cm x 6 cm) half-filled with water, with a foam rubber and a glass-plate on top covered by tissue paper, tissue paper in contact with the water. The test units were placed on the tissue paper.
Test units:	Formed by two cover slides (glass, 24 mm x 60 mm) fixed by gluing small cover slides (glass, 20 mm x 20 mm) to both side ends. A glue barrier was placed on the test unit to keep the mites on this test arena.
Replication:	3 units per treatment group (20 individuals per unit)
Duration:	14 days (reproduction was assessed after 7 days)
D.TEST CONDITIONS	
Test temperature:	24 - 25 °C
Relative humidity:	63 - 74 %
Lighting:	16 h light : 8 h dark (230 250 lux)

STUDY DESIGN AND METHODS

Experimental dates: 26 March 2019 to 27 May 2019

Test organism

T. pyri protonymphs were exposed on glass plates treated with S-2399 60 g/L EC. An untreated control and the toxic reference item Perfekthion were tested in parallel. Glass plates were sprayed with the respective treatment and dried for 30 to 40 minutes. Once dried, impartially selected mites were introduced to test units with a fine brush. The sex ratio for reproduction testing on day 7 was one male: five females at minimum.

Dose preparation and treatment calibration

Test item solutions were prepared by adding 0.0736, 0.184, 0.460, 1.15 and 2.88 g of test item to 250 mL deionised water. The reference item solution was prepared by dissolving 10 µL Perfekthion in 250 mL deionised water. Treatment calibration was performed by spraying a glass plate of known surface area with deionised water. The weight of the glass plate was determined immediately before and after application and the amount of spray deposit per cm² was calculated as the difference between the weight before and after spraying. The procedure was repeated 5 times in a row without changing the adjustment and every time the application rate was within 200 L/ha ± 10 %. The uniformity of the deposit distribution

was checked visually.

Measurement and observations
The numbers of living, dead and escaped mites were counted on days 3 and 7 after test initiation. Dead mites were removed, and any escaped mites were considered as dead. Test organisms were counted as dead animals when they were motionless even after touching them with a fine hairbrush or when they had an abnormal appearance. The number of eggs laid and number of live and dead juvenile stages per female was counted and removed afterwards on 3 assessment days from day 7 on with a maximum interval of 3 days up to day 14 (inclusively). Eggs laid until day 7 inclusively were removed from the test arena and were not counted. The reproduction assessment was performed when the corrected mortality was ≤ 50 %. No reproduction assessment was performed for the reference item.

Test conditions were recorded with suitable instruments. Short-term deviations (< 2 hours) are partly unavoidable due to handling and are not reported.

Data evaluation
Mortality was determined after Day 7 and corrected for control mortality using the formula of Abbott (1925), modified by Schneider-Orelli (1947):

$$M_{corr} = \frac{M_T - M_C}{100 - M_C} \times 100$$

M_{corr} = Corrected mortality [%]
M_C = Mortality in the control group [%]
M_T = Mortality in the test group [%]

The reproduction per replicate (Rpr) of the mites during the second week of the test was calculated as the ratio of eggs per female for each test unit. The number of eggs per female was determined by counting the number of females and eggs and juvenile stages at the 3 assessment days. The number of eggs per female during the reproduction period until day 14 (inclusive) was summed. Calculation was done per replicate according to the following formula:

$$Rpr = \frac{Ld9}{Fd7} + \frac{Ed9 + Ld11}{(\frac{Fd7 + Fd9}{2})} + \frac{Ed11 + Ld14}{(\frac{Fd9 + Fd11}{2})} + \frac{Ed14}{(\frac{Fd11 + Fd14}{2})}$$

Rpr = Reproduction per replicate
Fdx = number of females on day x
Edx = number of eggs on day x
Ldx = number of larvae on day x

The values obtained for each replicate were used to calculate the mean egg production per female (± Standard Deviation).

Statistical analysis

The LR_{50} (rate at which 50% of individuals die) of the mortality was calculated by applying logit analysis. Mortality data were analysed for significance using the Fisher's Exact Test, which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. For the analysis of the test item data the Bonferroni correction was applied (Bonferroni-Holm Fisher's Exact Test). Reproduction data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test (α = 0.05) and the Levene's test (α = 0.05), respectively. Because the reproduction data were normally distributed and not homogeneous, the Bonferroni-Welsh t-test (multiple comparison, one-sided, α = 0.05) was used. The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

II.RESULTS AND DISCUSSION

Biological effects

Effects of S-2399 60 g/L EC on mortality and reproduction are summarised in Table B.9.5.2 - 6.

Table B.9.5.2 - 6: Percent mortality and effects on reproduction in *Typhlodromus pyri* after exposure to S-2399 60 g/L EC

Application rate [mL product/ha]	Mortality		Escapees	Reproduction	
	[%] ± SD	[%] Corrected	[%]	No. eggs/female	Effect on reproduction [%]
Control	10.0 ± 5.0	n.a.	0.0 ± 0.0	8.6 ± 1.2	n.a.
64	6.7 ± 2.9	-3.7	1.7 ± 2.9	9.8 ± 1.5	-14.5
160	3.3 ± 5.8	-7.4	0.0 ± 0.0	7.6 ± 1.3	11.6
400	13.3 ± 5.8	3.7	0.0 ± 0.0	7.2 ± 0.4	16.2
1000	36.7 ± 5.8 [a]	29.6	20.0 ± 0.0	7.3 ± 3.4	14.1
2500	86.7 ± 2.9 [a]	85.2	36.7 ± 7.6	n.a.	n.a.
Reference Item (8.0 mL Perfekthion/ha)	100.0 ± 0.0 [b]	100.0	65.0 ± 13.2	n.a.	n.a.

SD: Standard Deviation; n.a. not applicable/available

Negative values indicate increased survival compared to controls
[a] Statistically significantly different from control (Bonferroni-Holm Fisher Exact Test; α = 0.05)
[b] Statistically significantly different from control (Fisher Exact Test; α = 0.05)

There was statistically significant mortality in the test item treatment groups of 1000 and 2500 mL product/ha. No significant effect on reproduction was found in all treatment groups assessed (i.e. up to 1000 mL product/ha).

Figure B.9.5.2-1 displays the concentration-response relationship for mortality effects.

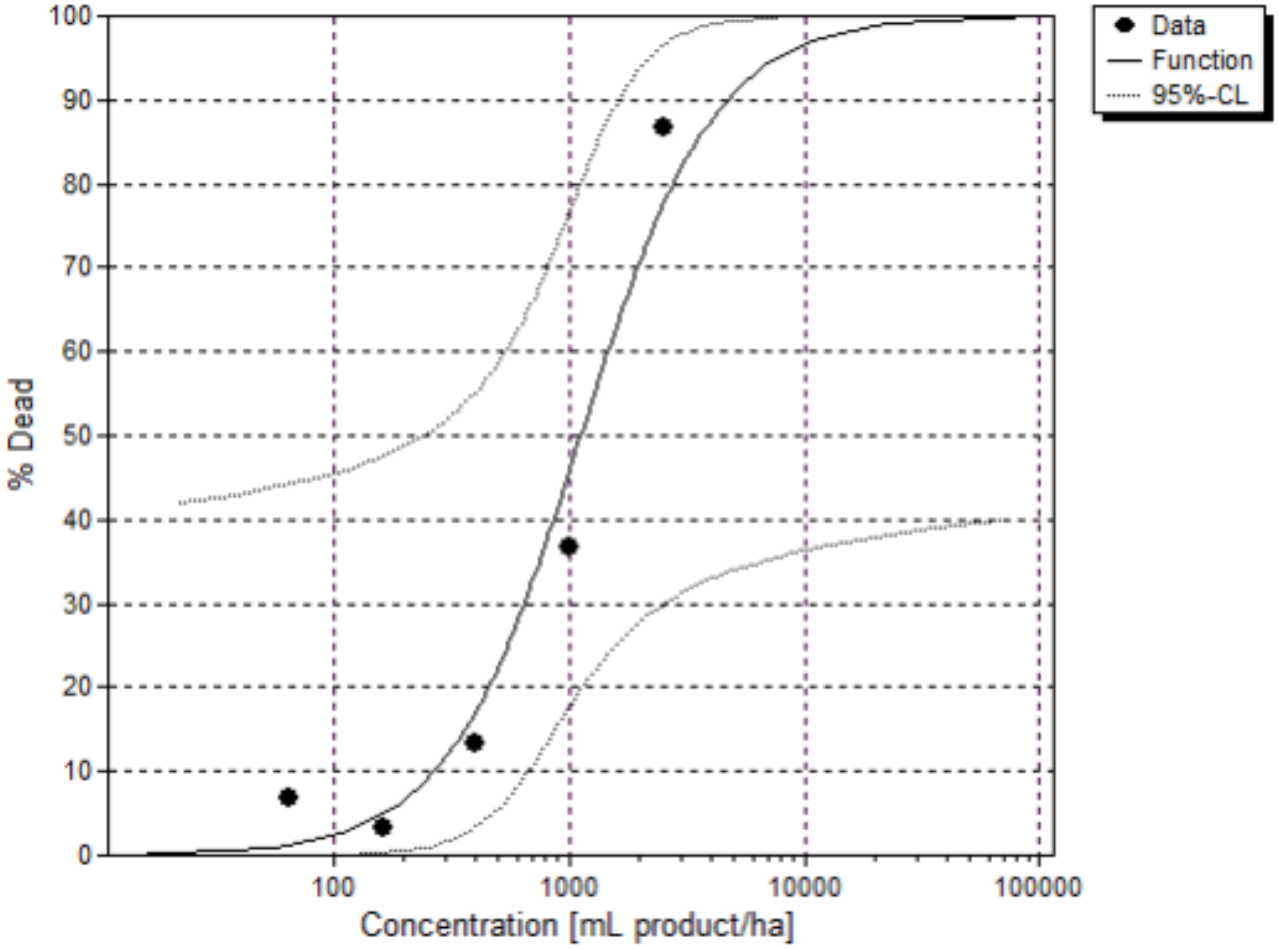

Figure B.9.5.2 - 1: Illustration of the dose-response relationship for mortality effects

Endpoints are summarised in Table B.9.5.2 - 7.

Table B.9.5.2 - 7: Table of endpoints

Endpoint	[mL product/ha] (95% confidence limit)	[g a.s./ha] (95% confidence limit)
Mortality LR_{50}	1116 (238 – n.d.)	67.7 (14.4 – n.d.)
Mortality NOER	400	24.3
Mortality LOER	1000	60.7
Reproduction ER_{50}	> 1000 (n.d.)	> 60.7
Reproduction NOER	≥ 1000	≥ 60.7
Reproduction LOER	> 1000	> 60.7

n.d. could not be determined due to mathematical reasons

Environmental conditions

Short-term deviations (< 2 hours) from the recommended ranges are partly unavoidable (e.g. due to handling of the set-ups) and will normally not result in major disturbances of the test performance. Therefore, short-term deviations were not reported.

Validity criteria

The validity criteria for the study were met according to Blümel et al. (2000).

Table B.9.5.2 - 8: Compliance with Blümel et al. (2000) validity criteria

Validity criterion	Required	Obtained
Control mortality (Day 7)	≤ 20 %	10 %
Control reproduction (eggs per female, Week 2)	≥ 4 eggs	8.6 eggs
Reference item toxicity (Day 7)	≥ 50 %	100 %

CONCLUSIONS

In this 14-day exposure toxicity test with S-2399 60 g/L EC, the 7-day mortality NOER was determined to be 400 mL product/ha (24.3 g a.i/ha) and LR_{50} was estimated at 1116 mL product/ha (67.7 g a.s/ha). The reproduction ER_{50} and NOER were both > 1000 mL product/ha (60.7 g a.s/ha).

HSE COMMENTS

The study was carried out according to and evaluated against the Blümel et al. (2000)

guideline. All validity criteria were satisfactorily met for the duration of the study. There were no significant deviations from the guideline. Treatment with the toxic reference (dimethoate) indicated the sensitivity of the mites and reliability of the test system was appropriate.

The following minor deviations were noted for Blümel et al. (2000):
Blümel et al. (2000) recommends an application rate of 9 to 15 mL of formulated product/ha for the dimethoate reference formulation. The study used 8 mL formulated product/ha. As reference item mortality adhered to the validity criteria this deviation was considered minor and acceptable. Moreover, Blümel et al. (2000) states, “it is inappropriate to use a toxic reference at a rate that consistently results in 100% mortality”. Reference item mortality was 100 %. Taken together, this suggests that the test system was potentially more sensitive than required. This is an acceptable deviation due to its conservative nature.

Blümel et al. (2000) states that the application timing of the glue barrier (before or after the application and drying of the treatment) should be reported. This was not reported. HSE considers this a minor reporting omission and acceptable.

The equation used for calculating cumulative number of eggs per female in this study selected different observation windows from the equation outlined in the guideline. Cumulative number of eggs per female was still calculated from Day 7 to 14. This is a minor deviation with no impact on the study results. HSE consider it acceptable.

Regarding statistical considerations, the study only used three replicates. This was considered acceptable as the main aim of this study was to estimate a LR_{50} for risk assessment. The guideline recommends the use of probit analysis. The study conductor analysed the data using logit analysis, which is a widely acceptable alternative. Why the study conductor deviated from probit analysis, however, was not discussed. This is particularly relevant as the logit model fitted failed to define an upper confidence interval for the LD_{50}. Therefore, there is low confidence in the estimated LD_{50}, although visual interrogation of the data and fitted model suggests the 1116 mL product/ha value estimated is plausible. Furthermore, the guideline requests the fit of the data to the model be statistically assessed. This was performed and reported in the appendix. The model failed a Chi^2 goodness-of-fit test, suggesting the model was a poor fit for the data. To address this uncertainty during risk assessment, HSE has decided to lower the LR_{50} to > 1000 mL product/ha.

Finally, although NOERs are not used in the current risk assessment procedure, there was a > 10 % effect on reproduction for > 160 mL product/ha. A 10 % effect is an oft quoted threshold for effects considered biologically relevant (implied from EC_{10} calculations). Above 160 mL product/ha, however, there was no clear concentration-response, which could indicate that control reproduction was elevated relative to historical control reproduction. This is not possible to confirm without historical data on control reproduction (number of

eggs per female). HSE notes this uncertainty. Given that NOER values are not used in the existing risk assessment framework for NTAs, this potential issue is not considered further.

The above study was conducted to GLP and considered valid.

The agreed endpoints suitable for use in the risk assessment are: 7-day LR_{50} > 1000 mL product/ha (60.68 g a.s./ha)

Reference:	KCP 10.3.2.1/03
Report Title:	Effects of Fungicides on Four Native Generalist Phytoseiid Species（Acari: Phytoseiidae)
Author(s) & year:	[redacted] et al. (2020)
Document No, Authority registration No:	Journal of the Japanese society of applied animal entomology (Oidokon) Vol. 64, No.4: 175 – 182 (2020)
Substance used:	Inpyrfluxam product FL (37 % w/w)
Method of analysis:	n/a
Guideline(s):	No guideline followed
Deviations:	N/A
GLP or GEP:	No
Acceptability:	Supplementary
Study relied upon:	Yes

Introduction

29 fungicides, including inpyrfluxam, were tested against 4 phytoseiid species native to Japan: *Amblyseius eharai* Amitai and Swirski, *Amblyseius tsugawai* Ehara, *Euseius sojaensis* Ehara, and *Typhlodromus vulgaris* Ehara (Acari: Phytoseiidae). Inpyrfluxam was tested at 37 g a.s./ha and categorised as harmless to the four mite species according to the IOBC/WPRS pesticide toxicity categorisation (< 30 % corrected mortality).

Method

The four mite species were collected from fruit trees or vegetation near orchards (at least 16 females per species) and raised cumulatively in the lab (22 – 23 °C, 16L:8D). 10 female adults were placed in test arenas (diameter 5 cm, height 0.8 cm) within 10 days of emergence and, 30 minutes later, sprayed with 4 mg/cm^2 of chemical solution, equating to 37 g a.s./ha, using a model painting airbrush. After air-drying, arenas were placed in incubators (25 °C, 16L:8D, 90 ± 10 % RH). After 48 hours, mortality and number of eggs laid was quantified. Three replicates of 10 adults were performed.

For the eggs, females were introduced to an egg collection device where eggs were laid on velvet cloth, cut into 1 cm x 0.5 cm strips. The number of eggs per velvet cloth was adjusted to 12 – 13 and then placed into test arenas. Eggs were sprayed with inpyrfluxam at 4 mg/cm^2 of chemical solution in the same manner as adult females. Eggs development was tracked all the way through to adult emergence and corrected mortality calculated. Three replicates of 12 – 13 eggs were performed.

The 4 mg/cm^2 spray rate could be expressed in g/ha using the 37 % AI concentration of the product and 4000-fold dilution. This was equal to 37 g/ha.

Results

The effects of inpyrfluxam for the four mite species are presented in the table below.

Table B.9.5.2 – 9: Results summary of a 37 g a.s./ha overspray exposure regime to four mite species

Species	Treatment	Corrected mortality adults	No. of eggs oviposited per 10 females (mean +- SE)	Corrected mortality eggs
A. eharai	control	0	27 ± 2	0
A. eharai	inpyrfluxam	6.7	23.3 ± 0.7	8.3
A. tsugawai	control	0	28.3 ± 1.9	0
A. tsugawai	inpyrfluxam	0	20.7 ± 3	2.8
E. sojaensis	control	0	26 ± 1.2	5.6*
E. sojaensis	inpyrfluxam	3.3	20.6 ± 3	20.6
T. vulgaris	control	0	27 ± 1	0
T. vulgaris	inpyrfluxam	6.9	25 ± 2.6	0

* Assumed reporting error

The reported effects are discussed in the context of the results from submitted studies conducted according to regulatory guidelines during risk assessment.

Relevance

[redacted] et al. (2020) is directly relevant to the effects on arthropods data requirements of Regulations 283/2013 and 284/2013. Inpyrfluxam was clearly defined as the test material and a rate could be calculated and expressed in terms of g/ha. The test species were mites found in orchard crops. Their sensitivity can directly inform on the general sensitivity of arthropods to inpyrfluxam. The duration and route of exposure (overspray) is possible in the field. The observation window for mortality and reproductive effects is short (2 days)

compared to studies conducted according to Blümel et al. (2000) (7 day mortality and 14 day egg laying). This complicates results interpretation (please refer to NTA risk assessment). Overall, the study was considered **relevant**. Consequently, the results are discussed in the context of the results from submitted studies conducted according to regulatory guidelines in the NTA risk assessment.

Reliability

A full assessment of the reliability of [redacted] et al. (2020) has not been conducted as the reported effects can be considered covered by the submitted *T. pyri* regulatory study and associated risk assessment. Please see the risk assessment below for further details. HSE Ecotoxicology does note that a corrected egg mortality of 5.6 % was reported for the *E. sojaensis* controls. This is not possible and raises the possibility that further reporting errors were made.

B.9.6 Risk assessment for arthropods

B.9.6.1 Risk assessment for bees

A summary of submitted studies and the associated endpoints can be found in Table 9.6-1 and key aspects are discussed below. Endpoints considered reliable for risk assessment are presented in bold. Other endpoints are discussed further in the text.

Table 9.6-1: Inpyrfluxam toxicity endpoints for bee risk assessment

Test Item	Study type	Species	Endpoint	Results	References
Acute adult					
Inpyrfluxam	48 h acute oral	*Apis mellifera*	LD_{50}	**>111.3 µg a.s./bee**	KCA 8.3.1.1.1/01 [redacted] 2015
Inpyrfluxam	48 h acute contact			**>100 µg a.s./bee**	
Inpyrfluxam	48 h acute oral	*Bombus terrestris*	LD_{50}	>95.1 µg a.s./bee	KCA 8.3.1.1.1/02 [redacted] 2016
Inpyrfluxam	48 h acute contact			>100 µg a.s./bee	
S-2399 60 g/L EC [a]	48 h acute oral	*Apis mellifera*	LD_{50}	274.95 µg product/bee **(17.99 µg a.s./bee)**	KCP 10.3.1.1.1/01 [redacted] 2019
	48 h acute contact			252.91 µg product/bee **(16.55 µg a.s./bee)**	

Test Item	Study type	Species	Endpoint	Results	References
S-2399 60 g/L EC [a]	48 h acute oral	*Bombus terrestris*	LD_{50}	> 532.69 µg product/bumblebee (>34.74 µg a.s./bumblebee)	KCP 10.3.1.1.1/02 [redacted] 2020
	48/72 h acute contact	*Bombus terrestris*		> 3066.67 µg product/bumblebee (>200 µg a.s./bumblebee)	
Chronic adult					
S-2399 60 g/L EC [a]	10 d chronic	*Apis mellifera*	LDD_{50}	133.83 µg product/bee/d (8.76 µg a.s./bee/day)	KCP 10.3.1.2/01 [redacted] 2021a
			NOEC	3157.27 mg product/kg (206.61 mg a.s./kg)	
			NOEDD	46.56 µg product/bee/day (3.05 µg a.s./bee/day)	
Larvae					
S-2399 60 g/L EC [a]	22 d chronic	*Apis mellifera*	ED_{50}	275.23 µg product/larva (18.01 µg a.s./larva)	KCP 10.3.1.3/01 [redacted] 2021b
			NOED	162.71 µg product/larva (10.65 µg a.s./larva)	

[a] Active substance content of the formulation; density 0.9273 g/mL, 60.68 g a.s./L (corresponding to 6.544% w/w)
Endpoints highlighted in **bold** are used in the risk assessment

B.9.6.1.1 Studies conducted with the active substance

The applicant submitted the following active substance studies on inpyrfluxam (S-2399):

- Honeybee adult acute contact and oral
- Bumblebee adult acute contact and oral (single concentration limit test)
- Honeybee larval 72 h acute.

B.9.6.1.1.1.1 Active substance honeybee adult acute study

The active substance acute oral and contact studies for honeybees were performed as limit

tests at 111.3 and 100 µg a.s./bee respectively. No mortality or behavioural effects were recorded across the 48-hour observation period. No deviations of concern were noted and the studies were considered suitable for use in risk assessment.

B.9.6.1.1.1.2 Active substance bumblebee adult acute study

The active substance acute oral and contact studies for bumblebees were performed as limit tests at 95.1 and 100 µg a.s./bee respectively. No analytical verification was performed, which introduces a degree of uncertainty into the reported test concentrations and doses. However, the study was performed before OECD 246 (2017) was finalised. The study conductor followed a draft version of this guideline, which did not require analytical verification. Furthermore, there was a lack of clarity surrounding the number of colonies used in the study and, if study individuals did originate from different colonies, if individuals originating from different colonies were evenly distributed between treatment groups. In addition, it should be noted that due to a lack of noted guidance this study is considered as supporting information only. Nevertheless, this study was considered valid.

B.9.6.1.1.1.3 Active substance honeybee larval acute study

HSE does not consider OECD 237 (2013) studies adequate to address Data point 8.3.1.3 set out in COMMISSION REGULATION (EU) No 283/2013, which states, "*the bee brood study shall provide sufficient information to evaluate possible risks from the active substance on honeybee larvae*". The single exposure design of OECD 237 (2013) is not considered worst-case, unlike the multiple exposure design of OECD 239. An OECD 239 study was submitted for the representative formulation. Its suitability and the potential for read-across from the representative formulation to the active substance is discussed below.

B.9.6.1.2 Studies conducted with the formulation

The applicant submitted the following formulation studies on inpyrfluxam (S-2399):

- Honeybee adult acute contact and oral
- Bumblebee adult acute contact and oral
- Honeybee adult chronic oral
- Honeybee larval 22-day test, repeated exposure

B.9.6.1.2.1.1 Formulation honeybee adult acute studies

The S-2399 60 G/L EC acute oral and contact study for honeybees was performed as a dose-response study. For the oral study, mortality was recorded 4, 24 and 48 hours after the start of feeding on test solutions instead of 4, 24 and 48 hours after the test solutions were replaced by unspiked sucrose solution. This resulted in the study ending 6 hours early for higher treatment levels that did not fully consume the spiked solution. For the 191.67 and 383.34 µg test item/bee treatment levels there were small increases (2 and 4 %) in mortality from 24 to 48 hours, which may have been greater if the observation period had continued for the additional six hours. This deviation may have resulted in a small underestimation of the estimated endpoints. There were transient sub-lethal effects at > 191.67 µg test item/bee

for the oral study. These were observed after 4 hours but reduced to a low incidence (5 – 6.3 %) after 48 hours. For the contact study, there were sub-lethal effects for all treatment levels (> 47.92 µg test item (Nominal)/bee) after 4 and 24 hours ranging from 10 to 62 % and 8 to 26 % respectively. After 48 hours these sub-lethal effects were still present in 14 and 26 % of individuals for the 47.92 and 95.83 µg test item (Nominal)/bee treatment levels. Finally, there was a clear reduction in oral consumption of spiked sucrose at > 191.67 µg test item/bee (> 43 %). This study was considered valid.

B.9.6.1.2.1.2 Formulation bumblebee adult acute studies

The S-2399 60 G/L EC acute oral and contact study for bumblebees was performed as a dose-response study. There was a clear repellency effect for the test item in the oral exposure study. This effect appeared approximately binary in nature with most bees either displaying a < 35 % or > 85 % consumption per bee. This resulted in several treatment groups excluding a high proportion of bees. The LD_{50} was set using a treatment group containing only 11 bees after HSE demonstrated that there is a > 99 % confidence that the true LD_{50} is higher given the sample size and level of observed mortality. This study is considered valid for use in risk assessment. Due to the lack of noted guidance, however, it will be considered as supporting information only.

B.9.6.1.2.1.3 Formulation honeybee adult chronic study

The S-2399 60 G/L EC chronic oral study for honeybees was performed as a dose-response study. There was a clear dose-response for the mass of feeding solution consumed per bee per day. There were also behavioural abnormalities reported for the two highest treatment levels throughout the study (Day 2 – 10 with 2.1 – 8.5 % of bees affected for 6945.99 mg test item/kg feeding solution and Day 1 - 10 with 2.4 – 26.9 % of bees affected for 15281.18 mg test item/kg feeding solution). These behavioural effects were presented at the end of the study, impacting 5.1 and 15.4 % of bees on Day 10 of the study for the 6945.99 and 15281.18 mg test item/kg feeding solution treatment levels respectively.

B.9.6.1.2.1.4 Formulation honeybee larval chronic development study

The S-2399 60 G/L EC larval chronic development study for honeybees was performed as a dose-response study. There were no deviations that added uncertainty to the estimated endpoints. The study was considered valid.

B.9.6.1.3 Acute risk assessment for honeybees

Assessment of the acute risk of inpyfluxam to bees is conducted in accordance with Regulation (EC) No. 1107/2009, and the noted Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002).

The first-tier risk assessment is based on a Hazard Quotient approach (Q_H) by calculating the ratio between the application rate (expressed in g a.s./ha) and the laboratory contact and oral LD_{50} (expressed in µg a.s./bee) for honeybees.

Q_H values are calculated using data from the studies performed with the active substance and with the formulation. Q_H values higher than 50 indicate the need of higher tiered activities to clarify the actual risk to honeybees.

$$Q_{HO/C} = \frac{max\ application\ rate}{LD_{50} oral/contact}$$

The risk assessment for bees for inpyrfluxam is based on the application rate stated in the GAP table of one application per season of 90 g a.s./ha to winter and spring cereals (BBCH 30-71).

For this acute risk assessment, data on the active substance and formulation are the key endpoints. The calculations are presented in Table 9.6-2 below.

Table 9.6-2: First-tier assessment of the risk for bees due to the use of S-2399 60 G/L EC in cereals

Substance	Endpoint	Application rate (g a.s./ha)	LD_{50} (µg a.s./bee)	Calculated Q_H	Acceptable Risk? ($Q_H \leq 50$)
Inpyrfluxam	Acute oral	90	>111.3	< 0.809	yes
	Acute contact	90	>100	< 0.900	yes
S-2399 60 G/L EC	Acute oral	90	17.99	5.00	yes
	Acute contact	90	16.55	5.44	yes

All calculations of Q_Hs for the acute oral and contact honeybee studies fell below the trigger value of 50, indicating an acceptable acute risk to honeybees for the active substance and formulation for the proposed use of up to 90 g a.s./ha.

B.9.6.1.4 Additional studies

Although chronic studies for adult and larvae honeybees are also available for the product S-2399 60 G/L EC , as they do not form part of the risk assessment scheme under the current SANCO/10329/2002 rev.2 guidance document they have not been used in the risk assessment. Acute studies with the active substance are also available for honeybee larvae and bumblebee workers but these endpoints, similarly, do not form part of the current SANCO guidelines and have not been used in the risk assessment.

Technically, Data points 8.3.1.2. (chronic toxicity to bees) and 8.3.1.3. (effects on honeybee development and other honeybee life stages) of COMMISSION REGULATION (EU) No 283/2013 are not satisfied by the current data package. To determine the impact of these omissions, HSE compared the toxicity of the representative formulation S-2399 60 G/L EC to technical inpyrfluxam for all honey bee studies that were conducted with both test items (Table 9.6-3 below).

Table 9.6-3: Comparison of technical active substance and representative formulation endpoints for available *Apis mellifera* studies

Species	Study	Technical endpoint (µg a.s./bee)	Formulation endpoint (µg a.s./bee)	Factor of difference (technical/formulation)
Apis mellifera	Acute oral	**> 111.3**	17.99	> 6.19
Apis mellifera	Acute contact	**> 100**	16.55	> 6.04

For both studies, the representative formulation S-2399 60 G/L EC is > 6 times more toxic than the technical active substance. This suggests that S-2399 60 G/L EC can be considered worst-case for the technical active substance and the data gaps for the active substance are addressed by the equivalent S-2399 60 G/L EC studies.

B.9.6.1.5 Sub-lethal effects

No sub-lethal effects were reported for any active substance study. Sub-lethal effects were detected in three of the four S-2399 60 G/L EC studies. For the acute contact and chronic oral *Apis mellifera* studies these sub-lethal effects were present after 48 hours: 1) a > 10 % frequency after acute exposure to 47.92 µg product/bee (3.13 µg a.s./bee), and 2) a > 10 % frequency after chronic exposure to 171.56 µg product/bee/day (11.23 µg a.s./bee/day).

1) The sub-lethal effects associated with acute contact exposure clearly decreased over time, strongly suggesting that the effects remaining after 48 hours would continue to reduce. Furthermore, there was no clear dose-response associated with the sub-lethal effects after 48 hours considering their low incidence at 191.67 and 383.34 µg product/bee. Taking the transient nature of these sub-lethal effects with the lack of a clear dose-response, HSE concludes that they do not raise enough concern to warrant additional consideration within the risk assessment.
2) For the sub-lethal effects associated with chronic oral adult exposure, a $NOED_{mortality}$ was provided of 46.56 µg product/bee/day, which can be considered protective of the two higher treatment doses where sub-lethal effects were sustained (> 90 µg product/bee/day).

Overall, HSE concludes that the sub-lethal effects observed across all bee studies do not raise sufficient concerns to warrant further consideration.

B.9.6.1.6 Overall conclusions for risk of inpyrfluxam to bees

There is an acceptable acute risk of inpyrfluxam to adult honeybees, as assessed using the hazard quotient approach. HSE considers an acceptable risk to honeybees can be

concluded for the proposed use.

B.9.6.2 Risk assessment for non-target arthropods other than bees

The following table summarises the data available for non-target organisms with the representative product S-2399 60 g/L EC .

Table B.9.6.2 - 1: Endpoints for non-target arthropods other than bees (NTA) and the representative product

Species	Test system	Test substance	Endpoint	Results	References
First tier studies					
Aphidius rhopalosiphi	Glass plate (2D)	S-2399 60 g/L EC [a]	**LR_{50}** ER_{50} NOER	809 mL product/ha **(49.1 g a.s./ha)** > 400 mL product/ha (24.3 g a.s./ha) < 64 mL product/ha (3.88 g a.s./ha)	CP 10.3.2.1/01 [redacted] 2019a
Typhlodromus pyri	Glass plate (2D)	S-2399 60 g/L EC [a]	**LR_{50}** ER_{50} NOER	> 1000 mL product/ha **(60.68 g a.s./ha)** > 1000 mL product/ha (> 60.7 g a.s./ha) 400 mL product/ha (24.3 g a.s./ha)	CP 10.3.2.1/02 [redacted] 2019b

[a] Active substance content of the formulation; density 0.9273 g/mL, 60.68 g a.s./L (corresponding to 6.544% w/w). Endpoints highlighted in **bold** used in the risk assessment

The risk assessment for non-target arthropods other than bees (NTA) is based on ESCORT II guidance[7].

Risk assessment according to ESCORT II is divided into tiers. The first tier requires laboratory 'glass plate' data on two indicator species, *A. rhopalosiphi* and *T. pyri*. The endpoint for mortality (LR50) for both species are compared to in- and off-field Predicted Environmental Rates (PER), to calculate a Hazard Quotient (HQ). If the HQ is lower than the trigger value of 2, an acceptable risk can be determined.

For Inpyrfluxam, the following uses are requested on the representative GAP:

Table B 9.6.2-2: Summary of uses requested for Inpyrfluxam

Crop	Growth stage	Max. no. of applications	Application method	Max. application rate (g a.s./ha)
Cereals	BBCH 30-71	1	Foliar spray	90

Inpyrfluxam is proposed solely for outdoor use; therefore, a full outdoor risk assessment according to ESCORT II is required.

Exposure

In-field exposure

Non-target arthropods inhabiting the crop can be exposed to residues of S-2399 60 g/l EC by direct contact, either as a result of overspray or through contact with residues on plants and soil or in food items. S-2399 60 g/l EC is applied at a maximum rate of 1 x 90 g a.s/ha to cereals (field crops). The risk assessment is thus carried out based on this worst-case field application rate.

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

$$PER_{in\text{-}field} = \text{Application rate [g/ha]} \times MAF$$

The MAF is the 'multiple application factor'. As the proposed GAP is for a single application, a default value of 1.0 is used (ESCORT II).

The maximum predicted environmental rates (PER) occurring within the field after the application of S-2399 60 g/L EC are presented in the table below.

[7] Guidance Document on regulatory Testing and Risk Assessment Procedures for Plant Protection Products with Non-Target Arthropods. From the ESCORT 2 Workshop (European Standard Characteristics of Non-target Arthropod Regulatory Testing). Candolfi *et al.*, 2000.

Table B 9.6.2. – 3: $PER_{in\text{-}field}$ values for application of S-2399 60 G/L EC in cereals

Crop	Application rate [g a.s /ha]	MAF	$PER_{in\text{-}field}$ [g a.s/ha]
Cereals	90	1	90

Off-field exposure

Risk assessment of off-field areas immediately surrounding the crop is considered important since these areas represent a natural environment for arthropod populations across all life stages. Exposure of non-target arthropods living in off-field areas to S-2399 60 g/l EC will mainly be due to spray drift from field applications.

Off-field foliar PER values were calculated from the following equation:

$$PER_{off\text{-}field} = \text{Application rate} \times MAF \times (\text{drift factor} / VDF) \times \text{Correction Factor}$$

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 studies. A dilution factor of 10 is recommended by ESCORT 2.

The drift factor is in ESCORT 2 table in Appendix IV. The drift value for one application at 1 m distance in field crops is 2.77% of the application rate (90^{th} percentile drift). The drift factor (% drift/100) is therefore 2.77/100 = 0.0277.

The resulting PER off-field values are shown in the table below.

Table B 9.6.2 -4: $PER_{off\text{-}field}$ values following application of S-2399 60 g/l EC in cereals

Study type	Exposure type	Maximum $PER_{off\text{-}field}$ [g a.s/ha]	Drift factor [% drift/100]	Vegetation distribution factor	$PER_{off\text{-}field}$ [L/ha]
Cereals	2D	90	0.0277	10	0.25

In-field risk assessment

The potential risk of S-2399 60 g/L EC to in-field non-target arthropods was assessed by calculation of the hazard quotient (HQ) using the $PER_{in\text{-}field}$ and the lowest lethal rate (LR_{50}) values according to the following equation:

$$HQ_{in\text{-}field} = PER_{in\text{-}field} / LR_{50}$$

The HQ trigger for Tier I laboratory studies is 2. When following the HQ approach for in-field assessments, a HQ value lower than the trigger value indicates a low risk to non-target arthropods. A quotient value equal to or greater than the trigger indicates a potential

hazard to non-target arthropods. The resulting $HQ_{in\text{-}field}$ values are presented in the table below.

Table B.9.6.2 - 5: First tier assessment of the in-field risk for non-target arthropods due to the use of S-2399 60 g/L EC in cereals

Product	S-2399 60 g/L EC		
Application rate (g a.s./ha)	1 × 90		
MAF	1.0		
Test species Tier 1	**LR_{50} (lab.) (g a.s./ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	60.68	90	1.48
Aphidius rhopalosiphi	49.1		1.83

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient.

The hazard quotients for the standard glass plate studies at Tier 1 are less than 2 indicating that S-2399 60 g/L EC poses an acceptable in-field risk to non-target arthropods from use in cereals.

Off-field risk assessment (Tier I)

In order to assess the potential risk of S-2399 60 g/L EC to off-field non-target arthropods, the $PER_{off\text{-}field}$ is compared to the toxicity endpoints according to the following equation:

$$HQ_{off\text{-}field} = PER_{off\text{-}field} / LR_{50}$$

The HQ trigger for Tier I laboratory studies is 2. Furthermore, ESCORT 2 recommends a correction factor of 10 for Tier I data in the off-field risk assessment to account for extrapolation from testing just two representative species to the species diversity expected in off-field crop areas.

Respective $HQ_{off\text{-}field}$ values are given in the table below.

Table 9.6.2 - 6: First-tier assessment of the off-field risk for non-target arthropods due to the use of S-2399 60 g/L EC in cereals

Product	S-2399 60 g/L EC					
Application rate (g a.s./ha)	90					
MAF	1.0					
VDF	10 (Tier 1)					
Test species Tier 1	**LR_{50} (lab.) (g a.s./ha)**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**Corrected $PER_{off\text{-}field}$ (g/ha)**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	60.68	2.77	0.25	10	2.5	0.04
Aphidius rhopalosiphi	49.1					0.05

MAF: Multiple application factor; vdf: Vegetation distribution factor; PER: Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient.

The hazard quotients are less than 2 indicating that S-2399 60 g/L EC poses an acceptable off-field risk to non-target arthropods from use in cereals.

Consideration of KCP 10.3.2.1/03

A study captured during the literature review tracked mortality, reproductive output and developmental effects after a direct overspray application of 37 g a.s./ha for four mite species native to Japan. The results are summarised in the table below.

Table 9.6.2 – 7: Summary of results from KCP 10.3.2.1/03, captured during the literature review

Species	Treatment	Corrected mortality adults	No. of eggs oviposited per 10 females (mean +- SE)	Corrected mortality eggs
A. eharai	control	0	27 ± 2	0
A. eharai	inpyrfluxam	6.7	23.3 ± 0.7	8.3
A. tsugawai	control	0	28.3 ± 1.9	0
A. tsugawai	inpyrfluxam	0	20.7 ± 3	2.8
E. sojaensis	control	0	26 ± 1.2	5.6
E. sojaensis	inpyrfluxam	3.3	20.6 ± 3	20.6
T. vulgaris	control	0	27 ± 1	0
T. vulgaris	inpyrfluxam	6.9	25 ± 2.6	0

There appears to be evidence for adverse reproductive and developmental effects. For *A. tsugawai* and *E. sojaensis*, a 26.9 % and 20.7 % reduction in egg laying was observed, respectively. Also, a 20.6 % corrected mortality for directly exposed eggs was observed for *E. sojaensis*. For the same species, negligible corrected adult mortality (max 3.3 %) was observed.

The four species tested above are commonly found in fruit orchards in Japan. Of the two species tested in the lower tier NTA risk assessment, *T. pyri* is the clear analogue, as it is from the same genus as *T. vulgaris*. Copied below are the results from the submitted *T. pyri* glass plate study:

Table 9.6.2 – 8: Percent mortality and effects on reproduction in *Typhlodromus pyri* after exposure to S-2399 60 g/L EC

Application rate [g a.s./ha]	Mortality		Escapees	Reproduction	
	[%] ± SD	[%] Corrected	[%]	No. eggs/female	Effect on reproduction [%]
Control	10.0 ± 5.0	n.a.	0.0 ± 0.0	8.6 ± 1.2	n.a.
3.88	6.7 ± 2.9	-3.7	1.7 ± 2.9	9.8 ± 1.5	-14.5
9.71	3.3 ± 5.8	-7.4	0.0 ± 0.0	7.6 ± 1.3	11.6
24.3	13.3 ± 5.8	3.7	0.0 ± 0.0	7.2 ± 0.4	16.2
60.7	36.7 ± 5.8 [a]	29.6	20.0 ± 0.0	7.3 ± 3.4	14.1
152	86.7 ± 2.9 [a]	85.2	36.7 ± 7.6	n.a.	n.a.
Reference Item (8.0 mL Perfekthion/ha)	100.0 ± 0.0 [b]	100.0	65.0 ± 13.2	n.a.	n.a.

SD: Standard Deviation; n.a. not applicable/available
Negative values indicate increased survival compared to controls
[a] Statistically significantly different from control (Bonferroni-Holm Fisher Exact Test; α = 0.05)
[b] Statistically significantly different from control (Fisher Exact Test; α = 0.05)

It should be noted that the different observation windows, exposure routes, and developmental stage of individuals at exposure make a direct comparison of the results challenging.

For mortality, the closest observation window comparison available is 2-day ([redacted] et al. (2020)) vs 3-day (KCP 10.3.2.1/02). However, the developmental stage was different at exposure start (adult ([redacted] et al. (2020)) vs protonymph (KCP 10.3.2.1/02)), as well as the exposure route itself. In [redacted] et al. (2020), adult females were directly oversprayed compared to the exposure to dried residues in KCP 10.3.2.1/02. How 2-day mortality for adults exposed via direct overspray compares to 3-day mortality for protonymphs exposed to dried residues is unclear. To answer this question, a comparison of adult to protonymph pesticide sensitivity would be required. Because of the uncertainties surrounding the mortality comparison, a qualitative approach is taken.

For the comparison of reproductive endpoints, similar uncertainties exist due the methodological differences. However, it is possible that the reproductive effects associated with direct overspray of an egg-laying adult are higher than protonymph exposure followed

by development before egg-laying. Again, due to the associated uncertainties a qualitative comparison is performed.

For mortality, adults from all four species tested exhibited low mortality two days after direct overspray (maximum 6.9 % at 37 g a.s./ha). Three days after dried residue exposure to *T. pyri* protonymphs, an 8.3 % mortality was observed at 24.3 g a.s./ha. This comparison suggests that the lethal effects of inpyfluxam for the four mite species tested in [redacted] et al. (2020) are broadly comparable to those for *T. pyri*. The mortality-based lower tier risk assessment in ESCORT II was validated using field and semi-field data. The HQ = 2 trigger was defined by comparing lower-tier HQ values with a 40 % semi-field and field effects threshold. Semi-field and field effects included sublethal and reproductive endpoints or direct population monitoring. Thus, based on the comparable mortality sensitivity of all tested mite species and *T. pyri*, both the mortality and reproductive effects reported in [redacted] et al. (2020) are likely covered by the *T. pyri* risk assessment. This includes the 20.7 and 26.9 % reductions in egg-laying at 37 g a.s./ha for *E. sojaensis* and *A. tsugawai*, respectively, and the 20.6 % correctly mortality observed for eggs directly exposed to 37 g a.s./ha.

In summary, two species of mite displayed elevated reproductive sensitivity to inpyrfluxam compared to the standard test species *T. pyri*. However, they displayed comparable mortality sensitivity, therefore, the first tier *T. pyri* risk assessment is considered protective of the effects observed for *A. tsugawai* and *E. sojaensis*. No further consideration is required.

Conclusion

The in-field and off-field risk for other non-target arthropods from the intended uses of the product S-2399 60 g/l EC in cereals is acceptable as the HQ does not exceed the trigger value for *T. pyri* or *A. rhopalosiphi* for in-field or off-field assessments at tier 1. The lower tier risk assessment for *T. pyri* is considered protective of effects observed in four further mite species, captured during the literature review. There is no need to consider extended laboratory testing, semi-field, field or alternate route of exposure studies as an acceptable risk was shown in the laboratory studies.

B.9.7 Effects on Non-Target soil meso- and macrofauna

B.9.7.1 Earthworms

Reference:	KCP 10.4.1.1/01
Report Title:	S-2399 60 g/L EC: Effects on Reproduction and Growth of Earthworms *Eisenia andrei* in Artificial Soil
Author(s) & year:	[redacted] (2019a)
Document No, Authority registration No:	Study No. 141471022, TPW-0108
Substance used:	S-2399 6 EC, V16-7L1901, 6.544 ± 0.024% w/w
Method of analysis:	n/a
Guideline(s):	OECD 222 (2016)
Deviations:	Yes, see HSE Comments
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test item:** S-2399 60 g/L EC /Emulsifiable Concentrate)
   Description: Slightly yellow liquid
   Lot/Batch: V16-7L1901
   Active substance content: Nominal: 60 g S-2399/L
   Analysed: 60.68 ± 0.23 g S-2399/L or 6.544 ± 0.024 %
   (w/w). Verified by certificate of analysis.
   Density: 0.9273 g/mL
   Solvent: None (Test uses 10 g of finely ground industrial quartz sand with a quantity of the test chemical necessary to achieve the test concentration)
   Expiry date:
2. **Control:** Untreated, moistened with deionised water
3. **Reference item:** Carbendazim (600 g/L)
   Reference test performed at least once a year in a separate test. Last test performed June-Sept. 2018 (Project no. 105684022)

B. STUDY DESIGN AND METHODS

1. **Test species:** Earthworm *Eisenia Andrei* (Annelida; Oligochaeta)
   Age/life stage: Adults (individuals not differing by more than

	4 weeks), approximately 5 months, with clitellum
Body weight:	312 – 598 mg
Source:	In-house culture. Bred under standardised conditions in ibacon laboratories in a breeding medium of cattle manure, peat, sand, calcium carbonate and straw, fed with cattle manure, stored at room temperature.
Acclimation:	1 day, in artificial soil, under test conditions
Diet:	Finely ground cattle manure. 5 g/container was scattered on the soil surface at day 1 after treatment application and was moistened with 5g deionised water. 5 g/container (moistened with 2 g deionised water) was added each week for the first 4 weeks of the experiment, when the food of the previous week had almost been consumed. If the food was not quite fully consumed, the added amount of food was adjusted to replace the visually estimated consumption. 4 weeks after application, the food was mixed into the substrate, following the removal of adults.

2. Test units:

Plastic boxes (18.3 cm × 13.6 cm × 6 cm, tapered towards the bottom, with a soil surface of approx. 16.5 cm × 11.5 cm = 189.75 cm²) with perforated transparent lids to enable exchange of air, to minimise evaporation from the artificial soil, and to prevent the earthworms from escaping. Each container was filled with 624.9 g of the prepared soil (500 g dry weight plus deionised water). The height of the soil layer in the containers was approximately 5 cm.

Test concentrations:
4.08, 7.35, 13.23, 23.81, 42.86, 77.16, 138.89 and 250 mg product/kg soil dry weight

No. earthworms per vessel: 10 (for test concentrations and control
No. of replicates: 4 (for test concentrations), 8 (for control)

Test substrate: 10% Sphagnum-peat, air-dried and finely ground (2 mm with no visible plant remains); 20% Kaolin clay (Kaolinite content >30%), 69.6% fine quartz-sand (F34) containing more than 50% by mass of particle size 0.05 mm to 0.2 mm; 0.4% Calcium carbonate (CaCO3) was added to adjust pH to 6.0 ± 0.5.The artificial soil was moistened to approximately half of the final water content 1 day before the application. The additional water required to achieve the final water content was added when applying the test item.

Maximum Water Holding Capacity: 48% of the dry weight of artificial soil.

3. Environmental conditions

A summary of the environmental conditions is shown in Table 9.7-1 below.

Table 9.7-1: Environmental conditions obtained in study of *E. andrei* exposed to S-2399 60EC

Variable	Required OECD 222 (2004)	Obtained
Temperature	20 ± 2 °C	18 – 22ºC
pH	Approx. 5.5 to 6.0	Test start: 5.5 – 5.7 Test end: 5.8 – 6.1
Soil water content	40 to 60 % of the maximum water holding capacity	Test start: 25.9 – 26.9% (54.0 – 56.1% of WHC_{max}) Test end: 26.8 – 28.9% (55.9 – 60.3% of WHC_{max})
Photoperiod	16 hours light and 8 hours dark	16 hours light and 8 hours dark
Light intensity	400 to 800 lux	400 to 800 lux

Study dates:
Experimental Starting Date: June 18, 2019
Experimental Completion Date: August 14, 2019

4. Test design and treatment:

The test was performed with eight nominal concentrations of 4.08, 7.35, 13.23, 23.81, 42.86, 77.16, 138.89 and 250 mg product/kg soil dry weight (corresponding to 0.27, 0.48, 0.87, 1.56, 2.80, 5.05, 9.09 and 16.36 mg a.s./kg soil dry weight, calculated based on an active substance content). An untreated control was tested in parallel. Carbendazim (600 g/L SC) was used as a toxic reference item, tested in a separate study (test facility study no. 105684022) at five nominal concentrations of 1.39, 2.00, 2.88, 4.16 and 6.00 mg test item/kg soil dry weight (corresponding to 0.695, 1.00, 1.44, 2.08 and 3.00 mg carbendazim/kg soil dry weight, respectively). Four replicates were used per test item treatment and eight were used for the control. Each replicate contained 10 earthworms.

All earthworms were washed with tap water, dried with dry paper towels and weighed individually and randomly assigned to groups of 10 earthworms. The different batches were sorted into four classes on the basis of the total weight and one batch of each weight class was assigned to each treatment group (two batches for the control), to ensure weights were homogenous. Earthworms were placed on the soil surface after treatment application. Adult earthworms were exposed for 4 weeks before removal. Juveniles were exposed for an additional 4 weeks.

5. Dose preparation:

A stock solution was prepared by weighing 1200.4 mg of S-2399 60 g/L EC and adding deionised water to a final net weight of 600 g (suspension with 2.0007 mg test item/g, corresponding to the highest test item treatment solution). A magnetic stirrer was used to

obtain a homogeneous dispersion. Test item concentrations of nominal 250, 138.89, 77.16, 42.86 and 23.81 mg product/kg soil dry weight (corresponding to 16.36, 9.09, 5.05, 2.80 and 1.56 mg a.s./kg soil dry weight, respectively) were obtained by adding 262.5, 145.8, 81.0, 45.0 and 25.0 g stock solution to 2100 g dry artificial soil, respectively.

Furthermore, for the three lowest test concentrations a dilution series were prepared by adding 30 g stock solution to 536.9 g water (dilution 1), 250 g of dilution 1 to 200 g water (dilution 2) and 150 g of dilution 2 to 120.2 g water. 262.5 (dilutions 1 and 2) or 262.6 g (dilution 3) were added to 2100 g dry artificial soil to obtain nominal treatment concentrations of 13.23, 7.35 and 4.08 mg product/kg soil dw (corresponding to 0.87, 0.48 and 0.27 mg a.s./kg soil dw, respectively).

There were no significant deviations to the nominal target concentrations. The control was left untreated.

6. Measurements and observations:

Total mortality (including any missing worms) and total number of adults affected (e.g. lack of movement, rigidity) were determined on day 28 after application. Mean body weights were determined at the start of the test and on day 28 after application.

Number of juveniles was assessed 56 days after application. Juveniles were removed by placing the test units in a water bath at 50 – 60ºC. All emerging earthworms were counted. The soil from each container was also emptied into a tray and checked visually for remaining juveniles.

7. Statistical analysis:

Mortality data were analysed for significance by using the Fisher's Exact Binomial Test (multiple comparison, with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). An LC_{50} value at day 28 was calculated by applying Probit-Analysis.

The body weight change data were tested for normal distribution and homogeneity of variance ($\alpha = 0.05$) using the Kolmogorov-Smirnov and the Levene´s test, respectively. As the data for body weight changes and reproduction were normally distributed and heterogeneous the Welsh t-test after Bonferroni-Holm was used to compare treatment and control values (multiple comparison, $\alpha = 0.05$, two-sided).

The reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.05$) using the Shapiro-Wilk´s test and the Cochran's test, respectively. Since the reproduction data were normally distributed and homogeneous and did follow a monotonicity trend (contrast trend) the Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller) was used to compare treatment and control values. The EC values and their 95% confidence limits for reproduction were calculated by applying Probit-Analysis.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

No mortality was observed in the control or any treatment group up to and including the test concentration of 138.89 mg test item/kg soil dry weight. At the concentration of 250 mg product/kg soil dry weight a mortality of 72.5% was observed, which was statistically significantly different compared to the control.

The body weight changes in the test item treated groups were not statistically significantly different compared to the control up to and including the highest test concentration of 250 mg product/kg soil dry weight.

The reproduction rates were not statistically significantly different compared to the control up to and including the test concentration of 42.86 mg product/kg soil dry weight. At the test concentration of 77.16 mg product/kg soil dry weight and above, reproduction was statistically significantly reduced compared to the control.

The results of the reference item test with carbendazim (test facility study no. 105684022) showed significant effects at ≥ 0.695 mg/kg soil dw; i.e. an $NOEC_{reproduction}$ of 0.695 mg/kg soil dw. The EC_{10} was 0.62 mg/kg soil dw and the EC_{50} for reproduction was calculated as 0.94 mg a.s./kg soil dw.

A summary of results is presented in Table 9.7-2.

Table 9.7-2: Effects of S-2399 60 g/L EC on earthworms (*Eisenia andrei*) in a 56-day reproduction study

Nominal soil concentration [mg/kg soil dw]	**Control**	**4.08**	**7.35**	**13.23**	**23.81**	**42.86**	**77.16**	**138.89**	**250**
[mg a.s./kg soil dw]		**0.27**	**0.48**	**0.87**	**1.56**	**2.80**	**5.05**	**9.09**	**16.36**
Mortality day 28 [%] (mean ± SD)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	72.5 ± 23.6
Statistical significance [a]	n.a.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
Body weight change day 28 [%] (mean ± SD)	8.4 ± 4.7	10.3 ± 5.0	16.8 ± 7.3	13.3 ± 5.3	11.5 ± 4.2	15.1 ± 2.4	21.1 ± 6.2	18.2 ± 4.2	24.5 ± 21.3
Statistical significance [b]	n.a.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
No. juveniles on day 56 (mean ± SD)	97 ± 22	92 ± 20	94 ± 9	83 ± 10	93 ± 21	83 ± 8	68 ± 3	72 ± 6	2 ± 2
Statistical significance [c]	n.a.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	*

Nominal soil concentration [mg/kg soil dw]	Control	4.08	7.35	13.23	23.81	42.86	77.16	138.89	250
[mg a.s./kg soil dw]		0.27	0.48	0.87	1.56	2.80	5.05	9.09	16.36
Reproduction in [%] of control, on day 56	n.a.	94.5	96.8	85.5	95.8	85.7	69.5	73.9	1.8
Food consumption [g added food] (mean ± SD)	23.6 ± 0.7	24.0 ± 0.0	24.0 ± 0.0	24.3 ± 0.5	24.0 ± 0.0	24.3 ± 0.5	24.3 ± 0.5	24.3 ± 0.5	13.5 ± 1.3
	Endpoints (95% confidence limits)								
	[mg product/kg soil dw]					**[mg a.s./kg soil dw]**			
NOEC (day 28 mortality)	138.89					9.09			
LOEC (day 28 mortality)	250					16.36			
LC_{50} (day 28 mortality) [d]	233.1					-			
NOEC (day 28 weight changes)	≥ 250					≥ 16.36			
LOEC (day 28 weight changes)	> 250					> 16.36			
NOEC (day 56 reproduction)	42.86					2.80			
LOEC (day 56 reproduction)	77.16					5.05			
EC_{10} (day 56 reproduction) [d]	58.5 (n.d.)					3.83			
EC_{20} (day 56 reproduction) [d]	79.0 (n.d.)					5.17			
EC_{50} (day 56 reproduction) [d]	140.7 (n.d.)					9.21			

SD: Standard Deviation
n.a. not applicable; n.d. not determined due to mathematical reasons
n.s. = not significantly different compared to the control
* Statistically significantly different compared to the control
[a] Fisher´s Exact Test, α = 0.05, one-sided greater
[b] Welsh t-test, α = 0.05, two-sided
[c] Williams t-test, α = 0.05, one-sided smaller
[d] Probit analysis

The concentration-response curve is shown in Figure 9.7.1-1 below:

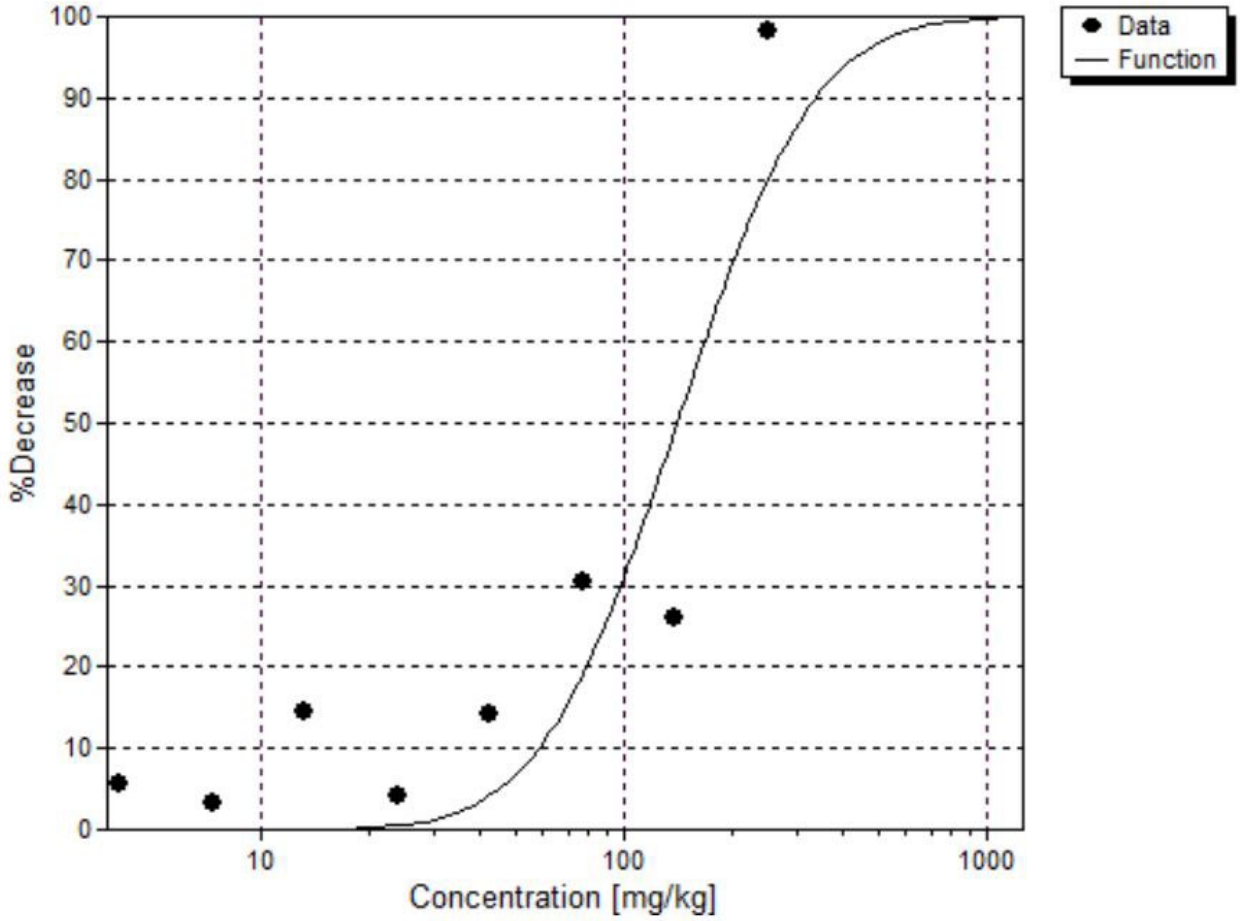

Figure 9.7-1: Concentration-response curve for 56d reproduction of *Eisenia andrei* exposed to S-2399 60EC

B. VALIDITY CRITERIA

The validity criteria were fulfilled according to the most recent version of the EU test guideline (OECD 222, 2016), as detailed below:

- Control mortality should not exceed 10% over the initial 4-week test period. Actual control mortality was 0%.
- Reproduction in the control should be ≥30 earthworms per replicate. Actual number of juvenile earthworms in the control was 69 to 137.
- Coefficient of variation of reproduction in the control should not exceed 30%. The actual coefficient of variation of control reproduction was 22.7%.

III. CONCLUSION

The overall 56-day NOEC for reproduction of earthworms (*Eisenia andrei*) exposed to S-2399 60 g/L was 42.86 mg product/kg soil dry weight and the EC_{10} for reproduction (at day 56) was determined to be 58.5 mg product/kg soil dry weight. The 56-day $EC_{20,\ 50}$ for

reproduction were determined at 79.0 and 140.7 mg product/kg soil dry weight, respectively.

HSE COMMENTS:

This study was conducted to GLP under OECD 222 (2016) guidelines and has been assessed against these same guidelines.

There are some slight deviations to OECD 222 (2016) guidelines notes in relation to the dose preparation of concentrations. Sand quartz was used to make up the required concentrations in this study. OECD 222 (2016) guidelines state that 10g quartz should be added to the test item, but this study used 20g quartz. There is no data available regarding the mean measured concentration, so it is unclear if this had any impact on test concentrations. OECD 222 (2016) guidelines recommend chemical analysis of the test substance at the start and the end of the test as the test where there is uncertainty in maintaining the nominal concentration. This is not referenced in the study report, so it cannot be determined whether the test concentrations were maintained over the duration of the study. However, as all validity criteria were met, the study is considered valid.

The reference test results showed significant effects at ≥ 0.695 mg/kg soil dw. This result is lower than the OECD 222 (2016) guideline recommendation of significant effects between 1 and 5 mg/kg soil dw, but still indicates appropriate levels of species sensitivity.

It is also noted that the worms were washed with tap water prior to being weighed at the start of the test. OECD 222 (2016) guidelines state that worms should be washed with deionised water at this stage. The validity criteria for the study have been met, so this does not invalidate the study.

The data does not display a clear concentration-response. HSE have amended the endpoints from the values provided by the applicant to be in line with the biological data. No 95% confidence limits have been provided by the applicant. They stated that this was due to ‘mathematical reasons’, but no further explanation has been provided. This is contradictory to the statement provided in the full study report that states ‘The EC values and their 95% confidence limits for reproduction were calculated by applying Probit-Analysis’ under statistical analysis. It is also noted a statistics report was provided for NOEC calculations, but not for EC_{10}.

HSE has taken a conservative approach and amended the NOEC to 23.81mg product/kg soil dry weight (1.56 mg a.s/kg soil dw). Due to the poor fit of the model to the data for the EC_{10} estimation, this endpoint will not be used in risk assessment.

The endpoints to use in risk assessment are:

- **56-day NOEC = 23.81mg product/kg soil dry weight (1.56 mg a.s/kg soil dw)**

B.9.7.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

Reference:	KCP 10.4.2.1/01
Report Title:	S-2399 60G/L EC: Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil
Author(s) & year:	[redacted] (2019b)
Document No, Authority registration No:	Study No. 141471089, TPW-0109
Substance used:	S-2399 6 EC, V16-7L1901, 6.544 ± 0.024% w/w
Method of analysis:	n/a
Guideline(s):	OECD 226 (2016)
Deviations:	Yes, see HSE Comments
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

1. Test item: S-2399 60 G/L EC (Emulsifiable Concentrate)

Description: Slightly yellow liquid

Lot/Batch: V16-7L1901

Active substance content: Nominal: 60 g S-2399/L
Analysed: 60.68 ± 0.23 g S-2399/L or 6.544± 0.024 % (w/w).
Confirmed via certificate of analysis.

Density: 0.9273 g/mL

2. Control: Untreated control soil moistened with deionised water

3. Reference item: BAS 152 11 I (dimethoate, 385 g/kg, 38.5% w/w, nominal) tested in a separate study

Active substance content: Nominal: 400.0 g/L Dimethoate
Analysed: 429.0 g/L Dimethoate
Reference test performed at least once a year in a separate test. Last test performed Nov./Dec. 2018 (Project no. 136532089)

B. STUDY DESIGN AND METHODS

1. Test species: Predatory mite *Hypoaspis aculeifer* (Canestrini 1883)

Age: Adult females (from a synchronized cohort) approximately 11 days after reaching the

adult stage

Source: In-house culture

Diet: 1 spatula cheese mites (*Tyrophagus putrescentiae*) at test start and on days 3, 5, 7, 10 and 12.

2. Test units: Glass containers (volume: 100 mL; Æ 5 cm), tight screw top closure to avoid water evaporation, filled with approximately 20 g ± 1.0 g artificial soil dry weight. The height of the soil layer in the containers was 1.5 to 2 cm.

Artificial soil: According to OECD 226: 5% sphagnum-peat (air-dried, finely ground to < 2 mm), 20% kaolin clay (Kaolinite content > 30%), 74.8% fine quartz sand and 0.2% calcium carbonate

Concentrations tested: 8.17, 14.7, 26.5, 47.6, 85.7, 154, 278 and 500 mg product/kg soil dry weight (dw)

No. of mites per replicate: 10 (for test concentration and control groups)

No. of replicates: 4 (for test concentration groups), 8 (for control groups)

Duration: 14 days

WHC_{max}: 53% of the dry weight

3. Environmental conditions

A summary of the environmental conditions are shown in Table 9.7-3 below:

Table 9.7-3: Environmental conditions obtained in the study of *H. aculeifer* exposed to S-2399 60 EC

Variable	Required OECD 226 (2016)	Obtained
Temperature	20 ± 2 °C	18 – 22ºC
pH	6.0 ± 0.5	Test start: 5.8 – 5.9 Test end: 5.7 – 5.8
Soil water content	Maintained throughout the test	Test start: 27.9 – 28.8% (52.7% - 54.3% of maximum water holding capacity) Test end: 26.1 – 27.4% (49.3% - 51.7% of maximum water holding capacity)
Photoperiod	16 hours light and 8 hours dark	16 h light : 8 h dark
Light intensity	400 – 800 lux	400 – 800 lux
Ventilation	aerated twice a week	Days 3, 5, 7, 10 and 12

Study dates:
Experimental Starting Date: August 09, 2019
Experimental Completion Date: August 26, 2019

4. Test organism and treatment:

Based on the results of a range finding test, eight nominal concentrations 8.17, 14.7, 26.5, 47.6, 85.7, 154, 278 and 500 mg product/kg soil dry weight (dw) were tested. An untreated control was tested in parallel. BAS 152 11 I (nominal content: 400.0 g dimethoate) was used as a toxic reference item, tested in a separate study (test facility study no. 136532089) at five nominal concentrations of 1.54, 2.23, 3.23, 4.69 and 6.80 mg a.s./kg soil dwt.

There were eight replicates for the control and four replicates per test item treatment group, each containing 10 adult female predatory mites. One additional container was set up per treatment to check the pH and water content of the test substrate after 14 days

Mites (collected with a fine brush) were placed onto the surface of the treated artificial soil within two hours after preparation of the final test substrate. The total duration of the test was 14 days.

For extraction of mites, the soil was filled into Millipore pots with attached plastic containers for collecting the escaping mites. These extraction units were placed in a heat extractor. The soil including the mites was exposed to a temperature of approximately 25°C to 30°C for approximately 3 days. Escaping mites were collected in a fixing liquid (containing glycol and a detergent), cooled at a temperature of approximately 16°C.

5. Dose preparation:

A stock solution (corresponding to the highest dosing solution) was prepared by weighing 350.2 mg S-2399 60 G/L EC and adding deionized water to obtain a final wet weight of 96.4 g resulting in a suspension of 3.628 mg test item/g. A dilution series was prepared by adding 39.9, 40.3, 39.9, 40.0, 41.3, 40.1 and 40.0 g water to 50 g stock solution or subsequent dilution (exception: 51.9 g of dilution 4) of the stock solution or the consecutive dilution to generate the other dosing concentrations of 2.0205, 1.1187, 0.6222, 0.3457, 0.1925, 0.1068 and 0.0593 mg/g, respectively. 30.3 g of the dosing solutions were added to artificial soil equivalent to 220 g dry weight to prepare target concentrations of 500, 278, 154, 85.7, 47.6, 26.5, 14.7 and 8.17 mg/kg soil dry weight (achieved: 500.3, 278.3, 154.1, 85.70, 47.61, 26.51, 14.71 and 8.174 mg/kg soil dw). The artificial soil was moistened to approximately half of the final water content 2 days before the application. The additional water required to achieve the final water content was added when applying the test item.

6. Measurements and observations:

Number of surviving adult female predatory mites was recorded 14 days after test initiation (counted after extraction). Missing adult predatory mites were recorded as dead. The surviving predatory mites were observed for differences in morphology or any abnormalities at experimental end.

Reproduction was assessed as the number of juvenile mites counted after extraction on day 14 after application.

pH and water content were checked at test start and test end according to ISO 10390 and ISO 11465. Besides, water content was checked on day 7 after application by reweighing the additional test containers. Loss of water was not compensated as it did not deviate by more than 2% from initial water content.

Mite extraction efficiency was checked separately in July 2019 resulting in an efficiency of 95.3%.

7. Statistical analysis:

Mortality data based on mean numbers of dead adult female mites for each treatment were statistically analysed using Fisher's Exact Binomial Test (multiple comparison, with Bonferroni Correction, α = 0.05, one-sided greater).

Reproduction data (mean number of juvenile mites for each treatment) were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene´s test (α = 0.05). Since the reproduction data were normally distributed and homogeneous and followed a monotonicity trend (contrast trend) the Williams t-test (multiple comparison, α = 0.05, one-sided smaller) was used to compare treatment and control values.

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The EC values were calculated by Probit Analysis.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

Mortality in the test item treated group ranged from 3% to 13% up to and including the test concentration of 154 mg product/kg soil dw. The values were not statistically significantly different compared to the control, where 1% of the adult mites died. At the test concentrations of 278 mg product/kg soil dw and 500 mg product/ kg soil dw, the observed mortality was 20% and 100%, respectively, which was statistically significantly increased compared to the control.

No differences in morphology of the mites between the test item treated groups and the control were observed.

The toxic reference item showed statistically significant treatment related effects on reproduction at a concentration of 3.23 mg dimethoate/kg soil and above. The EC_{50} for reproduction was 3.31 mg dimethoate/kg soil dw.

Effects of S-2399 60 G/L EC on mortality and reproduction and endpoints are summarised in Table 9.7-4.

Table 9.7-4: Summary of effects of S-2399 60 G/L EC on the predatory mite *Hypoaspis aculeifer*

Nominal soil concentration [mg product/kg soil dw]	Control	8.17	14.7	26.5	47.6	85.7	154	278	500
[mg a.s./kg soil dw]		0.53	0.96	1.73	3.11	5.61	10.1	18.2	32.7
Mortality ± SD [%]	1 ± 4	8 ± 5	13 ± 19	5 ± 10	10 ± 0	3 ± 5	5 ± 10	20 ± 18	100 ± 0
Statistical significance [a]	n.a.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*
Mean no. of juveniles ± SD	174 ± 37	161 ± 16	193 ± 46	172 ± 36	162 ± 29	162 ± 45	175 ± 26	124 ± 8	6 ± 5
Reproduction in [%] of control	n.a.	93	111	99	94	93	101	71	3
Statistical significance [b]	n.a.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*
Endpoints [mg product/kg soil dw] (95% confidence limits)									
NOEC (mortality)	154								
LOEC (mortality)	278								
LC_{50} (mortality)	420								
NOEC (reproduction)	154								
LOEC (reproduction)	278								
EC_{10}(reproduction)	233.0 (183.6 – 254.6)								
EC_{20}(reproduction)	259.7 (226.4 – 277.7)								
EC_{50}(reproduction)	319.5 (298.3 – 371.3)								

n.a. not applicable;
SD: Standard Deviation
n.s. = not significantly different compared to the control
* = significantly different compared to the control
[a] Fisher's Exact Test, α = 0.05, one-sided greater
[b] Williams t-test, α = 0.05, one-sided smaller

The dose-response curve for reproduction is shown in Figure 9.7.2-1 below.

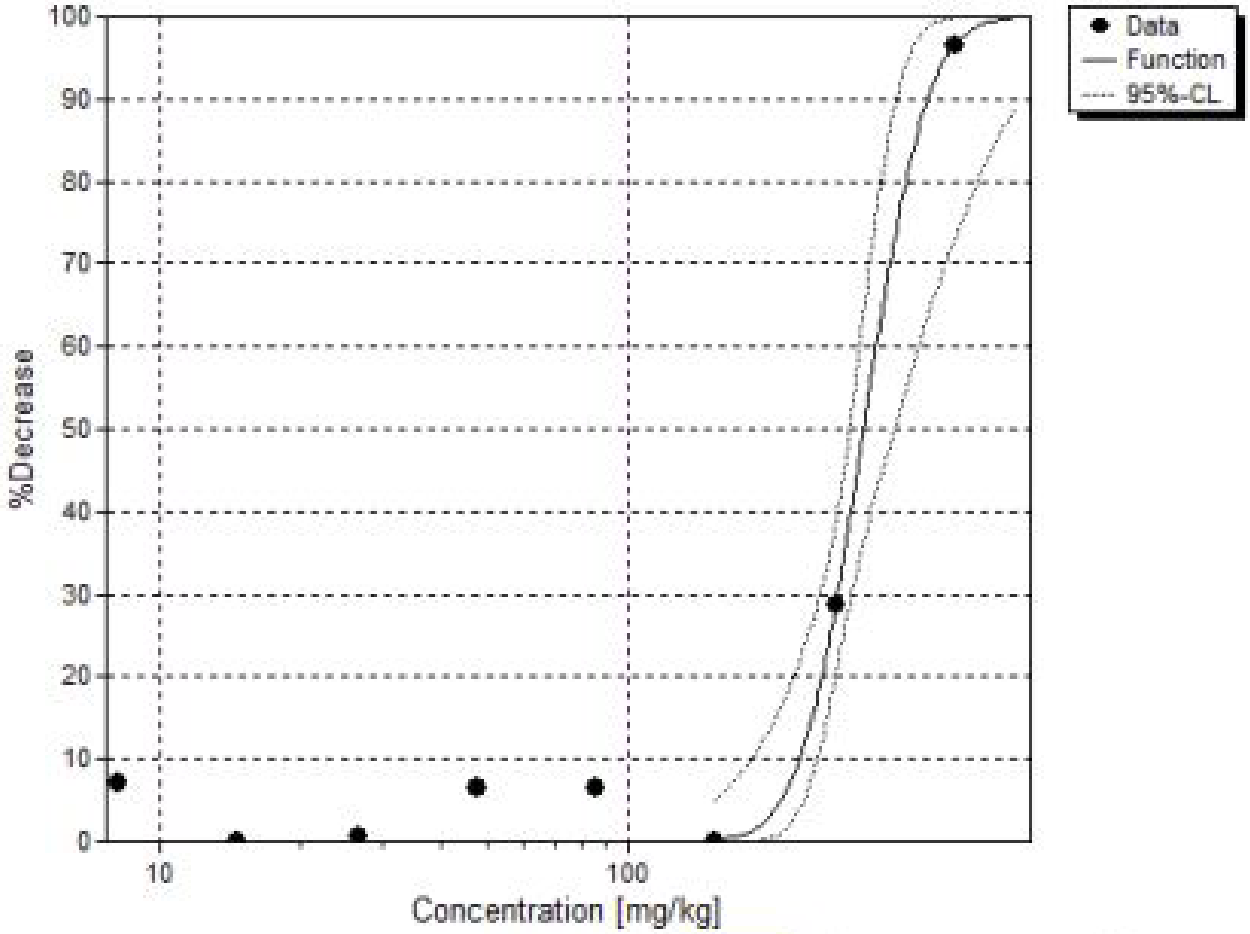

Figure 9.7-2 Dose-response curve of reproduction of *Hypoaspis aculeifer* exposed to S-2399 60 EC for 14 days

B. VALIDITY CRITERIA

The study fulfils the validity criteria outlined in the most recent version of the EU test guideline (OECD 226, 2016), as detailed below:

- Mean adult control mortality should not exceed 20% at the end of the test. Actual mean control mortality was 1%.
- Mean number of juveniles per control replicate (with 10 adult females introduced) should be ≥ 50 at the end of the test. The mean number of juvenile mites per replicate was 174± 37 in control replicates.
- The coefficient of variation for control reproduction should be ≤ 30% at the end of the test. The actual coefficient of variation of the control reproduction was 21.3.

III. CONCLUSION

For *Hypoaspis aculeifer* exposed to S-2399 60 G/L EC, the 14-day NOEC for mortality and reproduction was determined to be 154 mg product/kg soil dw. The 14-day LC_{50} was

determined to be 420 mg product/kg soil dw. The 14-day $EC_{10, 20, 50}$ values were determined to be 233.0, 259.7 and 319.5 mg product/kg soil dw, respectively.

HSE COMMENTS:
This study was conducted to GLP under OECD 226 (2016) guidelines. It has been assessed against these same guidelines.

It is noted that there is some overlap with the 95% confidence limits of the EC_{10} and EC_{20} values, which may affect the confidence of the results, but as the use of statistics were in line with OECD 226 (2016) guidelines and all validity criteria were met, the study is considered valid. The use of statistics is in line with OECD 226 (2016) guidelines. It is noted that the data for reproduction does not fit very well onto the dose-response curve, but a dose-response effect can be seen over the two highest concentrations.

An LC_{50} value of 420mg product/kg soil dw is presented under the biological effects. This value does fit the dose-response curve, but no 95% confidence limits have been provided. This does not impact the risk assessment.

The reference item test (dose response) is performed at least once a year at the test facility as a means of ensuring that the laboratory test conditions are adequate and to verify that the response of the test organisms does not change significantly over time. The GLP conducted experiment was performed in November/December 2018. According to the test guideline, the EC_{50} for dimethoate based on the number of juveniles should fall in the range between 3.0 and 7.0 mg/kg soil dw. The EC_{50} for reproduction was 3.31 mg dimethoate/kg soil. This demonstrates that the test system is sufficiently sensitive.

The agreed endpoints for use in risk assessment are:
NOEC = 154 mg product/kg soil dw
EC_{10} = 233.0 mg product/kg soil dw

Reference:	KCP 10.4.2.1/02
Report Title:	S-2399 60G/L EC: Effects on Reproduction of the Collembola *Folsomia candida* in Artificial Soil
Author(s) & year:	[redacted] (2019c)
Document No, Authority registration No:	Study No. 141471016, TPW-0110
Substance used:	S-2399 6 EC, V16-7L1901, 6.544 ± 0.024% w/w
Method of analysis:	n/a
Guideline(s):	OECD 232 (2016)
Deviations:	Yes, see HSE Comments
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

I. MATERIALS AND METHODS

A. MATERIALS

Test item:	S-2399 60 G/L EC (Emulsifiable Concentrate)
Description:	Slightly yellow liquid
Lot/Batch:	V16-7L1901
Active substance content:	Nominal: 60 g S-2399/L Analysed: 60.68 ± 0.23 g S-2399/L or 6.544 ± 0.024 % (w/w). Verified by certificate of analysis.
Density:	0.9273 g/mL
Control:	Untreated control soil moistened with deionised water
Reference item:	Boric acid (100.1%) Reference test performed at least once a year in a separate test. Last test performed Sept./Oct. 2018

B. STUDY DESIGN AND METHODS

1. Test species:	Collembola *Folsomia candida* (Willem 1902)
Age:	Juveniles, 10 -12 days old at test start
Source:	In-house culture
Diet:	2 mg granulated dried yeast provided at day 0 and after 14 days by spreading over the soil surface
Duration:	28 days
2. Test units:	Glass containers (volume: 100 mL; diameter: 5 cm), closed tightly to avoid water evaporation, filled with 30 g ± 1.0 g artificial soil dry weight. The height of the soil layer in the containers was 2 to 2.5 cm.
3. Test substrate:	Artificial soil, according to OECD 232: 5% sphagnum-peat (air-dried, finely ground to < 2 mm), 20% kaolin clay (Kaolinite content > 30%), 74.8% fine quartz sand and 0.2% calcium carbonate
WHC_{max}:	53% of the dry weight

4. Environmental conditions

A summary of the environmental conditions is shown in Table 9.7-5 below:

Table 9.7-5: Environmental conditions obtained in the study of *F. candida* exposed to S-2399 60EC

Variable	Required OECD 232 (2016)	Obtained
Temperature	20 ± 2˚C	18 – 22ºC
pH	6 ± 0.5	Test start: 5.8 – 5.9 Test end: 5.8 – 5.9
Water content	Maintained throughout test	Test start: 26.2 – 27.6% (49.9%-52.1% of maximum water holding capacity) Test end: 24.3 – 26.1% (45.8% - 49.3% of maximum water holding capacity)
Photoperiod	16h light: 8h dark	16 h light : 8 h dark
Light intensity	400 – 800 lux	400 – 800 lux
Ventilation	Twice a week	Days 2, 4, 7, 9, 11, 14, 16, 18, 21, 23 and 25

Study dates:
Experimental Starting Date: August 05, 2019
Experimental Completion Date: September 03, 2019

5. Test organism and treatment:

Based on the results of a range finding test, eight nominal concentrations 0.82, 1.47, 2.65, 4.76, 8.57, 15.4, 27.8 and 50.0 mg product/kg soil dry weight (dw) were tested. An untreated control was tested in parallel. Boric acid (100.1%) was used as a toxic reference item, tested in a separate study (test facility study no. 136521016) at five nominal concentrations of 30.5, 48.8, 78.1, 125 and 200 mg/kg soil dw.

There were eight replicates for the control and four replicates per test item treatment group, each containing 10 individuals per unit/replicate. One additional container was set up per treatment to check the pH and water content of the test substrate after 28 days.

Springtails (collected with an aspirator) were placed into a small glass tube, counted to ensure that 10 individuals are introduced and placed on the surface of the treated artificial soil. The total duration of the test was 14 days.

After the exposure (i.e. at day 28), the contents of the test containers were suspended in water and suspensions were tinted with dark ink and stirred with a fine brush. Adult animals were counted twice under binocular microscopes.

6. Dose preparation:

A stock solution (corresponding to the highest dosing solution) was prepared by weighing 30.0 mg S-2399 60 G/L EC and adding deionized water to obtain a final wet weight of 82.8 g resulting in a suspension of 0.3623 mg test item/g. A dilution series was prepared by

adding 39.9, 40.3, 39.8, 40.0, 39.8, 40.1 and 39.6 g water to 50 g stock solution or subsequent dilution of the stock solution or the consecutive dilution to generate the other dosing concentrations of 0.2015, 0.1116, 0.0621, 0.0345, 0.0192, 0.0107 and 0.0060 mg/g, respectively. 27.6 g of the dosing solutions were added to artificial soil equivalent to 200 g dry weight to prepare target concentrations of 50.0, 27.8, 15.4, 8.57, 4.76, 2.65, 1.47 and 0.82 mg/kg soil dw (achieved: 50.00, 27.81, 15.40, 8.573, 4.763, 2.652, 1.472 and 0.821 mg/kg soil dw). The artificial soil was moistened to approximately half of the final water content 3 days before the application. The additional water required to achieve the final water content was added when applying the test item.

7. Measurements and observations:

The numbers of surviving adult *F. candida* were recorded on day 28 after application. Missing adults were recorded as dead. Surviving *F. candida* were observed for any abnormal behaviour or conditions at day 28 after application, and the number of juvenile *F. candida* were counted at day 28 after application. Adults were counted once visually, and juveniles were counted twice using binocular microscopes.

Measurements of pH and water content were conducted at test start and test end according to ISO 10390 and ISO 11465. Besides, water content was checked on day 14 after the application by reweighing the additional test containers. Loss of water was not compensated as it did not deviate by more than 2% from initial water content.

Mite extraction efficiency was checked separately in October 2018 resulting in an efficiency of 97.5%.

8. Statistical analysis:

Mortality data were statistically analysed using Fisher's Exact Binomial Test (multiple comparison, with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). An LC_{50} value at day 28 was not determined statistically as no mortality above 50% was observed.

Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Cochran's Test ($\alpha = 0.05$). Since the reproduction data were normally distributed and homogeneous but did not follow monotonicity trend (contrast trend) the Dunnett´s t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller) was used to compare treatment and control values.

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The EC values could not be determined by statistical analysis since there was no adequate concentration response.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

Mortality of *Folsomia candida* was not statistically significantly different compared to the control up to and including the test concentration of 50 mg product/kg soil dw.

No abnormal behaviour was observed with the surviving Collembola.

There were no statistically significant effects on reproduction of *Folsomia candida* up to and including the concentration of 50 mg product/kg soil dw.

The toxic reference item test with boric acid showed statistically significant effects on reproduction at concentrations of ≥ 48.8 mg/kg soil dw. The EC_{50} for reproduction was calculated to be 100.2 mg/kg soil dw

Effects on mortality and reproduction are summarised in Table 9.7-6.

Table 9.7-6: Effect of S-2399 60 G/L EC on *Folsomia candida* in a 28-day reproduction study

Nominal soil concentration [mg product/kg soil dw]	**Control**	**0.82**	**1.47**	**2.65**	**4.76**	**8.57**	**15.4**	**27.8**	**50**
[mg a.s./kg soil dw]		**0.05**	**0.10**	**0.17**	**0.31**	**0.56**	**1.01**	**1.82**	**3.27**
Mortality ±SD [%]	20 ± 13	15 ± 6	20 ± 12	20 ± 22	23 ± 22	10 ± 8	10 ± 8	23 ± 5	8 ± 5
Statistical significance [a]	n.a.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Mean no. of juveniles ± SD	746± 86	798 ± 54	816 ± 26	824 ± 48	710 ± 100	748 ± 54	834 ± 102	752 ± 80	759 ± 152
Reproduction in [%] of control	n.a.	107	109	110	95	100	112	101	102
Statistical significance [b]	n.a.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Endpoints [mg product/kg soil dw] (95% confidence limits)									
NOEC (mortality)	50								
LOEC (mortality)	> 50								
LC_{50} (mortality)	> 50								
NOEC (reproduction)	50								
LOEC (reproduction)	> 50								

n.a. not applicable;
SD: Standard Deviation
n.s. = not significantly different compared to the control
* = significantly different compared to the control
[a] Fisher's Exact Test, α = 0.05, one-sided greater
[b] Dunnett´s t-test, α = 0.05, one-sided smaller

B. VALIDITY CRITERIA

The study fulfilled the validity criteria outlined in the most recent EU test guideline (OECD 232, 2016), as detailed below:

- In the controls, mean adult mortality should not exceed 20% at the end of the test. Actual mean mortality in the control was 20%.
- In the controls, the mean number of juveniles per vessel should be at least 100 at the end of the test. The actual mean number of juvenile collembola per control replicates was 746; therefore, the validity criterion was met.
- In the controls, the coefficient of variation calculated for the number of juveniles should be less than 30% at the end of the test. The actual coefficient of variation in control reproduction was 11.5%.

III. CONCLUSION

For the collembolan *Folsomia candida* exposed to S-2399 60 G/L EC, the overall 28-day NOEC was determined to be ≥ 50 mg product/kg soil dw (equivalent to ≥ 3.27 mg a.s./kg soil dw). The 28-day LC_{50} and EC_{50} were estimated to be > 50 mg product/kg soil dw (i.e. > 3.27 mg a.s./kg soil dw).

HSE COMMENTS:

This study was conducted under GLP and conducted to OECD 232 (2016) guidelines. It has been assessed against these same guidelines.

The extraction efficiency was tested separately approximately 10 months before this study began; therefore, two extraction units with untreated soil were prepared by adding 60 collembolans to each unit. An extraction efficiency of 97.5% was obtained. This is higher than the required 95% value.

For the reference test, the EC_{50} of 100.2 mg/kg soil dw is in line with the guideline which states that 100 mg/kg soil dw should reduce reproduction by 50% and therefore the test system can be concluded to be sufficiently sensitive.

The use of statistics is suitable for this study and meet the requirements of the OECD 232 (2016) guidelines. It is noted that the study author has not calculated any EC_{10}/EC_{20} values, however as < 10% effects on reproduction were demonstrated this does not impact the risk assessment.

The agreed endpoints for use in risk assessment are:

28 day NOEC (reproduction) = 50 mg product/kg soil dw
28 day EC_{10} > 50 mg product/kg soil dw

B.9.8 Risk assessment for non-target soil meso- and macrofauna

B.9.8.1 Earthworms

B.9.8.1.1 Toxicity endpoints

The available earthworm toxicity data for inpyrfluxam and its metabolites and S-2399 60 G/L EC are summarised in Table 9.8-1.

Table 9.8-1: Endpoints used in the earthworm risk assessment for S-2399, its metabolites and S-2399 60 G/L EC

Test item	Exposure system	Species	Endpoint	Results	References
S-2399 TG	Soil 56 d chronic 5% peat content	*Eisenia fetida*	EC_{10}	21.5 mg a.s./kg soil dw	KCA 8.4.1/01 [redacted] 2016a
			NOEC	6.25 mg a.s./kg soil dw	
			$NOEC_{corr}$	**3.125 mg a.s./kg soil dw**	
3'-OH-S-2840	Soil 56 d chronic 5% peat content	*Eisenia fetida*	EC_{10}	>100 mg/kg soil dw	KCA 8.4.1/02 [redacted] 2016a
			NOEC	100 mg/kg soil dw	
			$NOEC_{corr}$	**50 mg/kg soil dw**	
1'-COOH-S-2840	Soil 56 d chronic 5% peat content	*Eisenia fetida*	EC_{10}	52.4 mg/kg soil dw	KCA 8.4.1/03 [redacted] 2016b
			NOEC	**50 mg/kg soil dw**	
S-2399 60 G/L EC[a]	Soil 56 d chronic 10% peat content	*Eisenia andrei*	NOEC	23.81 mg product/kg soil dw (1.56 mg a.s./kg soil dw)	KCP 10.4.1.1/01 [redacted] 2019a
			$NOEC_{corr}$	11.91 mg product/kg soil dw (**0.78 mg a.s./kg soil dw**)	

[a] Active substance content of the formulation; density 0.9273 g/mL, 60.68 g a.s./L (corresponding to 6.544% w/w)

Endpoints highlighted in **bold** used in the risk assessment (lowest of the NOEC and EC_{10})

Due to log P_{OW} > 2 (log Pow = 3.65 for S-2399 at pH 7.1 – 7.3; log Pow = 2.53 for 3'-OH-S-2840 at pH 6.5), endpoints from earthworm studies conducted in artificial soil were corrected for S-2399 and 3'-OH-S-2840 to account for the difference in organic matter in agricultural soils.

Five earthworm studies were submitted for evaluation including a 14-day acute study using the active substance, and four 56-day chronic studies using the active substance (inpyrfluxam), product (S-2399 60 G/L EC) and two metabolites (3'-OH-S-2840, 1'-COOH-S-2840). All chronic studies were deemed valid and suitable for use in risk assessment. For the product study (KCP 10.4.1.1/01), the NOEC was lowered to 23.81 mg product/kg soil based on the argument that this is the last concentration before an approximate concentration-response begins. HSE is aware that this NOEC is uncertain, and arguments could be advanced for either 23.81 or for 42.86 mg product/kg soil. For this reason, the worst-case lower NOEC was selected. The impact of this decision will be assessed below.

It is noted that, although an acute earthworm toxicity study is available for the active substance, this was not considered by HSE as it is no longer a requirement under the current data requirements.

B.9.8.1.2 Exposure

The relevant PEC_{soil} values for risk assessment covering the proposed use pattern have been established in Section B.8 of this assessment report by the Environmental Fate and Behaviour specialist. According to the assessment of environmental-fate data, the $PEC_{soil, accumulation}$, consisting of the carryover concentration (from the previous season) plus the addition of a new application, is to be considered for the active substance and the metabolites. $PEC_{soil, accumulation}$ values for the a.s. and its metabolites are presented in Table 9.8-2.

Table 9.8-2: Plateau Max PECsoil values for the inpyrfluxam and its metabolites after a single 90 g/ha foliar application to cereals at BBCH 30-71 in spring

Compound	$PEC_{soil, accumulation}$
Inpyrfluxam	0.069
3'-OH-S-2840	0.030
1'-COOH-S-2840	0.087

B.9.8.1.3 Risk Assessment

Earthworm (*Eisenia fetida*) toxicity studies have been submitted in line with the reporting requirements in Commission Regulation (EU) No 283/2013. The studies investigated the impact of the a.s. inpyrfluxam (S-2399), its metabolites 3'-OH-S-2840, 1'-COOH-S-2840, and the formulated product S-2399 60 G/L EC on earthworms. All studies were evaluated to be valid with no major deviations to the relevant study guidelines.

The risk assessment was performed in accordance with the recommendations of the

"Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

Risk is assessed in terms of Toxicity Exposure Ratios (TERs), using the endpoints highlighted in bold from Table 9.8-1 above and calculated using the following equation:

$$TER = \frac{Study\ endpoint}{PEC_{soil}}$$

As the log P_{ow} values for inpyrfluxam and 3'-OH-S-2840 are > 2, endpoints from earthworm studies conducted in artificial soil were corrected by a factor of two to account for the likely higher organic matter content relevant to agricultural soils, which would lead to greater adsorption of the test chemical and lower bioavailability. This was performed for all studies that selected inpyrfluxam or 3'-OH-S-2840, regardless of organic matter (peat) content, as recommended by the Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA, 2015)[8] document.

The risk is considered acceptable if the TER is > 5. The resulting TERs for earthworms are summarised in Table 9.8-3.

Table 9.8-3: First-tier assessment of the acute and chronic risk for earthworms due to the use of S-2399 60 G/L EC in cereals

Chronic effects on earthworms			
Test compound	**NOEC/NOEC$_{corr}$ (mg/kg dw)**	**PEC$_{soil,\ accumulation}$ (mg/kg dw)**	**TER$_{lt}$ (criterion TER ≥ 5)**
S-2399 60 G/L EC (mg a.s./kg dw)	0.78	0.069	11.3
S-2399	3.125	0.069	45.3
3'-OH-S-2840	50	0.030	1667
1'-COOH-S-2840	50	0.087	575

All TER values are above the relevant trigger value of 5 for chronic effects on earthworms. There is considerable margin of safety for all test compounds. This highlights that the selection of the lower S-2399 60 G/L EC NOEC endpoint did not change the outcome or conclusions of the risk assessment. An acceptable risk from the intended uses of S-2399 60 G/L EC is concluded for earthworms. No further assessment is required.

[8] EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924. 62 pp.

B.9.8.2 Non-target soil meso- and macrofauna (other than earthworms)

B.9.8.2.1 Toxicity endpoints

The available toxicity data for S-2399, its metabolites and S-2399 60 G/L EC are summarised in Table 9.8-4.

Table 9.8-4: Summary of toxicity endpoints for S-2399 and its metabolites for non-target soil meso- and macrofauna other than earthworms

Test item	Exposure system	Species	Endpoint	Results	References
S-2399 TG	Soil 14 d, chronic 5% peat content	*Hypoaspis aculeifer*	EC_{10}	>100 mg a.s./kg soil dw	CA 8.4.2/04 [redacted] 2016c
			NOEC	100 mg a.s./kg soil dw	
			$NOEC_{corr}$	**50 mg a.s./kg soil dw**	
S-2399 TG	Soil 28 d, chronic 5% peat content	*Folsomia candida*	EC_{10}	>100 mg a.s./kg soil dw	CA 8.4.2/01 [redacted] 2016b
			NOEC	100 mg a.s./kg soil dw	
			$NOEC_{corr}$	**50 mg a.s./kg soil dw**	
3'-OH-S-2840	Soil 14 d, chronic 5% peat content	*Hypoaspis aculeifer*	EC_{10}	>100 mg/kg soil dw	CA 8.4.2/05 [redacted] 2016e
			NOEC	100 mg/kg soil dw	
			$NOEC_{corr}$	**50 mg/kg soil dw**	
3'-OH-S-2840	Soil 28 d, chronic 5% peat content	*Folsomia candida*	EC_{10}	>100 mg/kg soil dw	CA 8.4.2/02 [redacted] 2016c
			NOEC	100 mg/kg soil dw	
			$NOEC_{corr}$	**50 mg/kg soil dw**	
1'-COOH-S-2840	Soil 14 d, chronic 5% peat content	*Hypoaspis aculeifer*	EC_{10}	>100 mg/kg soil dw	CA 8.4.2/06 [redacted] 2016f
			NOEC	**100 mg/kg soil dw**	
1'-COOH-S-2840	Soil 28 d, chronic 5% peat content	*Folsomia candida*	EC_{10}	>100 mg/kg soil dw	CA 8.4.2/03 [redacted] 2016d
			NOEC	**100 mg/kg soil dw**	

Test item	Exposure system	Species	Endpoint	Results	References
S-2399 60 G/L EC [a]	Soil 14 d, chronic 5% peat content	*Hypoaspis aculeifer*	EC_{10}	233 mg product/kg soil dw	CP 10.4.2.1/01 [redacted] 2019b
			NOEC	154 mg product/kg soil dw (10.08 mg a.s./kg soil dw)	
			$NOEC_{corr}$	77 mg product/kg soil dw **(5.04 mg a.s./kg soil dw)**	
S-2399 60 G/L EC [a]	Soil 28 d, chronic 5% peat content	*Folsomia candida*	NOEC	50 mg product/kg soil dw (3.27 mg a.s./kg soil dw)	CP 10.4.2.1/02 [redacted] 2019c
			$NOEC_{corr}$	25 mg product/kg soil dw **(1.635 mg a.s./kg soil dw**)	

[a] Active substance content of the formulation; density 0.9273 g/mL, 60.68 g a.s./L (corresponding to 6.544% w/w)

Endpoints highlighted in **bold** used in the risk assessment (smallest of NOEC and EC_{10}, corrected)

Due to log P_{OW} > 2 (log Pow = 3.65 for S-2399 at pH 7.1 – 7.3; log Pow = 2.53 for 3'-OH-S-2840 at pH 6.5), endpoints from earthworm studies conducted in artificial soil were corrected for S-2399 and 3'-OH-S-2840 to account for the difference in organic matter in agricultural soils.

All studies were evaluated to be valid with no major deviations to the relevant study guidelines.

B.9.8.2.2 Risk assessment

The evaluation of the risk for other non-target soil meso- and macro-fauna was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

The relevant PEC_{soil} values for risk assessments covering the proposed use pattern have been established in Section B.8 of this assessment report by the Environmental Fate and Behaviour specialist. They relate to the $PEC_{soil,\ accumulation}$ values (carryover concentration

plus the addition of a new application) (Table 9.8-2).

Toxicity endpoints were again corrected by a factor of two in accordance with EFSA Supporting publication 2015:EN-924[8].

Table 9.8-5: First-tier assessment of the chronic risk for other non-target soil organisms (meso- and macro-fauna) due to the use S-2399 60 G/L EC in cereals

Chronic effects on other soil macro- and mesofauna			
Test compound	**NOEC/NOEC$_{corr}$ (mg/kg dw)**	**PEC$_{soil,\ accumulation}$ (mg/kg dw)**	**TER$_a$ (criterion TER ≥ 5)**
Folsomia candida			
S-2399 60 G/L EC (mg a.s./kg dw)	1.64	0.069	23.8
S-2399	50	0.069	725
3'-OH-S-2840	50	0.030	1667
1'-COOH-S-2840	100	0.087	1149
Hypoaspis aculeifer			
S-2399 60 G/L EC (mg a.s./kg dw)	5.04	0.069	73
S-2399	50	0.069	725
3'-OH-S-2840	50	0.030	1667
1'-COOH-S-2840	100	0.087	1149

The TER values were above the trigger value of 5 for exposure of *Hypoaspis aculeifer* and *Folsomia candida* to the formulation S-2399 60 G/L EC , S-2399 and its metabolites, considering a single application in cereals. For the *F.candida* studies, OECD 232 (2016) requires the extraction and counting method to be validated, with a juvenile extraction efficiency of 95 %. This was either not performed or reported. This omission was partly addressed by the performance of a third count when two juvenile counts diverged > 10 % of their mean. Given the large margin of safety in the above risk asesssement and alternative measures to provide reliable counts, HSE considers this uncertainty addressed.

An acceptable risk from the intended use of S-2399 60 G/L EC is concluded.

B.9.9 Effects on soil nitrogen transformation

No soil nitrogen transformation formulation studies were submitted for inpyrfluxam. Please refer to the 3CA B9 document for active substance and metabolite studies.

B.9.10 Risk assessment for soil nitrogen transformation

B.9.10.1 Toxicity endpoints

A summary of the available endpoints for S-2399 and its metabolites are summarised in Table 9.10-1.

Table 9.10-1: Summary of endpoints for inpyrfluxam (S-2399) and its metabolites

Test Item	Exposure system	Results	References
S-2399 TG	28 d natural soil	Effects < 25% after 28 days at 0.27 and **1.33 mg a.s./kg soil dw**	CA 8.5/01 [redacted] 2016a
3'-OH-S-2840	28 d natural soil	Effects < 25% after 28 days at 0.06 and **0.3 mg/kg soil dw**	CA 8.5/02 [redacted] 2016b
1'-COOH-S-2840	28 d natural soil	Effects < 25% after 28 days at 0.1 and **0.5 mg/kg soil dw**	CA 8.5/03 [redacted] 2016c

Endpoints highlighted in **bold** used in the risk assessment

In agreement with the HSE formulation data requirements guidance[9], which is based on the requirements outlined in regulation 284/2013, specific formulation testing is not required since the formulation S-2399 60 G/L EC only contains one active substance and therefore, it is possible to extrapolate effects from the nitrogen transformation study conducted with S-2399. The HSE formulation data requirements guidance states, "*Given that formulations are unlikely to remain 'intact' in soil over chronic timescales, long term effects from the formulation on soil micro-organism activity are considered unlikely. Therefore, specific formulation testing is not usually required for products that only contain one active substance. In most cases it should be possible to extrapolate effects data from studies conducted in support of the active substance data requirements*".

B.9.10.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

The relevant PEC_{soil} values for risk assessments covering the proposed use pattern have been established in Section B.8 of this assessment report by the Environmental Fate and Behaviour specialist and relate to the $PEC_{soil,\ accumulation}$ values (carryover concentration plus

[9] Formulation studies and combined risk assessment in ecotoxicology, Guidance on the need for studies and their use in risk assessment, February 2022

the addition of a new application).

Table 9.10-2: Assessment of the risk for effects on soil micro-organisms due to the use of S-2399 60 G/L EC in cereals

Test compound	Max. conc. with effects ≤ 25 % (mg/kg dw)	$PEC_{soil,\ accumulation}$ (mg/kg dw)	Risk acceptable?
S-2399 TG	1.33	0.069	Yes
3'-OH-S-2840	0.3	0.030	Yes
1'-COOH-S-2840	0.5	0.087	Yes

The results show that S-2399 had no effects of ≥25% compared to the control on soil microbial activity up to a maximum tested concentration of 1.33 mg a.s./kg soil, after 28 days, which is considerably higher than the $PEC_{soil,\ accumulation}$. No effects of ≥25% compared to the control were noted with any of the metabolites at concentrations exceeding the PEC values. This supports the conclusion that under field conditions, use of S-2399 60 G/L EC poses an acceptable risk to non-target soil micro-organisms.

B.9.11 Effects on terrestrial non-target higher plants

B.9.11.1 Summary of screening data

No screening data has been submitted for Inpyrfluxam or the representative product.

B.9.11.2 Testing on non-target plants

Reference:	KCP 10.6.2/01
Report Title:	S-2399 60 g/L EC: Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Test
Author(s) & year:	[redacted] and [redacted] (2020a)
Document No, Authority registration No:	Study No. 141471086, TPW-0127
Substance used:	S-2399 6 EC, V16-7L1901, 6.544 ± 0.024% w/w
Method of analysis:	UPLC-method with UV-detection
Guideline(s):	OECD 208
Deviations:	Yes, see HSE Comments
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

I. MATERIALS AND METHODS

A.MATERIALS

Test item: S-2399 60 g/L EC (Emulsifiable Concentrate)
Active substance content: Analysed: 60.68 ± 0.23 g/L or 65.44 ± 0.24 g/kg or 6.544 ± 0.024 % w/w (verified by certificate of analysis)
Description: Yellow liquid
Lot/Batch: V16-7L1901
Density: 0.9273g/mL
Reference item: None
Solvent: Deionised water
Control: Deionised water

B.STUDY DESIGN AND METHODS

1. Test species:

Table B.9.11.2 - 1: Test species

Species	Common name	Family	Sowing date
Monocotyledoneae			
Lolium perenne	Perennial ryegrass	Poaceae	September 24, 2019
Allium cepa	Onion	Amaryllidaceae	September 24, 2019
Dicotyledoneae			
Brassica napus	Oilseed rape	Brassicaeae	September 24, 2019
Glycine max	Soybean	Fabaceae	September 24, 2019
Lactuca sativa	Lettuce	Asteraceae	September 24, 2019
Beta vulgaris	Sugar beet	Amaranthaceae	September 24, 2019

2. Artificial soil

Composition: LUFA soil No. 2.3 (USDA: sandy loam)
Particle size distribution: All particles ≤ 0.2 mm
Organic carbon: 0.66 ± 0.07%
pH: 5.9 ± 0.5

3. Test units:

Test units: Commercial plastic flowerpots (15 cm)
Concentrations tested: 1500, 750, 375, 188 and 93.8 mL product/ha
Duration: 14-21 days

4. Environmental conditions

A summary of the environmental conditions are shown in Table B.9.11.2 - 2 below:

Table B.9.11.2 - 2: Environmental conditions obtained in the study of S-2399 60 g/l EC on seedling emergence

Variable	Required OECD (208) 2006	Obtained
Temperature	22˚C ± 10˚C	16.3 – 24.2˚C
Relative humidity	70 % ± 25 %	53 – 87%
Photoperiod	≥ 16hrs light	16hrs light. 8hrs dark
Light intensity	350 ± 50 µE/m2 /s	200–390 µE/m^2/s

Study dates: Experimental Starting Date: September 25, 2019
Experimental Completion Date: October 21, 2019

5. Test design and treatment:
Five to 10 pots per treatment were sown manually with at least 20 seeds per treatment group (No. of replicates/pots × no. of plants per pot: *Brassica napus*: 7 × 3, *Glycine max*: 10 × 2, *Lactuca sativa*: 7 × 3, *Beta vulgaris*: 10 × 2, *Lollium perenne*: 4 × 5, *Allium cepa*: 4 × 5) each pot representing one replicate.

Nominal rates of 1500, 750, 375, 188 and 93.8 mL product/ha (corresponding to 91.0, 45.5, 22.8, 11.4 and 5.69 g a.s./ha, respectively) in 75 L deionised water/ha and a control of 75 L deionised water /ha tested in parallel were sprayed onto soil using laboratory spraying equipment (Fa. Schachtner with TeeJet 8001 EVS nozzle at a distance of 40 cm from soil at spraying pressure of 2.00 bar and a spraying speed of 3.50 km/h). The spray equipment was calibrated and using a glass plate with filter paper sprayed with water and weighing before and after application. Uniformity of spray deposits was checked visually. Verification of each treatment rate was performed and deviations in spray deposits on the glass plate did not exceed ± 10% of nominal. Test item application was on September 25, 2019.

The plants were exposed for 14 or 21 days after 50% emergence in the control depending on the growth of the seedlings.

Bottom watering (through saucers) was done where necessary after a daily check. Water was given individually in order to assure optimal water supply of the plants. After development of the first true leaves, fertiliser was added to the water up to two times a week, depending on the development of the plants.

6. Dose preparation:
The spray mixture for the highest rate was prepared by diluting 37.1 g S-2399 60 g/L to 2000 mL with deionised water (18.55 g test item/L corresponding to 1500 mL test item/ha in 75 L deionised water/ha). Based on the stock solution (of 1500 mL test item/ha) further rates of 750, 375, 188 and 93.8 mL test item/ha (corresponding to 91.0, 45.5, 22.8, 11.4 and 5.69 g a.s./ha), respectively were prepared (dilution factor: 2 by diluting 500 g out of the next higher

concentration to 1000 g with deionized water). All six plant species were tested at these rates.

7. Measurements and observations:
Plant fresh weight and plant height of above ground parts of all individual survived plants were determined 14 or 21 (if species was exposed longer) days (and the height also after 7 days) after 50% seedling emergence in the control. Emergence was checked daily on weekdays (except weekends) until 50% of the control plants had emerged. Further checks were done weekly. Plants were checked for mortality and visible signs of phytotoxicity on days 7, 14 and 21 after 50% seedling emergence in the control. Growth stages at days 14 and 21 days after 50% control emergence were recorded according to BBCH-Monograph.

Analytical verification was performed for the stock solution and the control solution. Method validation data are presented in Document Part B, Section 5. Method for Determination: UPLC-method with UV-detection.

Test conditions were recorded with one mean measuring point every 15 minutes for temperature and relative humidity and once a week for light intensity with 5 different measuring points for each species.

8. Statistical analysis:
Fresh weight data and plant height data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test (α = 0.05) and the Levene's test (α = 0.05). If the data were normally distributed, homogeneous and showed no monotonic dose response the Dunnett's t-test (multiple comparison, one-sided smaller, α = 0.05) was used for comparing treatment groups and control. If the data were normally distributed and not homogeneous the Bonferroni-Welch t-test (multiple comparison, one-sided smaller, α = 0.05) was used. If the data were not normally distributed and showed no monotonic dose response the Bonferroni-Holm U-test (multiple comparison, one-sided smaller, α = 0.05) was used.

For the mortality and emergence data Fisher's Exact Binomial Test (with Bonferroni Correction, two sample comparison, one-sided greater, α = 0.05) was used.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. ANALYTICAL RECOVERY

The analytical recovery rate of the active ingredient S-2399 in the stock solution was 97% of nominal. In the control solution, no active substance was detected. LOD = 0.5 mg a.s./L.

LOQ = 12.5 g test item/L (diluted by factor 100). The analytical results are presented in the table below.

Table B.9.11.2 - 3: Analytical results

Sample description	Concentration				
	Found (mg a.s/L)	DF	Calculated (mg a.s/L)	Nominal	% of nominal
Biological control	<LOD	100	n.a	0	n.a
Biological stock solution	11.772	100	1177	1214	97

B. BIOLOGICAL EFFECTS

Biological results are presented in Table B.9.11.2 - 4 to Table B.9.11.2 -6.

Table B.9.11.2 - 4: Emergence (%), survival (%), phytotoxic effects and growth stages in plants following exposure to S-2399 60 g/L EC

Application rate [g a.s./ha]	Ryegrass	Onion	Oilseed rape	Soybean	Lettuce	Sugar beet
	Emergence [%] [a]					
Control	70	100	95	100	95	95
5.69	90	100	95	90	81	95
11.4	80	100	76	100	90	100
22.8	85	100	86	90	86	100
45.5	80	95	95	100	100	90
91.0	100	100	100	100	90	90
Mortality [%] [a]						
Control	0	0	0	0	0	0
5.69	0	0	0	0	0	0
11.4	0	0	0	0	0	0
22.8	0	0	0	6	6	0
45.5	0	0	5	0	0	0
91.0	0	0	0	0	0	0
Phytotoxicity [%] (day 7, day 14, day 21)						
Control	0, 0, 0	0, 0, 0	0, 0, n.a.	0, 0, n.a.	0, 0, 0	0, 0, n.a.
5.69	0, 0, 0	0, 0, 0	0, 0, n.a.	0, 0, n.a.	0, 0, 0	0, 0, n.a.
11.4	0, 0, 0	0, 0, 0	0, 0, n.a.	0, 0, n.a.	0, 0, 0	0, 0, n.a.
22.8	0, 0, 0	0, 0, 0	0, 0, n.a.	0, 5, n.a.	0, 0, 7	0, 0, n.a.
45.5	0, 0, 0	0, 0, 0	0, 5, n.a.	0, 0, n.a.	0, 0, 0	0, 0, n.a.
91.0	0, 0, 0	0, 0, 0	0, 3, n.a.	0, 0, n.a.	0, 0, 0	0, 0, n.a.
BBCH growth stage (day 14 or day 21)						
Control	22	12	13-16	12-13	19	12-13
5.69	22	12	13-16	12-13	19	12-13

Application rate [g a.s./ha]	Ryegrass	Onion	Oilseed rape	Soybean	Lettuce	Sugar beet
	Emergence [%] [a]					
11.4	22	12	13-16	12-13	19	12-13
22.8	22	12	13-16	12-13	19	12-13
45.5	22	12	13-16	12-13	19	12-13
91.0	22	12	13-16	12-13	19	12-13

n.a. not applicable
a Fisher´s Exact Binominal Test with Bonferroni Correction, α = 0.05; no effect or no statistical significance at all tested rates on all plant species

Beside necrosis due to mortality for one replicate each of one treatment group of *Brassica napus*, *Glycine max* and *Lactuca sativa*, only very slight growth reduction was observed for *Brassica napus* for three replicates at 91.0 g a.s./ha.

Table B.9.11.2 - 5: Shoot height and inhibition in plants following exposure to S-2399 60 g/L EC

Application rate [g a.s./ha]	Mean shoot height [mm ± SD] / % change compared to control											
	Ryegrass		Onion		Oilseed rape		Soybean		Lettuce		Sugar beet	
	[mm]	[%]	[mm]	[%]	[mm]	[%]	[mm]	[%]	[mm]	[%]	[mm]	[%]
Control	310 ± 56.0	n.a.	174± 21.0	n.a.	143± 13.2	n.a.	189± 42.2	n.a.	94± 15.7	n.a.	110± 13.2	n.a.
5.69	283± 20.2	- 8.7 [c]	182± 12.7	4.4 [c]	148± 11.9	3.7 [b]	179± 27.1	-5.5 [b]	96± 9.8	2.8 [b]	111± 19.4	1.4 [a]
11.4	284± 28.0	-8.3 [c]	186± 14.9	6.5 [c]	151± 13.9	5.9 [b]	174± 26.3	-8.2 [b]	96± 3.7	2.7 [b]	118± 11.8	7.5 [a]
22.8	312± 50.3	0.6 [c]	163± 38.1	-6.7 [c]	144± 16.2	0.8 [b]	187± 27.0	-1.1 [b]	96± 5.8	2.4 [b]	107± 21.5	-3.0 [a]
45.5	294± 50.5	-5.2 [c]	176± 13.1	0.8 [c]	150± 8.8	5.3 [b]	186± 45.7	-1.7 [b]	96± 6.2	2.3 [b]	104± 16.2	-5.7 [a]
91.0	300± 33.5	-3.3 [c]	189± 11.1	8.2 [c]	139± 10.2	-2.8 [b]	156± 51.3	-17.8 [b]	101± 3.1	8.1 [b]	116± 15.4	5.9 [a]

n.a. not applicable; - value indicates reduction compared to controls
Test for statistical significance / statistically significant difference:
[a] Multiple comparison Dunnett´s t-test, α = 0.05; not statistical significance
[b] Multiple comparison Bonferroni-Holm U-test, α = 0.05; not statistical significance
[c] Multiple comparison Bonferroni-Welch t-test, α = 0.05; not statistical significance

Table B.9.11.2 - 6: Fresh weight and inhibition in plants following exposure to S-2399 60 g/L EC

Application rate [g a.s./ha]	Mean Fresh weight [g] / % change compared to control											
	Ryegrass		Onion		Oilseed rape		Soybean		Lettuce		Sugar beet	
	[g]	[%]	[g]	[%]	[g]	[%]	[g]	[%]	[g]	[%]	[g]	[%]
Control	6.03± 1.45	n.a.	2.70± 0.38	n.a.	15.63± 0.96	n.a.	6.99± 1.90	n.a.	18.96± 4.72	n.a.	3.52± 0.65	n.a.

Application rate [g a.s./ha]	Mean Fresh weight [g] / % change compared to control											
	Ryegrass		Onion		Oilseed rape		Soybean		Lettuce		Sugar beet	
	[g]	[%]	[g]	[%]	[g]	[%]	[g]	[%]	[g]	[%]	[g]	[%]
5.69	7.22± 1.27	19.6 [a]	2.84± 0.42	5.4 [c]	15.80± 1.26	1.1 [b]	5.74± 1.55	-17.9 [a]	16.50± 5.84	-13.0 [a]	3.54± 1.30	0.5 [a]
11.4	7.14± 1.42	18.4 [a]	2.80± 0.30	3.6 [c]	15.21± 1.14	-2.6 [b]	5.92± 1.03	-15.3 [a]	15.46± 3.60	-18.5 [a]	4.00± 1.04	13.7 [a]
22.8	7.11± 2.29	17.9 [a]	2.21± 0.96	-18.0 [c]	15.33± 2.26	-1.9 [b]	6.24± 2.11	-10.8 [a]	15.27± 7.18	-19.4 [a]	3.27± 1.18	-7.2 [a]
45.5	7.06± 0.97	17.0 [a]	2.30± 0.44	-14.9 [c]	15.92± 2.46	1.9 [b]	6.99± 2.19	0.0 [a]	18.59± 4.44	-2.0 [a]	3.25± 1.13	-7.8 [a]
91.0	8.25± 1.07	36.7 [a]	2.77± 0.48	2.7 [c]	15.62± 1.14	-0.1 [b]	6.28± 1.71	-10.1 [a]	22.34± 3.20	17.8 [a]	3.73± 0.99	5.9 [a]

n.a. not applicable; - value indicates reduction compared to controls
Test for statistical significance / statistically significant difference:
[a] Multiple comparison Dunnett´s t-test, α = 0.05; not statistical significance
[b] Multiple comparison Bonferroni-Holm U-test, α = 0.05; not statistical significance
[c] Multiple comparison Bonferroni-Welch t-test, α = 0.05; not statistical significance

Endpoints are summarised in Table B.9.11.2-7.

Table B.9.11.2 - 7: Summary of endpoints

Endpoint [g a.s./ha]	Ryegrass	Onion	Oilseed rape	Soybean	Lettuce	Sugar beet
S-2399 (active substance)						
Emergence						
$ER_{50, 20, 10}$	> 91	> 91	> 91	> 91	> 91	> 91
LOER	> 91	> 91	> 91	> 91	> 91	> 91
Mortality						
$ER_{50, 20, 10}$	> 91	> 91	> 91	> 91	> 91	> 91
LOER	> 91	> 91	> 91	> 91	> 91	> 91
Shoot height						
$ER_{50, 20, 10}$	> 91	> 91	> 91	> 91	> 91	> 91
LOER	> 91	> 91	> 91	> 91	> 91	> 91
Fresh weight						
$ER_{50, 20, 10}$	> 91	> 91	> 91	> 91	> 91	> 91
LOER	> 91	> 91	> 91	> 91	> 91	> 91

C. VALIDITY CRITERIA

The validity criteria were fulfilled according to the most recent version of the EU test guideline (OECD 208, 2006) as detailed below.

- Emergence rate of control seeds of at least 70%. The actual emergence rate was 70-100%.
- Mean survival of emerged control plants ≥ 90%. There was 100% survival in the controls.

- The plants in the control group do not exhibit visible phytotoxic effects and only normal variation in growth and morphology. Control plants exhibited no visible phytotoxic effects.
- Environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media or substrate from the same source. Environmental conditions were identical for each species tested.

III. CONCLUSION

The effect of S-2399 60 g/L EC on seedling emergence and seedling growth was determined in the laboratory. The NOER for all species was determined to be ≥ 91 g a.s./ha and the ER_{50} to be > 91 g a.s./ha (corresponding to > 1500 mL product/ha).

HSE COMMENTS:
This study has been conducted to GLP under OECD 208 (2006) guidelines and has been assessed against these same guidelines.

The light intensity recorded during this study (200–390 µE/m^2/s) is below the range recommended in the guideline (350 ± 50 µE/m2 /s). However, as the validity criteria have been met, this does not invalidate the study.

No reference item was used to determine species sensitivity. However, this does not invalidate the study as this is a recommendation in the guidelines rather than a requirement.

The use of statistics is suitable for this study and is in line with OECD 208 (2006) guidelines. As there were < 50% effects demonstrated, the ER_{50} values were unable to be statistically determined.

It is noted that interrupted dose responses were observed for fresh weight, however as < 50% effects were demonstrated and the NOER is not required for the risk assessment, the NOER values have not been considered further by HSE under the current assessment. In addition, the light intensity during this study dropped below the minimum requirements set out in the guidelines. As the validity criteria have been met, this does not invalidate the study.

No % organic matter or salt content for the soil is provided in the study report. These are part of the reporting criteria outlined in OECD 208 (2006). However, as the validity criteria have been met, this does not invalidate the study.

The method of analysis used in the study was evaluated by HSE Chemistry. The conclusions of their evaluation are reproduced below. Please see Volume 3 CA, section B5 for more details.

"*The analytical method is not fully validated according to SANTE/2020/12830 rev. 1 for the determination of the active substance inpyrfluxam in deionised water as the matrix effects have not been determined. However, the study was generated prior to the implementation*

of SANTE 2020/12830 rev.1 and SANCO 3029 rev.4 did not require matrix effects to be addressed. As all other validation requirements have been met (including the minimum validation requirements outlined in SANTE 2020/12830 rev.1), the method is considered to be fit for purpose."

The agreed endpoint to use in risk assessment are:

- **ER_{50} > 91 g a.s./ha (corresponding to > 1500 mL product/ha).**
  No species recorded 50% phytotoxic effects in this study The endpoint is protective of 50% phytotoxicity.

Reference:	KCP 10.6.2/02
Report Title:	S-2399 60 g/L EC: Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test
Author(s) & year:	[redacted] and [redacted] (2020b)
Document No, Authority registration No:	Ibacon, Germany Study No. 141471087 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0117
Substance used:	S-2399 6 EC, V16-7L1901, 6.544 ± 0.024% w/w
Method of analysis:	UPLC-method with UV-detection
Guideline(s):	OECD 227
Deviations:	Yes, see HSE Comments
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

I.MATERIALS AND METHODS

A.MATERIALS

Test material	S-2399 6 EC
Lot/Batch:	V16-7L1901
Active Substance Content:	6.544 ± 0.024% w/w or 65.44 ± 0.24 g/kg or 60.68 ± 0.23 g/L (verified by certificate of analysis, 22 October 2019)
Description:	Yellow liquid
Density:	0.9273 g/mL
Expiry date:	17 May 2021
Storage on Receipt:	At 20 ± 5 °C, in the dark

B.TREATMENTS

Test concentrations:	1500, 750, 375, 188 and 93.8 mL test item/ha corresponding to 91.0, 45.5, 22.8, 11.4 and 5.69 g a.s./ha

	in 75 L deionised water/ha and a control of 75 L deionised water/ha
Analysis of test concentrations:	Yes, stock solution and control solution using UPLC-method with UV-detection (Limit of Detection = 0.5 mg a.s./L)
C.TEST ORGANISMS	
Species:	Oilseed Rape (*Brassica napus*), Soybean (*Glycine max*), Lettuce (*Lactuca sativa*), Sugar beet (*Beta vulgaris*), Perennial ryegrass (*Lolium perenne*), Onion (*Allium cepa*)
D.TEST DESIGN	
Plant pots:	Commercial plastic flower pots (Ø 15 cm)
Soil:	The soil was steam sterilized, delivered and analysed by the LUFA Speyer, Germany. Soil Type: LUFA 2.3 (USDA: sandy loam) Particle Size: all particles ≤ 0.2 cm C_{org}: 0.66 ± 0.07% pH: 5.9 ± 0.5
Replication:	5 to 10 pots per treatment group were treated. At least 20 plants were tested per treatment group
Duration:	21 days
Application equipment:	Laboratory-spraying equipment (Fa. Schachtner, D-71640 Ludwigsburg)
Spray nozzle type:	TeeJet 80015 EVS
Distance between spray nozzle and plants:	40 cm
Spraying pressure:	2.60 bar
Spraying speed:	4.00 km/h (control, 5.69, 11.4, 22.8 and 45.5 g a.s./ha) 3.50 km/h (91.0 g a.s./ha)
E.TEST CONDITIONS	
Test temperature:	16.1 - 23.3 °C (cultivation period, mean:19.6 °C) 15.9 – 24.0 °C (exposure period, mean:19.9 °C)
Humidity:	60 – 93 % (cultivation period, mean: 74 %) 56 – 90 % (exposure period, mean: 72 %)
Light intensity:	200 – 240 µE/m^2/s (cultivation period, mean: 217 µE/m^2/s) 200 - 330 µE/m^2/s (exposure period, mean: 260 µE/m^2/s)
Lighting:	16 h light:8 h dark
Fertiliser:	After development of the first true leaves, Ferty® 9 “Hydro” (Planta-Düngemittel GmbH) at 3 g/L and Terraflor®-AZ (Terraflor GmbH) at 0.4 g/L were added to the water up to two times a week, depending on the development of the plants.

Moisture check and irrigation:	Bottom watering (through saucers) was done where necessary after a daily check. Water was given individually in order to assure optimal water supply of the plants.

F.STUDY DESIGN AND METHODS

Experimental dates:
Experimental phase (biological): 29 August 2019 to 19 September 2019.
Experimental phase (analytical): 30 October 2019 to 30 October 2019.

Test design and treatment

Five to 10 pots per treatment were sown manually with at least 20 seeds per treatment group with each pot representing one replicate (No. of replicates/pots × no. of plants per pot: *Brassica napus*: 7 × 3, *Glycine max*: 10 × 2, *Lactuca sativa*: 7 × 3, *Beta vulgaris*: 10 × 2*, Lollium perenne*: 4 × 5, *Allium cepa*: 4 × 5).

Plants were grown until they had reached the 2 to 4 true-leaf stage prior to dosing. To account for the different development speed of the species the sowing was done on different dates, to ensure that all species were in the 2 to 4 true leaf stage at the application day. After sowing, pots were placed on saucers and watered.

For spray application calibration, a glass plate of known surface area with filter paper of the same size as the glass plate was sprayed with deionised water. The weight of the glass plate with the filter paper was determined immediately before and after application and the amount of spray deposit per cm² was calculated as the difference between the weight before and after spraying. The procedure was repeated 5 times in a row without changing the adjustment and every time the application rate was within 75 L/ha ± 10% (corresponding to 0.75 mg/cm²). The uniformity of the deposit distribution was checked visually.

For spray application verification, the calibration procedure was performed for each application rate. The 91.0 g a.s./ha application rate was not in the range of ± 10% of the nominal value. Therefore, the spraying speed was adjusted and the spraying amount verified by two further sprayings that were within ± 10% of the nominal value.

Plants were sprayed with the test item using laboratory spraying equipment, which simulates field application.

Dose preparation

The spray mixture for the highest rate was prepared by diluting 37.1 g S-2399 60 g/L EC to 2000 Ml with deionised water (18.55 g test item/L corresponding to 1500 Ml test item/ha in 75 L deionised water/ha). The subsequent concentrations were prepared by serial dilutions of 500 g out of the next higher concentration made to 1000 g with deionised water.

Measurement and observations

The fresh weight of the above ground part of all survived plants of a pot (each pot is considered as a replicate) was determined 21 days after application. The height of the above ground part of each individual surviving plant was recorded one day before application day and on days 7, 14 and on 21 days after application. The number of living and dead plants was recorded 7, 14 and 21 days after application. A plant was considered dead if no living tissue could be found on the leaves or shoots. All other plants were considered living. Visual phytotoxicity (e.g. chlorosis, necrosis, abnormal growth) was recorded 7, 14 and 21 days after application according to EPPO PP 1/135(4)[10]. Growth stages at the application day and 21 days after application were recorded according to BBCH-Monograph – Growth stages.

Test conditions were recorded with one mean measuring point every 15 minutes for temperature and relative humidity and once a week for light intensity with 5 different measuring points for each species.

Statistical analysis

Fresh weight and plant height data for 21 days after application were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test (α = 0.05) and the Levene's test (α = 0.05). If the data were normally distributed, homogeneous and showed no monotonic dose response the Dunnett's t-test (multiple comparison, one-sided smaller, α = 0.05) was used for comparing treatment groups and control. If the data were not normally distributed and showed no monotonic dose response the Bonferroni-Holm U-test (multiple comparison, one-sided smaller, α = 0.05) was used.

ER_{10}, ER_{20}, ER_{50} and the 95 percent confidence limits as well as the NOER and LOER in terms of fresh weight and height were calculated if possible.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

II.RESULTS AND DISCUSSION

Analytical results

The analytical recovery rate of the active substance S-2399 in the stock solution was 98% of the nominal value.

Biological effects

Survival, phytotoxic effects and growth stages throughout the study are presented in Table B.9.11.2 – 8.

[10] EPPO PP 1/135(4): Efficacy evaluation of plant protection products, Phytotoxicity assessment, 2014.

Table B.9.11.2 – 8: Survival (%), phytotoxic effects and growth stages in plants following exposure to S-2399 60 g/L EC

Application rate [g a.s./ha]	Ryegrass	Onion	Oilseed rape	Soybean	Lettuce	Sugar beet
	Mortality [%]					
Control	0	0	0	0	0	0
5.69	0	0	0	0	0	0
11.4	0	0	0	0	0	0
22.8	0	0	0	0	0	0
45.5	0	0	0	0	0	0
91.0	0	0	0	0	0	0
Phytotoxicity [%] (day 7, day 14, day 21)						
Control	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
5.69	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
11.4	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
22.8	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
45.5	0, 0, 0	0, 0, 0	0, 0, 0	1, 0, 0	0, 0, 0	2, 0, 0.4
91.0	0, 0, 0	0, 0, 0	0, 0, 0	1, 0, 0	0, 0, 0	4, 1, 0.4
BBCH growth stage (day 0 / day 21)						
Control	13– 4 / 23-24	13 / 15-16	13 / 18-19	12 / 60	12-13 / 19	12 /16-17
5.69	13– 4 / 23-24	13 / 15-16	13 / 18-19	12 / 60	12-13 / 19	12 /16-17
11.4	13– 4 / 23-24	13 / 15-16	13 / 16-19	12 / 60	12-13 / 19	12 / 14-15
22.8	13– 4 / 23-24	13 / 15-16	13 / 16-19	12 / 60	12-13 / 19	12 / 14-15
45.5	13– 4 / 23-24	13 / 15-16	13 / 16-19	12 / 60	12-13 / 19	12 / 14-15
91.0	13– 4 / 23-24	13 / 15-16	13 / 16-19	12 / 60	12-13 / 19	12 / 14-15

For phytotoxicity, after 21 days two replicates from the 45.5 and 91.0 g.a.s./ha treatment groups for *Beta vulgaris* displayed 2 % phytotoxicity (chlorosis), resulting in 0.4 % mean

phytotoxicity. For plant development, after 21 days there was a reduction from BBCH 16 – 17 (control) to BBCH 14 – 15 for treatment groups above 11.4 g a.s./ha for *Beta vulgaris*, indicative of delayed development. Delayed development was also observed for *Brassica napus*: after 21 days there was a reduction from BBCH 18 – 19 (control) to BBCH 16 – 19 for treatment groups above 11.4 g a.s./ha. For all other species and endpoints there were no observable effects after 21 days.

A summary of fresh weight per replicate and inhibition relative to the control is presented in Table B.9.11.2 -9.

Table B.9.11.2 - 9: Fresh weight and inhibition in plants following exposure to S-2399 60 g/L EC

Application rate [g a.s./ha]	Fresh weight [g] / Inhibition [%]											
	Ryegrass		Onion		Oilseed rape		Soybean		Lettuce		Sugar beet	
	[g]]	[%]	[g]	[%]	[g]	[%]	[g]	[%]	[g]	[%]	[g]	[%]
Control	14.0 ± 1.0	n.a.	16.7 ± 2.9	n.a.	43.2 ± 3.8	n.a.	28.1 ± 4.0	n.a.	41.3 ± 4.3	n.a.	24.5 ± 3.8	n.a.
5.69	14.6 ± 1.1	4.7	16.3 ± 2.5	-2.6	39.4 ± 6.1	-8.7	31.1 ± 3.4	10.4	41.2 ± 5.1	-0.2	23.5 ± 4.4	-4.0
11.4	14.3 ± 1.1	2.6	16.5 ± 1.5	-1.2	38.9 ± 4.0	-9.8	32.0 ± 3.1	13.7	40.9 ± 2.7	-1.1	24.3 ± 2.6	-0.5
22.8	14.1 ± 0.9	0.9	15.9 ± 1.0	-4.9	36.8 ± 4.6	-14.8 [a]	29.3 ± 3.1	4.0	43.1 ± 3.5	4.3	24.2 ± 1.5	-1.2
45.5	13.7 ± 0.7	-2.1	14.9 ± 0.8	-11.1	39.5 ± 3.5	-8.5	29.1 ± 3.8	3.5	42.3 ± 3.9	2.5	22.7 ± 2.8	-7.1
91.0	14.6 ± 1.0	4.2	15.4 ± 2.0	-7.7	39.6 ± 6.8	-8.3	28.6 ± 4.4	1.8	41.5 ± 4.5	0.4	25.1 ± 2.2	2.6

n.a. not applicable; - value indicates reduction compared to controls

[a] Multiple comparison Dunnett´s t-test, α = 0.05; statistically significantly different from contro

There was statistically significant reduction for oilseed rape at 22.8 g a.s./ha. This, however, did not appear to be treatment related due to the lack of a clear dose-response., with inhibitions at 45.5 and 91.0 g a.s./ha similar to treatment levels below 22.8 g a.s./ha.

Effects on shoot height are summarised in Table B.9.11.2-10.

Table B.9.11.2 - 10: Shoot height and inhibition in plants following exposure to S-2399 60 g/L EC

Application Rate [g a.s./ha]	Shoot height [mm ± SD] / Inhibition [%]											
	Ryegrass		Onion		Oilseed rape		Soybean		Lettuce		Sugar beet	
	[mm]	[%]	[mm]	[%]	[mm]	[%]	[mm]	[%]	[mm]	[%]	[mm]	[%]
Control	359 ± 20.6	n.a.	263 ± 23.1	n.a.	182 ± 10.9	n.a.	679 ± 131.7	n.a.	104.± 3.8	n.a.	180 ± 21.9	n.a.
5.69	381 ± 25.9	6.1	254 ± 9.1	-3.4	182 ± 9.6	0.0	755 ± 127.0	11.2	105 ± 3.1	0.7	174 ± 11.1	-2.9
11.4	371 ± 20.1	3.2	255 ± 11.5	-3.0	182 ± 13.7	-0.3	806 ± 125.8	18.6	101 ± 3.7	-2.7	178 ± 11.8	-1.1
22.8	341 ± 10.4	-5.1	250 ± 15.6	-4.7	181 ± 6.4	-0.7	709 ± 90.7	4.4	106 ± 3.5	1.6	178 ± 8.8	-0.8
45.5	352 ± 22.0	-1.9	258 ± 13.9	-1.7	180 ± 15.1	-1.2	714 ± 114.0	5.2	107 ± 6.3	2.7	181 ± 18.7	0.7
91.0	368 ± 23.4	2.4	251 ± 8.1	-4.6	178 ± 10.4	-2.5	717 ± 130.5	5.6	105 ± 5.6	1.1	174 ± 8.2	-3.3

n.a. not applicable; - value indicates reduction compared to controls

There were no significant effects for any species at any treatment level.

Endpoints are summarised in Table B.9.11.2-11.

Table B.9.11.2 – 11: Summary of endpoints

Endpoint [g a.s./ha]	Ryegrass	Onion	Oilseed rape	Soybean	Lettuce	Sugar beet
S-2399 (active substance)						
Mortality						
$ER_{50, 20, 10}$	> 91	> 91	> 91	> 91	> 91	> 91
NOER	91	91	91	91	91	91

Endpoint [g a.s./ha]	Ryegrass	Onion	Oilseed rape	Soybean	Lettuce	Sugar beet
LOER	> 91	> 91	> 91	> 91	> 91	> 91
Shoot height						
$ER_{50, 20, 10}$	> 91	> 91	> 91	> 91	> 91	> 91
NOER	91	91	91	91	91	91
LOER	> 91	> 91	> 91	> 91	> 91	> 91
Fresh weight						
$ER_{50, 20, 10}$	> 91	> 91	> 91	> 91	> 91	> 91
NOER	91	91	91	91	91	91
LOER	> 91	> 91	> 91	> 91	> 91	> 91
Phytotoxicity						
$ER_{50, 20, 10}$	> 91	> 91	> 91	> 91	> 91	> 91
NOER	91	91	91	91	91	91
LOER	> 91	> 91	> 91	> 91	> 91	> 91

Validity criteria

The validity criteria for the study were met according to OECD 227 (2006) (Table B.9.11.2-12).

Table B.9.11.2 - 12: Compliance with OECD 227 (2006) validity criteria

Validity criterion	Required	Obtained
Seedling emergence	≥ 70 %	93 – 99 %
21-day control mean plant survival	≥ 90 %	100 %
Control phytotoxicity, growth and morphology.	Plants should not exhibit phytotoxic effects and exhibit only normal variation in growth and morphology	No visible phytotoxic effects and the plants exhibited only normal variation in growth and morphology
Environmental conditions	Environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix,	Yes

Validity criterion	Required	Obtained
	support media, or substrate from the same source	

CONCLUSIONS

The effect of S-2399 60G/L EC on vegetative vigour was determined in the laboratory. The NOER for all species was determined to be 91 g a.s./ha and the ER_{50} to be > 91 g a.s./ha (corresponding to > 1500 mL product/ha).

HSE COMMENTS

The study was carried out according to and evaluated against the OECD 227 (2006) guideline. All validity criteria outlined in OECD 227 (2006) were satisfactorily met for the duration of the study.

The following deviations were noted:

OECD 227 (2006) § 2 states "*after the application, the plants are evaluated against untreated control plants for effects on vigour and growth at various time intervals through 21 - 28 days from treatment. A test period of 21 days can be sufficient for the 10 crop species listed in Annex 4*". Two of the species selected for this study, *Brassica napus* and *Beta vulgaris*, are not listed in Annex 4 and it is not clear if a 21-day treatment window is also suitable for these species.

OECD 227 (2006) § 6 covers the use of a reference substance or historical control data to verify the performance of the test system over time. Neither was provided, calling into question the sensitivity of the test system. The wording of the guideline, however, suggests that these measures are optional. Therefore, HSE considers the lack of a reference substance or control data acceptable.

OECD 227 (2006) § 7 lists the field soil characteristics that should be reported. Salt content as electronic conductivity of the final prepared soil was not reported. The high seedling emergence and control plant survival indicate that soil salt content was appropriate. HSE considers this a minor, acceptable deviation.

OECD 227 (2006) § 16 states, "*after the seeds have emerged, thinning should be completed so that there is only one plant per pot for larger-growing species, while for smaller growing species more than one plant per pot is allowed. Whether or not there will be one plant per pot or more than one plant per pot will be dependent on the size the plant will grow to by the end of the test period, and to avoid overcrowding. As much as is possible, there should be only one plant per pot*". For sugar beet and soybean, which are considered larger growing

species, there were more than one plant per 15 cm pot. All statistical analyses, however, were performed at the pot level. Also, overcrowding was less likely as the experiment was only for run for 21 days as opposed to 28. No pot overcrowding was reported. HSE considers this a minor acceptable deviation.

OECD 227 (2006) § 20 covers test conditions including light intensity. The guideline recommends a light intensity of 350 ± 50 µE/m^2/s for greenhouse testing. The study had light intensities of 200 – 240 µE/m^2/s and 200 - 330 µE/m^2/s for the cultivation and exposure periods respectively, although the test was performed in a growth chamber not a greenhouse. Control plant survival of 100 % alongside no phytotoxicity and normal growth and morphology strongly suggest that the lower than specified light intensity did not introduce any additional stress into the test system. HSE considers this a minor, acceptable deviation.

OECD 227 (2006) § 30 outlines what should be included in the test report. In relation to the test organism, it requires a detailed history of the seeds used including the name of the supplier, percentage germination, seed size class, batch or lot number, seed year or growing season collected and date of germination rating. This information was not provided beyond "*all uncoated seeds used in the test were from the same source and lot number*". Seedling emergence was 93 – 99 % for the six species. This indicates that seeds were of an acceptable quality. HSE considers these reporting omissions to be of minimal consequence and acceptable.

Finally, there was evidence of delayed development for *Beta vulgaris* at 11.4 g a.s./ha and above. This will be considered using a margin of safety approach at the risk assessment stage.

The method of analysis used in the study was evaluated by HSE Chemistry. The conclusions of their evaluation are reproduced below. Please see Volume 3 CA, section B5 for more details.

"*The analytical method is not fully validated according to SANTE/2020/12830 rev. 1 for the determination of the active substance inpyrfluxam in deionised water as the matrix effects have not been determined. However, the study was generated prior to the implementation of SANTE 2020/12830 rev.1 and SANCO 3029 rev.4 did not require matrix effects to be addressed. As all other validation requirements have been met (including the minimum validation requirements outlined in SANTE 2020/12830 rev.1), the method is considered to be fit for purpose.*"

The above study was conducted to GLP and considered valid.

The agreed endpoint suitable for use in the risk assessment is ER_{50} > 91 g a.s./ha (1500 mL test item/ha)

B.9.11.3 Extended laboratory studies on non-target plants

No data submitted or required.

B.9.11.4 Semi-field and field tests on non-target plants

No data submitted or required.

B.9.12 Risk assessment for terrestrial non-target higher plants

The following endpoints are available from the tests submitted by the applicant:

Toxicity

A summary of the available endpoints with non-target terrestrial plants for S-2399 60 g/L EC is presented in the following table.

Table B.9.12 - 1: Summary of toxicity endpoints of S-2399 60 g/L EC on seedling emergence and vegetative vigour

Test type	Test substance	Test species	Endpoint	Results (g a.s./ha)	References
21 d Seedling emergence	S-2399 60 g/L EC [a]	*Lolium perenne* [m] *Allium cepa* [m] *Brassica napus* [d] *Glycine max* [d] *Lactuca sativa* [d] *Beta vulgaris* [d]	ER_{50}	**≥ 91**	CP 10.6.2/01 [redacted] and 2020a
21 d Vegetative vigour	S-2399 60 g/L EC [a]	*Lolium perenne* [m] *Allium cepa* [m] *Brassica napus* [d] *Glycine max* [d] *Lactuca sativa* [d] *Beta vulgaris* [d]	ER_{50}	**≥ 91**	CP 10.6.2/02 [redacted] and 2020b

[a] Active substance content of the formulation; density 0.9273 g/mL, 60.68 g a.s./L (corresponding to 6.544% w/w) m: monocotyledoneae; d: dicotyledoneae

Endpoints highlighted in **bold** used in the risk assessment

Risk assessment

The evaluation of the risk to non-target plants was performed in accordance with the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). S- 2399 6EC is a fungicide and is therefore not expected to have significant

herbicidal activity. A preliminary assessment is conducted using the available screening data.

Summary of screening data

According to the Terrestrial Guidance Document, the risk to non-target terrestrial plants should be considered acceptable if less than a 50% effect on at least six species is seen at the highest nominal application rate (single application).

For S-2399, studies on seedling emergence and vegetative vigour of terrestrial higher plants with a maximum test rate of 91 g a.s./ha were conducted using the formulation S-2399 60 g/L EC . The results showed all ER_{50} values to be > 91 g a.s./ha for all plant species tested with <50% effects.

Conclusion

As the ER_{50} values for the active substance exceed the maximum proposed application rates of 90 g a.s./ha in cereals, an acceptable risk to non-target terrestrial plants for the intended uses of S-2399 60 g/L EC can be concluded. No further consideration is required.

B.9.13 Effects on other terrestrial organisms (Flora and Fauna)

No studies submitted.

B.9.14 Risk assessment for other terrestrial organisms (Flora and Fauna)

No studies submitted.

B.9.15 References Relied On

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate Study Y/N	Data Protection Claimed Y/N	Justification if Data Protection is claimed	Owner	Previous evaluation Y/N
KCP 10.2.1/01	[redacted]	2020a	S-2399 6 EC: Fish, Acute Toxicity Test [redacted], Study No. 3202418 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0120 GLP, unpublished	Y	Y	Data for the first approval	Sumitomo Chemical Co., Ltd.	N
KCP 10.2.1/02	[redacted]	2020b	S-2399 6 EC: Acute Toxicity to *Daphnia magna* Smithers ERS Limited Study No. 3202417 Sumitomo Chemical Agro Europe S.A.S.	N	Y	Data for the first approval	Sumitomo Chemical Co., Ltd.	N

			Report No. TPW-0116 GLP, unpublished					
KCP 10.2.1/03	[redacted]	2020c	S-2399 6 EC: Inhibition of Growth to the Alga *Raphidocelis subcapitata* (Formerly known as *Pseudokirchneriella subcapitata*) Smithers ERS Limited Study No. 3202416 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0119 GLP, unpublished	N	Y	Data for the first approval	Sumitomo Chemical Co., Ltd.	N
KCP 10.3.1.1.1 /01 and KCP 10.3.1.1.2/01	[redacted]	2019	S-2399 60 g/L EC: Acute Oral and Contact Toxicity to the Honey Bee, *Apis mellifera* L., under Laboratory Conditions Eurofins Scientific Study No. S19-00421	N	Y	Data for the first approval	Sumitomo Chemical Co., Ltd.	N

			Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0107 GLP, unpublished					
KCP 10.3.1.1.1 /02 and KCP 10.3.1.1.2/02	[redacted]	2020	S-2399 60 g/L EC: Acute oral and contact Toxicity to the Bumblebee *Bombus terrestris* L., under Laboratory Conditions Eurofins Scientific Study No. . S19-00422 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0121 GLP, unpublished	N	Y	Data for the first approval	Sumitomo Chemical Co., Ltd.	N
KCP 10.3.1.2/01	[redacted]	2021a	S-2399 60 g/L EC: Chronic Oral Toxicity Test (10-Day Feeding) to the Honey Bee (*Apis mellifera* L.) under Laboratory Conditions	N	Y	Data for the first approval	Sumitomo Chemical Co., Ltd.	N

			Eurofins Scientific Study No. S20-00800 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0135 GLP, unpublished					
KCP 10.3.1.3/01	[redacted]	2021b	S-2399 60 g/L EC: Honey Bee (*Apis mellifera* L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions Eurofins Scientific Study No. S20-00798 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0136 GLP, unpublished	N	Y	Data for the first approval	Sumitomo Chemical Co., Ltd.	N
KCP 10.3.2.1/01	[redacted]	2019a	S-2399 60G/L EC: Effects on the Parasitoid *Aphidius rhopalosiphi* in the Laboratory – Dose Response Test	N	Y	Data for the first approval	Sumitomo Chemical Co., Ltd.	N

			Ibacon GmbH Study No. 141471001 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0106 GLP, unpublished					
KCP 10.3.2.1/02	[redacted]	2019b	S-2399 60G/L EC: Effects on the Predatory Mite *Typhlodromus pyri* in the Laboratory – Dose Response Test Ibacon GmbH Study No. 141471063 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0105 GLP, unpublished	N	Y	Data for the first approval	Sumitomo Chemical Co., Ltd.	N
KCP 10.3.2.1/03	[redacted] et al.	2020	Effects of Fungicides on Four Native Generalist Phytoseiid Species （Acari: Phytoseiidae）	N	N	N/A	N/A	N/A

KCP 10.4.1.1/01	[redacted]	2019a	S-2399 60 g/L EC: Effects on Reproduction and Growth of Earthworms *Eisenia andrei* in Artificial Soil incl. 1st Amendment Ibacon, Germany Study No. 141471022 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0108 GLP, unpublished	N	Y	Data for the first approval	Sumitomo Chemical Co., Ltd.	N
KCP 10.4.2.1/01	[redacted]	2019b	S-2399 60G/L EC: Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil incl. 1st Amendment Ibacon, Germany Study No. 141471089 Sumitomo Chemical Agro Europe S.A.S.	N	Y	Data for the first approval	Sumitomo Chemical Co., Ltd.	N

			Report No. TPW-0109 GLP, unpublished					
KCP 10.4.2.1/02	[redacted]	2019c	S-2399 60G/L EC: Effects on Reproduction of the Collembola *Folsomia candida* in Artificial Soil incl. 1st Amendment Ibacon, Germany Study No. 141471016 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0110 GLP, unpublished	N	Y	Data for the first approval	Sumitomo Chemical Co., Ltd.	N
KCP 10.6.2/01	[redacted] and [redacted]	2020a	S-2399 60G/L EC: Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Test Ibacon, Germany Study No. 141471086 Sumitomo Chemical Agro Europe S.A.S.	N	Y	Data for the first approval	Sumitomo Chemical Co., Ltd.	N

			Report No. TPW-0127 GLP, unpublished					
KCP 10.6.2/02	[redacted] and [redacted]	2020b	S-2399 60G/L EC: Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test Ibacon, Germany Study No. 141471087 Sumitomo Chemical Agro Europe S.A.S. Report No. TPW-0117 GLP, unpublished	N	Y	Data for the first approval	Sumitomo Chemical Co., Ltd.	N

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