



Draft Assessment Report

Evaluation of Active Substances

Plant Protection Products

Prepared according to **Regulation (EC) 1107/2009**
as it applies in Great Britain

Pydiflumetofen

Volume 3 – B.9 (PPP) – Miravis Plus

Ecotoxicology

Great Britain

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B.9. ECOTOXICOLOGY DATA AND ASSESSMENT OF RISKS FOR NON-TARGET SPECIES

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

Background information

Pydiflumetofen (SYN545974) is a fungicide plant protection product. The representative formula is ‘Miravis Plus’, an EC formulation containing 62.5 g/l pydiflumetofen. The proposed use is on winter and spring cereals, and winter and spring oilseed rape.

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.9.1-1 significant pydiflumetofen metabolites

Environmental Compartment	Metabolite(s)
Soil	Pydiflumetofen
Groundwater	Pydiflumetofen
Surface water	Pydiflumetofen NOA449410 SYN548261
Sediment	Pydiflumetofen SYN545547
Air	Pydiflumetofen

Uses

The following table outlines the intended uses of SYN545794.

Table B.9.1.-2 Summary of proposed uses

Crop	Application		Application rate		
	Timing/growth stage	Max number a) Per use b) Per crop/season	L product/ha a) Max. rate per appl b) Max. total rate per crop/season	g a.s./ha a) Max. rate per appl b) Max. total rate per crop/season	Water L/ha Min/max
Barley, spring	BBCH 30-59	a) 1	a) 2.65	a) 166	100-300
		b) 1	b) 2.65	b) 166	
	BBCH 41-59	a) 1 b) 1	a) 2.65 b) 2.65	a) 166 b) 166	100-300
	BBCH 55-65	a) 1 b) 1	a) 3.2 b) 3.2	a) 200 b) 200	100-300
Barley, winter	BBCH 30-59	a) 1	a) 2.65	a) 166	100-300
		b) 1	b) 2.65	b) 166	
	BBCH 41-59	a) 1 b) 1	a) 2.65 b) 2.65	a) 166 b) 166	100-300
	BBCH 55-65	a) 1 b) 1	a) 3.2 b) 3.2	a) 200 b) 200	100-300
Durum wheat	BBCH 30-69	a) 1	a) 2.65	a) 166	100-300
		b) 1	b) 2.65	b) 166	
	BBCH 41-69	a) 1	a) 2.65	a) 166 b) 166	100-300

Crop	Application		Application rate		
	Timing/growth stage	Max number a) Per use b) Per crop/season	L product/ha a) Max. rate per aapl b) Max. total rate per crop/season	g a.s./ha a) Max. rate per appl b) Max. total rate per crop/season	Water L/ha Min/max
		b) 1	b) 2.65		
	BBCH 61-69	a) 1 b) 1	a) 3.2 b) 3.2	a) 200 b) 200	100-300
Oat Spring	BBCH 55-65	a) 1 b) 1	a) 3.2 a) 3.2	a) 200 b) 200	100-300
Oat winter	BBCH 55-65	a) 1 b) 1	a) 3.2 a) 3.2	a) 200 b) 200	100-300
Spelt	BBCH 30-69	a) 1 b) 1	a) 2.65 b) 2.65	a) 166 b) 166	100-300
	BBCH 41-69	a) 1 b) 1	a) 2.65 b) 2.65	a) 166 b) 166	100-300
	BBCH 61-69	a) 1 b) 1	a) 3.2 b) 3.2	a) 200 b) 200	100-300
Wheat, spring	BBCH 30-69	a) 1 b) 1	a) 2.65 b) 2.65	a) 166 b) 166	100-300
	BBCH 41-69	a) 1 b) 1	a) 2.65 b) 2.65	a) 166 b) 166	100-300
	BBCH 61-69	a) 1 b) 1	a) 3.2 b) 3.2	a) 200 b) 200	100-300
Wheat, winter	BBCH 30-69	a) 1 b) 1	a) 2.65 b) 2.65	a) 166 b) 166	100-300
	BBCH 41-69	a) 1 b) 1	a) 2.65 b) 2.65	a) 166 b) 166	100-300
	BBCH 61-69	a) 1 b) 1	a) 3.2 b) 3.2	a) 200 b) 200	100-300
Oilseed Rape, Spring	BBCH 57-69	a) 1 b) 1	a) 3.2 b) 3.2	a) 200 b) 200	100-300
Oilseed Rape, Winter	BBCH 57-69	a) 1 b) 1	a) 3.2 b) 3.2	a) 200 b) 200	100-300
Rye, spring	BBCH 61-69	a) 1 b) 1	a) 3.2 b) 3.2	a) 200 b) 200	100-300
Rye, Winter	BBCH 61-69	a) 1 b) 1	a) 3.2 b) 3.2	a) 200 b) 200	100-300
Triticale, spring	BBCH 61-69	a) 1 b) 1	a) 3.2 b) 3.2	a) 200 b) 200	100-300
Triticale, winter	BBCH 61-69	a) 1 b) 1	a) 3.2 b) 3.2	a) 200 b) 200	100-300

B.9.1.1. Effects on birds

Data have been submitted addressing the acute and long term/reproductive toxicity of the active substance SYN545794 (Pydiflumetofen) to birds. These studies address data requirements for the active substances as stated in retained EU regulation 283/2013. The studies are summarised and evaluated in Volume 3CA Part B9.

A study on the dietary (five-day) toxicity of the active substance shall only be required where the mode of action or results from mammalian studies indicate a potential for the dietary LD₅₀ measured by the short-term dietary toxicity study to be lower than the LD₅₀ based on an acute oral study.

EU retained regulation 283/2013 states that the avian dietary study is only required:

‘Where the mode of action or results from mammalian studies indicates a potential for the dietary LD₅₀ measured by the short-term dietary toxicity study to be lower than the LD₅₀ based on an acute oral study. The short-term dietary toxicity test shall not be conducted for any other purpose than to determine intrinsic toxicity through dietary exposure unless a justification of the need to do so is supplied.’

It is noted that two studies have been submitted, one using the Bobwhite Quail, and one using the Mallard Duck. This has been done to adhere to international data requirements. The LC₅₀/EC₅₀ from both of these studies are greater than the highest tested concentration. Given the degree to which it passes the risk assessment, and the fact that pydiflumetofen does not match any of the criteria highlighted in the regulation indicates that these studies were gratuitous and as a result have not been used in the risk assessment. The studies have been evaluated for completeness and can be found in CA B 9.1.1.

Table 9.1.1-1: Summary of SYN545794 toxicity endpoints relevant for the risk assessment for birds

Species	Substance	Exposure system	Endpoint	Reference (Author, date)
Acute toxicity				
<i>Colinus virginianus</i>	SYN545794	14 day acute oral study	LD ₅₀ > 2000 mg a.s./kg body weight	█ and █ (2013)
<i>Serinus canaria</i>	SYN545794	14 day acute oral study	LD ₅₀ > 2000 mg a.s./kg body weight	█ and █ (2013a)
Short-term dietary				
<i>Colinus virginianus</i>	SYN545794	8 day dietary study	EC ₅₀ = > 1258 mg a.s./kg body weight/day (nominal concentration)	█ et al. (2013)
<i>Anas platyrhynchos</i>	SYN545794	8 day dietary study (5 days exposure)	LC ₅₀ > 2437 mg a.s./kg bw/day (nominal concentration)	█ , █ & █ (2013a)
Reproductive toxicity				
<i>Colinus virginianus</i>	SYN545794	21 week dietary reproductive study	NOEC = 90.1 mg SYN545794 a.s./kg bw/day	█ et al., (2015)
<i>Anas platyrhynchos</i>	SYN545794	20 week dietary reproductive study	NOEC = 141 mg a.s./kg b.w. body weight/day ((nominal concentration))	█ et al., (2014)

Values in bold will be used in the risk assessment.

There were no metabolites found at ≥ 10% of parent level in edible crop parts in crop metabolism studies (see **M-CA Section 6, Residues**). Mammalian toxicity testing of the common SDHI metabolites SYN508272 and NOA449410 indicates that the metabolites are similarly toxic to parent SYN545794 (see **M-CA Section 5, Toxicology**). It can therefore be concluded that the risk to birds from metabolites formed in edible crop parts will be low and no further risk assessment has been conducted.

Selection of endpoints

Data have been submitted addressing the acute and long term/reproductive toxicity of the active substance SYN545794 (Pydiflumetofen) to birds. These studies address data requirements for active substances as stated

in retained EU regulation 283/2013. The studies are summarised and evaluated in Volume 3CA Part B9 and selection of the endpoints used in the risk assessment is considered below.

Acute oral toxicity endpoints

Two acute studies were submitted with the same endpoint. In the acute oral toxicity studies with SYN545974 no mortalities were observed and the LD₅₀ values were reported as > 2000 mg/kg bw, the highest tested dose. Under point 2.1.2. of the EFSA Bird and Mammal guidance Document (2009) a method has been proposed to extrapolate upwards the LD₅₀ value. The acute toxicity values have been extrapolated and are presented in the table below.

Table 9.1.1-2: Extrapolation of the acute oral toxicity values for SYN545974

Test type	Test substance	Test Species	Experimental LD ₅₀ (mg/kg bw)	Number of animals tested	Number of mortalities	Extrapolation factor	Corrected LD ₅₀ (mg/kg bw)
Acute oral	SYN545974	Bobwhite quail	> 2000	10	0	1.888	3776
		Canary	> 2000	10	0	1.888	3776

Formulation toxicity endpoint

Studies with the representative formulation have not been performed as direct exposure of birds to applications of formulation are considered unlikely. At the time of application and for a short period after, most birds will leave the immediate vicinity of spray operations in response to the human disturbance. Birds are typically exposed to dry residues on their food items following the dilution and spraying of the formulated product. During these processes, much of the formulation constituents are likely to be lost by volatilisation. This is also stated in 2013/283 and 2013/284.

Long-term/reproductive toxicity endpoint

Two reproductive studies were submitted, with a Bobwhite Quail and a Mallard Duck. Both studies were deemed to be reliable, the summaries can be found in Volume 3CA Part B9. The Bobwhite Quail toxicity study resulted in a more sensitive endpoint of 90.1 mg SYN545974/kg bw/day.

Summary of endpoints used for the risk assessment

The table below summarises the endpoints use in the risk assessment.

Table 9.1.1-3: Summary of endpoints used to assess risk from SYN545974 to birds

Test substance	Test type	Test Species	Endpoint	Value	Reference (Author, date)
SYN 545974	Acute Oral	Bobwhite quail (<i>Colinus virginianus</i>)	LD ₅₀ extrapolated	3776 mg a.s./kg bw	██████ and ██████ (2013)
	Dietary reproductive	Bobwhite quail (<i>Colinus virginianus</i>)	NOEC	90.1 mg a.s./kg bw/d	██████ <i>et al.</i> , (2015)

B.9.1.2. Effects on terrestrial vertebrates other than birds

Mammalian toxicity studies have been carried out with SYN545974 (acute, long term). Full details of these studies are provided in DAR section 6 (Toxicology).

Table 9.1.2-1: Summary of SYN545974 toxicity endpoints for mammals

Test type	Test substance	Test species	Endpoint	Value (ppm)	Value (mg a.s./kg bw/d)	Reference (Author, date)
Acute Oral	SYN545974	Rat	LD₅₀	-	> 5000	██████████ (2012)
Two generation reproduction	SYN545974	Rat	NOAEL (parental)	Male:750 Female: 450	Male: 46 Female: 31.6	██████████ (2015)
			NOAEL (reproduction)	Male: 750 Female: 450	Male: 46 Female: 31.6 36.1	
			NOAEL (offspring)	Male: 4500 Female: 1500	Male: 276.6 Female: 116	
Developmental toxicity	SYN545974	Rat	NOAEL (maternal)	-	30	██████████ (2015)
			NOAEL (developmental)	-	100	
		Rabbit	NOAEL (maternal)	-	500	██████████ (2015b)
			NOAEL (developmental)	-	10	
104 week carcinogenicity study with combined 52 week toxicity study	SYN545974	Rat	NOAEL (chronic)	Males: 200 Females: 450	Males: 9.9 Females: 31	██████████ (2015)
			NOAEL (carcinogenicity)	Males: 6000 Females: 1500	Males: 319 Females: 102	
80 week carcinogenicity study	SYN545974	Mouse	NOAEL (chronic)	Males: 75 Females: 375	Males: 9.2 Females: 48.4	██████████ (2015a)
			NOAEL (carcinogenicity)	Males: 75 Females: 2250	Males: 9.2 Females: 306	

Values in bold will be used in the risk assessment.

Selection of the SYN544974 acute mammalian endpoint to be used in the acute risk assessment

There is one acute active substance study submitted for this application. The acute endpoint of > 5000 mg a.s./kg bw/d will be used in the risk assessment.

Selection of the SYN544974 long term endpoint to be used in the chronic risk assessment

The table below outlines the chronic studies from toxicology on SYN544974. These were used to inform the relevant ecotoxicology endpoints.

Table 9.1.2-2: Information from the mammalian toxicology section, relevant to identify the ecotoxicologically relevant reproductive endpoint of mammals

Study & Acceptability	Mode of Dosing	Test material & Dose Levels	NO(A)EL (mg/kg bw/day)	LOAEL (mg/kg/day)	Effects at the LOAEL
Two generation reproductive toxicity study in the rat [REDACTED] , (2015) <i>Modern, valid, guideline study</i>	Dietary	Pydiflumetofen 98.5% Males: 0, 150, 750 & 4500 ppm Females: 0, 150, 450 & 1500 ppm	<i>Parental:</i> <u>Males</u> 750 ppm (46 mg/kg bw/d) <u>Females</u> 450 ppm (31.6 36.1 mg/kg bw/d)	<i>Parental:</i> <u>Males</u> 4500 ppm (276.6 mg/kg bw/d) <u>Females</u> 1500 ppm (116 mg/kg/d)	<i>Parental:</i> ↓(10%) bwg in males in P0 and F1; ↓(8%) food con in males in F1; ↑liver wt and associated hypertrophy in males and females in P0 and F1; ↑thyroid wt and associated hypertrophy in males in P0 and F1;
			<i>Reproduction:</i> <u>Males</u> 750 ppm (46 mg/kg bw/d) <u>Females</u> 450 ppm (31.6 36.1 mg/kg bw/d)	<i>Reproduction:</i> <u>Males</u> 4500 ppm (276.6 mg/kg bw/d) <u>Females</u> 1500 ppm (116 mg/kg/d)	<i>Reproduction</i> Delays in VO and PS in F1 pups
			<i>Offspring:</i> <u>Males:</u> 4500 ppm (276.6 mg/kg bw/d) <u>Females</u> 1500 ppm (116 mg/kg bw/d)	<i>Offspring:</i> <u>Males:</u> >4500 ppm (>276.6 mg/kg bw/d) <u>Females</u> >1500 ppm (>116 mg/kg bw/d)	<i>Offspring</i> No treatment-related effects
Main Developmental toxicity in the rat [REDACTED] , (2015) <i>Modern, guideline study but top dose inadequate</i>	Gavage	Pydiflumetofen 98.5% 0, 10, 30 & 100 mg/kg bw/d	<i>Maternal:</i> 30 mg/kg bw/d <i>Developmental:</i> 100 mg/kg bw/d	<i>Maternal:</i> 100 mg/kg bw/d <i>Developmental:</i> >100 mg/kg bw/d	<i>Maternal:</i> Marginal effects on bodyweight and food consumption during gestation days 6-9. <i>Developmental:</i> None.
Developmental toxicity in the rabbit [REDACTED] , (2015b) <i>Modern, guideline study but top dose inadequate</i>	Gavage	Pydiflumetofen 98.5% 0, 10, 100 & 500 mg/kg bw/d	<i>Maternal:</i> 500 mg/kg bw/d <i>Developmental:</i> 10 mg/kg bw/d	<i>Maternal:</i> >500 mg/kg bw/d <i>Developmental:</i> 100 mg/kg bw/d	<i>Maternal:</i> None. <i>Developmental:</i> Increased incidence of one skeletal variant (rib costal cartilage interrupted) at 100 and 500 mg/kg bw/d without clear dose response but incidence above the HCD

Study & Acceptability	Mode of Dosing	Test material & Dose Levels	NO(A)EL (mg/kg bw/day)	LOAEL (mg/kg/day)	Effects at the LOAEL
104 week rat carcinogenicity study with a combined 52 week toxicity study (██████████, 2015) <i>Modern, valid guideline study</i>	Dietary	Pydiflumetofen 98.5% <u>Males</u> 0, 200, 1000 & 6000 ppm; <u>Females</u> 0, 150, 450 & 1500 ppm	<i>Chronic toxicity</i> <u>Males</u> 200 ppm (9.9 mg/kg bw/d); <u>Females</u> 450 ppm (31 mg/kg bw/d) <i>Carcinogenicity</i> <u>Males</u> 6000 ppm (319 mg/kg bw/d) <u>Females</u> 1500 ppm (102 mg/kg bw/d)	<i>Chronic toxicity</i> <u>Males</u> 1000 ppm (51 mg/kg bw/d) <u>Females</u> 1500 ppm (102 mg/kg bw/d) <i>Carcinogenicity</i> <u>Males</u> >6000 ppm (>319 mg/kg bw/d) <u>Females</u> >1500 ppm (>102 mg/kg bw/d)	<i>Chronic toxicity</i> <u>1000 ppm</u> (mid-dose males): ↓ bw and bwg, food utilization, hepatocyte hypertrophy and ↑liver weight. <u>1500 ppm</u> (top-dose females): ↓ bw and bwg, food utilization, ↑liver weight associated with minimal hepatocellular hypertrophy <i>Carcinogenicity</i> No treatment related neoplastic findings.
80 week mouse carcinogenicity study (██████████, 2015a) <i>Modern, valid guideline study</i>	Dietary	Pydiflumetofen 98.5% 0, 75, 375 & 2250 ppm	<i>Chronic toxicity</i> <u>Males:</u> 75 ppm (9.2 mg/kg bw/d) <u>Females:</u> 375 ppm (48.4 mg/kg bw/d) <i>Carcinogenicity</i> <u>Males:</u> 75 ppm (9.2 mg/kg bw/d) <u>Females:</u> 2250 ppm (306 mg/kg bw/d)	<i>Chronic toxicity</i> <u>Males:</u> 375 ppm (45.4 mg/kg bw/d) <u>Females:</u> 2250 ppm (306 mg/kg bw/d) <i>Carcinogenicity</i> <u>Males:</u> 375 ppm (45.4 mg/kg bw/d) <u>Females:</u> >2250 ppm (>306 mg/kg bw/d)	<i>Chronic toxicity</i> <u>375 ppm (males):</u> ↑liver weight associated with hepatocellular hypertrophy <u>2250 ppm (females):</u> ↓ bw and bwg, food consumption, ↑liver weight. <i>Carcinogenicity</i> Liver tumours in males from 375 ppm. No tumours in females up to 2250 ppm

The proposed long-term endpoint is the NOAEL (reproduction) of **31.6 36.1** mg a.s./kg bw/d from the two generation reproduction study.

Two generation reproduction study

A two generation reproductive study was conducted in the rat ([REDACTED], 2015). Parental effects in the males showed a 10 % decrease in body weight in P and F1, and an 8 % decrease in food consumption in F1. Body weight is considered a relevant endpoint for reproduction in EFSA Bird and Mammal Guidance (2009), as it has potential to disrupt reproduction. Additionally, there was an increase in liver weight in males and females in P and F1, and an increase in thyroid weight in males in P and F1. It is not considered that these effects would go on to disrupt reproduction.

Reproduction effects from the two generation reproductive study showed delays in vaginal opening and balano-preputial separation in F1 pups at 31.6 36.1 mg/kg bw/d. This is a relevant endpoint for reproduction as it could lead to a decrease in the number of litters per year, disrupting reproduction.

There were no treatment related effects on offspring from the two generation reproduction study.

Developmental toxicity study

A developmental toxicity study was performed in both the rat and the rabbit.

The rat developmental toxicity study ([REDACTED], 2015) showed no developmental effects. There were marginal maternal effects on body weight and food consumption during gestation days 6-9. Changes in body weight is an indicator of parental effects with potential to disrupt reproduction, Bird and Mammal Guidance Document (2009), therefore this is a relevant endpoint for reproduction. The body weight changes were observed at the highest test concentration, from the first day of dosing (day 6) to day 10 of gestation, this was resolved by day 11 of gestation and the body weight at the highest dose level was subsequently similar to the controls. An initial reduction (-90 - -18 %) in food consumption was observed between day 6 and day 9 of gestation, however, intake was similar to controls thereafter. As the maternal effects were not long lasting, HSE ecotoxicology considers the endpoint of the two generation reproduction study to be more relevant for risk assessment.

The developmental toxicity study on rabbits ([REDACTED], 2015b) showed no maternal effects. There were developmental effects of an increased incidence of one skeletal variant in foetuses at 100 and 500 mg/kg bw/d. However, there was not a clear dose response and it is not considered that this endpoint is relevant to reproduction.

104 week rat carcinogenicity study

The lowest NOAEL resulted from the 104 week rat carcinogenicity study ([REDACTED], 2015). There were effects on the body weight and food utilisation in males, however, these effects occurred in older animals, and are therefore not considered to be relevant endpoints for reproduction when compare to the effects from other studies.

Selection

HSE has selected the NOAEL of 31.6 36.1 mg/kg bw/d (reproduction) from the two generation reproductive study as it is the endpoint most relevant to reproduction. Whilst there are effects on body weight of rats in the developmental toxicity study, HSE considers the delay in sexual maturity of pups resulting from the rat two generation study to be more relevant to population viability and reproductive performance.

Metabolites

There were no metabolites found at ≥ 10 % of parent level in edible crop parts in crop metabolism studies (see **M-CA Section 6, Residues**). Mammalian toxicity testing of the common SDHI metabolites SYN508272 and NOA449410 indicates that the metabolites are similarly toxic to parent SYN545974 (see **M-CA Section 5, Toxicology**). It can therefore be concluded that the risk to mammals from metabolites formed in edible crop parts will be low and no further risk assessment has been conducted.

Table 9.1.2-3: Summary of endpoints used to assess risk from SYN545974 to mammals

Test substance	Test type	Test Species	Endpoint	Value	Reference (Author, date)
SYN 545974	Acute Oral	Rat	LD ₅₀	> 5000 mg a.s./kg bw/d	[REDACTED] (2012)
	Two generation reproduction	Rat	NOAEL (reproduction)	31.6 36.1 mg a.s./kg bw/d	[REDACTED] (2015)

B.9.2. RISK ASSESSMENT FOR BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.2.1. Risk Assessment for birds

Exposure

Exposure of birds will be predominantly dietary, through the consumption of residues on food items. Exposure is calculated according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009). The proposed uses are summarised in table B 9.1-2.

Screening step

The proposed application is in Cereals and Oilseed Rape in growth stages between BBCH 30-69. The proposed use of SYN545974 is a single maximum application with a maximum application rate of 200 g a.s./ha. The results of the acute and reproductive screening risk assessments are summarised in the following tables.

Table 9.2.1-1: Screening assessment of the acute and long-term/reproductive risk for birds due to SYN545974 use in cereals using the worst-case application rate

Intended use	Cereals				
Active substance	SYN545974				
Application rate (kg/ha)	1 × 0.2				
Acute toxicity (mg/kg bw)	3776				
TER criterion	10				
Crop scenario	Indicator species	SV₉₀	MAF₉₀	DDD₉₀ (mg/kg bw/d)	TER_a
Cereals and Oilseed Rape	Small omnivorous bird	158.8	-	31.76	118.89
Reprod. toxicity (mg/kg bw/d)	90.1				
TER criterion	5				
Crop scenario	Indicator species	SV_m	MAF_m × TWA	DDD_m (mg/kg bw/d)	TER_{lt}
Cereals and Oilseed Rape	Small omnivorous bird	64.8	1 × 0.53	6.87	13.12

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 greater than the trigger value of 10, the reproductive TER value for the intended use is greater than the trigger value of 5. This indicates the risk to birds from consuming residues of SYN545974 on food items resulting from spray application is acceptable according to the representative GAP.

Higher tier risk assessment

A higher-tier risk assessment is not necessary as an acceptable acute and reproductive risk was shown with the screening step risk assessment for the worst-case scenario.

Risk to birds through drinking water

Leaf scenario

EFSA Bird and Mammal Guidance (2009) states the leaf scenario should be considered for leaf vegetables (forming heads) and other leaf vegetables with a morphology that facilitates collection of rain/irrigation water in reservoirs that are large enough and easily accessible to attract birds. Since SYN545974 is intended to be applied on cereals, which do not form heads or have a morphology to collect rain/irrigation, the leaf scenario does not need to 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 ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

SYN545974 has a K_{oc} of 2921, this makes it a more sorptive substance. The TER will be compared to a trigger value of 3000.

Table 9.2.1-2: Summary of the ratio of application rate to toxicity for risk to birds from SYN545974 by drinking water in puddles.

Test substance	K_{oc}	Max. application rate (g/ha)	Acute LD_{50} (mg/kg bw)	Ratio of AR_{eff} / LC_{50}	Long-term NOEL (mg/kg bw/d)	Ratio of $AR_{eff} / NOEL$	Ratio trigger
SYN545974	2921	200	3776	0.052	90.1	2.2	3000

The resulting ratio falls below the trigger of 3000 indicating that further assessment of the acute and long-term risk to birds from drinking water from puddles is not required for SYN545974.

In conclusion, the risk to birds via drinking water from the intended use of SYN545974 according to the proposed use pattern is acceptable.

Effects of secondary poisoning

According to the EFSA/2009/1438, substances with a $\log P_{ow} \geq 3$ have potential for bioaccumulation and should be assessed for the risk of biomagnification in aquatic and terrestrial food chains. The $\log P_{ow}$ of SYN545974 is 3.8, which triggers an assessment of the potential risk from secondary poisoning.

Metabolites

The $\log P_{ow}$ of the aquatic metabolite SYN545547 is 3.59, so further assessment is required for the secondary poisoning via fish, however, HSE Fate and Behaviour have reported that the metabolite is not present in high enough concentrations to trigger a risk assessment, therefore exposure is considered to be negligible. The $\log P_{ow}$ for the aquatic metabolite NOA449410 is 1.06 so the risk from secondary poisoning is considered to be low. The $\log P_{ow}$ for aquatic metabolite SYN548261 is not known, however as the risk from the parent substance and SYN545547 has shown to be low, and due to the low predicted environmental concentrations of SYN548261, risk from secondary poisoning is likely to be low.

Risk to earthworm eating birds

According to EFSA Bird and Mammal Guidance (2009), the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil which is based on experimental data. The maximum PEC_{soil} was used in the assessment, details of PEC soil calculations can be found in DAR Section 8 (fate). The resulting TER value is shown in the table below.

Table 9.2.1-3: Long-term risk from secondary poisoning to earthworm eating birds from SYN545974

Test substance	Max. PEC_{soil} (mg a.s./kg)	K_{ow}	f_{oc}	K_{oc}	$BCF_{earthworm}$	PEC_{worm} (mg a.s./kg)	DDD (mg a.s./kg bw/d)	NOEL (mg a.s./kg bw/d)	TER_{LT}
SYN545974	0.053	6310	0.02	2921	1.31	0.07	0.073	90.1	1234.25

The TER_{LT} for SYN545974 exceeds the long term trigger value of 5 for acceptability of effects, indicating an acceptable risk to earthworm-eating birds via secondary poisoning.

Risk to fish eating birds

According to EFSA/2009/1438, the risk for piscivorous birds is assessed for a bird of 1000 g body weight with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

The maximum PEC_{sw} was used to calculate the risk to fish eating birds. Details of PEC surface water calculations can be found in DAR Section 8 (fate).

Table 9.2.1-4: Long-term risk from secondary poisoning to fish eating birds from SYN545974

Test substance	PEC_{sw} (mg/L)	BCF	PEC_{fish} (mg/kg)	DDD (mg/kg bw/d)	Long-term NOEL (mg/kg bw/d)	TER_{LT}
SYN545974	0.00185	31.1	0.058	0.0092	90.1	9793.48

The TER_{LT} for SYN545974 exceeds the long-term trigger value of 5 for acceptability of effects, indicating an acceptable risk to fish eating birds via secondary poisoning.

Biomagnification in terrestrial food chains

Bioconcentration studies in fish showed rapid depuration of residues of the parent active substance.

Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

Overall conclusions

Overall, it can be concluded that the acute and long-term risk to birds from the application of SYN545974 according to good agricultural practice is acceptable with an application rate of 0.2 kg/ha in cereals.

B.9.2.2. Risk assessment for other terrestrial vertebrates

Exposure

Exposure of mammals will be predominantly dietary, through the consumption of residues on food items. Exposure is calculated according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009). The summary of proposed uses is shown in table B 9.1-2.

Screening step

The proposed application is in Cereals and Oilseed Rape in growth stages between BBCH 30-69. The proposed use of SYN545974 is a single maximum application with a maximum application rate of 200 g a.s./ha. The results of the acute and reproductive screening risk assessments are summarised in the following tables.

Table 9.2.2-1: Screening step- Acute and Reproductive risk to mammals from SYN545974

Intended use		Cereals				
Active substance		SYN545974				
Application rate (kg/ha)		1 × 0.2				
Acute toxicity (mg/kg bw)		> 5000				
TER criterion		10				
Crop scenario	Indicator species		SV ₉₀	MAF ₉₀	DDD ₉₀ (mg/kg bw/d)	TER _a
Cereals and Oilseed Rape	Small herbivorous mammal		118.4	1	23.68	211.15
Reprod. toxicity (mg/kg bw/d)		31.6 36.1				
TER criterion		5				
Crop scenario	Indicator species		SV _m	MAF _m × TWA	DDD _m (mg/kg bw/d)	TER _{It}
Cereals and Oilseed Rape	Small herbivorous mammal		48.9 48.3	1 x 0.53	5.18 5.12	6.1 7.1

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 greater than the trigger value of 10, indicating there is an acceptable risk to birds from the use of SYN545974 according to the proposed use pattern. The reproductive TER value is 6.97 7.1, above the trigger value of 5, at screening step.

Higher tier risk assessment

A higher-tier risk assessment is not necessary as an acceptable acute and reproductive risk was shown with the screening step risk assessment for the worst-case scenario.

Risks for mammals through drinking water

Based on the EFSA Guidance Document the puddle scenario is only considered relevant for assessing the risk to mammals from drinking water.

Puddle scenario

This applies for mammals drinking water from puddles formed on the soil surface of a field after rainfall following the application of a pesticide product to the crop or bare soil.

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 ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

SYN545974 has a K_{oc} of 2921, this makes it a more sorptive substance. The TER will be compared to a trigger value of 3000.

Table 9.2.2-2: Summary of the ratio of application rate to toxicity for risk to mammals from SYN545974 by drinking water in puddles.

Test substance	K _{oc}	Max. application rate (g/ha)	Acute LD ₅₀ (mg/kg bw)	Ratio of AR _{eff} / LC ₅₀	Long-term NOEL (mg/kg bw/d)	Ratio of AR _{eff} / NOEL	Ratio trigger
SYN545974	2941	200	> 5000	0.04	31.6 36.1	6.33	3000

The resulting ratio falls below the trigger of 3000 indicating that further assessment of the acute and long-term risk to mammals from drinking water from puddles is not required for SYN545974.

In conclusion, the risk to mammals via drinking water from the intended use of SYN545974 according to the proposed use pattern is acceptable.

Effects of secondary poisoning

According to the EFSA/2009/1438, substances with a $\log P_{ow} \geq 3$ have potential for bioaccumulation and should be assessed for the risk of biomagnification in aquatic and terrestrial food chains. The $\log P_{ow}$ of SYN545974 is 3.8, which triggers an assessment of the potential risk from secondary poisoning.

Metabolites

The $\log P_{ow}$ of the aquatic metabolite SYN545547 is 3.59, so further assessment is required for the secondary poisoning via fish. However, HSE Fate and Behaviour have reported that the concentration is below the trigger for risk assessment, therefore the exposure is considered to be negligible. The $\log P_{ow}$ for the aquatic metabolite NOA449410 is 1.06 so the risk from secondary poisoning is considered to be low. The $\log P_{ow}$ for aquatic metabolite SYN548261 is not known, however as the risk from the parent substance and SYN545547 has shown to be low, and due to the low predicted environmental concentrations of SYN548261, risk from secondary poisoning is likely to be low.

Risk to earth worm eating mammals

According to EFSA Bird and Mammal Guidance (2009), the risk for vermivorous mammals is assessed for a mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil which is based on experimental data. The maximum PEC_{soil} was used in the assessment, details of PEC_{soil} calculations can be found in DAR Section 8 (fate). The resulting TER value is shown in the table below.

Table 9.2.2-3: Long-term risk from secondary poisoning to earthworm eating mammals from SYN545974

Test substance	Max. PEC_{soil} (mg a.s./kg)	K _{ow}	f _{oc}	K _{oc}	BCF _{earthworm}	PEC _{worm} (mg a.s./kg)	DDD (mg a.s./kg bw/d)	NOEL (mg a.s./kg bw/d)	TER _{LT}
SYN545974	0.053	6310	0.02	2921	1.31	0.069	0.089	31.6 36.1	355.06 405.6

The TER value for SYN545974 is above the long-term trigger value of 5 indicating there is an acceptable risk to earth worm eating mammals from the proposed use.

Risk to fish eating mammals

Bioaccumulation in mammals is estimated based on predicted concentrations in surface water.

The maximum PEC_{sw} was used to calculate the risk to fish eating birds. Details of PEC_{sw} calculations can be found in DAR Section 8 (fate).

Table 9.2.2-4: Long-term risk from secondary poisoning to fish eating mammals from SYN545974

Test substance	PEC _{sw} (mg/L)	BCF	PEC _{fish} (mg/kg)	DDD (mg/kg bw/d)	Long-term NOEL (mg/kg bw/d)	TER _{LT}
SYN545974	0.00185	31.1	0.058	0.0082	31.6 36.1	3853.66 4402.4

The TER value for SYN545974 is above the long-term trigger value of 5 indicating there is an acceptable risk to fish eating mammals from the proposed use.

Biomagnification in terrestrial food chains

Bioconcentration studies in fish showed rapid depuration of residues of the parent active substance.

Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

Overall conclusions

Overall, it can be concluded that the acute and long-term risk to mammals from the application of SYN545974 according to good agricultural practice is acceptable in cereals at an application rate of 0.2 kg/ha

B.9.3. EFFECTS ON AQUATIC ORGANISMS**B.9.3.1. Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes**

Report:	K-CP 10.2.1 [REDACTED], (2019), Pydiflumetofen EC (A21857B) - Acute Toxicity to Fish (Rainbow trout), Static, 96 Hours. Report Number [REDACTED]. [REDACTED] (Syngenta file no. VV-619141)
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GUIDELINES

OECD Guidelines for Testing of Chemicals, Method 203: Fish, Acute Toxicity Test (1992)

Official Journal of the European Communities, Commission Regulation (EC) No 440/2008, Method C.1: Acute Toxicity for Fish (2008)

GLP: Yes

MATERIALS

Test material	Pydiflumetofen EC (A21857B)
Lot/Batch #:	JEA001-118-001
Purity:	5.62 % w/w, corresponding to 61.7 g/L
Density:	1097 kg/m ³
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 0.313, 0.625, 1.25, 2.50 and 5.00 mg A21857B/L
Solvent:	None
Analysis of test concentrations:	Samples from each concentration and the control analysed from fresh (0 hr) and aged test media (96 hr) by LC-MS/MS
Test organisms	
Species:	<i>Oncorhynchus mykiss</i> (Rainbow trout)
Source:	[REDACTED]
Acclimatisation period:	12 days < 5 % mortality during acclimatisation period
Treatment for disease:	None

Weight and length of dilution water control fish:	Average body length (measured at end of exposure): 5.81 cm Average body weight (measured at end of exposure): 2.21 g
Feeding:	Fish were not fed during the exposure
Loading:	0.774 g fish per litre test solution
Test design	
Test vessels:	Glass-aquaria holding 20 L test medium; covered with glass plates
Test medium:	Tap water of local origin; filtered on activated charcoal and aerated for at least 24 h to remove chlorine
Replication:	One replicate for each test concentration and the control
No of fish per tank:	Seven
Exposure regime:	Static
Aeration:	Gentle aeration was provided
Duration:	96 hours
Environmental conditions	
Test temperature:	15 °C constant in a range of ± 1 °C during the test
pH:	6.0 – 8.5
Dissolved oxygen:	≥ 87 % of air saturation value
Hardness of dilution water:	57 mg CaCO ₃ /L
Conductivity:	151 μ S/cm (measured quarterly)
Alkalinity:	0.60 mmol/L (measured quarterly)
Lighting:	Daily 16 hours photoperiod; 60 - 68 Lux

STUDY DESIGN AND METHODS

Experimental dates: 29 May 2017 – 06 June 2017

At the start of the test, a stock solution of 100 mg/L was prepared in dilution water. The stock solution was shaken manually at room temperature until the solution was homogenous, and then used to prepare the required test item concentrations by serial dilution. The test media were mixed with an ultraturrax (1 min, 17000 rpm). No auxiliary solvent or emulsifier was used. The control consisted of dilution water only.

At the start of the test seven fish were randomly allocated to the test concentrations and the dilution water control. Observations for mortalities and symptoms of toxicity were made at 4, 24, 48, 72 and 96 hours. Fish were considered dead if there was no visible movement and if touching of the caudal peduncle produced no reaction. Visible abnormalities such as loss of equilibrium, swimming behaviour, respiratory function etc. were recorded.

Daily measurements of the test solutions were undertaken throughout the 96-hour period for pH, temperature and dissolved oxygen concentration. Visual appearance of the test item in the test medium was assessed at every renewal interval. Light intensity on the surface of the test aquaria was measured at test start.

The test concentration was verified by chemical analysis of A21857B at 0- and 96-hours using LC-MS/MS.

The NOEC was determined directly from the raw data. The LC₅₀-value after 96 hours and the corresponding confidence intervals were determined by non-linear dose response regression, straight line analysis. The following software was used for calculations: Excel, Microsoft Corporation; GraphPad Prism, Graphpad Software, inc.

RESULTS AND DISCUSSION

At the start of the test (0 h), the measured concentrations of pydiflumetofen were between 90 and 97% of the nominal values. At the end of the test (96 h), the measured concentration of pydiflumetofen were in the range of 45 to 75 % of the nominal values. The limit of quantification was 0.250 mg A21857B/L. Nominal test item concentrations of Pydiflumetofen EC (A21857B) were used for the calculation and reporting of results.

Table 9.3.1-1: Analytical results

Nominal concentrations of A21857B (mg/L)	% of nominal measured at 0 hours	% of nominal measured at 96 hours
Dilution water control	< LOQ	< LOQ
0.313	94	49
0.625	97	54
1.25	90	45
2.50	93	52
5.00	95	75

LOQ: 0.250 mg test item/L

The LC₅₀ value after 96 hours and the corresponding confidence intervals were determined by non-linear dose response regression, straight line analysis. The NOEC and LOEC were provided based on observation of the test organisms, including sub lethal findings, without any statistical calculations. The mortality data, sublethal effects and estimated LC₅₀ values are shown in the table below:

Table 9.3.1-2: Effects of A21857B on the survival and behaviour of *Oncorhynchus mykiss*

Nominal concentration (mg A21857B /L)	Mortality observed (cumulative number of dead fish) (n = 7)				
	4 hours	24 hours	48 hours	72 hours	96 hours
Dilution water control	0	0	0	0	0
0.313	0	0	0	0	0
0.625	0	0	0	0	0
1.25	0	0	0	0	0
2.50	0	0 ^a	0 ^a	0 ^a	0 ^a
5.00	0 ^{b,c,d}	0 ^{d,e}	3 ^{d,e}	5 ^f	7
LC ₅₀ (mg/L)	> 5.00	> 5.00	> 5.00	4.07	3.54
95% confidence interval	n.d	n.d	n.d	2.50 – 5.00	2.50 – 5.00
NOEC _{mortality} (mg/L)	2.50				

a. 7 fish showing slow escape reflex. b. 3 fish showing loss of equilibrium. c. 4 fish missing escape reflex. d. all living fish showing hyperventilation. e. 7 fish lying on side. f. 2 fish showing lethargy.

n.d.: could not be determined - 95% confidence limits could not be calculated with the mortality data obtained.

During the exposure, there were no sublethal effects observed for the control and the treatment levels of 0.313, 0.625 and 1.25 mg/L. All fish were showing slow escape reflex at the treatment level of 2.50 mg/L and after 96 hours of exposure. At the 5.00 mg/L treatment level, sublethal effects included hyperventilation, loss of equilibrium, missing escape reflex, lethargy and lying on side.

VALIDITY CRITERIA

The validity criteria for the study were partially met:

Table 9.3.1-3: Compliance with OECD 283 validity criteria

Validity criterion	Required	Obtained
Mortality in the control(s)	≤ 10 %	0 %
Dissolved oxygen concentration	At least 60 % of the air saturation value throughout the test.	Observed > 87 %
Concentration of substance	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.	No- nominal concentrations have been used despite deviations of greater than 20 %.

CONCLUSIONS

Based on nominal concentrations, the 96-hour LC₅₀ for A21857B to rainbow trout (*Oncorhynchus mykiss*) was 3.54 mg A21857B/L and the 96-hour NOEC_{mortality} was 2.50 mg/L.

(██████, 2019)

HSE COMMENTS

This study was conducted in accordance with GLP and follows OECD 203 (1992), which has since been updated (OECD 203, 2019). This study has been evaluated against the most recent guideline, OECD 203 (2019).

OECD 203 (2019) states that where concentrations deviate more than 20 % from nominal, geometric mean measured concentrations should be used. In the aged test media, concentrations ranged from 45-75 % of nominal. Despite the test concentrations not being maintained within 20 % from nominal, study endpoints have been based on nominal concentrations. Study endpoints should be recalculated based on geometric mean-measured concentrations before use in risk assessment.

There is also some uncertainty with regard to the calculation of the 95 % confidence intervals, as they are not mentioned in the statistics section of the appendix. However, the LC₅₀ does appear to fit centrally between the two concentrations. The dose response is steep, changing from 0 to 100 % mortality between 2.5 and 5.0 mg A21857B /L. Although the factor used (2) was within the guideline recommendations (not exceeding 2.2), the guideline recommends the use of lower factors: “*For the definitive test with fish, at least five concentrations in a geometric series with a factor preferably not exceeding 2.2 are used; smaller separation factors of 1.6 to 1.8 should be used whenever possible*”.

Some additional deviations from the guideline are noted. The measured light intensity was only 60-68 lux, much below the guideline recommendation of 540-1000 lux. Additionally, the measured temperature (15 °C) slightly exceeded the guideline range of 10-14 °C. Since there was no mortality or abnormal behaviour in the control fish, these deviations are considered minor and are not thought to have affected the study outcome.

The analytical method has been evaluated by HSE Chemistry specialists in Vol. 3CP Part B5.1.2.5. The following was concluded for this method: “Acceptable method. LOQ: 0.25 mg test item/L, corresponding to 0.014 mg a.s./L in fish dilution water”.

This study does not currently fulfil the validity criteria stipulated in OECD 203 (2019) guidelines since mean-measured concentrations have not been used in calculating the endpoint. As such the 96-hour LC₅₀ of 3.54 mg A21857B /L is not suitable for use in risk assessment.

Following a request for additional information, the applicant has supplied re-calculated endpoints based on mean measured concentrations. The re-calculated results are presented in the table below.

Table 9.3.1-4: Endpoints based on geometric mean measured concentrations of A21857B

Parameter (mg A21857B/L)	Test duration								
	4 hours	24 hours	48 hours	72 hours			96 hours		
	LC ₅₀	LC ₅₀	LC ₅₀	NOEC	LOEC	LC ₅₀	NOEC	LOEC	LC ₅₀
	Based on geometric mean measured test item concentrations								
Value	> 4.24	> 4.24	> 4.24	1.75	4.24	4.10	1.75	4.24	2.84
Lower 95 %-cl	n.a.	n.a.	n.a.	n.a.	n.a.	4.09	n.a.	n.a.	1.29
Upper 95 %-cl	n.a.	n.a.	n.a.	n.a.	n.a.	4.11	n.a.	n.a.	4.06

Cl: confidence limits; n.a.: not applicable.

Therefore, the endpoint suitable for use in risk assessment is:

- 96 h LC₅₀ = 2.84 mg A21857B/L.

Report:	K-CP 10.2.1 [REDACTED], (2019a), Pydiflumetofen EC (A21857B) - Toxicity to the Water Flea <i>Daphnia magna</i> Straus under Laboratory Conditions (Acute Immobilization Test – Static), Report Number 160713SF / DAI17425, Noack Laboratorien GmbH Käthe-Paulus-Straße 1, 31157 Sarstedt Germany, (Syngenta File No. VV-725187)
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Guideline(s): OECD Guidelines for Testing of Chemicals, Method 202: *Daphnia* sp., Acute Immobilisation Test (2004)

Official Journal of the European Communities, Commission Regulation (EC) No 440/2008 Method C.2 (30 May 2008): “*Daphnia* sp. Acute Immobilization Test

GLP: Yes

Duplication (if vertebrate study) Not applicable

MATERIALS

Test Material

Name/code:	Pydiflumetofen EC (A21857B)
Lot/Batch #:	JEA001-118-001
Purity:	Pydiflumetofen / 5.62 % w/w, corresponding to 61.7 g/L
Density:	1,097 kg /m ³
Description:	Light yellow liquid
Stability of test compound:	Stable at <30 °C
Reanalysis/Expiry date:	30 June 2019

Treatments

Test concentrations:	0.625 - 1.25 - 2.50 - 5.00 - 10.0 mg A21857B /L
Solvent:	None
Negative control:	Dilution water without test item
Positive control:	Potassium dichromate at 0.5, 1.0, 2.0, and 4.0 mg /L
Analysis of test concentrations:	Verified via LC-MS/MS in fresh media at the start and end of exposure: Fresh media (0 hours) ranged 93 - 111% of nominal Expired media (48 hours) ranged 74 - 88% of nominal.

Test organisms

Species:	<i>Daphnia magna</i> STRAUS (Clone 5), less than 24 hours old daphnids from a healthy stock. No first brood progeny was used for the test.
Source:	Bred in Noack Labs, Origin: Institut für Wasser-, Boden- und Lufthygiene (WaBoLu), 14195 Berlin, Germany
Feeding:	5 times per week <i>ad libitum</i> with a mix of unicellular green algae, e.g. <i>Pseudokirchneriella subcapitata</i> and <i>Desmodesmus subspicatus</i> , with an algae cell density of $> 10^6$ cells/mL.

Test design

Test vessels:	Glass beakers (4 (ID) x 7 (H) cm), 50 mL capacity, loosely covered with watch glasses
Test medium:	Elendt M4, according to OECD 202
Replication:	4 replicates per group, with 5 daphnids each.
Exposure regime:	Static conditions
Duration:	48 hours

Environmental conditions

Test temperature:	Incubator temperature ranged from 19 – 20 °C during the definitive test.
pH range:	7.25-8.30
Dissolved oxygen:	6.04-10.1 mg/L
Total hardness of dilution water:	165 mg CaCO ₃ /L
Lighting:	16/8 hours light/dark cycle - Diffuse light, light intensity of max. 1500 lux

STUDY DESIGN AND METHODS

Experimental dates: 08 March 2017 to 11 March 2017

The acute toxicity of pydiflumetofen EC (A21857B) to *Daphnia magna* was determined under static conditions. Daphnids were exposed to nominal concentrations of 0.625, 1.25, 2.50, 5.00 and 10.0 mg A21857B /L alongside a dilution water control.

The test medium of the highest nominal concentration of 10.0 mg A21857B /L was prepared by dissolving 5.090 mg of the test item completely in 500 mL of test water. The stock solution was mixed thoroughly by manual agitation until the solution was visually clear. Using this stock solution, the remaining nominal test concentrations were prepared by serial dilution. The control consisted of dilution water only. Test solutions were added to the test vessels and the *Daphnia* added without conscious bias. *Daphnia* used in the experiment were all < 24 hours old, and from a healthy stock.

The immobility of the daphnids was determined by visual observations after 24 and 48 hours of exposure. Organisms unable to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile. Any abnormal behaviour or appearance was also recorded.

The pH, temperature and dissolved oxygen were measured at the start and end of the test in each test concentration and the control. Observation of the appearance of the test media was carried out at 0, 24 and 48 hours. The concentration of the test item was analytically verified for all test concentrations, by chemical analysis of A21857B at 0 and 48 hours using LC-MS/MS detection.

The EC₅₀-values after 24 and 48 hours were calculated by sigmoidal dose-response regression. The respective 95 % confidence limits were calculated from the standard error and the t-distribution. All calculations were carried out from the best-fit values with the software GraphPad Prism. All data were computer-processed and rounded

for presentation. Consequently, minor variations may occur from the original figures if manual calculations based on the original figures are made subsequently.

RESULTS AND DISCUSSION

Validity criteria

Table 9.3.1-5 : Compliance with OECD 202 validity criteria

Validity criterion	Required	Obtained
Immobilisation in the controls	≤ 10 % immobilisation or other signs of disease or stress in the control.	Dilution water control: 0 %
Dissolved oxygen concentration	The dissolved oxygen concentration at the end of the test should be ≥ 3 mg /L in control and test vessels.	Dissolved oxygen concentration was > 6.04 mg /L throughout the test.

The measured concentrations of Pydiflumetofen EC (A21857B) in the fresh media (0 hours) were in the range of 93 to 111% of the nominal values. The measured test item concentrations in the old media (48 hours) were in the range of 74 to 88% of the nominal values. Since all initial measured concentrations were between 80-120 % of nominal, the results have been calculated using the nominal concentrations.

Table 9.3.1-6: Analytical results

Nominal concentrations (mg A21857B /L)	Determined Concentration at 0 hours	Determined Concentration at 48 hours	Mean measured concentration (mg A21857B /L) 0 - 48 hours
Control	< LOQ	< LOQ	n.a.
0.625	0.695	0.548	0.6215
1.25	1.24	0.926	1.083
2.50	2.39	1.86	2.125
5.00	4.63	3.89	4.26
10.0	9.53	7.46	8.495

n.a.: not applicable

LOQ = limit of quantification of the analytical method (0.250 mg test item /L)

There was no immobility observed in the dilution water control, or in the lowest tested concentration of 0.625 mg /L. No behavioural effects, or symptoms of stress, disease, or toxicity were noted. Immobility data and estimated EC₅₀ values are shown in table 9.3.1-7 below, the 48 hour concentration-effect curve is displayed graphically in Figure 9.3.1-1.

Table 9.3.1-7: Effects of A21857B on *Daphnia magna* following exposure for 48-hours in a static test

Nominal concentration (mg A21857B /L)	Immobilised daphnids after 24 hours		Immobilised daphnids after 48 hours	
	Number	%	Number	%
Control	0 / 20	0	0 / 20	0
0.625	0 / 20	0	0 / 20	0
1.25	0 / 20	0	1 / 20	5

Nominal concentration	Immobilised daphnids after 24 hours		Immobilised daphnids after 48 hours	
2.50	9 / 20	45	13 / 20	65
5.00	9 / 20	45	16 / 20	80
10.0	17 / 20	85	20 / 20	100
EC ₅₀ mg/L (95% Confidence limits)	3.94 (2.69 – 6.25)		2.25 (1.98 – 2.54)	
NOEC mg/L	1.25		1.25	
Potassium dichromate reference item EC ₅₀ mg/L (95% Confidence limits)	2.05 (2.00 – 3.71)			

95% Confidence limits are presented in brackets.

The NOEC-values after 24 and 48 hours were empirically derived from the observed immobilization rates. The EC₅₀ values after 24 and 48 hours were derived by sigmoidal-dose response regression.

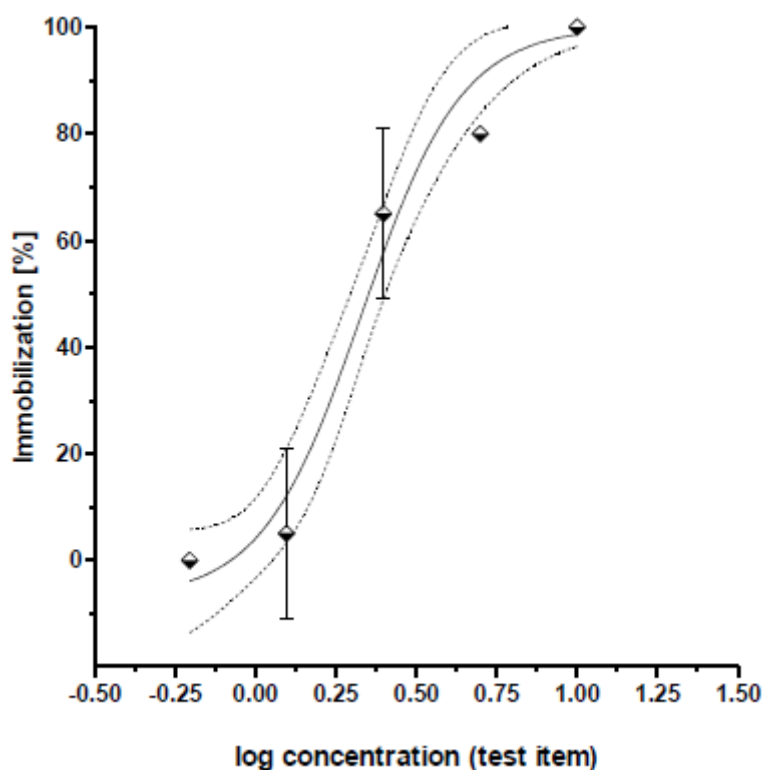


Figure 9.3.1-1: Concentration-effect relationship of pydiflumetofen EC (A21857B) after 48 hours.

CONCLUSIONS

The acute toxicity of A21857B to *Daphnia magna* was determined under static conditions. Daphnids were exposed to nominal concentrations of 0.625, 1.25, 2.50, 5.00 and 10.0 mg A21857B /L alongside a dilution water control. Based on nominal concentration the 48-hour EC₅₀ was 2.25 (1.98 – 2.54) mg A21857B /L and the NOEC was 1.25 mg A21857B /L.

(■■■■, 2019a)

HSE COMMENTS

This study was carried out to GLP and conducted in accordance with OECD 202 (2004): *Daphnia* sp. Acute Immobilisation Test. The following deviation to the guideline (OECD 202) was noted:

Nominal test item concentrations were used for the analysis and reporting of data. The applicant has justified this as “all initial measured concentrations were between 80-120 % of the nominal”. However, the measured concentrations at test completion varied from 74 – 88 % of the nominal values, and so according to the OECD 202 (2004) guidelines, mean measured concentrations should have been used instead.

The study authors state that the media temperature was continuously monitored throughout the test period, although no raw data was provided. It was reported that the incubator temperature ranged from 19-20 °C during the definitive test, and that the temperature of the dilution water was 19.8 °C. As the validity criteria were met, and there were no adverse effects noted in the control condition, this is unlikely to influence the reliability of the data.

The statistical methods used to analyse the data are in line with the guidelines, and visual inspection of the data in Figure 9.3.1-1 supports the calculated endpoints.

The analytical method has been evaluated by HSE Chemistry specialists in Vol. 3CP Part B5.1.2.5. The following was concluded for this method: “Acceptable method. LOQ: 0.25 mg test item/L, corresponding to 0.014 mg a.s./L in daphnia dilution water”. Based on the nominal concentrations, the 48 hour EC₅₀ for A21857B to *Daphnia magna* was 2.25 mg /L, with 95% confidence intervals of 1.98 – 2.54 mg /L. The 48-hour NOEC was 1.25 mg A21857B /L.

Following a request for additional information, the applicant has provided re-calculated endpoints based on mean measured concentrations. These are presented in the table below.

Table 9.3.1-8: Endpoints based on geometric mean measured concentrations of A21857B

Parameter (mg A21857B/L)	Test duration					
	24 hours			48 hours		
	NOEC	LOEC	EC ₅₀	NOEC	LOEC	EC ₅₀
Based on geometric mean measured test item concentrations						
Value	1.07	2.11	3.33	1.07	2.11	1.90
Lower 95 %-cl	n.a.	n.a.	2.27	n.a.	n.a.	1.67
Upper 95 %-cl			5.28			2.15

CI: confidence limits; n.a.: not applicable

Therefore, the endpoint suitable for use in risk assessment is:

- **48 hour EC₅₀ = 1.90 mg A21857B/L**

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

No studies submitted.

B.9.3.3. Further testing on aquatic organisms

Report: K-CP 10.2.1 [REDACTED], (2019b), Pydiflumetofen EC (A21857B) - Acute Toxicity to *Pseudokirchneriella subcapitata* in a 96-Hour Algal Growth Inhibition Test. Report Number 160713SF / SPO17425, Noack Laboratorien GmbH, Käthe-Paulus-Straße 1, 31157 Sarstedt, Germany. (Syngenta File No. VV-619320)

Guideline(s): OECD Guidelines for Testing of Chemicals, Method 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2011)

Council Regulation (EC) No. 266/2016, Method C.3., Official Journal of the European Union (2016)

Deviations: No

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) Not applicable

MATERIALS**Test Material****Name/Code:**

A21857B
Pydiflumetofen EC (062.5)

Lot/Batch #:

JEA001-118-001

Actual content of active ingredient:

Pydiflumetofen: 5.62 % w/w corresponding to 61.7 g/L

Description:

Light yellow liquid

Stability of test compound:

Stable under standard conditions

Reanalysis/expiry date:

30 June 2019

Density

1097 kg/m³

Treatments**Test concentrations:**

Test medium control and nominal concentrations of 1.00, 3.16, 10.0, 31.6 and 100 mg A21857B/L, corresponding to initial measured concentrations of 0.823, 2.59, 7.26, 24.4 and 71.3 mg A21857B/L

Solvent:

None

Positive control:

Potassium dichromate tested on 11 October 2016 – 14 October 2016, (Study number SPO71610)

Analysis of test concentrations:

Yes, analysis of pydiflumetofen at 0 and 96 hours using LC-MS/MS

Test organism**Species:**

Pseudokirchneriella subcapitata HINDÁK, SAG 61.81

Source:

Original cultures were obtained from Sammlung von Algenkulturen (SAG), Pflanzenphysiologisches Institut der Universität Göttingen, Nikolausberger Weg 18, D-37073 Göttingen

Test design**Test vessels:**

250 mL Erlenmeyer flasks sealed with cotton wool plugs, containing 100 mL of test medium

Test medium:

OECD medium

Replication:

6 replicates for the control and 3 replicates for each test concentration

Starting cell density:

Approximately 5×10^3 - 10^4 cells/mL

Exposure regime:

Static

Aeration:

None

Duration:

96 hours

Environmental conditions

Test temperature:	22.0 – 23.0 °C
pH:	Test start: 7.72 – 8.16 Test end: 7.96 – 9.38
Lighting:	Continuous illumination, 4903 – 6327 lux

STUDY DESIGN AND METHODS

Experimental dates: 13 February 2017 to 23 February 2017

A stock solution with a nominal concentration of 100 mg A21857B/L was prepared with dilution water and homogenised by manual shaking. The stock solution was diluted with OECD medium to prepare four additional test solutions at target nominal concentrations of 1.00, 3.16, 10.0 and 31.6 mg A21857B/L. The control consisted of culture medium only.

Algae were added to each test vessel with an initial cell density of approximately $5 \times 10^3 - 10^4$ cells/mL. The flasks were positioned randomly and repositioned daily. Test containers were placed on a rotary shaker and oscillated at approximately 70 rpm. The test was conducted under continuous illumination.

Test medium samples were collected from each replicate of the treatment and control groups at approximately 24-hour intervals during the 96-hour exposure period. The algal cell densities in these samples were measured by chlorophyll a-fluorescence. In addition, after 96 hours of exposure, samples were taken from the control and from all test concentrations. The shape of the algal cells was examined microscopically in these samples.

The pH was measured at the start and end of the test. The room temperature was monitored continuously. The light intensity was measured prior to the start of exposure.

The test concentrations were verified by chemical analysis of pydiflumetofen at 0 and 96 hours, using LC-MS/MS. The algal cell densities were measured at 24, 48, 72 and 96 hours and the mean biomass, growth rate and yield calculated. The 96-hour E_bC_{50} , E_rC_{50} and E_yC_{50} values (defined as the concentration resulting 50 % reduction of each parameter) and their 95 % confidence intervals were calculated using sigmoidal dose-response regression. For the determination of the 72 and 96-hour NOECs, the calculated growth rates, biomass and yields at each test concentration were tested for significant differences compared to the control values by Multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm ($p < 0.05$).

RESULTS AND DISCUSSION

At the start of the test, the measured concentrations of pydiflumetofen were in the range 71 to 82 % of the nominal values and at the end of the test were in the range 53 to 84 % (see table below). The limit of quantification in this study was 0.250 mg A21857B/L corresponding to 0.0141 mg pydiflumetofen/L. The initial measured concentrations were used for the reporting of results.

Table 9.3.3-1: Analytical results

Nominal concentrations (mg A21857B/L)	Nominal concentrations (mg pydiflumetofen/L)	Determined Concentration at 0 hours (mg pydiflumetofen/L)	% of nominal measured at 0 hours	Determined Concentration at 96 hours (mg pydiflumetofen/L)	% of nominal measured at 96 hours
Control	Control	< LCL	-	< LCL	-
1.00	0.0562	0.0463	82	0.0441	78
3.16	0.178	0.146	82	0.150	84
10.0	0.562	0.408	73	0.438	78
31.6	1.78	1.37	77	1.40	79
100	5.62	4.01	71	2.96	53

Lowest calibration level (LCL) = 0.001 mg/L of the standard

Limit of quantification (LOQ) = 0.250 mg A21857B/L corresponding to 0.0141 mg pydiflumetofen/L

Algal Biomass

The algal biomass at 0, 24, 48, 72 and 96 hours were calculated for each replicate culture and the means are shown below.

Table 9.3.3-2: Values for the control and test item treatment of A21857B for the density of algal cultures at 24, 48, 72 and 96 hours for *Pseudokirchneriella subcapitata*

Initial measured concentrations (mg A21857B/L)	Density of algal cells (cells/mL)				
	0 h	24 h	48 h	72 h	96 h
Control	6839	35755	389101	1909551	3148195
0.823	6839	33068	397782	2041894	2682141
2.59	6839	24749	244592	1755068	2717331
7.26	6839	11524	27289	103964	724725
24.41.6	6839	3549	n.a.	n.a.	4053
71.3	6839	n.a.	n.a.	n.a.	2980

n.a. = not applicable as cell density < LOQ of the cell density (2736 cells/mL)

Growth rate, yield and biomass (area under the growth curve)

Table 9.3.3-3: Mean values for the control and test item treatment of A21857B for the percent inhibition of growth rate and AUC at 72 hours for *Pseudokirchneriella subcapitata*

Initial measured concentrations (mg A21857B/L)	0 to 72 h					
	Biomass (d ⁻¹)	Percentage inhibition of biomass	Growth rate (d ⁻¹)	Percentage inhibition of growth rate	Yield (cells/mL)	Percentage inhibition of yield
Control	1362534	-	1.88	-	1902712	-
0.823	1434699	-5	1.90	-1	2035055	-7
2.59	1129777*	17	1.85*	1	1748229*	8
7.26	73697*	95	0.903*	52	97125*	95
24.4	n.a.*	100	n.a.*	100	n.a.*	100
71.3	n.a.*	100	n.a.*	100	n.a.*	100

* = Denotes a statistically significant change in comparison to the control at p < 0.05 according to Dunnett's

Multiple t-test for growth rates and Williams Multiple t-test for yield and biomass

n.a. = not applicable

Negative value represents stimulation compared to the control

Table 9.3.3-4: Mean values for the control and test item treatment of A21857B for the percent inhibition of growth rate and AUC at 96 hours for *Pseudokirchneriella subcapitata*

Initial measured concentrations (mg A21857B/L)	0 to 96 h					
	Biomass (d ⁻¹)	Percentage inhibition of biomass	Growth rate (d ⁻¹)	Percentage inhibition of growth rate	Yield (cells/mL)	Percentage inhibition of yield
Control	3884568	-	1.53	-	3141356	-
0.823	3789877	2	1.49	3	2675302	15
2.59	3359138*	14	1.50*	2	2710492*	14
7.26	481203*	88	1.16*	24	717886*	77
24.4	n.a.*	100	n.a.*	100	n.a.*	100
71.3	n.a.*	100	n.a.*	100	n.a.*	100

* = Denotes a statistically significant change in comparison to the control at p < 0.05 according to Dunnett's

Multiple t-test for growth rates and Williams Multiple t-test for yield and biomass

n.a. = not applicable

Table 9.3.3-5: Summary of biological results for toxicity of A21857B to *Pseudokirchneriella subcapitata* after 72 and 96 hours

Parameter	after 72 h (mg A21857B/L)			after 96 h (mg A21857B/L)		
	Growth rate	Yield	Biomass	Growth rate	Yield	Biomass
EC ₅₀	7.12	3.93	3.65	8.21	6.28	4.35
95% CI	6.94 – 7.28	3.50 – 4.58	3.29 – 4.28	4.00 – 17.6	3.11 – 7.24	3.89 – 4.84
EC ₂₀	4.98	3.02	2.69	7.06	4.91	2.98
95% CI	4.41 – 5.47	2.78 – 3.43	2.48 – 2.97	3.56 – 16.7	2.56 – 7.22	2.64 – 3.42
EC ₁₀	4.07	2.67	2.32	6.40	n.c.	2.32
95% CI	3.51 – 4.73	2.44 – 2.99	2.05 – 2.56	3.23 – 16.4		1.89 – 2.77
NOEC	2.59	2.59	0.823	2.59	n.d.	0.823
LOEC	7.26	7.26	2.59	7.26	0.823	2.59

n.c. = not calculable

n.d. = not determined

All NOEC/ LOEC values were determined using Multiple Sequentially-rejective Welsh-t-test after Bonferroni-Holm

Determination of EC-values was done by sigmoidal-dose response analysis

VALIDITY CRITERIA

The test was considered valid:

- The cell density in the control cultures had a 279 fold increase within 72 hours and a 460 fold increase within 96 hours (must be ≥ 16 within 72 hours and 96 hours).
- The mean coefficient of variation for section-by-section specific growth rates (days 0 – 1, 1 – 2 and 2 – 3) in the control were 25.1 % after 72 hours and 51.4 % after 96 hours (must be ≤ 35 %).
- The coefficient of variation of average specific growth rates in replicate control cultures were 2.36 % after 72 hours and 1.11 % after 96 hours (must be ≤ 7 %).

CONCLUSIONS

The toxicity of A21857B to the green alga *Pseudokirchneriella subcapitata* was investigated in a 96-hour static test. Algae were exposed to nominal concentrations of 1.00, 3.16, 10.0, 31.6, and 100 mg A21857B/L alongside a culture medium control. Based on initial measured concentrations, the 96-hour E_rC₅₀, E_bC₅₀ and E_yC₅₀ were 8.21, 4.35 and 6.28 mg A21857B/L, respectively. The 96-hour NOEC values for growth rate and biomass were 2.59 and 0.823 mg A21857B/L, respectively. The NOEC based on yield could not be determined.

(■■■■■, 2019b)

HSE COMMENTS**Table 9.3.3-6 : Compliance with OECD 201 validity criteria**

Validity criteria OECD 201 (2011)	Required	Obtained
Biomass in the control(s)	Increased by a factor of ≥ 16 within 72 hours	Cell growth increased by a factor of 279 after 72 hours (specific growth rate 1.88 day ⁻¹).
Coefficient of variation for section-by-section specific growth rates in control	Must not exceed 35 %	25.1 % (72 h)
Coefficient of variation of average specific growth rates in the control(s)	Must not exceed 7 % in tests with <i>Pseudokirchneriella subcapitata</i> .	2.36 % (72 h)

The above table shows validity criteria for the study. The 72 hour endpoints have all met the validity criteria. The mean coefficient of variation for section by section specific growth rates in the controls exceeded 35 % for 96 hour control values (51.4 %). As EU studies use the 72 hour control values this does not invalidate the study.

The study was carried out to GLP and follows guidance document OECD 201 (2006). The study was reviewed according to OECD 201 (2011). There were no deviations to the guidelines.

To calculate the EC_x values, sigmoidal dose-response regression was used. The LOEC and NOEC were checked for normal distribution using Shapiro-Wilk's test and checked for homogeneity with Levene's test. The test concentration was compared to the control with Multiple sequentially-rejective Welch t-test after Bonferroni-Holm. The analysis used is mentioned in OECD 201 and the endpoints calculated appeared to be in-line with the experimental data. Therefore, HSE considers the statistical analysis conducted appropriate.

The analytical method has been evaluated by HSE Chemistry specialists in Vol. 3CP Part B5.1.2.5. The following was concluded for this method: "Acceptable method. LOQ: 0.25 mg test item/L, corresponding to 0.014 mg a.s./L in algae dilution water. It should be noted a significant decline in the content of the test item is observed after 96 hours". The initial measured concentrations were not within ± 20 % of the nominal. Additionally, at the highest test concentration, levels were not maintained within 20 % of the initial concentration (73.8 %). Therefore, EC_x values should be based on geometric mean measurements.

Following a request for additional information, the applicant has provided recalculated endpoints based on geometric mean measured concentrations. These are presented in the table below.

Table 9.3.3-7: 72 and 96 hour endpoints based on geometric mean measured values of A21857B

Parameter (mg A21857B/L)	Growth rate			Yield			Biomass		
	ErC10	ErC20	ErC50	EyC10	EyC20	EyC50	EbC10	EbC20	EbC50
	Based on geometric mean measured values of A21857B								
	Values after 72 hours of exposure								
Value	4.20	5.14	7.38	2.70	3.07	4.01	2.34	2.73	3.72
Lower 95 %-cl	3.59	4.53	7.19	2.46	2.81	3.57	2.06	2.50	3.34
Upper 95 %- cl	4.90	5.66	7.54	3.04	3.49	4.69	2.59	3.01	4.38
NOEC	2.62			2.62			0.803		
LOEC	7.52			7.52			2.62		
	Values after 96 hours of exposure								
Value	6.62	7.31	8.52	n.d.	5.02	6.48	2.35	3.02	4.45
Lower 95 %-cl	3.43	3.80	7.54	n.a.	2.59	3.17	1.90	2.67	3.97
Upper 95 %- cl	16.5	16.8	17.7	n.a.	7.47	7.50	2.81	3.48	4.96
NOEC	2.62			< 0.803			0.803		
LOEC	7.52			0.803			2.62		

Cl: confidence limits; n.a.: not applicable; n.d.: not determined.

Therefore, the endpoint suitable for use in risk assessment is:

- 72 hour E_rC_{50} = 7.38 mg A21857B/L

B.9.4. RISK ASSESSMENT FOR AQUATIC ORGANISMS

The following risk assessment has been conducted according to the EFSA (2013)¹ guidance document.

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
SYN545547	Sediment
SYN548261	Water
NOA449410 (M700F001)	Water

Toxicity

Active substance (pydiflumetofen)

The data available to address the toxicity of the active substance, pydiflumetofen, 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 pydiflumetofen

Test substance	Test organism	Test system	Endpoint (mg a.s./L)		Reference
Acute toxicity to fish					
Pydiflumetofen SYN545974	<i>Lepomis macrochirus</i>	96-hours, flow-through	LC ₅₀	0.48 (m.m)	[REDACTED] (2014)
			NOEC	0.2 (m.m)	
Pydiflumetofen SYN545974	<i>Oncorhynchus mykiss</i>	96-hours, flow-through	LC ₅₀	0.18 (m.m)	[REDACTED] (2012)
Pydiflumetofen SYN545974	<i>Pimephales promelas</i>	96-hours, flow-through	LC ₅₀	0.35 (m.m)	[REDACTED] (2013)
			NOEC	0.24 (m.m.)	
Pydiflumetofen SYN545974	<i>Cyprinus carpio</i>	96-hours, flow-through	LC ₅₀	0.33 (m.m)	[REDACTED] (2013a)
			NOEC	0.13 (m.m)	
Pydiflumetofen SYN545974	<i>Cyprinodon variegatus</i>	96-hours, flow-through	LC ₅₀	0.66 (m.m)	[REDACTED] (2013b)
			NOEC	0.48 (m.m)	
Long-term toxicity to fish					
Pydiflumetofen SYN545974	<i>Pimephales promelas</i>	32-days, flow-through	EC ₁₀	0.13 (body weight)	[REDACTED] (2020)
			EC ₂₀	0.32	
			NOEC	0.025 (length, survival and body weight) (m.m)	

¹ EFSA Journal 2013;11(7):3290

Test substance	Test organism	Test system	Endpoint (mg a.s./L)		Reference
Pydiflumetofen SYN545974	<i>Cyprinodon variegatus</i>	34-days, flow-through	NOEC	0.17 (m.m)	(2015) ; (2016a) (statistical reanalysis)
			EC ₁₀	0.34 (CI 0.12-0.58) (m.m)	
Bioconcentration in fish					
Pydiflumetofen SYN545974	<i>Lepomis macrochirus</i>	26-days, flow-through	Steady state bioconcentration factor (BCF _{ss}) whole fish	27.7 L/kg	(2017)
			Lipid normalised steady state bioconcentration factor (BCF _{ssl}) whole fish	31.1 L/kg	
			Depuration half-life whole fish	0.41 days	
Acute toxicity to invertebrates					
Pydiflumetofen (SYN545974)	<i>Daphnia magna</i>	48-hours, static	EC ₅₀	0.42 (m.m)	(2017)
Pydiflumetofen SYN545974	<i>Chaoborus crystallinus</i>	48-hours, static	EC ₅₀	2.489 (m.m)	(2015)
			NOEC	1.59 (m.m)	
Pydiflumetofen (SYN545974)	<i>Cloeon dipterum</i>	48-hours, static	EC ₅₀	>5.01(m.m)	(2015a)
			NOEC	1.59 (m.m)	
Pydiflumetofen (SYN545974)	<i>Crassostrea virginica</i>	96-hours, flow-through	EC ₅₀ (shell deposition)	0.31 (m.m)	(2014a) Not suitable for use in risk assessment
Pydiflumetofen SYN545974	<i>Cyclops agilis speratus</i>	48-hours, static	EC ₅₀	4.168 (m.m)	(2015b)
			NOEC	1.94 (m.m)	
Pydiflumetofen SYN545974	<i>Asellus aquaticus</i>	48-hours, static	EC ₅₀	4.209 (m.m.)	(2015)
			NOEC	1.94 (m.m)	
Pydiflumetofen SYN545974	<i>Crangonyx pseudogracilis</i>	48-hours, static	EC ₅₀	1.226 (m.m.)	(2015b)
			NOEC	0.333 (m.m.)	
Pydiflumetofen SYN545974	<i>Lumbriculus variegatus</i>	48-hours, static	EC ₅₀	4.651 (m.m.)	(2015c)
			NOEC	3.14 (m.m.)	
Pydiflumetofen SYN545974	<i>Lymnaea stagnalis</i>	48-hours, static	EC ₅₀	>7.30 (m.m)	(2015d)
			NOEC	7.30 (m.m)	
Pydiflumetofen SYN545974	<i>Chironomus riparius</i>	48-hours, static	EC ₅₀	0.691 (m.m)	(2015a)
			NOEC	0.351 (m.m)	
Pydiflumetofen SYN545974	<i>Hyaella azteca</i>	48-hours, static	EC ₅₀	0.12 (m.m)	(2015) et al

Test substance	Test organism	Test system	Endpoint (mg a.s./L)		Reference
Pydiflumetofen SYN545974	<i>Americamysis bahia</i>	96-hours, static	LC ₅₀	0.16 (m.m)	(2016)
Long-term toxicity to invertebrates					
Pydiflumetofen (SYN545974)	<i>Americamysis bahia</i>	28-days, flow-through	NOEC	0.037 (nom.)	(2015a)
			EC ₂₀	n.d.	
			EC ₁₀	n.d.	
Pydiflumetofen SYN545974	<i>Daphnia magna</i>	21-days, static-renewal	NOEC	0.042 (m.m)	(2016a)
			EC ₁₀ (reproduction)	0.085 (m.m)	
Toxicity to sediment-dwelling organisms					
Pydiflumetofen (SYN545974)	<i>Chironomus dilutus</i>	59-days, static spiked sediment	NOEC (59-day percent emergence)	15 mg a.s./kg sediment (m.m)	(2015) Not suitable for use in risk assessment
Pydiflumetofen (SYN545974)	<i>Hyalella azteca</i>	42-days, static spiked sediment (with surface water renewal)	NOEC (42-day reproduction)	88 mg a.s./kg sediment (m.m)	(2015a)
			NOEC (28, 35 and 42 day survival)	36 mg a.s./kg sediment (m.m)	
			EC ₂₀ survival (28 days)	> 88 mg a.s./kg sediment (m.m)	
			EC ₂₀ growth (28 and 42 days)	> 88 mg a.s./kg sediment (m.m)	
			EC ₁₀ growth (28 and 42 days)	> 88 mg a.s./kg sediment (m.m)	
Pydiflumetofen (SYN545974)	<i>Leptocheirus plumulosus</i>	10-days, static	LC ₅₀	> 89 mg a.s./kg sediment(g.m)	(2015b)
Toxicity to algae					
Pydiflumetofen (SYN545974)	<i>Skeletonema costatum</i>	96-hours, static	ErC ₅₀	2.7 g.m. (72 h)	(2014)
			ErC ₂₀	2.5 g.m. (72 h)	
			ErC ₁₀	2.5 g.m. (72 h)	
			E _v C ₅₀	2.7 g.m. (72 h)	
			E _v C ₂₀	2.5 g.m. (72 h)	
			E _v C ₁₀	2.5 g.m. (72 h)	
			NOEC	2.4 g.m. (72 h)	
Pydiflumetofen (SYN545974)	<i>Anabaena flos-aquae</i>	96-hours, static	ErC ₅₀	3.4 (m.m)	(2013)
			E _v C ₂₀	3.1 (m.m)	
			E _b C ₁₀	3.4 (m.m)	
			NOEC (growth rate)	0.28 (m.m)	
			ErC ₅₀ (72 h)	3.6 (m.m)	
			ErC ₂₀ (72 h)	3.0 (m.m)	
			ErC ₁₀ (72 h)	2.8 (m.m)	
			E _v C ₅₀ (72 h)	3.5 (m.m)	
			E _b C ₅₀ (72 h)	3.6 (m.m)	
			NOEC(72 h)	2.7 (m.m)	

Test substance	Test organism	Test system	Endpoint (mg a.s./L)		Reference
Pydiflumetofen SYN545974	<i>Pseudokirchneriella subcapitata</i>	96-hours, static	ErC ₅₀	> 5.9 (m.m.) (72 h)	<div></div> (2013)
			ErC ₂₀	5.7 (m.m.) (72 h)	
			ErC ₁₀	2.3 (m.m.) (72 h)	
			NOEC	0.9 (m.m.) (72 h)	
Pydiflumetofen SYN545974	<i>Navicula pelliculosa</i> , strain 661	96-hours, static	ErC ₅₀ (72 h)	1.6 (m.m)	<div></div> (2015)
			ErC ₂₀ (72 h)	1.1 (m.m)	
			ErC ₁₀ (72 h)	0.97 (m.m)	
			E _y C ₅₀ (72 h)	1.5 (m.m)	
			E _y C ₂₀ (72 h)	0.97 (m.m)	
			E _y C ₁₀ (72 h)	0.68 (m.m)	
			NOEC (72 h growth and yield)	0.89 (m.m)	
			ErC ₅₀ (96 h)	1.5 (m.m)	
			E _y C ₅₀ (96 h)	1.1 (m.m)	
			NOEC (96 h growth and yield)	0.31 (m.m)	
Toxicity to aquatic macrophytes					
Pydiflumetofen SYN545974	<i>Lemna gibba</i>	7-days, semi-static (media replaced on D3 & D5)	E _y C ₅₀	> 6.3 (m.m.)	<div></div> (2015a) Not suitable for use in risk assessment
			E _y C ₂₀	> 6.3 (m.m.)	
			E _y C ₁₀	> 6.3 (m.m.)	
			ErC ₅₀	> 6.3 (m.m.)	
			ErC ₂₀	> 6.3 (m.m.)	
			ErC ₁₀	> 6.3 (m.m.)	
			NOEC	6.3 (m.m.)	

nom. = nominal; m.m. = arithmetic mean measured; g.m. = geometric mean measured.

Bold values are recommended for use in risk assessment.

Selection of endpoints for Tier 1 risk assessment for the active substance Pydiflumetofen

The following endpoints were selected for use in the tier 1 risk assessment for Pydiflumetofen.

Acute toxicity to fish: Five acute toxicity studies were conducted with fish using the following species: *Onchyrinchus mykiss*, *Lepomis macrochirus*, *Pimephales promelas*, *Cyprinus carpio* and *Cyprinodon variegatus*. All five studies were considered valid for use in risk assessment. As such, the lowest endpoint from the study with *O. mykiss*, an **LC₅₀ value of 180 µg a.s./L (m.m.)**, has been used in the risk assessment.

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 and were conducted in accordance with OECD 210 guidelines. The study with *P. promelas* resulted in the lowest endpoints, with an EC₁₀ of 0.15 mg a.s./L (based on body length) or 0.13 mg a.s./L (based on body weight). As the most critical value, the **EC₁₀ based on body weight of 130 µg a.s./L (m.m.)** has been used in the risk assessment.

Acute toxicity to aquatic invertebrates: A total of 12 acute aquatic invertebrate toxicity studies with the active substance were conducted. According to the data requirements under EU Regulation 283/2013, data on *Daphnia magna* are always required, and, for active substances which show insecticidal activity a second species should be tested, for example with Chironomid larvae or Mysid shrimps. To fulfil this data requirement, a study with *Daphnia magna* and a study with *Americamysis bahia* were submitted. The remaining studies with additional invertebrate species were conducted for refinement, following risks identified at tier 1. All studies were considered valid, excluding the study with *Crassostrea virginica*, which failed to meet the validity criteria for mean shell

deposition in the control. Although not a standard species, the study with *Hyaella Azteca* resulted in the lowest endpoint, an **EC₅₀ of 120 µg a.s./L (m.m)**, which has been used in the risk assessment.

Chronic toxicity to aquatic invertebrates: two chronic studies with aquatic invertebrates were conducted; one with *D. magna* and one with *A. bahia*. Both studies were considered valid. For the study with *A. bahia*, the applicant calculated a 28-day NOEC of 76 µg a.s./L. This has been refined by the HSE assessor to a more conservative NOEC of 37 µg a.s./L, due to a reduction in the number of offspring produced at 76 µg a.s./L compared to lower concentrations. Although not statistically significant, this reduction is considered to potentially be biologically relevant. EC_{10/20} values were requested from the applicant during the EU evaluation of Pydiflumetofen, however could not be calculated due to the lack of treatment-related effects. As the lowest endpoint, the **28-day NOEC of 37 µg a.s./L (nom.)** from the study with *A. bahia* has been used in the risk assessment.

Toxicity to sediment-dwelling organisms: three studies (two chronic and one acute) were conducted with the following sediment-dwelling organisms: *Chironomus dilutus*, *Hyaella azteca* and *Leptocheirus plumulosus*. The data requirements under point 8.2.5.3/4 from 283/2013 require an EC_{10/20} and NOEC for sediment dwellers. As such, the acute study with *Leptocheirus plumulosus* has not been considered in this risk assessment. The lowest endpoint from the chronic studies was from the study with *Chironomus dilutus* however, the validity criteria were not fully satisfied as the oxygen concentration in the overlying water was not maintained at > 60 % ASV. Therefore, this study endpoint has not been considered in the risk assessment. The chronic study with *Hyaella Azteca* was valid and resulted in a **42-day NOEC (survival) of 36000 µg a.s./ kg sediment (m.m.)**, which has been used in the risk assessment. EC_{10/20} values were calculated by the applicant but were not considered reliable due to missing or overly wide confidence intervals.

Toxicity to algae: Four studies were conducted with the active substance to assess toxicity to algae, one using the green algae *Pseudokirchneriella subcapitata* and three using the following additional algal species; *Skeletonema costatum*, *Anabaena flos-aquae*, and *Navicula pelliculosa*. The study with *Anabaena flos-aquae* did not meet the validity criterion for section-by-section growth rate coefficient of variation, marginally exceeding the target by 5 %. This was attributed to the filamentous growth structure of this species, which differs from the growth patterns of green algae. Since the other validity criteria were met in this study, including the overall coefficient of variation, HSE considers this study to be valid. All other studies were considered valid, and the endpoint from the study with *Navicula pellicula* was the lowest. Therefore, this endpoint, a **72 hr E_rC₅₀ of 1600 µg a.s./L (m.m)**, has been used in the risk assessment.

Toxicity to aquatic macrophytes: only one study assessing the toxicity to aquatic macrophytes with the active substance is available, using *Lemna gibba*. The validity criteria were met, however, the test item concentration was not maintained within ± 20 % of the nominal value in the 10 mg/L treatment and additional analytical measurements were not taken at each subsequent media renewal. This generates uncertainty in the endpoints derived, especially considering significant effects were seen at the second highest treatment level (3.1 mg a.s./L) but not the highest (10 mg a.s./L), where test item concentration was not maintained. Following a request for additional information, the applicant provided the following statement: “A decrease of exposure concentrations was not foreseen and therefore, no additional sampling dates were planned. Explanation regarding the possible cause of the decrease is given in the report. No negative effects were observed in this study. The endpoints are given based on mean measured concentrations providing the worst-case”. This is not considered adequate justification for the lack of additional sampling. Since the test concentration cannot be verified across the study duration, the endpoints are not considered reliable for use in risk assessment. However, this will not form a data gap, since, according to EU Regulation 283/2013, laboratory tests with *Lemna* are only required for herbicides, plant growth regulators and where there is evidence from studies with non-target plants that the substance has herbicidal activity. The available data from non-target plants (section 9.12) do not indicate that Pydiflumetofen has herbicidal activity, therefore the absence of a reliable *Lemna* study does not constitute a data gap.

As per EFSA (2013), the risk from the active substance will be assessed using the appropriate PEC values for the proposed uses of Pydiflumetofen. Endpoints are compared to an Assessment Factor (AF) and the values used (100 for acute endpoints and 10 for chronic endpoints) are taken from Commission Implementing Regulation 546/2011. RAC values will be compared to the relevant PEC 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. The most sensitive endpoint for each species has been used in calculating the RAC.

Table 9.4-3: Regulatory acceptable concentrations (RAC) for Pydiflumetofen for each organism group

Test species:	Fish		Invertebrates		Sediment-dwelling organisms	Algae
	Acute <i>O. mykiss</i>	Chronic <i>P. promelas</i>	Acute <i>H. azteca</i>	Chronic <i>A. bahia</i>	Chronic <i>H. azteca</i> ¹	<i>N. pelliculosa</i>
Endpoint	LC ₅₀	EC ₁₀	EC ₅₀	NOEC	NOEC	E _r C ₅₀
[µg a.s./L]	180	130	120	37	36000	1500
AF	100	10	100	10	10	10
RAC [µg a.s./L]	1.8	13	1.2	3.7	3600	150

¹ Spiked sediment study; value is expressed as µg/kg sediment

Metabolites of Pydiflumetofen

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

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

Test substance	Test organism	Test system	Endpoint (mg a.s./L)		Reference
Acute toxicity to fish					
SYN545547	<i>Oncorhynchus mykiss</i>	96-hours, static	LC ₅₀	1.4 (g.m.)	[REDACTED] (2015)
			NOEC	0.2 (g.m.)	
SYN548261	<i>Oncorhynchus mykiss</i>	96-hours, static	LC ₅₀	>100 (nom.)	[REDACTED] and [REDACTED] (2016)
M700F001 (NOA449410)	<i>Oncorhynchus mykiss</i>	96-hours, static	LC ₅₀	>100 (nom.)	[REDACTED] (2009)
			NOEC	100 (nom.)	
Acute toxicity to invertebrates					
SYN545547	<i>Daphnia magna</i>	48-hours, static	EC ₅₀	7.3 (m.m.)	[REDACTED] (2015a)
			NOEC	2.5 (m.m.)	
SYN548261	<i>Daphnia magna</i>	48-hours, semi-static	EC ₅₀	>100 (nom.)	[REDACTED] and [REDACTED] (2016a)
			NOEC	100 (nom.)	
M700F001 (NOA449410)	<i>Daphnia magna</i>	48-hours, static	EC ₅₀	>100 (nom.)	[REDACTED] (2009a)
			NOEC	100 (nom.)	
Toxicity to sediment-dwelling organisms					
SYN545547	<i>Chironomus riparius</i>	28-days, static	NOEC (male development)	7.2 (m.m.)	[REDACTED] (2015)
Toxicity to algae					
SYN545547	<i>Pseudokirchneriella subcapitata</i>	96-hours, static	E _r C ₅₀	4.0 (m.m.)	[REDACTED] (2015)
			E _y C ₅₀	2.4 (m.m.)	
			E _b C ₅₀	2.6 (m.m.)	
			NOEC	1.0 (m.m.)	
			E _r C ₅₀ (72h)	4.1 (m.m.)	
			E _y C ₅₀ (72h)	2.9 (m.m.)	
			E _b C ₅₀ (72h)	3.0 (m.m.)	
			NOEC (72h)	2.0 (m.m.)	

M700F001 (NOA449410)	<i>Pseudokirchneriella subcapitata</i>	72-hours, static	E _r C ₅₀	36.31 (nom.)	[REDACTED] (2009b)
			E _r C ₂₀	25.03 (nom.)	
			E _r C ₁₀	20.61 (nom.)	
			E _y C ₅₀	26.42 (nom.)	
			E _y C ₂₀	21.60 (nom.)	
			E _y C ₁₀	19.43 (nom.)	
SYN548261	<i>Pseudokirchneriella subcapitata</i>	96-hours, static	E _r C ₅₀ (72 h)	> 100 (nom.)	[REDACTED] and [REDACTED] (2016b)
			E _y C ₅₀ (72 h)	> 100 (nom.)	
			NOEC (72 h growth rate and yield)	> 100 (nom.)	

nom. = nominal; m.m. = arithmetic mean measured; g.m. = geometric mean measured.

HSE Environmental Fate and Behaviour have indicated that the metabolite SYN545547 only triggers assessment in sediment. Therefore, the endpoints for fish, aquatic invertebrates and algae have been listed here for completeness but will not be considered in the risk assessment since these studies do not consider exposure via spiked sediment. The corresponding RACs for use in risk assessment are detailed in Table 9.4-5 below.

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

Test species:	Fish (Acute)		Aquatic invertebrates (Acute)		Sediment dwelling organisms	Algae	
	<i>O. mykiss</i>		<i>D. magna</i>		<i>Chironomus riparius</i>	<i>Pseudokirchneriella subcapitata</i>	
Metabolite:	M700F001 (NOA449410)	SYN548261	M700F001 (NOA449410)	SYN548261	SYN545547	M700F001 (NOA449410)	SYN548261
Endpoint [µg metabolite/L]	>100,000	>100,000	>100,000	>100,000	7200	36310	>100,000
AF	100	100	100	100	10	10	10
RAC [µg metabolite/L]	1000	1000	1000	1000	720	3631	10,000

Formulation ‘Miravis Plus’

Toxicity data, regarding the representative formulation ‘Miravis Plus’, available for use in risk assessment are summarized in Table 9.4-6 below.

Table 9.4-6: Summary of toxicity data related to the representative formulation ‘Miravis Plus’.

Test substance	Test organism	Test system	Endpoint in mg product/L [mg a.s./L]		Reference
Acute toxicity to fish					
Miravis Plus A21857B	<i>Onchyrhynchus mykiss</i>	96-hours, static	LC ₅₀	2.84 (g.m.) [0.160]	█ (2019)
Miravis Plus A21857B	<i>Daphnia magna</i>	48 hrs, static	EC ₅₀	1.90 (g.m.) [0.107]	█ (2019a)
Miravis Plus A21857B	<i>Pseudokirchneriella subcapitata</i>	96-hours, static	72 hr E _r C ₅₀	7.38 (g.m.) [0.415]	█ (2019)b

g.m. geometric mean measured concentration

The formulation data for fish is presented here for completeness but, as discussed below, will not be considered in the risk assessment.

HSE has considered the toxicity of the formulation in comparison with the toxicity of the active substance alone. The table below compares the endpoints (expressed in terms of active substance content) from acute fish, acute invertebrate and algal studies using the active substance, to like-for-like studies using the formulation, Miravis Plus (A21857B). In line with SANCO Technical Equivalence guidance², 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.

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) ¹	Factor difference
<i>Onchyrhynchus mykiss</i>	LC ₅₀	0.18 (m.m)	0.160 (gm)	1.125
<i>Daphnia magna</i>	EC ₅₀	0.42 (m.m)	0.107 (gm)	3.925
<i>Pseudokirchneriella subcapitata</i>	E _r C ₅₀	> 5.9 (m.m.) (72 h)	0.415 (gm)	14

¹Based on active substance content of 5.62 % w/w.

For acute invertebrates and algae, the formulation is a factor of three, or more, times the toxicity of the active substance alone. Therefore, a separate assessment for formulation spray drift is required. This is considered in more detail below. 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 to be covered by the risk assessment for the active substance and a formulation specific risk assessment is not required. Regarding aquatic invertebrates, it is noted that *Daphnia* was not the most sensitive species when considering toxicity of the active substance. *Hyallela* was the most sensitive species tested, with an EC₅₀ of 0.12 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.

² 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

Table 9.4-8: Regulatory acceptable concentrations (RAC) for ‘Miravis Plus’ for each organism group.

Test species:	Invertebrates	Algae
	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint [µg/L]	EC ₅₀	72 hr E _r C ₅₀
	1900	7380
AF	100	10
RAC [µg formulation/L]	19.00	738

Risk assessment- Active substance (Pydiflumetofen)

Risk assessment for the active substance (Pydiflumetofen) is summarised in Table 9.4-9. The PEC values used relate to the worst-case GAP use of Pydiflumetofen on oilseed rape at 200 g a.s./ha. These values are protective of the risk from all other proposed uses.

Table 9.4-9: First-tier risk assessment for exposure to the active substance (Pydiflumetofen) due to use on oilseed rape at 200 g a.s./ha

Scenario	PEC (µg/L)	Fish acute	Fish long-term	Aquatic invertebrates acute	Aquatic invertebrates long-term	Algae	Accumulated PEC sed (µg/L)	Sediment dwelling invertebrate
		<i>O. mykiss</i>	<i>P. promelas</i>	<i>H. azteca</i>	<i>A. bahia</i>	<i>N. pelliculosa</i>		<i>H. azteca</i>
		RAC (LC ₅₀)	RAC (EC ₁₀)	RAC (EC ₅₀)	RAC (NOEC)	RAC (E _r C ₅₀)		RAC (NOEC)
		1.8	13	1.2	3.7	150		3600
Spray-drift (1 m)	1.847	1.026	0.142	1.539	0.499	0.012	30.121	0.0084
Drainflow	0.062	0.034	0.005	0.05	0.017	0.0004	1.002	0.00028

Values in **bold** are above the trigger of 1

Conclusion: For the proposed use on cereals and oilseed rape at 200 g a.s./ha, there is an unacceptable acute risk to fish and aquatic invertebrates via spraydrift. Therefore, further consideration is required. An acceptable risk from drainflow can be concluded for all organism groups. Refinement of the risk assessment for these groups is considered in the tier 2 risk assessment below.

Higher tier risk assessment- Tier 2**Fish:**

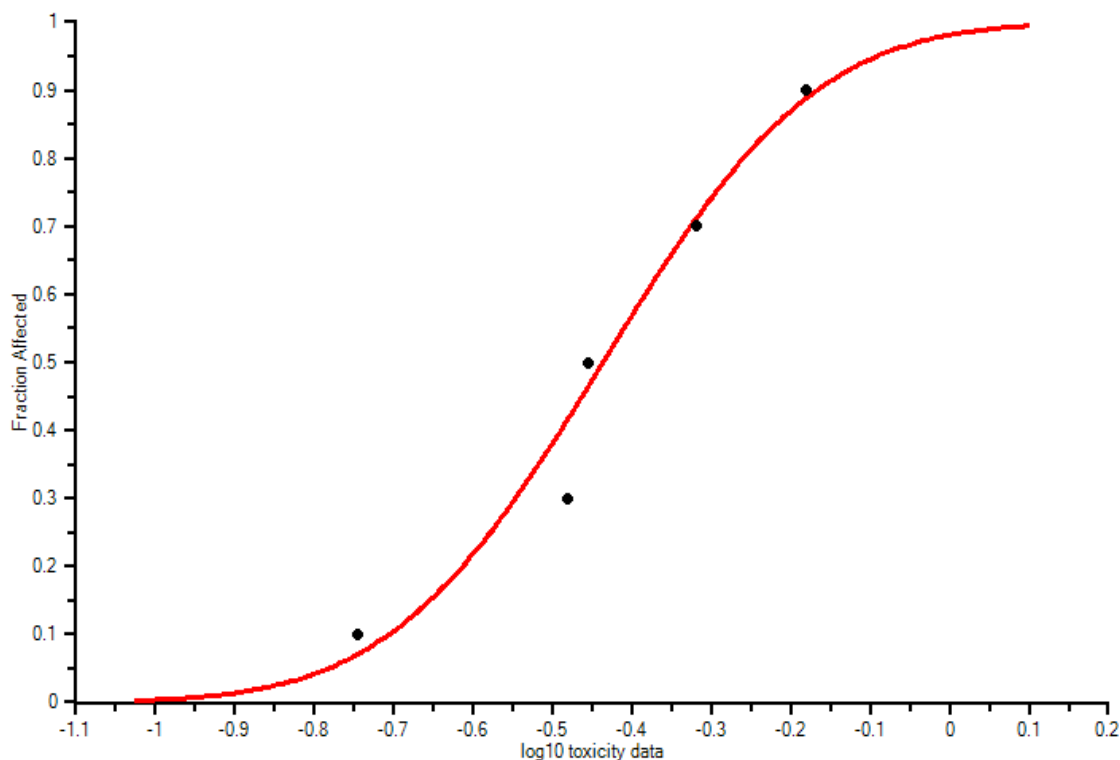
Five acute fish studies are available for use in a species sensitivity distribution (SSD) to refine the acute fish endpoint. Use of additional data to refine the endpoint is not desirable due to the increased vertebrate testing. According to Article 62 of EU legislation 1107/2009: “Testing on vertebrate animals for the purposes of this Regulation shall be undertaken only where no other methods are available”. HSE does not consider alternative methods to have been fully explored and does not support the use of additional vertebrate studies. Nonetheless, SSDs have been considered using both LC₅₀ and NOEC values, and the most conservative of these SSDs will form the basis of the risk assessment. The endpoints are displayed in Table 9.4-10 below.

Table 9.4-10: Studies considered in the acute fish SSD

Species	Exposure type and duration	Endpoint type	Endpoint (95 % confidence intervals) [mg SYN545974/L]
<i>Lepomis macrochirus</i>	96-hours flow-through	LC ₅₀	0.48 (0.38-0.61)
		NOEC	0.20
<i>Oncorhynchus mykiss</i>	96-hours flow-through	LC ₅₀	0.18 (0.15-0.21)
		NOEC	0.12
<i>Pimephales promelas</i>	96-hours flow-through	LC ₅₀	0.35 (0.26-0.46)
		NOEC	0.24
<i>Cyprinus carpio</i>	96-hours flow-through	LC ₅₀	0.33 (0.28-0.40)
		NOEC	0.13
<i>Cyprinodon variegatus</i>	96-hours flow-through	LC ₅₀	0.66 (0.52-0.83)
		NOEC	0.48

LC₅₀ SSD:

Fish LC₅₀ data have been combined in an acute fish SSD, calculated using ETX and shown in Figure 9.4-1 below:

**Figure 9.4-1: SSD distribution for acute fish LC₅₀ endpoints**

The resulting HC₅ is 154.75 µg SYN545974/L (47.87- 246.48). A normal distribution was confirmed for log-transformed data with the following goodness of fit tests at a significance level of 0.05; Anderson-Darling; Kolmogorov-Smirnov and Cramer von Mises.

NOEC SSD:

Fish NOEC data have been combined in an acute fish SSD, calculated using ETX and shown in Figure 9.4-2 below:

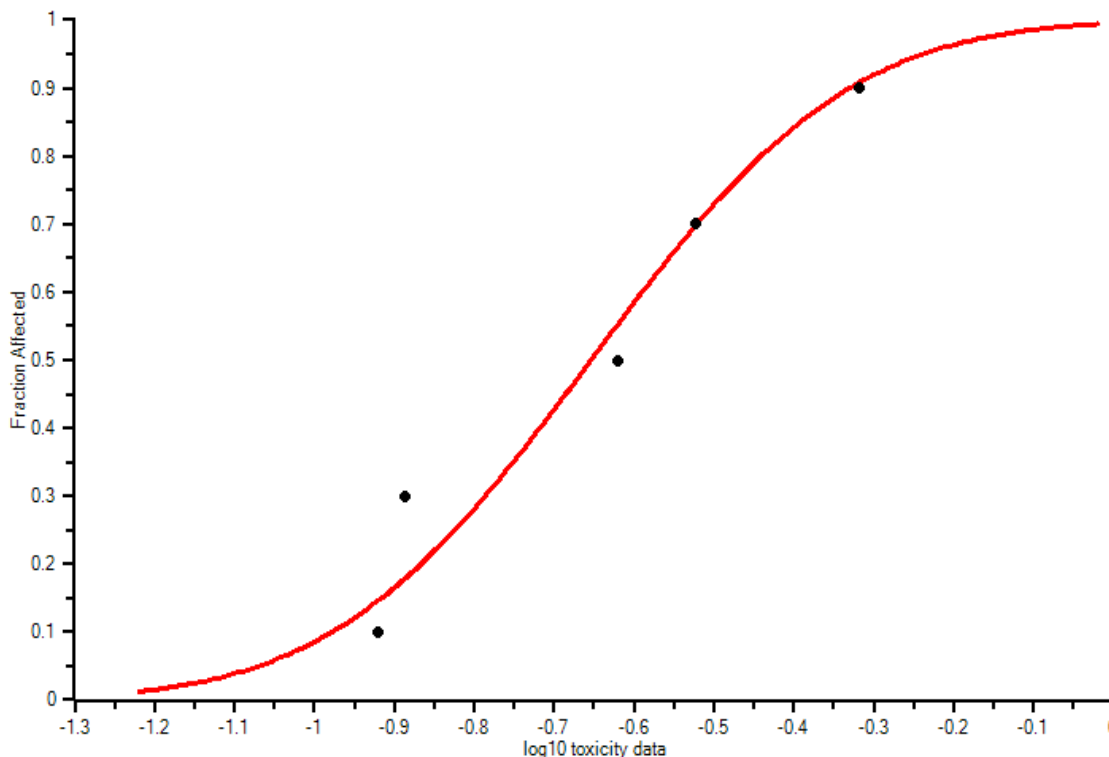


Figure 9.4-2: SSD distribution for acute fish NOEC endpoints

The resulting HC₅ is 78.78 µg SYN545974/L (19.21-137.89). The distribution was shown to be normal at the 0.05 significance level, when considering the following goodness of fit tests; Anderson-Darling; Kolmogorov-Smirnov and Cramer von Mises.

Determination of assessment factor:

As per EFSA (2013) aquatic guidance, the HC₅ is used to derive a RAC by applying an assessment factor (AF). The guidance document states the following:

It is recommended that the following hazardous concentrations and AFs are used to derive a RAC for fish and other aquatic vertebrates. The rationale behind the suggested AFs is an extrapolation from the AFs used for invertebrates, which has been calibrated with micro-/mesocosm experiments. However, for fish, a more stringent protection level has been adopted for the acute RA (avoiding visible mortality of individuals) and for that reason the PPR Panel proposes to apply an AF of 3 on the median HC₅ from an SSD constructed with acute NOEC/EC₁₀ values for fish. In order to also derive an SSD-RAC for vertebrates based on acute LC₅₀ values (since these data are usually reported in the dossiers) the PPR Panel assumes an overall difference of 3 between acute LC₅₀ and acute LC₁₀/NOEC values for fish resulting in an AF of 9. For the ratio between the acute LC₅₀ and chronic NOEC/L(E)C₁₀, usually a factor of 10 is assumed (see, for example, [REDACTED] et al., 2000). Taking this into account, assuming a factor of 3 for the ratio between the acute LC₅₀ and acute NOEC/LC₁₀ for fish seems to be appropriate. Furthermore, traditionally, an AF of 10 has been attributed to the variation in sensitivity between species (for the acute RA) and hence an AF of 9 harmonises to this assumption. Nevertheless it is acknowledged that the method proposed needs calibration.

Therefore, an assessment factor of 3 would be applied to the SSD conducted with NOEC endpoints and an assessment factor of 9 would be applied to the SSD conducted with LC₅₀ data. This would result in an SSD RAC of 26.26 µg SYN545974/L for NOEC data and an SSD RAC of 17.19 µg SYN545974/L for LC₅₀ data. However, Table 28 of EFSA (2013) aquatic guidance highlights the need to consider latency of effects when determining an appropriate assessment factor, stating that:

“Information of possible latency of effect may be obtained on the basis of knowledge on the specific toxic mode of action and, read across information. To avoid unnecessary testing with aquatic vertebrates for animal welfare considerations the conduct of prolonged acute toxicity tests to demonstrate latency is not considered”.

No consideration of latency of effects has been provided to support the assessment factor determination. Therefore, since a suitable assessment factor cannot be determined, it is considered more appropriate to refine the risk assessment using the geometric mean approach. **This results in a geomean RAC of 3.66 µg SYN545974/L, based on a geomean of 366 µg SYN545974/L and an assessment factor of 100. This geomean RAC has been considered in the tier 2 risk assessment in Table 9.4-13 below.**

Invertebrates:

The applicant has proposed the following endpoints from the acute aquatic invertebrate dataset for consideration in an SSD to refine the acute aquatic invertebrate endpoint.

Table 9.4-11: Studies considered in the acute aquatic invertebrate SSD

Species	Exposure type and duration	Endpoint type	Endpoint and 95 % confidence intervals (mg SYN545974/L)
<i>Daphnia magna</i>	48-hours, static	EC ₅₀	0.42 (m.m) 0.36-0.49
<i>Chaoborus crystallinus</i>	48-hours, static	EC ₅₀	2.489 (m.m) 1.76- 3.52
<i>Cloeon dipterum</i>	48-hours, static	EC ₅₀	>5.01(m.m) n.d.
<i>Crassostrea virginica</i>	96-hours, flow-through	EC ₅₀ (shell deposition)	0.31 (m.m) 0.24-0.39
<i>Cyclops agilis speratus</i>	48-hours, static	EC ₅₀	4.168 (m.m) 4.04-5.67
<i>Asellus aquaticus</i>	48-hours, static	EC ₅₀	4.209 (m.m.) 3.49-5.08
<i>Crangonyx pseudogracilis</i>	48-hours, static	EC ₅₀	1.226 (m.m.) 0.89-1.64
<i>Lumbriculus variegatus</i>	48-hours, static	EC ₅₀	4.651 (m.m.) 3.88-5.58
<i>Chironomus riparius</i>	48-hours, static	EC ₅₀	0.691 (m.m) 0.57-0.84
<i>Lymnaea stagnalis</i>	48-hours, static	EC ₅₀	>7.30 (m.m) n.d.
<i>Hyalella azteca</i>	48-hours, static	EC ₅₀	0.12 (m.m) 0.06-0.21
<i>Americamysis bahia</i>	96-hours, static	LC ₅₀	0.16 (m.m) 0.15-0.17

The study with *D. magna* was required as part of the tier 1 data requirements. Only one additional species is required under the tier 1 data requirements, however studies with an additional 11 species were conducted in order to refine the tier 1 acute toxicity endpoint. Of these 11 additional studies, only the studies with *C. virginica*, *H. azteca* and *A. bahia* were conducted in accordance with species-specific guidelines. The remaining studies were conducted with non-standard species and therefore no specific guidelines were available, however OECD 202 (acute *Daphnia* study) and OECD 235 (acute *Chironomus* study) guidelines were followed where possible. As such, no positive control data is available for these studies, raising some uncertainty as to the sensitivity of the test systems and their ability to detect effects of the test item. Additionally, it was not possible to confirm the validity of these studies, although the studies were checked against the validity criteria for OECD 202/OECD 235 as a substitute, and these were satisfied for the vast majority of studies. In the study with *Chaoborus crystallinus*, the applicant consulted OECD 202 (2004) *Daphnia* guidelines. The validity criterion for control immobility was not met (required < 10 %, observed 12.5 %). However, OECD 202 is not designed for use with *Chaoborus* and the validity criteria in the OECD 235 guideline may be a more appropriate comparison as both *Chironomus* and *Chaoborus* are species of midge. The acceptable control mortality for *Chironomus* is 15 %, therefore the mortality of 12.5 % observed in the *Chaoborus* study may be considered acceptable. Therefore, in the absence of an agreed guideline, since the validity criteria of OECD 235 were met, the study is considered valid and the endpoint suitable for use in risk assessment.

The study with *Crassostrea virginica* did have a species-specific study guideline, however the validity criteria were not met, since an overall mean of at least 2 cm of new shell growth was not observed in each control group. Additionally, the EC₅₀ is based on shell deposition, rather than immobility and is not considered comparable to the EC₅₀ endpoints from other invertebrate studies. Therefore, this study has not been considered in the SSD calculation. The studies with *Cloeon dipterum* and *Lymnaea stagnalis* generated unbound (>) endpoints, as effects of 50 % were not reported at the maximum test concentration. This indicates that these species are less sensitive to SYN545974 than others tested. EFSA (2013) states that “If a greater- or lower-than value relates to a species for which no other data are available, this value should only be used (without the < or > sign) in the SSD where it is outside the range of all other available toxicity values for other species or taxa”. Since the endpoints derived from these studies are outside the range of toxicity data for all other species, an SSD was conducted including the highest unbound value. The results of this SSD differed very little from the SSD conducted without the unbound values, and the HC₅ value was higher. It was therefore considered most appropriate to exclude unbound values and use the more conservative HC₅ value. The SSD is therefore based on endpoints from 9 of the 12 aquatic invertebrate studies conducted.

The SSD was calculated in ETX and the resulting curve is shown in Figure 9.4-3 below:

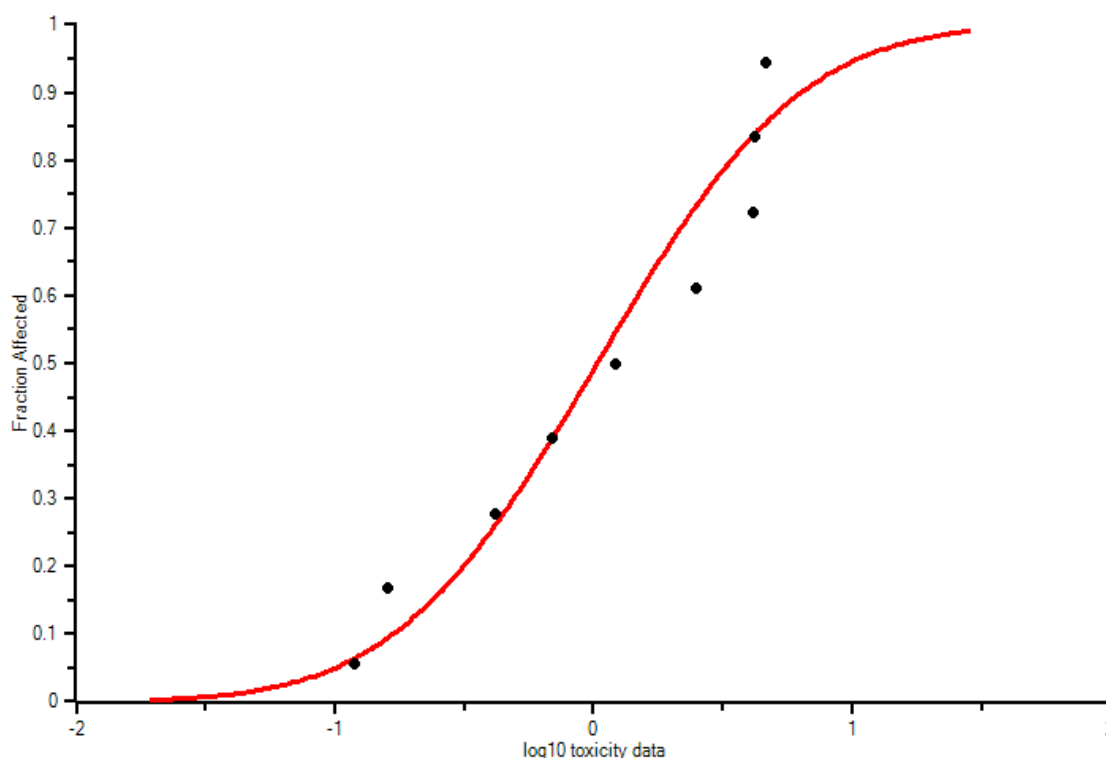


Figure 9.4-3: SSD distribution for acute invertebrate EC₅₀ endpoints:

The resulting HC₅ is 92.01 µg SYN545974/L (14.13-254.98). The following goodness of fit tests confirmed the normality of the distribution at a significance level of 0.05: Anderson-Darling; Kolmogorov-Smirnov and Cramer von Mises.

Determination of assessment factor:

Consideration of an appropriate assessment factor for the acute invertebrate SSD has been conducted according to criteria specified in EFSA 2013 and is detailed in Table 9.4-12 below:

Table 9.4-12: Consideration of assessment factor according to EFSA (2013) criteria:

Point from EFSA (2013)	HSE consideration	Assessment factor proposed																														
<p>The quality of the acute toxicity data used to construct the SSD.</p> <p>If the toxicity data used to construct the SSD comprise several different genera/families/orders of the potential sensitive taxonomic group, including EPT taxa (Ephemeroptera/Plecoptera/Trichoptera), a lower AF in the proposed range may be selected. However, if another valid SSD can be constructed with a more limited dataset containing the most sensitive species, and the HC₅ derived from this SSD curve is lower than that of the SSD curve using toxicity data for a wider array of taxa, a higher AF in the proposed range may be selected to be applied to the SSD from the wider set.</p>	<p>The SSD has been constructed with data derived from a range of taxa, representing 7 orders. A study with <i>Ephemoptera</i> was available but was not included in the SSD due to the endpoint being unbound. No studies with <i>Plecoptera</i> or <i>Trichoptera</i> were available. The taxonomic groups covered by the data are shown in the table below.</p> <table><tr><th>Species</th><th>Order</th><th>Family</th></tr><tr><td><i>Daphnia magna</i></td><td>Cladocera</td><td>Daphniidae</td></tr><tr><td><i>Chaoborus crystallinus</i></td><td>Diptera</td><td>Chaoboridae</td></tr><tr><td><i>Cyclops agilis speratus</i></td><td>Cyclopoida</td><td>Cyclopidae</td></tr><tr><td><i>Asellus aquaticus</i></td><td>Isopoda</td><td>Asellidae</td></tr><tr><td><i>Crangonyx pseudogracilis</i></td><td>Amphipoda</td><td>Crangonyctidae</td></tr><tr><td><i>Lumbriculus variegatus</i></td><td>Lumbriculada</td><td>Lumbriculadae</td></tr><tr><td><i>Chironomus riparius</i></td><td>Diptera</td><td>Chironomidae</td></tr><tr><td><i>Hyalella azteca</i></td><td>Amphipoda</td><td>Hyalellidae</td></tr><tr><td><i>Americamysis bahia</i></td><td>Mysida</td><td>Mysidae</td></tr></table> <p>In the absence of suitable studies with EPT taxa, a higher assessment factor is appropriate.</p>	Species	Order	Family	<i>Daphnia magna</i>	Cladocera	Daphniidae	<i>Chaoborus crystallinus</i>	Diptera	Chaoboridae	<i>Cyclops agilis speratus</i>	Cyclopoida	Cyclopidae	<i>Asellus aquaticus</i>	Isopoda	Asellidae	<i>Crangonyx pseudogracilis</i>	Amphipoda	Crangonyctidae	<i>Lumbriculus variegatus</i>	Lumbriculada	Lumbriculadae	<i>Chironomus riparius</i>	Diptera	Chironomidae	<i>Hyalella azteca</i>	Amphipoda	Hyalellidae	<i>Americamysis bahia</i>	Mysida	Mysidae	<p>A higher assessment factor is appropriate.</p>
Species	Order	Family																														
<i>Daphnia magna</i>	Cladocera	Daphniidae																														
<i>Chaoborus crystallinus</i>	Diptera	Chaoboridae																														
<i>Cyclops agilis speratus</i>	Cyclopoida	Cyclopidae																														
<i>Asellus aquaticus</i>	Isopoda	Asellidae																														
<i>Crangonyx pseudogracilis</i>	Amphipoda	Crangonyctidae																														
<i>Lumbriculus variegatus</i>	Lumbriculada	Lumbriculadae																														
<i>Chironomus riparius</i>	Diptera	Chironomidae																														
<i>Hyalella azteca</i>	Amphipoda	Hyalellidae																														
<i>Americamysis bahia</i>	Mysida	Mysidae																														
<p>The lower limit value of the HC₅.</p> <p>If the lower limit HC₅ derived from the curve is less than one-third of the median HC₅, a higher AF in the proposed range may be warranted.</p>	<p>The lower limit HC₅, derived from the curve of the SSD, is less than one-third of the median HC₅.</p> <table><tr><th>HC₅ median (mg/L)</th><th>HC₅ lower limit (mg/L)</th><th>Proportion of median</th></tr><tr><td>0.092</td><td>0.014</td><td>0.15</td></tr></table>	HC ₅ median (mg/L)	HC ₅ lower limit (mg/L)	Proportion of median	0.092	0.014	0.15	<p>A higher assessment factor is appropriate.</p>																								
HC ₅ median (mg/L)	HC ₅ lower limit (mg/L)	Proportion of median																														
0.092	0.014	0.15																														
<p>The lower tier RACs on the basis of standard toxicity data (tier 1), standard and additional toxicity data (Geomean approach) and tier 3 data</p> <p>The size of the AF should ideally not result in an SSD-RAC_{sw;ac} higher than the tier 3 RAC derived from effect class 1 and 2 of micro-/mesocosms studies nor lower than the tier 1 RAC_{sw;ac} on the basis of standard test species and/or the</p>	<p>No micro- or mesocosm studies have been conducted. Applying assessment factors in the range 3-6 results in the following RAC values when using the HC₅ derived in the SSD. The RAC values have been compared to the Tier 1 RAC and the geomean.</p> <table><tr><th>AF</th><th>SSD µg a.s./L</th><th>Tier 1 µg a.s./L</th><th>Geomean µg a.s./L</th></tr><tr><td></td><td>RAC</td><td>RAC</td><td>RAC</td></tr></table>	AF	SSD µg a.s./L	Tier 1 µg a.s./L	Geomean µg a.s./L		RAC	RAC	RAC	<p>An assessment factor in the range 3-6 is appropriate.</p>																						
AF	SSD µg a.s./L	Tier 1 µg a.s./L	Geomean µg a.s./L																													
	RAC	RAC	RAC																													

Point from EFSA (2013)	HSE consideration				Assessment factor proposed
<i>Geomean-RAC_{sw;ac} and/or method 3 to 5 (EFSA, 2006a) on the basis of the same toxicity data that were used to construct the SSD. The Geomean approach aims to achieve the same average level of protection as in the tier 1 effect assessment but can be predicted more accurately because of the availability of additional toxicity data for the relevant taxonomic groups.</i>					As shown above, the SSD RACs are higher than the Tier 1 RAC and the geomean RAC. Therefore, an assessment factor in the range 3-6 is appropriate.
	3	30.67	1.2	10.37	
	4	23.00			
	5	18.40			
	6	15.33			
<i>The position of the toxicity data in the lower tail of the SSD (around the HC₅).</i> <i>If in the lower tail the toxicity data overall are positioned on the right side of the SSD curve, the derived HC₅ estimate may be considered relatively conservative for the most sensitive species. This may be a reason to adopt a lower AF from the proposed range. In contrast, if in the lower tail, the toxicity data overall are positioned on the left side of the SSD curve, this may be a reason to adopt a higher AF from the proposed range.</i>	Of the bottom third of the data points, one sits clearly to the left of the SSD curve, one slightly to the left, touching the curve and the third sits slightly to the right, touching the curve. As the lower toxicity data overall are positioned on the left side, a higher assessment factor may be appropriate.				A higher assessment factor may be appropriate.
<i>The steepness of the SSD curve.</i> <i>In the case of a relatively steep SSD curve (e.g. less than a factor of 100 between lowest and highest L(E)C₅₀ value used to construct the SSD curve) a higher AF from the proposed range is recommended since exposure concentrations that exceed the RAC_{sw;ac} may have ecotoxicological consequences for a larger number of taxa.</i>	The factor between lowest and highest EC ₅₀ value is 38.76, indicating a steep SSD slope. Therefore, a higher assessment factor is appropriate.				A higher assessment factor is appropriate.
<i>Read-across information for compounds with a similar toxic mode of action.</i> <i>For a PPP with a well-known mode of action sufficient higher tier information on related compounds (e.g. organophosphates) may be available that allow the evaluation of the predictive value of the median HC₅ and/or lower limit HC₅ for possible effects in micro-/mesocosms. This information may be used to select an appropriate AF within the proposed range</i>	The applicant has stated: “Read across to comparable substances could be considered, however the acute data provided here represents a range of aquatic invertebrates and meets the criteria specified in the EFSA guidance for the calculation of HC5 values and so is considered acceptable to assess the risk to aquatic invertebrates.” HSE does not consider the range of aquatic invertebrates an adequate substitute for read-across information for compounds with a similar mode of action. In the absence of read-across information, a higher assessment factor is appropriate.				A higher assessment factor is appropriate.
<i>Considering information on chronic effects.</i> <i>If the acute to chronic ratio (acute EC₅₀/chronic EC₁₀) is larger than 10, then an AF in the higher range may be warranted.</i>	The acute to chronic ratios for <i>Daphnia magna</i> and <i>Americamysis bahia</i> are 4.9 and 4.3, respectively. Both values are < 10.				A median assessment factor is appropriate.

Point from EFSA (2013)	HSE consideration	Assessment factor proposed

Consideration of latency of effects:

According to EFSA (2013) section 8.4.4 Table 27, for the median HC5 from standard acute toxicity tests to be used, no latency of effects must be demonstrated:

“This has to be demonstrated by the applicant, see further section 4.5.1. For example, by read-across for substances with similar toxic mode of action, prolonged acute toxicity tests, and information from micro-/mesocosm studies for similar compounds with a longer-term observation period after exposure”.

Following a request for additional information, the applicant has provided the following information regarding latency of effects:

“Looking at the mysid, the acute: chronic ratio is approximately 4 and there is little increase in mortality over the 28 day chronic exposure compared to the 4 day acute exposure, suggesting no latency of effect. There is 5% mortality at 4 days at 0.13 mg/L, 95% mortality at 0.25 mg/L and in the chronic study no difference to control mortality at 0.076 mg/L, the highest concentration tested after 28 d.

Similarly with Daphnia, where the acute: chronic ratio is 10, looking at mortality, there is again little increase over 21 day chronic exposure compared to the 48 hour acute. There is 65% mortality after 48 hours at 0.48 mg/L, no difference to control mortality at 0.3 mg/L, the highest concentration tested, after 21 d.

Alternatively, as described in the Section MCP-10, an acute geomean RAC of 10.37µg/L can be used to refine the risk assessment, where an expectation of latency of effects does not need consideration, according to the AGD.”

HSE does not consider the acute : chronic ratio sufficient to demonstrate a lack of latency of effects. As stated above, the EFSA (2013) guidance suggests that lack of latency of effects must be demonstrated either experimentally, or via read-across from similar compounds. Further to this Section 4.5.1 states the following:

“In longer term studies, latency may result from delays in the chain of events between exposure and expression of effects (e.g. in the case of moulting inhibiting insecticides and substances suspected of endocrine disrupting effects). To demonstrate latency, it may even be required to make observations on the responses of the offspring. It is advised to address latency if, through analogy to similar substances or knowledge of mechanisms of action, it is expected to occur. In cases where latency is known not to occur in PPPs with a similar toxic mode of action, it might be disregarded.”

Lack of latency of effects has not been demonstrated experimentally, nor has information on read-across to similar PPPs been provided that would allow latency to be disregarded. Therefore, HSE considers there to be insufficient information to derive an appropriate assessment factor to determine an SSD RAC for aquatic invertebrates. Therefore it is proposed to use the geomean approach, as described below.

Conclusion on consideration of assessment factor:

Consideration of an appropriate assessment factor for the acute invertebrate SSD has been conducted according to criteria specified in EFSA 2013 in Table 9.4-12 above. Based on these criteria, a higher assessment factor of 6 would be warranted, which would result in an SSD RAC of 15.335. However, given that insufficient evidence has been provided to demonstrate no latency of effects, it is not possible to determine a suitable assessment factor. Instead, the geomean approach has been considered. **This results in a geomean RAC of 10.37 µg SYN545974/L, based on a geomean of 1037 µg SYN545974/L and an assessment factor of 100. This RAC has been considered in the tier 2 risk assessment in Table 9.4-13 below.**

Risk assessment:

The spray drift risk assessment for acute fish and acute aquatic invertebrates using Tier 2 SSD RAC values has been conducted in Table 9.4-13 below:

Table 9.4-13: Refined risk assessment for acute fish and acute aquatic invertebrates using geomean RACs due to use on oilseed rape at 200 g a.s./ha

Scenario	PEC _{sw} (µg/L)	Fish acute	Invertebrates acute
		Geomean RAC (LC ₅₀)	Geomean RAC (EC ₅₀)
		3.66 µg/L	10.37 µg/L
Spray-drift (1 m)	1.847	0.50	0.18

Conclusion: For the proposed use on cereals and oilseed rape at 200 g a.s./ha, the acute risk to fish and aquatic invertebrates from spray drift has been resolved using the geomean RAC values. No further consideration is required.

Risk assessment- metabolites of Pydiflumetofen

Risk assessments for the metabolites SYN545547, M700F001 (NOA449410) and SYN548261 are summarised for the critical GAP (200 g a.s./ha on oilseed rape) in Tables 9.4-14 to 9.4-16 below:

Table 9.4-14: First tier risk assessment for exposure to SYN545547 due to use on oilseed rape at 200 g a.s./ha

Scenario	Accumulated PEC _{sed} (µg/kg)	Sediment dwelling organisms
		<i>Chironomus riparius</i> ¹
		RAC (NOEC)
		720
Spraydrift (1 m)	4.353	0.006

¹ Spiked sediment study; value is expressed as µg/kg sediment

Table 9.4-15: First tier risk assessment for exposure to M700F001 (NOA449410) due to use on oilseed rape at 200 g a.s./ha

Scenario	PEC _{sw} (µg/L)	Fish acute	Aquatic invertebrates (acute)	Algae
		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>
		RAC (LC ₅₀)	RAC (EC ₅₀)	RAC (E _r C ₅₀)
		1000	1000	3631
Spraydrift (1 m)	0.041	0.000041	0.000041	0.00001

Table 9.4-16: First tier risk assessment for exposure to SYN548261 due to use on oilseed rape at 200 g a.s./ha

Scenario	PEC _{sw} (µg/L)	Fish acute	Aquatic invertebrates (acute)	Algae
		<i>Oncorhynchus mykiss</i>	<i>Daphnia magna</i>	<i>Pseudokirchneriella subcapitata</i>
		RAC (LC ₅₀)	RAC (EC ₅₀)	RAC (E _r C ₅₀)
		1000	1000	10,000
Spraydrift (1 m)	0.092	0.000092	0.000092	0.000092

Conclusion: For the proposed worst-case use on oilseed rape at 200 g a.s./ha, an acceptable risk to aquatic organisms can be concluded for all relevant metabolites of Pydiflumetofen.

Risk assessment- Formulation ‘Miravis plus’

Risk assessment for the formulation ‘Miravis plus’ is summarised in Table 9.4-17. The PEC values used relate to the worst-case GAP use of ‘Miravis Plus’ on oilseed rape at 200 g a.s./ha. These values are protective of the risk from all other proposed uses.

Table 9.4-17: First tier risk assessment for exposure to ‘Miravis Plus’ due to use on oilseed rape at 200 g a.s./ha

Scenario	PEC (µg formulation/L)	Aquatic invertebrates	Algae
		<i>D. magna</i>	<i>P. subcapitata</i>
		RAC (EC ₅₀)	RAC
		19.00 µg/L	738 µg/L
Spraydrift (1 m)	32.413	1.7	0.044
Spraydrift (5 m)	6.670	0.35	-

An acceptable risk to algae is concluded at 1 m. When considering formulation data for aquatic invertebrates, an acceptable risk can be concluded providing a 5 m buffer zone is implemented.

Multispecies data from 12 aquatic invertebrate species were available to refine the risk from the active substance. Both an SSD and geomean approach were considered but the geomean RAC of 10.37 µg/L was used in the active substance risk assessment. As previously mentioned, *Daphnia* was not the most sensitive species when considering active substance data. The most sensitive species was *Hyalella* (EC₅₀ of 0.12 mg a.s./L vs EC₅₀ of 0.42 mg a.s./L for *Daphnia*), however no formulation data was available with this species. This raises concern as to whether the formulation risk assessment is sufficiently protective of the risk to all aquatic invertebrates. In order to address the risk of the formulation to the most sensitive aquatic invertebrate species, HSE has taken into account the multispecies active substance data as follows.

Considering both active substance and formulation data for *Daphnia*, the formulation appears to be 3.925 times more toxic than the active substance (see Table 9.4-7). HSE has applied this factor to the aquatic invertebrate geomean RAC (10.37 µg/L), in order to better approximate the toxicity of the formulation to aquatic invertebrates by accounting for a wider range of species sensitivities. The resulting RAC is 2.64 µg/L (expressed in terms of a.s.). This RAC is compared to the active substance PEC_{sw} at 1 m in Table 9.4-18.

Table 9.4-18: Risk assessment for aquatic invertebrates considering active substance multispecies data.

Scenario	PEC (µg a.s./L)	Aquatic invertebrates
		Multispecies data
		RAC
		2.64 µg/L
Spraydrift (1 m)	1.847	0.70

An acceptable risk to aquatic invertebrates can be concluded at 1 m. This approach is considered more suitable than using the *Daphnia* formulation endpoint, since it accounts for a wider range of species sensitivities and can therefore be considered protective of species more sensitive than *Daphnia*. The risk of the formulation to aquatic invertebrates can therefore be resolved at 1 m with no risk mitigation required.

Conclusion: An acceptable risk to aquatic organisms can be concluded for the worst-case GAP use of Miravis Plus. No further consideration is required.

Bioaccumulation risk assessment

The log Pow of Pydiflumetofen is 3.8. Therefore, bioaccumulation in fish has been addressed by the applicant with a BCF study on *Lepomis macrochirus* (■■■■■, 2017). The study was evaluated and considered suitable for use in risk assessment. The steady state BCF value was 31.1 L/kg when corrected for lipid content and the depuration half life was 0.41 days. As stated in EFSA aquatic guidance 2013 biomagnification must be considered for compounds where the BCF is > 1000 and the elimination of radioactivity during the 14-day depuration phase in the bioconcentration study is < 95 % and the substance is stable in water or sediment (DegT₉₀ > 100 days). These triggers were not met, so further consideration is not required.

B.9.5. EFFECTS ON ARTHROPODS**B.9.5.1. Effects on bees**

Report: K-CP 10.3.1.1, ■■■■■, (2016), Pydiflumetofen EC (A21857B) – Acute Oral and Contact Toxicity to the Honey Bee, *Apis mellifera* L. under Laboratory Conditions, Report Number S16-05072. Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Eutingen Str. 24, 75223 Niefern-Öschelbronn, Germany. (Syngenta file No. VV-466570).

Guidelines

- OECD Guidelines No. 213. Honeybees, acute oral toxicity test (1998a)
- OECD Guidelines No. 214. Honeybees, acute contact toxicity test (1998b)

GLP: Yes, with the exceptions of honey bee colony inspections and stock keeping

Materials

Test Material	A21857B (formulation) SYN545974 EC (062.5) Pydiflumetofen EC (062.5)
Lot/Batch #:	JEA001-116-001
Actual content of active ingredients:	SYN545974: 5.69 % w/w corresponding to 62.4 g/L as stated on certificate of analysis from study sponsor.
Description:	Light yellow clear liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	28 February 2019
Density:	1097 kg/m ³
Treatments	
Test rates:	Contact (nominal): 62.5, 125, 250, 500 and 1000 µg A21857B/bee Oral (nominal): 62.5, 125, 250, 500 and 1000 µg A21857B/bee Oral (consumed): 50.6, 123, 234, 245 and 423 µg A21857B/bee
Control:	Contact: Deionised water Oral: 50 % (w/v) aqueous sucrose solution
Toxic standard:	Perfekthion BAS 152 11 I (active ingredient: 420.3 g dimethoate/L) Contact (nominal): 0.13, 0.17, 0.22 and 0.29 µg a.i./bee Oral (nominal): 0.06, 0.08, 0.11 and 0.14 µg a.i./bee Oral (consumed): 0.05, 0.08, 0.08 and 0.13 µg a.i./bee
Administration:	Contact: topical application to the dorsal thorax Oral: ingestion in aqueous sucrose solution
Test organisms	
Species:	Adult worker bees of species <i>Apis mellifera</i> L. (Hymenoptera: Apidae)
Source:	Test facility stock: from healthy and queen-right hive.
Food:	50 % w/v aqueous sucrose solution
Test design	

Test cage description:	Stainless steel cages (approx. 8 x 4 x 6 cm) with a transparent window and a perforated steel base. The test cage was lined with filter paper.
Replication:	4
No. of bees/arena :	10
Duration of test:	48 hours
Environmental test conditions	
Temperature:	24.6 – 25.1 °C
Humidity:	58.0 – 61.7 %
Photoperiod:	Constant darkness, except at the start of the experimental phase in the oral toxicity test (feeding of the bees), start of the experimental phase in the contact toxicity test (topical application) and during the assessments

Study Design and Methods

Experimental dates: 13 September 2016 to 16 September 2016

Honeybees (*Apis mellifera*) were exposed to A21857B via two routes of administration: contact and oral ingestion. To immobilise the bees during the course of the contact treatment, they were anaesthetised using CO₂.

Contact test procedures: Bees were treated with one 2 µL droplet of the test solution, control or toxic standard applied to the dorsal surface of the thorax using a micro applicator. The bees were returned to the test unit and fed with 50 % w/v aqueous sucrose solution *ad libitum*.

Oral test procedures: Bees were starved for 2 hours until treatment. Each group of bees was offered 220 µL (equivalent to 22 µL/bee) of the test material or toxic standard dispersed in aqueous sucrose solution.

Treatments were calculated so that the target dose was contained in 200 µL, however 220 µL was actually provided per test unit. This was to ensure sufficient consumption of the test material so that the target dose was achieved. The doses were measured into the feeding tubes and the weights of these were recorded before the doses were made available to the bees. The test solutions offered for a maximum of six hours. After the feeding period the bees were supplied *ad libitum* with untreated 50 % aqueous sucrose solution.

In both the contact and oral tests there were 4 replicates per treatment and control consisting of 10 bees each. Mortality and sublethal effects were assessed at 4, 24 and 48 hours for the test material and toxic standard for both oral and contact tests.

For each concentration, the corrected mortality (in cases of mortality in the respective control) was calculated according to Abbott (1925) modified by [REDACTED] (1947). The LD₅₀ with 95 % confidence limits were calculated for both oral and contact toxic reference item tests by means of a trimmed Spearman Karber test. The statistical program ToxRat Professional 3.2.1 was used for statistical analysis. The data from the test item was unsuitable for statistical analysis so the LD₅₀ was estimated by extrapolating from the data.

Results and Discussion

In the oral toxicity test, no behavioural abnormalities were observed during the entire 48 hour observation period and in all doses tested. At the two highest dose levels of 500 and 1000 µg A21857B/bee the bees consumed less than half of the test solution offered; therefore a repellent effect of the test item at these dose levels can be assumed. Oral mortality data for A21857B are summarised in the table below:

Table 9.5.1-1: Summary of acute oral toxicity of formulation A21857B to the honeybee

Treatment (µg A21857B/bee)		Treatment (µg a.s./bee)*		Mortality after 24 hours	Mortality after 48 hours
Nominal	Consumed	Nominal	Consumed	Mean (%)	Mean (%)
Control		Control		0.0	0.0
62.5	50.6	3.56	2.88	0.0	0.0
125	123	7.11	7.00	0.0	0.0
250	234	14.2	13.3	0.0	0.0

Treatment (µg A21857B/bee)		Treatment (µg a.s./bee)*		Mortality after 24 hours	Mortality after 48 hours
Nominal	Consumed	Nominal	Consumed	Mean (%)	Mean (%)
500	245	28.5	13.9	0.0	0.0
1000	423	56.9	24.1	2.5	2.5
LD ₅₀ (µg A21857B/bee, consumed)				> 423	> 423

*Calculated from formulation amount using 5.69 % w/w as stated on certificate of analysis from study sponsor.

In the contact toxicity test, affected and single moribund bees were observed at the four highest dose levels after 4 hours. At the dose levels of 125, 250 and 500 µg A21857B/bee single affected and moribund bees were recorded after 24 hours. However, most of the affected bees were able to recover by the end of the observation period. Contact data for A21857B is summarised in the table below:

Table 9.5.1-2: Summary of acute contact toxicity of A21857B to the honeybee

Table 10.1.2. Summary of acute contact toxicity of A21857B to the honey bee									
Treatment (µg A21857B/ bee)	Treatment (µg a.s./ bee)	Observations after 4 hours		Observations after 24 hours			Observations after 48 hours		
		Mean mortality (%)	Behav. ¹ (%)	Mean mortality (%)	Corrected mortality (%)	Behav. ¹ (%)	Mean mortality (%)	Corrected mortality (%)	Behav. ¹ (%)
Control		0	M: 0 A: 0	2.5	-	M: 0 A: 0	2.5	-	M:0 A: 0
62.5	3.56	0	M: 0 A: 0	0.0	-2.6	M: 0 A: 0	0.0	-2.6	M: 0 A: 0
125	7.11	0	M: 2.5 A: 2.5	2.5	0.0	M: 0 A: 2.5	5.0	2.6	M: 0 A: 2.5
250	14.2	2.5	M: 5 A: 15	10.0	7.7	M: 2.5 A: 5	12.5	10.3	M: 0 A: 0
500	28.5	0	M: 0 A: 22.5	0.0	-2.6	M: 2.5 A: 0	2.5	0.0	M: 0 A: 0
1000	56.9	0	M: 0 A: 22.5	0.0	-2.6	M: 0 A: 0	0.0	-2.6	M: 0 A: 0
LD ₅₀ (µg A21857B/ bee)		-	-	> 1000		-	> 1000		-

¹ Mean observed behavioural abnormalities (%). Percentages calculated by HSE from available raw data. 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); A: affected (bees still upright and attempting to walk but showing signs of reduced coordination).

Validity criteria

The validity criteria are listed below:

- The mortality in both the contact and oral control were 2.5 and 0 %, respectively (must be ≤ 10 %)
- The oral 24 hour LD₅₀ for the reference item was 0.10 (95 % C.I. 0.09 – 0.11) µg dimethoate/bee (recommended 0.10 – 0.35 µg a.s./bee)
- The contact 24 hour LD₅₀ for the reference item was 0.16 (95 % C.I. 0.15 – 0.17) µg dimethoate/bee (recommended 0.10 – 0.30 µg a.s. /bee)

Conclusions

The acute oral and contact toxicity of A21857B to honeybees was assessed over 48 hours. In the contact toxicity test, the LD₅₀ after 48 hours was > 1000 µg A21857B/bee. In the oral toxicity test, the LD₅₀ after 48 hours was > 423 µg A21857B/bee.

At 4 and 24 hours after the start of the contact toxicity test, affected and moribund were observed at tested dose rates of 125 µg A21857B/bee and above when compared to the control group. However, most of these bees were able to recover until the end of the observation period. In the oral toxicity test, no behavioural abnormalities were observed when compared to the control during the entire 48 hour observation period.

(██████, 2016)

HSE Comments

This was a GLP study and was conducted to the OECD 213 and 214 (1998) guidelines. There were no deviations from the guideline with the exception that the contact dosing volume was 2 µl per bee instead of 1 µl. This is a common acceptable deviation that is justified by the authors because it ensures a ‘*more reliable dispersion of the test item*’.

The study met all validity criteria for these guidelines.

It is noted that behavioural abnormalities were observed in the contact toxicity test, but these did not appear to be dose responsive and some recovery was seen. The effects were observed at a maximum mean of 22.5 % of bees, so the endpoint is protective of these effects. It is also noted that there did appear to be some repellency/unpalatability observed at the two highest tested oral concentrations, as less than half of the treated food was consumed.

The acceptable endpoints for use in risk assessment are:

- **Oral 24h and 48h LD₅₀: > 423 µg A21857B formulated product/bee (consumed concentration), equivalent to > 24.07 µg a.s./bee.**
- **Contact 24h and 48h LD₅₀: > 1000 µg A21857B formulated product/bee (nominal concentration), equivalent to > 56.9 µg a.s./bee.**

Please note that the following study was conducted with the EU representative formulation (A19649B); further details are given in the risk assessment.

Report: K-CP 10.3.1.1.1, [REDACTED], (2015), SYN545974 SC (A19649B) - Acute Oral and Contact Toxicity to the Honey Bee, *Apis mellifera* L. under Laboratory Conditions, Report Number S14-04061. Eurofins Agrosience Services EcoChem GmbH Eutingen Str. 24 75223 Niefern-Öschelbronn, Germany (Syngenta file No. A19649B_10036).

Guidelines

- OECD Guidelines No. 213. Honeybees, acute oral toxicity test (1998a)
- OECD Guidelines No. 214. Honeybees, acute contact toxicity test (1998b)

GLP: Yes.

Materials

Test Material	A19649B (formulation)
Lot/Batch #:	SMU2JP001
Actual content of active ingredients:	SYN545974: 18.6 % w/w corresponding to 204 g/L as stated on certificate of analysis from study sponsor
Description:	Off-white liquid
Stability of test compound:	Stable under standard conditions.
Reanalysis/ Expiry date:	29 February 2016
Density:	1096 kg/m ³
Treatments	
Test rates:	Oral (nominal): 62.5, 125, 250, 500 and 1000 µg A19649B (formulation)/bee Oral (consumed): 72.2, 143, 297, 568 and 1132 µg A19649B (formulation)/bee Contact: 62.5, 125, 250, 500 and 1000 µg A19649B (formulation)/bee
Control:	Oral: 50 % (w/v) aqueous sucrose solution Contact: mineral water

Toxic standard:	Perfekthion (a.s. dimethoate)
Administration:	Contact: cuticular absorption following the application of droplets to the dorsal body surface Oral: ingestion in aqueous sucrose solution.
Test organisms	
Species:	<i>Apis mellifera</i> (Hymenoptera: Apidae)
Source:	Beekeeper. Carlos Feuerriegel, Ciudad Jardin 44, S-46620 Ayora, Spain
Food:	50 % w/v aqueous sucrose solution
Test design	
Test cage description:	Stainless steel chambers (approx. 4 x 8 x 6 cm) with a glass front and a perforated plate at the rear. A hole at the top of the test unit accommodated the feeding tube. The base of the cage was covered with filter paper.
Replication:	4
No. of bees/arena:	10
Duration of test:	48 hours
Environmental test conditions	
Temperature:	24.6 – 25.7 °C
Humidity:	58.0 – 65.1 %
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 29 July to 14 August 2014

Honeybees (*Apis mellifera*) were exposed to A19649B via two routes of administration: contact and oral ingestion. To immobilise the bees during the course of treatment, they were anaesthetised using short bursts of CO₂.

Contact test procedures: Bees were treated with a single 2 µl droplet of the test solution, control or toxic standard applied to the dorsal surface of the thorax using a micro applicator. The bees were returned to the test unit, allowed to recover and kept in the CE room with a continuous supply of 50 % w/v aqueous sucrose solution.

Oral test procedures: Bees were starved from the time they were collected from the hives for 2 hours until treatment, to ensure that the bees were equal in terms of their gut contents at the start of the test.

Each group of bees was offered 250 µL (equivalent to 25 µL/bee) of the test material or toxic standard dispersed in aqueous sucrose solution. Treatments were calculated so that the target dose was contained in 200 µL, however 250 µL was actually provided per test unit. This was to ensure sufficient consumption of the test material so that the target dose was achieved. The doses were measured into the feeding tubes and the weights of these were recorded before the doses were made available to the bees.

Once all test solutions were consumed, or six hours had elapsed since these were made available (whichever was achieved first), the feeding tubes were replaced with similar tubes containing approximately 1.5 mL of 50 % w/v aqueous sucrose solution. All feeding tubes with test solutions were weighed in order to calculate actual mean consumption per bee for each treatment.

In both the contact and oral tests there were 4 replicates per treatment. Mortality and sublethal effects were assessed at 24 and 48 hours for the test material and toxic standard for both oral and contact tests.

For each concentration, the corrected mortality (in cases of mortality in the respective control) was calculated according to Abbott (1925) modified by [REDACTED] (1947). The LD₅₀ was calculated for both oral and contact tests using Probit analysis.

Results and Discussion

Data for oral test on A19649B are summarised in the table below:

Table 9.5.1-3: Summary of acute oral toxicity of A19649B (formulation) to the honeybee

Treatment (µg formulation/bee)		Treatment (µg a.s./bee)*		Mortality after 4 hours	Sub-lethal effects after 4 hours ¹	Mortality after 24 hours	Sub-lethal effects after 24 hours ¹	Mortality after 48 hours	Sub-lethal effects after 48 hours ¹
Nominal	Consumed	Nominal	Consumed	Mean (%)	Mean (%)	Mean (%)	Mean (%)	Mean (%)	Mean (%)
0 (Control)		0 (Control)		0	0	0	0	0	0
62.5	72.2	11.6	13.4	0	2.5	5	0	5	0
125	143	23.3	26.6	0	2.5	5	0	5	2.5
250	297	46.5	55.2	0	5	0	0	2.5	5
500	568	93.0	106	0	2.5	0	0	0	5
1000	1132	186.0	210.6	0	10	2.5	7.5	2.5	7.5
LD ₅₀ (µg formulation/bee, consumed)				0	n.d.	> 1132	n.d.	> 1132	n.d.

n.d. not determined

*Calculated from formulation amount using 18.6 % w/w as stated on certificate of analysis from study sponsor.

¹ Sub-lethal effect/behaviour percentage calculated by HSE from available raw data

Data for contact test on A19649B is summarised in the table below. In terms of sublethal effects, two single affected bees were observed at the dose levels of 62.5 and 250 µg product/bee during the entire test period, which is therefore not considered to be treatment related.

Table 9.5.1-4: Summary of acute contact toxicity of A19649B (formulation) to the honeybee

Treatment (µg formulation/bee)	Treatment (µg a.s./bee)	Mortality after 24 hours		Mortality after 48 hours	
		Mean (%)	Corrected (%)	Mean (%)	Corrected (%)
0 (Control)	0 (Control)	2.5	-	2.5	-
62.5	11.6	0.0	-2.6	0.0	-2.6
125	23.3	0.0	-2.6	0.0	-2.6
250	46.5	0.0	-2.6	0.0	-2.6
500	93.0	0.0	-2.6	0.0	-2.6
1000	186.0	0.0	-2.6	0.0	-2.6
LD ₅₀ (µg formulation/bee)		> 1000		> 1000	

Mortality corrected with the corresponding control mortality according to SCHNEIDER-ORELLI, O. (1947); negative values mean lower mortality in the test or reference item treatment compared to the control group

Validity Criteria

The validity criteria are listed below:

- The mortality in both the contact and oral control was 2.5 and 0.0 % respectively (must be < 10 %).
- The oral 24 hour LD₅₀ for the reference item (dimethoate) was 0.11 µg /bee (recommended 0.10 – 0.30 µg a.s./bee).
- The contact 24 hour LD₅₀ for the reference item (dimethoate) was 0.12 µg /bee (recommended 0.10 – 0.35 µg a.s. /bee).

Conclusions

In the control group of the oral toxicity test (untreated aqueous sucrose solution) no mortality was observed during the 48 hour observation period.

In the control group of the contact toxicity test (mineral water) 2.5 % mortality was observed at the end of the 48 hours observation period.

The 24-hour oral and contact LD₅₀ values for the reference item were 0.11 and 0.12 µg dimethoate/bee, respectively. Consequently, validity criteria for both control and reference item mortality were met and the test was deemed valid.

In conclusion, the 48-hour oral LD₅₀ value for A19649B is > 1132 µg formulation/bee and the 48-hour contact LD₅₀ is >1000 µg formulation/bee.

(██████████, 2015)

HSE Comments

This study was conducted to GLP. Overall the study had no major deviations from the OECD 213 and 214 (1998) guidelines and the study met all validity criteria for these guidelines.

The following minor points are noted but have no impact on the outcome of the study:

- The contact dosing volume was 2 µl per bee instead of 1 µl as mentioned in the guideline but the authors justify the use of the larger volumes as it “*ensures a more reliable dispersion of the test item*” which is acceptable.

It is noted that the authors report sublethal effects for several single bees at all concentrations in the oral toxicity test, with increasing numbers at higher treatment levels. However, low mortality still supports the LD₅₀ endpoints of the study.

Two instances of abnormal effects are noted for the contact test in single bees, and the effect does not continue throughout the test. These small numbers of sublethal effects and absence of mortality in the contact test still support the final endpoint conclusions.

The authors do not fully specify their statistical analysis methods for determining LD₅₀ in the full report though they do note the use of “*probit analysis using the statistical program ToxRat Professional 2.10*” for the reference item treatment. Due to absence of or low mortality levels in the test treatments, the specified endpoints for representative product are deemed acceptable, as it is not possible to statistically derive robust LD₅₀ values.

The acceptable endpoints for use in risk assessment are:

- Oral 24-hour and 48-hour LD₅₀: > 1132 µg formulation A19649B /bee, equivalent to >210.6 µg a.s./bee (consumed concentration)
- Contact 24-hour and 48-hour LD₅₀: >1000 µg formulation A19649B /bee, equivalent to >186 µg a.s./bee (nominal concentration)

Please note that the following study was conducted with the EU representative formulation (A19649B); further details are given in the risk assessment.

Report:	K-CA 8.3.1.2, ██████████, (2014), A19649B - Chronic Toxicity to the Honeybee <i>Apis mellifera</i> L. in a 10 Day Continuous Laboratory Feeding Study. Report Number 14 10 48 004 B BioChem agrar Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6 04827 Gerichshain, Germany. (Syngenta File No. A19649B_10055)
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GUIDELINES

██████ *et al.* (2005)

██████ *et al.* (2001)

AFPP method CEB No. 230 (2012)

EFSA Guidance Document (2013)

Ring test protocol of the AG-Bienenschutz (2014)

GLP: Yes.

MATERIALS

Test Material	A19649B
Lot/Batch #:	SMU2JP001
Actual content of active ingredients:	SYN545974: 18.6 % w/w corresponding to 204 g a.s./L
Density:	1096 kg/m ³
Treatments	
Test rates:	Nominal: 150.1, 100.2, 67.0, 44.7 and 29.9 µg a.s./bee/day corresponding to 822.4, 549.3, 367.0, 245.1 and 163.7 µg A19649B/bee/day Consumed: 138.168, 89.073, 63.978, 49.525, and 28.271 µg a.s./bee/day corresponding to 757.2, 488.1, 350.6, 271.4 and 154.9 µg A19649B/bee/day
Control:	50 % (w/v) sucrose solution.
Toxic standard:	Dimethoate EC 400 (LC ₅₀ = 0.485 mg a.s./kg food)
Administration:	Ingestion in aqueous sucrose solution
Test organisms	
Species:	<i>Apis mellifera</i> L. (subspecies <i>carnica</i>)
Age and sex:	Young female workers 1-4 days old (collected at 1 day old).
Source:	Beekeeper Bienenfarm Kern GmbH, Rehbacher Anger 10, 04249 Leipzig, Germany
Collection:	Brood combs with capped cells were taken from outside hives and different colonies. Frames were placed without adult worker bees in a “five comb hive body” and incubated under controlled environmental conditions in an incubator at 33 ± 2 °C in darkness.
Acclimatisation:	Newly hatched bees were held in test cages for 24 ± 2 h at 33 ± 2 °C and 60 ± 15 % rH and were provided with sugar solution for acclimatization to the test conditions.
Food:	50 % w/v aqueous sucrose solution provided <i>ad libitum</i> via syringes
Test design	
Test cage description:	Aluminium cages (20 x 10 x 15 cm) with holes in the lateral walls for sufficient air supply and ventilation and two glass plates (one in front and one in the back) for observations of the bees
Replication:	3 test units per treatment/control group
No. of bees/arena:	20
Duration of test:	10 days

Environmental test conditions

Temperature:	32.6 – 33.1 °C
Humidity:	56.5 – 62.0 %
Photoperiod:	Constant darkness (diffuse artificial light during handling and assessment)

STUDY DESIGN AND METHODS

Experimental dates: 12 to 22 August 2014

Honeybees (*Apis mellifera*) were exposed to A19649B via oral ingestion for a period of 10 days. A stock solution of the highest test concentration was prepared weighing 0.623 g test substance and making up to 25 mL with 50% (w/v) sucrose solution. All lower test solutions were freshly prepared daily, by appropriate dilution of stock solution with 50 % (w/v) aqueous sucrose solution.

The treated/untreated food was provided *ad libitum* in a plastic syringe, which had been weighed before application. The actual dietary consumption was determined by reweighing the syringe containing the remaining test solution each day after removal from the test units. Any unconsumed food was rejected. Observations for mortality, sublethal effects and behavioural abnormalities were recorded daily.

Due to their social feeding behaviour, the honeybees of a distinct group are assumed to share the application solution (trophallaxis) and thus receive similar doses of the applied respective item.

Descriptive statistics; Fisher's Exact Binomial test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$); Probit analysis using linear maximum likelihood regression for calculation of the LD₅₀/LC₅₀ value of the reference item.

RESULTS AND DISCUSSION

Mortality data for the test substance and control are summarised in the table below.

Table 9.5.1-5: Summary of chronic toxicity of A19649B to the honeybee (*Apis mellifera* L)

Treatment		Mortality after 10 days (%)	Corrected 10-day mortality (%)
Nominal (µg a.s./bee/day)	Consumed (µg a.s./bee/day)		
Control	-	1.7	-
150.1	138.168	3.3	1.6
100.2	89.073	0.0	0.0
67.0	63.978	0.0	0.0
44.7	49.525	1.7	0.0
29.9	28.271	1.7	0.0
Toxic reference (ng/bee/day)			
27.3	17.458	86.7*	86.4
16.4	11.373	26.7*	25.4
9.8	8.111	11.7	10.2
5.9	5.694	0.0	0.0
10-d LD₅₀		> 138.2 µg a.s./bee/day	
10-d LC₅₀		> 3.854 g a.s./kg food	
NOED		138.2 µg a.s./bee/day	
NOEC		3.854 g a.s./kg food	

Results are averages based on 3 replicates, containing 20 bees each; Calculations are performed with non-rounded values

* statistically significant difference in pairwise comparison between treatment and untreated control (Fisher's Exact Binomial Test with Bonferroni Correction; $\alpha=0.05$; one sided greater)

VALIDITY CRITERIA

The validity criteria outlined in OECD 245 (2014) were met:

Validity criterion	Required	Observed
Average mortality in control groups at end of test	< 15 %	1.7 %
Average mortality in the reference substance treated group at end of test	$\geq 50\%$	LC ₅₀ = 0.485 mg a.s./kg food

CONCLUSIONS

The chronic toxicity of A19649B was tested on honeybees under laboratory conditions.

The 10-day LD₅₀ was determined to be >138.2 µg consumed a.s./bee/day. The 10-day NOED was determined to be 138.2 µg consumed a.s./bee/day. The 10-day LC₅₀ was determined to be >3.854 g a.s./kg food. The 10-day NOEC was determined to be 3.854 g a.s./kg food.

(██████, 2014)

In accordance with Commission Regulation (EU) No 283/2013, estimation of LC10 and LC20 values was attempted. As less than 2% corrected mortality occurred at all test concentrations the LC10 and LC20 values were empirically estimated to be > 138.2 µg a.s./bee/day.

HSE COMMENTS

This study was conducted in accordance with GLP and has been assessed against OECD 245 (2017) test guidelines. The reference item test was conducted with a series of test concentrations, rather than a single dose between 0.5 and 1.0 mg a.i./kg feeding solution, as recommended in OECD 245 (2017). However, the LC₅₀ for the reference item was 0.485 mg a.s./kg food, which indicates that the sensitivity of the test system is slightly greater than expected and is therefore acceptable.

The validity criteria outlined in OECD 245 (2017) have been satisfactorily met, however HSE does not consider the study fully reliable and uncertainties are noted, due to the following deviations from the test guideline.

Firstly, OECD 245 (2017) guidelines require that analytical determination of the test item concentrations is performed. No analytical methods are provided with this study and no samples of the test item appear to have been taken for use at a later date. Furthermore, OECD 245 requires that losses due to evaporation are considered when calculating the amount of diet consumed. No measurements of evaporative loss have been conducted in this study. As a result of these omissions, there is uncertainty surrounding the reliability of the study results, since the dose of test item per bee cannot be confirmed.

An additional minor deviation to the guideline is noted: 20 bees were used per replicate, rather than 10 as stipulated in OECD 245 (2017). Since the test cage size was adequate to house this number of bees, this deviation is not thought to have affected the study outcome.

Due to the lack of analytical measurements and uncertainty surrounding the dose of test item received per bee, this study cannot be considered fully reliable, and will be considered as supporting information at risk assessment.

The following study was conducted to fulfil the EU data requirement in accordance with **Commission Regulation (EU) No 283/2013** determine effects on honeybee development.

Please note that the following study was conducted with the EU representative formulation (A19649B); further details are given in the risk assessment.

Report:	K-CA 8.3.1.3, [REDACTED], (2015), A19649B – Chronic toxicity to the honeybee larvae <i>Apis mellifera</i> L. under laboratory conditions (<i>in vitro</i>). Report Number 14 10 48 005 B. BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany. (Syngenta file No. A19649B_10076).
Report:	K-CA 8.3.1.3, [REDACTED] (2016a) Pydiflumetofen – Statistical re-analysis: SYN545974 SC (A19649B) – Chronic toxicity to the honeybee larvae <i>Apis mellifera</i> L. under laboratory conditions (<i>in vitro</i>). Report Number CEA.1832. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK (Syngenta file No. A19649B_10296).

Guidelines

OECD DRAFT Guidance Document for testing chemicals: Honey bee (*Apis mellifera*) larval toxicity test, repeated exposure (February 2014)

OECD 237 Guidelines for testing chemicals: Honey bee (*Apis mellifera*) larval toxicity test, single exposure (2013)

GLP: Yes

Materials

Test Material	A19649B
Lot/Batch #:	SMU2JP001
Actual content of active ingredients:	SYN545974: 18.6 % w/w corresponding to 204 g /L
Description:	Off-white suspension
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	29 February 2016
Density:	1096 kg /m ³

Treatments

Test rates:	37.6, 75.3, 150.6, 301.1 and 602.3 µg A19649B /larva 6.9, 13.7, 27.5, 55.0 and 109.9 µg SYN545974 /larva 0.043, 0.087, 0.174, 0.347 and 0.695 g SYN545974 /kg diet
Control:	Untreated aqueous sugar solution
Toxic standard:	Dimethoate Tech. (BAS 152 I), purity 99.8 % w/w
Administration:	Oral application using a sterile pipette

Test organisms

Species:	Worker honey bee larvae <i>Apis mellifera</i> L. subspecies <i>carnica</i> P. (Hymenoptera, Apoidea)
Age:	First instar (L1) during grafting
Source:	Obtained from Bienenfarm Kern GmbH, Rehbacher Anger 10, 04249 Leipzig, Germany. The bee colonies producing the larvae were held under field conditions.

Food:	An aqueous sugar solution mixed with royal jelly, at a ratio of 1:1 based on (w/w) Composition of sugar solution, based on (w/v): Diet A: 12 % Glucose, 12 % Fructose, 2 % Yeast Diet B: 15 % Glucose, 15 % Fructose, 3 % Yeast Diet C: 18 % Glucose, 18 % Fructose, 4 % Yeast
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Test design

Test cage description: Crystal polystyrene grafting cells (internal diameter 9 mm) place in 48 well plates filled up to 1/3 with a piece of dental roll. The grafting cells were placed on the wetted and disinfected dental rolls.

Replication: 3
No. of bee larvae /replicate: 12

Environmental test conditions

Temperature*: 34.0 – 35.0 °C
Humidity*: 90 – 97 % (RH)
Photoperiod: Constant darkness
Duration of test: Pre-grafting (*in vivo*): 4 days (D-3 to D0)
Grafting: 1 day (D1)
Pre-exposure (*in vitro*): 3 days (D1 to D3)
Application: 4 days (D3 to D6)
Post exposure (*in vitro*): 2 days (D7 to D8)

* Deviations < 2 hours are not reported.

Study Design and Methods

Experimental dates: 29 August 2014 to 05 September 2014

Honeybee larvae *Apis mellifera* L. were exposed to repeated oral application of A19649B (active ingredient SYN545974) at 6.9, 13.7, 27.5, 55.0 and 109.9 µg SYN545974 /larva (equivalent to 0.043, 0.087, 0.174, 0.347 and 0.695 g SYN545974 /kg diet) in an *in vitro* test. One control group was included in the test. Larvae used in this experiment were taken from a hive that had not received treatments with chemical substances for at least one month. The larvae of the control treatment were fed with untreated artificial diet, which otherwise served as a vehicle for the test item and reference item. The test item was administered daily from Day 3 to Day 6 (inclusive). The toxic reference item was applied once on Day 3.

The bees used during the test were taken from three queen right colonies, which were treated in the same way. In order to ensure uniformity of the larvae, on day -3 (D-3) the queen was caged in an excluder cage, where she placed eggs solely on this comb. The caging time was approximately 30 hours. On day -2 (D-2), the queen was released from the excluder, and the comb was checked for the presence of freshly laid eggs. On Day 1, C-shaped, normally-developed larvae were transferred from combs to the grafting cells using a suitable grafting tool (e.g. grafting needle Swiss type) and placed on the surface of untreated artificial diet (Diet A) within the cells. Cells were placed in 48 well plates filled up to 1/3 with a piece of dental roll.

Each replicate unit consisted of 12 larvae, and there were 3 replicates per treatment and control. On Days 3 and 4, the test item or reference item was mixed into aqueous sugar solutions, and prepared according to Diets B (Day 3) and C (Day 4), to yield a stock solution. To achieve the final test concentrations, further appropriate sugar solution dilutions of the stock solutions were prepared, and royal jelly added at a ratio of 1:1, based on (w/w). The stock solutions prepared on Day 4 were stored in the fridge for further use on Days 5 and 6. Each larva was fed separately using a sterile pipette, with the food drop placed next to the larvae. The dead larvae were counted and removed daily from Day 4 to Day 8, and notes were taken of any other observations (such as small body size). A larva was defined as dead when it was observed to be immobile, and there was no reaction to the contact

of the grafting tool or paintbrush. Or alternatively, when a larva does not show signs of respiration under a stereomicroscope. Any unconsumed food was recorded on Day 7 and Day 8. After the last assessment (Day 8) the culture plates with all organisms were placed in a freezer. All observations were made in comparison to the control larvae.

For each concentration, the corrected mortality was calculated according to Abbott (1925), and further modified by Schneider-Orelli (1947). The precise LD₅₀ value could not be statistically determined, and was therefore stated to be greater than the highest tested dose. It was not possible to calculate a reliable LD₁₀ value, as the relationship between dose and response was not significant (■■■■■ 2016a). The statistical significance of the mortality values and the NOEC/NOED values were calculated using Fisher's Exact Binomial Test with Bonferroni Correction ($p \leq 0.05$).

Results and Discussion

Validity Criteria

The validity criteria according to OECD 237 Guidelines for testing chemicals: Honey bee (*Apis mellifera*) larval toxicity test, single exposure (2013); and OECD 239 Guidelines for testing chemicals: Honey bee (*Apis mellifera*) larval toxicity test, repeated exposure (2016) are displayed below:

Table 9.5.1-6: Validity criteria

Validity criterion	Required	Obtained
Mortality in the controls	Cumulative control larval mortality from D3 to D8 should be ≤ 15 % across all replicates.	8.3 %
Control emergence rate	Adult emergence in the control plate(s) on D22 should be ≥ 70 % across all replicates.	Please refer to the CA comments below regarding this point.
Positive control mortality	When using dimethoate, larval mortality should be ≥ 50 % on D8 across all replicates.	Absolute larval mortality rate was 72.2 %. When corrected for control mortality, the rate was 69.7 %.

Analytical results

Analytical verification of the concentration of active substance in the test solution was conducted using HPLC coupled with UV detection. The measured concentration of active substance in analysed stock solution ranged from 99 – 104 % of the nominal concentrations, and so the nominal concentrations were used for the analysis and reporting of data. The limit of quantification (LOQ) was defined as 832.1 mg /L a.s..

Biological results

Mortality data for the test material and control are summarised in Table 9.5.1-7 below:

Table 9.5.1-7: Summary of chronic toxicity of A19649B to honeybee larva (*Apis mellifera*)

Item applied	Dosage ($\mu\text{g SYN545974 /larva}$)	Concentration (g SYN545974 /kg diet)	Day 8		
			Mortality mean %		OO
			Absolute	Correct.	Mean %
Control	-	-	8.3	-	8.6
Test item	6.9	0.043	8.3	0.0	2.8
	13.7	0.087	8.3	0.0	8.6
	27.5	0.174	2.8	0.0	5.6

Item applied	Dosage (µg SYN545974 /larva)	Concentration (g SYN545974 /kg diet)	Day 8		
			Mortality mean %		OO
			Absolute	Correct.	Mean %
	55.0	0.347	8.3	0.0	20.8
	109.9	0.695	47.2*	42.4	35.1
Reference item	6.2	0.039	72.2	69.7	33.3
Treatment	Endpoints		Day 8		
Test item doses	LD ₁₀ (g SYN545974 /larva) (95 %-CL/lower-upper)		n.d.		
	LD ₅₀ (µg SYN545974 /larva) ² (95 %-CL/lower-upper)		>109.9 (263.8 – 345.1)		
Test item concentrations	NOEC (g SYN545974 /kg /diet) ¹		0.347		
Reference item	LD ₅₀ (µg Dimethoate /larva) (95 %-CL/lower-upper)		n.r.		

Results are averages based on 3 replicates containing 12 larvae each.

OO: Other observations (large quantities of remaining food, smaller body size of larvae)

*Statistically significant difference in pairwise comparison between treatment and untreated control (Fisher's Exact Binomial Test with Bonferroni Correction; $\alpha = 0.05$; one sided greater)

¹ Fisher's Exact Binomial Test with Bonferroni Correction; $\alpha = 0.05$; one sided greater

² All test item doses are based on a sum of applications on D3, D4, D5 and D6.

n.d.: not determined

n.r.: not reported

Conclusions

The 8 day NOEC was determined to be a food concentration of 0.347 g SYN545974 /kg (NOED = 55 µg SYN545974 /larva). The 8 day LD₁₀ was not determined, and the 8 day LD₅₀ was determined to be > 109.9 µg SYN545974 /larva, the highest concentration tested.

(██████████, 2015)

(██████████, 2016a)

HSE comments

This study was conducted using GLP and was in accordance with OECD 237 (2013) and OECD 239 (2016). Both validity criteria from OECD 237 (2013) were met, but one of the three validity criteria from OECD 239 (2016) was not, this is discussed further below:

It is stated that in the control plate(s), the adult emergence rate on D22 should be ≥ 70 % across all replicates. This study was ended on D8, and as a result, no measurements of the adult emergence rate were taken on D22. The OECD 239 guideline states that “Data are summarised (e.g. in a tabular form), showing for each treatment group, as well as control and reference chemical groups, the number of larvae used, mortalities and adverse effects: larval mortalities from D3 to D8, pupal mortalities from D8 to D15 and emergence rate on D22.” This issue with the larval study has not been pursued further at this stage as there is currently no noted guidance scheme to use the endpoints from these studies in the risk assessment.

It is noted that the study was conducted in 2015, prior to the publication of the OECD 239 guideline in 2016. The validity criterion regarding larval mortality on day 8 was met. The reference substance dimethoate was tested at 6.20 µg a.s. /larvae, 0.039 g a.s. /kg diet. A 69.7 % mortality rate was obtained on D8, at a slightly lower concentration than that recommended in the guideline – demonstrating the sensitivity of the test system to detect effects on larval mortality. Therefore, larval mortality endpoints can be derived from this study.

The study authors had initially provided the LD₅₀ value, but had not supplied the LD₁₀ value or any justification as to why it was not calculated. The calculation of an LD₁₀ value or justification for its omission is required in accordance with the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA 2015), and so a separate supporting document containing statistical reanalysis was also provided to address this (██████████, 2016a). The supporting document explains that statistical analyses of the available data for number of surviving larvae after 8 days of exposure revealed that the LD₁₀ and LD₂₀ values could not be determined, as the relationship between dose and response was not significant.

No LD₅₀ value was provided for the dimethoate reference item. This is unlikely to impact the reliability of the data, as the validity criterion for the reference item has been met.

Experimental conditions and feed composition were in line with OECD 237 (2013).

At the end of the test, deviations to the normal food consumption and therefore a corresponding reduction in larval development occurred in the remaining larvae, which had been treated with the two highest test concentrations of 109.9 or 55.0 µg a.s. (35 % and 21 % of remaining larvae on D8). Larvae which had received 27.5, 13.7 or 6.9 µg a.s. only showed an unusual feeding behaviour at the rate of the control or below.

NOEC/NOED were determined using Fisher's exact binomial test with Bonferroni correction. It was not possible to statistically determine the LD₅₀, and so it was determined to be greater than the highest tested concentration of 109.9 µg SYN545974 /larva in total developmental period. The statistical procedures were conducted in accordance with the recommendations from OECD 237 (2013). The statistics were not in line with the OECD 239 (2016) guidelines, as the duration of the experiment was 8 days instead of the 22 days required by OECD 239 (2016).

The analytical method has been evaluated by HSE Chemistry specialists in Vol. 3CA Part B5.1.2.6. The following was concluded for this method: "Acceptable method. LOQ: 832 mg/L in test diet (aqueous sugar solution)".

Based on nominal concentrations, the 8-day NOEC was determined to be a food concentration of 0.347 g SYN545974 /kg diet. The 8-day NOED was determined to be a total dose of 55.0 µg SYN545974 /larva². The 8-day LD₅₀ was estimated to be > 109.9 µg SYN545974 /larva in total developmental period.

Please note that the following study was conducted with the EU representative formulation (A19649B); further details are given in the risk assessment.

Report:	K-CA 8.3.1.3 ██████████ (2015a) SYN545974 SC (A19649B) - A laboratory study to determine the chronic effects on the brood of the honey bee <i>Apis mellifera</i> L. (Hymenoptera: Apidae). Report Number 037SRFR15C07, SynTech Research France SAS 613 route du Bois de Loyse 71570 La Chapelle de Guinchay, France (Syngenta file No. A19649B_10184 updated to include Amendment 1)
Report:	K-CA 8.3.1.3 ██████████ (2016) Pydiflumetofen - Statistical re-analysis: A laboratory study to determine the chronic effects of SYN545974 SC (A19649B) on the brood of the honey bee <i>Apis mellifera</i> L. (Hymenoptera: Apidae). Report Number CEA.1831. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK (Syngenta file No. A19649B_10294)
Report:	K-CA 8.3.1.3 ██████████ (2016) SYN545974: Response to ANSES comments regarding the bee brood toxicity test with honeybees (<i>Apis mellifera</i>) (██████████, 2015a) (Syngenta File No. SYN545974_10462)

Guidelines

OECD Guidelines for Testing of Chemicals, DRAFT method: Honey bee (*Apis mellifera*) larval toxicity test, repeated exposure (2014)

GLP: Yes

Materials

Test Material	Formulation A19649B
Lot/Batch #:	SMU2JP001
Actual content of active ingredients:	SYN545974 (pydiflumetofen): (204 g/L) equivalent to 18.6 % w/w as stated on certificate of analysis from study sponsor.
Description:	Off-white suspension
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	End of February 2016
Density:	1.096 g/mL

Treatments

Test rates:	0.34, 1.0, 3.0, 9.0, 27.0, 81.0 and 243 µg formulation./larva across the entire rearing period (corresponding to 0.06, 0.19, 0.56, 1.67, 5.02, 15.07 and 45.22 µg a.s./larva), equivalent to concentrations of 2.429, 7.143, 21.43, 64.29, 192.9, 578.6 and 1736 mg formulation /L diet (corresponding to 0.45, 1.33, 3.99, 11.96, 35.90, 107.67 and 323.07 mg a.s./L diet).
Control:	Untreated
Blank formulation:	Product formulation without test item. A21676A (Batch No. MNV001-076-003). Tested at concentration equivalent to highest dose of test item (243 µg f.p./larva)
Toxic standard:	ROGOR PLUS (Dimethoate 400 g/L, equivalent to 37.9 % w/w), tested at 116.9 mg f.p./L diet or 44.3 mg a.s./L diet (0.044 µg a.s./µL diet). Equivalent to 16.37 µg f.p./larva or 6.2 µg a.s./larva.
Application method:	Oral application via artificial diet

Test organisms

Species:	Honey bee <i>Apis mellifera</i> L. (Hymenoptera: Apidae)
Age:	First instar (L1) during grafting
Source:	Maintained at test facility
Food:	Artificial diet containing 50 % royal jelly and 50 % aqueous solution (D-glucose, D-fructose, yeast extract) supplemented with formulation A19649B.

Test Design

Test cage description:	1 individual cell (queen starter). 48 cells per culture plate. Each well of the culture plate was half filled with a piece of dental roll.
Replication:	3 (one colony per replicate was used)
No. of larvae/replicate:	12

Environmental test conditions

Temperature:	23.2 to 25.6 °C
Humidity:	48 to 72 %
Photoperiod:	Constant darkness
Duration of test:	22 days

Study Design and Methods

Experimental dates: 8 to 29 June 2015

The study comprised an untreated control, a blank formulation equivalent to the maximum dose of the test item, a toxic reference item and seven doses of the test item treatment: 0.34, 1.0, 3.0, 9.0, 27.0, 81.0 and 243 µg formulation/larva (equivalent to concentrations of 2.429, 7.143, 21.43, 64.29, 192.9, 578.6 and 1736 mg formulation/L diet). Exposure to the treatments occurred via the diet during the larval rearing period. The toxic reference item was dimethoate applied at a dose equivalent to 16.37 µg f.p./larva during the entire rearing period and should result in a corrected mean mortality of between 50 % and 100 % at day-8 of the test. A blank

formulation control (A21676A) equivalent to the highest dose of test item (243 µg formulation/larva) was included in the study to determine any potential effects of the formulation components to the larvae.

Treatments were applied on days 3, 4, 5 and 6 of the larval rearing period (chronic exposure): using a calibrated micropipette.

The number of dead larvae was recorded on Day 4, Day 5, Day 6, Day 8 (plus uneaten food) and, Day 15. On Day 22 the number of emerged adult bees and number of dead pupae (pupal mortality) was counted. Behaviour and development were also recorded.

Results (except toxic reference results) were analysed with the statistical software Minitab® Release 14 (Fisher test with Bonferroni correction) to determine any significant differences between treatments and control. The EPA Probit analysis program V1.5 () was used to determine a rate-response relationship and calculate LD/EC₅₀ and LD/EC₁₀. Behavioural observations were not evaluated for statistical significance due to the non-quantitative nature of the observations.

In an additional report (, 2016), the data was further analysed using software ToxRat Professional version 3.2.1 to carry out Probit analysis with linear maximum likelihood regression, in order to try and determine LD₂₀ and EC₂₀ values.

Mortality results were corrected for control mortality using an adaptation of Abbott's formula (1925):
Corrected mortality [%] = (Treatment mortality [%] – control mortality [%]) / (100 – control mortality [%])

Results and Discussion

The mean control-corrected mortality rate in the toxic reference item treatment (applied at 16.37 µg f.p./larva) was at day 8 was 60.61 %, which meets the validity criteria.

Mortality data and other observations for the test material are summarised in the table below. There were no behavioural effects detected.

Endpoints are also shown in the table below as calculated in this report (LD₅₀/ED₅₀, LD₁₀/ED₁₀), or in the statistical re-analysis by (, 2016 (LD₂₀/ED₂₀).

The LD/ED₁₀ values are considered unreliable according to OECD criteria for the following reasons:

- Confidence intervals should not span more than two test concentrations
- Confidence intervals should not contain zero
- EC_x should not be extrapolated outside the range of tested concentrations (e.g. 25 % below the lowest or 25% above the highest concentration)

In (, 2016, neither endpoint (LD₂₀/ED₂₀) could be reliably determined because the 95 % confidence limits were either too wide (outside of two test concentrations) and/or were extrapolated outside of the tested range.

In a further report (, 2016), the authors discuss the 8-day NOED in more detail, justifying the observed biological effects at the lowest three treatment levels as being variable across replicates with a lack of dose-response relationship.

Table 9.5.1-8: Summary of chronic toxicity of test material to honey bee larvae

Test item					SYN545974 SC (A19649B)					
Test organism / Exposure					Honey bee larvae / Repeated exposure (Chronic)					
Application rate					8-day cumulative mean larval mortality [%]		Pupal mortality ^[1] [%] 22 days		22-day cumulative effects on adult emergence ^[4] [%]	
[µg f.p./larva]	[µg a.s./larva]	[mg a.s./kg diet]	[µg f.p./larva/day]*	[µg a.s./larva/day]*	Abs.	Corr.	Abs.	Corr.	Abs. ^[3]	Corr.
Control					8.333	-	3.030	-	11.11	-
0.34	0.06	0.409	0.085	0.015 ^f	19.44	12.12	10.34	7.543	27.78	18.75
1.0	0.19	1.209	0.25	0.048 ^f	22.22	15.15	14.29	11.61	33.33	25.0
3.0	0.56	3.627	0.75	0.14	22.22	15.15	17.86	15.29	36.11	28.13
9.0	1.67	10.87	2.25	0.42	27.78	21.21 (s)	15.38	12.74	38.89	31.25
27.0	5.02	32.64	6.75	1.26	38.89	33.33 (s)	13.64	10.94	47.22	40.63 (s)
81.0	15.07	97.89	20.25	3.77	41.67	36.36 (s)	28.57	26.34	58.33	53.13 (s)
243	45.02	293.7	60.75	11.31	58.33	54.55 (s)	46.67	45.0	77.78	75.0 (s)
'Blank' formulation equivalent to co-formulants at 243 µg f.p./larva (no a.s.) ^[2]					25.00	18.18	11.11	8.333	33.33	25.0
LD₅₀/ED₅₀ (95% confidence limits)			[µg f.p./larva]		> 243 ^e		-		41.06 (10.91 – 162.50)	
			[µg a.s./larva]		> 45.24 ^e		-		7.64 (2.03 – 30.25)	
			[µg f.p./larva/day]		> 60.75 ^e		-		10.27 (2.73 – 40.63)	
			[µg a.s./larva/day]		> 11.31 ^e		-		1.91 (0.51 – 7.56)	
NOED			[µg f.p./larva]		3.0		-		9.0	
			[µg a.s./larva]		0.56		-		1.68	
			[µg f.p./larva/day]		0.75		-		2.25	
			[µg a.s./larva/day]		0.14		-		0.42	
LD₁₀/ED₁₀ (95% confidence limits) ^a			[µg f.p./larva]		0.580 (0.001 – 4.462)		-		0.148 (0.001 – 1.133)	
			[µg a.s./larva]		0.10 (0.00018 – 0.80) ^c		-		0.028 (0.00019 – 0.211) ^d	
			[µg f.p./larva/day]		0.145 (0.000 – 1.116)		-		0.037 (0.000 – 0.283)	
			[µg a.s./larva/day]		0.026 (0.00 – 0.20) ^c		-		0.0069 (0.000 – 0.053) ^d	
LD₂₀/ED₂₀ (95% confidence limits) ^b			[µg f.p./larva]		0.872 (0.055 – 2.951)		-		0.165 (0.008 – 0.672)	
			[µg a.s./larva]		0.16 (0.0099 – 0.53) ^c		-		0.031 (0.0015 – 0.125) ^d	
			[µg f.p./larva/day]		0.218 (0.014 – 0.738)		-		0.041 (0.002 – 0.168)	
			[µg a.s./larva/day]		0.039 (0.0025 – 0.13) ^c		-		0.0076 (0.00037 – 0.0312) ^d	

f.p. = formulated product; **a.s.** = active substance; **Abs.** = Absolute value; **Corr.** = value corrected from untreated control according to Abbott (1925).

Treatment groups significantly different from the control (ANOVA plus Fisher test with Bonferroni correction, after Log transformation) are marked with (s).

* Mean value of daily consumed dose.

^[1] The pupal mortality is the inverse of the emergence effect from the day 8 to day 22. It is calculated from the number of dead pupae compared to the number of entering larvae on day 8. No statistical tests were performed on this data.

^[2] Blank formulation is the formulation components only, without the test substance. The concentration of the formulation components is equivalent to that which would be in the highest dose of test item (243 µg f.p./larva or 60.75 µg f.p./larva/day).

^[3] Calculated by HSE from the day 22 adult emergence data available in study report. For example, a 22 day mean adult emergence of 88.89 % gives a 22-day cumulative effect on adult emergence of 100 % - 88.89 % = 11.11 %.

^[4] 22-day cumulative effects show the impact on 22-day adult emergence. For example, a 22-day cumulative effect of 75 % means 75 % of adults did not successfully emerge by day 22.

^a Considered unreliable according to OECD criteria.

^b Calculated in [REDACTED], 2016. 95 % confidence intervals are wide (i.e. not within two test concentrations) and/or have been extrapolated outside of the tested range, therefore LD₂₀/ED₂₀ should be treated with caution.

^c Conversion from µg formulated product to µg a.s. stated in report by [REDACTED], 2016.

^d Conversion from µg formulated product to µg a.s. calculated by HSE from µg f.p. endpoints, using 18.6 % w/w a.s. in formulation.

^e Actual calculated LC₅₀ as stated in statistical Appendix of full study report: 266.8 (65.3 – 5221) µg f.p./larva and 66.7 (16.3 – 1305) µg f.p./larva/day; equivalent to 49.62 (12.14 – 971) µg a.s./larva and 12.4 (2.25 – 180.6) µg a.s./larva/day as calculated using 18.6 % w/w a.s. in formulation.

^f Value updated by HSE based on total dose per larva divided by 4 (number of days dose applied) to 3 decimal places (previous figure was rounded to 2 decimal places which causes loss of detail at these low concentrations).

Analytical Verification

The actual analysed concentration of 3.1640 g a.s./L, was within the required range of 80-120 % of the nominal concentration (actual values: -2.1 %), thus confirming correct preparation of the analysed test item stock solution.

Validity Criteria

The validity criteria for the test were met;

- The control cumulative larval mean mortality from day 4 to day 8 was 8.33 % (must not exceed 15 % across all replicates).
- The control adult mean emergence on day 22 was 88.89 % (must be ≥ 70 % across all replicates).
- The cumulative mortality in the toxic reference item (dimethoate) on day 8 was 60.61 % (must be ≥ 50 %).

Conclusions

The objective of the study was to determine the lethal and sublethal effects of SYN545974 on the brood of the honey bee *Apis mellifera* L. (Hymenoptera: Apidae), when mixed with artificial diet and fed to larvae.

There was no significant difference in larval mortalities and emergence between the control and the blank formulation.

The NOED during larval development was 0.75 µg formulation/larva/day (corresponding to 0.14 µg a.s./larva/day) and the LD₅₀ during larval development was estimated to be > 60.75 µg formulation/larva/day (corresponding to > 11.31 µg a.s./larva/day).

The NOED for the entire development period was 2.25 µg formulation/larva/day (corresponding to 0.42 µg a.s./larva/day) and the LD₅₀ for the entire development period was estimated to be 41.06 µg formulation/larva (95 % confidence limits: 10.91 – 162.50 µg formulation/larva) (corresponding to 1.91 µg a.s./larva/day; 95 % confidence limits: 0.51 – 7.56 µg a.s./larva/day).

LD/ED₁₀ and LD/ED₂₀ values were determined for both timepoints but were considered unreliable due to large confidence limits and/or the resulting values being extrapolated outside the range of tested concentrations.

([REDACTED], 2015a)

([REDACTED], 2016)

([REDACTED], 2016)

HSE Comments

This study was conducted to GLP with the exception of diet, hive history and preparation of plastic cells. The study was carried out according to the draft OECD guideline for repeated exposure larval toxicity test (2014), however the most recent version is OECD 239 (2016), so the study was assessed against the more recent guideline.

The authors verified the concentration of the test substance in the stock solution was within ± 20 % of nominal and therefore use nominal concentrations when reporting results.

The analytical method has been evaluated by HSE Chemistry specialists in Vol. 3CA Part B5.1.2.6. The following was concluded for this method: “Acceptable method. LOQ: 0.26 mg/L in test diet (aqueous sugar solution)”.

Overall the study met all validity criteria and there were no major deviations from the guideline. The following minor points are noted for information:

- The authors conducted a reference test with dimethoate tested at 0.044 $\mu\text{g a.s./}\mu\text{L}$ diet and the 8-day mortality results met the validity criteria of the guideline. The OECD guideline specifies the use of 0.053 $\mu\text{g a.s./}\mu\text{L}$ diet for dimethoate reference treatment. The use of the lower test concentration is acceptable since it is more conservative but still successfully demonstrated the sensitivity of the tests system.
- There is a minor deviation in the relative humidity from day 15 onwards. The guideline states the relative humidity should be maintained at 50-80 %, whereas in the study it was slightly below at 45-55 %. This small difference has not affected the study controls meeting the validity criteria, so is of no concern.

Note on Blank Formulation

In addition to the negative control, an additional ‘blank formulation’ was tested, consisting of the product formulation only, without the active substance. The amount of formulation components in this blank is equivalent to the concentration of formulation components present in the highest treatment level (243 $\mu\text{g f.p./larva}$). Although there are clearly some biological effects are observed with this blank formulation (8-day mortality: 25 % absolute, 18 % control-corrected; 22-day effects 33 % absolute, 25 % control-corrected), these are not shown to be statistically significant (Table 9.5.1-8).

In a further report produced in response to an RMS query during the EU evaluation of this study (██████████, 2016), the authors note that the results from the blank formulation should not have an impact on determining the NOED, since the purpose of the blank formulation dose was to ‘*determine any potential effects of the formulation components on the larvae at the **maximum** level of exposure in the study*’. The authors further note that at their proposed 8-day NOED of 0.56 $\mu\text{g a.s./larva}$ (0.14 $\mu\text{g a.s./larva/day}$), the levels of co-formulants are 80-fold lower than in the blank formulation dose, but that ‘*the formulation components may be contributing to the toxicity at the higher test concentrations*’.

HSE agrees that the results of the blank do not prevent determination of endpoints such as NOED from being derived in this study, though HSE has issues with the proposed NOEDs for other reasons as detailed below. It is however noted, that the impact of the formulation components on toxicity introduces some uncertainty at higher test concentrations. This is because despite the results of the blank being statistically insignificant, there are clear biological effects observed, with both the 8-day and 22-day absolute results for the blank exceeding the control validity criteria thresholds.

Notes on LD/ED endpoints (8-Day and 22-Day)

The study authors note that the determined LD/ED₁₀ and LD/ED₂₀ are unreliable due to having large 95 % confidence intervals and/or resulting in a value extrapolated outside the tested range of concentrations.

HSE notes that the LD/ED₅₀ also have wide confidence limits for 22-day mortality, which adds some uncertainty to these endpoints as well. Additionally, the calculated 8-day LD₅₀ is presented as an unbounded value ($> 60.75 \mu\text{g f.p./day}$) due to being extrapolated beyond the highest tested concentration, and has very large confidence limits (Table 9.5.1-8 note e). This compares to a 54.55 % 8-day mortality at the tested concentration of 60.75 $\mu\text{g f.p./day}$. Although the 8-day LD₅₀ of $> 60.75 \mu\text{g f.p./day}$ is generally in line with the data, HSE deems the use of LD₅₀ = 60.75 $\mu\text{g f.p./day}$ more appropriate in this case rather than the unbounded value.

Note on proposed NOEDs (8-Day and 22-Day)

The author’s proposed NOEDs are 0.75 $\mu\text{g f.p./larva/day}$ for 8-day larval mortality and 2.25 $\mu\text{g f.p./larva/day}$ for 22-day cumulative emergence effects, which were based on the statistical analysis. HSE does not agree with these

endpoints, because there appear to be potentially relevant biological effects observed at concentrations equal to and lower than these NOEDs, which although are not statistically significant, are relevant for risk assessment. Additionally, the absolute observed effects at these NOEDs both exceed the respective control validity criteria, suggesting that the observed effects are not within natural variation for this test:

- The 8-day control validity criteria is ≤ 15 % mortality; at the author's proposed 8-day NOED of 0.75 $\mu\text{g f.p./larva/day}$, absolute mortality is 22.22 % compared to 8.33 % in the control.
- The 22-day control validity criteria is ≤ 30 % mortality; at the author's proposed 22-day NOED of 2.25 $\mu\text{g f.p./larva/day}$, absolute mortality is 38.89 % compared to 11.11 % in the control.

HSE proposes alternative NOEDs as follows:

- 8-day NOED is not determinable [n.d., $< 0.085 \mu\text{g f.p./larva/day}$]. This is because:
 - The 8-day absolute mortality at the lowest test concentration of $0.085 \mu\text{g f.p./larva/day}$ is 19.44 %, which exceeds the control validity criteria of 15 % background mortality, suggesting this cannot be attributed to natural variation alone. Additionally, considering the control-corrected value of 12.12 % mortality, this could be considered a potentially biologically relevant effect.
- 22-day NOED is $0.085 \mu\text{g f.p./larva/day}$, equivalent to $0.015 \mu\text{g a.s./larva/day}$. This is because:
 - At this test concentration, the observed absolute mortality of 27.78 % is within the 30 % control validity criteria, suggesting it could be within natural variation for this study design. However, it is still noted that considering the control-corrected mortality is 18.75 %, there may still be potentially relevant biological effect at this test concentration.

Agreed endpoints:

The agreed endpoints for consideration in risk assessment are presented below, noting that wide confidence limits and/or extrapolation beyond tested concentrations mean all calculated LD/ED_x should be treated with caution:

- 8-day LD₅₀ = 45.24 $\mu\text{g a.s./larva}$ or 11.31 $\mu\text{g a.s./larva/day}$ (consumed nominal concentration)
- 8-day NOED = n.d. ($< 0.06 \mu\text{g a.s./larva}$ or $< 0.015 \text{ a.s./larva/day}$ (consumed nominal concentration))
- 22-day ED₅₀ = 7.64 $\mu\text{g a.s./larva}$ or 1.91 $\mu\text{g a.s./larva/day}$ (consumed nominal concentration)
- 22-day NOED = 0.06 $\mu\text{g a.s./larva}$ or 0.015 $\mu\text{g a.s./larva/day}$ (consumed nominal concentration)

Please note that the following study was conducted with the EU representative formulation (A19649B); further details are given in the risk assessment.

Report:	K-CP 10.3.1.5, [REDACTED] (2017) Pydiflumetofen SC (A19649B) - A Semi-Field Study to Evaluate the Side Effects on Honeybees (<i>Apis mellifera</i> L.) in <i>Phacelia tanacetifolia</i> in Germany 2016. Report number S16-000293. Eurofins Agrosience Services EcoChem GmbH/Eurofins Agrosience Services Ecotox GmbH, Eutinger Str. 46, 75223 Niefern-Öschelbronn, Germany (Syngenta file No. A19649B_10312)
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Guidelines

OECD Guidance Document No. 75 (2007) and current recommendations of the AG Bienenschutz ([REDACTED] et al., 2012).

OEPP/EPPO, Guideline No. 170 (4), (2010) EU Guideline 7029/VI/95 rev. 5: General recommendations for the design, preparation and realization of residue trials (1997).

GLP: Yes

Materials

Test item	A19649B
Lot/Batch #:	CWA002-104-001
Formulation type:	SC (soluble concentrate)
Appearance	White liquid

Nominal content of active ingredients:	Pydiflumetofen 200 g /L (18.4% w/w)
Stability of test compound:	Sufficient for the test purpose (at least 1 h)
Reanalysis/Expiry date:	31 March 2017
Density:	1.093 g/cm ³
Reference Item:	Insegar (Fenoxycarb 25% w/w)
Application method:	Portable boom sprayer (Schachtner), reduced drift fan nozzle, at a pressure of 2.5 - 2.6 bar.
Test item application rates:	75 (T1), 125 (T2) and 200 g a.s. /ha (T3).
Reference item application rate:	300 g a.s./ha
Duration of each stage:	Bee colonies were installed in the tunnels 6 days before application. Colonies were kept in the tunnels for a further 7 days after application. Colonies were moved to the monitoring site 8 days after application, and were periodically assessed until 63 days after application.
Total test duration:	69 days
Test design Location:	Stutensee, near Karlsruhe in the region of Baden-Württemberg, Germany
Tunnel specifications:	Floor area was approximately 100 m ² , tunnels were 3.5 m high in the center, tunnels were covered with a 1.5 x 1.5 mm mesh.
Replication:	Four tunnels per treatment (control, test item and reference item) with one honey bee colony per tunnel. Four additional tunnels (Cs, T1s, T2s, and T3s) with one colony per tunnel (control and test item) for residue sampling
Crop:	<i>Phacelia tanacetifolia</i>
Test organism:	Honey bee, <i>Apis mellifera</i> L.
Honey bee colonies:	Single box colonies with 10 combs per colony (Zander type).
Environmental conditions:	Weather data was obtained from a data logger in the field and from two nearby weather stations (in Niefern and Enzberg).

Study Design and Methods

Experimental dates: 14th June 2016 – 7th April 2017

The objective of the study was to determine potential effects of exposure of honey bees to flowering *Phacelia tanacetifolia* treated once at start of flowering with pydiflumetofen (applied as formulation A19649B) under semi-field conditions. The formulated test item pydiflumetofen (A19649B) was applied to flowering *Phacelia tanacetifolia* with the target application rates of 75, 125 and 200 g a.s./ha (T1, T2, T3) during daily honey bee flight.

Honey bee colonies were placed in the tunnels at the start of flowering. The mortality, foraging activity, behaviour of the bees, development of the bee brood assessed in individually marked cells and condition of the colonies were examined prior to and post application. All colonies used in the experiment were free of symptoms of noseiosis, varroosis, foulbrood and other bee diseases. Colonies were all queen-right, and all brood stages were present at the start of the test. At each colony assessment, colonies were assessed for bee diseases according to standard beekeeping practice. Accordingly, any unusual occurrences (e.g. presence of dead bees, dark “bald”

bees, “crawlers” or flightless bees, unusual brood patterns or brood age structure) and clear symptoms of disease or pests were recorded. Total numbers of bees per colony were first recorded at the first colony assessment (9DBA), and then at each subsequent assessment day.

The test item, reference item, and control were applied to flowering *Phacelia tanacetifolia* (BBCH 65) once, during honey bee flight, using a calibrated portable boom sprayer with a reduced drift fan nozzle. The spray volume was 400 L /ha for all conditions. Honey bee colonies inside the tunnel were covered with plastic during application, and the water sources were moved out of the tunnel to avoid direct contamination. No rainfall occurred within the 2 hours following application.

The influence of Pydiflumetofen SC was evaluated by comparing the data of the assessments of the three test item groups T1, T2 and T3 to the reference item group R and the control group C, and by comparing the pre-application data to the post-application data.

Samples of forager bees (for preparation of pollen and nectar), leaves, flowers and samples of soil were collected during the exposure phase. Samples of pollen and nectar (in-hive products), pollen (from pollen trap) and dead bees (from dead bee traps and from the hive bottoms) were collected during the monitoring phase of the study. Samples of pollen and nectar prepared from forager bees, leaves, flowers, samples of in-hive products and pollen from pollen trap were analysed for residues of pydiflumetofen. Soil samples were collected on 2DBA but were not analysed for residues since pydiflumetofen was not found in any of the untreated samples of pollen, nectar, flowers and leaves. In addition, it was possible to obtain the full pesticides/maintenance history for the test field showing what applications had been made from 2013 to 2016. Since no dead bee sample contained > 100 bees /day (criteria set above which analysis would have taken place), samples were not analysed.

After the last evaluation of mortality and foraging activity in the tunnels, in the evening of 7DAA, the colonies were relocated and maintained until 63DAA at a monitoring site for the monitoring phase (distance between field site and monitoring site: 22 km). The two monitoring sites were placed in an area without intensive agriculture or flowering crops nearby (radius of 3 km) which might be attractive to honey bees. Both hives were placed in the Baden-Württemberg region of Germany. Monitoring site 1 was 22 km from the field site, at coordinates: 8°46'58.22" E, 48°53'12.26" N; monitoring site 2 was placed 24 km from the field site, at coordinates: 8°49'09.06" E, 48°59'23.92" N. The hives were placed at a minimum distance of 3 m from each other to prevent robbing during monitoring phase.

The software “SAS®”, version 9.3, was used for the statistical analysis of mortality (except male bee mortality), foraging activity data, colony data (number of bees, food cells and brood cells) and the brood termination rates, brood indices and compensation indices resulting from the photographic assessments. No statistical evaluation of dead male bees and male pupae was carried out due to their rare occurrence during this study and the low informative value of those records.

For all tests, the significance level was set as $\alpha = 0.05$. For the pre-application period all tests were conducted in a two-sided approach whereas for the data assessed after application one-sided tests (“upper” for mortality and termination rate, “lower” for flight activity, brood and compensation index, colony assessment data and colony strength) were conducted. For evaluation of mortality, the number dead bees on the linen sheets, in the dead bee traps and on the hive floor (bottom drawer) were summarized per replicate.

Data from the test item treatments T1, T2, T3 and the control were checked for normality using Shapiro-Wilks test. If the distribution of the data fitted the normal distribution well (Shapiro-Wilks test, $p \geq 0.2$) then Bartlett's test was used to check for homoscedasticity of data, in the other cases Levene's test was used. If logarithmic transformation of data solved problems with normality or homoscedasticity transformed data were used for analysis to enable use of tests with higher statistical power. If normality and also homoscedasticity were proven, Dunnett's t-test was used for analysis of the data. If normality was met but homoscedasticity was disturbed, the Bonferroni-Holms corrected Satterthwaite t-test (same as Welch test) was used for analysis. If data were not normal, the Bonferroni-Holms corrected U-test was used.

Data of the reference item treatment and corresponding data of the control were tested for normality using Shapiro-Wilks Test and for homoscedasticity using the Folded F-Test. Log-transformed data were used for analysis if these data allowed the use of tests with higher power. Student's t-Test (pooled) was used for data meeting normal distribution and homoscedasticity. In case of no homoscedasticity but proven normality Satterthwaite's t-test was used. In case of no normal distribution of data, the Mann-Whitney Exact Test was used.

Results and Discussion

Number of bees per colony

The number of bees per colony was recorded at each assessment day. The results of the first colony size assessment are displayed in Table 9.5.1-9 below. 6 of the 24 colonies had fewer than the 6,000 bees recommended by OECD 75 (2007), however, all but one of these had increased to at least 6,000 bees by the next colony size assessment at 1DBA.

Table 9.5.1-9: Colony size at 1st colony assessment

	Total number of honey bees per colony at the 1 st colony assessment (9DBA)				
	C	T1	T2	T3	R
Replicate a	4550	4420	4680	4420	4160
Replicate b	6175	7280	4225	5265	5525
Replicate c	7085	6565	7150	7605	5655
Replicate d	6955	6370	7670	8060	8775
Mean	6191	6159	5931	6338	6029
St. Dev.	1166	1223	1731	1770	1952

Mortality of adult worker bees

During the exposure period (0DAA to 7DAA) and the monitoring phase (8DAA to 27DAA) no test item related effects were observed. On 0DAA, (before treatment) the mean mortality in all treatment groups was higher than the days before, and in test item treatment group T2, the mean mortality calculated for 0DBA was statistically different compared to the control, but was still within the acceptable level of mean mortality in a tunnel tent. The study authors state that this is due to both the high temperatures on the day of application, and the adaption to the restricted conditions in the tunnels, and they also state that this is not unusual at this time of the trial. This may be the case, however, it reduces the statistical power of any analysis designed to demonstrate the presence of treatment-related mortality.

Overall mean adult worker bee mortality rates during the pre-exposure and exposure periods are displayed in Table 9.5.1-10 below. Mortality data is also displayed graphically in Figures 9.5.1-1 and 9.5.1-2.

Table 9.5.1-10: Overall mean adult worker bee mortality during the pre-exposure and exposure period

Date	Timing	Mortality (dead adult worker bees) [Mean ± STD] ¹⁾				
		C	T1	T2	T3	R
19 Jun 2016	4DBA	11.3 ± 8.3	11.0 ± 6.6	6.8 ± 3.1	14.5 ± 7.1	9.8 ± 4.3
20 Jun 2016	3DBA	17.8 ± 8.0	20.0 ± 5.9	37.0 ± 20.8	21.0 ± 8.3	24.0 ± 5.9
21 Jun 2016	2DBA	23.8 ± 8.8	17.8 ± 5.1	26.5 ± 6.2	26.3 ± 6.1	25.8 ± 11.3
22 Jun 2016	1DBA	33.8 ± 20.6	28.3 ± 8.8	26.0 ± 11.0	25.0 ± 7.9	29.8 ± 14.7
23 Jun 2016	0DBA	41.5 ± 13.6	64.3 ± 14.9	75.8* ± 17.1	50.3 ± 18.7	50.0 ± 11.9
Mean pre-exposure		25.6 ± 9.5	28.3 ± 5.2	34.4 ± 10.1	27.4 ± 6.4	27.9 ± 9.0
Sum 0DAA		46.5 ± 7.1	53.3 ± 12.8	60.0 ± 18.1	43.8 ± 18.1	65.3 ± 26.7
24 Jun 2016	1DAA	10.0 ± 6.5	15.5 ± 0.6	12.0 ± 5.8	10.0 ± 2.3	16.8* ± 1.7
25 Jun 2016	2DAA	42.8 ± 9.5	61.0 ± 13.3	74.3 ± 32.0	67.3 ± 22.6	69.0 ± 38.3
26 Jun 2016	3DAA	52.8 ± 21.4	38.3 ± 3.3	63.3 ± 38.5	54.5 ± 14.2	73.3 ± 36.4
27 Jun 2016	4DAA	48.5 ± 10.0	49.3 ± 10.0	95.8* ± 61.8	55.8 ± 7.1	77.5 ± 29.7
28 Jun 2016	5DAA	51.0 ± 30.3	42.5 ± 20.2	49.8 ± 22.6	60.0 ± 24.1	57.5 ± 26.7
29 Jun 2016	6DAA	35.5 ± 19.5	26.0 ± 5.4	50.3 ± 9.7	50.5 ± 12.1	39.0 ± 16.8

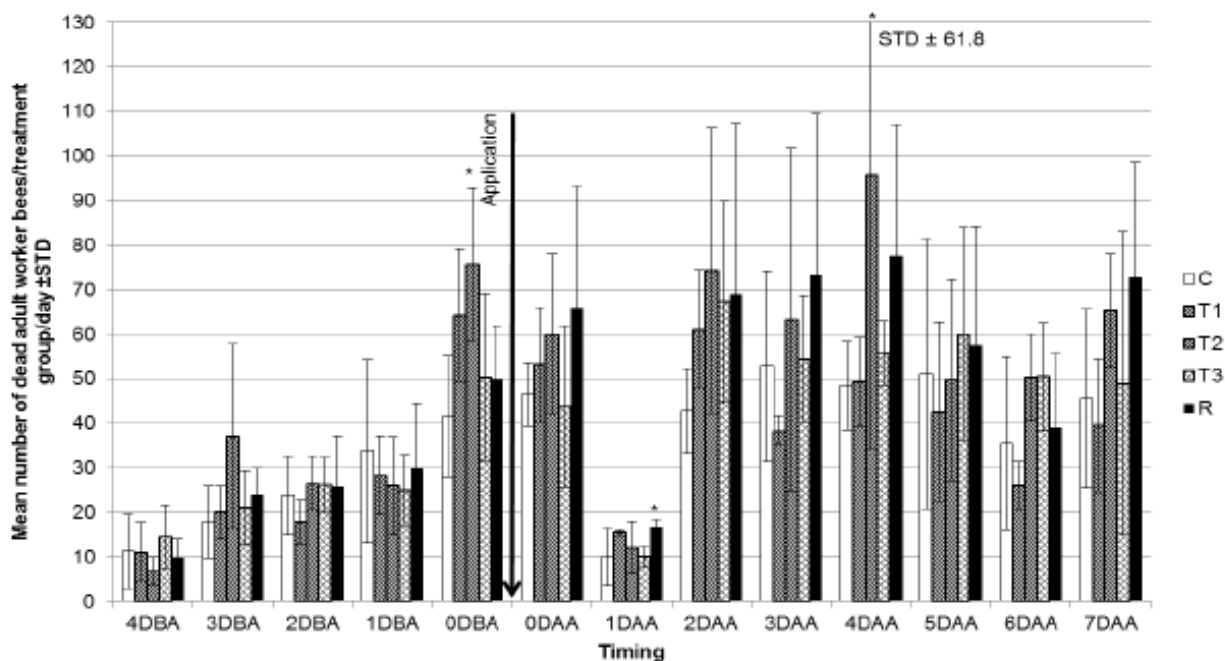
30 Jun 2016	7DAA	45.5 ± 20.0	39.5 ± 15.1	65.3 ± 12.7	49.0 ± 34.0	72.8 ± 25.8
Mean 0DAA to 7DAA		41.6 ± 14.3	40.7 ± 7.9	58.8 ± 22.7	48.8 ± 6.9	58.9* ± 21.6
Mean 8DAA to 27DAA		10.1 ± 5.6	11.4 ± 2.6	11.1 ± 5.8	11.2 ± 1.9	14.9 ± 4.6

¹⁾ Recorded by counting the dead adult honey bees in the dead bee traps in front of the hives, on the hive floor (drawer) and on the linen sheets which were spread out in the tunnels.

* = Statistically significant difference from the control (Student's t-test (pooled), Dunnett's or Satterthwaite t-test, one-sided, $p \leq 0.05$).

DBA/DAA = Days before/after application

STD = Standard deviation



DBA = Days before application; DAA = Days after application

* Statistically significant different from the control (t-test (pooled)(R), or T2: Dunnett's t-test one-sided, $p \leq 0.05$)

Figure 9.5.1-1: Mean number of dead adult worker bees counted per colony in the dead bee traps and on the linen sheets of the control group C, the treatment groups T1, T2, and T3, and the reference item group R from 4DBA to 7DAA.

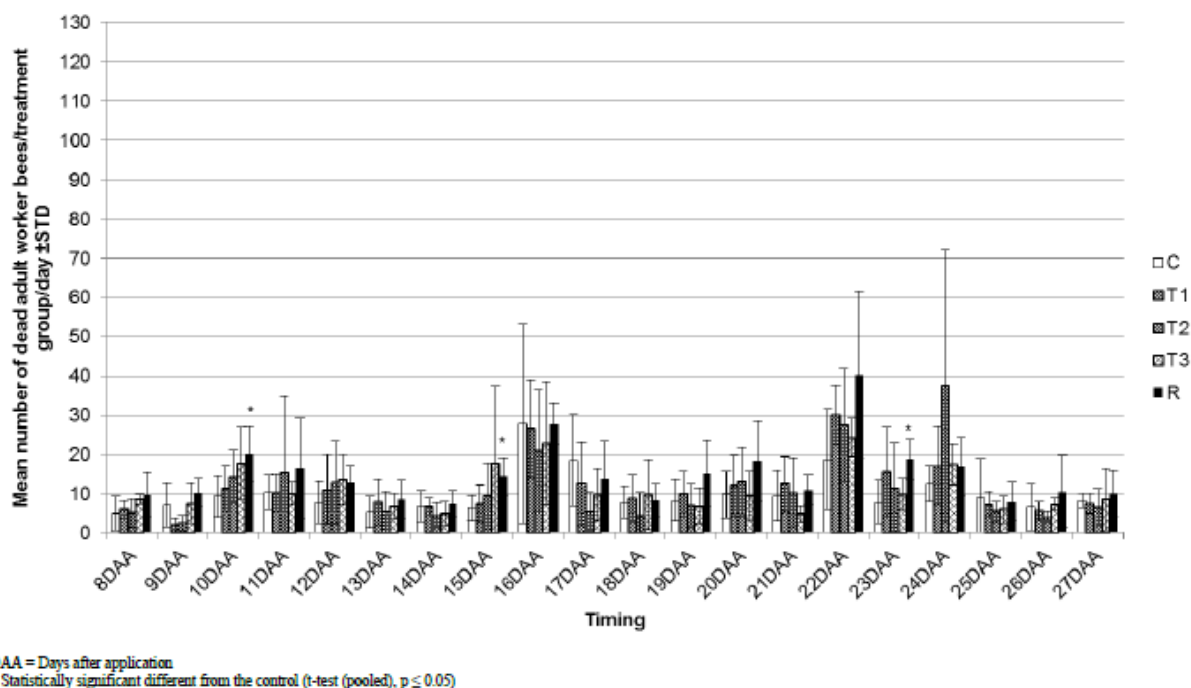


Figure 9.5.1-2: Mean number of dead adult worker bees counted per colony in the dead bee traps of the control group C, the treatment groups T1, T2, and T3, and the reference item group R from 8DAA to 27DAA

Foraging activity

On the day of application, foraging activity was recorded once shortly before application, 4 times during the first hour after application, and at 2 hours, 4 hours, and 6 hours after application. No test item related effect on honey bee foraging activity was noticed from 0DAA to 7DAA.

Aspects of foraging behaviour correlated with the weather conditions (Weather data shown in Table 9.5.1-12 below). For example, there was a heavy rainfall event 2DAA, which corresponded with a reduction in foraging activity on 2DAA and 3DAA. Foraging data is displayed in tabular form in Table 9.5.1-11 below, and graphically in Figure 9.5.1-3 below.

Table 9.5.1-11: Overall daily mean bee foraging activity during the pre-exposure and exposure period.

Date	Timing	Foraging activity [mean number of honey bees/m ² ± STD]				
		C	T1	T2	T3	R
19 Jun 2016	4DBA	1.4 ± 1.3	2.3 ± 2.1	3.7 ± 3.0	1.9 ± 1.6	3.0 ± 1.3
20 Jun 2016	3DBA	8.8 ± 5.1	10.1 ± 2.3	13.2 ± 2.5	13.2 ± 3.1	9.6 ± 2.9
21 Jun 2016	2DBA	5.1 ± 3.6	6.9 ± 3.5	6.4 ± 3.8	8.1 ± 3.6	7.1 ± 5.2
22 Jun 2016	1DBA	18.5 ± 4.2	22.6 ± 6.4	21.4 ± 5.2	20.0 ± 6.1	19.3 ± 4.5
23 Jun 2016	0DBA	16.4 ± 2.3	13.8 ± 1.7	15.8 ± 2.6	20.7 ± 3.1	17.6 ± 5.1
Mean pre-exposure		10.0 ± 1.5	11.1 ± 2.1	12.1 ± 1.9	12.8 ± 0.6	11.3 ± 2.8
Sum 0DAA		18.8 ± 3.4	19.5 ± 3.4	26.5 ± 5.0	21.2 ± 0.8	17.3 ± 2.3
24 Jun 2016	1DAA	16.6 ± 1.9	20.7 ± 3.4	21.4 ± 3.1	26.6 ± 1.0	22.5 ± 3.1
25 Jun 2016	2DAA	9.2 ± 4.4	6.0 ± 5.8	4.2 ± 5.1	6.8 ± 5.9	4.1 ± 4.5
26 Jun 2016	3DAA	5.8 ± 2.7	13.3 ± 1.3	8.6 ± 2.6	7.7 ± 3.5	5.7 ± 2.5
27 Jun 2016	4DAA	12.0 ± 1.4	7.6* ± 3.3	14.6 ± 1.3	10.9 ± 1.4	14.1 ± 2.6
28 Jun 2016	5DAA	0.0 ± 0.0 ^{a)}	3.6 ± 2.2	10.0 ± 2.3	7.7 ± 1.2	10.1 ± 1.6
29 Jun 2016	6DAA	9.6 ± 2.5	5.5 ± 3.6	5.6 ± 3.7	12.6 ± 2.2	11.7 ± 2.5
30 Jun 2016	7DAA	11.7 ± 1.3	12.5 ± 4.9	10.9 ± 2.0	13.1 ± 3.9	10.7 ± 2.1
Mean 0DAA to 7DAA		10.5 ± 0.6	11.1 ± 1.2	12.7 ± 1.6	13.3 ± 1.1	12.0 ± 1.4

a) Rain during assessment in C

* = Statistically significant reduction compared to the control (Dunnett's t-test, $p \leq 0.05$)

DBA/DAA = Days before/after application

STD = Standard deviation

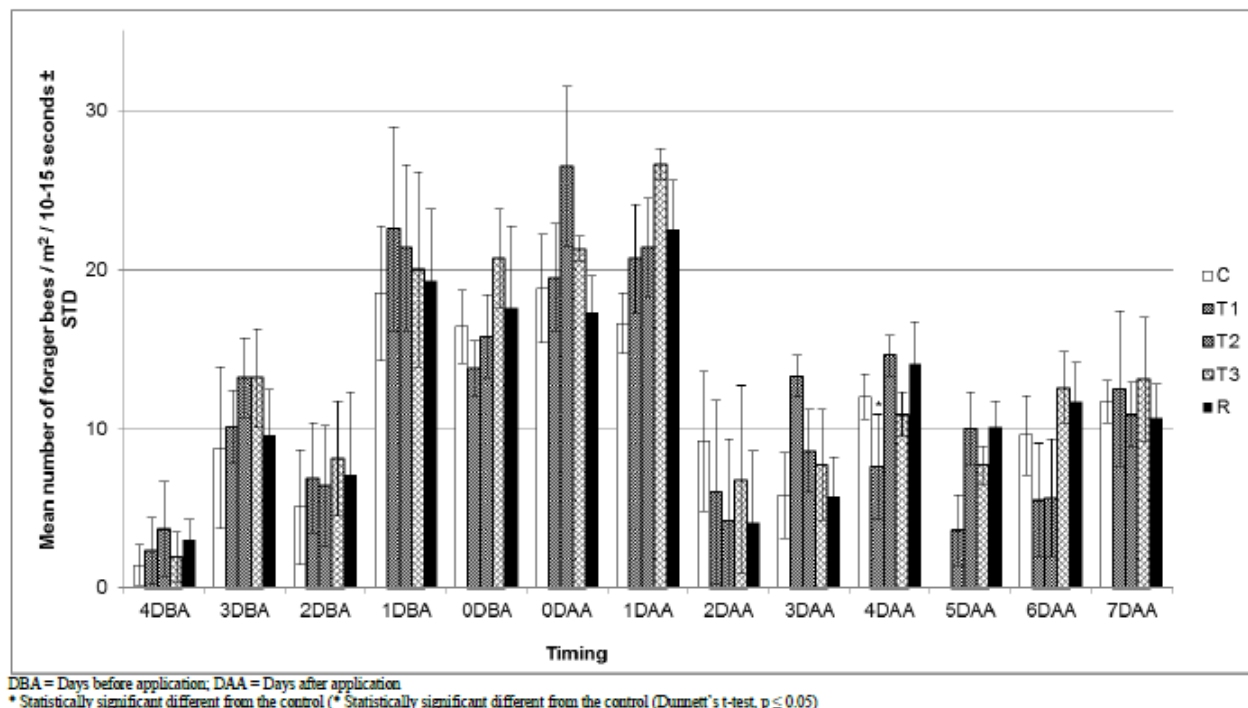


Figure 9.5.1-3: Mean number of honey bees foraging per m² per 10-15 seconds in the control group C, the treatment groups T1, T2, and T3, and the reference item group R from 4DBA to 7DAA.

Weather data

A summary of the weather data and BBCH growth stages during the exposure period at the field site is provided in Table 9.5.1-12 below. It is noted that the *Phacelia* plants were already halfway through the flowering period at the time of application, and were approaching the end of flowering at 4DAA.

Table 9.5.1-12: Weather data during the exposure period at the field site

Date	Timing	Temperature ¹⁾ (min/max) [°C]	Rel. Humidity ¹⁾ (min/max) [%]	Precipitation ²⁾ [mm]	BBCH ²⁾
18 Jun 2016	5DBA	9.2 / 20.1	58.5 / 100	7.0	63-64
19 Jun 2016	4DBA	11.6 / 18.6	57.5 / 98.5	1.0	63-64
20 Jun 2016	3DBA	8.6 / 23.5	41.0 / 100.0	0.0	63-65
21 Jun 2016	2DBA	13.1 / 22.9	59.0 / 100.0	0.0	63-65
22 Jun 2016	1DBA	17.5 / 28.2	48.5 / 100.0	4.0	63-65
23 Jun 2016	0DAA	17.0 / 33.7	41.0 / 98.5	0.0	65
24 Jun 2016	1DAA	18.3 / 33.1	44.0 / 100.0	0.0	65
25 Jun 2016	2DAA	17.0 / 25.6	63.0 / 100.0	28.0	65
26 Jun 2016	3DAA	11.6 / 22.2	46.0 / 100.0	7.0	65
27 Jun 2016	4DAA	11.3 / 21.9	51.0 / 94.5	0.5	65-67
28 Jun 2016	5DAA	14.7 / 21.4	65.5 / 98.5	0.5	65-67

Date	Timing	Temperature ¹⁾ (min/max) [°C]	Rel. Humidity ¹⁾ (min/max) [%]	Precipitation ²⁾ [mm]	BBCH ²⁾
29 Jun 2016	6DAA	16.5 / 24.8	50.0 / 100.0	3.0	65-67
30 Jun 2016	7DAA	15.2 / 25.6	49.0 / 100.0	0.0	65-67

DBA = Days before application

DAA = Days after application

¹⁾ = Data was recorded by a data-logger

²⁾ = Data were recorded during the evaluations of mortality and foraging activity or using a rain gauge

Behaviour

No unusual behaviour was observed from 4DBA to 0DBA. After the application unusual behaviour (mainly locomotion problems, in few cases also cramping and trembling) was observed in the test item treatments and in the reference item treatment, mostly in ≤ 5 bees per replicate per assessment day. A higher number of inactive bees were observed 4 hours after the application in test item treatment T3 (replicate c: ~100 and replicate d: ~30). However, two hours later these bees were not observed and the number of dead bees in replicate c and d were not significantly higher compared to replicates a and b. Clustering at the hive entrance was observed approximately 6 hours after application and in the evening in T2, T3 and R. The study authors state that this is not a behaviour typically observed after the application of the reference item, Insegar. However, the authors suggest that since it was a hot day (max 33.7 °C) clustering at the hive entrance could have been caused by this. Overall, no test item related changes in behaviour were observed.

Photographic Evaluation of Brood Development

The results of the photographic assessments of the brood development 22 days after the brood area fixing day showed a mean termination rate of 22.26 for eggs in C, 37.55 in T1, 36.55 in T2 and 30.55 in T3. For the reference item treatment, a mean termination rate of 79.95 was determined. The brood and compensation indices and termination rates for eggs, young larvae and old larvae in T1, T2 and T3 were not statistically different from the control on any assessment date. Statistically significant differences were only detected for the reference item treatment R for eggs and old larvae. The brood termination rates for eggs, young larvae, and old larvae are shown in Table 9.5.1-13 below:

Table 9.5.1-13: Brood termination rate for eggs, young larvae, and old larvae.

Life stage	Replicate	Data type	No. of marking cells	Termination rate (%) at x days after brood area fixing day (BFD)			
				+ 5	+ 10	+ 16	+ 22
Eggs	Control	Range	214 – 226	2.79 – 42.06	7.91 – 49.07	8.37 – 49.53	8.84 – 50.93
		Mean (STD)	220.0 (n/a)	16.31 (17.61)	21.12 (18.84)	21.79 (18.73)	22.26 (19.31)
	T1	Range	233 – 266	4.14 – 66.67	16.92 – 69.51	17.67 – 71.14	18.62 – 71.14
		Mean (STD)	248.0 (n/a)	28.54 (27.35)	36.36 (24.79)	37.06 (25.23)	37.55 (24.87)
	T2	Range	234 – 254	11.74 – 47.64	18.22 – 57.09	18.22 – 57.87	18.22 – 57.87
		Mean (STD)	245.8 (n/a)	30.53 (14.74)	36.15 (15.96)	36.35 (16.31)	36.55 (16.28)
	T3	Range	228 – 266	8.65 – 46.93	12.03 – 66.67	12.41 – 66.67	13.53 – 66.67
		Mean (STD)	245.8 (n/a)	21.16 (17.66)	29.16 (25.51)	30.05 (25.11)	30.55 (24.67)
	Reference item	Range	220 – 268	4.10 – 99.63	6.56 – 100.0	15.57 – 100.0	22.54 – 100.0
		Mean (STD)	247.5 (n/a)	73.31 (46.20)	75.96 (46.28)	78.21* (41.78)	79.95* (38.30)
Young larvae	Control	Range	215 – 253	0.00 – 70.75	0.00 – 71.15	0.79 – 71.15	0.79 – 71.15

Life stage	Replicate	Data type	No. of marking cells	Termination rate (%) at x days after brood area fixing day (BFD)			
				+ 5	+ 10	+ 16	+ 22
		Mean (STD)	239.3 (n/a)	21.45 (33.28)	22.22 (33.07)	22.96 (32.66)	22.96 (32.66)
		Range	208 – 249	2.01 – 68.15	2.81 – 68.15	2.81 – 68.95	2.81 – 68.95
	T1	Mean (STD)	232.3 (n/a)	30.65 (27.52)	31.07 (27.17)	32.84 (27.46)	32.84 (27.46)
		Range	232 – 262	6.07 – 82.33	6.88 – 82.33	6.88 – 82.76	6.88 – 82.76
	T2	Mean (STD)	248.0 (n/a)	58.72 (35.75)	59.21 (35.49)	59.42 (35.66)	59.42 (35.66)
		Range	221 – 281	0.82 – 89.68	0.82 – 89.68	1.22 – 89.68	1.22 – 89.68
	T3	Mean (STD)	245 (n/a)	43.8 (39.41)	44.03 (39.54)	44.45 (39.18)	44.55 (39.11)
		Range	203 – 254	8.23 – 100.0	9.09 – 100.0	9.96 – 100.0	12.99 – 100.0
	Reference item	Mean (STD)	232.3 (n/a)	71.98 (43.56)	72.29 (43.17)	72.51 (42.74)	73.48 (41.32)
		Range	206 – 257	0.39 – 10.70	1.56 – 10.70	1.56 – 10.70	-
	Control	Mean (STD)	232.3 (n/a)	3.36 (4.95)	3.95 (4.50)	3.95 (4.50)	-
		Range	205 – 243	0.00 – 2.90	0.00 – 3.86	0.00 – 3.86	-
Old larvae	T1	Mean (STD)	223.0 (n/a)	0.83 (1.39)	1.53 (1.65)	1.53 (1.65)	-
		Range	217 – 257	0.39 – 10.60	1.56 – 12.90	1.56 – 12.90	-
	T2	Mean (STD)	241.0 (n/a)	3.68 (4.70)	4.86 (5.43)	4.86 (5.43)	-
		Range	221 – 235	0.00 – 3.17	0.00 – 3.60	0.00 – 3.60	-
	T3	Mean (STD)	225.8 (n/a)	1.8 (1.33)	2.25 (1.61)	2.25 (1.61)	-
		Range	241 – 264	2.03 – 56.82	23.05 – 87.12	23.46 – 87.12	-
	Reference item	Mean (STD)	248.5 (n/a)	27.36 (27.77)	56.17* (26.29)	56.69* (26.09)	-
		Range	206 – 257	0.39 – 10.70	1.56 – 10.70	1.56 – 10.70	-
	Control	Mean (STD)	232.3 (n/a)	3.36 (4.95)	3.95 (4.50)	3.95 (4.50)	-
		Range	205 – 243	0.00 – 2.90	0.00 – 3.86	0.00 – 3.86	-
	T1	Mean (STD)	223.0 (n/a)	0.83 (1.39)	1.53 (1.65)	1.53 (1.65)	-
		Range	217 – 257	0.39 – 10.60	1.56 – 12.90	1.56 – 12.90	-

STD = Standard deviation

- = No data available

* Statistically significant difference from the control (one-sided pooled t-test, $p \leq 0.05$)

Colony Strength and Brood Development

Mean colony strength in the control and test item treatment groups T1, T2, and T3, followed the natural course of honey bee colony development for summer and autumn. Statistically significant differences were detected regarding mean colony strength for the reference item treatment group R in comparison to the control (on 30DAA, 45DAA, and 52DAA).

The results of the colony assessments show a decrease in the mean number of brood cells from 1DBA to 9DAA for all treatments which is described by the study authors as likely to be due to the confinement of the colonies in the tunnels. The colonies of the reference item treatment group showed the lowest number of brood cells followed by test item treatment T1. Overall mean values for eggs, larvae, pupae, nectar and pollen in T1, T2 and T3 were not significantly different when compared to the control. On one occasion at the colony assessment for T1 on 9DAA (2 Jul 2016), the mean number of cells containing nectar was significantly lower compared to the control.

However, the study authors state that this is likely to be due to the higher mean number of bees per colony, consuming more nectar, and due to the restricted resources available in the preceding confinement in the tunnels.

Statistically significant differences were only detected for the reference item treatment, R.

Food Stores

All colonies had sufficient food (nectar and pollen) throughout the test period. During the monitoring period, colonies were supplied with artificial nectar twice (BFD+16, 15DAA and on 41DAA) due to the colonies being on the point of starving (nectar resources <15 % and Pollen resources < 7 %). All colonies were fed in the same way with 2.5 kg artificial nectar each (Api Invert). Each colony consumed all sucrose solution within few days. Natural food supply tends to be lower in August however it was further exacerbated by the dry weather conditions. Feeding of colonies at this time is usual and common beekeeping practice in this region of Germany.

Residue Analysis

Residues of Pydiflumetofen

Samples of flowers, leaves, pollen and nectar from forager bees were sampled 2DBA, 0DAA, 1DAA, 4DAA, 6DAA, 38DAA, and 52DAA. Nectar from the combs and pollen from pollen traps were also analysed for residues of pydiflumetofen (SYN545974) resulting from application of pydiflumetofen SC (A19649B), on 38DAA and 52DAA. Flowers, leaves, pollen and nectar samples of untreated groups and the three treated groups (T1, T2 and T3) with the different application rates (T3>T2>T1) were analysed.

No residues were detected above the defined LOD (0.0015 mg/kg for the analyte pydiflumetofen (SYN545974)) in any of the untreated pollen and nectar from forager bees, flowers and leaves specimens or in any of the samples taken before application.

In the samples collected at the monitoring site, no residues of other pesticides were detected.

Residue data from the first 6 days after application is shown in Table 9.5.1-14 below:

Table 9.5.1-14: Residues of pydiflumetofen detected in the first 6 days after application.

Sample type	Condition	0 days after application	1 day after application	4 days after application	6 days after application
		mg /kg			
Pollen samples from forager bees	T1	9.83	1.14	0.105	0.0519
	T2	33.3	2.03	0.165	0.0398
	T3	26.3	2.05	0.278	0.145
	Range (Mean)	9.83 – 33.3 (23.14)	1.14 – 2.05 (5.22)	0.105 – 0.278 (0.183)	0.0398 – 0.145 (0.0789)
Nectar samples from forager bees	T1	0.0403	0.0120	n.d.	< LOD
	T2	0.111	< LOQ	n.d.	< LOQ; > LOD
	T3	0.107	< LOQ	n.d.	< LOQ; > LOD
	Range (Mean)	0.0403 – 0.111 (0.0861)	< LOQ – 0.0120 (n.d.)	n.d.	< LOD - < LOQ ; > LOD (n.d.)
Treated flower specimens	T1	18.8	4.91	0.526	0.370
	T2	28.9	9.75	1.01	0.427
	T3	30.6	21.9	1.40	0.883
	Range (Mean)	18.8 – 30.6 (26.1)	4.91 – 21.9 (12.19)	0.526 – 1.40 (0.98)	0.370 – 0.883 (0.56)
Treated leaf specimens	T1	14.6	11.2	2.16	5.80
	T2	18.9	27.1	4.69	4.42
	T3	33.4	34.5	1.73	5.80

	Range (Mean)	14.6 – 33.4 (22.3)	11.2 – 34.5 (24.27)	1.73 – 4.69 (2.86)	4.42 – 5.80 (5.34)
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LOD = 0.0015 mg /kg ; LOQ = 0.005 mg /kg

n.d. – not detectable

Treated Pollen Specimens

Pollen from forager bees: Residues of pydiflumetofen (SYN545974) were 9.83 mg/kg in the treated group T1, 33.3 mg/kg in the treated group T2 and 26.3 mg/kg in the treated group T3 at 0 days after application and decreased to 0.0519 mg/kg in the treated group T1, 0.0398 mg/kg in the treated group T2 and 0.145 mg/kg in the treated group T3 within 6 days after application.

Pollen from traps and combs: No residues of pydiflumetofen (SYN545974) were detected above the defined LOD (0.0015 mg/kg) in pollen from traps and combs at sampling times 38 DAA and 52 DAA at the monitoring location.

Treated Nectar Specimens

Nectar from forager bees: Residues of pydiflumetofen (SYN545974) were 0.0403 mg/kg in the treated group T1, 0.111 mg/kg in the treated group T2 and 0.107 mg/kg in the treated group T3 at 0 days after application and decreased to non-detectable levels in the treated group T1 and between the limit of detection and below the limit of quantification in the treatment groups T2 and T3 within 6 days after application.

Nectar from combs: No residues of pydiflumetofen (SYN545974) were detected above the defined LOD (0.0015 mg/kg) in nectar from combs at sampling times 38 DAA and 52 DAA at the monitoring location.

Treated Flowers Specimens

Residues of pydiflumetofen (SYN545974) were 18.8 mg/kg in the treated group T1, 28.9 mg/kg in the treated group T2 and 30.6 mg/kg in the treated group T3 shortly after application and decreased to 0.370 mg/kg in the treated group T1, 0.427 mg/kg in the treatment group T2 and 0.883 mg/kg in the treatment group T3 within 6 days after application.

Treated Leaves Specimens

Residues of pydiflumetofen (SYN545974) were 14.6 mg/kg in the treated group T1, 18.9 mg/kg in the treated group T2 and 33.4 mg/kg in the treated group T3 at 0 days after application and decreased to 5.80 mg/kg in the treated group T1, 4.42 mg/kg in the treatment group T2 and 5.80 mg/kg in the treatment group T3 within 6 days after application.

Conclusion

The objective of the study was to determine potential effects of exposure of honey bees to flowering *Phacelia tanacetifolia* treated once at start of flowering with Pydiflumetofen SC (A19649B) under semi-field conditions. The test item Pydiflumetofen SC (A19649B) was applied to flowering *Phacelia tanacetifolia* with the target application rates of 75, 125, and 200 g a.s. /ha (T1, T2, T3) during daily honey bee flight.

During the post-application period, 0DAA to 27DAA, no effect on honey bee mortality was observed in the test item treatment groups T1, T2 and T3 compared to the control. No test item related effects were observed regarding foraging activity. Slight, but not test item related behavioural changes were observed during the post-application period (0DAA to 27DAA). The brood and compensation indices and termination rates for eggs, young larvae and old larvae in T1, T2 and T3 were not statistically different from the control on any assessment date. The overall honey bee colony development in the test item treatment groups T1, T2 and T3, measured as mean number of cells covered with the different types of brood (eggs, larvae and pupae) or food (nectar, pollen) per colony were not significantly different when compared to the control (except mean amount of nectar, T1, DAA9).

Residues of pydiflumetofen (SYN545974) were found in leaves, flowers and in pollen and nectar samples from forager bees at 0DAA in all treatment groups, decreasing within 6 days after application. No residues of pydiflumetofen (SYN545974) were detected above the defined LOD (0.0015 mg /kg) in pollen from traps and combs and in nectar from combs at sampling times 38 DAA and 52 DAA. No residues were found in any of the samples taken from the control or in T1 to T3 taken before application.

(██████, 2017)

HSE Comments

This study was conducted according to GLP, and follows ‘OECD 75 (2007): Honey Bee Brood Test Under Semi-Field Conditions’, as well as the current recommendations of the AG Bienenschutz (PISTORIUS, J. et al., 2012) and the OEEP/EPPO Guideline No. 170 (4) (2010) and the Commission Working Document 7029/VI/95 Rev. 5, General Recommendations for the Design, Preparation and Realisation of Residue Trials, July 22, 1997. The following deviations from the guidelines were noted:

The temperature on the day of application was in the range of 27.3 to 30.8 °C, at times marginally above the recommended limit of 30 °C. OECD 75 (2007) states that daytime temperatures of > 30 °C may prevent flight activity in the crop. The applicant has stated that the best day possible was chosen for application, due to the wet conditions prior to the application date, and the already advanced growth stage of the crop. The high temperature on the application date is unlikely to be of concern, as the honey bee foraging rates were quantified prior to application, and all treatment groups displayed a mean foraging activity of > 13.8 honey bees /m² (≥ 10 honey bees /m² recommended by OECD 75 (2007)).

The OECD 75 (2007) guidelines recommend that each colony should consist of approximately 6,000 worker bees at the start of the experiment. However, 6 of the 24 colonies contained fewer than this (Colony Ca (4550), T1a (4420), T2a (4680), T2b (4225), T3a (4420) and Ra (4160)). The applicant has stated that this was due to the long, cold and wet spring, meaning that the colony development was not as expected. The applicant also stated that this should not have any adverse influence on the reliability of the study, as all colonies were assessed with a high number of brood cells and all colonies except one (colony Ra) increased to approximately 6000 bees /colony at the following colony assessment 1DBA. However, due to the large variation in starting colony size, the sensitivity of the study is likely to be quite low.

On 0DAA, (before treatment) the mean mortality in all treatment groups was higher than the days before, and in test item treatment group T2, the mean mortality calculated for 0DBA was statistically different compared to the control, but was still within the acceptable level of mean mortality in a tunnel tent. The study authors state that this is due to both the high temperatures on the day of application, and the adaption to the restricted conditions in the tunnels, and they also state that this is not unusual at this time of the trial. This may be the case, however, it reduces the statistical power of any analysis designed to demonstrate the presence of treatment-related mortality.

Additionally, the foraging behaviour of the bees after application was reduced, as there was an incidence of heavy rainfall 2DAA. This also means that the test substance may have been washed away, reducing the exposure of the bees to the active substance. The foraging activity then remained low, likely due to the advanced growth stage of the plants. On the date of application, the *Phacelia* plants were at growth stage 65, meaning that 50 % of the flowers are open, and the first petals may have fallen. 4 days after application, the plants ranged from growth stage 65 to 67, meaning that the flowering period was finishing, resulting in fewer flowers for the bees to forage from, further reducing exposure to the test item.

Once the colonies were transferred to the monitoring site, they were not subject to normal conditions conducive to healthy growth and development. Additional feeding was necessary on two separate occasions, as the colonies were on the verge of starvation. The study authors have justified this by stating that the ‘natural food supply tends to be lower in August, and it was further exacerbated by the dry weather conditions. Feeding of colonies at this time is usual and common beekeeping practice in this region of Germany’. No sources or additional information were provided to support this statement, and as a result, this may further reduce the reliability of the results.

The statistical procedures used in this experiment were in line with those recommended in the OECD 75 (2007) guidelines. The analytical methods have been evaluated by HSE Chemistry specialists in Vol. 3CP Part B5.1.2.5. The following was concluded for this method: “Acceptable method. LOQ: 0.005 mg/kg in nectar, pollen, flowers and leaves”.

No effects on honey bee mortality or foraging behaviour were observed in the post-application period (0DAA to 27DAA) in any of the test item treatment groups, however, it is noted that the standard deviations for adult worker bee mortality rates were very large for certain datapoints, resulting in a greater level of uncertainty when interpreting the mortality data. Slight behavioural changes were observed in the post-application period, but these were determined not to be treatment related.

The brood termination rate, brood index, and the compensation index rates for eggs, young larvae and old larvae in the test conditions were not statistically different from the control on any assessment date.

Overall honey bee colony development, which was measured as mean number of cells per colony covered with the different types of brood (eggs, larvae and pupae) or food (nectar or pollen), was not significantly different when compared to the control (except mean amount of nectar, T1, 9DAA).

Residues of pydiflumetofen (SYN545974) were found in leaves, flowers and in pollen and nectar samples from forager bees at 0DAA in all treatment groups, decreasing within 6 days after application. No residues of pydiflumetofen (SYN545974) were detected above the defined LOD (0.0015 mg /kg) in pollen from traps and combs and in nectar from combs at sampling times 38 DAA and 52 DAA.

Please note that the following study was conducted with the EU representative formulation (A19649B); further details are given in the risk assessment.

Report: K-CP 10.3.1.5, [REDACTED] (2017) Pydiflumetofen SC (A19649B) - A Semi-Field Study to Evaluate the Side Effects on Honeybees (*Apis mellifera* L.) in *Phacelia tanacetifolia* in Germany 2016. Report number S16-04919. Eurofins Agrosience Services EcoChem GmbH/Eurofins Agrosience Services Ecotox GmbH, Eutinger Str. 46, 75223 Niefern-Öschelbronn, Germany (Syngenta File No. A19649B_10314)

Guidelines

OECD Guidance Document No. 75 (2007) and current recommendations of the AG Bienenschutz ([REDACTED] *et al.*, 2012)

OEPP/EPPO, Guideline No. 170 (4), (2010) EU Guideline 7029/VI/95 rev. 5: General recommendations for the design, preparation and realization of residue trials (1997)

GLP: Yes

Executive Summary

The objective of the study was to determine potential effects of exposure of honey bees to flowering *Phacelia tanacetifolia* treated once at start of flowering with pydiflumetofen (A19649B) under semi-field conditions. The test item pydiflumetofen (A19649B) was applied to flowering *Phacelia tanacetifolia* with the target application rates of 75, 125 and 200 g a.s /ha (T1, T2, T3) during daily honey bee flight.

Mortality, foraging activity, behaviour, conditions of the colonies, and development of the bee brood were observed.

Seven days after test application the colonies were moved to a new site and monitored.

Materials

Test item	A19649B
Lot/Batch #:	CWA002-104-001
Formulation type:	SC (soluble concentrate)
Nominal content of active ingredients:	Pydiflumetofen 201 g/L(18.4% w/w) from the Certificate of Analysis (23 Oct 2014)
Density:	1.093 g/cm ³
Reference Item:	Insegar (fenoxycarb 25% w/v)
Lot/Batch #:	SMO4D0025

Formulation type:	WG
Test Item application rates:	75 (T1), 125 (T2) and 200 (T3) g a.s./ha
Reference item application rate:	300 g a.s./ha
Test design	
Test organism:	Honey bee, <i>Apis mellifera</i> L. Sourced from Eurofins Agrosience Services EcoChem GmbH, Germany
Colonies:	5330 – 8385 bees per colony at the start of the test. Colonies Cs, T1s, T2s, T3s were only used for residue sampling. They contained 8775, 8580, 8775, 9555 honey bees respectively. The corresponding queens all originated from one breeding line. The following criteria for each colony were met (in line with OECD 75): <ul style="list-style-type: none"> - 4-7 brood combs containing eggs, larvae and capped cells. - 4 – 10 combs containing honey and pollen. - The colonies were free of symptoms of nosemosis, varroosis, foulbrood and other bee diseases. - Colonies were queen-right and all brood stages were present at the start of the test.

Study Design and Methods

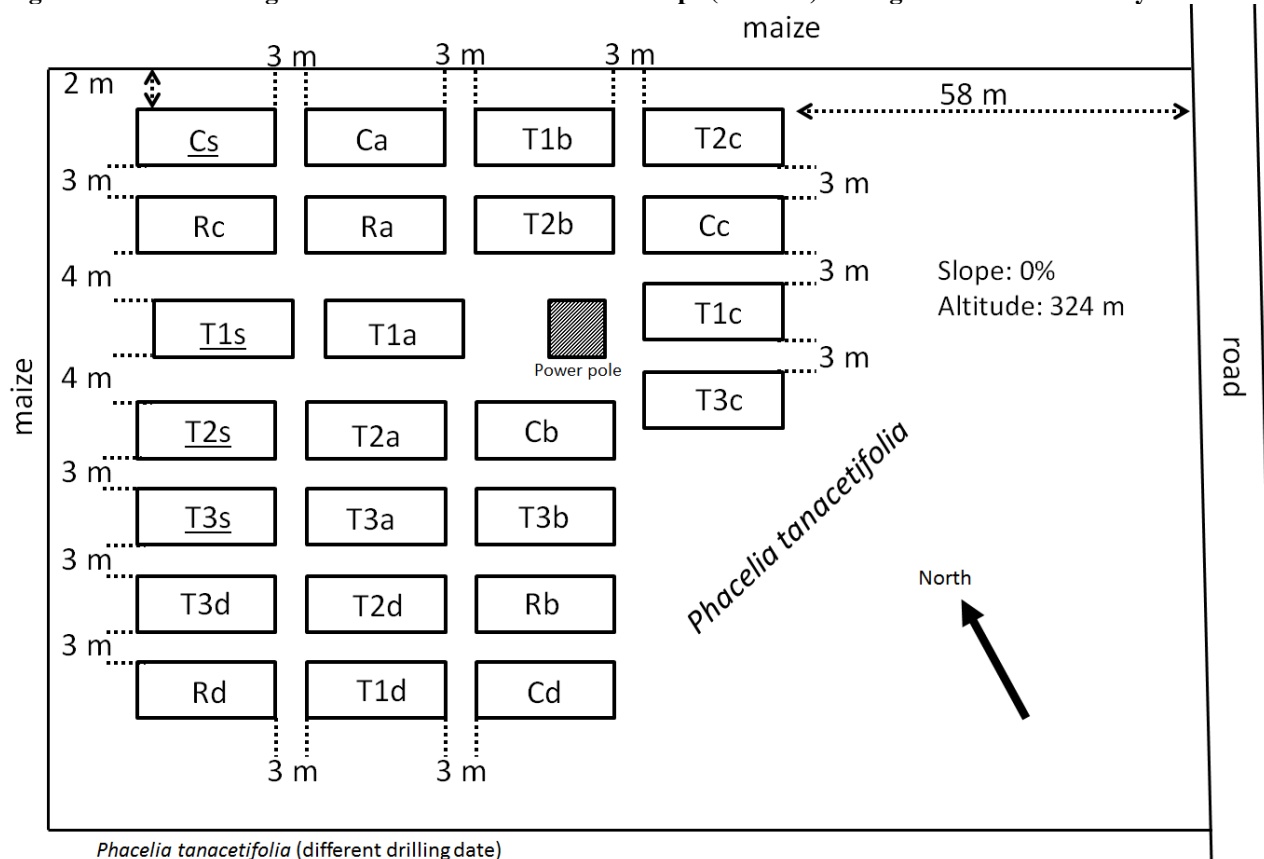
Experimental dates: 10th August 2016 – 7th April 2017

Site

The semi-field study was carried out in Pforzheim, Baden-Württemberg, Germany.

The field site was sown with *Phacelia tanacetifolia*. Twenty tunnels were installed prior to moving the colonies to the experimental field. Four additional tunnels were installed for residue sampling only (Cs, T1s, T2s, and T3s). Figure 1 shows the arrangement of the tunnels in the field area.

Figure 9.5.1-4: Arrangement of different Treatment Groups (Tunnels) during the Semi-Field Study



Five days before application one small commercial honey bee colony was introduced to each tunnel in the evening after daily flight activity. A container with water was placed in each tunnel, with floatable material to prevent the honey bees from drowning.

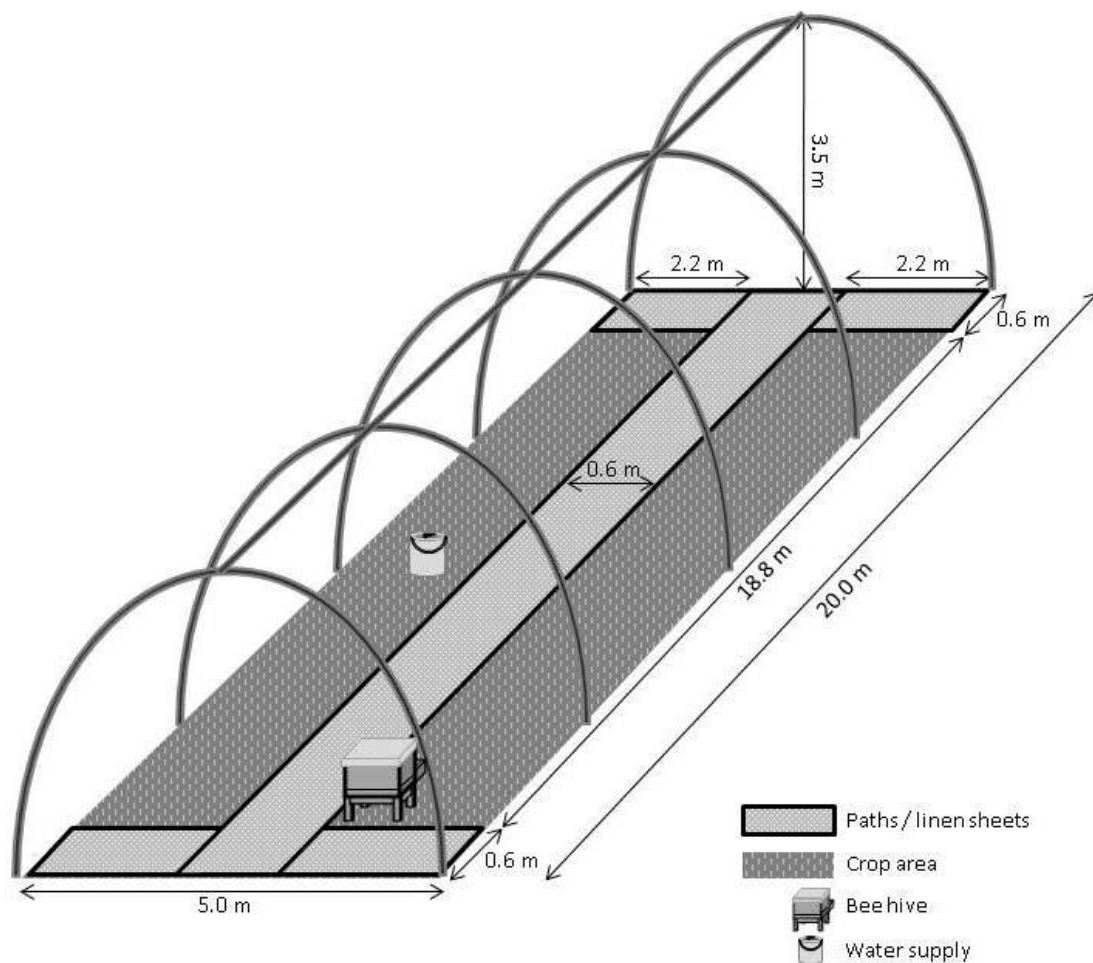
After the last evaluation of mortality and foraging activity in the tunnels on 7DAA, the colonies were relocated and maintained at the monitoring site 14.2 km away. The hives were placed at a minimum distance of 5 m away from each other.

The monitoring site was a mixed area next to a small village. There were forests, vineyards and agricultural areas without flowering main crops in the near surroundings which might be attractive to honey bees.

Tunnels

The tunnel area was approximately 100 m², the dimensions were 20 m long, 5 m wide and approximately 3.5 m high in the centre. There were two crop areas within each tunnel measuring 2.2 m x 18.8 m each. There was at least 3 m between each tunnel. Before the start of the test paths were created in each tunnel by removal of the plants and smoothing the ground. These paths were covered in linen sheets for the assessment of dead honey bees in the crop area. The layout of the tunnel is shown in figure 2. The tunnels were assigned to different treatments as shown in figure 1. Honey bee colonies were placed in the tunnels at the start of flowering, five days before application.

Figure 9.5.1-5: Design of a tunnel



Application

Application was performed using a calibrated portable boom sprayer. The application had a nominal spray volume of 400 L/ha, with a mean spray volume of 402.2, 404.1, 400.8, 403.8 and 402.0 L/ha for test groups C, T1, T2, T3 and R respectively.

The test item solution was prepared shortly before the application. The following conditions were met during application:

- Crop at full flowering BBCH 63-65.
- Honey bees were actively foraging during the application (14.8 bees/m² in C, 16.2 bees/m² in T1, 16.6 bees/m² in T2, 16.3 bees/m² in T3, 20.4 bees/m² in R).
- Maximum wind speed was 2.0 m/s.
- Air temperature was 19.1 °C – 29.7 °C.
- Spray tolerance was within ± 10 %.
- No rainfall occurred on the day of the application.

During the application the colonies were covered with plastic sheets and the water supply was moved out of the tunnel.

Replications

Four tunnels per treatment (control, test item and reference item) with one honey bee colony per tunnel. Additional tunnels with one colony per tunnel (control and test item) for residue sampling

There were 3 m between each tunnel in the field site. Seven days after exposure the colonies were moved to the monitoring site. The colonies were placed 5 m away from each other.

Observations

The colonies Cs, T1s, T2s, and T3s were used for residue sampling only, no biological assessments were conducted in these replicates. The parameters assessed during the study include mortality, foraging activity, behaviour, conditions of the colonies, and development of the bee brood.

Assessments were made prior to and post application. The colonies were monitored at a remote location for two further brood cycles following the initial detailed brood assessments (first brood cycle). The influence of pydiflumetofen was evaluated by comparing the data of the assessments of the three test item groups T1, T2 and T3 to the reference item group R and the control group C, and by comparing the pre-application data to the post-application data.

Additionally, the brood index, compensation index and brood termination rate was calculated.

Data of the test item treatments T1, T2, and T3 and the control were checked for normality using Shapiro-Wilks test. If the distribution of the data fitted the normal distribution very well then Bartlett's test was used to check for homoscedasticity of data, in the other cases Levene's test was used. If logarithmic transformation of data solved problems with normality or homoscedasticity transformed data were used for analysis to enable use of tests with higher statistical power.

If normality and also homoscedasticity were proven Dunnett's t-test was used for analysis of the data. If normality was met but homoscedasticity was disturbed, the Bonferroni-Holms corrected Satterthwaite t-test (same as Welch test) was used for analysis. If data were not normal, the Bonferroni-Holms corrected U-test was used.

Data of the reference item treatment and corresponding data of the control were tested for normality using Shapiro-Wilks Test and for homoscedasticity using the folded F-test. Log-transformed data were used for analysis if these data allowed the use of tests with higher power.

Student's t-Test (pooled) was used for data meeting normal distribution and homoscedasticity. In case of no homoscedasticity but proven normality Satterthwaite's t-test was used. In case of no normal distribution of data, the Mann-Whitney Exact Test was used.

Weather

During the pre-exposure and exposure period meteorological data in the form of air temperature and relative humidity was provided by the EAS weather station 0.1 km from the field site. The data recorded consisted of air temperature (daily minimum/maximum) and Relative air humidity (daily minimum/maximum). Daily precipitation was measured by a rain gauge in tunnel Ca. During the application and assessments (mortality and foraging) cloud cover, air temperature, relative air humidity and wind speed was recorded at the field site.

On application day the temperatures ranged from 19.1°C to 29.7°C and relative humidity ranged from 30.7 % to 59.3 %. The cloud cover during applications was between 0 % and 80 % and no rainfall occurred in the night before the day of application up until 3DAA.

During the presence of the colonies in the tunnels favourable foraging conditions were observed. The temperature ranged from 8.9°C to 32.6°C, the humidity was between 32.7 % and 100 % and rainfall was recorded only once (3.5 mm between 3DAA and 4DAA).

During the monitoring period the meteorological data was provided by a weather station 8.7 km from the monitoring site.

During the monitoring period favourable foraging conditions were observed at the site during most of the time. Cooler periods (< 20°C) were recorded from 30DAA to 35DAA and after 45DAA. The temperature ranged between 1.8°C and 36.5°C, the air humidity was between 31.2 % and 100 % and the rainfall was up to 25.0 mm

per day (total rainfall during period: 82.4 mm). Heavy rainfall (> 10 mm/day) was recorded on 32DAA and 41DAA.

Table 9.5.1-15: Weather data during the exposure period at the field site

Timing	Temperature ¹⁾ (min/max) [°C]	Rel. Humidity ¹⁾ (min/max) [%]	Precipitation ¹⁾ [mm]	Cloud cover ³⁾ [%]
5DBA	11.4 / 23.8	63.9 / 100.0	n.a.	n.a.
4DBA	10.9 / 28.4	47.7 / 100.0	0.0	0 – 10
3DBA	10.7 / 30.5	39.7 / 100.0	0.0	0 – 10
2DBA	10.4 / 32.3	40.4 / 100.0	0.0	0
1DBA	10.9 / 31.1	39.9 / 100.0	0.0	10 – 75
0DBA/0DAA	9.1 / 30.7	32.7 / 100.0	0.0	0 – 90
1DAA	9.0 / 25.4	56.2 / 100.0	0.0	70 – 100
2DAA	10.5 / 27.4	55.8 / 100.0	0.0	35 – 90
3DAA	9.8 / 27.5	56.2 / 100.0	0.0	90 – 100
4DAA	8.9 / 22.4	45.7 / 100.0	3.5	40 – 80
5DAA	10.8 / 23.1	60.1 / 100.0	0.0	60 – 100
6DAA	11.3 / 31.4	40.9 / 100.0	0.0	0 – 5
7DAA	12.9 / 32.6	38.1 / 100.0	0.0	0

¹⁾ Temperature and humidity data were recorded by an EAS weather station at the field site at Katharinentaler Hof (Pforzheim), approximately 0.1 km away from the tunnels (non-GLP record)

²⁾ Rainfall was recorded by a rain gauge in one of the tunnels (read and emptied once per day in the morning at the time of the daily assessments of mortality, GLP record)

³⁾ Data was recorded during the applications and evaluations of mortality and foraging activity.

Table 9.5.1-16: Weather data at the monitoring site

Timing	Temperature ¹⁾ (min/max) [°C]	Rel. Humidity ¹⁾ (min/max) [%]	Precipitation ¹⁾ [mm]
5DAA	13.8 / 30.3	48.3 / 100.0	0.2
8DAA	14.8 / 31.9	41.4 / 94.9	0.0
9DAA	14.1 / 35.1	41.9 / 100.0	0.0
10DAA	15.3 / 36.5	31.2 / 100.0	0.0
11DAA	17.4 / 32.7	38.1 / 95.4	0.0
12DAA	18.5 / 25.1	40.2 / 83.3	0.0
13DAA	12.0 / 25.9	50.2 / 100.0	0.0
14DAA	11.8 / 28.9	48.9 / 97.7	0.0
15DAA	15.8 / 26.9	43.7 / 96.4	0.0
16DAA	11.4 / 25.9	50.2 / 100.0	0.0
17DAA	12.8 / 28.3	45.0 / 99.1	0.0
18DAA	14.3 / 22.7	64.4 / 94.3	1.6
19DAA	13.9 / 19.9	72.1 / 96.3	0.0
20DAA	8.9 / 25.2	48.6 / 100.0	0.0
21DAA	11.8 / 24.9	55.1 / 95.8	0.0
22DAA	10.8 / 29.2	43.0 / 100.0	0.0
23DAA	15.1 / 28.2	52.9 / 98.36	0.0
24DAA	11.7 / 30.7	40.3 / 100.0	0.0
25DAA	15.9 / 29.6	44.2 / 96.0	0.0
26DAA	13.2 / 31.4	45.7 / 100.0	0.0
27DAA	13.3 / 32.3	31.2 / 100.0	0.0
28DAA	13.5 / 30.9	35.2 / 99.8	0.0
29DAA	16.3 / 24.3	66.5 / 90.1	0.0
30DAA	11.1 / 19.6	71.5 / 100.0	0.2
31DAA	11.6 / 20.9	63.2 / 100.0	5.2
32DAA	12.0 / 15.3	100.0 / 100.0	22.6
33DAA	11.3 / 17.2	73.1 / 100.0	0.0

Timing	Temperature ¹⁾ (min/max) [°C]	Rel. Humidity ¹⁾ (min/max) [%]	Precipitation ¹⁾ [mm]
34DAA	11.4 / 18.6	75.0 / 100.0	0.0
3 DAA	6.2 / 18.7	58.2 / 100.0	0.2
36DAA	4.8 / 20.5	53.3 / 100.0	0.2
37DAA	6.9 / 21.4	52.1 / 100.0	0.2

¹⁾ Data were recorded by an EAS weather station in Mühlacker-Enzberg approximately 8.7 km away from the monitoring site (non-GLP record).

Mortality of adult worker bees

Mortality of the honey bees was recorded by counting the number of dead honey bees in the dead bee traps, in the bottom drawer inside the hives and on the linen sheets spread out in the tunnels. These were differentiated into adult worker bees, pupae, and larvae, as well as dead male bees and male brood. Dead bees were removed after each assessment. To calculate mean mortality the dead bees on the linen sheets were added to the dead bees in the dead bee traps and on the bottom drawers and counted as one value. Mortality during the pre-exposure, exposure and post exposure period was assessed once a day in the morning up to noon, if possible, at the same time. Mortality was also assessed shortly before the start of application, 2 hours, 4 hours and 6 hours after application and in the evening after flight activity on the day of application.

Mortality of the honey bees was recorded as the sum of the dead honey bees found in the dead bee traps, the bottom drawer inside the hives and on the linen sheets. The mortality was checked for normality using Shapiro-Wilks test. Treatment group mortality was compared to the control with one-sided Dunnett's t-test or Bonferroni U-test (exact), ($p \leq 0.05$)

The overall mean adult worker bee mortality during pre-exposure was a similar level across all treatment groups. During exposure (0DAA – 7DAA) there was no effect on the mean adult worker bee mortality and no statistically significant differences in T1, T2, T3 or R compared to the control. There was no statistically significant mortality in any of the individual replicates in the treatment groups compared to the control. Figure 3 shows the mean number of dead honey bees counted from 4DBA to 7DAA. Figure 3 shows that from 3DAA to 5DAA the mean mortality in the control is higher than T1, T2 and T3, but from 3DAA – 7DAA the mean mortality in the reference group is higher than the control.

Table 9.5.1-17: Overall mean adult worker bee mortality during the pre-exposure and exposure period

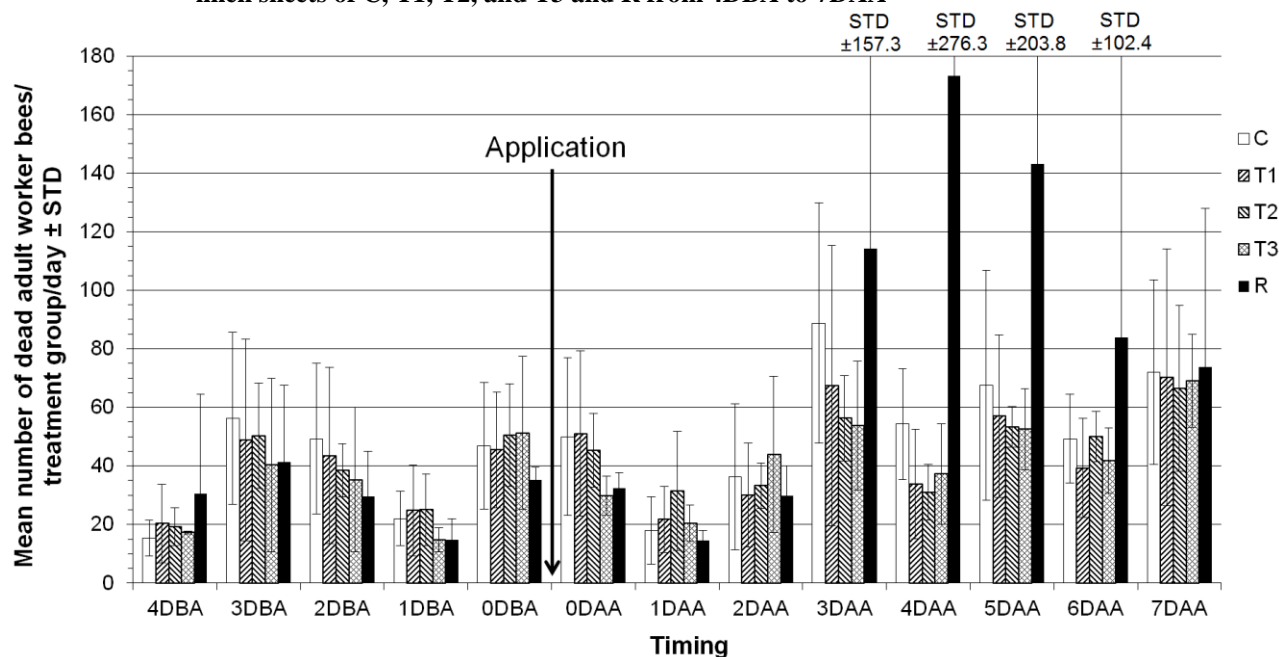
Date	Timing	Mortality (dead adult worker bees) [Mean \pm STD] ¹⁾				
		C	T1	T2	T3	R
13 Aug 2016	4DBA	15.3 \pm 6.1	20.3 \pm 13.4	19.3 \pm 6.4	17.3 \pm 0.5	30.5 \pm 34.0
14 Aug 2016	3DBA	56.3 \pm 29.4	48.8 \pm 34.4	50.3 \pm 17.9	40.3 \pm 29.6	41.3 \pm 26.3
15 Aug 2016	2DBA	49.3 \pm 25.8	43.5 \pm 30.1	38.5 \pm 9.0	35.3 \pm 24.7	29.5 \pm 15.5
16 Aug 2016	1DBA	22.0 \pm 9.3	24.8 \pm 15.5	25.0 \pm 12.3	14.8 \pm 4.2	14.8 \pm 7.2
17 Aug 2016	0DBA	46.8 \pm 21.6	45.5 \pm 19.8	50.5 \pm 17.6	51.3 \pm 26.1	35.3 \pm 4.3
Mean pre-exposure		37.9 \pm 16.8	36.6 \pm 19.5	36.7 \pm 10.1	31.8 \pm 15.6	30.3 \pm 6.9
17 Aug 2016	Sum 0DAA	50.0 \pm 26.9	51.0 \pm 28.2	45.3 \pm 12.5	29.8 \pm 6.7	32.5 \pm 5.2
18 Aug 2016	1DAA	18.0 \pm 11.5	21.8 \pm 11.3	31.5 \pm 20.4	20.5 \pm 6.2	14.5 \pm 3.4
19 Aug 2016	2DAA	36.3 \pm 25.0	30.0 \pm 17.8	33.3 \pm 7.8	44.0 \pm 26.7	29.8 \pm 10.2
20 Aug 2016	3DAA	88.8 \pm 41.1	67.5 \pm 47.8	56.3 \pm 14.6	53.8 \pm 22.0	114.3 \pm 157.3
21 Aug 2016	4DAA	54.3 \pm 18.9	33.8 \pm 18.6	31.0 \pm 9.6	37.3 \pm 17.2	173.3 \pm 276.3
22 Aug 2016	5DAA	67.5 \pm 39.2	57.0 \pm 27.7	53.3 \pm 6.9	52.5 \pm 13.8	143.0 \pm 203.8
23 Aug 2016	6DAA	49.3 \pm 15.2	39.3 \pm 16.9	50.0 \pm 8.6	41.8 \pm 11.1	83.8 \pm 102.4
24 Aug 2016	7DAA	72.0 \pm 31.5	70.3 \pm 43.8	66.5 \pm 28.4	69.0 \pm 15.9	73.8 \pm 54.2
Mean 0DAA to 7DAA		54.5 \pm 23.5	46.3 \pm 24.8	45.9 \pm 10.6	43.6 \pm 13.3	83.1 \pm 99.8

DBA/DAA = Days before/after application STD = Standard deviation

¹⁾ recorded by counting the dead adult honey bees in the dead bee traps in front of the hives, on the hive floor (drawer) and on the linen sheets which were spread out in the tunnels

There were no statistically significant differences between the test item or reference item treatments compared to the control during the pre-exposure and exposure period.

Figure 9.5.1-6: Mean number of dead honey bees counted per colony in the dead bee traps, hive floor and linen sheets of C, T1, T2, and T3 and R from 4DBA to 7DAA



During the monitoring period (8DAA – 27DAA) the mean daily adult worker bee mortality was not statistically different from the control. On individual days there were statistically significant differences from the control observed in T1 (19DAA, 21DAA, and 27DAA), T2 (21DAA), and T3 (14DAA, 16DAA, 19DAA and 21DAA). Figure 4 shows the mean number of dead honey bees counted from 8DAA to 27DAA. On 10DAA the mean mortality in T1 and T3 is higher than the control. On 11DAA - 14DAA, 16DAA – 24DAA and 27DAA the mean mortality in all treatment groups is higher than the control. However, excepting 16DAA, 22DAA, 24DAA and 25DAA the mean mortality in all treatment groups is at a similar level. Apart from 15DAA, 25DAA, and 26DAA the mean mortality in R was higher than the control.

Table 9.5.1-18: Overall mean adult worker bee mortality during the post exposure period

Date	Timing	Mortality (dead adult worker bees) [Mean ± STD] ¹⁾				
		C	T1	T2	T3	R
25 Aug 2016	8DAA	35.3 ± 16.5	14.5 ± 9.7	12.8 ± 10.8	22.0 ± 20.8	91.0* ± 36.0
26 Aug 2016	9DAA	78.5 ± 36.4	54.8 ± 27.1	26.0 ± 13.6	22.3 ± 9.1	81.0 ± 38.0
27 Aug 2016	10DAA	33.5 ± 18.5	39.0 ± 21.6	27.3 ± 17.2	52.0 ± 30.5	44.0 ± 30.8
28 Aug 2016	11DAA	5.5 ± 5.1	7.3 ± 4.4	6.8 ± 9.5	13.3 ± 7.9	20.5 ± 16.0
29 Aug 2016	12DAA	6.3 ± 6.4	8.0 ± 3.4	6.8 ± 6.7	18.3 ± 11.4	36.3* ± 29.7
30 Aug 2016	13DAA	6.0 ± 1.4	6.0 ± 2.2	3.3 ± 2.8	8.8 ± 4.3	7.8 ± 7.8
31 Aug 2016	14DAA	4.5 ± 3.4	5.5 ± 3.4	5.0 ± 1.8	11.8* ± 6.3	9.3 ± 6.8
01 Sept 2016	15DAA	10.8 ± 4.8	12.3 ± 5.4	8.8 ± 4.6	10.8 ± 5.0	8.5 ± 4.7
02 Sept 2016	16DAA	12.3 ± 9.8	16.5 ± 12.2	15.0 ± 11.6	42.0* ± 19.3	40.3* ± 18.3
03 Sept 2016	17DAA	10.5 ± 3.7	17.3 ± 5.9	10.3 ± 12.1	13.8 ± 5.7	20.5 ± 9.9
04 Sept 2016	18DAA	5.0 ± 4.1	10.5 ± 2.5	8.3 ± 9.5	10.8 ± 5.0	7.3 ± 2.8
05 Sept 2016	19DAA	2.8 ± 1.9	11.0* ± 3.2	6.5 ± 3.1	9.3* ± 3.9	7.8* ± 1.0
06 Sept 2016	20DAA	0.0 ± 0.0	2.3 ± 2.6	0.8 ± 1.5	2.8 ± 5.5	14.5 ± 13.1
07 Sept 2016	21DAA	0.5 ± 1.0	6.5* ± 6.6	6.3* ± 5.9	7.0* ± 3.5	8.5* ± 4.7
08 Sept 2016	22DAA	34.5 ± 12.1	38.5 ± 17.1	30.3 ± 10.3	50.3 ± 9.2	40.5 ± 9.7

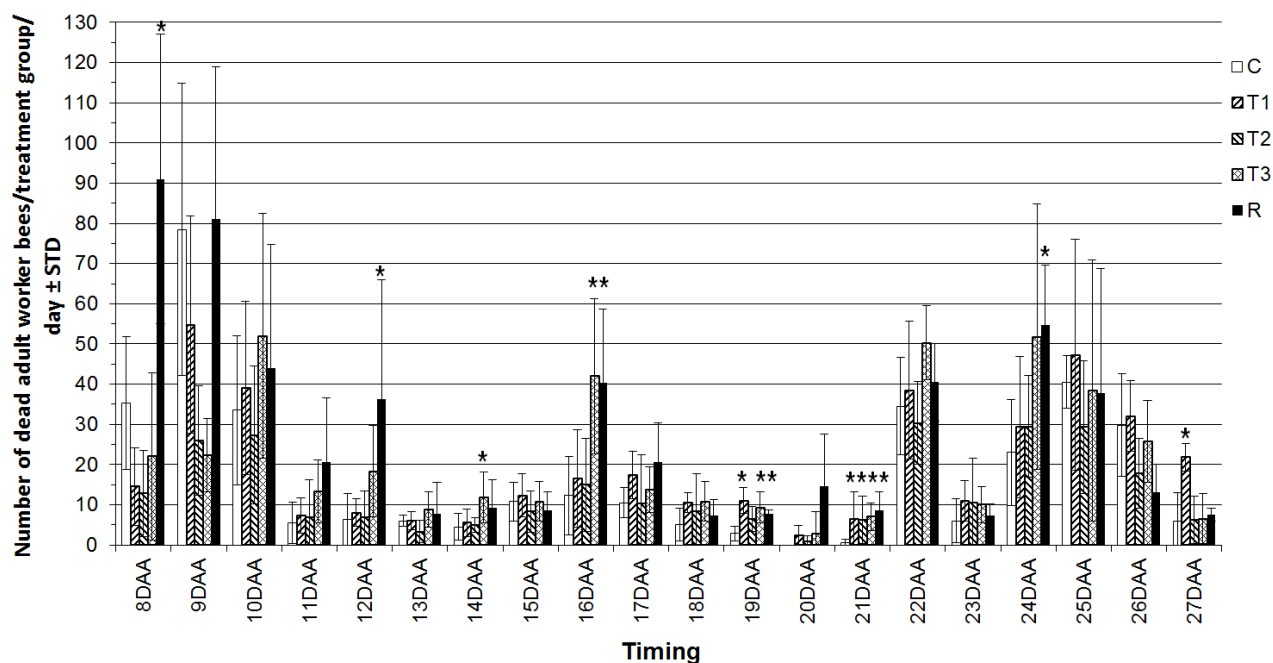
Date	Timing	Mortality (dead adult worker bees) [Mean \pm STD] ¹⁾				
		C	T1	T2	T3	R
09 Sept 2016	23DAA	6.0 \pm 5.5	11.0 \pm 5.0	10.5 \pm 11.1	10.0 \pm 4.5	7.3 \pm 2.8
10 Sept 2016	24DAA	23.0 \pm 13.2	29.3 \pm 17.6	29.5 \pm 12.7	51.8 \pm 33.0	54.8* \pm 14.8
11 Sept 2016	25DAA	40.5 \pm 6.5	47.3 \pm 28.8	29.3 \pm 16.6	38.3 \pm 32.5	37.8 \pm 30.9
12 Sept 2016	26DAA	29.8 \pm 12.8	32.0 \pm 8.8	17.8 \pm 8.7	25.8 \pm 10.2	13.0 \pm 7.0
13 Sept 2016	27DAA	5.8 \pm 7.2	21.8* \pm 3.5	6.3 \pm 5.9	6.5 \pm 6.2	7.5 \pm 1.7
Mean 8DAA to 27DAA		17.6 \pm 5.0	19.6 \pm 2.9	13.4 \pm 6.4	21.4 \pm 7.2	27.9* \pm 6.3

DBA/DAA = Days before/after application STD = Standard deviation

¹⁾ Mortality data from 12DAA to 27DAA may include fragmented honey bees which resulted from vespine wasps foraging on bees and carcasses in the bee traps. In these cases, only one type of fragment (the most frequent type: head, thorax or abdomen) was recorded to avoid double counting of individual bees.

* Statistically significantly different from the control (one sided Dunnett's t-test or Bonferroni U-test (exact), $p \leq 0.05$)

Figure 9.5.1-6: Mean number of dead honey bees counted per colony in the dead bee traps and hive floor in C, T1, T2, T3 and R from 8DAA to 27DAA



Mortality of larvae and pupae

Mean mortality per replicate and day of the worker larvae and pupae in the test item treatments was ≤ 1 over the whole study period. There were no treatment related effects or statistically significant differences to the control. The larvae and pupae mortality in R was significantly higher than the control from 8DAA – 13DAA, 17DAA, 20DAA and 27DAA. The mean value from 8DAA – 27DAA was 22.9 dead pupae/day. This is expected from the mode of action of this reference substance.

Foraging activity

Observations of foraging activity started one day after installation of the colonies in the tunnel (4DBA) and were carried out until 7DAA. At each assessment time the number of bees that were foraging on flowers in the assessment areas or flying over the crop were counted on three foraging assessment areas of 1 m² per tunnel for approximately 10 to 15 seconds. The location of the assessment areas was chosen randomly prior to each assessment. During pre-exposure foraging activity was assessed once a day during flight activity of the bees. On the day of application foraging activity was assessed once shortly before application, four times in the first hour after application (15 min, 30 min, 45 min, 1 hour) and then 2 hours, 4 hours, and 6 hours after application. 1DAA foraging activity was assessed three times during flight activity of the bees (morning, midday, afternoon). From 2DAA – 7DAA the foraging activity was assessed once a day during the flight activity of the bees.

The data for foraging activity was checked for normality using Shapiro-Wilks test and homogeneity using Bartlett's or Levene's test. Treatment group foraging activity was compared to the control with one-sided Dunnett's t-test or Student's t-test (pooled), $p \leq 0.05$).

The overall daily mean foraging activity during the pre application period was similar in all treatments groups at 14.5, 14.5, 15.9, 15.0, and 15.4 bees/m²/day in C, T1, T2, T3 and R respectively. On 0DBA the mean foraging activity was 14.8, 16.2, 16.6, 16.3 and 20.4 bees/m²/day indicating the bees had adapted to the tunnel conditions, and they were well exposed during application.

After application on 0DBA the foraging activity slightly increased compared to the assessments on the same day before application at 22.4, 28.6, 26.0, 25.5 and 26.8 bees/m²/day in C, T1, T2, T3 and R respectively. Foraging activity was high in all treatments on all days throughout the study period. The mean foraging activity during the post application period (0DAA – 7DAA) was 22.4, 24.3, 24.9, 25.5, and 23.9 bees/m²/day for C, T1, T2, T3 and R. None of these differences were statistically significant and there was no test item related effect on honey bee foraging activity.

Table 9.5.1-19 Overall daily mean bee foraging activity during the pre-exposure and exposure period

Date	Timing	Foraging activity [mean number of honey bees/m ² ± STD]				
		C	T1	T2	T3	R
13 Aug 2016	4DBA	4.9 ± 1.5	4.4 ± 1.9	6.4 ± 1.2	9.5 ± 4.3	5.9 ± 2.1
14 Aug 2016	3DBA	19.6 ± 3.1	18.6 ± 7.2	21.4 ± 5.6	17.1 ± 5.7	17.2 ± 2.7
15 Aug 2016	2DBA	20.6 ± 3.7	20.2 ± 4.4	23.1 ± 3.1	20.2 ± 4.1	20.9 ± 2.3
16 Aug 2016	1DBA	12.5 ± 2.1	12.9 ± 1.4	12.2 ± 3.8	12.0 ± 2.0	12.4 ± 0.4
17 Aug 2016	0DBA	14.8 ± 1.6	16.2 ± 4.0	16.6 ± 2.2	16.3 ± 1.2	20.4 ± 2.3*
Mean pre-exposure period (4DBA to 0DBA)		14.5 ± 1.5	14.5 ± 3.1	15.9 ± 2.7	15.0 ± 2.5	15.4 ± 1.2
17 Aug 2016	Mean 0DAA	22.4 ± 1.2	28.6 ± 3.9	26.0 ± 2.1	25.5 ± 1.6	26.8 ± 1.9
18 Aug 2016	Mean 1DAA	21.0 ± 1.1	23.4 ± 2.3	24.5 ± 2.7	24.3 ± 1.2	18.6 ± 3.1
19 Aug 2016	2DAA	25.6 ± 2.3	26.4 ± 1.4	28.3 ± 2.1	26.5 ± 1.6	25.8 ± 0.6
20 Aug 2016	3DAA	22.6 ± 2.5	21.3 ± 2.0	25.3 ± 2.0	20.8 ± 1.8	17.1 ± 1.1
21 Aug 2016	4DAA	15.7 ± 1.0	19.9 ± 3.3	23.2 ± 5.8	26.4 ± 3.3	27.8 ± 2.6
22 Aug 2016	5DAA	17.1 ± 1.1	22.3 ± 1.7	23.7 ± 2.5	24.1 ± 3.2	25.9 ± 5.9
23 Aug 2016	6DAA	28.8 ± 3.2	27.3 ± 2.2	26.0 ± 2.5	31.8 ± 2.0	26.1 ± 8.0
24 Aug 2016	7DAA	26.0 ± 2.8	25.4 ± 3.0	22.7 ± 1.2	24.3 ± 1.9	23.2 ± 4.3
Mean exposure period (0DAA to 7DAA)		22.4 ± 0.8	24.3 ± 1.5	24.9 ± 1.5	25.5 ± 0.8	23.9 ± 2.8

DBA/DAA = Days before/after application STD = Standard deviation

* foraging activity in R significantly higher than in C (Student's t-test (pooled), two-sided, $p \leq 0.05$) on 0DBA

Behaviour

Behaviour of the honey bees was assessed during the assessments for mortality and foraging activity. Behaviour types assessed included:

- Intensive cleaning
- Trembling
- Cramping
- Locomotion problems
- Inactive bees
- Filtering bees
- Flying without landing on the crop
- Hanging bees
- Clustering at the hive entrance

During the pre-application period (4DBA to 0DBA) a few bees with locomotion problems were observed in all tunnels except T3. In the control, other behavioural patterns (mainly locomotion problems and inactivity) were also observed on the following days during the confinement period in the tunnels (0DAA to 5DAA) and also on 9DAA/10DAA at the monitoring location. In most cases these observations were recorded for only 1-2 individual bees in one of the four replicates per assessment date which is considered a normal level for these behaviour patterns.

During exposure to the treated crop (0DAA to 7DAA), a slight increase in the number of bees with locomotion problems was observed in T1 and T3. In T3, there was also a slight increase in the number of cramping bees during this period, indicating a slight effect of the test item treatment on honey bee behaviour in these groups. Four hours after exposure 65 bees were observed clustering at the hive entrance in T2, when behaviour was monitored again at 6HAA there were no bees clustering so this effect can be considered to be transient.

Observations in T1 and T2 at the monitoring site (8DAA to 27DAA) were similar to those in the control. In T3, locomotion problems, cramping and trembling were observed slightly more often compared to C, T1 and T2. However this could be partly due to interference from wasps which were preying on and occasionally stinging bees at the hive entrances, which may result in similar symptoms. No other information was provided by the applicant.

Development of the bee brood (photographic assessment)

The development of the bee brood was assessed in individually marked brood cells; the fixed brood areas were photographed during each brood stage assessment. At the assessment before application (Brood fixing day = BFD) one or several brood combs were taken out of each colony to mark areas containing at least 200 eggs, 200 young larvae and 200 old larvae on the comb. The selected combs were uniquely identified.

The exact positions of the markers on the wooden frames and of each cell were defined on the computer in the digital image. This means each selected cell could be identified and the contents of the cell was evaluated on the digital picture. The assessments were conducted on BFD (1DBA), 6 days after BFD, 10 days after BFD, 16 days after BFD, and 22 days after BFD.

The results of the photographic assessments of brood development 22 days after the brood area fixing day (BFD0) showed mean termination rates of 34.90% for eggs in C, 37.16% in T1, 18.91% in T2, 41.31% in T3 and 79.88% in R. There were no statistically significant differences of brood termination rates of eggs, young larvae or old larvae in T1, T2 and T3 compared to C on any assessment date. Statistically significant differences were only detected for the reference item treatment R for eggs (all assessment dates from BFD+6 through BFD+22), young larvae (BFD+16 and BFD+22) and old larvae (BFD+16).

The brood indices and compensation indices for eggs, young larvae and old larvae in T1, T2 and T3 were not statistically different from the control on any assessment date. In the reference item R, the brood indices and compensation indices of cells initially containing eggs on BFD0 were significantly different from the control on all assessment dates (BFD+6, BFD+10, BFD+16, BFD+22). For young larvae, the compensation index in R was significantly different from the control on BFD+16 and the brood index was significantly different on BFD+16 and BFD+22. For old larvae, the brood index in R was significantly different from C on BFD+16.

Conditions of the colonies and colony development

The condition of the colonies and the development of the honey brood were checked twice before start of exposure and nine times afterwards. These assessments took place before installation of the colonies at the test site (7DBA), the day before application, 5DAA and eight times at the monitoring site. The colonies used for residue sampling were assessed once on 7DBA. To assess the effects of the test item and reference item compared to the control the following parameters were assessed:

- Colony strength (number of bees)
- Presence of a healthy queen (e.g., presence of eggs)
- Pollen storage area and area with nectar or honey
- Area containing cells with brood stages

At each assessment the comb area containing bees and cells with nectar, pollen, eggs, larvae, and capped cells was estimated per comb slide. The total number of bees and the number of cells containing the single brood stages, pollen and nectar was calculated for each colony. The mean values were then calculated for each treatment and

assessment date. The calculation of the area containing brood and food stages based on a comb size of 800 cm² per comb side and assuming 400 cells per 100 cm². For the calculation of colony strength, 130 honey bees per 100 cm² were assumed as full coverage.

At each colony assessment colonies were assessed for bee disease, according to standard bee keeping practice, any unusual occurrences and clear symptoms of disease or pests were recorded.

Colony strength

At the start of the study (7DBA and 1DBA), mean colony sizes were on a similar level in all treatment groups. The mean colony strength on 7DBA was 7020, 7053, 7361, 7313 and 6906 in C, T1, T2, T3, and R respectively. During the summer and autumn, mean colony strength in the control and test item treatment groups T1, T2 and T3 followed the natural cycle of honey bee colony development for this period. The biggest colony sizes were observed at the first assessment at the monitoring site (9DAA). The following development of the mean colony sizes was essentially similar in C, T1, T2 and T3, with only minor fluctuations and no statistically significant differences to the control.

However, a clear impact on colony size was observed in the colonies in the reference item treatment R (significantly different from the control at all assessment dates from 15DAA to 63DAA). Test treatment groups were compared to the control using the Student's t-test.

Brood development

All control and test item treatment colonies (T1, T2, T3) showed all brood stages (eggs, larvae, pupae) at all assessments throughout the study, except for the lack of some of these brood stages in several hives at the end of the observation period in October 2016 (54DAA, 63DAA), which coincided with the natural end of the honey bee brood rearing season and the colonies getting ready to overwinter.

In all treatment groups, the mean amount of brood was highest at the first assessment (7DBA) and slowly decreased, with only minor fluctuations, during the following assessments until 63DAA (19 Oct 2016). This slow decrease of the total amount of brood is not unusual at this time of the year since the natural end of the honey bee brood rearing season was approaching and the colonies were preparing for overwintering.

None of the slight differences in total amount of brood or the amount of the different brood stages (eggs, larvae, pupae) in the test item treatments T1, T2 and T3 were statistically different from the control, except in T3 where a slightly reduced number of eggs was recorded on 28DAA and a slightly reduced number of larvae was recorded on 44DAA. Since there were no other unusual observations regarding brood development in this treatment, it is unlikely that this temporary reduction of eggs or larvae was related to the treatment but rather due to the late season (mid to end of September) and the upcoming natural end of brood rearing in all colonies.

In the reference item there was a clear impact of the treatment on the total amount of brood during all assessments from 9DAA to 44DAA reflecting the significantly reduced number of eggs, larvae and pupae in this treatment.

Food Stores

At the start of the test the mean number of nectar cells per colony was 12000, 8600, 10400, 15000 and 8700 in C, T1, T2, T3 and R. Initial differences on 7DA were compensated by moderate feeding of the relevant hives on 7DBA before the installation of the colonies in the tunnels. At the assessments in the tunnels shortly before application (1DBA) the food stores of the different treatment groups were as homogenous as possible. The mean number of nectar cell per colony was 10900, 1110, 11550, 11050, and 13250 in C, T1, T2, T3 and R, the mean number of pollen cells per colony was 3000, 2300, 2450, 2200, and 1150 in C, T1, T2, T3 and R.

During the assessments, all colonies had pollen and nectar during the entire study, except for a lack of pollen in one replicate of treatment R (28DAA) and in one replicate of treatments T2 (54DAA) and T3 (63DAA), which was clearly due to the late timing of this assessment and the scarcity of natural pollen sources during October.

On 5DAA in T1 and R and 15DAA in R there was a statistically significant reduction of pollen stores, however these were temporary and recovery was observed afterwards. Pollen stores were still at an acceptable level.

Due to scarcity of natural nectar sources in autumn, nectar stores decreased in the control and all treatment groups during the monitoring period. Critically low levels of nectar (<15 %) with most hives reaching the point of starvation were observed on 21DAA. According to good beekeeping practice at this time of the season (preparation of the colonies for overwintering and depletion of natural flowering resources at the monitoring

location) and due to most of the colonies having very low levels of nectar (<15%), all colonies were moderately fed 5 kg sucrose solution in September 2016 (23DAA).

Residue analysis

Specimens of nectar, pollen and dead bees were analysed for residues of various commonly used fungicides and insecticides with different limits of quantification (LOQ). Samples were successfully analysed for residues using high performance liquid chromatography with triple quadruple mass spectrometry determination (LC-MS/MS). Samples of pollen and nectar prepared from forager bees, leaves, flowers, samples of in-hive products (pollen and nectar from combs) and pollen from pollen traps were analysed for residues of pydiflumetofen.

Samples for residue analysis were only taken from colonies Cs, T1s, T2s, and T3s. Immediately after sampling all samples were divided into two sub-samples; A for analysis and R for retention. In each sampling the control sample was collected first, or by different personnel with different equipment. Samples were kept deep-frozen (typically at $\leq 18^{\circ}$).

During the exposure phase samples of forager bees (prepared for pollen and nectar), leaves, flowers and samples of soil were collected. During the monitoring phase samples of pollen and nectar (in-hive products), pollen (pollen trap) and dead bees (bee traps and hive bottoms) were collected.

Due to increased mortality in Cc and T1a on 9DAA (shortly after installation of the colonies at the monitoring location), the samples of dead bees collected in these colonies on this day will be analysed for residues of various pesticides together with the in-hive products (pollen and nectar) and the pollen from the pollen traps collected from the monitoring location.

Sampling dead worker bees from dead bee traps and bottom drawer

Dead worker bees from the dead bee trap and bottom drawer were taken every assessment day after the assessment of mortality during the exposure and monitoring period. Each sample contained all the dead worker bees from one colony excluding pupae and larvae. The honey bees were collected by hand, or using tweezers. Individual samples were taken from each of the colonies for biological assessments except the reference.

Dead bee samples of Cc and T1 on 9DAA with ≥ 94 dead bees per day and replicate were additionally analysed for residues of various other pesticides.

Sampling pollen samples from pollen trap

Pollen traps were used to collect pollen from forager bees returning to hives Cs, T1s, T2s and T3s on 37DAA and from 57DAA – 61DAA. The grid of the pollen trap was inserted during the time of foraging activity of the honey bees and was kept in place for 7 hours (sampling S6, 37DAA) or several days (sampling S7, 57DAA – 61DAA). Sampling S7 extended over several days of good weather after an earlier attempt failed to obtain this sample (54DAA) due to poor foraging conditions and seasonal scarcity of natural flowering pollen sources.

After the respective collection period, the grid was removed, and the collected pollen was sampled. During sampling S6 only A-samples could be obtained due to naturally low availability of pollen sources and small sample sizes of approximately 2g or less.

Sampling of pollen and nectar samples from combs

Pollen from combs was sampled with a special pollen sampling device on 37DAA and on 54DAA. Each sample was generated by sampling from various comb locations.

Sampling forager bees for preparation of pollen and nectar for residue analysis

Forager bees for the preparation of nectar from honey stomachs and pollen from pollen loads for residue analysis were collected on 3DBA, 0DAA, 2DAA, 4DAA, and 6DAA. At each sampling the colony entrances were sealed before the sampling and the forager bees returning to the colonies were subsequently collected using modified hoovers and dry ice. After sampling the colonies were reopened.

On each sampling day > 200 forager bees per tunnel were collected from the colonies for the A-sample, and > 150 forager bees for the R-sample.

The honey stomachs were prepared as follows:

- The total number of bees were counted
- At least 50 % of the bees in A-sample were prepared
- If the minimum could not be obtained, then sample-R was prepared and added to sample-A until the requested amount was achieved.
- The duration of the samples remaining outside the freezer did not exceed 2 hours.

The pollen loads were detached from the legs of the forager bees and transferred into a vial. Bees were fixed at the thorax and the abdomen was separated with tweezers. The honey stomach content was transferred directly into a vial and stored deep frozen immediately. The minimum sample amount was 200 mg for pollen and 200 mg for nectar.

Sampling for flowers for residue analysis

Phacelia tanacetifolia flowers for residue analysis were taken on 3DBA, 0DAA, 2DAA, 4DAA and 6DAA. The terminal inflorescences with open flowers were cut at the end of the stem and the open flowers were bagged. A pooled sample of flowers was collected from at least 12 different locations across the plot.

Sampling leaves for residue analysis

Phacelia tanacetifolia leaves were cut or pinched by hand and bagged. A pooled sample was collected from at least 12 different locations across the plot.

Sampling soil cores from the test field

Soil samples were collected on 3DBA but were not analysed for residues since pydiflumetofen was not found in any of the untreated samples of pollen, nectar, flowers and leaves. In addition, it was possible to obtain the full pesticides/maintenance history for the test field showing what applications had been made from 2013 to 2016.

Residue Analysis

Samples of pollen and nectar from forager bees, pollen and nectar from combs, pollen from pollen traps, *Phacelia* flowers and *Phacelia* leaves were analysed for residues of pydiflumetofen resulting from one application of Pydiflumetofen (A19649B) during flowering and honey bee flight activity. Untreated samples and the three treated groups (T1, T2 and T3) with different application rates ($T3 > T2 > T1$) were analysed in the current study.

There were no detectable residues of pydiflumetofen above the defined LOD (0.0015 mg/kg for this analyte) in any of the samples of nectar, pollen, leaves and flowers taken in C throughout the study period and in T1, T2 and T3 prior to application in these treatments (3DBA).

Treated pollen specimens

Pollen from forager bees (exposure phase): Residues of pydiflumetofen (SYN545974) in pollen were 7.37 mg/kg in T1, 24.0 mg/kg in T2 and 29.5 mg/kg in T3 at 0 days after application and decreased to 0.110 mg/kg in T1, 0.433 mg/kg in T2 and 0.383 mg/kg in T3 within 6 days after application.

Pollen from traps and combs (monitoring phase): Residues of pydiflumetofen (SYN545974) in treated pollen samples were not detectable except for the two comb pollen samples from T3 with residues of 0.108 mg/kg and 0.560 mg/kg, and one pollen trap sample from T3 and one comb pollen sample from T1 with residues between the LOD (0.0015 mg/kg) and the LOQ (0.005 mg/kg).

Treated nectar specimens

Nectar from forager bees (exposure phase): Residues of pydiflumetofen (SYN545974) were 0.0409 mg/kg in T1, 0.165 mg/kg in T2 and 0.156 mg/kg in T3 at 0 days after application and decreased to values below the limit of detection ($< LOD$) within 6 days after application.

Nectar from combs (monitoring phase): No residues of pydiflumetofen (SYN545974) were detected above the defined LOD (0.0015 mg/kg) in nectar from combs at the sampling dates 37DAA and 54DAA, except for the nectar sample from the T1 group at 37 DAA where residues were detected at 0.0100 mg/kg.

Table 9.5.1-20: Residues of pydiflumetofen found in pollen and nectar from treated specimens

Time	Residues of pydiflumetofen (mg/kg)								
	Pollen						Nectar		
	T1		T2		T3		T1	T2	T3
Exposure Phase						Exposure Phase			
0DAA	7.37		24.0		29.5		0.0409	0.165	0.156
6DAA	0.110		0.433		0.383		< LOD	< LOD	< LOD
Monitoring Phase						Monitoring Phase			
	PT	PC	PT	PC	PT				PC
37DAA	n.d.		n.d.		0.108	n.d.	0.0100	< LOD	< LOD
54DAA	n.d.	< LOQ	n.d.		0.560	< LOQ	< LOD	< LOD	< LOD

LOD: 0.0015 mg/kg, LOQ: 0.005 mg/kg

PT pollen from traps, PC pollen from combs

n.d.: none detected

Treated *Phacelia* flowers specimens (exposure phase)

Residues of pydiflumetofen (SYN545974) were 12.2 mg/kg in T1, 22.5 mg/kg in T2 and 31.8 mg/kg in T3 at 0 days after application and decreased to 1.74 mg/kg in T1, 2.45 mg/kg in T2 and 3.48 mg/kg in T3 within 6 days after application.

Treated *Phacelia* leaves specimens (exposure phase)

Residues of pydiflumetofen (SYN545974) were 10.8 mg/kg in T1, 17.4 mg/kg in T2 and 41.4 mg/kg in T3 at 0 days after application and decreased to 5.78 mg/kg in T1, 13.2 mg/kg in T2 and 17.4 mg/kg in T3 within 6 days after application.

Table 9.5.1-20: Residues of pydiflumetofen found on flowers and leaves of *Phacelia* from treated specimens

Time	Residues of pydiflumetofen (mg/kg)					
	<i>Phacelia</i> Flowers			<i>Phacelia</i> leaves		
	T1	T2	T3	T1	T2	T3
0DAA	12.2	22.5	31.8	10.8	17.4	41.4
6DAA	1.74	2.45	3.48	5.78	13.2	17.4

LOD: 0.0015 mg/kg, LOQ: 0.005 mg/kg

n.d.: none detected

Multi-residue analysis

There were no residues of any of the other pesticides above the LOQ in samples of pollen from combs, nectar from combs or pollen from traps, except the sample of pollen from traps of hive T1s on 37DAA where quantifiable residues of phosphonic acid were detected (0.198 mg/kg).

Dead bees collected from control hive Cc on 9DAA had low levels of residues of metrafenone (0.0120 mg/kg). No residues were found in dead bees from hive T1a collected on the same day (this sample analysed as >100 dead bees observed; the criteria set in the study plan).

Conclusion

The objective of the study was to determine potential effects of exposure of honey bees to flowering *Phacelia tanacetifolia* treated once at start of flowering with pydiflumetofen (A19649B) under semi-field conditions. The test item pydiflumetofen (A19649B) was applied to flowering *Phacelia tanacetifolia* with the target application rates of 75, 125 and 200 g a.i./ha (T1, T2, T3) during daily honey bee flight.

There was no test item related effect on mortality of adult worker honey bees or honey bee larvae and pupae. There was no test item related effect on honey bee foraging activity. Slight behavioural changes were observed during the post-application period (0DAA to 7DAA) in T1 and T3. There was no negative impact of the test item treatment on the brood index, compensation index and brood termination rates of individually marked cells containing eggs, young larvae or old larvae at BFD0 (one day before application of the test item) through to the completion of their 22 day development cycle. There was no negative impact of the test item treatment on the condition of the colonies (number of bees per hive, amount of brood, presence and amount of brood of different stages, amount of nectar and pollen).

There were no detectable residues of pydiflumetofen in any of the samples taken in the control group throughout the study period or in the samples from the test item treatment groups T1, T2 and T3 taken prior to application. During the exposure phase in the tunnels, residues of pydiflumetofen (A19649B, SYN545974) were found in leaves, flowers and in pollen and nectar samples from forager bees after application at 0DAA in all treatment groups and decreased within 6 days after application. During the monitoring phase at the remote location, residues of pydiflumetofen above the LOD were found in pollen from the pollen traps in T3 on 57-61DAA (<LOQ) and in comb pollen in T1 on 54DAA (<LOQ) and in T3 on 37DAA and 54DAA. Residues of pydiflumetofen above the LOD were found in comb nectar in T1 on 37DAA.

(██████████, 2017)

HSE comments

This semi-field study is conducted to OECD Guidance document No. 75 (2007), and EPPO 170 (4) (2010), with no deviations to the guidelines. Statistical analysis was in line with OECD guidelines.

The following conditions were met during application. The crops were flowering and the bees were actively foraging, the wind speed was less than 2 m/s, the air temperature was less than 30°C and there was no rain. The mean spray tolerance was ± 10 %. This follows recommendations in OECD Guidance document No. 75 (2007).

During the exposure period the weather was favourable for foraging resulting in goods exposure of the bees to the test treatments. There was no rain immediately after application (rainfall on 3DAA), so exposure to the test treatment should be accurate. Due to the timing of the study (August to April), during the monitoring period the temperature declined after 45DAA, and the rainfall increased to 25.0 mm/day. As this was during the monitoring phase, it will not have affected the exposure of the honey bees to the treatment.

The highest application rate in the study was 200 g a.s/ha, equivalent to the highest proposed application in the GAP table. This is in line with recommendations in OECD Guidance document No. 75 (2007). The treatment was applied to *Phacelia tanacetifolia*. This is a bee attractive crop, in line with OECD 75 recommendations.

Fenoxycarb was used alongside the test substance as a toxic reference item. This was applied at 300 g a.s/ha, OECD Guidance document No. 75 (2007) recommends at least 150 g a.s./ha. There was a statistically significant higher rate of larvae and pupal mortality in the post exposure period for the toxic reference. There was a high egg termination rate in the reference item, the brood indices and compensation indices of cells initially containing eggs were significantly different from the control. HSE agrees this shows an appropriate level of sensitivity for the study.

The factors assessed during the study included; mortality, behaviour, flight intensity, general colony assessments (colony strength, food status, colony development) and the development of the bee brood. There were no adverse effects on any of the parameters assessed at any of the application rates compared to the control, except for slight behavioural changes post application in T1 and T3 corresponding with 75 g a.s./ha and 200 g a.s./ha. The brood developed as expected, and the compensation indices in the treatment groups were not statistically different from the control indicating the ability for the brood to recover. It was noted in the control group a dead Queen was found at six days post application. However, a second Queen was observed in the colony so HSE does not consider this to have had an impact on the colony condition.

The behaviour reported in the control and treatment groups during the study included locomotive issues, inactivity, cramping, clustering and trembling. The incidences of locomotion problems, inactivity, and trembling occurred at similar levels across C, T1, T2 and T3, in 1 or 2 individual bees during each observation, therefore, are not considered to be treatment related as there is no clear dose response. The increased incidence of cramping in the bees in T3 (14 total) indicate a slight treatment related effect compared to C, however, this still only occurred in

a maximum of 3 bees at each observation. Four hours after application 65 bees were recorded clustering at the hive entrance in T2, however, as they were not present 6 hours later this is not considered to be treatment related.

The colony size decreased for all treatment groups, C and R during the course of the study. This is due to the natural cycle of honey bee colony development during the study period (August-April). As this occurred in both the control and the treatment groups the reduction in colony size is not considered to be treatment related. Additionally, there was less than 15 % nectar in the colonies, with all hives reaching the point of starvation when observed 21 days after application. According to good bee keeping practice, the colonies were fed 5kg of sucrose solution from 23DAA. As this supplementary feeding took place during the monitoring period, it is not considered to have had an effect on the exposure of the bees to the treatment.

Overall HSE considers this study to show there are no treatment related effects of pydiflumetofen on mortality, foraging activity, colony strength and colony development from the proposed use.

The analytical method has been evaluated by HSE Chemistry specialists in Vol. 3CP Part B5.1.2.5. The following was concluded for this method: "Acceptable method. LOQ: 0.005 mg/kg in nectar, pollen, flowers and leaves".

This study is considered reliable and will be discussed further in the risk assessment.

Please note that the following study was conducted with the EU representative formulation (A19649B); further details are given in the risk assessment.

Report:	K-CP 10.3.1.5. [REDACTED], (2018), Pydiflumetofen SC (A19649B) - A semi-field study to evaluate the side effects on the honey bee <i>Apis mellifera</i> L. in Germany in 2017. Report Number 17 48 BTB 0003. BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany (Syngenta file no A19649B_10349)
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Guidelines

OECD Guidance document No.: 75 (2007)

GLP: Yes

Key Abbreviations: DAT: Days After Treatment; BFD: Brood Fixing Day.

Executive Summary

The purpose of the study was to determine potential effects of A19649B on the honeybee (*Apis mellifera* L.) after single foliar application on full-flowering Phacelia (*Phacelia tanacetifolia* B.) under semi-field conditions.

A19649B was applied once at a rate of 375 mL (equivalent to 75 g a.s./ha), 625 mL (equivalent to 125 g a.s./ha) and 1000 mL product/ha (equivalent to 200 g a.s./ha) in a semi-field study (bee brood tunnel study according to OECD Guidance No. 75) on full-flowering *Phacelia tanacetifolia* during daily bee flight. The exposure of the bees to the test item treatments was proven by the assessment of the foraging activity and residue analysis of the test item in flowers, foliage and bee food sources (pollen and nectar) collected on several sampling days after application.

The application conditions as set OECD Guidance No. 75 were met. Following the application there were no treatment related effects on adult and pupal bee mortality (monitored for 28 days after application) or foraging activity (monitored for 7 days after application), and there was no effect on the behaviour of the bees (monitored for 29 days after application).

Colony strength (no. bees/colony), food and brood development by comb area were observed between the test item treatments and control up to 62 days after application. There were occasions of significantly lower mean colony strength during the first brood cycle only, for the highest two test concentrations, after this there were no significant differences. There were significantly reduced food stores from 9 days after application onwards for the highest two test concentrations or from 34 days after application onwards for the lowest test item concentration. There were no significant differences in brood development by comb area across any test item concentration during the whole test (up to 62 days after application).

The specific evaluation of the detailed bee brood development by photo documentation of initially labelled eggs, young larvae and old larvae during the first brood cycle (21 days after application) revealed no statistically significant differences in the test item treatments on eggs, young larvae and old larvae during this time.

In contrast to this, following the application of the reference item, effects on colony strength, detailed and general brood and food development were observed in the reference item treatment up to 62 days after application. These observations demonstrate the sensitivity of the test system.

Overall, based on the results of this study, the test item does not adversely affect the development and survival of the honeybee colonies.

Materials and Methods

Experimental dates: 27th May 2017 – 1st August 2017.

Test material details

Test item name	Pydiflumetofen SYN545974 SC (formulation A19649B)
Lot/Batch #:	SMU6AP004
Purity:	18.5 % w/w, corresponding to 201 g a.s./L according to certificate of analysis from study sponsor. Appearance: Off-white liquid. Density: 1086 kg/m ³ .
Reanalysis/Expiry date:	Certificate of Analysis of 5 th April 2016; Recertification Date end of March 2019

Treatment rates of test item and reference item

Test rates:	A19649B applied at 375, 625 and 1000 mL product/ha; equivalent to 75, 125 and 200 g a.s./ha. These concentrations may be referred to as Test Item I, II and III, respectively.
Control:	Tap water
Toxic standard (reference item):	Insegar 25 WG (fenoxycarb) applied at 1200 g product/ha; equivalent to 300 g a.s./ha

Test crop details

Crop:	<i>Phacelia tanacetifolia</i> (Balo), sowing density 10kg seed/ha.
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Test cage/tunnel details

The exposure phase of the study was carried out in tunnels; each covered an area of 108 m² (18 m x 6 m). The tunnel was enclosed by metal frames (maximum height of 2.5 m) and covered with fine plastic gauze with a mesh size less than 3 mm. This is in line with the requirements of OECD 75 (Table 9.5.1-21). Each tunnel was equipped with water supply. The distance between each tunnel was at least 3 m and the test cages were arranged in a randomised pattern.

Prior to installing the colonies, to facilitate the counting of dead bees (mortality), the crop was removed from 3 x 0.5 m wide paths along the first and last metal frame and in the middle of the plot of the tunnel. The total effective plot size with flowering *Phacelia* was 93.5 m², which comprising two sub-plots each of 2.75 m x 17 m = 46.75 m². Gauze sheets (twice: 6 m x 0.5 m and once: 18 m x 0.5 m) were spread out over these “pathed” areas to further aid the collection and counting of dead bees on the ground (Figure 9.5.1-7).

Each colony was also fitted with a dead bee trap for mortality measurements. The dead bee traps were constructed in such a way that a bee could not leave the colony carrying a dead bee or brood without dropping it into the trap (■■■■ et al. 2002). All bees leaving the colony must exit through the dead bee trap (22 cm x 18 cm x 12 cm) covered with metal gauze lids (mesh width 1.2 cm) (Figure 9.5.1-7). The dead bees dropped onto a bottom slide, which could be withdrawn in order to count and remove the bees collected in each trap.

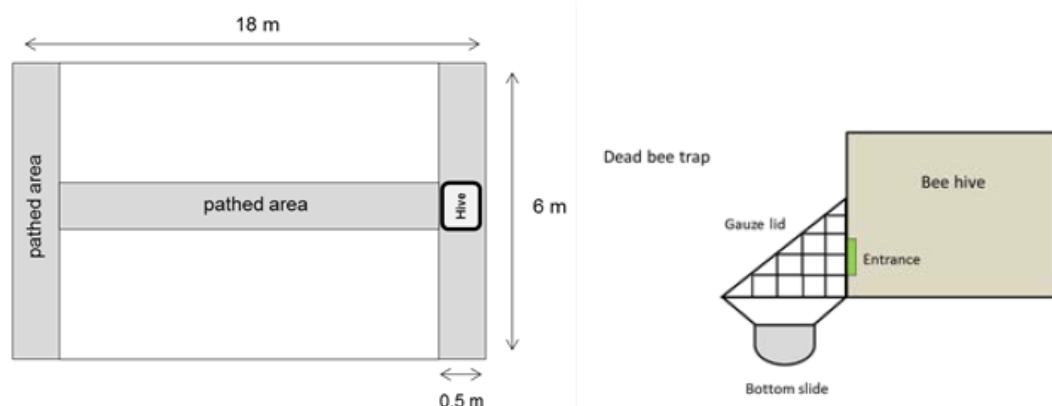


Figure 9.5.1-7. Design of the tunnel (left) and dead bee trap (right)

Test organism details

Species:	Honey bee, <i>Apis mellifera</i> L. Buckfast
Age:	Adult and juvenile bees and all brood stages
Source:	BioChem agrar GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany; apiary number (location Brandis): 14729-070-006

Test colony details

General information

The colonies used were healthy and queen-right containing sister queens of 2016. The use of sister queens and queen right colonies is a requirement of the OECD 75 guideline and this has been met.

Bee colonies were regularly inspected by the responsible beekeepers (project personnel) according to Good Apicultural Practice to avoid them from swarming, robbery and to check sufficiency of food.

Description of hive

Each colony was equipped with one polystyrene hive body consisting of 11 combs of “German standard-size” with comb size of 37 cm × 22.3 cm = 825.1 cm² per comb side and total comb area of 18152 cm² per colony.

Health

The health of the bee colonies was in line with the OECD 75 guideline (see Table 9.5.1-21) apart from the presence of deformed wing virus observed in a single replicate in the control. No clinical symptoms of disease were recorded.

Table 9.5.1-21 Health details for the colonies used in the test

Information point and OECD 75 guideline requirement, if stated		Value in Study				
		Control	75 g a.s./ha	125 g a.s./ha	200 g a.s./ha	Reference item
The colony should be healthy. Bees should be free of clear clinical symptoms of disease.	General health	The health of the colonies was checked about 2 weeks before the start of the study for presence of diseases. The health of the colony was also confirmed by colony assessment the day before application (DAT - 1/BFD 0).				
	Virus test ^[1]	Colony 1.3: DWV	Negative	Negative	Negative	Negative
	<i>Varroa</i> ^[2]	Absent	Absent	Absent	Absent	Absent
	No. replicates with <i>Nosema</i> . ^[3]	Low: 2 Absent: 2	Absent: 4	Low: 1 Absent: 3	Low: 2 Absent: 2	Low: 1 Absent: 3
Medical treatment (none allowed within 4 weeks before test start)		None stated	None stated	None stated	None stated	None stated

Information point and OECD 75 guideline requirement, if stated	Value in Study				
	Control	75 g a.s./ha	125 g a.s./ha	200 g a.s./ha	Reference item
Queen right colonies (required)	Yes				
Colonies from sister queens (required)	Yes, sister queens of 2016				

^[1] health certificate (non-GLP) for diverse viruses (DWV (deformed wing virus), SBV, ABPV, KBV, CBPV, BQCV, IAPV) by the Länderinstitut für Bienenkunde in Hohen Neuendorf, Germany, sampling data 17th May 2015).

^[2] examination of *Varroa destructor* mite infestation via bottom slides (non-GLP; check for 3 days shortly before experimental start by BioChem agrar GmbH.

^[3] examination of *Nosema* sp. (non-GLP by BioChem agrar GmbH): level of infestation 0-3 (0-absent, 1=low), assessed according to [REDACTED] 1996.

Colony size, brood and food status

The colony requirements are compared with that of the OECD 75 guideline in the Table 9.5.1-22 below. Although the colony size is smaller than the guideline example as scaled up for a crop area of 93.5 m², the ratio of brood to food is well within guideline requirements (Table 9.5.1-22).

Table 9.5.1-22. Colony size, brood and food status

Information point and OECD 75 guideline requirement, if stated	Value in Study				
	Control	75 g a.s./ha	125 g a.s./ha	200 g a.s./ha	Reference item
Tunnel design: guideline minimum 40 m ² floor space, minimum height 2.5 m. Covering gauze maximum 3 mm mesh size.	Tunnel 18 m x 6 m x 2.5 m; effective crop area 93.5 m ² . Metal frame covered with fine plastic gauze with mesh size < 3 mm. This meets the guideline requirements.				
Size of colony (no. of bees on BFD 0). Guideline: 14,025 bees ^[1]	9506 ± 863	10828 ± 249	9591 ± 1247	9956 ± 771	9731 ± 1025
Estimated total brood area (eggs, larvae & pupae) (cm ² /colony) ± SD Guideline: 1755 cm ² ^[1]	8251 ± 971	9321 ± 1151	8754 ± 2450	8767 ± 1042	7529 ± 672
Estimated total nectar and pollen area (cm ² /colony) ± SD Guideline: 2.34 combs (size not stated)	4641 ± 750	5067 ± 1398	5208 ± 1744	5660 ± 1287	6343 ± 1386
Ratio of Brood : Food (should not exceed 4:1)	1.78 : 1	1.84 : 1	1.68 : 1	1.55 : 1	1.19 : 1
No. combs containing brood on BFD 0	6-10				
No. combs containing honey on BFD 0	4-11				
No. combs containing pollen on BFD 0	3-7				

^[1] The guideline (OECD75) states that the size of the colonies should be chosen based on the available crop area per tunnel. Guideline example (OECD 75): A 40 m² crop area should have a colony with approximately 3000 brood cells respectively 750 cm² with brood in all stages, 1 food comb (size not stated) with honey and pollen and approximately 800 g (6000 individuals) worker bees. With the available crop area in this study of 93.5 m², the equivalent parameters scaled up from 40 m² (by a factor of 93.5/40=2.34) is 1755 cm² in all brood stages, 2.34 (size not stated) food combs and approximately 14,040 worker bee individuals.

Feeding

Colonies were well fed at the start of the test and did not exceed the maximum brood to food ratio as stated in the OECD 75 guideline (see Table 9.5.1-22).

A water feeder was placed into each tunnel during the pre-exposure and exposure phase (DAT -3 to DAT 7); this was covered with a lid during application.

The food status of all colonies was acceptable throughout the course of the study, therefore, no artificial food was offered, and all assessments of colony represented the natural state. To keep the food status as long as possible on a high level, honey was not harvested.

Test replication

The minimum number of replicates according to OECD 75 (2007) is three. In this study, the guideline requirements are met: there are four replicate tunnels with one colony per tunnel for biological measurements per control, treatment and reference, each containing one honeybee colony.

One additional tunnel was added to the control and test item groups only, from which flowers, leaves (foliage), pollen and nectar were collected for residue analysis.

Duration of test and experimental dates

Experimental dates: 27th May 2017 – 1st August 2017.

The total duration of the test covered three honey bee brood cycles, lasting for 62 days after treatment (DAT) to BFD 63 (DAT 62).

The colonies were introduced to the tunnels 3 days prior (DAT-4) to application at flowering of Phacelia (BBCH 63-65) to ensure that bees become familiar with the new environmental conditions. This is within the guideline recommendation of 2-3 days acclimatisation. The bees were maintained in the tunnels for seven days after application (guideline requirements: seven days) which formed the exposure phase.

After the exposure phase (in the evening of DAT 7) bee colonies were removed from the tunnels and transferred to the monitoring site (details below), for the subsequent assessments of the remaining brood cycle and a further two consecutive brood cycles.

The dates of the three experimental phases are as follows:

- **Pre-exposure phase:** 3 days (DAT -3 to DAT -0ba; 28.05.2017 – 31.05.2017)
- **Exposure phase:** 7 days following test item application (DAT 0 to DAT 7; 31.05.2017 – 07.06.2017)
- **Post-exposure phase:** 54 days (DAT 8 to DAT 62; 08.06.2017 – 01.08.2017)

The test site details including location and vegetation cover

Tunnel test site location: Hirschfeld near Leipzig (Saxony, Germany), 126m above sea level, Latitude 51°19'38.30"N, Longitude: 12°31'28.44"E (Figures 9.5.1-8, 9.5.1-9 and 9.5.1-10).

Post-exposure monitoring site location: Itenbach near Leipzig (Saxony, Germany), 10.5 km from test site, 130 m above sea level, Latitude: 51°21'14.73"N, Longitude: 12°40'07.18"E (Figures 9.5.1-8 and 9.5.1-11). There were no main crops or intensive agriculture at the monitoring site. 10.5 km from the test site, with an access to natural nectar and pollen sources (no main crops or intensive agriculture)

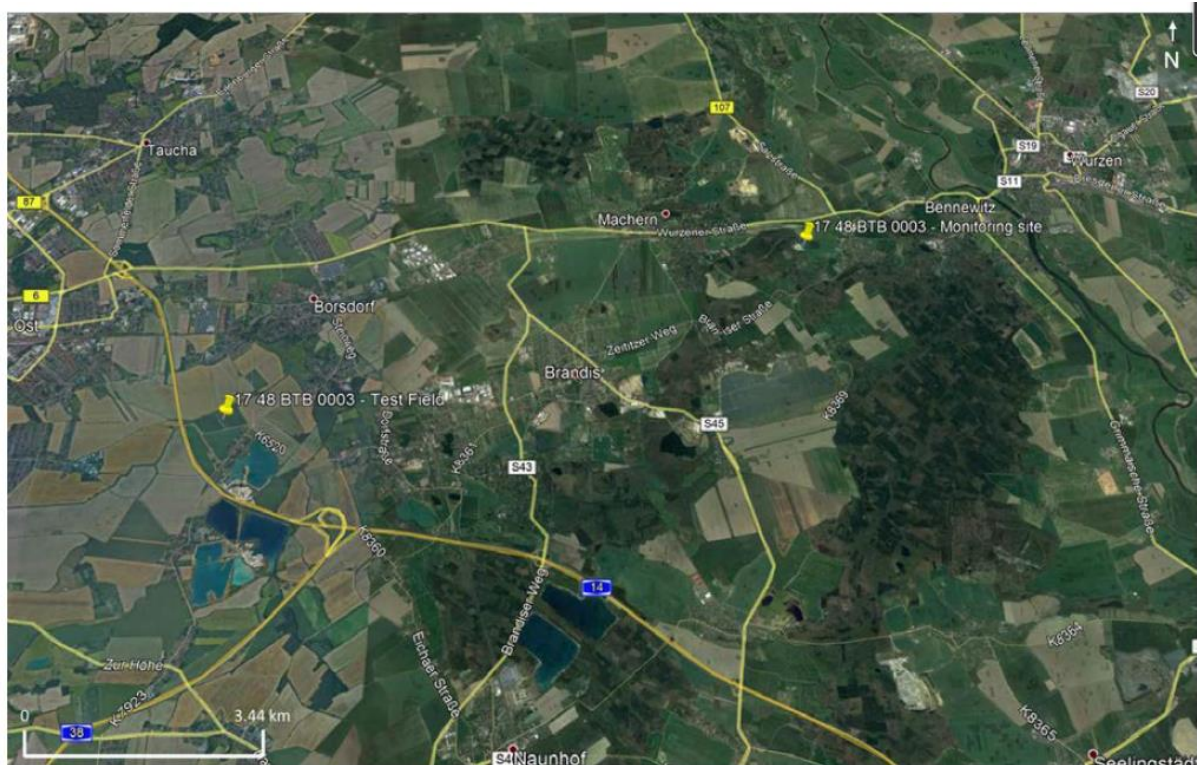


Figure 9.5.1-8. Map showing location of the test and monitoring sites (image date not stated).



Figure 9.5.1-9. Test site location (near centre of image) (image date not stated).

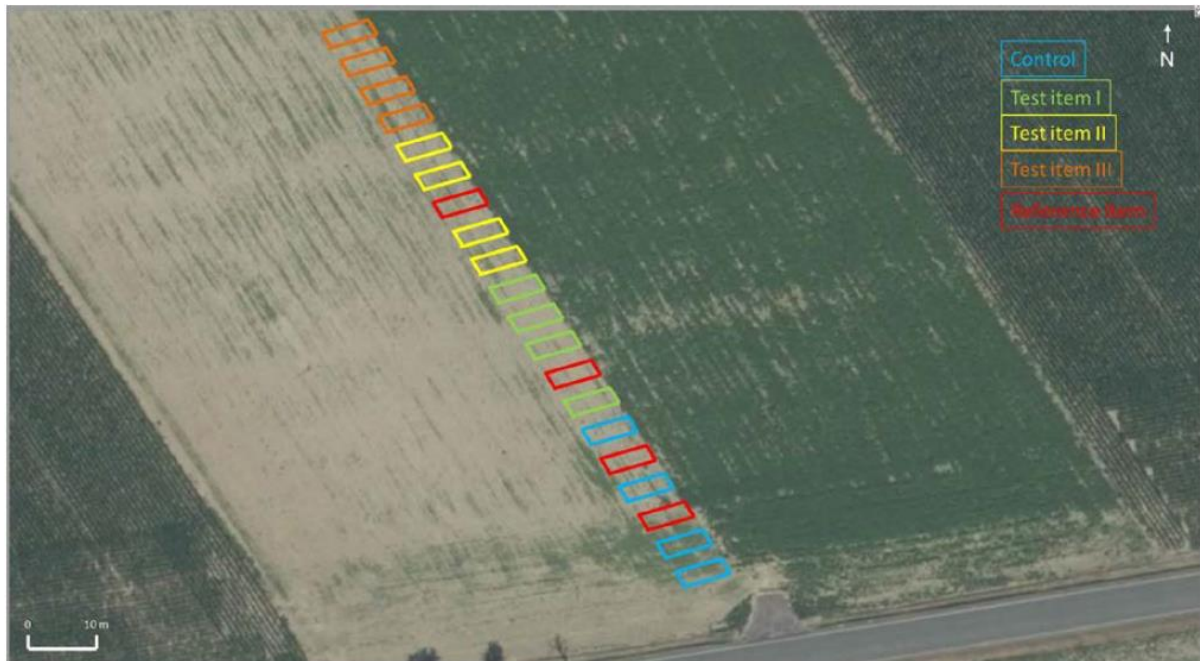


Figure 9.5.1-10. Detailed map of tunnel set up at test site (image date not stated).

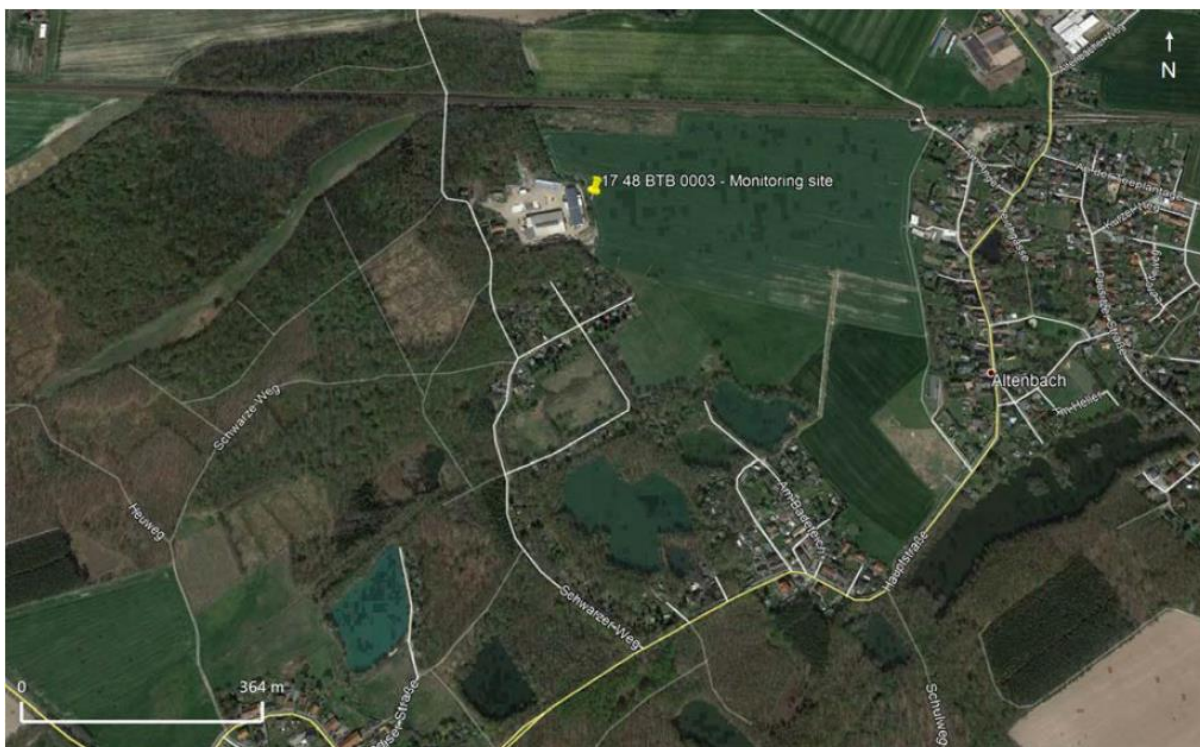


Figure 9.5.1-11. Close up map of the monitoring site (image date not stated).

Test item preparation

The required amounts of the test item and reference item were measured out in the laboratory on day of application. Until the application all items were stored and transported under chilled conditions in a polystyrene box. Immediately before application, the respective required spray solution was prepared by mixing the test or reference item with the necessary volume of tap water.

Spray details for test item application

The test item was applied as a single spray application once in full-flowering during bee flight (bee flight detail discussed later). During application the colonies as well as the water suppliers were protected from direct spray residues by covering with plastic sheets.

All applications were in 400 L tap water/ha. For the application procedure itself, two identical sprayers (designated BC 902 and BC 939) with the following specification were used: plot-sprayer PSG T3 (Schachtner Gerätetechnik, Ludwigsburg) with ten TEEJET 110015 VS nozzles spaced 0.25 m apart, 2.75 m spraying width, 0.25 MPa spray pressure. The plot sprayer was calibrated three times with tap water before application after adjusting the application speed and spray pressure.

The order of application for sprayer BC 902 was first the water control, followed by the test item II treatment and for sprayer BC 939 was first the test item I treatment followed by the test item III treatment and the reference item treatment. The sprayers were cleaned using Mucosal®, alcohol and tap water to remove residues before changing the treatment application.

The actual applied spray solution was determined after each application to confirm a valid application rate: the overall spray deviation varied from +1.1 to +9.6 % of the target spray volume, which is within the ± 10 % stated as acceptable in the OECD 75 guideline.

Observations and Assessments

Details of observations and associated methods are provided in the results section. Each observation or assessment was performed in the same manner and approximately the same time. The assessments of mortality, foraging activity, behaviour of bees were conducted separately each one after the other and they were performed in the following manner: the mortality was observed first followed by the foraging activity and the behaviour of bees for each replicate of all treatment groups. Other assessments include colony strength, brood/colony development and food status. Table 9.5.1-23 below details the observations and assessments taken in the study.

Table 9.5.1-23. List of observations and assessments taken during the semi-field bee study

Observation/Assessment	Method Summary	Timepoints taken
Meteorological data	Non-GLP, recorded at a weather station (Uni Klima 7) located 5.0 km to the test site at SKW Piesteritz, LAF Cunnersdorf 04451, Germany: <ul style="list-style-type: none"> air temperature air humidity precipitation, post-exposure phase GLP, recorded by a data logger at the test field and monitoring site for the whole study: <ul style="list-style-type: none"> air temperature humidity GLP, measured directly at test site during daily assessments and at application: <ul style="list-style-type: none"> precipitation, during pre- and exposure phase. cloud cover, to DAT 28 GLP, measurement taken at application ¹ : <ul style="list-style-type: none"> wind speed 	Continuous hourly recordings. Daily measurements. Wind velocity measured directly before application of each tunnel (within 15 s before application).
Adult and Pupal Mortality	Dead bee trap or dead be trap and gauze sheets during tunnel phase	Once daily for one brood cycle to BFD 29/DAT28
Foraging activity of bees	Assessed on 3 subplots/tunnel each 1 m ² as the number of bees/m ²	Once daily from DAT -3 to BFD 8/DAT 7 (pre-exposure and exposure tunnel phase) with additional assessments at application.

Observation/Assessment	Method Summary	Timepoints taken
Bee behaviour	Sublethal effects recorded manually	Once daily for one brood cycle to BFD 29/DAT 28
Colony strength (number of bees)	As estimated by the area covered by bees according to [REDACTED] <i>et al.</i> (1987), where one comb side of 825.1 cm ² is covered by a maximum of 900 bees.	Whole test period (three brood cycles to BFD 63/DAT 62 every 4-6 days.
General brood and food development by comb area	Estimating the comb area covered by each brood stage and food type according to [REDACTED] <i>et al.</i> (1987), where this estimation is based on dividing the comb into eighths.	Whole test period (three brood cycles to BFD 63/DAT 62 every 4-6 days.
Detailed brood development for calculation of:	Digital imaging of 300-500 cells of initially labelled eggs, young larvae or old larvae and assigned number score to each stage.	for one brood cycle on: <ul style="list-style-type: none"> • BFD 4 (DAT 3) • BFD 10 (DAT 9) • BFD 15 (DAT 14) • BFD 22 (DAT 21) • except initially labelled old larvae where the last measurement day was BFD 15 (DAT 14).
Brood Termination Rate (BTR)	a measure of the failure of individual eggs or larvae to develop: the percentage of brood cells which had not reached the expected life stage or been terminated	
Brood Index (BI)	an indicator of brood development to allow comparison between treatments	
Brood Compensation Index (BCI)	an indicator for brood development including recovery; includes cells which were refilled with eggs after termination	
Residue analysis of Pydiflumetofen (A19649B)	in flowers, leaves (foliage), pollen from pollen traps at hive entrance and nectar extracted from honey sacs obtained from forager bees at the hive entrance	<ul style="list-style-type: none"> • DAT -1 (before application) • DAT 0 (within 4 hours after application) • DAT 1 • DAT 4 • DAT 7
	from in-hive pollen and nectar from combs at the monitoring site	<ul style="list-style-type: none"> • DAT 28 • DAT 40 • DAT 47 • DAT 62

¹ No further details of wind velocity measurement methodology provided.

Any statistical analysis was performed using software Easy Assay 4.0 (RATTE, H.T. 1998) and ToxRat Professional 3.2.1 (RATTE, M. 2015). Statistical significance when comparing treatment against control was tested via Student t-test, Welch t-test at post-application period; one-sided (greater): mortality, brood termination rate; one-sided (less): foraging activity, brood index and brood compensation index. The %-values of the brood termination rate were arcsine- transformed to ensure the homogeneity of the data before conducting the t-test procedure.

Results and Discussion

Application conditions

Environmental conditions during application

The time of application was between 10:15 to 12:25. The plant height was 70-80 cm, growth stage BBCH 65, 100 % ground cover with good crop condition, few weeds.

At application the following climatic data (GLP) was recorded (see Table 9.5.1-24 for detailed data):

- Air temperature [°C]: 22.9-24.5 °C (warm temperature)
- Relative air humidity [%]: 51.0-61.0 %
- Wind velocity measured directly before (15 s) application of each tunnel [m/s]: 0.2-0.9 m/s; this meets the OECD 75 criterion of < 2 m/s wind velocity.
- Cloud cover [%]: 40 %
- Precipitation [mm]: 0 mm

These conditions can be regarded as favourable for foraging of bees.

Table 9.5.1-24. Detailed weather data from time of application of test substance formulation A19469B to the crop.

Date of application		31.05.2017, time 10:15 to 12:25														
Treatment		Control					75 g a.s./ha					125 g a.s./ha				
Replicate		1	2	3	4	5 ¹⁾	1	2	3	4	5 ¹⁾	1	2	3	4	5 ¹⁾
Air temp. [° C]	Min	23.7	23.0	23.8	22.9	23.0	23.2	23.4	23.9	24.2	23.6	23.8	24.2	23.8	24.1	23.4
	Max	24.0	23.7	24.1	23.5	23.1	23.6	23.8	24.2	24.5	24.0	24.1	24.5	24.1	24.4	23.7
Relative humidity [%]	Min	53.5	58.7	58.7	53.3	56.2	51.9	51.0	52.9	55.6	56.6	52.5	57.1	58.1	53.7	57.3
	Max	55.7	61.0	59.3	55.8	57.1	53.3	53.0	53.4	57.1	58.0	53.7	58.7	58.9	57.1	58.7
Wind velocity [m/s]		0.3	0.8	0.5	0.5	0.4	0.9	0.7	0.5	0.5	0.8	0.6	0.7	0.5	0.5	0.5
Cloud cover [%]		40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Precipitation [mm] DAT 0		0														
Treatment		200 g a.s./ha					Reference item									
Replicate		1	2	3	4	5 ¹⁾	1	2	3	4						
Air temp. [° C]	Min	23.6	23.9	23.9	24.1	24.1	23.6	23.8	23.4	23.7						
	Max	23.9	24.2	24.3	24.4	24.5	24.1	24.2	23.8	24.0						
Relative humidity [%]	Min	56.6	58.4	57.2	58.0	58.4	55.4	56.7	57.1	59.1						
	Max	58.4	59.1	58.0	58.6	59.0	57.3	59.0	59.2	60.7						
Wind velocity [m/s]		0.4	0.3	0.3	0.4	0.4	0.7	0.3	0.2	0.2						
Cloud cover [%]		40	40	40	40	40	40	40	40	40						
Precipitation [mm] DAT 0		0														

¹⁾ Additional replicate used for residue analysis only – no biological measurements taken.

n.d. – no data

Bee flight/foraging activity during application

The application took place at full flowering during daily bee flight. Bee flight was confirmed to be ‘full’ (>10 bees/m²) in line with the OECD 75 guideline: on DAT 0 immediately before application (‘DAT 0bef’, foraging activity was (as shown in Table 9.5.1-27 in the results section):

- 10-14 (mean 12.3) bees/m² for control
- 11-14 (mean 12.4) bees/m² for 75 g a.s./ha (test item I)
- 12-14 (mean 13.0) bees/m² for 125 g a.s./ha (test item II)
- 12-15 (mean 13.2) bees/m² for 200 g a.s./ha (test item III)
- 12-14 (mean 12.8) bees/m² for the reference item.

This indicates that bees were well exposed during the test substance application. The following OECD 75 criteria were fulfilled during application:

- *Phacelia tanacetifolia* B. growth stage BBCH 65 (full flowering)
- Mean foraging activity of ≥ 10 bees/m² on the *Phacelia tanacetifolia* B (as shown in Table 9.5.1-27).
- Wind speed directly before application of each tunnel ≤ 2 m/s (as shown in Table 9.5.1-24)
- Spray tolerance of ± 10 % (as described in methods)

Meteorological data from test and monitoring sites

Weather data for the whole study is summarised in Table 9.5.1-25. The mean air temperature during exposure phase was within a range of 15.1 – 20.8 °C, which can be regarded as favourable for good foraging of honeybees.

Overall weather during the entire test (DAT -3 to DAT 62) from the field datalogger (GLP): mean air temperature 19.4 °C (full range 8.8 – 36 °C); mean relative air humidity 74.4 % (daily mean range 53.4 – 96 %, full range 20.8 – 99.9 %) (Table 9.5.1-25). Total precipitation over entire test period (non-GLP) was 195.0 mm.

Table 9.5.1-25. Climatic data from the semi-field bee study for A19649B.

Weather		Pre-exposure period (DAT -3 to DAT -1)	At application (DAT 0, 10:15 to 12:25)	Exposure period (DAT 0 to DAT 7)	Post-exposure period DAT 8 – DAT 28	Post-exposure period DAT 29 – DAT 62
Precipitation [mm]	Data	DAT -3: 0 mm DAT -2: 11 mm DAT -1: 0 mm	0 mm	DAT 0-1: 0 mm DAT 2: 13 mm DAT 3: 5 mm DAT 4: 1 mm DAT 5: 2 mm DAT 6: 2 mm	0 – 8.6 mm	0 – 55.2 mm
	Source	GLP, direct measurement at test site	GLP, direct measurement at test site	GLP, direct measurement at test site	Non-GLP, weather station ¹	Non-GLP, weather station ¹
Air temperature [°C]	Mean	21.6 – 25.1	-	15.1 – 20.8	17.3 – 22.4	14.7 – 23.5
	Min	9.7	22.9	8.8	10.0	8.8
	Max	36.0	24.5	34.6	35.3	33.6
	Source	GLP, field datalogger	GLP, field datalogger	GLP, field datalogger	GLP, field datalogger	GLP, field datalogger
Relative humidity [%]	Mean	53.4 – 73.3	-	68.8 – 88.9	61.2 – 78.3	65.8 – 96.0
	Min	20.8	51.0	24.1	31.1	37.8
	Max	97.2	61.0	99.9	97.8	99.7
	Source	GLP, field datalogger	GLP, field datalogger	GLP, field datalogger	GLP, field datalogger	GLP, field datalogger
Wind velocity [m/s]	Data	n.d.	0.2 – 0.9 m/s	n.d.	n.d.	n.d.
	Source	n.d.	GLP, measured 15 s before application	n.d.	n.d.	n.d.
Cloud cover [%]	Data	DAT -3: 0 % DAT -2: 0 % DAT -1: 80 %	40 %	DAT 0: 10-70 % DAT 1-2: 0 % DAT 3: 70 % DAT 4: 20 % DAT 5: 90 % DAT 6: 50 % DAT 7: 40 %	0 – 80 %	n.d.
	Source	GLP, direct measurement at test site	GLP, direct measurement at test site	GLP, direct measurement at test site	GLP, direct measurement at test site	n.d.

¹ weather station (model Uni Klima 7) located 5.0 km to the test site at SKW Piesteritz, LAF Cunnersdorf 04451, Germany

Adult and pupal mortality to BFD 29/DAT 28

Assessments of mortality were carried out daily at least once by collecting and removing dead bees found on the gauze sheets (pathed areas) and in dead bee trap attached in front of the colony. Thus, the number of adult and juvenile (pupae or larvae), worker bees as well as drones were assessed and recorded separately. The mortality during pre-exposure (DAT -3 to DAT 0ba) and exposure phase (DAT 0 to DAT 7) comprised by dead bees found in the dead bee trap and on the gauze sheets, whereas, the mortality during post-exposure phase (DAT 8 to DAT 28) was determined by the dead bee trap, only. All dead bees collected from the gauze sheets within one tunnel were pooled. On the day of application (DAT 0), one additional assessment was conducted in order to assess any impact after application. Additionally, assessments were carried out three times (morning, noon and in the evening after bee flight) on the first day after application (DAT 1).

Pupal mortality to BFD 29/DAT 28

During the pre-exposure phase no dead pupae were observed in any replicate of the treatment groups. During the exposure and post-exposure phase, no dead pupae were found in the control or in any replicate of the test item treatments. Therefore, with respect to the application of A19649B, no adverse effect occurred on pupal mortality during the entire course of the study.

For the reference item effect on pupal mortality:

- during exposure phase no dead pupae were observed in any replicate of the control and reference item treatment, respectively.
- In the post-exposure phase, dead pupae were observed in all replicates treated with Insegar 25 WG.
- The sum pupal mortality per colony between DAT 8 to DAT 28 ranged from 127 to 271 dead pupae (8.6 dead pupae/colony/day).
- The effect of increased pupal mortality after application of Insegar 25 WG confirms the sensitivity of the test system.

Adult mortality to BFD 29/DAT 28

Summary data for adult bee mortality is presented below in Table 9.5.1-26 and Figure 9.5.1-12 and 9.5.1-13. A description of the results:

- *Pre-exposure phase (DAT -3 to DAT 0bef):* comparable overall mean adult bee mortality of 22.1, 14.8, 12.8, 12.4 and 21.3 dead bees/colony in the control, test item I, test item II, test item III and reference item treatment, respectively.
 - No significant differences were observed between the treatment groups, based on overall comparison before application (Tukey-test, two-sided, $p > 0.05$).
 - This indicates that the new environmental conditions (i.e. enclosed in a tunnel) had no adverse effects on bee mortality and the bee colonies can therefore be regarded as well adapted.
- *Exposure and post exposure phase – treatments I and II:* In general, after application the mortality of test item I and test item II groups was lower compared to the control in the pre-exposure an exposure phase, and similar in the post-exposure phase (Table 9.5.1-26), but there was no statistically significant differences (Student t-test, one-sided (greater), $p > 0.05$).
- *Exposure and post exposure phase – treatment III:* No significant differences in mortality were observed post-exposure between test item III when compared to the control, with the exception of DAT 27 for the where there was 2.0 and 4.0 dead bees/colony in the control and test item III, respectively (Student t-test, one-sided (greater), $p > 0.05$). This difference has to be regarded as not test item related as the mortality in the test item III group was not elevated above the natural variation and moreover, not increased compared to the exposure and pre-exposure phase.
- *Exposure and post-exposure phase – reference item:* slightly increased mortality on DAT0, but this was not statistically significant different (Welch t-test, one sided (greater), $p > 0.05$). No significant difference DAT 0 to DAT 28 except on DAT 7 where there was a significant difference of 19.3 and 22.8 dead bees/colony in the control and reference item, respectively. (Student t-test, one-sided (greater), $p > 0.05$).

Table 9.5.1-26 Mortality of adult bees exposure to formulation A19649B.

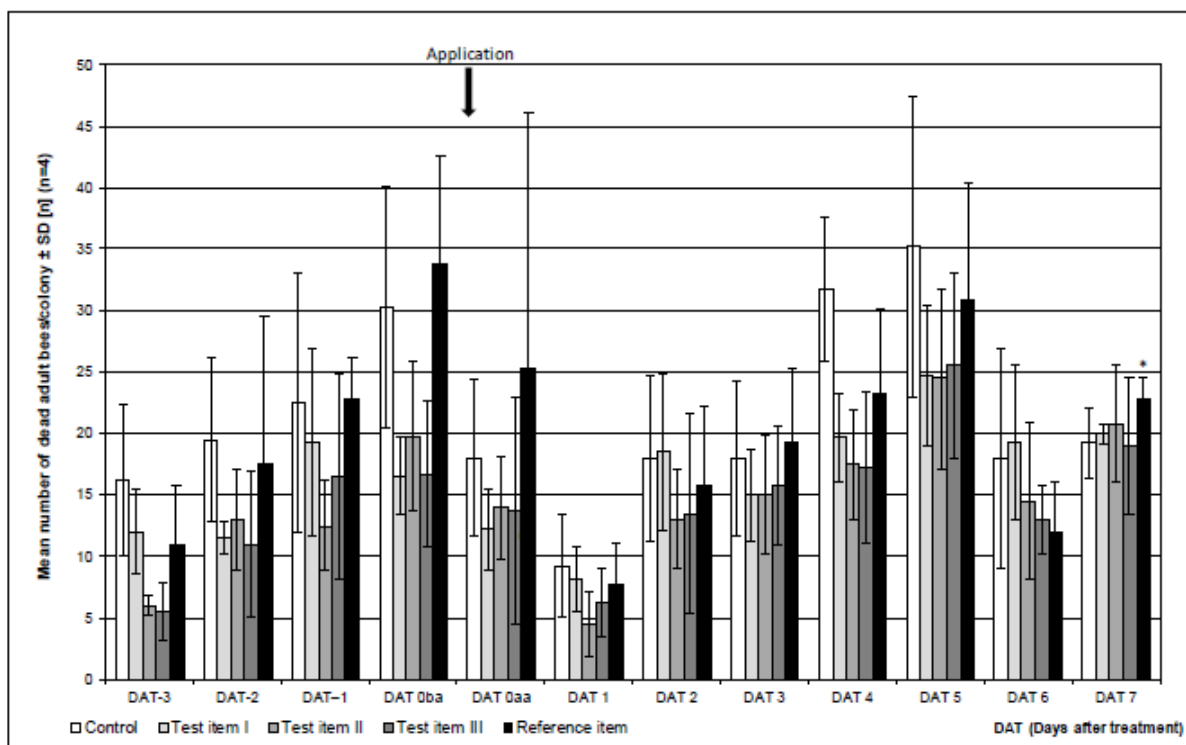
Assessment day	Mortality [no. of dead adult bees]									
	Control		Test item I (75 g a.s./ha)		Test item II (125 g a.s./ha)		Test item III (200 g a.s./ha)		Reference item	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
DAT -3	16.3 ^b	6.2	12.0 ^{a,b}	3.5	6.0 ^a	0.8	5.5 ^a	2.4	11.0 ^{a,b}	4.7
DAT -2	19.5 ^{a,b}	6.6	11.5 ^a	1.3	13.0 ^{a,b}	4.1	11.0 ^a	5.9	17.5 ^{a,b}	12.1
DAT -1	22.5 ^{a,b}	10.5	19.3 ^{a,b}	7.5	12.5 ^a	3.7	16.5 ^a	8.4	22.8 ^{a,b}	3.4
DAT 0bef	30.3 ^{a,b}	9.8	16.5 ^a	3.1	19.8 ^{a,b}	6.1	16.8 ^a	5.9	33.8 ^b	8.9
Mean DAT -3 to 0bef	22.1^{a,b}	7.6	14.8^{a,b}	3.2	12.8^a	2.8	12.4^a	5.3	21.3^{a,b}	6.5
DAT 0aa +2 h	6.0	2.9	5.5	2.1	6.5	2.4	6.3	5.3	12.5	9.9
DAT 0aa +4 h	2.8	1.7	1.5	1.0	2.0	1.8	3.8	1.9	6.3	5.7

Assessment day	Mortality [no. of dead adult bees]									
	Control		Test item I (75 g a.s./ha)		Test item II (125 g a.s./ha)		Test item III (200 g a.s./ha)		Reference item	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
DAT 0aa +6 h	2.8	2.9	2.8	1.0	3.8	1.0	2.8	2.1	7.5	6.6
DAT 0 after bee flight	6.5	4.8	4.0	0.8	3.8	2.2	4.8	3.1	5.3	4.4
Mean DAT 0	18.0	6.4	13.8	3.0	16.0	4.1	17.5	10.1	31.5	26.1
DAT 1	9.3	4.2	8.3	2.6	4.5	2.6	6.3	2.8	7.8	3.3
DAT 2	18.0	6.7	18.5	6.4	13.0	4.0	13.5	8.1	15.8	6.5
DAT 3	18.0	6.3	15.0	3.7	15.0	4.9	15.8	4.8	19.3	6.0
DAT 4	31.8	5.9	19.8	3.6	17.5	4.5	17.3	6.1	23.3	6.9
DAT 5	35.3	12.3	24.8	5.7	24.5	7.3	25.5	7.5	30.8	9.6
DAT 6	18.0	9.0	19.3	6.2	14.5	6.4	13.0	2.8	12.0	4.1
DAT 7	19.3	2.9	20.0	0.8	20.8	4.8	19.0	5.5	22.8 *	1.9
Mean DAT 0 to 7	20.9	1.6	17.2	1.2	15.5	1.3	15.5	2.5	19.6	5.7
DAT 8	6.5	3.8	2.5	3.1	6.3	2.2	4.3	2.6	8.0	4.4
DAT 9	7.3	3.0	3.8	3.9	5.0	3.5	3.5	2.4	6.8	6.1
DAT 10	0.8	1.0	1.5	1.0	1.8	1.7	1.0	1.4	3.0	2.8
DAT 11	1.5	1.7	1.0	2.0	2.5	2.5	1.3	1.0	1.5	1.0
DAT 12	1.3	1.5	1.5	0.6	2.3	1.9	2.3	0.5	2.0	1.4
DAT 13	4.3	2.4	5.5	1.9	5.5	1.7	3.8	1.0	9.5	6.7
DAT 14	6.0	5.0	6.0	3.2	5.8	5.0	4.0	1.8	8.5	4.7
DAT 15	2.0	1.6	1.8	2.2	1.8	0.5	1.8	1.0	5.0	4.1
DAT 16	4.5	3.1	7.0	4.2	3.3	3.3	8.0	4.7	6.8	4.6
DAT 17	1.8	1.0	2.8	1.7	1.0	0.8	0.8	0.5	1.8	1.0
DAT 18	1.8	1.0	3.0	1.4	1.5	1.7	1.3	1.0	1.5	1.0
DAT 19	1.5	1.7	2.0	2.2	1.3	0.5	1.3	1.3	2.3	1.7
DAT 20	1.8	1.3	2.0	2.7	1.5	1.3	2.3	1.7	2.5	1.3
DAT 21	4.0	3.2	4.3	1.7	3.8	2.2	4.0	2.9	3.0	2.7
DAT 22	2.3	2.5	4.5	2.4	1.0	1.4	1.0	1.2	4.3	2.2
DAT 23	3.3	2.6	3.5	2.9	1.5	1.7	0.8	0.5	3.3	2.2
DAT 24	1.5	1.3	1.8	1.0	2.0	0.8	1.8	1.7	2.0	0.8
DAT 25	3.3	2.2	4.0	1.8	3.0	2.4	2.3	1.3	1.3	1.0
DAT 26	4.0	1.2	3.8	1.3	3.5	2.1	4.3	2.6	3.8	3.0
DAT 27	2.0	0.8	2.0	0.8	2.5	1.0	4.0 *	1.2	2.8	1.5
DAT 28	4.3	2.6	3.3	1.0	2.3	1.0	3.5	1.3	2.8	1.5
Mean DAT 8 to 28	3.1	0.8	3.2	0.4	2.8	1.0	2.7	0.7	3.9	1.3
Mean DAT 0 to 28	8.0	0.5	7.1	0.4	6.3	1.0	6.2	0.8	8.2	2.5

^{a,b}: same letters indicate that groups are not statistically significant different (Tukey-test, $\alpha=0.05$) at pre-application period

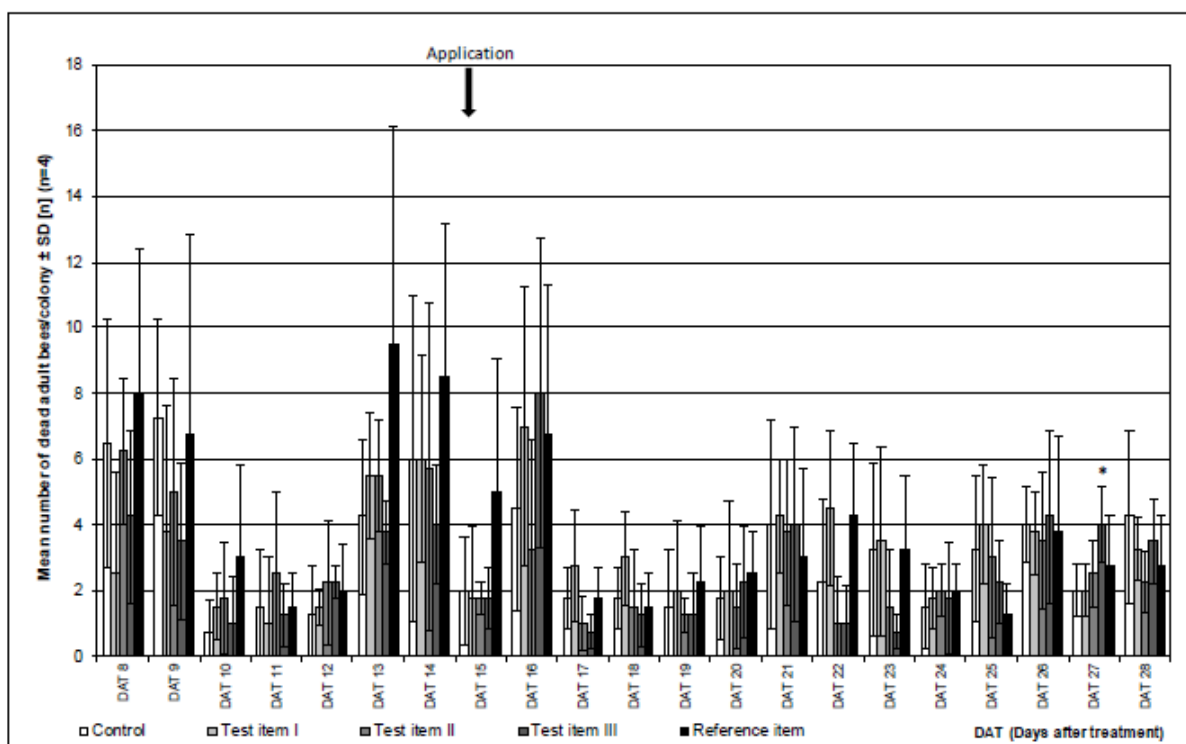
* statistically significant different when comparing treatment against control via Student t-test at post-application period; one- sided (greater).

0bef: The day of treatment application before the treatment was applied.



* significantly different comparing treatment against control via Student t-test at post-application period; one-sided (greater).

Figure 9.5.1-12. Mortality of adult bees (pre- and exposure phase). Test Item I, II and III are pydiflumetofen (A19649B) applied at equivalent of 75, 125 and 200 g a.s./ha, respectively. Reference item Insegar 25 WG (fenoxycarb) applied at equivalent of 300 g a.s./ha.



* significantly different comparing treatment against control via Student t-test at post-application period; one-sided (greater).

Figure 9.5.1-13 Mortality of adult bees (post-exposure phase) Test Item I, II and III are pydiflumetofen (A19649B) applied at equivalent of 75, 125 and 200 g a.s./ha, respectively. Reference item Insegar 25 WG (fenoxycarb) applied at equivalent of 300 g a.s./ha.

Foraging activity to BFD 8/DAT 7

During pre-exposure and exposure phase (DAT -3 to DAT 7), the number of active foraging bees was recorded at least once per day during daily bee flight [bees/m²] on three squares/tunnel (each 1 m²). This was generally between 11 am and 3 pm. On the day of application and on the first day after application (DAT 0), additional assessments were conducted in order to confirm any immediate impact of the application. On the first day after application assessments were carried out two times within the first hour after application and once at 2, 4 and approximately 6 hours after application. On DAT 1 three foraging assessments were carried out during daily main bee flight activity (09:03-09:36, 11:21-12:14 and 16:42-17:01).

The detailed results are shown in the Table 9.5.1-27, whereas a summary table across experimental phases can be seen in Table 9.5.1-28. Any calculations were performed with non-rounded values. Foraging activity was similar between all treatments, reference item and control at all assessment times. No significant differences in foraging activity were observed post-exposure between test item or reference item when compared to the control (Student t-test, one-sided smaller, $p > 0.05$). A summary of results:

- During pre-exposure phase, the overall mean foraging activity was 13.9, 14.3, 14.4, 14.4 and 14.1 bees/m²/day in the control, test item I, test item II, test item III and reference item, respectively. Statistical analysis revealed no significant differences among treatment groups (Tukey test, two-sided, $p > 0.05$) indicating that bees had adapted well to the new environmental conditions in the tunnels.
- On DAT 0bef, immediately before application, foraging activity was between 10-14 (mean 12.3), 11-14 (mean 12.4), 12-14 (mean 13.0), 12-15 (mean 13.2) and 12-14 (mean 12.8) bees/m² in the control, test item I, test item II, test item III and reference item respectively, indicating that bees were well exposed during the application. Thus, the requested criterion and exposure during application was achieved.
- On the day of application (DAT 0aft) following the application, no decrease in foraging activity of bees occurred in the control, the test item treatment and the reference item treatment, rather an increase was noted compared to pre-exposure (not statistically significant, Student t-test, one-sided smaller, $p > 0.05$).
- During the entire exposure phase, no reduction of foraging activity in the test item treatments and reference item group was observed at any day when compared to the control. Consequently, the overall daily mean foraging activity was on a comparable level to the pre-exposure phase, amounted to 14.7, 15.0, 15.0, 14.7 and 14.3 bees/m²/day in the control, test item I, test item II, test item III and reference item treatment, respectively. There were no statistically significant differences (Student t-test, one-sided smaller, $p > 0.05$).

Table 9.5.1-27: Summary on honeybee foraging activity during exposure to pydiflumetofen (A19649B)

Assessment Day	Foraging activity [no. of bees/m ²]									
	Control		Test item I A19649B 75 g a.s./ha		Test item II A19649B 125 g a.s./ha		Test item III, A19649B 200 g a.s/ha		Reference item Insegar 25 WG	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
DAT -3	13.8	1.3	13.8	1.0	13.0	1.5	12.8	1.2	14.4	0.8
DAT -2	14.8	1.1	15.9	0.8	16.3	0.8	16.3	0.7	14.9	2.0
DAT -1	14.6	1.0	15.0	0.9	15.2	1.1	15.3	1.0	14.5	1.2
DAT 0ba	12.3	1.2	12.4	1.0	13.0	0.7	13.2	0.8	12.8	0.8
Mean DAT -3 to 0bef	13.9 ^a	0.6	14.3 _{a,b}	0.2	14.4 _{a,b}	0.4	14.4 ^{a,b}	0.4	14.1 ^a	0.6
DAT 0aft +1/2 h	14.8	0.6	17.5	0.8	17.1	0.8	17.5	0.7	16.4	0.9
DAT 0aft +1 h	17.1	0.7	17.1	0.8	17.6	0.8	15.9	1.0	16.8	0.6
DAT 0aft +2 h	4.2	1.4	5.3	1.9	5.0	2.0	9.5	2.3	4.8	2.1
DAT 0aft +4 h	16.3	1.4	16.5	1.9	16.5	2.0	15.4	1.4	15.2	1.7

Assessment Day	Foraging activity [no. of bees/m ²]									
	Control		Test item I A19649B 75 g a.s./ha		Test item II A19649B 125 g a.s./ha		Test item III, A19649B 200 g a.s./ha		Reference item Insegar 25 WG	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
DAT 0aft +6 h	17.2	1.4	18.0	1.6	17.5	1.7	17.0	1.5	16.0	1.0
Mean DAT 0	13.9	0.4	14.9	1.0	14.7	0.4	15.1	0.2	13.9	0.8
DAT 1 (morning)	10.4	2.3	8.7	2.1	8.9	2.1	9.1	1.9	9.3	1.6
DAT 1 (noon)	13.4	3.2	16.6	1.4	15.2	1.9	15.1	1.6	15.2	2.0
DAT 1 (afternoon)	17.3	2.1	16.8	2.3	16.8	2.3	17.8	2.7	15.7	2.1
Mean DAT 1	13.7	1.3	14.0	0.5	13.6	0.7	14.0	0.7	13.4	0.5
DAT 2	16.7	2.2	16.0	1.8	17.3	1.4	15.9	1.6	15.4	1.3
DAT 3	13.8	1.6	13.7	1.2	13.8	1.7	12.7	1.1	12.8	1.6
DAT 4	14.3	2.4	14.8	1.8	14.3	2.6	14.2	2.0	14.0	2.2
DAT 5	14.9	2.1	15.7	1.9	15.6	1.7	15.1	2.2	14.3	2.5
DAT 6	15.3	2.6	15.2	2.5	15.8	2.6	15.0	2.0	15.0	2.0
DAT 7	15.2	1.3	15.8	1.7	15.1	2.1	15.9	1.5	15.8	1.7
Mean DAT 0 to 7	14.7	1.1	15.0	0.5	15.0	0.5	14.7	0.5	14.3	0.4

^{a,b}: same letters indicate that groups are not statistically significant different (Tukey-test, $\alpha=0.05$) at pre-application period

0bef: The day of treatment application before the treatment was applied.

0aft: The day of treatment application after the treatment was applied.

Detailed brood analysis to BFD 22/DAT 21: Indices of BTR, BI and BCI

Detailed brood development for one brood cycle was assessed via photo documentation of eggs, young larvae and old larvae representing the main endpoint for the study. 300-500 eggs/young larvae/old larvae were initially labelled on BFD 0 (DAT -1). The analysis was subsequently performed on BFD 4 (DAT 3), BFD 10 (DAT 9), BFD 15 (DAT 14) and BFD 22 (DAT 21). Photo documentation was analysed using the “Honeybee Brood Logger” digital image analysis tool. Each cell was assigned a numerical code from 1-5 relating to the brood stage (egg to successful hatch) The following calculations were performed:

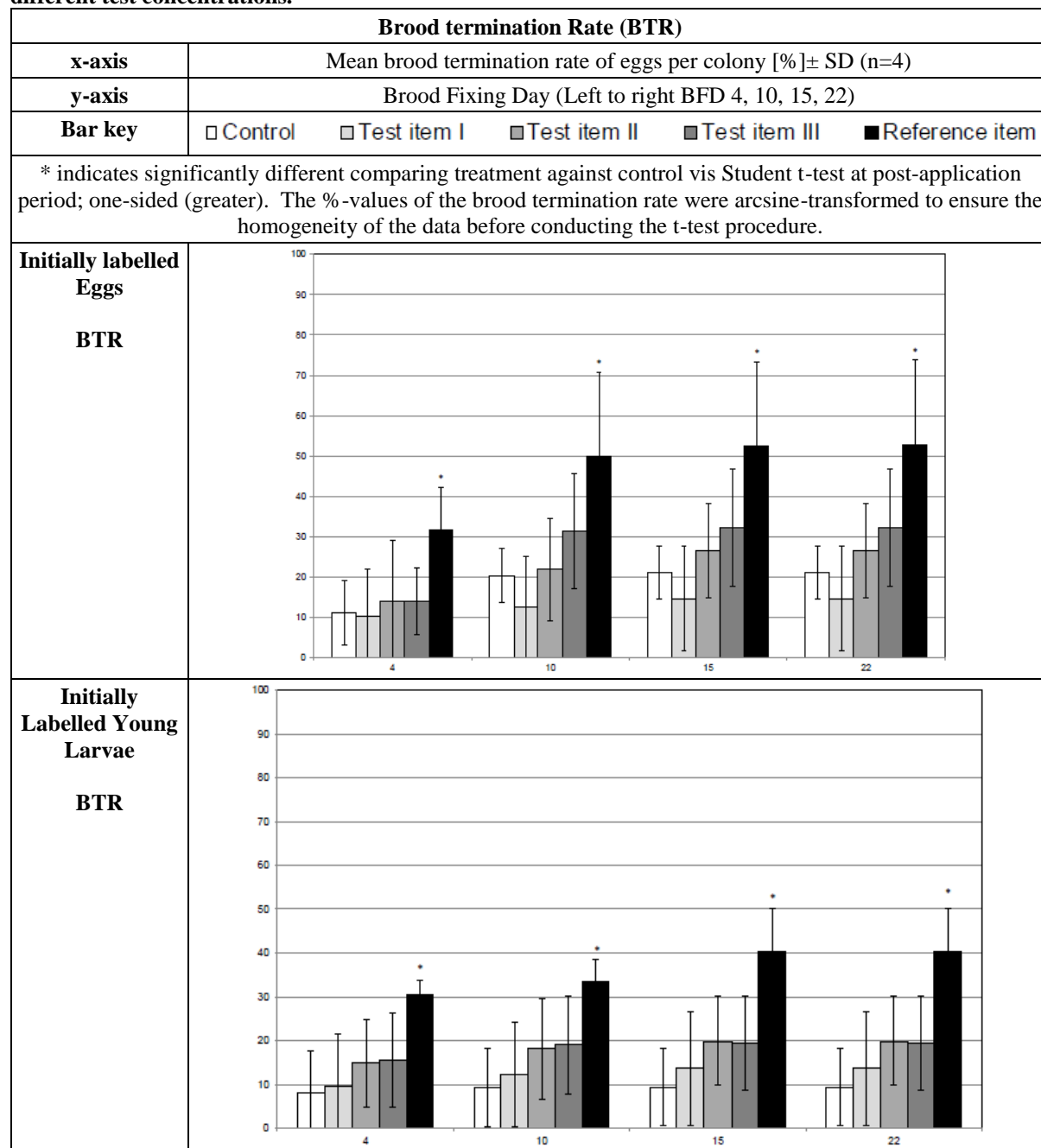
- *Brood termination rate (BTR) (%)*: the percentage of initially marked cells which had been terminated (cells which had not reached the expected developmental stage at each assessment day). According to the lifecycle of a worker honeybee which normally averages 21 (± 1) days it can be assumed that young bees from cells with eggs had hatched between BFD 15 and BFD 22. Concerning the time after a successful hatch the marked brood cells were scored with 0 (unsuccessful = terminated) or 1 (successful) for calculation of brood termination.
- *Average Brood-index (BI)*: this is an indicator of the bee brood development and facilitates a comparison between different treatments. This is an average of the numerical brood stage codes for each treatment on each assessment day, where a value of ‘0’ is assigned to a terminated brood on the assessment day and following assessment days.
- *Average brood compensation-index (BCI)*: The brood compensation-index is an indicator for brood development including recovery after termination of brood in the marked cells. This is an average of the numerical brood stage codes for each treatment on each assessment day, with numerical brood stage codes solely based on the identified growth stage on the assessment days rather than by comparison with expected growth stage. It includes cells which were refilled with eggs after termination.

A summary of results on the final measurement day of BFD 22 (DAT 21) can be seen in Table 9.5.1-28. Detailed results per measurement day can be seen in Figure 9.5.1-14a, b and c. Statistical significance was tested with Student t-test, one-sided (greater), or one-sided (less), $p < 0.05$. No significant differences were observed between treatment rates and control with the sporadic exception of BCI on BFD 15 for Test Item II. The reference item

displays significant reduction across all indices, which demonstrates the sensitivity of the test system. A summary of the results:

- *Brood termination Rate (BTR)*: The %-values of the brood termination rate were arcsine-transformed to ensure the homogeneity of the data before conducting the t-test procedure.
 - For initially labelled eggs and young larvae, the BTR increased with increasing test concentration, however none of these were significantly different from the control. Contrastingly, the reference item had significantly increased BTR for initially labelled eggs and young larvae at all measurement days (Figure 9.5.1-14a).
 - For initially labelled old larvae, there were no differences between treatments and control for test treatments, whereas the reference item was significantly lower.
- *Brood Index (BI)*:
 - The brood index of initially labelled eggs was typically reduced as the test item concentration increased, however there were no significant differences observed for any treatment level compared to the control (Figure 9.5.1-14b). Contrastingly, the reference item had significantly reduced BI on BFD 10, 15 and 22.
 - The brood index of initially labelled young larvae was generally reduced in test treatments compared to the control, but there were no statistically significant differences. Contrastingly, the reference item had significantly reduced BI on all four days assessed.
 - The brood index of initially labelled old larvae was generally similar between test treatments and the control, whereas the reference item had significantly reduced BI on BFD 10 and 15.
- *Brood Compensation Index (BCI)*
 - For initially labelled eggs, the brood compensation Index generally decreased with increasing test concentration, however none of these were statistically significantly different from control. Contrastingly, the reference item had significantly reduced BCI on BFD 10, 15 and 22 (Figure 9.5.1-14c).
 - For initially labelled young larvae, test item BCI was generally reduced compared to the control, but there were no significant differences apart from Test item II on BFD 15. As this is a sporadic result which is not dose responsive this exception can be attributed to natural variation between the colonies rather than to the action of the test item. Contrastingly, the reference item had significantly reduced BCI on all assessed days.
 - For initialled labelled old larvae, the BCI was similar across all treatment rates and the control and there were no significant differences. Contrastingly, the reference item had significantly reduced BCI on BFD 10 and 15.

Figure 9.5.1-14a. Average Brood termination Rate (BTR) of bee colonies exposed to pydiflumetofen at three different test concentrations.



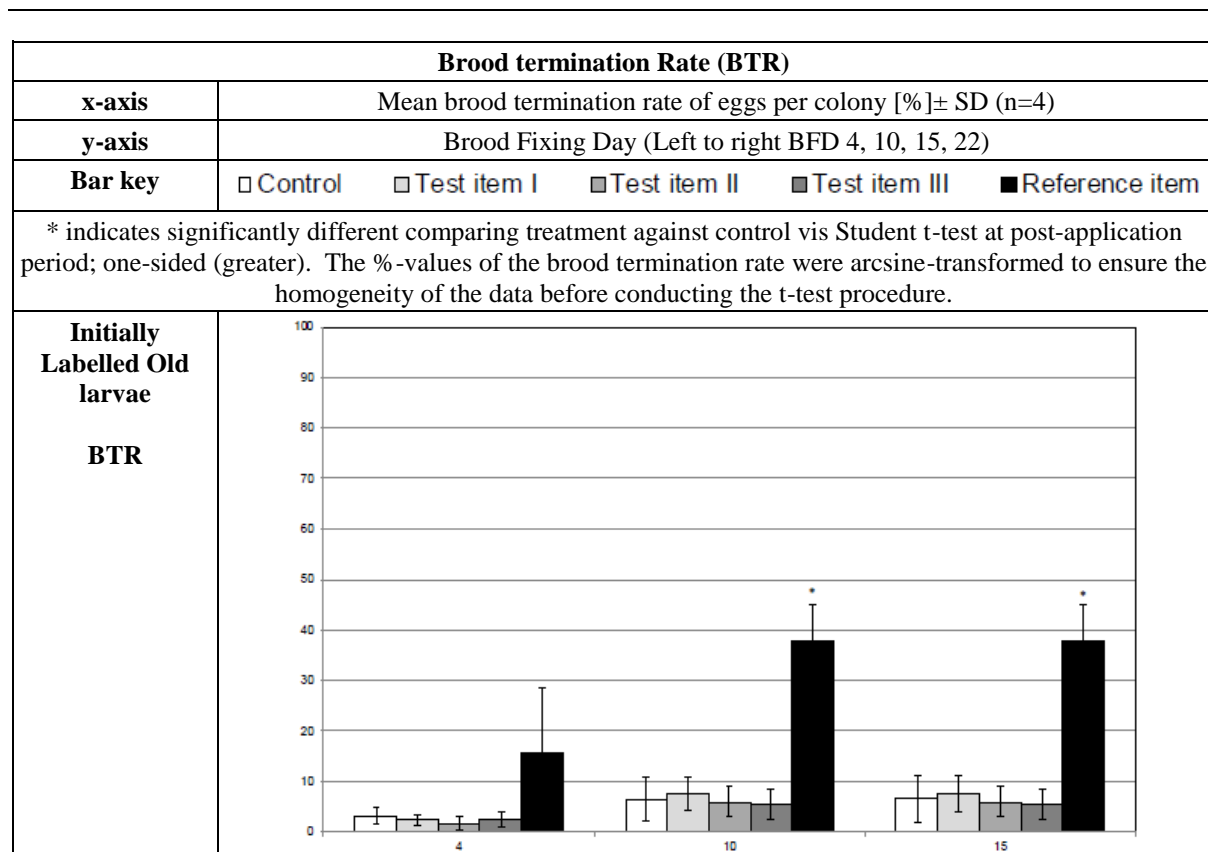
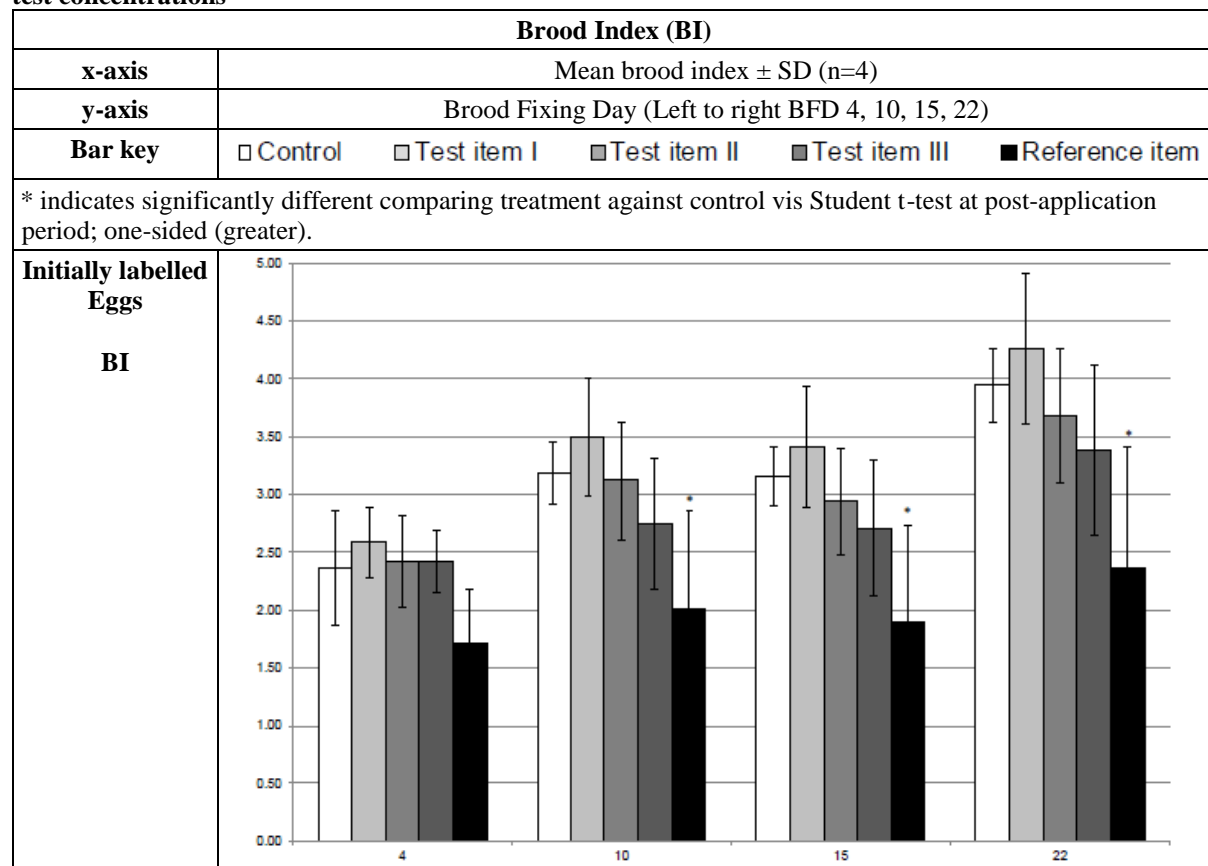


Figure 9.5.1-14b. Average Brood Index (BI) of bee colonies exposed to pydiflumetofen at three different test concentrations



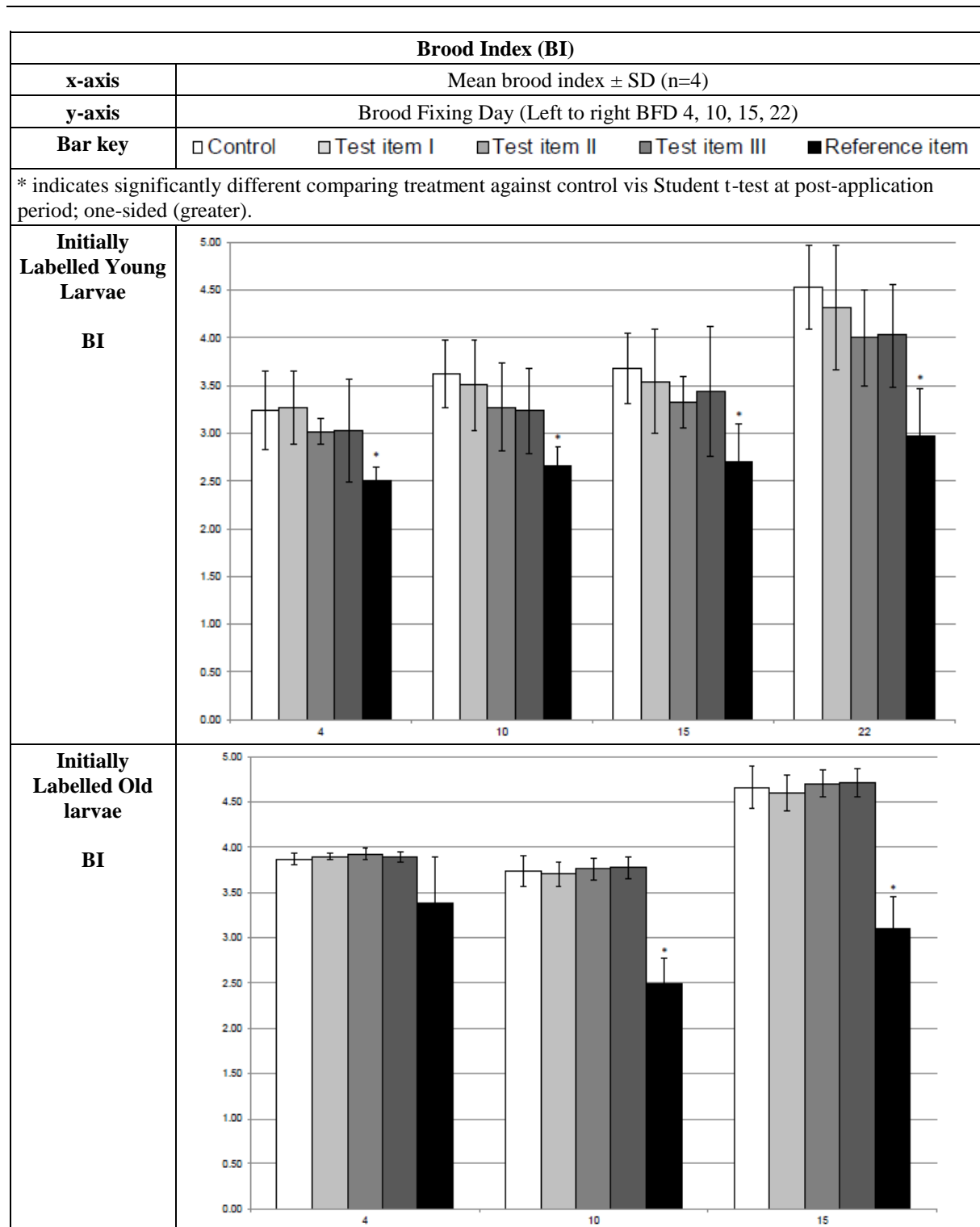
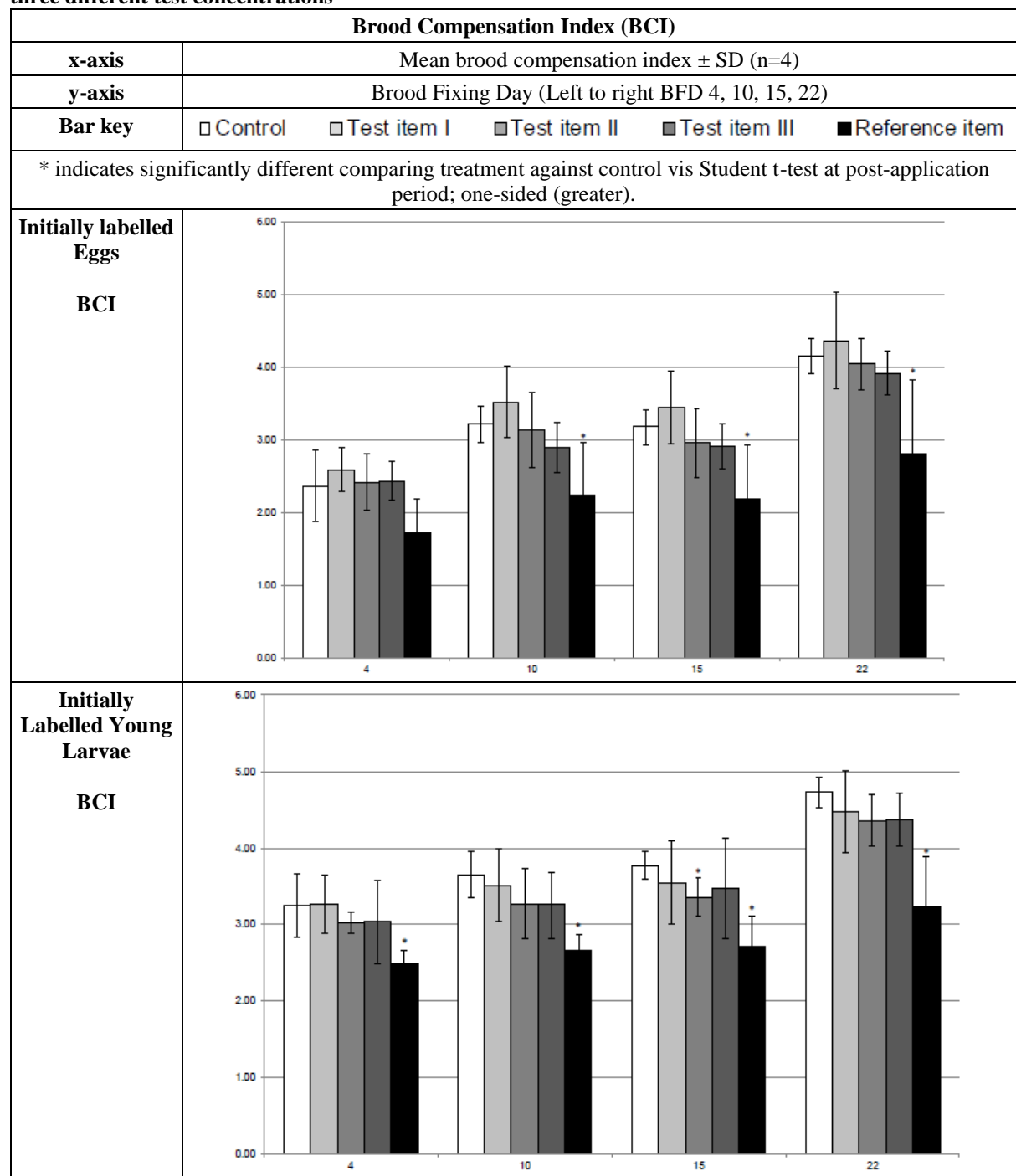


Figure 9.5.1-14c. Average Brood Compensation Index (BCI) of bee colonies exposed to pydiflumetofen at three different test concentrations

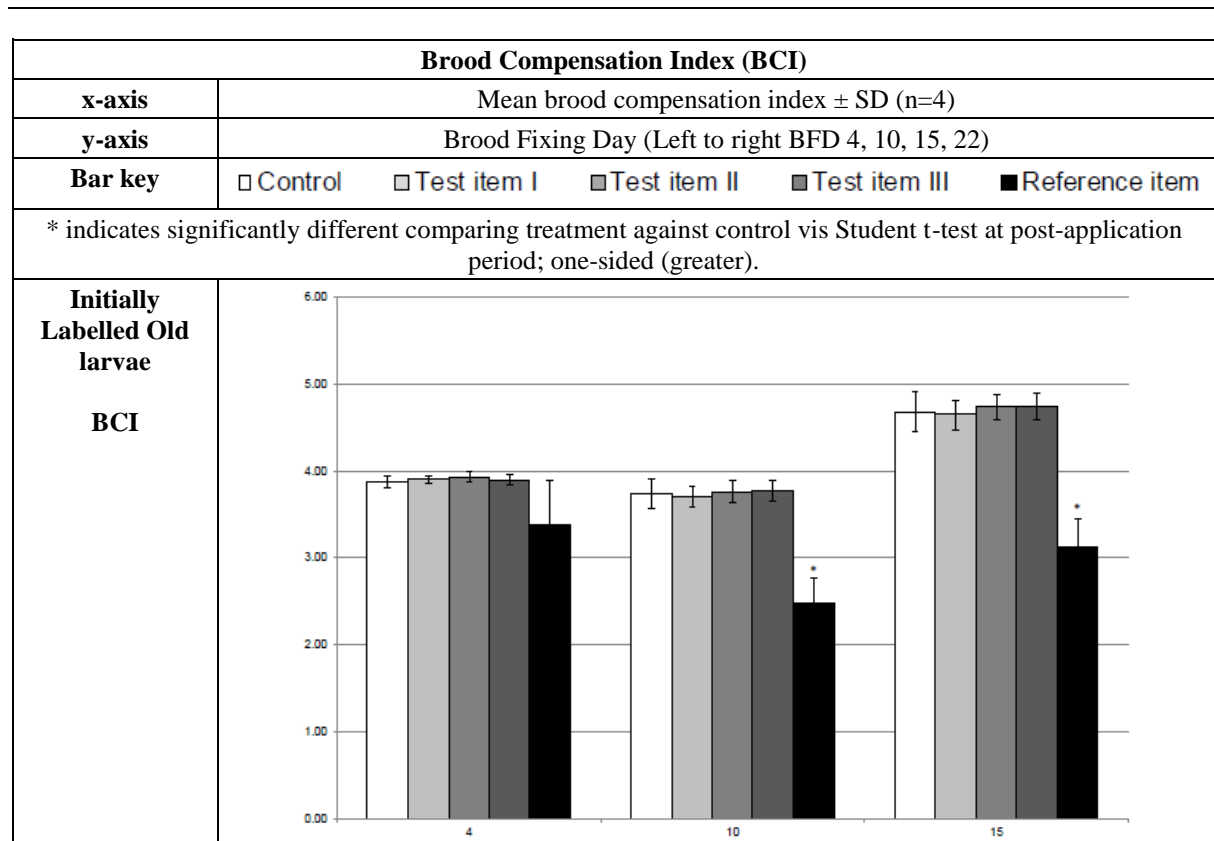


Table 9.5.1-28: Overview of main endpoints for control, test item and reference treatments by the final assessment date.

Evaluation / Assessment			Treatment group									
			Control		Test item I A19649B 75 g a.s./ha		Test item II A19649B 125 g a.s./ha		Test item III, A19649B 200 g a.s/ha		Reference item Insegar 25 WG	
					mean ¹	SD	mean ¹	SD	mean ¹	SD	mean ¹	SD
Adult mortality to BFD 29 (DAT 28) [bees/colony/day]	Pre-exposure ² (DAT -3 to 0 _{bef})	22.1 ^{a,b}	7.6	14.8 ^{a,b}	3.5	12.8 ^a	2.8	12.4 ^a	5.3	21.3 ^{a,b}	6.5	
	Exposure phase ² (DAT 0 _{aft} to 7)	20.9	1.6	17.2	1.2	15.5	1.3	15.5	2.5	19.6	5.7	
	Post-exposure phase ³ (DAT 8 to 28)	3.1	0.8	3.2	0.4	2.8	1.0	2.7	0.7	3.9	1.3	
	Overall after application (DAT 0 to 28)	8.0	0.5	7.1	0.4	6.3	1.0	6.2	0.8	8.2	2.5	
Pupal mortality to BFD 29 (DAT 28) [bees/colony/day]	Pre-exposure ² (DAT -3 to 0 _{bef})	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	Exposure phase ² (DAT 0 _{aft} to 7)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	Post-exposure phase ³ (DAT 8 to 28)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.4	11.2	
	Overall after application (DAT 0 _{aft} to 28)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.1	10.3	
Foraging activity to BFD 8 (DAT 7) [bees/m ² /colony/day]	Pre-exposure phase (DAT -3 to 0 _{bef})	13.9 ^a	0.6	14.3 ^{a,b}	0.2	14.4 ^{a,b}	0.4	14.4 ^{a,b}	0.4	14.1 ^a	0.6	
	DAT 0, before application (DAT 0 _{bef})	12.3	1.2	12.4	1.0	13.0	0.7	13.2	0.8	12.8	0.8	
	DAT 0, after application (DAT 0 _{aft})	13.9	0.4	14.9	1.0	14.7	0.4	15.1	0.2	13.9	0.8	
	Whole exposure phase (DAT 0 _{aft} to 28)	14.7	1.1	15.0	0.5	15.0	0.5	14.7	0.5	14.3	0.4	
Brood termination rate [%]⁴	Initially labelled eggs on BFD 0 (DAT -1)	21.1	6.5	14.7	13.0	26.5	11.6	32.3	14.7	52.8*	21.1	
Brood-index⁴		3.9	0.3	4.3	0.7	3.7	0.6	3.4	0.7	2.4*	1.1	
Brood compensation index⁴		4.2	0.2	4.4	0.7	4.0	0.4	3.9	0.3	2.8*	1.0	
Brood termination rate [%]⁴	Initially labelled young larvae on BFD 0 (DAT -1)	9.3	8.9	13.7	13.0	19.9	10.2	19.4	10.8	40.4*	9.8	
Brood-index⁴		4.5	0.4	4.3	0.6	4.0	0.5	4.0	0.5	3.0*	0.5	
Brood compensation index⁴		4.7	0.2	4.5	0.5	4.4	0.3	4.4	0.3	3.2*	0.6	
Brood termination rate [%]⁴	Initially labelled old larvae on BFD 0 (DAT -1)	6.7	4.6	7.7	3.7	5.9	3.0	5.6	3.1	37.8*	7.0	
Brood-index⁴		4.7	0.2	4.6	0.2	4.7	0.2	4.7	0.2	3.1*	0.4	
Brood compensation index⁴		4.7	0.2	4.6	0.2	4.7	0.1	4.7	0.2	3.1*	0.3	

BFD – Brood Fixing Day; DAT – Days After Treatment; DAT 0_{bef} – day of treatment application, before application of test item. DAT 0_{aft} – day of treatment application, after application of test item.

¹⁾ mean of four replicates. ²⁾ sum of dead honeybees found in dead bee trap and on gauze sheets in the tunnels. ³⁾ dead honeybees found in dead bee trap only. ⁴⁾ Based on final detailed measurement on BFD 22 (DAT 21), after one brood cycle, with the exception of initially labelled old larvae, where the final assessment is based on BFD 15.

^{a,b}: same letters indicate that groups are not statistically significant different (Tukey-test, two-sided, $\alpha=0.05$). * = significantly different when comparing treatment against control via Student t-test (for variance homogeneous data) or Welch t-test (for variance inhomogeneous data) at $\alpha=0.05$ significance level for the post-application period (one-sided (greater): mortality, brood termination rate; one-sided (less): foraging activity, brood index and brood compensation index). The %-values of the brood termination rate were arcsine-transformed to ensure the homogeneity of the data before conducting the t-test procedure.

Bee behaviour to DAT 28/BFD 29

Any abnormal behaviour or deviation to the normal behaviour in comparison to the control was recorded (e.g. aggression, intensive bee flight without landing on the crop, accumulation of bees at the hive entrance, bees no longer producing pollen balls, swarming etc.).

The application did not result in behavioural abnormalities of honeybees immediately after application or during the exposure and post-exposure phase when compared to the control. Bees were observed actively foraging (collecting nectar and pollen), were calm and furthermore, showed no symptoms of apathy, intensified cleaning or intoxication. In the reference item treatment, no behavioural changes of honeybees in the crop, at the bee hive, in the dead bees traps or on the linen sheets were observed before and after application compared to the control.

Colony strength to BFD 63

Colony strength was checked by estimating the number of bees per colony upon manual examination of the combs, including the presence of a healthy queen. The colony strength observation was carried out by estimating how many eighths of a comb were covered by bees, according to the methods in [REDACTED], 1987 and [REDACTED], 1999: the assessment is based on the assumption that a “German standard-size” (“Deutsch Normalmass”) comb with an area of 825.1 cm² could be covered in maximum by 900 bees per comb side (=1800 bees per comb and 1/8 equal to 112.5 bees). Therefore, the maximum number of bees per colony consisting of one super with a total of 11 combs and two bounding hive walls could be theoretically 21600 (19800 bees on combs and 1800 bees on walls). Each comb side was separated in 8 equal parts. During assessments the number of parts (eighths) covered by bees was assessed per comb side (1/8 to 8/8). Based on number of eighths covered by bees per comb in an 11 comb colony with two bounding hive walls, the number of bees per colony (colony strength) was determined using the conversion that 1/8 of a comb side covered by bees is equivalent to 112.5 bees. The total comb area per colony based on a frame size of 37 cm x 22.3 cm = 825.1 cm² per comb side (total comb area per colony (11 combs: 18152 cm²); 1/8 of a comb are equivalent to 103.1 cm²).

Colony strength data is shown in the Table 9.5.1-29 below. Summary of results:

- *Pre-exposure:* The mean estimated colony strength before application was appropriate in relation to the available crop area and statistical analysis revealed no differences among treatment groups (Tukey test, two-sided, $p > 0.05$).
- *Post exposure:* During the course of the study, bee colonies of the test item groups generally developed in a comparable way to the control, with the following notes:
 - A significant decrease in colony strength was observed for test item II at a single time point of DAT 14. This is regarded as natural variation since for the remaining course of the study the colony strength was comparable.
 - Replicate 3 of test item III group was found to be queenless at BFD 29 and colony strength of this replicate decreased over time to 5738 and 5625 bees/colony on BFD 57 and BFD 63, respectively, from 16313 at BFD 29. Statistical analysis revealed significant differences between control and test item III groups during the first brood cycle on DAT 9 (BFD 10), DAT 14 (BFD 15) and DAT 21 (BFD 22) (Student t-test, one-sided (smaller), $p < 0.05$). However, no statistically significant differences were observed during the second and third brood cycle from BFD 29 to 63, despite the decrease in strength from the single queenless replicate colony.
- *By the end of the test (DAT 62):* The estimated average number of bees was 18309 (+93 %), 19238 (+78 %), 16622 (+73 %), 13950 (+40 %) bees/colony DAT 62 (BFD 63) in the control, test item I, test item II and test item III, respectively (see table below). By the end of the test, on DAT 62 (BFD 63), statistical analysis revealed no differences between all treatment groups (Student t-test, one-sided (smaller), $p > 0.05$).
- *Reference item:* In contrast, the mean estimated colony strength of the reference item colonies decreased to 5541 bees/colony (-43 %) on DAT 62 (BFD 63) and significantly decreases in colony strength compared to control occurred from DAT 9 (BFD 10) onwards during the further course of the study (Student t-test, one-sided (smaller), $p < 0.05$).

Table 9.5.1-29. Mean colony strength of honeybee colonies exposed to Pydiflumetofen

Assessment Day	Mean Colony Strength (estimated average number of bees/colony)									
	Control		Test item I		Test item II		Test item III		Reference item	
	Mean (± SD)	% change from DAT- 1 ¹⁾	Mean (± SD)	% change from DAT- 1 ¹⁾	Mean (± SD)	% change from DAT- 1 ¹⁾	Mean (± SD)	% change from DAT- 1 ¹⁾	Mean (± SD)	% change from DAT- 1 ¹⁾
DAT -1 (BFD 0)	9506 ^a (863)	-	10828 _{a,b} (249)	-	9591 ^a (1247)	-	9956 ^{a,b} (771)	-	9731 ^{a,b} (1025)	-
DAT 3 (BFD 4)	8719 (653)	-8	9731 (620)	-10	9225 (559)	-4	9394 (782)	-6	9169 (195)	-6
DAT 9 (BFD 10)	13584 (1024)	+43	15047 (1119)	+39	12459 (1775)	+30	12291 * (489)	+23	9759 * (1185)	0
DAT 14 (BFD 15)	15469 (1119)	+63	15891 (652)	+47	12656 * (1355)	+32	13809 * (1073)	+39	9450 * (2760)	-3
DAT 21 (BFD 22)	18197 (829)	+91	19266 (1563)	+78	16256 (2454)	+70	15413 * (1328)	+55	8297 * (2049)	-15
DAT 28 (BFD 29)	14822 (336)	+56	15609 (612)	+44	14344 (1822)	+50	15019 (1307)	+51 ^q	10041 * (1471)	+3
DAT 34 (BFD 35)	15609 (1234)	+64	16875 (630)	+56	15019 (1307)	+57	14344 (1041)	+44 ^q	7200 * (2097)	-26
DAT 40 (BFD 41)	16959 (1145)	+78	17381 (1156)	+61	16228 (2736)	+69	16847 (645)	+69 ^q	10406* (1634)	+7
DAT 47 (BFD 48)	19041 (2476)	+100	18591 (902)	+72	16228 (2641)	+69	17381 (1843)	+75 ^q	8269 * (5397)	-15
DAT 56 (BFD 57)	18169 (1430)	+91	19238 (400)	+78	18366 (3773)	+91	15694 (7082)	+58 ^q	7228 * (8246)	-26
DAT 62 (BFD 63)	18309 (3106)	+93	19238 (1602)	+78	16622 (5106)	+73	13950 (5810)	+40 ^q	5541 * (5835)	-43

^{a,b}: same letters indicate that groups are not statistically significant different (Tukey test, two-sided, $p > 0.05$) at pre-application period.

*statistically significant different when comparing treatment against control via Student t-test at post-application period; one- sided (less), $p < 0.05$.

¹⁾ relative change in comparison to DAT -1 calculated from the respective mean values

^q From BFD 29 replicate 3 of Test Item II group was found to be queenless

Brood and food development monitored by comb area to BFD 63/DAT 62

Brood and food development observations were carried out by estimating how many eighths of a comb were covered by eggs, larvae, pupae, pollen and honey, according to the same methods employed for colony strength as described in [REDACTED], 1987 and [REDACTED], 1999: the area of each frame side covered with the different stages of brood (including eggs, larvae and capped cells), honey, pollen and empty cells were assessed by dividing the comb into 8 equal parts and counting number of eighths per frame side in each category. The total comb area per colony based on a frame size of 37 cm x 22.3 cm = 825.1 cm² per comb side; 1/8 of a comb are equivalent to 103.1 cm² (total comb area per 11-comb colony 18152 cm²). The number of eighths of brood or food were converted to cm², taking into account that 1/8 of a comb is equivalent to 103.1 cm².

Brood development by comb area to BFD 63/DAT 62

The brood development by comb area can be seen in Table 9.5.1-30 and Figures 9.5.1-15 for the whole brood, 9.5.1-16 for eggs, 9.5.1-17 for larvae and 9.5.1-18 for pupae. During the course of the study, the mean estimated brood areas of the control and test item treatments developed within the range of natural variability and in a comparable way. A description of the results is as follows:

- *Total brood pre-exposure:* The mean estimated total brood area occupied by eggs, larvae and pupae before application was on a comparable level in all treatment groups (8251, 9321, 8754, 8767 and 7529 cm²/colony for the control, test item I, test item II, test item III and reference item, respectively (Figure 9.5.1-15). No significant differences were observed between the treatment groups, based on overall comparison of the entire mean brood area before application (Tukey-test, two-sided, $p > 0.05$).
- *Total brood to BFD 22/DAT 21:* At the end of the first brood cycle (BFD 22) the mean brood area/colony was 11590 (+40 % compared to BFD 0), 12222 (+31 %), 11526 (+32 %) and 12235 cm² (+40 %) in the control, test item I, test item II and test item III, respectively (figure 9.5.1-15, table 9.5.1-30). There were no statistically significant differences (Student t-test, one-sided (less), $p > 0.05$).
- *Total brood to BFD 63/DAT 62:* At the end of the test (BFD 63), the average total brood area per colony amounted to 9979 (+21 %), 9695 (+4 %), 8896 (+2 %) and 6214 (-29 %) in the control, test item I, test item II and test item III. As replicate 3 of test item III group was queenless, the mean brood area of test item III is lower compared to the other treatment groups (Figure 9.5.1-15, Table 9.5.1-30). All treatments have less of an increase in total brood area from BFD 48 to 63 than the control. However, statistical analysis revealed no significant differences between the control and the test item groups at any point (Student t-test, one-sided (less), $p > 0.05$).
- *Total brood in reference item:* Contrastingly, the reference item displayed a strong decline in all brood stages, i.e. eggs, larvae and pupae. At the end of exposure phase, the mean entire brood area declined by -73 % (9.5.1-15, table 9.5.1-30). Statistical analysis revealed significant differences between control and reference item on all assessment days during the course of the study (Student t-test, one-sided (smaller), $p < 0.05$). This demonstrates the sensitivity of the test system.
- At BFD 4 (DAT 3), there was reduced eggs in all test items and the reference item compared to BFD 0, whereas the control had an increase (+13 %, -37 %, -13 %, -31 % and -38 % for control, test item I, test item II, test item III and the reference item, respectively). However, this observation was not dose responsive and the differences were not statistically significant. There was a corresponding greater increase in the area taken up by pupae at BFD 4 in all treatments apart from the reference item, compared to the control. Additionally, at BFD 10 (DAT 9) the number of eggs was increased compared to BFD, in line with the control (Table 9.5.1-30).
- At BFD 10 (DAT 9), there was reduced larvae compared to BFD 0 in all treatments, whereas the control had a similar number of larvae (+1 %, -23 %, -14 %, -26 % and -56 % for control, test item I, test item II, test item III and the reference item, respectively). On this day overall the total brood area did not grow as much as the control, however none of these differences were statistically significant (Table 9.5.1-30)

Table 9.5.1-30 Summary of honeybee brood development by comb area compared to BFD 0.

		Mean area on BFD 0 ± SD (cm ² /colony)	[%] deviation of comb area compared to BFD 0 ¹									
Brood Fixing Day (BFD):		0	4	10	15	22	29	35	41	48	57	63
Eggs**	Control	1096 ± 415	13	71	133	51	32	36	7	55	91	72
	Test item I	1289 ± 399	-37	72	98	12	56	60	37	62	42	94
	Test item II	1444 ± 776	-13	39	100	39	50	50	43	57	41	-4
	Test item III	1495 ± 352	-31	60	76	33	26 ^a	-12 ^a	21 ^a	24 ^a	31 ^a	24 ^a
	Reference item	954 ± 398	-38	-38	-45	-76	-70	-81	-84	-51	-49	-86
Larvae**	Control	2024 ± 570	-10	1	26	41	43	40	53	2	4	39
	Test item I	2694 ± 608	-3	-23	13	30	21	11	16	-16	-28	-14
	Test item II	2475 ± 689	-17	-14	13	45	6	4	-16	-15	-23	10
	Test item III	2295 ± 464	-16	-26	62	54	17 ^a	-2 ^a	-34 ^a	-11 ^a	-73 ^a	-34 ^a
	Reference item	1135 ± 365	20	-56	-43	-75	-59	-45	-45	-59	-45	-43
Pupae**	Control	5131 ± 1066	8	10	-2	38	55	43	29	50	18	3
	Test item I	5337 ± 464	12	10	2	36	45	42	39	41	16	-9

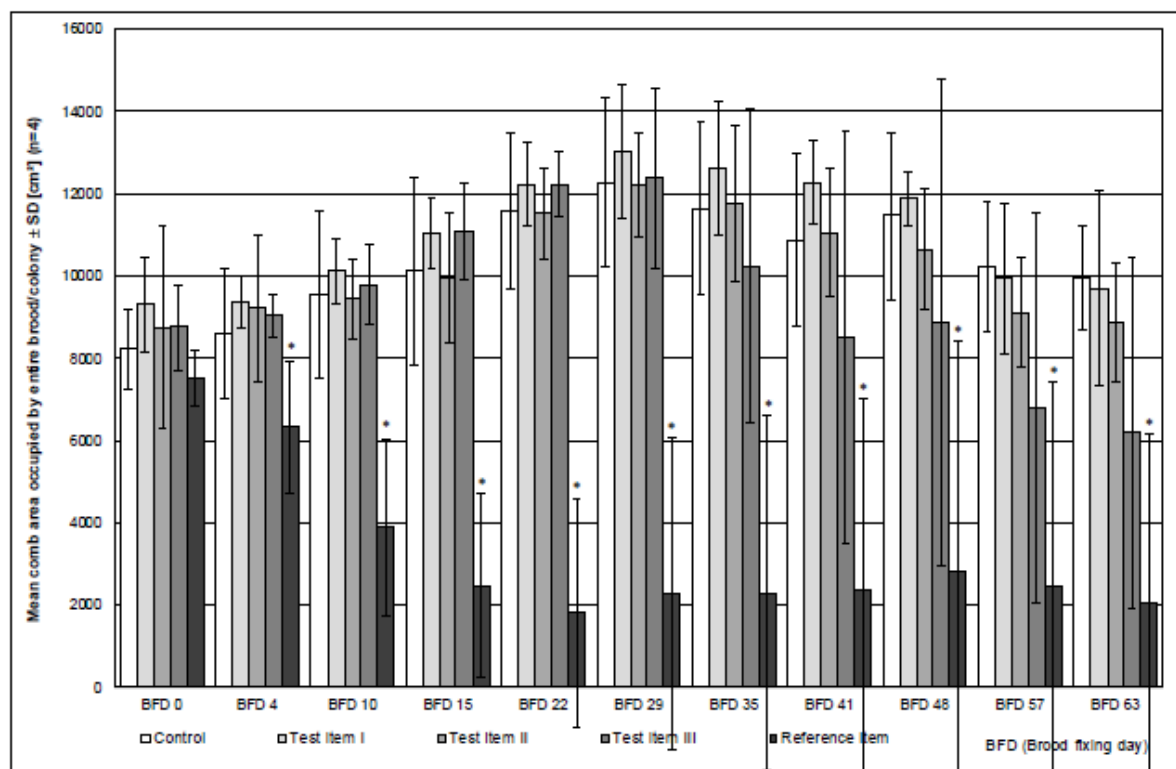
		Mean area on BFD 0 ± SD (cm ² /colony)	[%] deviation of comb area compared to BFD 0 ¹									
Brood Fixing Day (BFD):		0	4	10	15	22	29	35	41	48	57	63
	Test item II	4835 ± 1392	22	10	-11	23	54	46	43	30	7	-1
	Test item III	4976 ± 1159	22	15	-5	35	57 ^q	34 ^q	5 ^q	0 ^q	-15 ^q	-43 ^q
	Reference item	5441 ± 760	-20	-48	-76	-76	-72	-72	-70	-65	-75	-76
Whole Brood (Eggs + Larvae + Pupae)	Control	8251 ± 971 a	4	16	23	40	49	41	32	39	24	21
	Test item I	9321 ± 1151 a,b	1	9	19	31	40	36	32	28	7	4
	Test item II	8754 ± 2450 a,b	5	8	14	32	40	35	26	22	4	2
	Test item III	8767 ± 1042 a,b	3	12	27	40	41 ^q	17 ^q	-3 ^q	1 ^q	-23 ^q	-29 ^q
	Reference item	7529 ± 672 a	-16*	-48*	-67*	-76*	-70*	-69*	-68*	-63*	-67*	-73*

¹ Positive values indicate an increase compared to BFD 0, negative values indicate a decrease.

*statistically significant different when comparing treatment against control via Student t-test at post-application period; one- sided (less). ** Statistical analysis performed on Whole Brood only.

a,b: same letters indicate that groups are not statistically significant different (Tukey-test, two-sided, $\alpha=0.05$) at pre-application period

^q From BFD 29 replicate 3 of Test Item II group was found to be queenless



* significantly different comparing treatment against control via Student t-test at post-application period; one-sided (less).

Figure 9.5.1-15. Brood development of comb area occupied by entire brood (eggs + larvae + pupae).

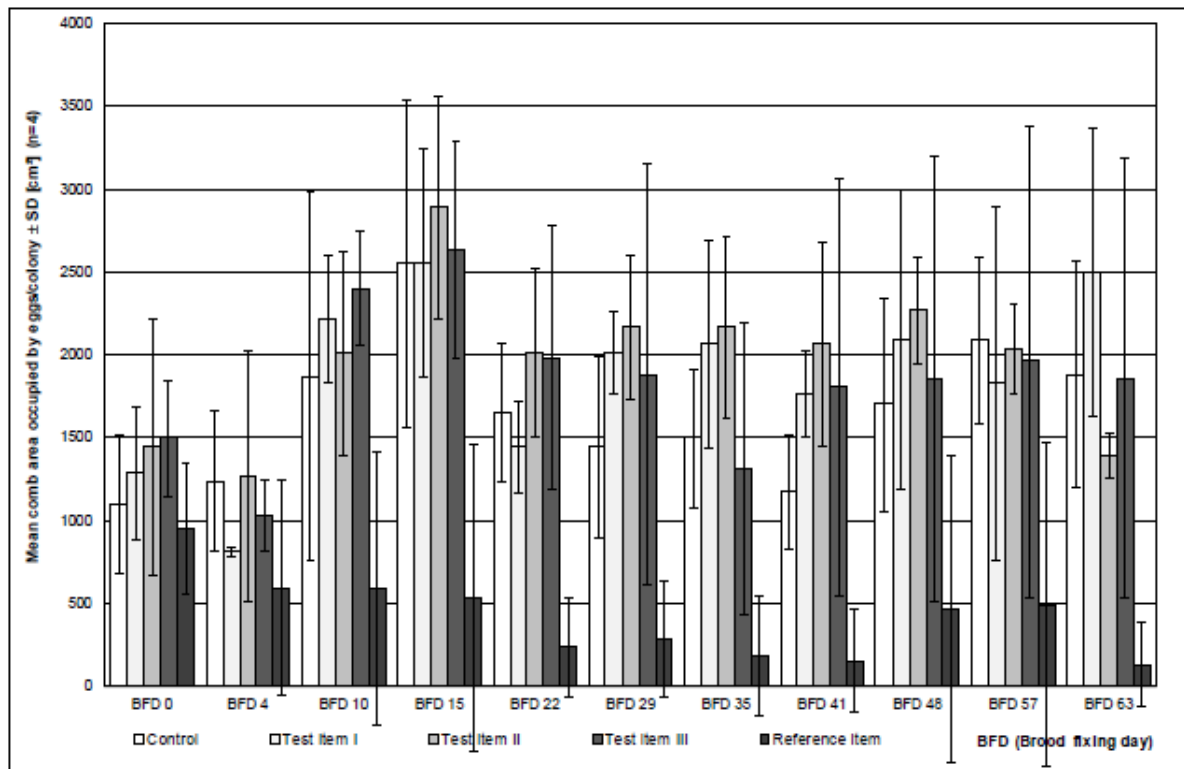


Figure 9.5.1-16. Brood development of comb area occupied by eggs.

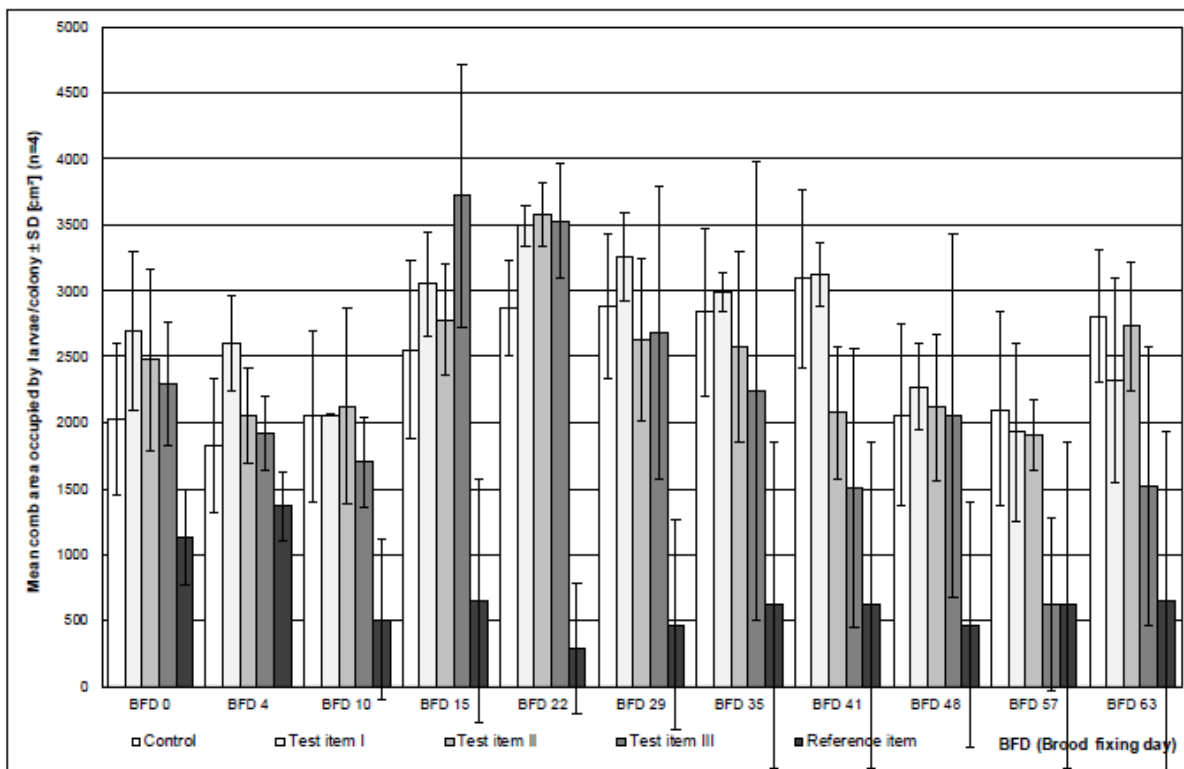


Figure 9.5.1-17. Brood development of comb area occupied by larvae

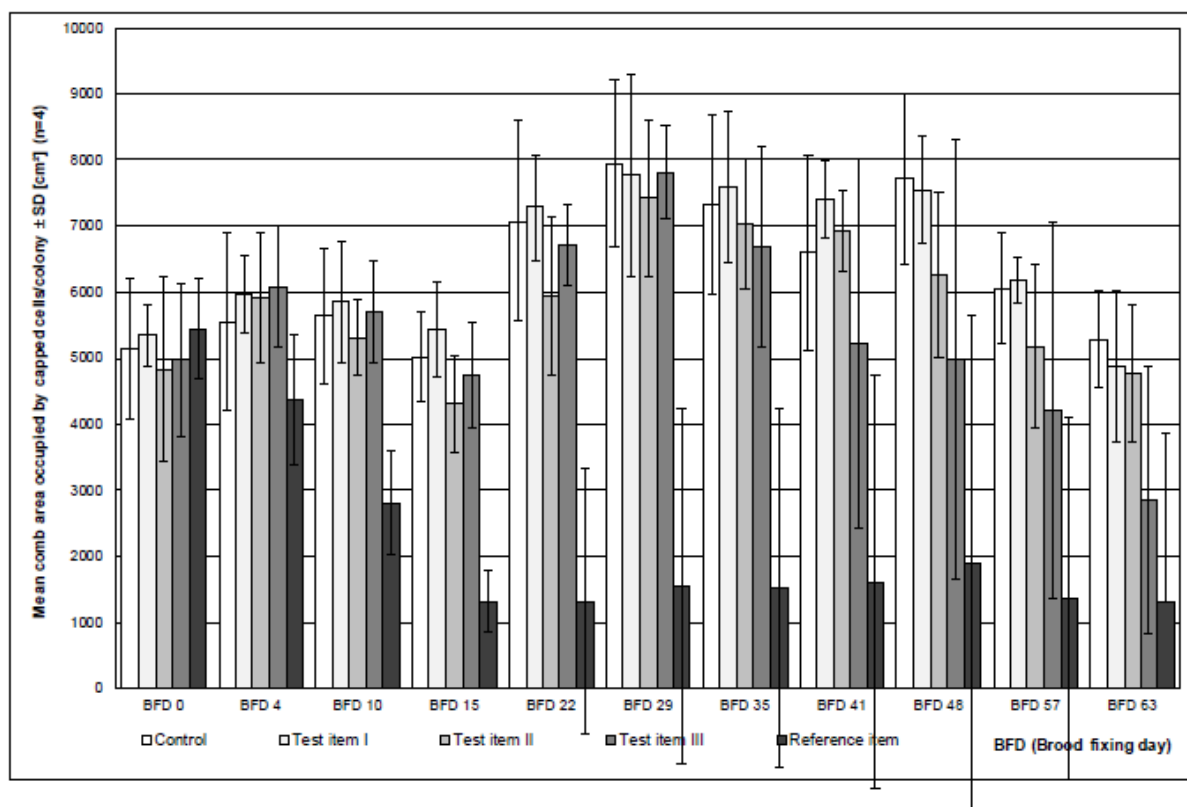


Figure 9.5.1-18. Brood development of comb area occupied by pupae.

Food development by comb area to BFD 63/DAT 62

The food development by comb area can be seen in Table 9.5.1-31 and Figures 9.5.1-19 for nectar and 9.5.1-20 for pollen. The assessment of the areas covered with pollen and nectar/honey before application (BFD 0) ranged from 4641 – 6343 cm²/colony (table 10.3.1.5-11), demonstrating comparable levels (no significant differences) and a sufficient supply of all colonies with food (Tukey test, two-sided, $p > 0.05$). During the course of the study, the mean area covered with nectar and pollen developed in a different way in all treatment groups and the reference item. A description of the results is as follows:

- At the last assessment on BFD 63 the average total food area per colony had not increased as much in the test item treatments compared to the control: 17482 (+277 %), 14052 (+177 %), 7297 (+40 %), 4989 (-12 %) and 6601 cm² (+4 %) in the control, test item I, test item II, test item III and reference item, respectively (table 10.3.1.5-11).
- Statistical analyses revealed significantly lower food stores in test item I compared to the control on BFD 35, BFD 41, BFD 48 and BFD 57, respectively (Student t-test, one-sided (less), $p < 0.05$).
- For test item II and test item III, significantly lower food stores occurred on every assessment day with the exception on BFD 4 in both treatment groups (Student t-test, one-sided (less), $p < 0.05$).

Table 9.5.1-31. Summary of honeybee food development by comb area compared to BFD 0

		Mean Comb area at BFD 0 ± SD (cm ² /colony)	[%] deviation of comb area compared to BFD 0 ¹									
Brood Fixing Day (BFD):		0	4	10	15	22	29	35	41	48	57	63
Nectar**	Control	2514 ± 1291	-2	113	162	472	663	611	629	580	548	533
	Test Item I	3094 ± 1506	1	73	87	313	431	377	356	322	294	295
	Test Item II	3442 ± 2023	12	-14	-4	179	241	206	158	124	67	71
	Test Item III	3752 ± 475	-7	-8	-9	68	124	78	74	29	-32	-20
	Reference Item	4770 ± 1560	-5	6	32	37	51	11	22	-43	-70	-74
Pollen**	Control	2127 ± 682	-5	-45	-4	70	43	25	47	-10	-23	-26
	Test Item I	1973 ± 165	5	-69	-34	34	22	-24	13	-49	-29	-7
	Test Item II	1766 ± 327	-8	-75	20	20	-14	-23	-3	-47	-57	-20
	Test Item III	1908 ± 861	-9	-82	-83	-43	-73	-34	-2	-36	-22	3
	Reference Item	1573 ± 477	33	-27	-2	67	152	167	232	246	232	239
Nectar + Pollen	Control	4641 ± 750 a	-3	40	86	288	379	342	363	310	286	277
	Test Item I	5067 ± 1398 a	3	18	40	204	272	221*	222*	177*	168*	177
	Test Item II	5208 ± 1744 a,b	5	-35*	4*	125*	154*	129*	104*	66*	25*	40*
	Test Item III	5660 ± 1287 a,b	-7	-33*	-34*	31*	58*	40*	48*	7*	-29*	-12*
	Reference Item	6343 ± 1386 a,b	4	-2	23	44	76*	50*	74*	29*	5*	4*

¹ Positive values indicate an increase compared to BFD 0, negative values indicate a decrease.

*statistically significant different when comparing treatment against control via Student or Welch t-test at post-application period; one-sided (less), $p < 0.05$).

**statistical analysis performed on total food stores (nectar + pollen) only.

a,b: same letters indicate that groups are not statistically significant different (Tukey-test, two-sided, $\alpha = 0.05$) at pre-application period.

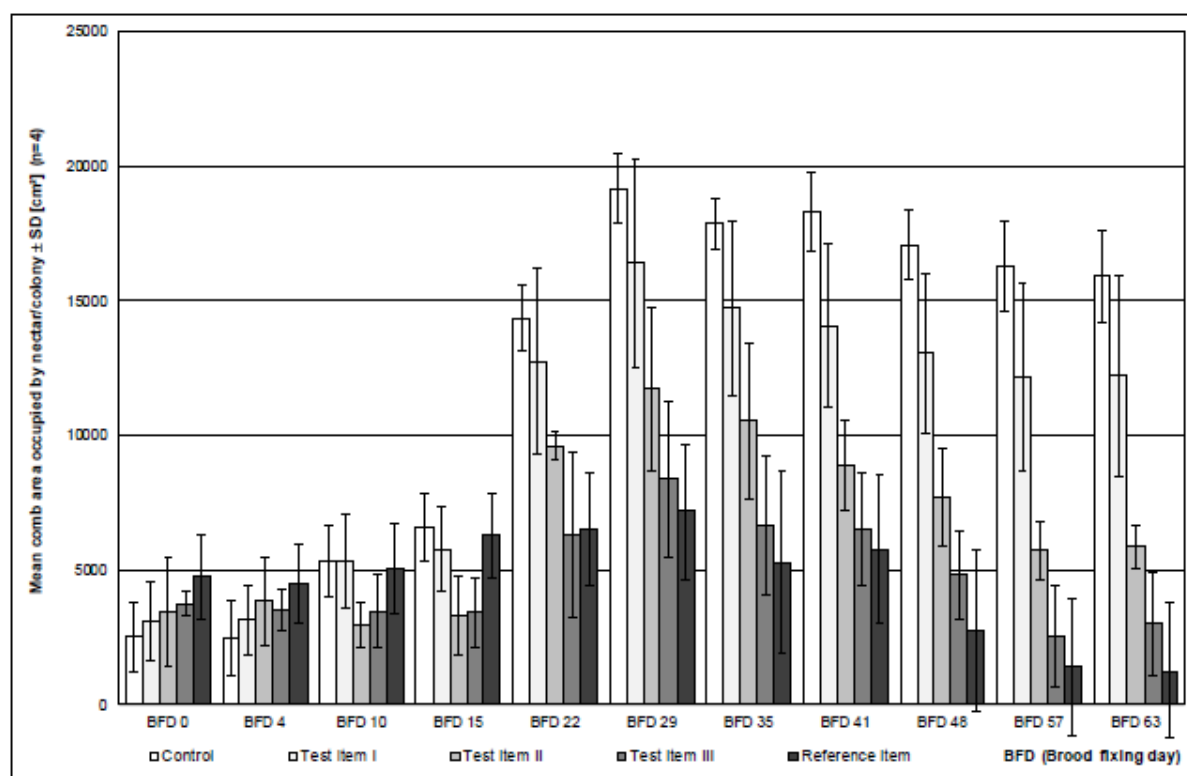


Figure 9.5.1-19. Food development of comb area occupied by nectar

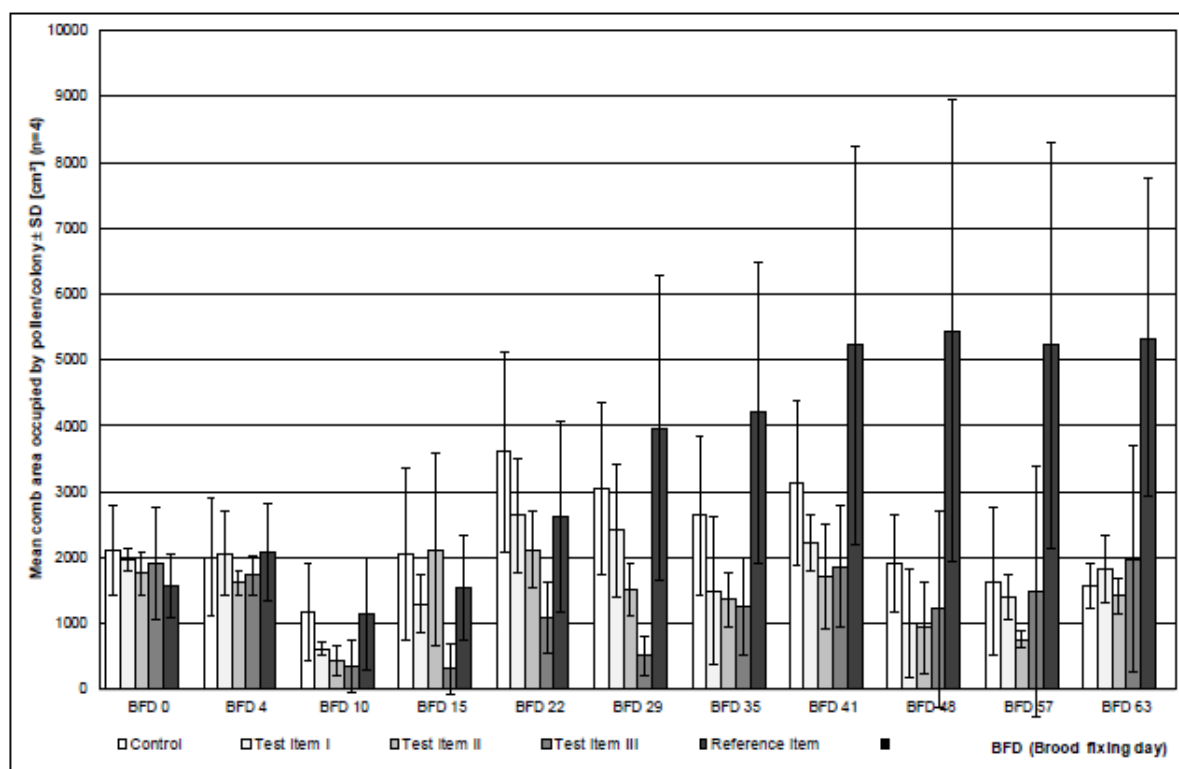


Figure 9.5.1-20. Food development of comb area occupied by pollen.

Residue analysis of flowers, leaves (foliage), pollen and nectar to BFD 8/DAT7

- Residue analysis of Pydiflumetofen (A19649B) in flowers, leaves (foliage), pollen from pollen traps at hive entrance and nectar extracted from honey sacs obtained from forager bees at the hive entrance, from DAT -1 before application, DAT 0 (within 4 hours after application), DAT 1, DAT 4 and DAT 7.
- Residue analysis of in-hive pollen and nectar from combs at the monitoring site on DAT 28, DAT 40, DAT 47 and DAT 62

Residues of A19649B were measured in flowers, foliage, pollen and nectar from one additional tunnel/colony per treatment group, which had also been treated with tap water (control) or A19649B (test item) at each of the three rates.

- Flowers and foliage were collected from different locations within the tunnel and pooled per replicate, with a target amount of 5 g sampled flowers and 5 g samples foliage.
- Pollen was collected from pollen traps attached to the front of each hive for approximately 4 hours. The target amount of sampled pollen was at least 1 g. Pollen was collected from inside the residue hives (target amount 5 g) at the monitoring site on BFD 29, BFD 41, BFD 48 and BFD 63 for multi residue analysis (non-GLP).
- Nectar was obtained by collecting returning forager bees by closing the entrance hole of the hive for several minutes during daily bee flight and collecting returning forager bees using a sweeping net and freezing them immediately by placing them into liquid nitrogen. One sample comprised at least 200 bees to provide enough material (target amount at least 0.5 g) for analysis. Nectar was extracted from the stomach of the frozen bees. Nectar was also collected from inside the residue hives (target amount 5 g) at the monitoring site on BFD 29, BFD 41, BFD 48 and BFD 63 for multi residue analysis (non-GLP).
- All samples were stored at $\leq -18^{\circ}\text{C}$ before analysis at the analytical laboratory using a HPLC MS-MS method with a limit of quantification (LOQ) for Pydiflumetofen of 0.005 mg/kg.

No residues of Pydiflumetofen were detected in any of the control specimens or in specimens taken one day before application (DAT -1). Residue data are displayed in the Table 9.5.1-32 below, and show that there was adequate exposure to the test item.

Table 9.5.1-32 Residues of Pydiflumetofen in pollen, nectar, flowers and foliage

Treatment group	Sampling day	Pollen analysed conc. of a.s. [mg/kg]	Nectar analysed conc. of a.s. [mg/kg]	Flowers analysed conc. of a.s. [mg/kg]	Foliage analysed conc. of a.s. [mg/kg]
Control	DAT -1	n.d.	n.d.	n.d.	n.d.
	DAT 0 aa	n.d.	n.d.	n.d.	n.d.
	DAT 1	n.d.	n.d.	n.d.	n.d.
	DAT 4	n.d.	n.d.	n.d.	n.d.
	DAT 7	n.d.	n.d.	n.d.	n.d.
Test item I (nominally 75 g a.s./ha)	DAT -1	n.d.	n.d.	n.d.	n.d.
	DAT 0 aa	6.81	0.105	3.06	4.30
	DAT 1	4.53	0.0366	2.65	2.23
	DAT 4	0.667	0.0765	0.501	4.14
	DAT 7	0.452	0.0048	0.0298	1.71
Test item II (nominally 125 g a.s./ha)	DAT -1	n.d.	n.d.	n.d.	n.d.
	DAT 0 aa	7.74	0.178	3.41	7.11
	DAT 1	5.00	0.0962	3.08	5.68
	DAT 4	1.49	0.0266	0.975	3.18
	DAT 7	0.726	0.0043	0.145	2.15
Test item III (nominally 200 g a.s./ha)	DAT -1	n.d.	n.d.	n.d.	n.d.
	DAT 0 aa	17.7	0.352	5.96	8.71
	DAT 1	5.35	0.0896	4.24	8.06
	DAT 4	1.61	0.0280	1.85	7.76
	DAT 7	0.477	0.0252	0.460	2.07

DAT – Day after treatment, aa – after application. Limit of quantification (LOQ) for Pydiflumetofen was 0.005 mg/kg.

Validity Criteria

The following criteria were fulfilled during application:

- *Phacelia tanacetifolia* B. growth stage BBCH 65 (full flowering)
- Mean foraging activity of ≥ 10 bees/m² on the *Phacelia tanacetifolia* B.
- Wind speed directly before application of each tunnel ≤ 2 m/s
- Spray tolerance of ± 10 %

The test was considered valid:

- the mean brood termination of initially marked eggs of the reference item treatment was 52.8 % on BFD 22 and was therefore significantly higher compared to the control which was 21.1 %,
- the individual brood termination rate of the control replicates ranged from 17.0 % to 30.7 % and was therefore below 40 %
- the mean pupal mortality in the reference item treatment was significantly increased for the duration of the post-exposure phase compared to the control.
- the mean foraging activity before application amounted to 12.3, 12.4, 13.0, 13.2 and 12.8 bees/m² in the control, test item I, test item II, test item III and reference item treatment, respectively (requirement ≥ 10 bees/m²).

These data confirm the sensitivity of the test system and therefore, exposure of bees to evaluate potential effects on the bee brood.

Conclusions

A19649B was applied once at a rate of 375 mL (equivalent to 75 g a.s./ha), 625 mL (equivalent to 125 g a.s./ha) and 1000 mL (equivalent to 200 g a.s./ha) in a semi-field study (bee brood tunnel study according to OECD Guidance No. 75) on full-flowering *Phacelia tanacetifolia* during daily bee flight. The exposure of the bees to the test item treatments was proven by the assessment of the foraging activity and residue analysis of the test item in flowers, foliage and bee food sources (pollen and nectar) collected on several sampling days after application.

Key findings:

- *Adult mortality, to BFD 29:* There was no effect of the test item on adult mortality. The single statistically significant result at the highest test concentration on one day (4 dead bees/colony on DAT 27 for highest treatment level compared to 2 dead bees/colony in the control) is considered to not be treatment related due to the low numbers and lack of effects on other days.
- *Pupal mortality, to BFD 29:* There was no effect of the test item on pupal mortality.
- *Foraging Activity/Bee flight, to BFD 8:* no effect of the test item on foraging activity.
- *Bee behaviour to BFD 29:* no effect of the test item on behaviour.
- *Detailed brood analysis by photo documentation of initially labelled cells to BFD 22:*
 - BTR increases with increasing test concentration for initially labelled eggs and young larvae, though there were no statistically significant difference from the control.
 - BI of initially labelled eggs and young larvae was typically reduced compared to the control, though there were no statistically significant differences from the control.
 - BCI of initially labelled eggs and young larvae was typically reduced compared to the control and with increasing test concentration for initially labelled eggs, but not dose-responsive for initially labelled young larvae. There was a single sporadic statistically significant reduction for Test Item II at BFD 15 which was not dose responsive and can be attributed to natural variation.
 - BTR, BI and BCI of initially labelled old larvae was similar across all treatment concentrations with no statistical differences from the control.
- *Food analysis by comb area, to BFD 63:* significantly reduced food stores in all test item treatments from BFD 35 for Test item I or BFD 10 for Test Item II and III.
- *Brood analysis by comb area, to BFD 63:* All treatments have less of an increase in total brood area from BFD 48 to 63 than the control. However, statistical analysis revealed no significant differences between the control and the test item groups at any point (Student t-test, one-sided (less), $p > 0.05$). Brood area was reduced in test item III at BFD 63 compared to BFD 0 due to a single replicate becoming queenless at BFD 29. However, there were no statistically significant differences.
- *Colony strength, to BFD 63:*
 - Significantly lower mean colony strength (no. bees/colony) for Test item II on BFD 15 only
 - Significantly lower mean colony strength (no. bees/colony) for Test item III on BFD 10, 15 and 22 only
 - One replicate of Test item III group was found to be queenless at BFD 29.
- *Residue analysis* of the test item concentrations and control was performed in one additional tunnel for pollen, nectar, flowers and foliage and demonstrated exposure to the test item.
- *The reference item* resulted in:
 - Significantly increased BTR and significantly reduced BI and BCI compared to the control.

- Significantly lower colony strength compared to the control from BFD 10 to BFD 63.
- Significantly reduced brood area from BFD 4 to 63.
- Significantly reduced food area from BFD 29 to BFD 63.
- This successfully demonstrates the sensitivity of the test system.

(██████, 2018)

HSE Comments

This semi-field honey-bee brood study was evaluated according to OECD guidance document 75 (2007). The minimum length for this type of study is 28 days after BFD according to the OECD guideline. This study provides additional long term monitoring data to BFD 63. There were no significant deviations from the guideline and the test is considered valid.

Residue analysis showed that bees were exposed to the test item. The analytical method has been evaluated by HSE Chemsitry specialist in Vol. 3CA Part B5.1.2.6. The following was concluded for this method: “The method is acceptably validated and is suitable for the determination of SYN545974 in feeding solutions, nectar, pollen, flower and foliage samples with an LOQ of 0.005 mg/kg.”

The following minor methodological points are noted for reference:

- *Meteorological data:* The OECD guideline states that during the whole testing period the temperature, relative humidity and rainfall “*should be recorded daily (ideally from inside the tunnel)*”, with additional measurements of cloudiness during assessment and wind speed during application both inside and outside the tunnel. In this study, climatic measurements are performed both at the test site (GLP data) and from a weather station located 5 km from the test site (non-GLP data – precipitation DAT 8 to 62 only). Therefore, there is some uncertainty with the applicability of the weather station data, but since it is only the precipitation data from DAT 8 onwards this is less important. The key climatic condition in the OECD guideline line is the wind speed criterion during test application. Although it is not specified whether the wind measurement is taken from inside or outside the tunnel, it is stated that it was within 15s of application, therefore the measurement is applicable. The criterion of windspeed being < 2 m/s during application was met, and as there were no obvious adverse effects on the colonies, the impact of the weather and climatic conditions on the outcome of the study can be concluded to be minimal.
- *Reference item concentration:* It is noted that the reference item is tested at a concentration of 300 g a.s./ha whereas the guideline states 150 g a.s./ha. This is not deemed to be an issue with the study as the higher test rate still demonstrates the sensitivity of the test system and there is no validation data for comparison between studies.
- *Monitoring site location:* It is noted that the post-exposure monitoring site is in a different location 10 km away from the pre and post-exposure tunnel site. There was adequate climactic data from both sites from a datalogger placed at each site, and the move does not appear to have had a negative impact on the colonies as seen by the control data. Therefore, this move does not affect the endpoints of the study.

The following points regarding the findings of the study are noted:

- The single statistically significant result for adult morality is not considered to be treatment related – HSE agrees based on examination of the data.
- There was a single sporadic statistically significant result in the middling test item for brood compensation index (BCI) of initially labelled young larvae – HSE agrees that based on the available data this can be attributed to natural variation.
- Although results for Brood termination rate (BTR), brood index (BI) and brood compensation index (BCI) are not significantly significant compared to the control, there does appear to be a potential dose-response for initially labelled eggs, which could be biologically relevant. This will be considered further at risk assessment.

- There were significantly lower food stores in all test item treatments compared to control, throughout the course of the test
- There was some effect on colony strength for the highest two test concentrations, and food stores in all test concentrations, in addition a queenless colony discovered in the highest test concentration. This will be considered further at risk assessment.

The results from this test will be considered further in risk assessment.

B.9.5.2. Effects on non-target arthropods other than bees

Report: K-CP 10.3.2.1, [REDACTED], 2018, Pydiflumetofen EC (A21857B) – A laboratory bioassay of the effects of fresh residues on the parasitic wasp *Aphidius rhopalosiphii* (Hymenoptera, Braconidae). Report Number: SYN-18-19. Mambo-Tox Ltd. 2 Venture Road, University Science Park, Southampton SO16 7NP, United Kingdom. (Syngenta file No. VV-469963).

GUIDELINES

Mead-Briggs *et al.* A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphii* (2000).

GLP: Yes.

MATERIALS

Test Material	A21857B Pydiflumetofen EC (062.5) SYN545974 EC (062.5)
Lot/Batch #:	JEA001-116-001
Actual content of active ingredients:	SYN545974: 5.69 % w/w corresponding to 62.4 g/L
Density:	1097 kg/m ³
Treatments	
Test rates:	375, 750, 1500, 3000 and 6000 mL A21857B/ha
Control:	Purified water
Toxic standard:	BAS 152 11 I (nominally 400 g dimethoate/L) in purified water, applied at a rate of 10 mL product in 400 L water/ha
Spray volume rate:	200 L spray solution/ha
Application method:	Schachtner track sprayer (3 bar pressure, 80° flat fan nozzle)
Spray calibration:	Sprayer calibrated using purified water to confirm deposition rate of 2 mg deposit/cm ² . Three consecutive applications used to confirm rate.
Test organisms	
Species:	<i>Aphidius rhopalosiphii</i> De Stefani-Perez. (Hymenoptera: Braconidae)
Age:	Adult wasps, used within 48 hours of emergence
Source:	Culture maintained at Test Facility (originally: Katz Biotech AG, Baruth, Germany)
Rearing	Wasps were reared on mixed-species culture of cereal aphids containing <i>Rhopalosiphum padi</i> and <i>Metopolophium dirhodum</i> . Emergence chambers stored at 21-22 °C, 66-73 % relative humidity, 16 h photoperiod of 1991 lux.

Feeding:	1:3 v/v solution of honey and water
Test design - Mortality phase	
Arenas:	Treated glass plates fitted to a square frame (10 cm x 10 cm external dimensions) made from metal casing. Three holes (10 mm diameter) drilled through each of the side walls of the frame provided ventilation, covered with discs of fine-gauge, stainless steel mesh. One hole was left uncovered as access for the introduction of the parasitoids before being sealed with a cotton wool bung. Air was forced through units using a small pump to prevent pesticide vapour build-up.
Replication:	Four replicate arenas
No. of wasps/arena:	10 wasps (total 40 wasps per treatment)
Duration	48 hours
Test design - Fecundity phase	
Arenas:	Clear acrylic cylinders (9 cm diameter, 20 cm high, tops covered with nylon netting) were placed over pots containing 15 barley seedlings (<i>Hordeum vulgare</i> L. var. Sienna). The untreated barley had been infested eight days previously with host aphids (>100 adults and nymphs of <i>Metopolophium dirhodum</i> and <i>Rhopalosiphum padi</i>).
Replication:	15 female wasps/treatment
No. of wasps/arena:	1
Duration of test:	Initiated at 48 hours; observation of mummies developing 10 days after adult removal
Environmental test conditions	
Temperature:	Mortality assessment phase: 19-21 °C Fecundity assessment phase: 20-22 °C
Humidity:	Mortality assessment phase: 69-83 % RH
Photoperiod:	Mortality assessment phase: 16 h photoperiod (1022 lux) Fecundity assessment phase: 16 h photoperiod (4286 lux)

STUDY DESIGN AND METHODS

Experimental dates: 11 April 2018 to 24 April 2018.

Mortality phase:

Dilutions of test item were prepared in purified water shortly before use and the solutions were thoroughly agitated to ensure their homogeneity. Treatments were applied to glass plates using a laboratory track-sprayer, calibrated in advance to deliver a deposition rate of 2 mg deposit/cm² (actual mean measured deposition 200 L/ha ± 5 %). Treatments were applied in order of control, ascending rate order treatments and finally the toxic reference item. The sprayer was flushed and wiped down prior to the toxic reference item application. Once dry the glass plates were used to construct the test arenas. The wasps (10 per arena, minimum 5 female) were introduced into these arenas using an aspirator and their behaviour and mortality was assessed after 2, 24 and 48 h. Insects were classed as being: Live, alive and apparently unaffected; Affected, upright, attempting to walk but with reduced coordination or inactive; Moribund, on their back or side, twitching slightly or Dead, not moving. At 48 h, any moribund wasps were included with the dead insects for calculations of percentage mortality.

Fecundity phase:

To assess any sub-lethal effects, reproduction assessments were then carried out for the control and for the only treatment rate of the test item resulting in < 60% corrected mortality (375 ml/ha). Female wasps were confined individually over untreated aphid-infested barley plants for 24 h in a controlled-environment room, before being removed. Female wasps are distinguished by their abdomen being longer and more pointed than in males. The plants were left for a further 10 days before recording the number of aphid ‘mummies’ (pupal wasps) that had developed on plants where wasps had been found alive after the 24-h oviposition period.

Statistical analysis:

The percentage mortality of the insects in the bioassay over 48 h was calculated. Mortality was defined as the numbers of *moribund* and *dead* insects combined. The corrected percentage mortality (taking into account any control treatment losses) was derived using Abbott’s formula. In order to derive a value for the *median lethal rate* (LR₅₀), a Probit regression analysis was performed on the 48-h mortality data. The dose rates were log₁₀-transformed, prior to being imported into the SPSS software, and the control data were entered as a zero dose rate. The level of background mortality in the data was estimated by the software, taking account of all available data (i.e. the *natural response rate estimate*). The 95% confidence intervals for the LR₅₀ value were calculated and a Chi-square test for goodness of fit ($\alpha = 0.05$) performed on the Probit line. Where there was treatment mortality at 48 h, this was compared to mortality in the control using Fisher’s Exact Test ($\alpha = 0.05$). For the reproduction assessments, a square root transformation was carried out on the data prior to analysis. The data set from the treatment were then checked for normality (Shapiro-Wilk test, $\alpha = 0.05$) and homogeneity of variance (Levene’s test, $\alpha = 0.05$), prior to comparison by independent samples t-test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Mortality and reproduction are summarised in the table below.

Table 9.5.2-1: Effects of fresh residues of A21857B on mortality and reproduction of *Aphidius rhopalosiphii*, when exposed under extended laboratory test conditions.

Treatment	Mean % mortality at 48 h ^a	Mean % corrected mortality at 48 h (M-value) ^b	Number females successfully assessed for reproduction	Mean number mummies per surviving female ^c	% Effect on reproduction compared to control (R-value) ^d
Control	2.5	-	15	16.0	-
6000 mL A21857B/ha	100 *	100	~	~	~
3000 mL A21857B/ha	100 *	100	~	~	~
1500 mL A21857B/ha	100 *	100	~	~	~
750 mL A21857B/ha	97.5 *	97.4	~	~	~
375 mL A21857B/ha	32.5 *	30.8	15	17.6	-10.0
Toxic reference	100 *	100	~	~	~

a) Individual treatments were compared to the control using Fisher’s Exact Test ($\alpha = 0.05$). Significant differences are indicated by an asterisk (*).

b) Derived using Abbott’s formula.

c) Individual treatments were compared to the control by t-test for independent samples ($\alpha = 0.05$), but there were no significant differences.

d) Percentage effect on reproduction. A negative value indicates an increase relative to the control.

~ Not assessed.

VALIDITY CRITERIA

The test was considered valid;

Validity criterion	Required	Observed
Control mortality after 48 h	$\leq 13 \%$	2.5 %
Mortality with toxic reference item after 48 h	$> 50 \%$	100 %
Number of aphid mummies in control	≥ 5 per female	16
Number of females producing zero aphid mummies in control	< 2	0

CONCLUSIONS

In a laboratory test to determine the effects of A21857B on the parasitic wasp *Aphidius rhopalosiphi*, the 48-h LR₅₀ was estimated to be 433.1 mL product/ha (95% confidence limits of 384.5 and 487.0 mL product/ha. The NOER with respect to wasp survival was < 375 mL product/ha. In terms of effects on the reproductive performance of surviving wasps, the ER₅₀ was estimated to be > 375 mL product/ha and the NOER was 375 mL product/ha.

(██████████, 2018)

HSE COMMENTS

This study was conducted in accordance with GLP and uses the following test guidance: Mead-Briggs *et al.* A laboratory test for evaluating the effects of plant protection products on the parasitic wasp *Aphidius rhopalosiphi* (2000). The guideline validity criteria have been fulfilled and there were no major deviations to the protocol. The toxic reference item resulted in 100 % mortality but was applied at a rate of 0.10 ml product/ ha, slightly below the guideline recommended rate of 0.3 ml product/ ha. This application rate was said to be based on annual GLP compliant studies performed at the test facility, and since the validity criteria for the reference item were met, it is deemed acceptable.

The LR₅₀ and 95 % confidence intervals were derived using Probit analysis, which is in line with statistical procedures suggested in the guidelines. However, the three highest treatment rates, in which 100 % mortality was observed, were excluded from this analysis. This may have reduced the accuracy of the LR₅₀ estimation due to there being only two data points used in the Probit. Whilst the proposed LR₅₀ of 433.1 ml product/ ha is plausible considering the data, due to the uncertainty surrounding its accuracy, a more conservative LR₅₀ estimate of > 375 ml product/ ha is considered most appropriate for use in risk assessment.

The following endpoints are suitable for use in risk assessment:

- 48 hr LR₅₀ (mortality) = > 375 ml A21857B /ha
- Rate at which $< 50 \%$ effect on reproduction occurred > 375 ml A21857B /ha

Report	KCP 10.3.2.1 - ██████████ (2018). Pydiflumetofen EC (A21857B) – A laboratory bioassay to determine the effects of fresh residues on the predatory mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae). Report Number SYN-18-20. Mambo-Tox Ltd. 2 Venture Road, University Science Park, Southampton SO16 7NP, United Kingdom (Syngenta file no. VV-470237)
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Guideline(s): Blümel et al. (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) for regulatory testing of plant protection products.

GLP: Yes

Duplication (if vertebrate study) Not applicable

Materials

Test Material Pydiflumetofen EC (062.5) (A21857B)

Lot/Batch #: JEA001-116-001

Actual content of active ingredients: 5.69 % w/w (62.4 g /L)

Description: Light-yellow clear liquid

Stability of test compound: Stable under standard conditions

Density: 1,097 kg /m³

Reanalysis/Expiry date: End of February 2019

Treatments

Test rates: 5000, 2500, 1250, 625, and 312.5 mL product /ha

Control: Purified water

Toxic standard: Dimethoate (BAS 152 11 I) (an EC formulation containing nominally 400 g a.s. /L), applied at a rate of 15 mL product /ha

Spray volume rate: 200 L /ha

Application method: Calibrated laboratory track-sprayer (Spray pressure 3 bar, single 80° flat-fan nozzle: Teejet 8003EVS)

Test organisms

Species: *Typhlodromus pyri*

Age: < 24-hr-old protonymphs

Source: In-house culture, originally obtained in April 1995 from P.K. Nützlingszuchten, Welzheim, Germany and was supplemented with further mites from the same source in 1996 and 1997.

Feeding: Tree pollen (1:1 v/v almond (*Prunus* sp. var. a mix of Aldrich, Nonpareil and Wood colony) and apple (*Malus* sp. var. Red Delicious) pollen). Mites were also occasionally fed freshly-collected pollen of the dwarf broad bean, *Vicia faba* L., var. Sutton Dwarf.

Test design

Arenas: Glass plates, with an oblong ring of ‘non-drying sticky insect gel’ drawn onto each plate to make a 3 cm x 4 cm arena in which to confine mites.

Replication: 3 per treatment

No. of mites/arena: 20 protonymphs

Duration of test: 14 days

Environmental test conditions

Temperature: 24.4 - 25.7 °C

Humidity: 69 - 83 %

Photoperiod: 16 h (450 - 1350 lux)

Study Design and Methods

Experimental dates: 4 June 2018 to 18 June 2018

The effects of A21857B on the predatory mite *Typhlodromus pyri* were assessed in a laboratory test. Dilutions were prepared in purified water, shortly before applications were made and the solutions were thoroughly agitated to ensure their homogeneity. Treatments were sprayed onto glass plates which were left to dry and then used to construct the test arenas (Based on the ‘open method’ described by Blümel et al., (2000)). Mites were then introduced to the arenas and their condition was assessed at approximately 24 hours after application, and then after a period of 7-days. Mites were recorded as being: *alive*, *dead*, *stuck*, or *drowned*. Mites that were missing at each assessment were grouped with the *dead* mites for the purposes of data analysis. After this initial 7 days, the mites in the control were now adult. The sex of the adult mites was determined, and the sex ratio was confined to ensure a male to female ratio of at least 1 : 5 males for every female. Any eggs that were produced prior to 7 days after treatment (DAT) were discarded, and the mites were then left *in situ* for a further 7 days, so that their reproduction could be assessed 7, 9, 11, and 14 DAT. The mean number of eggs produced per female between 7 and 14 DAT was calculated.

The sprayer was calibrated in advance of applications using purified water, to confirm a deposition rate at target level equivalent to 200 L/ha (i.e. 2 mg deposit /cm² with an actual range of within $\pm 10\%$ of the target rate, and a mean range of within $\pm 5\%$ of the target rate). Calibration procedures involved spraying and weighing the deposits delivered onto glass plates (10 cm x 10 cm) placed along the spray platform. Three pre-weighed plates were sprayed using purified water and were then re-weighed so that the rate of deposition could be determined.

Once three consecutive applications had delivered the correct deposition rate, treatments were applied in the order of control, test item (in ascending rate order) and finally the toxic reference item. The sprayer was flushed through with purified water before the toxic reference treatment was applied. Once the residues on the treated glass plates had dried, within an hour of application, twenty protonymphal mites were placed on each glass plate with the use of a fine brush.

The mean percentage mortality after 7 days was calculated for the individual treatments and then corrected for any losses in the control treatment using Abbott’s formula. In order to determine the *no-observed-effect rate* (NOER) in terms of mite survival, the percentage mortality in each treatment was compared to that in the control using Fisher’s Exact Test ($\alpha = 0.05$). In order to derive a value for the *median lethal rate* (LR₅₀), a Probit regression analysis was performed on the 7-day mortality data.

In order to determine the NOER for reproduction, the results for eggs per female in each replicate were compared statistically. The data were checked for normality (Shapiro-Wilk, $\alpha = 0.05$) before being compared to the control by Mann-Whitney *U*-test ($\alpha = 0.05$). The *median effect rate* (ER₅₀) for reproduction was estimated by extrapolation from the results.

Results and Discussion

Validity Criteria

The validity criteria were met according to Candolfi et al., (2000): Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products:

Table 9.5.2-2: Validity criteria

Validity criterion	Required	Obtained
Mortality in the controls	< 20 %	0 %
Corrected mortality in the toxic reference control	Corrected mortality rate of > 50 %	95 %

Reproduction in the control	Mean cumulative number of eggs produced in the control from D7 to D14 should be ≥ 4.0 per female.	6.3
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Mortality and fecundity are summarised in the table below:

Table 9.5.2-3: Effects of A21857B on mortality and fecundity of *Typhlodromus pyri*, when exposed under laboratory test conditions

Treatment	Mean % mortality at 7 DAT ^{a)}	Mean corrected % mortality at 7 DAT ^{b)}	Mean eggs/female from 7 to 14 DAT ^{c)}	% Effect on reproduction compared to control ^{d)}
Control	0	-	6.3	-
5000 mL A21857B /ha	100 *	100	~	~
2500 mL A21857B /ha	87 *	87	~	~
1250 mL A21857B /ha	18 *	18	5.0 *	21.4
625 mL A21857B /ha	3	3	4.8	24.7
312.5 mL A21857B /ha	2	2	8.9	-40.5
Toxic reference	95 *	95	~	~

^a Individual treatments were compared to the control using Fisher's Exact Test ($\alpha = 0.05$). Treatments that differed significantly from the control are indicated with an asterisk (*).

^b Calculated using Abbott's formula.

^c The individual test-item treatments were compared to the control by Mann-Whitney *U*-test ($\alpha = 0.05$). The treatment that differed significantly from the control is indicated with an asterisk (*).

^d Egg production, relative to the control. A positive value indicates a decrease and a negative value indicates an increase.

The 7-day LR₅₀ was calculated to be 1667.2 mL product/ha, with 95% confidence limits of 1448.8 and 1862.1 mL product /ha. The 7-day mortality probit curve is displayed in Figure 9.5.2-1 below:

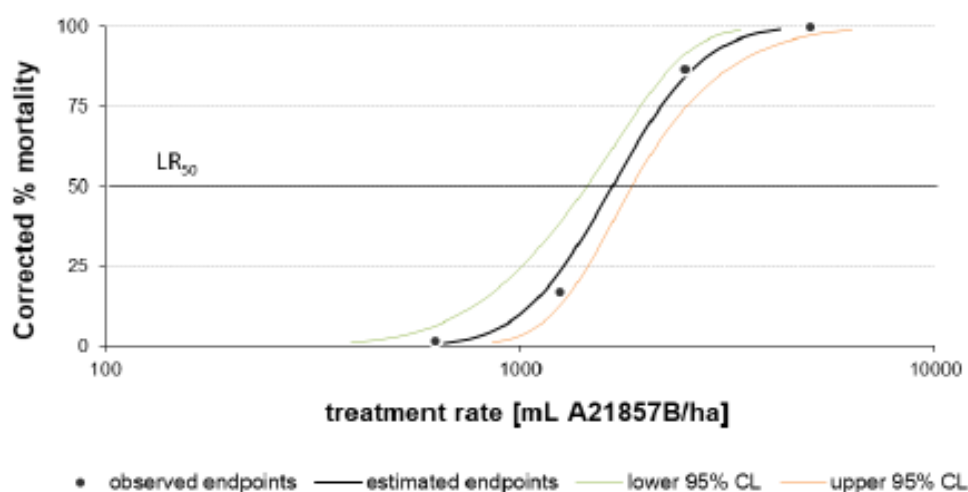


Figure 9.5.2-1: Probit graph for effects of A21857B on *T. pyri* mortality.

The percentage mortality of mites at 7-day, plotted against treatment rate of A21857B (log scale). The data points (●) indicate the mean actual mortality values obtained for each treatment, corrected for the natural response rate estimate of 1.6 %. The curves show the estimated LR_x values, along with their 95 % confidence limits.

Conclusions

In a laboratory test in which the predatory mite *Typhlodromus pyri* was exposed to fresh dry residues of A21857B, the 7-day LR₅₀ was calculated to be 1667.2 mL product/ha with 95% confidence limits of 1448.8, and 1862.1 mL product/ha. The NOER with respect to mite survival was 625 mL product/ha. For the reproduction assessments, the ER₅₀ was estimated to be > 1250 mL product/ha. The NOER with respect to reproduction was 625 mL product/ha.

(██████████, 2018)

HSE COMMENTS

This study was conducted according to GLP, and was based on Candolfi et al., (2000): Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products. All validity criteria were met. The following deviation from the guidelines was noted:

Candolfi et al., (2000) stipulates that a total of 5 replicates per treatment should be used. However, in the current experiment, only 3 replicates were used per treatment. No explanation or justification was provided for this, however, the LR₅₀ 95 % confidence intervals are narrow enough that this shouldn't have any major implications for the validity of the study.

The study authors state that 'when compared statistically, only the 1250 mL product /ha treatment differed adversely from the control (Mann-Whitney U-test, $\alpha = 0.05$)'. However, the 625 mL product /ha condition showed a marginally lower number of mean eggs /female from 7 to 14 DAT than the 1250 mL condition, which resulted in a greater difference when compared to the control result, but it wasn't considered statistically significant. As such, the raw data and statistical procedures were considered. The Mann-Whitney U-test which was used for statistical analysis compares the median values of all the replicates within each condition, and so is more susceptible to influence from anomalous results than if the mean values were used. These statistical procedures used in this study were in line with those recommended by Candolfi et al., (2000).

Based on the nominal concentrations, the 7-day LR₅₀ was calculated to be 1667.2 mL product /ha. The NOER with respect to both mite survival and reproduction was 625 mL product /ha. The ER₅₀ with respect to reproduction was estimated to be > 1,250 mL product /ha.

Report:	K-CP 10.3.2.2, ██████████, (2017), Pydiflumetofen EC (A21857B) – A rate-response extended laboratory bioassay of the effects of fresh residues on the parasitic wasp <i>Aphidius rhopalosiphii</i> (Hymenoptera, Braconidae). Report Number SYN-16-45. Mambo-Tox Ltd., 2 Venture Road, University Science Park, Southampton SO16 7NP, United Kingdom. (Syngenta file No. VV-466923).
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Guideline(s):	Mead-Briggs et al. An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, <i>Aphidius rhopalosiphii</i> (2009)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials

Test Material	A21857B Pydiflumetofen EC (062.5)
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Lot/Batch #:	SYN545974 EC (062.5) JEA001-116-001
Actual content of active ingredients:	SYN545974: 5.69 % w/w corresponding to 62.4 g/L
Description:	Light yellow clear liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	28 February 2019
Density:	1097 kg/m ³
Treatments	
Test rates:	952.6, 1714.7, 3086.4, 5555.6 and 10000 mL A21857B/ha
Control:	Purified water
Toxic standard:	BAS 152 11 I (nominally 400 g dimethoate/L) in purified water, applied at a rate of 10 mL product in 400 L water/ha
Spray volume rate:	400 L spray solution/ha
Application method:	Schachtner track sprayer (3 bar pressure, 80° flat fan nozzle)
Test organisms	
Species:	<i>Aphidius rhopalosiphi</i> De Stefani-Perez. (Hymenoptera: Braconidae)
Age:	< 48 hours
Source:	Culture maintained at Test Facility (originally: Katz Biotech AG, Baruth, Germany)
Feeding:	10 % w/v solution of fructose in water during the test
Test design - Mortality phase	
Arenas:	Clear acrylic cylinders (8 cm diameter, 20 cm high, tops covered with nylon netting) placed over pots containing barley seedlings (<i>Hordeum vulgare</i> L. var. Sienna)
Replication:	6
No. of wasps/arena:	5
Test design - Fecundity phase	
Arenas:	Clear acrylic cylinders (9 cm diameter, 20 cm high, tops covered with nylon netting) were placed over pots containing 15 barley seedlings (<i>Hordeum vulgare</i> L. var. Sienna). The untreated barley had been infested seven days previously with host aphids (>100 adults and nymphs of <i>Metopolophium dirhodum</i> and <i>Rhopalosiphum padi</i>).
Replication:	15 female wasps/treatment
No. of wasps/arena:	1
Duration of test:	Mortality assessment: 48 hours Fecundity assessment: 24 hours Observation of mummies developing: 10 days after adult removal
Environmental test conditions	
Temperature:	Mortality assessment phase: 21 °C Fecundity assessment phase: 21 °C
Humidity:	Mortality assessment phase: 71 – 75 % RH
Photoperiod:	Mortality assessment phase: 16 h photoperiod (1239 lux) Fecundity assessment phase: 16 h photoperiod (4884 lux)

Study Design and Methods

Experimental dates: 11 January 2017 to 24 January 2017

Treatments were applied to barley plants which, once dry, were used to construct the arenas. The wasps were introduced to these arenas and their behaviour and mortality was assessed 2, 3 (behaviour only), 24 and 48 h later.

To assess any sub-lethal effects, reproduction assessments were then carried out for the control and for the test material treatment rates of 1714.7, 3086.4 and 5555.6 mL A21857B/ha. Female wasps were confined individually over untreated aphid-infested barley plants for 24 h, before being removed. The plants were left for a further 10 days before the number of aphid mummies that had developed on plants where wasps had been found alive after the 24-h oviposition period was recorded.

Mortality in the individual treatments was compared to that in the control using Fisher's Exact Test (two-sided, $\alpha = 0.05$). The median lethal rate (LR_{50}) was determined by Probit regression analysis. Prior to analysis, the dose rates were \log_{10} -transformed.

Repellency for each treatment was calculated by obtaining a mean value for each replicate. These values were angularly transformed (square root arcsine) and were checked for normality (Shapiro-Wilk test, $\alpha = 0.05$) and for homogeneity of variance (Levene's test, $\alpha = 0.05$). Treatments were then individually compared to the control by one-way ANOVA & Dunnett's t-test ($\alpha = 0.05$) or by Mann-Whitney U-test ($\alpha = 0.05$), for normally and non-normally distributed data, respectively.

The numbers of mummies produced per female found alive after the 24-h parasitism period were analysed by one-way ANOVA and Dunnett's t-test or the Mann-Whitney U-test, for normally or non-normally distributed data, respectively, of the square root-transformed data. Treatments were considered statistically significantly different where $P < 0.05$. The percentage change in numbers of mummies produced in individual test item treatments, relative to the control, was also calculated.

Results and Discussion

Mortality and reproduction are summarised in the table below.

9.5.2-4: Effects of fresh residues of A21857B on mortality, wasp repellence, and reproduction of *Aphidius rhopalosiphi*, when exposed under extended laboratory test conditions

Treatment (mL A21857B/ha)	Mean % mortality at 48 h ^a	Mean % corrected mortality at 48 h (M-value) ^b	% observations where wasps recorded to be settled on the treated plants during initial 3 h ^c	Number females successfully assessed for reproduction	Mean number mummies per surviving female ^c	% Effect on reproduction compared to control (R-value) ^d
Control	0.0	-	41.3	14	20.6	-
952.6	0.0 [#]	0.0	36.0	n.d.	n.d.	n.d.
1714.7	6.7	6.7	44.0	14	19.6	4.5
3086.4	3.3	3.3	34.7	14	22.9	-11.1
5555.6	10.0	10.0	37.3	15	19.9	3.1
10000	73.3 [*]	73.3	36.0	n.d.	n.d.	n.d.
Toxic reference	86.7 [*]	86.7	24.7	n.d.	n.d.	n.d.
LR ₅₀ value (mL A21857B/ha)	8087.1 (95 % confidence limits 6758.4 – 10296.8)		n.d.	n.d.		
NOER value (mL A21857B/ha)	5555.6		n.d.	5555.6		

^a The results for individual treatments were compared to the control using Fisher's Exact Test ($\alpha = 0.05$) and an asterisk (*) indicates where they differed significantly

^b Derived using Abbott's formula

^c The data were analysed either by one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$), or by Mann-Whitney U-test ($\alpha = 0.05$); none of the treatments differed significantly from the control

^d Percentage effect on reproduction; a negative value indicates an increase relative to the control, a positive value a decrease

[#] The result for the 952.6 mL/ha treatment rate, in which 0 % mortality was observed, was excluded from the Probit regression analysis

n.d. = not determined

Validity criteria

The validity criteria are listed below:

- Mortality within the control treatment at 48 hours was 0 % (should not exceed 10 % (i.e. 3 wasps from 30))
- Mortality within the toxic-reference treatment at 48 hours was 86.7 % (should exceed 50 %)
- The mean number of mummies in the control treatment was 20.7 (must be > 5.0 per female)
- There were no zero values in the control treatment (should not exceed 2)

Conclusions

In an extended laboratory test to determine the effects of A21857B on the parasitic wasp *Aphidius rhopalosiphi*, the 48-h LR₅₀ was 8087.1 mL A21857B/ha. Based on statistical comparison with the control, the NOER for mortality was 5555.6 mL A21857B/ha.

The median effect rate (ER₅₀) for A21857B could not be determined. Based on statistical comparison with the control, the NOER for reproduction was 5555.6 mL A21857B/ha.

(████████, 2017)

HSE Comments

Validity Criteria (Mead-Briggs <i>et al</i> 2009)	Required	Obtained
Mortality in the control treatment	≤ 10 %	0 %
Mortality (corrected) in the toxic reference	> 50 %	86.7 %
Mean number mummies per female in control	> 5	20.7
Number of zero values for mummies in the control	< 2	0

This study was carried out according to GLP and followed Mead-Briggs *et al.* (2009) with no deviations to the guideline. All validity criteria outlined in Mead-Briggs *et al.* have been satisfactorily met.

The toxic reference item dimethoate was tested alongside the treatment concentrations. The application rate was 10 mL product/400 L water/ha. This is in line with guidance in Mead-Briggs *et al.* (2009). Mortality in the toxic reference was 86.7 %, within the recommended range. HSE accepts that the species shows appropriate sensitivity.

Before application of test treatments, the sprayer was calibrated on three plates, in line with Mead-Briggs *et al.* (2009) guidelines.

There was no need to correct mortality as the mortality in the control was 0 %. 48-hour mortality was compared to the control using Fisher's Exact Test, the dose rates were log₁₀ transformed and then Probit analysis was performed in order to determine the LR₅₀. This meets the guidance in Mead-Briggs *et al.* (2009) which states a suitable comparison procedure should be performed. Repellency was calculated using a mean value for each replicate. The data was angularly transformed and checked for normality and homogeneity using Shapiro-Wilks and Levene's test. ANOVA and Dunett's test and Mann-Whitney U-test was used for comparison to the control. This is within recommended guidelines in Mead-Briggs *et al.* (2009).

The agreed endpoints suitable for use in the risk assessment is;

- < 50 % mortality and reproductive effects compared to control at 5555.6 mL product/ha
- 48h LR₅₀ 8087 mL product/ha

Report: K-CP 10.3.2.2, [REDACTED], (2017), Pydiflumetofen EC (A21857B) – A rate-response extended laboratory bioassay of the effects of fresh residues on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae). Report Number SYN-16-44. Mambo-Tox Ltd., 2 Venture Road, University Science Park, Southampton SO16 7NP, United Kingdom. (Syngenta file No. VV-467035)

Guidelines

- Blümel *et al.* (2000) Laboratory residual contact test with the predatory mite *Typhlodromus pyri* for regulatory testing of plant protection products. In: Candolfi *et al.*, (2000).

GLP: Yes

Materials

Test Material	A21857B (formulation) Pydiflumetofen EC (062.5)
Lot/Batch #:	JEA001-116-001
Actual content of active ingredients:	SYN545974: 5.69 % w/w corresponding to 62.4 g/L as stated on Certificate of Analysis from study sponsor
Description:	Light yellow clear liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	28 February 2019
Density:	1097 kg/m ³
Treatments	
Test rates:	312.5, 625, 1250, 2500 and 5000 mL A21857B/ha
Control:	Purified water
Toxic standard:	BAS 152 11 I (420.3 g dimethoate/L), applied at a rate of 37.5 mL product/ha
Spray volume rate:	200 L spray solution/ha
Application method:	Laboratory track sprayer, 3 bar spray pressure, 80° flat-fan nozzle (Teejet 8003EVS). Calibrated by weighing the deposition of purified water in 3 runs of 3 plates (mean percentage of target rate 101.8 – 102.8 %).
Test organisms	
Species:	<i>Typhlodromus pyri</i> (Acari: Phytoseiidae)
Age:	Less than 24 h old protonymphs
Source:	Culture maintained at Test Facility (originally: P.K. Nützlingszuchten, Welzheim, Germany)
Feeding:	1:1 v/v almond (<i>Prunus</i> sp. var. Butte) and apple (<i>Malus</i> sp. var. Red Delicious) pollen
Test design	
Arenas:	Leaf discs, 5 cm diameter (cut from the flattened sections of the first true leaves of dwarf French bean plants <i>Phaseolus vulgaris</i> var. The Prince). Discs were mounted on damp cotton wool with a ring of a sticky gel drawn around the edge to create a circular arena in which the mites were confined. Spray was applied to the adaxial (upper) leaf surface of the discs.
Replication:	3
No. of mites/arena:	20
Duration of test:	14 days
Environmental test conditions	
Temperature:	24 to 26 °C
Humidity:	52 to 78 % RH ¹
Photoperiod:	16 h photoperiod, 550 to 1400 lux

¹ The relative humidity fell below the intended range for two separate periods of less than two hours; this did not affect the controls meeting the validity criteria so is not considered a significant deviation.

Study Design and Methods

Experimental dates: 06 December 2016 to 20 December 2016

Treatments were applied to the leaf discs and the bioassay was initiated within 1 h of application, once residues had dried. The leaf discs were placed onto damp cotton wool and a ring of a sticky non-drying gel drawn around the edge of each to create circular arenas in which mites were confined. Approximately 1 mg of untreated pollen was sprinkled on each leaf disc and this was replenished with untreated pollen daily thereafter. The water level in the dishes was topped up every day so that the cotton wool did not dry out. The survival of the mites was assessed at approximately 24 hours and 7 days after treatment (DAT). Any eggs that were produced prior to 7 DAT were removed and discarded. The sex of the adult mites was determined and they were then left in situ so that their reproduction could be assessed at 10, 13 and 14 DAT. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated.

The percentage mortality (including any stuck, drowned or missing mites) at each treatment rate was corrected for mortality in the control treatment using Abbott's formula (Abbott, 1925). The data for mortality at 7 days were analysed by Probit regression analysis, to determine the median lethal rate (LR₅₀). Prior to analysis, the dose rates were log₁₀-transformed. The level of background mortality was estimated by the software taking account of all available data. The 95 % confidence intervals for the LR₅₀ value were calculated and a Chi-square test for goodness of fit ($\alpha = 0.05$) was performed on the Probit line.

Mortality in the individual test item treatments was compared to that in the control treatment using Fisher's Exact Test ($\alpha = 0.05$). The data for mite reproduction were first checked for normality (Shapiro-Wilk test, $\alpha = 0.05$) and for homogeneity of variance (Levene's test, $\alpha = 0.05$), and were then statistically analysed by one-way Analysis of Variance and Dunnett's t-test ($\alpha = 0.05$). All values were calculated using the original raw data and were not based on rounded values.

Statistical analyses were performed using computer software SPSS (2013).

Results and Discussion

Mortality and fecundity are summarised in the table below. When compared statistically, none of the test-item treatments differed significantly from the control (one-way ANOVA and Dunnett's t-test, $\alpha = 0.05$).

Table 9.5.2-5: Effects of formulation A21857B on mortality and fecundity of *Typhlodromus pyri*, when exposed under extended laboratory test conditions

Treatment (mL A21857B/ha)	Mean % mortality at 7 DAT ^a	Mean corrected % mortality at 7 DAT ^b	Mean eggs/female from 7 to 14 DAT ^c	% Effect on reproduction compared to control ^d
Control	13	-	7.6	-
312.5	12	-2	-	-
625	15	2	-	-
1250	22	10	9.0	-18.8
2500	32*	21	6.8	9.9
5000	57*	50	5.1	33.3
Toxic reference	100*	100	-	-
LR ₅₀ value (mL A21857B/ha) ^e	5152.3 (95 % CI 3140.5 – 34673.7)		n.d.	

^a Individual treatments were compared to the control using Fisher's Exact Test ($\alpha = 0.05$); treatments that differed significantly from the control are indicated with an asterisk (*)

^b Calculated using Abbott's formula (Abbott 1925)

^c The test-item treatments were compared to the control by one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$); none of the results differed significantly

^d Egg production relative to the control; a positive value indicates a decrease and a negative value indicates an increase

^e Median lethal rate derived by Probit regression analysis (see methods). Chi-square test for goodness of fit ($\alpha = 0.05$) was performed on the Probit line: χ^2 with 12 d.f. 19.231 ($p > 0.05$).

n.d. = not determined

Validity criteria

For the test to be deemed valid, the protocol indicated that:

- Mortality in the control treatment was 13 % (must not be > 20 %)
- Mortality in the toxic reference treatment was 100 % (must be over 50 %)
- The mean cumulative number of eggs produced from 7 to 14 days was 7.6 per female in the control treatment (must be ≥ 4.0)

Conclusions

The 7-day LR_{50} for effects of A21857B on mortality of *Typhlodromus pyri* under extended laboratory test conditions was calculated to be 5152.3 mL A21857B/ha.

The test item did not have more than 50 % effects on the reproduction of the surviving mites at treatment rates of up to and including 5000 mL A21857B/ha.

(██████████, 2017)

HSE Comments

This study was conducted to GLP and follows guideline Blümel *et al.* (2000) with the exception that the test substance was sprayed onto a natural leaf substrate rather than glass plates. This deviation is acceptable as it is to allow for extended study test conditions as set out by the data requirements regulation 284/2013.

Additionally, a higher reference item test concentration is used than recommended in the guideline: 37.5 ml formulated product/ha was tested compared to the recommended 9-15 mL product/ha. However, the recommended rate is from ring-tests on glass plates rather than leaf substrate, and there is no ring-test data available for *T. pyri* on leaf substrates. The test rate in this study was justified by the authors as being based on internal GLP-compliant validation studies. Therefore, the higher test rate is acceptable for demonstrating the sensitivity of the test system, and any uncertainty surrounding the behaviour of the test and reference items on leaf substrates compared to glass plates is unavoidable.

No reproduction data is provided for the lowest two tested concentrations of 312.5 and 625 mL A21857B/ha and no reason is given as to why this was not assessed. However, the data for the remaining test concentrations of 1250, 2500 and 5000 mL A21857B/ha all show effects under 50 %, and therefore there is enough information to provide an endpoint to this effect level.

The authors statistically determined a LR_{50} of 5152.3 (95 % CI 3140.5 – 34673.7) from the mortality data. Due to unacceptably large confidence limits, and the LR_{50} value being extrapolated above the highest tested concentration, this statistically derived endpoint is not considered appropriate for use in risk assessment. Instead, the LR_{50} below is estimated by HSE from the data in Table 9.5.2-5, which shows a control-corrected mean 7-day mortality of 50 % at the 5000 mL A21857B/ha treatment level.

The agreed endpoints for use in risk assessment are:

- **LR_{50} : 5000 mL A21857B formulated product/ha (not statistically determined – estimated from highest tested concentration) (nominal concentration)**
- **Highest rate with < 50 % effects on reproduction: 5000 mL A21857B formulated product/ha (nominal concentration)**

Report: K-CP 10.3.2.2 [REDACTED], (2019), Pydiflumetofen EC (A21857B) – A Rate-Response Extended Laboratory Study to Evaluate the Effects of Fresh Residues on the Green Lacewing, *Chrysoperla carnea* (Neuroptera, Chrysopidae). Report Number SYN 19 26. Mambo-Tox, A Division of Cawood Scientific Ltd., 2 Venture Road, University Science Park, Southampton SO16 7NP, United Kingdom. (Syngenta file no VV-732035)

GUIDELINES

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

GLP: Yes

MATERIALS

Test Material	Pydiflumetofen EC A21857B
Lot/Batch #:	JEA002-052-001
Actual content of active ingredients:	Pydiflumetofen: 5.66% w/w (62.1 g/L)
Density:	1097 kg/m ³
Treatments	
Test rates:	3200, 1600, 800, 400 and 200 mL product/ha
Control:	Purified water
Toxic standard:	Dimethoate (an EC formulation containing nominally 400 g a.s./L), applied at a rate of 80 mL product/ha
Spray volume rate:	200 L spray solution/ha
Application method:	Calibrated laboratory track-sprayer (Chr. Schachtner, Ludwigsburg, Germany), 80° flat-fan nozzle (Teejet 8003EVS), spray pressure 3 bar.
Spray calibration:	Sprayer calibrated using purified water to confirm deposition rate. Three consecutive applications used to confirm rate.
Test organisms	
Species:	<i>Chrysoperla carnea</i> Steph. (Neuroptera: Chrysopidae)
Age:	2-3 days at start of test
Source:	Lacewing eggs obtained from culture maintained at Test Facility
Feeding:	Larvae: UV light-killed eggs of <i>Sitotroga cerealella</i> , provided every 2-3 days. Adults: artificial diet, honey water and purified water, provided 3 times per week.
Test design - Mortality phase	
Arenas:	Excised French bean leaf sandwiched between 7.5 cm x 7.5 cm glass plate and Perspex sheet, with 5-cm-diameter plastic collar treated with Fluon to confine larva. Ventilated lid placed on top.
Replication:	40 per treatment rate
No. of larvae/arena:	1
Test design - Fecundity phase	
Arenas:	Polystyrene box (15 cm x 27 cm x 10 cm) with close fitting lid. Fibrous tissue placed under each lid as oviposition site.

Replication:	2 boxes per treatment analysed (not considered as replication for statistical purposes).
No. of lacewings/arena:	16-20 adults.
Duration of test:	40 days
Environmental test conditions	
Temperature:	23.8-25.9 °C
Humidity:	63-78 %
Photoperiod:	16-hour photoperiod 2700-4000 lux.

STUDY DESIGN AND METHODS

Experimental dates: 28 August 2019 – 07 October 2019

Mortality phase:

Dilutions of the test item were prepared in purified water, shortly before treatments were applied, and the solutions were thoroughly agitated to ensure their homogeneity. Treatments (3200, 1600, 800, 400 and 200 mL A21857B/ha; control and toxic reference) were sprayed onto the target leaves (first true leaves of *Phaseolus vulgaris*) using a laboratory track-sprayer. The sprayer was calibrated in advance using purified water to confirm a deposition rate at the target level (2 mg deposit/ cm², with an actual range within ± 10 % of the target rate and mean range of within ± 5 % of the target rate). A visual inspection of the leaves was made, to confirm that an even deposition had been achieved across them. Once dry, the leaves were used to line the floor of simple test arenas. A single larva was confined in each arena, with 40 replicates (i.e. a total of 40 larvae) prepared for each treatment. Assessments of treatment effects were made every 1-3 days until the larvae pupated. Larvae were characterised as *Alive*- apparently healthy and unaffected. *Abnormal pupa*- larvae pupating without spinning a cocoon or appearing different from norm. *Dead*- no longer moving. *Pupated*- larvae having pupated. As pupae developed, they were collected before hatching of the adults, but were not removed from the surface to which they were attached. The number of successfully emerging adult lacewings was then recorded.

Reproductive phase:

To assess sub-lethal effects on reproduction, assessments were then carried out for the control and for the test item treatment rates of 3200, 1600 and 800 mL/ha, where corrected mortality was ≤ 50 %. The adults in the individual treatments used for the assessments emerged within 7 days. The sex of the adult lacewings was determined by eye, based on abdomen shape, and they were transferred to polystyrene oviposition boxes. They were divided between two boxes per treatment, simply for the convenience of processing the assessments, since too many insects in one box makes the process unwieldy. The ratio of males to females was 9:9/8:9; 10:7/10:6; 6:10 and 9:11/8:10 in replicates A and B of the control, 3200 ml, 1600 ml and 800 ml treatment groups respectively. Assessments commenced approximately 9 days after the majority (> 75 %) of adult lacewings had emerged in the control, 7 days after egg laying had first been noted. Eggs were sampled and counted by removing and replacing the fibrous tissue used to line the lids of the boxes over two 24-h periods. The eggs were then maintained for a further 7 days in order to assess the number that successfully hatched.

Statistical analysis:

The percentage pre-imaginal mortality of the insects in the bioassay was calculated. Pre-imaginal mortality was defined as the numbers of insects that did not successfully reach adulthood. The corrected percentage mortality (taking into account any control treatment losses) was derived using Abbott's formula. The *median lethal rate* (LR₅₀) was estimated by extrapolation from the data, since corrected mortality did not exceed 50% in any of the test-item treatments. Where there was test item treatment mortality, this was also compared to mortality in the control using Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm (one-sided, $> \text{control}$, $\alpha = 0.05$). Analyses were performed with validated computer software- ToxRatPro® (ToxRat Solutions GmbH, 2015). For the reproduction assessments, effects on lacewing reproduction in the individual test-item treatments are normally assessed on the basis of 'triggers', as specified in the guideline of [REDACTED] *et al.* (2000). Namely, if treatment effects are to be deemed harmless, there should be a mean of ≥ 15 eggs produced per female per day ($n = 2$) and the mean egg-hatching rate should be ≥ 70 %.

RESULTS AND DISCUSSION

Pre-imaginal mortality and reproduction are summarised in the table below.

Table 9.5.2-6: Effects of fresh residues of A21857B on mortality and reproduction of *Chrysoperla carnea*, when larvae exposed under extended laboratory test conditions.

Treatment	Mean % pre-imaginal mortality ^a	Mean % corrected pre-imaginal mortality (M-value) ^b	Mean number eggs/female/day ^c	Mean % egg viability ^d	Mean viable eggs/female/day
Control	10.0	-	35.5	90.0	31.9
3200 mL A21857B/ha	15.0	5.6	34.7	90.0	31.2
1600 mL A21857B/ha	17.5	8.3	31.1	90.8	28.2
800 mL A21857B/ha	2.5	-8.3	29.5	89.3	26.3
400 mL A21857B/ha	5.0	-5.6	-	-	-
200 mL A21857B/ha	7.5	-2.8	-	-	-
Toxic reference	100 *	100	-	-	-

^a The results for individual test item treatments were compared to the control using Multiple Sequentially rejective Fisher's Exact Test after Bonferroni-Holm (one-sided, > control, $\alpha = 0.05$). The result for the toxic reference treatment was compared to the control using Fisher's Exact Binomial test (one-sided, control, $\alpha = 0.05$). Significant differences are indicated by an asterisk (*).

^b Derived using Abbott's formula.

^c Based on two 24-h long assessments made for each oviposition box in each treatment.

^d Based on all eggs laid on the fibrous tissue sheet lining the lid of each oviposition box.

- Treatment not assessed.

VALIDITY CRITERIA

The test was considered valid;

Validity criterion	Required	Observed
Pre-imaginal mortality within the control treatment	$\leq 20 \%$	10 %
Pre-imaginal mortality within the toxic-reference treatment	$> 50 \%$	100 %
Mean egg production per female per day in the control treatment	≥ 15 per female	35.5
Mean egg viability in the control treatment	$\geq 70 \%$	90.0 %

CONCLUSIONS

In an extended laboratory test in which the foliar-active predator *Chrysoperla carnea* was exposed to freshly-dried foliar residues of A21857B, the LR₅₀ was > 3200 mL product/ha, the highest rate evaluated. The NOER with respect to lacewing survival was deemed to be 3200 mL product/ha. With respect to lacewing reproduction, the ER₅₀ was > 3200 mL product/ha and the NOER was 3200 mL product/ha. The overall NOER was 3200 mL product/ha.

(██████████, 2019)

HSE COMMENTS

This study was an extended laboratory study, adapted from the test guideline of [REDACTED], et al. (2000) for use with a natural test substrate; leaves of the French bean, *Phaseolus vulgaris* L. The study was performed in accordance with GLP and the validity criteria outlined in the study guideline have been satisfactorily fulfilled. Excluding the use of leaf discs over glass plate substrates, no major protocol deviations are noted. The toxic reference item Dimethoate was applied at a rate of 80 ml product/ha and resulted in 100 % mortality. This application rate exceeds that suggested in the guideline (30-45 ml product/ha, resulting in 60 % mortality) and may raise concern over the sensitivity of the test system. However, the guideline recommended rate is optimized for use with glass-plates, rather than leaf substrate and the rate used of 80 ml product/ ha was said to be based on historical data from GLP compliant studies. Taking this into consideration and the fact that the validity criteria for control and reference treatments were met, the reliability of the results is not thought to have been affected.

The LR₅₀ could not be calculated statistically due to < 50 % corrected mortality being observed in the treatments and was therefore estimated to be > 3200 ml product/ha, the highest treatment level tested. The NOER was determined statistically using Multiple sequentially-rejective Fisher Tests after Bonferroni-Holm, which is deemed suitable based on guideline recommendations. Effects on reproductive performance were assessed qualitatively. There were no treatment-related effects with regard to reproductive performance, as ≥ 15 eggs were produced per female per day (actual; 35.5) and mean egg-hatching rate was ≥ 70 % (actual; 90.0 %). Therefore, the following endpoints are concluded suitable for risk assessment:

- LR₅₀ > 3200 ml A21857B/ha
- NOER (survival and reproduction) = 3200 ml A21857B/ha

B.9.6. RISK ASSESSMENT FOR ARTHROPODS

B.9.6.1. Risk assessment for bees

B.9.6.1.1. Toxicity

A summary of submitted studies and the associated endpoints can be found in Table 9.6.1-1 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 9.6.1-1. Toxicity endpoints for Pydiflumetofen for risk assessment for bees (all studies on *Apis mellifera*)

insecticide)

Test substance	Study type	Endpoint	Value	Reference
Acute Adult Studies				
Pydiflumetofen SYN545974 (active substance)	Oral, limit test	24h & 48h LD ₅₀	>116 µg a.s./bee (consumed)	[REDACTED], (2012)
	Contact, limit test	24h & 48h LD ₅₀	>100 µg a.s./bee	
A19649B	Oral	24h & 48h LD ₅₀	>1132 µg A19649B/bee (consumed) >210.6 µg a.s./bee ^c (consumed)	[REDACTED], (2015)
	Contact	24h & 48h LD ₅₀	>1000 µg A19649B/bee >186 µg a.s./bee ^c	
A21857B (Miravis Plus)	Oral	24h & 48h LD ₅₀	>423 µg A21857B/bee ^b (consumed) >24.07 µg a.s./bee ^{b,f} (consumed)	[REDACTED], (2016)
	Contact	24h & 48h LD ₅₀	> 1000 µg A21857B/bee >56.9 µg a.s./bee ^f	
Chronic Adult Studies				
A19649B	Adult 10d chronic ^{a,b}	10d NOED ^{a,b}	757 µg A19649B/bee/day (consumed) 138.2 µg a.s./bee/day (consumed) 3854 mg a.s./kg diet	[REDACTED], (2014)

Test substance	Study type	Endpoint	Value	Reference
		10d LD ₅₀ ^{a,b}	>757 µg A19649B/bee/day (consumed) >138.2 µg a.s./bee/day (consumed) >3854 mg a.s./kg diet	
Chronic Larval Studies				
Pydiflumetofen SYN545974 (active substance)	Larval 22d, repeat exposure, limit test ^h	8d & 22d LD/ED ₅₀	>0.014 µg consumed a.s./larva ⁱ >0.0035 µg consumed a.s./larva/day ⁱ >0.09 mg a.s./kg diet ^{e,i}	[REDACTED], (2015)
		8d & 22d NOED	n.d. (unbounded) <0.014 µg consumed a.s./larva ⁱ <0.0035 µg consumed a.s./larva/day ⁱ <0.09 mg a.s./kg diet ^{e,i}	
A19649B	Larval 22d, repeated exposure	8d LD ₅₀	45.24 µg consumed a.s./larva ^d 11.31 µg consumed a.s./larva/day	[REDACTED], (2015a); Further statistics and discussion: [REDACTED], (2016); [REDACTED], (2016)
		22d ED ₅₀	7.64 µg consumed a.s./larva ^d 1.91 µg consumed a.s./larva/day	
		8d NOED	n.d. (unbounded) <0.06 µg consumed a.s./larva <0.015 µg consumed a.s./larva/day <0.409 mg a.s./kg diet	
		22d NOED	0.06 µg consumed a.s./larva 0.015 µg consumed a.s./larva/day 0.409 mg a.s./kg diet	
A19649B	Larval 8d, repeated exposure	8d NOED	55 µg a.s./larva ^g 13.75 µg a.s./larva/day 347 mg a.s./kg diet	[REDACTED], (2015); Further statistics: [REDACTED], (2016a)
		8d LD ₅₀	> 109.9 µg a.s./larva ^g >27.48 µg a.s./larva/day > 695 mg a.s./kg diet	
Semi-field and Colony Feeding Studies				
Pydiflumetofen SYN545974 (active substance)	Chronic brood colony feeding, field conditions, [REDACTED] 1992, OEPP/EPPO 1992. Pydiflumetofen via oral exposure to honeybees (tested up to 32.0 mg a.s./kg diet) does not adversely affect colony development and survival. See text for further discussion.			[REDACTED], (2018)
A19649B	Semi-field bee brood tunnel flower spray test, at concentrations of 375, 625			[REDACTED], (2017)
A19649B	and 1000 mL product/ha; equivalent to 75, 125 and 200 g a.s./ha. No significant adverse effects of test item on the colonies. See text for further			[REDACTED], (2017)
A19649B	discussion.			[REDACTED], (2018)

n.d.: not determinable/unbounded

^a Note there is uncertainty in reliability of endpoint for this study as there were no analytical measurements were provided and there were no corrections for evaporative loss.

^b Repellence/unpalatability observed in the higher test concentrations; this has been taken into account in the endpoint by using consumed values.

^c Calculated by HSE from formulation amount using a.s. content of 18.6 % w/w as stated on certificate of analysis from study sponsor.

^d There is some uncertainty with these ED/LD₅₀ endpoints due to wide confidence intervals. LC and EC_{10/20} were also calculated in the study, but were unreliable due to extrapolation outside tested concentrations and wide confidence intervals.

^e calculated by HSE from stated dose of 0.1 mg a.s./L diet, where diet was stated to be a density of 1.1 mg/µl. Therefore, 1 L diet = 1.1 kg, so 0.1 mg a.s./L diet = 0.1/1.1 mg a.s./kg diet = 0.09 mg a.s./kg diet.

^f Calculated by HSE from formulation amount using a.s. content of 5.69 % w/w as stated on certificate of analysis from study sponsor.

^g Endpoint does not take into consideration repellence/unpalatability at the two highest test concentrations of 109.9 or 55.0 µg a.s./larva: left over food and corresponding reduction in larval development observed in 35 % and 21 % of remaining larvae on day 8, respectively).

^h As this was a single dose study, no EC_{10/20} could be calculated.

ⁱ Endpoints use measured concentration, as the analytical measurements of the test solutions were outside the 80-120 % range of nominal (actual : -33.3 and -57.3 % of nominal).

B.9.6.1.1.1. Studies conducted with the active substance

The applicant submitted the following active substance studies on Pydiflumetofen SYN545974:

- Adult acute contact and oral (single concentration limit test)
- Larval 22-day test, repeated exposure (single concentration limit test)
- Higher tier chronic brood colony feeding, field conditions (multiple concentrations tested)

B.9.6.1.1.1.1. Active substance adult acute studies

An acute adult oral and acute contact study on the active substance Pydiflumetofen SYN545974 for the honeybee *Apis mellifera* (██████, 2012), was performed as a limit test, and was deemed to be a reliable study after evaluation with the endpoints suitable for use in risk assessment (see Volume 3CA-B9). The oral endpoint was expressed as consumed dose. No unpalatability was observed at this test concentration.

B.9.6.1.1.1.2. Active substance larval study

A 22-day laboratory study on honeybee larvae with repeated oral exposure (██████, 2015) was submitted and was considered valid after evaluation. This study was conducted as a limit test based on the maximum achievable solubility of the active substance in water. There were issues regarding the concentration of the active substance in the test solution, hence the measured analytical concentration is used in the endpoints. A statistically significant effect was observed for both 8-day mortality and 22-day emergence (control-corrected 21.2 % and 38.7 % respectively) at the measured concentration of the limit dose tested, of 0.014 µg a.s./larva (0.0035 µg a.s./larva/day). Therefore, the NOED, which is the key endpoint to this study, is an unbounded value of <0.0035 µg a.s./larva/day (Table 9.6.1-1) which means that it is not possible to use it in either a quantitative or qualitative risk assessment. It is however noted that the study does raise concerns that there are potential effects at a low dose of the active substance.

B.9.6.1.1.1.3. Active substance colony-feeding study

A brood colony feeding study under field conditions (██████, 2018), broadly based on ‘The Oomen method’ was submitted, investigating repeated oral exposure (for 9 days) of the test item in five different concentrations of the active substance from 2.5 – 32.0 mg a.s./kg food. This study was conducted in late summer (23 Jun – 28 Aug 2017). Significantly higher adult mortality was found in four out of five test item treatments, however, there was no clear dose response and the results were attributed to natural variation (see Volume 3CA B9 for further details). There are no specific validity criteria for this type of study, however it has been concluded that the endpoint was deemed applicable for use in risk assessment.

No statistical tests were carried out for pupal mortality which introduces some uncertainty, but the pupal mortalities were low (0.1-1.3 pupae/colony/day in the post-exposure phase DAT 10-26 across all treatment groups, compared to 0 and 10.8 pupae/colony/day for the control and reference item, respectively). The brood termination rate (BTR), brood index (BI), brood compensation index (BCI) indices were measured for initially labelled eggs only, and found no significant differences compared to the control. Based on the results of this study, the HSE evaluator concluded that pydiflumetofen via oral exposure to honeybees (tested up to 32.0 mg pydiflumetofen/kg food) does not adversely affect colony development and survival.

B.9.6.1.1.2. Studies conducted with formulations

Data is available for two formulations: A21857B (Miravis Plus), which is the representative product for the GB assessment of this active, and A19649B which was the representative product for the EU assessment of this active.

For A21857B (Miravis Plus), only an adult acute contact and oral study were submitted. Other data are available for the alternative formulation A19649B. This A19649B laboratory dataset includes an adult acute oral and contact study (██████, 2015), an adult 10-day chronic study (██████, 2014), a larval repeated exposure test for 22 days (██████, 2015a) and a larval repeated exposure test for 8 days (██████, 2015). A further three semi-field

bee brood tunnel tests with exposure to sprayed flowering crop were also submitted, which included residue data (██████, 2017; ██████, 2017; ██████, 2018).

The two formulations are not considered chemically comparable (see confidential dossier Volume 4 section C.1.3.5). Additionally, the differences between the two would be defined as major changes according to the ecotoxicology CRD formulation guidance 2022. In light of this, HSE has attempted to determine if it is appropriate to use data on A19649B to support the assessment of Miravis Plus A21857B, on the basis of comparable acute toxicity data.

B.9.6.1.1.2.1. Justification for use of A19649B data to assess risk for Miravis Plus A21857B

Both the adult acute oral and contact studies for formulation A19649B (██████, 2015) and the representative product A21857B (Miravis Plus) are considered valid. On the basis of the acute toxicity data, both formulations are of low toxicity to bees (see overall endpoints in Table 9.6.1-1 above, and more detail in Table 9.6.1-2, Table 9.6.1-3 below). By considering the effects within each study (Table 9.6.1-2 below), it is seen that mortalities do occur during the study and that these are similar in each study. However, it is further noted that both LD₅₀ endpoints are unbounded, making direct comparison difficult.

Table 9.6.1-2. Acute adult honeybee oral and contact toxicity dataset comparison between formulations A19649B and Miravis Plus (A21857B).

Consumed oral treatment (µg a.s./bee)	Mean control-corrected mortality (oral test) after 48 hours (%)		Contact treatment (µg a.s./bee)	Mean control-corrected mortality (contact test) after 48 hours (%)	
	A19649B ██████ (2015)	A21857B ██████ (2016)		A19649B ██████ (2015)	A21857B ██████ (2016)
0 (Control)	0	0	0 (Control)	2.5	2.5
2.88	-	0	3.56	-	0.0
7.00	-	0	7.11	-	5.0
13.3	-	0	11.6	0.0	-
13.4	5	-	14.2	-	12.5
13.9	-	0	23.3	0.0	-
24.1	-	2.5	28.5	-	2.5
26.6	5	-	46.5	0.0	-
55.2	2.5	-	56.9	-	0.0
106	0	-	93.0	0.0	-
210.6	2.5	-	186.0	0.0	-
LD ₅₀	>210.6	>24.1	LD ₅₀	>186	>56.9

Table 9.6.1-3 Acute adult honeybee oral and contact toxicity study comparison summary between formulations A19649B and Miravis Plus A21857B

Type of study	Study parameter	A19649B (██████ (2015))	A21857B Miravis Plus (██████ (2016))
Adult acute oral	Maximum oral test concentration, nominal	1000 µg nominal prod/bee = 186 µg nominal a.s./bee	1000 µg nominal prod/bee = 56.9 µg nominal a.s./bee
	Maximum oral test concentration, consumed	1132 µg consumed prod/bee = 210.6 µg consumed a.s./bee	423 µg consumed prod/bee = 24.1 µg consumed a.s./bee
	LD ₅₀ 48 hr (oral)	>1132 µg consumed prod/bee >210.6 µg consumed a.s./bee	>423 µg consumed prod/bee >24.1 µg consumed a.s./bee
	Actual control-corrected mortality 48 hr at highest test conc. (%) (oral)	2.5 %	2.5 %

	Sub-lethal effects at highest test concentration	Some single bees apathetic/affected (max 10 %) until end of test (48 hours), but endpoint protective	No behavioural abnormalities, but there was unpalatability/ food avoidance (see nominal vs consumed conc.)
Adult acute contact	Maximum contact test concentration (nominal)	1000 µg prod/bee = 186 µg a.s./bee	1000 µg prod/bee = 56.9 µg a.s./bee
	LD ₅₀ 48 hr (contact)	>1000 µg prod/bee >186 µg a.s./bee	>1000 µg prod/bee >56.9 µg a.s./bee
	Actual control-corrected mortality 48 hr at highest test conc. (contact)	0 %	0 %
	Sub-lethal effects at highest test concentration	none	Max 22.5 % of bees ^A , did not persist beyond 4 hours

^A Bees recorded as ‘affected’: bees still upright and attempting to walk but showing signs of reduced coordination.

In the contact tests, sub-lethal effects on bees were only observed sporadically in a few individual bees and were not deemed to be treatment related. However, for the Miravis Plus A21857B contact test, behavioural abnormalities were observed in a dose-responsive pattern, in a maximum of 22.5 % of bees after 4 hours (Table 9.6.1-3). However, this effect was transient and no behavioural abnormalities were observed at 24 or 48 hours apart from non-treatment related sporadic effects in individual bees. The toxicity endpoint is protective of these effects.

When examining sub-lethal effects in the oral test, for A19649B affected bees were observed in the highest test concentration only, in 10 % of bees after 4 hours, decreasing to 7.5 % after 24 and 48 hours. There was no food avoidance or unpalatability, as demonstrated by comparison of the nominal and consumed doses (Table 9.6.1-3). However, for the Miravis Plus A21857B oral test, although no sub-lethal effects on bees were observed, there was food avoidance at the highest two test concentrations, where less than half the food was consumed (500 and 1000 µg A21857B/bee nominal in diet resulted in consumption of 245 and 423 µg A21857B/bee) (Table 9.6.1-3). This is taken into account in the toxicity endpoints as they are expressed in terms of consumed dose.

It is noted that the unpalatability/food avoidance observed is not unique to formulation Miravis Plus A21857B, as it is also seen in the chronic adult study (██████, 2014) and one of the chronic larval studies (██████, 2015) on A19649B. This is taken into account since endpoints are presented as consumed doses (with the exception of the ██████ (2015) larval study, as noted). Additionally, there is no evidence of this avoidance being relevant at the field scale: in the semi-field studies on A19649B, only one study reported significantly reduced food stores in test item treatments compared to the control (██████, 2018), whereas the others (██████, 2017; ██████, 2017) found no significant differences between treatments and control despite utilising the same treatment concentrations. In the colony-feeding study using the technical a.s. (██████, 2018), no significant differences in food stores between treatments and control were observed and no food avoidance of feed solutions containing the active substance. Additionally, none of these studies reported any significant behavioural observations during test item exposure, and all demonstrated adequate foraging on the test crop (semi-field studies) or consumption of the test item (colony-feeding study). Therefore, although there is some noted food avoidance in the laboratory studies, this is taken into account by expressing results as consumed dose, and there is no evidence that this is specific to either formulation, or that it is cause for concern at a larger scale.

Therefore, whilst not chemically comparable, on the basis of the acute contact and oral studies, it is concluded that the formulations are of **comparable toxicity and hence can be deemed to be ecotoxicologically equivalent low toxicity**. Additionally, although there is some noted food avoidance in the laboratory studies, this is taken into account by expressing toxicity endpoints as consumed dose, and there is no evidence that this avoidance is specific to either formulation. On this basis, it is proposed that the studies on the A19649B can be used to support Miravis Plus A21857B. Furthermore, an acute risk assessment has been carried out for both formulations below, and demonstrates **comparable acceptable** risk.

Following discussion at the ECP meeting, the ECP advised that it is incorrect to take the view that the two formulations (EU formulation Miravis A19649B and UK formulation Miravis Plus A21857B) are of comparable toxicity to bees based on evidence from the unbounded acute toxicity values, although the Committee agreed that both formulations do not appear to be very toxic based on the acute toxicity dataset. However, it is not possible to determine if one formulation is more or less toxic than the other, based on the data. The Committee did accept the interpretation and use of semi-field data to support the conclusion on honeybee larvae, given the uncertainties in

the laboratory dataset for honeybee larvae. Therefore, the discussion of these studies has been left in below for reference.

Discussion of the remaining studies in the dataset on formulation A19649B is presented below.

B.9.6.1.1.2.2. A19649B adult chronic study

There is uncertainty in the endpoints of the adult 10-day chronic bee study on A19649B (████████, 2014) due to lack of analytical measurements of the test item and absence of correction for evaporative loss (see section B.9.5.1 for study evaluation). The reason this analysis was not conducted was because the study was carried out in 2014, prior to the adult chronic OECD GD 245 being available (TG published Oct 2017) where the requirement for analytical verification and adjustment for evaporation was laid out. However, as there is no other adult chronic laboratory study available, this study will be considered as a source of information during risk assessment, noting the uncertainties. It is also noted that in terms of food consumption, the consumed dose is less than the nominal for the top three doses: the nominal doses were 67.0, 100.2 and 150.1 µg a.s./bee/day whereas the consumed doses were 63.978, 89.073 and 138.168 µg a.s./bee/day. However, the endpoint does take this into account as it is expressed as consumed dose.

B.9.6.1.1.2.3. A19649B chronic larval development studies

Two larval studies have been submitted, one by ██████████ (2015a) and one by ██████████ (2015). The former study was conducted over a 22-day period and gave a 22-day NOED of 0.06 µg a.s./larva (0.015 µg a.s./larva/day) and an unbounded 8-day NOED of <0.06 µg a.s./larva (<0.015 µg a.s./larva/day), and the latter study was conducted over an 8-day period with a resulting 8-day NOED of 55 µg a.s./larva (13.75 µg a.s./larva/day). It can be seen that the 8-day mortality endpoints for both studies are conflicting, with the ██████████ (2015) 8-day NOED three orders of magnitude higher than the ██████████ (2015a) 8-day NOED, noting that the ██████████ (2015a) endpoint is actually unbounded so is not a true NOED value (see Table 9.6.1-1).

Both studies were considered valid and utilised the same test design for the first 8 days, with the only notable difference being that the bees were from an alternative source, as would be expected for two different studies. However, it is noted in the ██████████ (2015) study evaluation (see section B.9.5.1), that deviations in food consumption and a corresponding reduction in larval development were observed in 35 % and 21 % of larvae at test concentrations of 109.9 µg a.s./larva (27.5 µg a.s./larva/day) and 55.0 µg a.s./larva (13.75 µg a.s./larva/day), respectively. However, the endpoints of ██████████ (2015) were not expressed in terms of consumed dose, so are likely to be an overestimate of the true dose. Contrastingly, the ██████████ (2015a) study does not mention any lack of food consumption, and the dose per day is stated to be the average daily consumed dose. Nevertheless, even at the test concentration of 27.5 µg a.s./larva (6.88 µg a.s./larva/day) in the ██████████ (2015) study, which is the highest test concentration with no sub-lethal effects, this is still two orders of magnitude higher than the test rate of 0.06 µg a.s./larva (0.015 µg a.s./larva/day) in ██████████ (2015a), which resulted in 12 % control-corrected mortality.

For consideration in risk assessment, the worst-case endpoint of the two studies on A19649B will be used, which is the 22-day NOED of 0.06 µg a.s./larva (0.015 µg a.s./larva/day) from ██████████ (2015a) (Table 9.6.1-1). However, it is noted that this value is not protective of the endpoint from the larval study on the technical active substance (████████, 2015) from which the unbounded NOED of <0.0035 µg a.s./larva/day was derived. Given the mismatch between the endpoints of the two larval toxicity studies with formulation A19649B, and that the overall worst-case larvae NOED from the study on the technical a.s. is an unbounded value which cannot be used in risk assessment, then there is limited confidence in the endpoints of any of the three larval studies. Therefore, more weight will be given to the semi-field studies on formulation A19649B and colony-feeding study on the technical active substance in this instance. These are discussed below.

B.9.6.1.1.2.4. A19649B semi-field tunnel studies

Three semi-field tunnel studies on bee brood were submitted for A19649B (████████, 2017; ██████████, 2017; ██████████, 2018). There were no major concerns with any of these studies in the evaluations (see section B.9.5.1), although some sources of uncertainty were identified. Further discussion and consideration is given below, as there are no discrete endpoints for this type of study.

The three studies were conducted to OECD 75 (2007) and the highest application rate in these three studies was 200g a.s./ha which is equivalent to the highest proposed use.

Treatments were applied by spray application to full-flowering *Phacelia tanacetifolia*, which is considered an acceptable surrogate for the proposed crop (noting that application will be made during flowering, i.e., BBCH 57-69). The studies were carried out at different times of year: 27 May – 01 Aug 2017 (■■■■■, 2018), 10 Aug – 19 Oct 2016 (■■■■■, 2017) and 14 Jun – 25 Aug 2016 (■■■■■, 2017).

In the study by ■■■■■, (2018), no effects were observed on adult mortality, pupal morality, foraging activity or bee behaviour. There were significantly reduced food stores compared to the control in all test concentrations, however this did not appear to have any immediate effect on colony survival, colony strength or brood area for the remainder of the study (up to 63 days after treatment). No supplementary feeding of the colonies took place. One colony in the highest test item treatment was found to be queenless. Some increase in brood termination rate (BTR) and reduction in brood index (BI) and brood compensation index (BCI) for eggs and young larvae was observed in the test item treatments compared to the control, but this was not statistically significant.

In the study by ■■■■■, (2017), there were no effects on honeybee morality, foraging behaviour, brood termination rate (BTR), brood index (BI), brood compensation index (BCI) or overall brood development and food stores in the whole post-application period. There were some uncertainties noted by the evaluator in section B.9.5.1 with this study in that 6 out of 24 colonies were particularly small (4160 – 4680 worker bees per colony compared to the approximately 6,000 recommended the OECD 75 (2007) guideline), which reduces the sensitivity of the study. Additionally, there was heavy rainfall 2 days after test item application, which influenced bee foraging behaviour and may have influenced exposure of the test item. There was also high variation indicated by large standard deviations in worker honeybee mortality. Supplementary feeding of all colonies took place twice (15 days and 41 days after treatment) in accordance with good beekeeping practice appropriate to the time of year, to prevent starvation of colonies.

In the study by ■■■■■, (2017), the test item applied at a maximum of 200 g a.s./ha had no effect on mortality, foraging activity, colony strength or colony development, BTR, BI and BCI. Supplementary feeding took place in accordance with good beekeeping practice, which occurred 23 days after the application of the test item. It is noted that some behavioural effects were observed in the 7 days after treatment application, but they are not considered to be treatment related as there is no dose response, effects were also seen in the control, and were limited in numbers of individual bees.

Overall, the semi-field studies indicated no adverse effects on mortality, foraging activity, colony strength or brood development during the enclosed period of the study. It is not possible to draw any conclusion regarding long-term impacts of the test item on honeybee development due to the nature of semi-field studies in that the colonies are small and it is not possible to monitor issues such as over-wintering success. These studies are considered further at risk assessment.

B.9.6.1.2. Acute risk assessment

Assessment of the acute risk of pydiflumetofen 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).

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.

$$\text{Hazard Quotient, oral: } Q_{HO} = \frac{\text{max. appl. rate}}{LD_{50} \text{ oral}} = \frac{[\text{g a.s./ha or g total substance/ha}]}{[\mu\text{g a.s./bee or } \mu\text{g total substance/bee}]}$$

$$\text{Hazard Quotient, contact: } Q_{HC} = \frac{\text{max. appl. rate}}{LD_{50} \text{ contact}} = \frac{[\text{g a.s./ha or g total substance/ha}]}{[\mu\text{g a.s./bee or } \mu\text{g total substance/bee}]}$$

The risk assessment for bees for pydiflumetofen is based on the application rate stated in the GAP table of one application per season of 200 g a.s./ha to spring and winter cereals (BBCH 30-69) and oilseed rape (BBCH 57-69).

For this acute risk assessment, data on the active substance and the data for the representative product A21857B (Miravis Plus) are the key endpoints. In addition to the risk assessment for the a.s. and Miravis Plus, an assessment of the risk from A19649B has been carried out, since data from this formulation is used to inform the risk assessment. Whilst Miravis Plus and A19649B are not chemically comparable, there is an argument, see above, that they are ecotoxicologically comparable. To further advance this argument an acute risk assessment has been carried out using the formulation endpoints. It can be seen that the risk from both formulations is comparable, further adding to the argument that data on A19649B can be used to determine the risk from the use of Miravis Plus. The calculations are presented in Table 9.6.1-4 below.

Table 9.6.1-4: HQ calculations for honeybees: contact and oral exposure for the application in cereals and oilseed rape (1 x 200 g a.s./ha).

Substance	Endpoint	Application rate (g/ha)	LD ₅₀ (µg a.s./bee)	Calculated HQ	Acceptable Risk? (Trigger <50)
Pydiflumetofen	Acute oral	200	>116	<1.724	yes
	Acute contact	200	>100	<2.000	yes
Miravis Plus A21857B	Acute oral	200	>24.07	<8.309	yes
	Acute contact	200	>56.9	<3.515	yes
Formulation A19649B	Acute oral	200	>210.6	<0.950	yes
	Acute contact	200	>186	<1.075	yes

In conclusion, all calculations of HQs 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 both formulations for the proposed use of up to 200 g a.s./ha.

Whilst the acute risk is acceptable, HSE has considered the chronic risk and available field studies further below.

B.9.6.1.3. Chronic risk assessment

Currently, there is no agreed guidance that can be used to assess the chronic risk to honeybees, hence whilst these data are required for both the active substance and the formulation, it is not possible to undertake a quantitative risk assessment. HSE has attempted however, in the absence of agreed guidance to carry out a qualitative assessment.

To aid discussion of the chronic honeybee data for risk assessment, two approaches are used to consider a margin of safety, noting that there is no agreed trigger value for comparison:

1. **Dietary concentration margin of safety:** a comparison of the larval and adult toxicity endpoints expressed as mg a.s./kg diet with the dietary exposure via residues (from semi-field studies) as concentration of a.s. in food (expressed as nectar and sugar).
2. **Daily-dose margin of safety:** a comparison of larval and adult chronic toxicity endpoints expressed as mg a.s./bee/day or mg a.s./larva/day to estimated daily exposure. The estimated daily exposure is calculated using residue information from semi-field studies and estimates of honeybee sugar consumption values.

B.9.6.1.3.1. Estimating exposure using residue data from semi-field studies

Both the dietary concentration and daily-dose margin of safety approaches utilise residue data to estimate exposure. Three semi-field studies on the formulation A19649B (██████, 2017; ██████, 2017; ██████, 2018) conducted residue analysis of the test substance in nectar, pollen, flowers and foliage. In this chronic risk assessment, only residues in nectar from these studies will be used to determine potential exposure of bees to the a.s., rather than also including pollen. This is because it is noted that for the laboratory studies and for the colony-feeding study,

the test substance was assessed via the consumption of a diet of 50 % sugar. Therefore, it is potentially more appropriate to compare the residue values in nectar than pollen for exposure.

The worst-case residue value will be used in the following assessment due to the limited size of the residue dataset (three studies, each with single replicate measurements). The residue datasets are detailed in the study summaries found in section B.9.5.1 (██████, 2017; ██████, 2017; ██████, 2018). ██████ (2018) had the highest (worst-case) residue value of 0.352 mg a.s./kg nectar, and this will be used in the following assessment. It is noted that there is uncertainty in extrapolating the residue data from *Phacelia* to the crops (cereals and oilseed rape) of the proposed use.

Appendix J1 of EFSA (2013) bee guidance (not currently noted or adopted by GB) states that sugar consumption rather than nectar consumption is most relevant for risk assessment; also, daily consumption values in the guidance document are in terms of sugar per bee rather than nectar per bee. Additionally, the toxicity endpoints are from dosing of the a.s. or formulation in 50 % (w/v) aqueous sugar. Therefore, the a.s. residue figures from the semi-field studies, which are in mg a.s./kg nectar, need to be converted to mg a.s./kg sugar. The worst-case sugar content of the nectar is stated in Appendix J1 to be 15 % for honeybees for risk assessment when foraging on crop plants or 30 % for weed and field-margin scenarios, noting that they acknowledge that further research is needed into these values. Therefore, to convert the residue value in nectar to sugar, the value of **0.352 mg a.s./kg nectar** is divided by 0.15 to give **2.347 mg a.s./kg sugar**.

B.9.6.1.3.1.1. Estimating worst-case dietary concentration exposure

The worst-case estimate of dietary exposure of pydiflumetofen to honeybees, which will be used to calculate the dietary margin of safety, is the highest residue value from the semi-field studies, of 0.352 mg a.s./kg nectar (equivalent to 2.347 mg a.s./kg sugar, assuming 15 % sugar content of nectar), as discussed above.

B.9.6.1.3.1.2. Estimating worst-case daily-dose exposure

To estimate worst-case daily exposure of pydiflumetofen to honeybees, which will be used to calculate the daily-dose margin of safety, consideration of honeybee sugar consumption is required. Values of honeybee consumption of sugar were taken from Appendix Table J1 in EFSA (2013)³. The value for forager honeybees was 32–128 mg sugar/bee/day. The worst case of **128 mg sugar/bee/day** was used in subsequent calculations. The consumption value for honeybee larvae of 59.4 mg sugar/larva/5 days (overall consumption) was divided by 5 to give the daily consumption value of **11.88 mg sugar/larva/day**.

Worst case daily exposure values were determined by calculating the amount of active substance that would be consumed each day by each individual bee or larva, using the daily bee food consumption values in terms of sugar, and the worst-case residue value of the active substance from the semi-field studies. The calculation is shown in Table 9.6.1-5 below. The worst-case daily exposure for adult bees was estimated to be **0.3004 µg a.s./bee/day** and for larvae was estimated to be **0.02788 µg a.s./larva/day**.

Table 9.6.1-5 Calculation of worst-case daily exposure of pydiflumetofen applied up to 200 g a.s./ha to adult and larvae honeybee.

Life stage	Adult honeybees	Honeybee larvae
Type of food	Sugar	Sugar
Food consumption from EFSA, 2013 ^{A),3}	128 mg sugar/bee/day (forager bees)	11.88 mg sugar/larva/day
Worst case residue from semi-field studies ^{B)}	0.352 mg a.s./kg nectar =2.347 mg a.s./kg sugar (assuming 15 % sugar content of nectar) ^{C)}	
Worst case daily exposure	The worst-case residue of 2.347 mg a.s./kg sugar = 2.347 ng a.s./mg sugar. Therefore,	The worst-case residue of 2.347 mg a.s./kg sugar = 2.347 ng a.s./mg sugar.

³ European Food Safety Authority, 2013. EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus spp.* and solitary bees). EFSA Journal 2013;11(7):3295, 268 pp., doi:10.2903/j.efsa.2013.3295

	<p>in 128 mg sugar (forager bee daily consumption) there is $2.347 \times 128 = 300.4$ ng a.s.</p> <p>Therefore, the worst-case daily exposure for adult honeybees, based on daily food consumption and a.s. residue values in sugar, is:</p> <p>0.3004 µg a.s./bee/day</p>	<p>Therefore, in 11.88 mg sugar (larval bee daily consumption) there is $2.347 \times 11.88 = 27.88$ ng a.s.</p> <p>Therefore, the worst-case daily exposure for honeybee larvae, based on daily food consumption and a.s. residue values in sugar, is:</p> <p>0.02788 µg a.s./larva/day</p>
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^{A)} Consumption values from Appendix Table J1 in EFSA Guidance Document on the risk assessment of plant protection products on bees, 2013.

^{B)} Worst case residue data takes the highest observed residue from data of the three semi-field studies (see text).

^{C)} Where nectar is 15 % sugar (Appendix J1 EFSA 2013), and it is assumed that all the a.s. is in the sugar, then the mg a.s./kg sugar = mg a.s./kg nectar divided by 0.15 (see text).

B.9.6.1.3.2. Calculate crude margin of safety

The margins of safety were calculated by dividing the toxicity endpoints derived from the first-tier adult and larval laboratory studies by the worst-case concentration in diet (dietary) or daily-dose exposures as described in the sections above. The selection of toxicity endpoints from first tier laboratory studies for use in risk assessment has been discussed above:

- The adult chronic endpoint is a 10-day LD₅₀ of >138.2 µg a.s./bee/day (3854 mg a.s./kg food) from [REDACTED], 2014, noting some uncertainty due to lack of analytical measurements of the test item in the test diet.
- As indicated above, the available larval studies, whilst reliable, are contradictory and hence it is proposed to not rely on them in the risk assessment and put more weight on the semi-field studies. However, for illustrative purposes, all larval endpoints are used in the following margin-of-safety assessment.

Additionally, the study conducted to [REDACTED] *et al* ([REDACTED], 2018), which involved dosing a colony with sucrose solution containing the active substance, is considered in the following assessment. It is not possible to determine a dose-based endpoint from such a study, however, it is possible to compare the concentration at which no effects occurred in this study with the exposure in the field.

The margins of safety for both the dietary and daily-dose approaches for each of the toxicity endpoints, are shown in Table 9.6.1-6 below.

Table 9.6.1-6. Comparison of chronic adult and larval honeybee toxicity of pydiflumetofen with worst-case daily exposure values and worst-case concentration in diet values.

Daily exposure values and worst-case concentration in diet values.							
Study	End-point Type	Endpoint [mg a.s./kg diet]	Exposure [mg a.s./kg]	Dietary margin of safety	Endpoint [µg a.s./ bee/day]	Exposure [µg a.s./ bee(or larva)/day]	Daily exposure margin of safety
Chronic adult honeybee							
([REDACTED], 2014) A19649B	10d LD ₅₀	>3854	0.352 (nectar) 2.347 (sugar)	>10948 (nectar) >1642 (sugar)	>138.2	0.3004	>460
	10d NOED	3854		10948 (nectar) 1642 (sugar)	138.2		460
Chronic larval honeybee							

Study	End-point Type	Endpoint [mg a.s./kg diet]	Exposure [mg a.s./kg]	Dietary margin of safety	Endpoint [µg a.s./bee/day]	Exposure [µg a.s./bee(or larva)/day]	Daily exposure margin of safety
(██████████, 2015) Technical a.s.; limit test	8d & 22d NOED	<0.09	0.352 (nectar) 2.347 (sugar)	unbound* <0.256 (nectar) <0.038 (sugar)	<0.0035	0.02788	unbound* <0.126
(██████████, 2015a) A19649B	8d NOED	<0.409		unbound* <1.16 (nectar) <0.174 (sugar)	<0.015		unbound* <0.538
	22d NOED	0.409		1.16 (nectar) 0.174 (sugar)	0.015		0.538
(██████████, 2015) A19649B	8d NOED 1)	347		986 (nectar) 148 (sugar)	13.75		493
Chronic whole honeybee colony							
Colony-feeding field study; technical a.s. (██████████, 2018)	No effect 2)	32	0.352 (nectar) 2.347 (sugar)	90.9 (nectar) 13.63 (sugar)	Not possible to determine dose per larvae or adult bee for this study		

Values in **bold** indicate that there is no margin of safety.

*unbound values are not applicable for this type of margin of safety calculation but are included for reference to aid discussion.

a.s. = active substance.

¹⁾ There is uncertainty regarding this endpoint as it does not take into consideration the consumed dose (see discussion of endpoints section).

²⁾ Note there is not a clearly defined endpoint for this type of study

B.9.6.1.3.3. Assessing chronic risk to adult honeybees

Adult bees appear to have a large margin of safety, both when comparing the laboratory toxicity endpoint to crude dietary exposure (>10948 considering a.s. in nectar; >1642 considering a.s. in sugar) and daily exposure (>460) (Table 9.6.1-6). Noted uncertainties for the adult margin of safety include that for the laboratory toxicity endpoint used in the calculation, the test substance was A19649B rather than Miravis Plus A21857B, and that there were no analytical measurements of the test item in the study due to it being conducted before this was a requirement of the test guideline. Although some food avoidance was observed in the lab study, this was taken into account in the toxicity endpoint as it is expressed in terms of consumed dose.

The semi-field study findings do support the large margin of safety. For example, effects on mortality, behaviour and foraging activity in adults in the semi-field studies are either absent (██████████, 2018 and ██████████, 2017), or are sporadic and transient (██████████, 2017), which were not considered to be treatment related. Additionally, the colony-feeding study (██████████, 2018) supports the large margin of safety, as although some statistically significant effects on adult mortality were seen, these were small effects, were not dose-responsive, and were not deemed to be treatment related.

Therefore, on the basis of all the information, HSE concludes that there is an acceptable chronic risk of pydiflumetofen to adult honeybees at the proposed rate.

B.9.6.1.3.4. Assessing chronic risk to larval honeybees

As explained previously, there is limited confidence in the endpoints from the three first-tier larval studies due to issues with solubility in the limit test on the technical active substance (██████████, 2015), and the large variation in 8-day endpoints between two studies of the same design on formulation A19649B (██████████, 2015a; ██████████, 2015). However, when considering the 22d NOED from ██████████ (2015a), although a small dietary margin of safety (1.16) is seen when considering the a.s. in nectar, there is no dietary margin of safety when considering a.s. in sugar (<0.174), and no margin of safety for daily dose (0.538) (Table 9.6.1-6). Additionally, in light of the

presence of the lower unbound NOED from the a.s. study (██████, 2015), this indicates that there may be further risk to larvae. Therefore, on the basis of the toxicity data from larval first-tier laboratory studies, it is concluded there is no margin of safety for larvae.

Since data is available for three semi-field studies and an Oomen-based colony feeding study which can provide useful information regarding all bee life stages, more weight will be given to these studies to assess larval risk in light of the uncertainties with the first-tier laboratory studies.

B.9.6.1.3.4.1. Consideration of semi-field studies to assess risk to honeybee larvae

Three semi-field flower-spray tunnel studies, which exposed bees for 7 days within a tunnel to flowering *Phacelia tanacetifolia* sprayed once with the test item up to a maximum of 200 g a.s./ha, are considered in additional detail (██████, 2017; ██████, 2017; ██████, 2018). These studies provide an indication of any potential gross effects over a short time scale. Additionally, the colony-feeding study (██████, 2018), which fed the bee colony with technical a.s. up to a concentration of 32 mg a.s./kg diet repeatedly for 9 days, provides indication of effects across the whole colony from the repeated exposure, with effects monitored for approximately three brood cycles.

Looking to the three flower-spray tunnel semi-field studies (██████, 2017; ██████, 2017; ██████, 2018) and the colony-feeding field study (██████, 2018), none of the studies indicated a cause for concern for honeybee larvae (or indeed any life stage), as detailed below:

- In the ██████ (2017) study, the mean mortality per replicate per day of the worker larvae and pupae in the test item treatments was < 1 over the whole study period. There were no treatment related effects or statistically significant differences to the control in any of the brood indices (brood termination rate, brood index, brood compensation index).
- In the ██████ (2017) study, the rate of mortality for larvae and pupae was not significantly different from that observed in the control condition at any tested concentration, for both the periods spanning 0-7 days after test item application (DAA) and 8-27 DAA. There were no statistically significant differences of the brood indices, compensation indices, or brood termination rate of young or old larvae in any of the test item treatments compared to the control.
- In the ██████ (2018) study, there was a slight increase in brood termination rate (BTR) and reduction in brood index (BI) and brood compensation index (BCI) of initially labelled eggs and young larvae across the test item treatments as observed up to 21 days after treatment (DAT), but none of these were statistically significant from the control, with the exception of reduced BCI on DAT 14, which was not dose responsive and was attributed to natural variation. Additionally, there was no effect of the test item on pupal mortality.
- In the ██████ (2018) colony-feeding study, larval brood area was not statistically different from control in any of the treatment groups. Additionally, there were no statistically significant differences in and of the brood indices between treatments and control. There was no pupal mortality during the 9-day exposure phase of the study in any of the treatment groups. During the post-exposure phase (DAT 10-26), pupal mortality was generally low and did not occur consistently across replicates in the treatment groups. No statistical testing was carried out for pupal mortality, which introduces some uncertainty, but effects remained minimal compared to control (0.1-1.3 pupae/colony/day in the post-exposure phase DAT 10-26 across all treatment groups, compared to 0 and 10.8 pupae/colony/day for the control and reference item, respectively).

At this point, it is worth noting the general limitations of semi-field studies that are inherent in the OECD 75/EPPO 170 style of test design. Semi-field studies can provide detailed assessments of forager mortality and bee behaviour from contact exposure, however, limitations in the size of the colonies and the duration of exposure mean it is less straightforward to detect impacts on colony strength and colony development over a longer term as treatment related effects may be masked by successful recovery of colonies in the pesticide-free areas after exposure. Despite these shortcomings, the studies do provide information on the development of brood over three brood cycles.

In addition to general limitations, there are some specific uncertainties relating to the semi-field studies, namely:

- The study timing of [REDACTED] (2017) was late in the season (Aug – Oct 2016), which meant supplementary feeding was required.
- [REDACTED] (2017) was performed between Jun – Aug 2016, and also had supplementary feeding according to standard beekeeping practice for the region, due to natural food supply declining in August which was further exacerbated by dry weather.

Although necessary and not unjustified, this supplementary feeding could reduce the sensitivity of the test to potential effects of the test item, by providing an extra recovery condition for the bee colony after test item exposure.

Finally, however, it should be noted that the semi-field studies do represent a worst-case situation in terms of exposure, as, for the duration of the enclosed part of the study, the bees are forced to forage on a crop containing test item residues, rather than a more varied diet including plants which have not been treated.

B.9.6.1.3.4.2. Summary for larval honeybee risk

In summary, none of the semi-field studies indicate concern with regards to risk to larval honeybees and nor does the colony-feeding study. Taken together, the data suggests that the lack of margin of safety for honeybee larvae as calculated from first-tier study data is potentially over-conservative (noting uncertainties in this proposed approach and lack of agreed guidance).

The available data indicates that pydiflumetofen poses low risk to honeybee colonies through all life-stages, as tested up to 200 g a.s./ha or 32 mg a.s./kg food. It should be noted there are several uncertainties that have been identified by HSE when conducting the bee risk assessment. These have been summarised below.

B.9.6.1.4. Evaluating lines of evidence in the bee risk assessment and their uncertainty

A consideration of all lines of evidence and their uncertainties are considered in the Table 9.6.1-7 below to aid the risk assessment of pydiflumetofen to honeybees.

Table 9.6.1-7. Evaluation of uncertainties affecting risk assessment of pydiflumetofen to honeybees. The +/- symbols indicate whether each source of uncertainty has the potential to overestimate (+) or underestimate (-) risk, compared to the true risk.

Source of uncertainty	Potential effect on risk estimate	Explanation
‘+’: potential overestimate of risk; ‘-’: potential under-estimate of risk; ‘?’: unknown effect on estimate of risk		
Consideration of toxicity endpoints from studies on formulation A19649B for chronic adult, larval and semi-field flower-spray tunnel studies, rather than representative product Miravis Plus A21857B	?	<p>The two formulations are chemically not comparable, though both are shown to have low oral and contact acute toxicity to adult bees with unbounded (>) LD₅₀ endpoints (see Table 9.6.1-1, 9.6.1-2, 9.6.1-3). When used in a risk assessment, the endpoints indicated comparable low acute risks. Therefore, the two formulations were considered ecotoxicologically comparable.</p> <p>Food avoidance/unpalatability was seen in the acute oral test for A21857B. For A19649B, food avoidance was not seen in the acute oral test, but was in the chronic adult test and one of the larval studies. Endpoints were expressed as consumed dose which mitigates this issue, but it could still contribute some uncertainty into the read-across of A19649B data for A21857B, as although unpalatability is not specific to either formulation, there is limited information on this.</p>

Source of uncertainty	Potential effect on risk estimate	Explanation
‘+’: potential overestimate of risk; ‘-’: potential under-estimate of risk; ‘?’: unknown effect on estimate of risk		
Use of worst-case residue data from A19649B to estimate exposure (used in chronic adult and larval margin of safety calculations) ^A	+	<p>Residue data were based on a single worst-case value of the a.s. in nectar across three semi-field studies, from spray application at the highest rate in the GAP table of 200 g a.s./ha. This worst-case residue occurred at the closest measurement time after spray application (4 hours), whereas in reality, the residues decline over time (noting this is likely to be influenced by environmental conditions), and bees would not be exposed to this highest residue for extended periods. This is in line with the GAP table which is for a single spray application. Therefore, the use of the worst-case residue to calculate exposure represents a conservative approach, noting that ideally more residue studies would be preferable. ^A</p> <p>It is further noted that there is uncertainty in the extrapolation between residues in nectar from <i>Phacelia</i> to the crops of the proposed use (cereals and oilseed rape). Whether this would result in an overestimate or underestimate of risk is unknown.</p>
Use of worst-case sugar content in nectar to estimate daily-dose exposure (used in chronic adult and larval margin of safety calculation)	+	Daily-dose exposure was estimated using residue values from nectar, converted into residue values in sugar, using a worst-case sugar content of nectar of 15 %. This 15 % value is from Appendix J1 of EFSA (2013). This guidance is not currently noted or adopted by GB and it was noted that further research is needed into these values. Therefore, the use of this value may lead to an overestimate of exposure and hence overestimate of risk.
Use of worst-case daily food consumption values to estimate daily-dose exposure (used in chronic adult and larval margin of safety calculation)	+	The daily food consumption values for adult forager honeybees and honeybee larvae, taken from Appendix J1 of the EFSA (2013). This guidance is not currently noted or adopted by GB. These represent worst-case values, therefore are likely to contribute to an overestimate of risk rather than an underestimate of risk.
Larval toxicity endpoints for calculation of chronic larval margin of safety	?	<p>There is limited confidence in all three of the toxicity endpoints from the first-tier larval studies. Firstly, the two studies conducted on A19649B (██████████, 2015; ██████████, 2015a) had conflicting 8-day NOEDs, which spanned three orders of magnitude despite having identical study design. Secondly, of the three studies, only the ‘██████████ 2015a’ study has a usable 22-day NOED. However, a third study (██████████, 2015) on the technical a.s., produced an unbound ‘less-than’ 22-day NOED which indicates a greater toxicity than ██████████ 2015a, but there were issues with solubility of the technical a.s., which may make the results unreliable. This means that the 22-day NOED from ██████████ 2015a may, or may not, represent the worst-case toxicity.</p> <p>Therefore, the risk assessment relies more on the results of the semi-field and colony-feeding studies.</p>

Source of uncertainty	Potential effect on risk estimate	Explanation
‘+’: potential overestimate of risk; ‘-’: potential under-estimate of risk’; ‘?’: unknown effect on estimate of risk		
Adult chronic toxicity endpoint for calculation of adult chronic margin of safety.	?	The chronic adult toxicity endpoint was derived from a study which did not have analytical measurement of the test item in the supplied test diet, due to the study being performed before this was a requirement of the test guideline. Therefore, the actual applied test item dose could be higher or lower than stated. However, this uncertainty is minor and there are not expected to be any issues as there are not problems with the test item in other ecotoxicological studies using A19649B. Food avoidance was taken into account as endpoints were presented as consumed dose.
Consideration of evidence from semi-field tunnel studies on bee brood using A19649B to support conclusions	+	The semi-field study is representative of a worst-case situation in which bees are forced to forage on a crop containing test item residues, rather than a more varied diet including plants which have not been treated.
	-	The sensitivity of these studies is limited, though this is inherent in the test design (see above)
	?	Tested on A19649B rather than Miravis Plus A21857B (see explanation in rows above).
Consideration of evidence from colony-feeding study on technical a.s. to support conclusions	-	No statistical testing for pupal mortality, but effects remained minimal compared to control (0.1-1.3 pupae/colony/day in the post-exposure phase DAT 10-26 across all treatment groups, compared to 0 and 10.8 pupae/colony/day for the control and reference item, respectively).

^A Note that compared to other areas of ecotoxicological risk assessment, such as for birds and mammals, a minimum of four studies are required to move away from default residue values. There is no default value for bees, hence in this risk assessment the worst-case residue value is used.

It is clear that there is an acceptable acute risk of pydiflumetofen to adult honeybees, with little uncertainty in this conclusion: All acute studies were conducted according to the appropriated guidance documents with no concerns noted. Endpoints were derived for the technical active substance, formulation A19649B, and the representative product Miravis Plus A21857B. All endpoints were unbounded (>) LD₅₀ values, demonstrating low toxicity, and were protective of sub-lethal effects. Food avoidance observed for Miravis Plus A21857B was taken into account as endpoints were presented as consumed dose. The resulting HQs from risk assessment are well below the trigger value demonstrating low acute risk of pydiflumetofen to adult honeybees

As outlined in the table above, uncertainty arises in the assessment of chronic risk to honeybee adults and larvae, which is compounded by the lack of noted/adopted risk assessment guidance. Chronic risk was therefore considered using a combined approach, with a qualitative consideration of laboratory studies and semi-field studies, and semi-quantitatively with a margin-of-safety calculation from comparing estimated exposure in diet and by daily-dose with the relevant toxicity endpoints from laboratory studies.

One of the main sources of uncertainty lies in the consideration of toxicity endpoints from studies on the alternative and chemically non-comparable formulation A19649B, rather than that of the representative product Miravis Plus A21857B, for which there is only an acute dataset. As outlined earlier, on the basis of low **and comparable** acute toxicity of both A19649B, A21857B, and the technical active substance, it was considered feasible to use the A19649B data to support the risk assessment. Although this uncertainty is noted for the chronic adult, larvae and semi-field studies, the acute risk assessment, which is currently the only fully noted (SANCO 2002) part of honeybee risk assessment, is within the trigger value.

Following discussion at the ECP meeting, the ECP advised that it is incorrect to take the view that the two formulations (EU formulation Miravis A19649B and UK formulation Miravis Plus A21857B) are of comparable toxicity based on evidence from unbounded toxicity values, although the Committee agreed that both formulations do not appear to be very toxic based on the acute toxicity dataset. However, it is not possible to determine if one formulation is more or less toxic than the other, based on the data. The Committee accepted the interpretation and use of semi-field data to support the conclusion on honeybee larvae, given the uncertainties in the laboratory

dataset for honeybee larvae. The availability of the colony-feeding field study using the technical active substance rather than the EU formulation, adds weight to the conclusion.

The chronic risk assessment approach outlined above, yielded a margin of safety for chronic exposure of pydiflumetofen to adult bees on the basis of first-tier data. Additional information from the semi-field studies (noting their shortcomings outlined above) added some weight to this conclusion.

Due to the contradictory nature of the larval dataset, there was no reliable larval endpoint and therefore this part of the risk assessment has relied on the semi-field studies. It should be noted that an illustrative assessment was carried out using the range of endpoints with the outcome that one endpoint indicated a margin of safety, whilst with the others there was no margin of safety. On the basis of the semi-field and colony feeding studies, the following can be concluded: The semi-field and colony feeding studies saw no adverse effects on bee colonies up to the maximum test concentrations of 32 mg a.s./kg diet (colony-feeding study) or 200 g a.s./ha (semi-field tunnel studies). It is also noted that since the margin of safety was a crude calculation based on multiple worst-case values (worst-case residue from semi-field studies, worst case daily food consumption for adult forager bees, worst-case sugar content of nectar, worst-case toxicity endpoints), when taken together, the data supports a conclusion that there is an acceptable chronic risk to honeybees at all life-stages, as tested up to 200 g a.s./ha or 32 mg a.s./kg food.

This is also in-line with the SANCO (2002) guidance on bee risk assessment, which notes that in higher tier risk assessment ‘it is important to consider any effects observed in relation to the overall survival and productivity of the hive’. Therefore, although there are some small observations and noted uncertainties in the semi-field studies and colony-feeding study, the absence of adverse effects on the whole colony as seen across multiple separate semi-field studies not just a single study, supports a conclusion that there is an acceptable risk to honeybees across at least one brood cycle and at all life-stages, as tested up to 200 g a.s./ha or 32 mg a.s./kg food.

The advice of the ECP was sought regarding the risk to bees. Overall, based on the evidence put before it, the ECP advised that bees are not driving the risk assessment (bees are not the most sensitive organism group), and that based on the data available, are not a cause for concern.

B.9.6.1.5. Overall conclusions for risk of pydiflumetofen to bees

There is an acceptable acute risk of pydiflumetofen to adult honeybees, as assessed using the hazard quotient approach. There is an acceptable acute and chronic risk of pydiflumetofen to all honeybee life-stages (larval, adult), as concluded from a qualitative assessment of available data, which was carried out in the absence of GB noted/adopted guidance in this area but which requires consideration. Overall, when considering both the lower and higher tier risk assessment, GB (HSE CRD) 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 evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2.

A summary of the proposed GAP table for the representative product A21857B, containing 62.5 g pydiflumetofen/L, for consideration in the risk assessment is provided in the table below. The full proposed GAP table can be found in dRR Part B Section 0, A21857B.

Table 9.6.2-1 Summary GAP table for A21857B

Crop	Timing of application (range)	Number of applications per season	Application interval [days]	Maximum application rate per treatment [g a.s./ha]	Maximum application rate per treatment [L product/ha]
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Cereals* (spring and winter)	BBCH 30-69	1	n/a	200	3.2
Oilseed Rape (spring and winter)	BBCH 57-69	1	n/a	200	3.2

*cereals include: spring and winter barley, durum wheat, spring and winter oat, spelt, spring and winter wheat, spring and winter rye, spring and winter Triticale

B.9.6.2.1. Toxicity data/Endpoints

Tier I and Tier II toxicity tests on non-target arthropods *Aphidius rhopalosiphi* and *Typhlodromus pyri* have been carried out on A21857B. An additional extended laboratory test on *Chrysoperla carnea* has been submitted for A21857B. There are no studies on the active substance.

Table 9.6.2-2 shows the available ecotoxicological endpoints for non-target arthropods other than bees. All endpoints relevant endpoints for risk assessment are highlighted in bold. Summaries of these studies are provided in the Section B.9.5.2 of this 3CP dossier.

Table 9.6.2-2: Summary of SYN545974 (A21857B) toxicity endpoints for non-target arthropods other than bees.

Test substance	Test organism	Study type	Endpoint	Reference
A21857B	<i>Aphidius rhopalosiphi</i>	Tier 1 glass plate	LR₅₀ > 375 mL product/ha^a (23.4 g a.s./ha)^b Highest rate with < 50 % effect on reproduction: 375 mL product/ha	(2018)
A21857B	<i>Typhlodromus pyri</i>	Tier I glass plates	7d LR₅₀ 1667 mL product/ha (104 g a.s./ha)^b Highest rate with < 50 % effect on reproduction: 1,250 mL product/ha	(2018)
A21857B	<i>Typhlodromus pyri</i>	Extended laboratory study on leaf discs (2D)	LR ₅₀ 5000 mL product/ha (312 g a.s./ha) ^b Highest rate with < 50 % effects on reproduction: 5000 mL product/ha (312 g a.s./ha)^b	(2017)
A21857B	<i>Aphidius rhopalosiphi</i>	Extended laboratory study on sprayed barley plants (3D)	48h LR ₅₀ 8087 mL product/ha (504 g a.s./ha) ^b Highest rate with < 50 % effect on mortality and reproduction: 5555.6 mL product/ha (346.7 g a.s./ha)^b	(2017)
A21857B	<i>Chrysoperla carnea</i>	Extended laboratory study on leaf discs (2D)	LR ₅₀ > 3200 mL product/ha (198.7 g a.s./ha) ^c Highest rate with < 50 % effect on reproduction: 3200 mL product/ha (198.7 g a.s./ha)^c	(2019)

^a Applicant calculated LR₅₀ to be 433.1 mL product/ha using probit, but had removed the top 3 concentrations that resulted in 100 % mortality from the analysis. It is possible that the accuracy of the endpoint was affected by this, as only two data points were included in the probit analysis. To address the uncertainty a conservative estimate of > 375 mL product/ha is recommended.

^b Conversion to g a.s./ha calculated using active substance content of 62.4 g/L as stated in study report (taking into account a.s. content of 5.69 % w/w and density of 1.097 g/cm³).

^c Conversion to g a.s./ha calculated using active substance content of 62.1 g/L as stated in study report (taking into account a.s. content of 5.66 % w/w and density of 1.097 g/cm³).

Values in bold used in risk assessment

B.9.6.2.2. Exposure

B.9.6.2.2.1. In-field exposure

Non-target arthropods inhabiting the crop can be exposed to residues of A21857B by direct contact, either as a result of overspray or through contact with residues on plants and soil or in food items. A21857B is applied at a maximum rate of 1 x 3.2 L product/ha to cereals and oilseed rape (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:

$PER_{in-field} = \text{Application rate [L/ha]} \times \text{MAF}$

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 application of A21857B are presented in Table B.9.6.2-3.

Table B.9.6.2-3: $PER_{in-field}$ values for application of A21857B in oilseed rape and cereals (worst case use).

Crop	Worst case application rate in cereals and oilseed rape [L/ha]	MAF	$PER_{in-field}$ [L/ha]
Cereals and oilseed rape	3.2	1.0	3.2

B.9.6.2.2.2. Off-field exposure

Risk assessment of areas immediately surrounding the crop is considered important since these areas represent a natural reservoir for immigration, emigration and reproduction of arthropod populations. Exposure of non-target arthropods living in off-field areas to A21857B will mainly be due to spray drift from field applications. Off-field areas are assumed to be densely vegetated and thus spray drift is unlikely to reach bare ground. Therefore, evaluation of exposure via soil residues in off-field areas was not considered.

Off-field foliar PER values were calculated from in-field foliar PERs in conjunction with drift values published by the BBA [90th percentile drift according to BBA (2000): Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden] as shown in the following equation:

$$PER_{off-field} = \frac{\text{maximum } PER_{in-field} \times (\% \text{ drift}/100)}{\text{vegetation distribution factor}}$$

The model used to estimate spray drift was developed for drift onto a two-dimensional water surface and, as such, does not account for interception and dilution by three-dimensional vegetation in off-crop areas. Therefore, a vegetation distribution or dilution factor is incorporated into the equation when calculating PERs to be used in conjunction with toxicity endpoints derived from two-dimensional (glass plate, leaf disc or sand) studies. A dilution factor of 10 is recommended by ESCORT 2. For 3-dimensional studies, i.e. where spray treatment is applied onto whole plants, the dilution factor of 10 is not used, as any dilution over the 3-dimensional vegetation surface is accounted for in the study design.

The drift 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 (90th percentile drift). The drift factor (% drift/100) is therefore 2.77/100 = 0.0277.

The resulting PER off-field values are shown in Table B.9.6.2-4.

Table B.9.6.2-4: $PER_{off-field}$ values following application of A21857B in cereals and oilseed rape (worst case use)

Study type	Study type [Exposure scenario]	Maximum $PER_{in-field}$ [L/ha]	Drift factor [% drift/100]	Vegetation distribution factor (VDF)	$PER_{off-field}$ [L/ha]
Cereals and oilseed rape	2D	3.2	0.0277	10	0.008864
	3D			--	0.08864

B.9.6.2.3. Risk Assessment

B.9.6.2.3.1. Tier I in-field risk assessment

The potential risk of A21857B to in-field non-target arthropods was assessed by calculation of the hazard quotient (HQ) using the $PER_{in-field}$ and the lowest lethal rate (LR_{50}) values according to the following equation:

$$HQ_{in-field} = PER_{in-field} [L/ha] / LR_{50} [L/ha]$$

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 $HQ_{in-field}$ values are presented in Table B.9.6.2-5.

Table B.9.6.2-5: $HQ_{in-field}$ for non-target arthropods exposed to A21857B in cereals and oilseed rape (worst case use)

Species	LR_{50} [L/ha]	$PER_{in-field}$ [L/ha]	$HQ_{in-field}$	Trigger value
<i>Aphidius rhopalosiphi</i> Tier I, 2D exposure scenario	0.375	3.2	8.53	2
<i>Typhlodromus pyri</i> Tier I, 2D exposure scenario	1.667		1.91	2

PER = predicted environmental rate.

HQ values shown in **bold** is above the relevant trigger.

The calculated HQ value for *A. rhopalosiphi* was above the trigger of 2; however, the calculated value for *T. pyri* was below the trigger. Therefore, the application of A21857B to oilseed rape and cereals poses a potential risk to *A. rhopalosiphi* but indicates a low risk to *T. pyri*. Further consideration of potential in-field risk was therefore necessary via second tier (Tier II) risk assessment.

B.9.6.2.3.2. Tier I off-field risk assessment

In order to assess the potential risk of A21857B to off-field non-target arthropods, the $PER_{off-field}$ (as determined in table B.9.6.2-4) is compared to the toxicity endpoints according to the following equation:

$$HQ_{off-field} = \frac{PER_{off-field} [L/ha]}{LR_{50} [L/ha]} \times \text{Correction factor}$$

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-crop areas.

Respective $HQ_{off-field}$ values are given in the table below.

Table B.9.6.2-6: $HQ_{off-field}$ values for non-target arthropods exposed to A21857B in cereals and oilseed rape (worst case use)

Species	LR_{50} [L/ha]	$PER_{off-field}$ [L/ha]	Correction factor	$HQ_{off-field}$	Trigger value
<i>Aphidius rhopalosiphi</i> . Tier I, 2D exposure scenario	>0.375	0.008864	10	<0.236	2
<i>Typhlodromus pyri</i> , Tier I, 2D exposure scenario	1.667			0.0531	2

PER = predicted environmental rate.

The calculated $HQ_{off-field}$ values for *A. rhopalosiphi* and *T. pyri* fall below the trigger value of 2, indicating that the application of A21857B to cereals and oilseed rape poses a low risk to non-target arthropods in off-field situations. Therefore, no further consideration or risk mitigation is required for the off-field scenario.

B.9.6.2.3.3. Tier II in-field Risk Assessment

Due to only the in-field assessment failing at Tier I for *A. rhopalosiphi*, only a single additional crop-relevant species is required. Three extended laboratory studies were submitted to study the sublethal effects of A21857B on three non-target arthropods using natural substrates. This included a study on leaf discs (2D) for *T. pyri*, sprayed barley plants (3D) for *A. rhopalosiphi* and a study on leaf discs (2D) for the additional species *Chrysoperla carnea*. In this case, the submission of the extended laboratory study on *T. pyri* is not strictly necessary, but has been considered in risk assessment nevertheless. The applicant has addressed the data requirements according to ESCORT II.

In the extended laboratory studies, risk assessment is based on 50 % effects rather than the HQ approach. The trigger value is based on a 50 % effect compared with the control (either the LR₅₀ for lethal effects, or ER₅₀ for sublethal effects). Where the LR₅₀/ER₅₀ is greater than the PER_{in-field}, a low risk to non-target arthropods can be concluded. If the PER_{in-field} is exceeded, then further consideration of risk would be necessary.

The LR₅₀ and ER₅₀ values relating to sublethal effects on the reproduction of the non-target arthropods are reported in Table B.9.6.2-7.

Table B.9.6.2-7: Lethal and sublethal effect levels for non-target arthropods exposed to A21857B in cereals and oilseed rape (worst case use)

Species	LR ₅₀ [L A21857B/ha]	Highest rate with < 50 % effect on reproduction [L A21857B/ha]	PER _{in-field} [L/ha]
<i>Aphidius rhopalosiphi</i> Tier II, 3D exposure scenario	8.087	5.5556	3.2
<i>Typhlodromus pyri</i> Tier II, 2D exposure scenario	5.0	5.0	
<i>Chrysoperla carnea</i> Tier II, 2D exposure scenario	> 3.2	3.2	

PER = predicted environmental rate.

n.d. = not determined.

Based on the reported values, the 50 % effect levels for both non-target arthropod species are greater than the in-field PER. It is noted that for the additional species of *Chrysoperla carnea* the 50 % effect and LR₅₀ values are either equal to, or are an unbounded value that is equal to the in-field PER, and are therefore close to the trigger value. Examination of the data from the *C. carnea* study (2019) shows that at the maximum application rate of 3.2 L A21857B/ha there was a control-corrected mortality of 5.6 % and no effects were observed for reproduction as the mean number of eggs produced per female per day and the mean egg-hatching rate both exceeded the minimum values specified in the study guideline by Vogt *et al.*, (2000) (≥ 15 eggs were produced per female per day (actual; 35.5) and mean egg-hatching rate was ≥ 70 % (actual; 90.0 %)). Therefore, these effects show that the unbounded value safely exceeds the PER_{in-field} and it is concluded that there is a low in-field risk to non-target arthropods following application of A21857B to cereals and oilseed rape.

B.9.6.2.4. Conclusion

The in-field and off-field risk for other non-target arthropods from the intended uses of the product A21857B in oilseed rape and cereals is acceptable. The off -field risk is indicated to be acceptable based on the available data without the necessity to account for risk mitigation measures.

B.9.7. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.7.1. Earthworms

Report	KCP 10.4.1.1 - (2017) Pydiflumetofen EC (A21857B) - Sublethal Toxicity to the Earthworm <i>Eisenia foetida</i> in Artificial Soil with 5 % peat, Report Number 160713SF / RBR17425. Noack Laboratorien GmbH, Käthe-Paulus-Straße 1, 31157 Sarstedt, Germany. (Syngenta file No. VV-468032).
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Guideline(s):	OECD Guideline No. 222: Earthworm Reproduction Test (<i>Eisenia foetida</i> / <i>Eisenia andrei</i>) (2004)
GLP:	Yes
Duplication (if vertebrate study)	Not applicable

Materials

Test Material	Pydiflumetofen EC (A21857B)
Lot/Batch #:	JEA001-118-001
Actual content of active ingredients:	Pydiflumetofen: 5.62 % w/v corresponding to 61.7 g /L
Description:	Light yellow liquid
Stability of test compound:	Not specified
Reanalysis/Expiry date:	30 June 2019
Density:	1097 kg /m ³
Treatments	
Test rates:	16, 29, 53, 95, 171, 309, 556 and 1000 mg product /kg (spacing factor 1.8)
Control:	Artificial soil moistened with demineralised water without test or reference item.
Toxic standard:	Carbendazim is tested annually at concentrations of 0.5, 1, 2 and 4 mg a.s. /kg soil dry weight. Carried out from 21/01/16 to 18/03/2016 (Study No. RBR1601).
Test organisms	
Species:	<i>Eisenia foetida</i> (Annelida, Lumbricidae)
Age and weight range at test start:	6 to 7 months old, with clitellum, at 0.36 to 0.56 g body weight
Source:	Bred at test facility
Feeding:	Air dried cattle manure; at test start and day 28 mixed carefully into the soil, on the other days placed onto the soil surface.
Test design	
Vessels:	Round plastic trays (15 cm diameter, 14 cm height, 177 cm ² surface area) with transparent, perforated lids.
Substrate:	Artificial soil comprising 5 % peat, 20 % kaolinite clay (kaolinite content > 30 %), 74 % air dried quartz sand (> 50 % of the particles between 0.05 mm and 0.2 mm) and 0.2 % calcium carbonate. About 600 g dry weight, of artificial soil was added to each test vessel.
Replication:	8 control replicates and 4 replicates per test item condition
No. of worms/arena:	10 adult worms
Duration of test:	56 days
Environmental test conditions	
Temperature:	20 ± 2 °C

pH of soil:	6 ± 0.5
Water content of soil:	27 % WHC _{max} prior to application, post application, the humidity of the soil was adjusted to 54 % of the WHC _{max} using demineralised water.
Photoperiod:	16 h
Light intensity	667 ± 81 lux

Study Design and Methods

Experimental dates: 16 November 2016 to 11 January 2017

Approximately 24 hours prior to test start, the artificial soil was prepared and deionised water was added to the dry components to adjust the water content to approximately 40-60 % of its maximum water holding capacity (WHC). The worms were acclimatised in a separate batch of the untreated artificial substrate for approximately 24 hours before test start. The test concentrations were prepared by dispersing an exactly weighed amount of the test material in deionised water to make a stock solution. This stock solution was diluted with deionised water for each concentration and was thoroughly mixed with the artificial soil using a laboratory mixer, achieving a final nominal water content of 40-60 % of WHC. The acclimatised test animals were washed, gently dried on a paper towel, weighed and randomly placed onto the test substrate (10 animals per test vessel).

After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined. Four weeks later, the number of surviving juveniles and any morphological alterations were recorded. Observations of behavioural and pathological symptoms were recorded weekly.

At the experiment start and completion, pH-value and moisture content of the test medium were determined in every treatment and the control from pooled samples of all replicates. The water content of the test containers was checked weekly by weighing. The weight loss was replenished with the appropriate amount of demineralised water.

The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). The arithmetic mean and the standard deviation per treatment and per control for reproduction, mortality and biomass were calculated. The statistical analysis was performed with the software; Excel, Microsoft, Sigma Plot, Spss Inc. and ToxRat Professional 3.2, ToxRat Solutions GmbH. No EC_x/LC_x values for mortality and growth were calculated as no significant effects were observed. For identifying the NOEC values the Williams Multiple Sequential t-test Procedure was used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

Results and Discussion

Table 9.7.1-1: Validity criteria

Validity criterion	Required	Obtained
Control reproduction	Each control replicate (containing 10 adults) should have produced ≥ 30 juveniles by the end of the test	Between 30 to 43 juveniles per replicate
Reproduction coefficient of variation in the controls	The coefficient of variation of reproduction in the controls should be ≤ 30 %	14.4 %
Control mortality	Control adult mortality over the initial 4 weeks of the test should be ≤ 10 %	5 %

Pathological and Behavioural Symptoms

No significant pathological symptoms or changes in behaviour were observed in the control or at any tested concentration. No evident abnormal worm behaviour was observed on any of the days on which they were assessed (Days 0, 1, 7, 14, 21, and 55).

Mortality and Reproduction Assessment

The mortality and reproduction results are summarised in Table 9.7.1-2 below:

Table 9.7.1-2: Effect of A21857B on mortality, growth and reproduction of *Eisenia foetida*

Endpoints	Treatment groups (mg formulated product /kg soil dry weight) [mg a.s. /kg soil dry weight]								
	Control	16	29	53	95	171	309	556	1000
Mean adult mortality at 28 days (%)	5	5	7.5	2.5	5	2.5	0	0	2.5
Mean % biomass change of adults from 0-28 days	40.3	40.7	31.7	42.9	38.3	37.7	40.1	43	38.9
Mean number of juveniles after 8 weeks	36	27	34	39	45	20	36	16	15
Coefficient of variation for reproduction (cv %)	14.4	11.9	14.1	45.7	54	48.9	13.1	39	20.7
% difference in reproduction relative to the control	-	25	5.56	-8.33	-25	44.4	0	55.6	58.3
NOEC (mortality)	≥ 1000 [56.2]								
NOEC (biomass)	≥ 1000 [56.2]								
NOEC (reproduction)	309 [17.37]								
LC ₅₀	-								
EC ₁₀ (reproduction) (95 % confidence intervals)	194 (84.5 - 327) [10.9 (4.75 – 18.38)]								
EC ₂₀ (reproduction) (95 % confidence intervals)	302 (170 - 449) [16.97 (9.55 – 25.23)]								
EC ₅₀ (reproduction) (95 % confidence intervals)	703 (561 - 878) [39.51 (31.53 – 49.34)]								

The dose-response regression of reproduction after eight weeks of exposure to the test item is displayed in Figure 9.7.1-1 below:

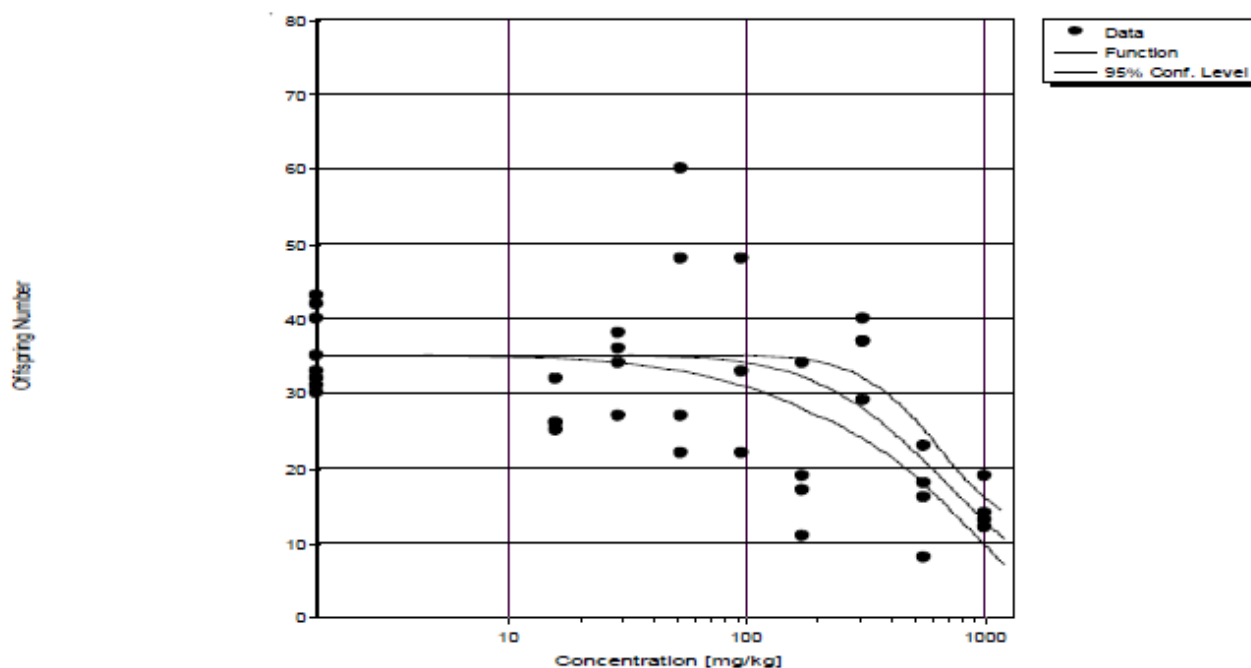


Figure 9.7.1-1 Dose-response regression of reproduction after eight weeks of exposure to the test item-

Conclusions

The NOEC of the test item concerning mortality and growth was determined to be ≥ 1000 mg /kg soil dry weight. The EC₅₀ value for mortality and growth was not calculated since no reduction exceeding 50 % occurred; the EC₅₀ value for mortality is therefore determined as > 1000 mg test item /kg soil dry weight.

The NOEC for reproduction was determined to be 309 mg /kg soil dry weight. The EC₅₀ value for reproduction was determined to be 703 mg /kg soil dry weight.

(██████████ 2017a)

HSE COMMENTS

This study was conducted according to GLP, and was in accordance with OECD 222 (2004): Earthworm reproduction test. For the purposes of this evaluation, it was also assessed against the updated earthworm reproduction test, OECD 222 (2016). All validity criteria were met, there were some minor deviations from the guideline:

The soil moisture deviated by more than 10 % from the initial value in all treatments. The mean deviation from the initial value was 34.0 % (range: 29.0 – 40.7 %). The mean soil moisture at test end was 65.2 % of the WHC_{max} (range 63.2 – 68.1 %). Therefore the soil moisture at test end increased and exceeded the required 40 – 60 % from the WHC. The physical-chemical properties of the artificial soil used require comparably high soil moisture contents for ensuring optimal conditions for the earthworms. Additionally, food was provided on day 0 (test start) instead of day 1, as the performing laboratory had previously had good experiences with this altered procedure.

These deviations are considered to have no impact on quality and integrity of the study, as the guideline validity criteria for the study were well met.

No dose-response relationship was observed at the lower test concentrations for the mean number of juveniles produced after 8 weeks. However, the 171 mg /kg condition showed a 44.4 % reduction in the number of mean juveniles when compared to the control. As the next highest concentration (309 mg /kg) produced the same mean number of juveniles to the control, the raw data were considered. The presence of 3 lower replicate values, with

one replicate value similar to the control results, and the resulting high coefficient of variation in the 171 mg /kg condition would indicate that this result was anomalous, as opposed to the results from the 309 mg /kg condition. This would support the applicant's predicted endpoints, and demonstrates a weak dose-response relationship for 8-week mean juvenile production at the higher test concentrations.

The statistical procedures used were in line with those recommended by OECD 222 (2016).

Based on the nominally administered concentrations, the NOEC_(reproduction) was 17.37 mg a.s. /kg soil, the EC_{50 (reproduction)} was 39.51 mg a.s. /kg soil with 95 % confidence intervals of 31.53 to 49.34 mg a.s. /kg soil and the EC_{10 (reproduction)} was 10.9 mg a.s./kg soil with 95 % confidence intervals of 4.75 – 18.38 mg a.s./kg soil.

B.9.7.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

Report: K-CP 10.4.2.1, [REDACTED], (2017) Pydiflumetofen EC (A21857B) - Effects on the Reproduction of the Collembolan *Folsomia candida*, Report Number 160713SF / ICR17425. Noack Laboratorien GmbH, Käthe-Paulus-Straße 1, 31157 Sarstedt, Germany (Syngenta file No. VV-467124).

Guideline(s):	OECD Guidelines No. 232. Collembolan Reproduction test in soil (2009)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not applicable

Materials

Test Material	Pydiflumetofen EC (A21857B)
Lot/Batch #:	JEA001-118-001
Actual content of active ingredients:	Pydiflumetofen / 5.62 % w/w, corresponding to 61.7 g/l
Description:	Light yellow liquid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	2019-06-30
Density:	1097 kg/m ³
Treatments	
Test rates:	16, 29, 53, 95, 171, 309, 556 and 1000 mg/kg soil dry weight
Control:	untreated
Toxic standard:	Boric Acid
Application method:	Agitation
Test organisms	
Species:	<i>Folsomia candida</i>
Age:	10 days
Source:	Culture maintained at Test Facility
Feeding:	Springtails were fed with 5 mg granulated dry yeast (without emulsifier) per replicate at test start and with 8 mg granulated dry yeast (without emulsifier) per replicate after 14 days.
Test design	
Arenas:	Glass beakers with a volume of 100 mL (inner Ø = 4.3 cm) were used and covered with PARAFILM.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74 % industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm)

Replication:

and 0.2% calcium carbonate. 5309 g wet weight soil, corresponding to about 4832 g dry weight, of artificial soil was added to each test vessel. 8 replicates for the control and 4 replicates for each test item concentration of the biological part (with test organisms). Additionally, 1 replicate per treatment for determination of the pH-value on day 0 and on day 28 (without test organisms). Additionally, 1 replicate per treatment for determination of the soil moisture on day 0 and 28 (without test organisms). Additionally, 2 replicates for determination of the soil moisture on day 14 (without test organisms).

No./arena :

10

Duration of test:

28 days

Environmental test conditions**Temperature:**

16.5 – 22 °C

pH of soil:

6.0 ± 0.5

Water content of soil:

60% WHC

Photoperiod:

12 hrs light/dark, 482 ± 52.3 lux

Study Design and Methods

Experimental dates: 11 November 2016 to 19 December 2016

The test concentrations were prepared by dispersing an exactly weighed amount of the test item in demineralised water to make a stock solution. This stock solution was diluted with deionised water for each test concentration and was thoroughly mixed with the artificial soil using a mixing machine, achieving a final nominal water content of 40-60 % of WHC. The control was treated with demineralised water only.

Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an exhaustor. 4 replicates (+ 4 replicates not loaded with collembolans for measurement purposes) were used per test concentration and 8 for control. Springtails were fed with 5 mg granulated dry yeast (without emulsifier) per replicate at test start and with 8 mg granulated dry yeast (without emulsifier) per replicate after 14 days. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

Determination of mean mortality in % per treatment. Mortality rates of test item will be corrected versus control according to formula 1 (SCHNEIDER-ORELLI, 1947). Determination of the mean number of juveniles per treatment. The reduction of reproduction compared to the control was calculated.

Significant differences in mortality and reproduction were determined in comparison to the control group using different tests.

Mortality: The Chi2 2x2 table test with Bonferroni Correction ($\alpha=0.05$) was carried out to determine statistically significant differences compared to the control. Before a qualitative trend analysis by contrasts was carried out.

Reproduction: The Williams multiple sequential t-test procedure ($\alpha=0.05$) was carried out to determine statistically significant differences compared to the control. Prior to the test a Normality Test and an Equal Variance Test were run.

The LC₅₀ and EC_{10, 20, 50} values were not calculated since no significant inhibitory effects occurred.

All calculations were carried out using software Excel, MICROSOFT CORPORATION Toxrat, Professional, TOXRAT©SOLUTIONS GMBH

Results and Discussion

There was no statistically significant effect of the test item on the mortality rate determined up to and including 1000 mg test item/kg soil dry weight. There was no statistically significant effect of the test item on reproduction rate at any test item concentration (noting relatively high stimulatory effects occurred in some test concentrations).

Mortality and fecundity are summarised in the table below.

Table 9.7.2-1. Effects of residues of A21857B on mortality and reproduction of *Collembola candida*

Endpoint	Treatment group (mg A21857B/kg soil d.w.)								
	Control	16	29	53	95	171	309	556	1000
% Mortality of parental collembolans after 4 weeks	8.75 ± 8.35	5.00 ± 10.0	2.50 ± 5.00	2.50 ± 5.00	5.00 ± 5.77	2.50 ± 5.00	5.00 ± 10.0	10.0 ± 11.6	12.5 ± 18.9
% corrected mortality (Abbott)	-	-4.11	-6.85	-6.85	-4.11	-6.85	-4.11	1.37	4.11
Mean number of juveniles after 4 weeks	436 ± 72.6	584 ± 131	428 ± 145	546 ± 167	618 ± 138	608 ± 88.7	574 ± 64.0	593 ± 95.6	524 ± 100
CV %	16.6	22.5	33.9	30.6	22.4	14.6	11.2	16.1	19.2
% reduction compared to control	-	-33.9	1.83	-25.2	-41.7	-39.5	-31.7	-36	-20.2
NOEC (mortality)	≥ 1000								
NOEC (biomass)	> 1000								
NOEC (reproduction)	≥ 1000								
LC ₅₀	n.d.								
EC ₁₀	n.d.								
EC ₂₀	n.d.								
EC ₅₀	n.d.								

No statistically significant differences compared to the control were detected

n.d. = not determined

(negative percentage values indicate an increase in comparison to the control)

Validity criteria

The validity criteria are as follows:

- Control treatment mortality was 8.75% (must be < 20%)
- The mean number of juvenile recorded in the control treatment was 436 (must be > 100 per replicate)
- The coefficient of variation of reproduction in the control was 16.6% (must not be > 30%)

Conclusions

The NOEC for mortality of the parental collembolans and for reproduction were both determined to be ≥ 1000 mg test item /kg soil dry weight. The LC₅₀ value for mortality and the EC₁₀, EC₂₀ and EC₅₀ values based on reproduction could not be calculated, but were all determined to be > 1000 mg test item/kg soil dry weight (the maximum rate tested).

(██████████, 2017a)

HSE comments

The study was carried out according to GLP and follows OECD 232 (2016) with no significant deviations to the guideline or the study plan. All validity criteria outlined in OECD 232 (2016) have been satisfactorily met.

Validity Criteria (OECD 232 (2016))	Required	Obtained
Mortality in the control	< 20 %	8.75 %
Mean number of juveniles in the control	> 100 per replicate	436
Coefficient of variation of reproduction in the control	< 30 %	16.6 %

A separate test with the reference item boric acid determined an EC₅₀ of 134 mg/kg soil dry weight for reproduction. This is within recommendations in OECD 232 (2016) of a 50 % reduction in reproduction at about 100 mg/kg soil dry weight.

In order to obtain the NOEC for mortality the Chi 2 x 2 test with Bonferroni Correction was used to compare the data to the control. Reproduction data was compared to the control using The Williams multiple sequential t-test procedure. This approach is in-line with OECD 232 (2016).

The EC_{10/20} values could not be statistically determined as there were less than 10 % mortality/reproductive effects at the highest test concentration.

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

- **28-day NOEC reproduction = > 1000 mg product/kg soil dry weight**
- **28-day NOEC mortality = > 1000 mg product/kg soil dry weight**

Report: K-CP 10.4.2.1, [REDACTED] (2017a) Pydiflumetofen EC (A21857B) - Effects on the Reproduction of the Predatory mite (*Hypoaspis aculeifer*), Report Number 160713SF / IHL17425. Noack Laboratorien GmbH, Käthe-Paulus-Straße 1, 31157 Sarstedt, Germany. (Syngenta file No. VV-467137).

Guidelines

- OECD Guideline 226: Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil (2008)

GLP: Yes

Materials

Test Material	Pydiflumetofen EC (formulation A21857B)
Lot/Batch #:	JEA001-118-001
Actual content of active ingredients:	Pydiflumetofen 5.62 % w/w, corresponding to 61.7 g/l, according to certificate of analysis from study sponsor
Density:	1.097 g/cm ³
Description:	Light yellow liquid
Treatments	
Test rates:	1000, 556, 309, 171, 95, 53, 29 and 16 mg A21857B/kg soil (dw)
Control:	Untreated artificial soil
Toxic standard:	Boric Acid (CAS 10043-35-3)
Test organisms	
Species	<i>Hypoaspis aculeifer</i> (Canestrini). Synchronised females.
Source:	KATZ BIOTECH AG (An der Birkenpfuhlheide 10, D-15837 Baruth)
	Mites delivered 4 days before start of test. Adaptation to test conditions (temperature, light intensity, photoperiod) 3 days before test.
Food:	The mites were fed <i>ad libitum</i> with springtails species <i>Folsomia candida</i> .
Age at test start:	28 – 31 days after start of the egg laying period
Test design	
Vessels:	Transparent plastic (polystyrene) vessels with lid (Ø = ca. 5 cm, height = ca. 6 cm, volume 125 mL) were used.
Substrate:	Artificial soil comprising: 5 % air-dried finely ground (2 mm) sphagnum peat, 20 % kaolinite clay (kaolinite >30 %), 74 % air dried industrial quartz sand (> 50 % particles 0.05 – 0.20 mm) and 0.2 % calcium carbonate. Maximum water holding capacity (WHC _{max}): 36.1 g H ₂ O/100 g dw of soil. Amount of soil per vessel: 40 g soil wet weight at 60 % WHC _{max} (this corresponds to approx. 32.9 g soil dw, as calculated by HSE using the stated WHC _{max}), without compression.
Replication:	Control group: 8

	Treated group: 4
	Additionally, 1 replicate per treatment for determination of the pH-value and water content on day 0 and day 14 (without test organisms).
	Additionally, 2 replicates for the determination of the temperature in soil during the heat/light extraction.
No. of mites/arena:	10
Duration of test:	14 days
Environmental test conditions	
Temperature:	18 - 22 °C
pH of soil:	5.87
Water content of soil:	60 % of maximum WHC
Photoperiod:	16 h light : 8 h dark, 619 ± 58.4 lux
Aeration	Three times a week by briefly opening the vessels

Study Design and Methods

Experimental dates: 21 November 2016 to 09 December 2016

The test concentrations were 1000, 556, 309, 171, 95, 53, 29 and 16 mg A21857B/kg soil dry weight. As a control, artificial soil without test item was used. Boric acid (300 mg/kg DW) was used as a reference item.

The test item was applied as a liquid: application solutions at the required concentrations were prepared by dilution from a stock solution and making up volume with demineralised water. The respective application solution was added to the artificial soil which had been pre-moistened to 30 % WHC_{max}, resulting in a final soil moisture of 60 % WHC_{max}. The test soil was subsequently thoroughly mixed with a blender to ensure homogenous distribution of the test item and the water.

After application, ten adult females of *Hypoaspis aculeifer* (28 - 31 days old) were placed onto the artificial soil per each replicate. Eight control and reference replicates and 4 replicates for each test item concentration were tested. During exposure, mites were fed with springtails species *Folsomia candida*.

Soil moisture in test containers was monitored throughout the test by weighing.

The exposure ended after 14 days followed by an approximate 54.5 h heat/light extraction. The extraction efficiency (combined for all developmental stages) is validated once within 12 months. The most recent validation resulted in an extraction efficiency of 84.3 % (January 2016). Juveniles and adults were counted on day 17 and 18. Observed parameters were the number of surviving adults (mortality) and the number of hatched juvenile mites (reproduction).

Mortality rates of the test item were control-corrected according to Schneider-Orelli, 1947. The reduction of reproductive output (Rr) for the treatment groups was calculated in comparison to the control.

Significant differences in mortality and reproduction were determined in comparison with the control group using the following statistical analysis:

- Mortality: A qualitative tre A Chi² 2x2 Table Test with Bonferroni Correction ($\alpha=0.05$) was carried out to determine statistically significant differences compared to the control. Before a Qualitative Trend Analysis by Contrasts were run.
- Reproduction: The Multiple Sequentially-rejective Welsh-t-test after Bonferroni-Holm ($\alpha=0.05$) was carried out to determine statistically significant differences compared to the control. Prior to this a Normality Test and an Equal Variance Test were run (data were found to be normally distributed but with heterogeneity of variance).
- The LCx / ECx-value could not be calculated, due to the lack of treatment-related effects observed during the study.

Calculations were carried out using software: Excel, (Microsoft Corporation) and TOXRAT 3.2.1 (ToxRat Solutions GmbH).

Results and Discussion

The pH of the soil as measured at the start and end of the test ranged from 5.27 – 5.55 across all replicates and control, demonstrating a stable pH. The range of soil moisture was 52.6 – 56.8 % WHC, which is within the recommended maximum 10 % variation of the guideline.

Mortality and reproduction are summarised in the table below. For reproduction, all treatment rates showed no statistically significant differences compared to the control (Multiple Sequentially-rejective Welsh- t-test procedure, $\alpha=0.05$). For mortality, no statistically significant difference in mortality rate was observed in all test item concentrations compared to the control (Chi² 2x2 Table Test with Bonferroni Correction, $\alpha=0.05$).

The reference item boric acid (300 mg/kg DW) resulted in a 99.3 % reduction in number of juveniles after 14 days, demonstrating the sensitivity of the test system.

Table 9.7.2-2: Effects of residues of A21857B on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg A21857B/kg soil d.w.)									Reference item
	Control	16	29	53	95	171	309	556	1000	
	Mortality of adult mites after 14 days									
% mortality ± SD	17.5 ± 11.7	37.5 ± 28.7	37.5 ± 12.6	12.5 ± 9.57	32.5 ± 27.5	37.5 ± 43.5	25.0 ± 12.9	30.0 ± 18.3	35.0 ± 19.2	16.3 ± 10.6
% corrected mortality	-	24.2	24.2	-6.06	18.2	24.2	9.09	15.2	21.2	-1.52
	Number of juveniles after 14 days									
Mean no. progeny per replicate ± SD	94.5 ± 17.5	76.0 ± 28.2	105 ± 40.0	172 ± 40.1	113 ± 49.5	102 ± 80.2	59.3 ± 20.5	107 ± 81.1	68.8 ± 15.3	0.63 ± 0.92
CV%	18.5	37.4	38.1	23.3	43.8	78.8	34.6	76.2	22.2	146
% reduction compared to control	-	19.6	-11.1	-82.3	-19.6	-7.67	37.3	-12.7	27.3	99.3
NOEC (mortality)	≥ 1000									-
LOEC	> 1000									-
NOEC (reproduction)	≥ 1000									-
EC ₅₀ / EC ₂₀ / EC ₁₀ (reproduction)	n.d.									-

No statistically significant differences compared to the control were detected.

n.d. = not determined

(Negative percentage values indicate an increase in comparison to the control)

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females: ≤ 20 % (observed: 17.5 %)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 94.5)
- Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (calculated: 18.5 %)

Conclusions

The NOEC for mortality of the parental mites and for reproduction were both determined to be ≥ 1000 mg test item/kg soil dry weight. The LC₅₀ value for mortality and the EC₁₀, EC₂₀ and EC₅₀ values based on reproduction could not be calculated, but were all determined to be > 1000 mg test item/kg soil dry weight (the maximum rate tested).

(████████ 2017b)

HSE Comments

The study was conducted to GLP. The study was conducted to OECD 226 (2008) but the study has been assessed to the more recent OECD 226 (2016) guideline.

The study meets all validity criteria and there are no major deviations from the guideline. The statistical procedures have been adequately described.

It is noted that the efficiency of the extraction method is 84.3 % whereas the guideline states this should be > 90 %. This adds some uncertainty to the results, which will be considered at risk assessment.

Additionally, 40 g wet (at 60 % WHC_{max}) artificial soil was used for each replicate (calculated by HSE to correspond to 32.9 g soil dry weight based on 60 % of stated WHC_{max} of 36.1 g/100 g dw) instead of 20 g dry mass as recommended by the OECD 226 guideline. This deviation is justified by the authors as '*due to good experience with the procedure*'. It is also noted that the measured pH of 5.27 – 5.55 is low compared to the pH 6.0 ± 0.5 detailed in the guideline. However, as the control validity criteria are met these are minor deviations which do not affect the outcome of the study.

It is noted that the % mortality was higher in all treatment groups than control, apart from the middling test concentration of 53 mg A21857B/kg soil dw. However, there is no dose-response relationship, and the results are not statistically significant between any treatments and the control, so HSE agrees with the mortality endpoint being at the highest tested concentration.

The reference item demonstrates the sensitivity of the test system for reproduction (99.3 % reduction in reproduction compared to control tested at 300 mg/kg DW, see table 9.7.2-2) but not for mortality (less mortality observed in reference item than control, see table 9.7.2-2). This is in line with the guideline, which states that the EC₅₀ of boric acid based on number of juveniles should fall between 100 – 500 mg/kg dw soil, and that if the reference substance is tested at a single concentration it should show an effect of > 50 % reduction in offspring. The guideline does not mention expected results for mortality. The obtained result for reproduction is within the expected range of the guideline.

The statistical procedures used have been considered:

- For the mortality data, the authors determined by trend analysis that the data was non-monotonic and selected a Chi² 2x2 Table Test with Bonferroni Correction. The data was not transformed prior to testing. This pair-wise non-parametric procedure is listed in the OECD Statistics Guidance Document 54 as a suitable method for determining NOEC for quantal data.
- For the reproduction data, the authors checked the normality and homogeneity of variance of the data, and found the data was normally distributed but had heterogeneity of variance. The data was not transformed prior to testing. The selected statistical test of multiple sequentially-rejective Welch-t-test after Bonferroni-Holm is a parametric pairwise comparison procedure suitable for data with unequal variance and is in line with the OECD Guidance Document 54.

The implications of the uncertainties noted above will be discussed in the risk assessment.

The agreed endpoints for use in risk assessment are:

- **EC₅₀ (reproduction): > 1000 mg formulation A21857B/kg soil dw (nominal concentration)**
- **LC₅₀: > 1000 mg formulation A21857B/kg soil dw (nominal concentration)**
- **NOEC (reproduction and mortality): ≥ 1000 mg formulation A21857B/kg soil dw (nominal concentration)**

B.9.8. RISK ASSESSMENT FOR NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.8.1. Risk assessment for earthworms


Toxicity

No earthworm studies were carried out using the active substance, only the representative product A21857B (Miravis Plus). In this case, the study carried out using the representative product 'Miravis Plus' was used to fulfil the active substance data requirements, and the risk assessment (calculation of TER values) was based on the endpoints calculated from the study performed with the representative product Miravis Plus (A21857B).

The study which was used in the risk assessment was deemed valid for regulatory purposes with no significant deviations from the study guidelines.

Endpoints in terms of the active substance content are presented in parentheses in Table 9.8.1-1 below.

Table 9.8.1-1: Endpoints used in risk assessment

Table 9.8.1-1: Endpoints used in risk assessment				
Test substance	Test organism	Endpoint mg product/kg soil d.w. (mg a.s./kg soil d.w.)		Reference
Chronic toxicity to earthworms				
A21857B (Miravis Plus)	<i>Eisenia foetida</i>	NOEC _{Mortality}	> 1000 (56.2) nom.	 (2017) KCP 10.4.1.1
		NOEC _{Reproduction}	309 (17.37) nom.	
		EC ₁₀ (reproduction)	194 (10.9) nom.	
		EC ₅₀ (reproduction)	703 (39.51) nom.	
		NOEC _{Mortality} CORR ¹⁾	> 500 (28.1) nom.	
		NOEC _{Reproduction} CORR ¹⁾	154.5 (8.69) nom.	
		EC ₁₀ (reproduction) CORR ¹⁾	97 (5.45) nom.	
		EC ₅₀ (reproduction) CORR ¹⁾	351.5 (19.76) nom.	

¹⁾Corrected by a factor of 2 due to $\log_{POW} > 2$

nom.: endpoints based on nominal concentrations

Soil d.w. = dry weight, a.s. = active substance

Endpoints in terms of the active substance are presented in parentheses, and were calculated based on the analysed content of pydiflumetofen in the formulation (5.62 % w/v, corresponding to 61.7 g a.s. /L).

Endpoints considered in the risk assessment are highlighted in **bold**.

Exposure

Estimates of the maximum predicted environmental concentrations in soil (PEC values) of pydiflumetofen, and the formulation 'Miravis Plus', have been established in Section B.8 of this assessment report by the Environmental Fate and Behaviour specialist. The relevant PEC values considered for toxicity exposure ratio (TER) calculations based on the proposed use in cereals are summarised in Tables 9.8.1-2 and 9.8.1-3 below. Maximum values are used for risk assessments.

Two sets of PEC_{soil} values were provided by CRD Environmental Fate and Behaviour, one set was calculated with an SFO DT₅₀ of 1,310 days (Table 9.8.1-2), and the other set was calculated using a DFOP DT₅₀ of 8,540 days, with a DT₉₀ of > 10,000 days (Table 9.8.1-3). For the purposes of this risk assessment, the worst-case values were selected, which were those calculated using a DFOP DT₅₀ of 8,540 days, with a DT₅₀ of > 10,000 days. The initial accumulated PEC values were calculated using an assumed soil mixing depth of 5 cm, however, due to the very long time period taken to reach a plateau in soil a.s. concentration (in some cases over 100 years), CRD Environmental Fate and Behaviour concluded that it is unreasonable to expect that the soil would not be cultivated deeper than 5 cm at all over this time period. For this reason, the final accumulated residue was recalculated over a 20 cm depth (Environmental Fate and Behaviour Section B8 3CA/CP dossier).

Table 9.8.1-2PECsoil values (with SFO DT₅₀ 1310 days)

Compound	GAP uses	PECsoil, ini [mg/kg]	PECsoil, accu [mg/kg]	PECsoil, max [mg/kg]
Pydiflumetofen	BBCH 30 Cereals 1x 166 g a.s. /ha with 80 % interception	1-28y - 0.044	'Steady state (20y)' 0.202 'Steady state (36y)' 0.052	'Peak' (20y) 0.247 'Peak' (36y) 0.096
	BBCH 55 Cereals 1x 200 g a.s. /ha with 90 % interception	1-7y – 0.027	'Steady state (20y)' 0.122 'Steady state (32y)' 0.031	'Peak' (20y) 0.149 'Peak' (32y) 0.058
	BBCH 57 Oilseed rape 1x 200 g a.s. /ha with 80 % interception	0-28y – 0.053	<u>Annual application</u> 'Steady state (20y)' 0.244 'Steady state (39y)' 0.063	<u>Annual application</u> 'Peak' (20y) 0.297 'Peak' (39y) 0.116
			<u>Application every 3rd year</u> 'Steady state (22y)' 0.067 'Steady state (28y)' 0.017	<u>Application every 3rd year</u> 'Peak' (22y) 0.120 'Peak' (28y) 0.070
'Miravis Plus' formulation	Cereals 2,907 g product/ha 80 % interception	0.775		
	Cereals 3,510.4 g product/ha 90 % interception	0.468		
	Oilseed rape 3,510.4 g product/ha 80 % interception	0.936		

Table 9.8.1-3PECsoil values (with DFOP DT₅₀ of 8540 days; DT₉₀ > 10,000 days)

Compound	GAP uses	PECsoil, ini [mg/kg]	PECsoil, accu [mg/kg]	PECsoil, max [mg/kg]
Pydiflumetofen	BBCH 30 Cereals 1x 166 g a.s. /ha with 80 % interception	0.044	'Steady state (20y); 5 cm depth': 0.567 'Steady state (final); 20 cm depth': 0.526	'Peak' (20y) 0.611 'Peak' (final) 0.570
	BBCH 55 Cereals 1x 200 g a.s. /ha with 90 % interception	0.027	'Steady state (20y); 5 cm depth': 0.341 'Steady state (final); 20 cm depth': 0.317	'Peak' (20y) 0.368 'Peak' (final) 0.344
	BBCH 57 Oilseed rape 1x 200 g a.s. /ha with 80 % interception	0.053	'Steady state (20y); 5 cm depth': 0.683 'Steady state (final); 20 cm depth': 0.634	'Peak' (20y) 0.736 'Peak' (final) 0.687
'Miravis Plus' formulation	Cereals 2,907 g product/ha 80 % interception	0.775		
	Cereals	0.468		

	3,510.4 g product/ha 90 % interception			
	Oilseed rape 3,510.4 g product/ha 80 % interception	0.936		

Bold values: worst case considered in risk assessment

Risk assessment for earthworms

Earthworm (*Eisenia foetida*) toxicity studies have been submitted in accordance with the reporting requirements in Commission Regulation (EU) No 283/2013. The assessment of the chronic risk to earthworms has been conducted according to SANCO/10329/2002 guidance. Risk is assessed in terms of Toxicity Exposure Ratios (TERs), using the endpoints from Table 9.8.1-1 above and is calculated using the following equation:

$$\text{TER} = \text{Study endpoint} / \text{PEC}_{\text{soil}}$$

As the log Pow value for pydiflumetofen is > 2, correction of the study endpoints is required to account for differences in the organic matter content of the test soil in comparison to artificial soils.

The risk is considered acceptable if the TER_{LT} is >5.

The resulting TERs for earthworms are summarised in Table 9.8.1-4 below.

Table 9.8.1-4 TER calculations for earthworms for each GAP use of pydiflumetofen

Compound	Species, study type	Endpoint	GAP uses	PEC _{soil} , max [mg/kg]	TER _{LT}	Trigger
Active substance risk assessment ^a						
A21857B (Miravis Plus)	<i>Eisenia foetida</i> , reproduction	5.45 mg a.s./kg	BBCH 30 Cereals 1x 166 g a.s./ha with 80 % interception	0.611	8.9	5
			BBCH 55 Cereals 1x 200 g a.s. /ha with 90 % interception	0.368	14.8	5
			BBCH 57 Oilseed rape 1x 200 g a.s. /ha with 80 % interception	0.736	7.4	5
Formulated product ‘Miravis Plus’ risk assessment ^a						
A21857B (Miravis Plus)	<i>Eisenia foetida</i> , reproduction	97 mg product/kg soil d.w.	BBCH 30 Cereals, 2,907 g/ha, 80% interception	0.775	125.2	5
			BBCH 55 Cereals, 3,510.4 g/ha, 90% interception	0.468	207.3	5
			BBCH 57 Oilseed rape, 3,510.4 g/ha, 80% interception	0.936	103.6	5

^a Both risk assessments were carried out using the same study - the active substance risk assessment used the study endpoints represented in terms of the active substance content (5.62 % w/v in the formulation) along with the active substance PEC_{soil} values, and the formulated product risk assessment used formulated product endpoints along with the PEC_{soil} values for the formulated product.

Conclusion:

All TER values exceed the trigger value of 5 with a large margin of safety, indicating that no unacceptable adverse effects on earthworms are to be expected from the intended uses of 'Miravis Plus' at the proposed application rates. As the product 'Miravis Plus' only contains one active substance, and a high margin of safety is demonstrated in the risk assessment, it is likely that the formulation risk assessment is protective of the risk from the active

substance. In order to demonstrate a potential risk from the active substance, the active would have to demonstrate a substantially higher level of toxicity in an active-only study, which is unlikely, given the formulation results. No further consideration or refinement is required.

B.9.8.2. Risk assessment for non-target soil meso- and macrofauna (other than earthworms)

Toxicity

No studies were carried out on non-target soil meso- and macrofauna (other than earthworms) using the active substance pydiflumetofen, studies were only submitted which tested the effects of the representative product A21857B (Miravis Plus). In this case, the studies carried out using the representative product 'Miravis Plus' were used to fulfil the active substance data requirements. Two risk assessments were carried out, one using the formulated product endpoints and formulation PEC_{Soil} values, and the other using active substance PEC_{Soil} values, and active substance endpoints which were derived from the formulation endpoints, these were calculated based on the analysed content of pydiflumetofen in the formulation (5.62 % w/v, corresponding to 61.7 g a.s. /L).

The studies used in the risk assessment were deemed valid for regulatory purposes with no significant deviations from the study guidelines. A summary of the endpoints used in the risk assessment is provided in Table 9.8.2-1 below. Endpoints in terms of the active substance content are presented in parentheses.

Table 9.8.2-1: Endpoints for use in the risk assessment:

Test substance	Test organism	Endpoint mg product/kg soil d.w. (mg a.s./kg soil dw)		Reference
Chronic toxicity to other soil macro-organisms				
A21857B (Miravis Plus)	<i>Hypoaspis aculeifer</i>	EC ₅₀ (reproduction)	> 1000 mg product /kg soil d.w. (nom); equivalent to 56.2 mg a.s./kg soil d.w.	██████████ (2017a) KCP 10.4.2.1
		LC ₅₀	> 1000 mg product /kg soil d.w. (nom); equivalent to 56.2 mg a.s./kg soil d.w.	
		NOEC (reproduction and mortality)	≥ 1000 mg product /kg soil d.w. (nom); equivalent to 56.2 mg a.s./kg soil d.w.	
		EC ₅₀ (reproduction) _{CORR} ¹⁾	> 500 mg product /kg soil d.w. (nom); equivalent to 28.1 mg a.s./kg soil d.w.	
		LC ₅₀ _{CORR} ¹⁾	> 500 mg product /kg soil d.w. (nom); equivalent to 28.1 mg a.s./kg soil d.w.	
		NOEC _{CORR} ¹⁾ (reproduction and mortality)	≥ 500 mg product /kg soil d.w. (nom); equivalent to 28.1 mg a.s./kg soil d.w.	
	<i>Folsomia candida</i>	28-day NOEC (reproduction)	≥ 1000 mg product /kg soil d.w. (nom); equivalent to 56.2 mg a.s./kg soil d.w.	██████████ (2017) KCP 10.4.2.1
		28-day NOEC (mortality)	≥ 1000 mg product /kg soil d.w. (nom); equivalent to 56.2 mg a.s./kg soil d.w.	
		28-day NOEC (reproduction) _{CORR} ¹⁾	> 500 mg product /kg soil d.w. (nom); equivalent to 28.1 mg a.s./kg soil d.w.	
		28-day NOEC (mortality) _{CORR} ¹⁾	≥ 500 mg product /kg soil d.w. (nom); equivalent to 28.1 mg a.s./kg soil d.w.	

¹⁾ Endpoints are corrected by a factor of 2 (due to log_{Pow} > 2) for use in the risk assessment.

nom: nominal concentration

Endpoints in terms of the active substance were calculated based on the analysed content of pydiflumetofen in the formulation (5.62 % w/v, corresponding to 61.7 g a.s. /L).

Endpoints considered in the risk assessment are highlighted in **bold**.

Exposure

Estimates of the maximum predicted environmental concentrations in soil (PEC values) of pydiflumetofen, and the formulation, 'Miravis Plus', have been established in Section B.8 of this assessment report by the Environmental Fate and Behaviour specialist, and are summarised above, in Tables 9.8.1-2 and 9.8.1-3 of this document for reference. Maximum values are used for risk assessments.

Risk assessment for non-target soil meso- and macrofauna (other than earthworms)

Studies with *Hypoaspis aculeifer* and *Folsomia candida* have been submitted in line with the reporting requirements in Commission Regulation (EU) No 283/2013. The studies investigate the impact of the active substance pydiflumetofen, as the formulated product 'Miravis Plus' on soil meso- and macrofauna. In the absence of pydiflumetofen active substance studies, the risk from pydiflumetofen to *Hypoaspis aculeifer* and *Folsomia candida* could not be directly assessed. However, given that the representative product 'Miravis Plus' contains only one active substance, it is likely that the formulation assessment is protective of the risk from the active. The risk assessment was conducted according to the SANCO/10329/2002 guidance on Terrestrial Ecotoxicology for the proposed application rate of pydiflumetofen.

All studies were deemed valid for regulatory purposes with no significant deviations from the study guidelines. A minor source of uncertainty came from the [REDACTED] (2017a) study, which is considered further in the risk assessment below.

Calculation of TERs

TER values for non-target soil meso- and macro-fauna (other than earthworms) were calculated as above in the earthworm risk assessment. As the log P_{ow} value for pydiflumetofen is > 2 , correction of the study endpoints is required to account for differences in the organic matter content of the test soil in comparison to artificial soils.

PEC_{soil} values have been compared to the study endpoints to determine TERs in Table 9.8.2-2 below.

Table 9.8.2-2: TER calculations for non-target soil meso- and macro-fauna (other than earthworms) for each GAP use of pydiflumetofen

Use of pyrimethanil						
Compound	Species	Endpoint	GAP uses	PECsoil, max* [mg/kg]	TER _{LT}	Trigger
Active substance risk assessment ^a						
A21857B (Miravis Plus)	<i>Hypoaspis aculeifer</i>	28.1 mg a.s./kg	BBCH 30 Cereals 1x 166 g a.s./ha with 80 % interception	0.611	46.0	5
			BBCH 55 Cereals 1x 200 g a.s. /ha with 90 % interception	0.368	76.4	
			BBCH 57 Oilseed rape 1x 200 g a.s. /ha with 80 % interception	0.736	38.2	
A21857B (Miravis Plus)	<i>Folsomia candida</i>	28.1 mg a.s./kg	BBCH 30 Cereals 1x 166 g a.s./ha with 80 % interception	0.611	46.0	
			BBCH 55 Cereals 1x 200 g a.s. /ha with 90 % interception	0.368	76.4	
			BBCH 57 Oilseed rape 1x 200 g a.s. /ha with 80 % interception	0.736	38.2	
Formulated product ‘Miravis Plus’ risk assessment ^a						
A21857B (Miravis Plus)	<i>Hypoaspis aculeifer</i>	500 mg product/kg soil d.w.	BBCH 30 Cereals, 2,907 g/ha, 80% interception	0.775	645.2	5
			BBCH 55 Cereals, 3,510.4 g/ha, 90% interception	0.468	1068.4	
			BBCH 57	0.936	534.2	

			Oilseed rape, 3,510.4 g/ha, 80% interception			
A21857B (Miravis Plus)	<i>Folsomia candida</i>	500 mg product/kg soil d.w.	BBCH 30 Cereals, 2,907 g/ha, 80% interception	0.775	645.2	
			BBCH 55 Cereals, 3,510.4 g/ha, 90% interception	0.468	1068.4	
			BBCH 57 Oilseed rape, 3,510.4 g/ha, 80% interception	0.936	534.2	

^a Both risk assessments were carried out using the same two studies - the active substance risk assessment used the study endpoints represented in terms of the active substance content along with the active substance PEC_{soil} values, and the formulated product risk assessment used formulated product endpoints along with the PEC_{soil} values for the formulated product.

There are two sources of uncertainty to be considered as part of the risk assessment. One is the extrapolation of formulated product endpoints to reach conclusions on the risk posed by the active substance. This is considered acceptable as the representative product 'Miravis Plus' only contains one active substance, and a high margin of safety is demonstrated in the risk assessment, indicating that the active substance alone would have to be significantly more toxic than the formulation in order for an unacceptable risk to be demonstrated. As such, it can be assumed that the formulation risk assessment is protective of the risk from the active substance.

The other uncertainty surrounds the extraction method from the [REDACTED] (2016a) study carried out on *Hypoaspis aculeifer*, where the efficiency of the extraction method was 84.3 % - slightly lower than the minimum OECD 226 (2016) recommendation of > 90 %. As this is only slightly below the minimum recommended extraction efficiency, and a large margin of safety has been demonstrated by the risk assessment, there is no reason to suggest that there is any negative impact on the reliability of the risk assessment conclusions as a result.

Conclusion:

All TER values exceed the trigger value of 5 with a large margin of safety, indicating that no unacceptable adverse effects on soil meso- and macro-fauna (other than earthworms) are to be expected from the intended uses of pydiflumetofen at the proposed application rates.

B.9.9. EFFECTS ON SOIL NITROGEN TRANSFORMATION

Report: K-CP 10.5/01 [REDACTED], (2017a) Pydiflumetofen EC (A21857B) - Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests), Report Number 160713SF /TBM17425 Noack Laboratorien GmbH, Käthe-Paulus-Straße 1, 31157 Sarstedt, Germany. (Syngenta file No. VV-467942).

GUIDELINES

OECD guidelines 217, Soil Microorganisms: Carbon Transformation Test (2000)
OECD guidelines 216, Soil Microorganisms: Nitrogen Transformation Test (2000)

GLP: Yes

MATERIALS

Test Material	Pydiflumetofen EC (A21857B)
Lot/Batch #:	JEA001-118-001
Actual content of active ingredients:	Pydiflumetofen: 5.62 % w/w corresponding to 61.7 g/L

Density:	1097 kg/m ³
Treatments	
Test rates:	5 mg test item/kg dry soil and 25 mg test item/kg dry soil
Control:	Untreated soil
Toxic standard:	Dinoterb for carbon transformation, tested once within twelve months at concentration of 18.3 mg/kg soil dw. Cyanoguanidine for nitrogen transformation, tested once within twelve months at concentrations of 50 and 100 mg/kg soil dw.
Test design	
Soil type:	LUFA soil 2.3. Organic carbon content: 0.759 % ; microbial biomass of total organic carbon: 3.0 %; soil texture: silty sand; maximum water holding capacity: 35.6 ± 1.4
Test units:	Plastic boxes (volume 6.25 L (carbon transformation) and 1.0 L (nitrogen transformation), food grade) with perforated tops to enable gas exchange.
Replication:	Triplicates per application rate and control Separate replicates for nitrogen and carbon formation were prepared.
Sampling intervals:	Nitrogen transformation test: 5:45 h, 7, 14 and 28 days after application Carbon transformation test: 0:15 h, 7, 14 and 28 days after application
Duration of test:	28 days
Environmental test conditions	
Temperature:	20 ± 2 °C
pH of soil:	5.9 ± 0.6
Soil moisture content:	45 % WHC _{max} (water losses 0.1-0.2 % in carbon transformation and 0.4-0.9 in nitrogen transformation test; compensation with demineralised water).
Photoperiod:	Constant darkness

STUDY DESIGN AND METHODS

Experimental dates: 09 December 2016 to 09 January 2017

Soil samples were treated with A21857B at two doses, 5 and 25 mg test item/kg dry soil.

The test item was mixed with deionised water and the test solution was subsequently mixed with the soil in the laboratory mixer. Water was added to the soil to achieve a water content of approximately 45 % of WHC. The water content of the soil in each test vessel was determined at test start (after application) and adjusted once a week to the required range of 40 - 50 % of WHC.

Three replicate soil samples were prepared for each treatment rate and the control for the nitrogen transformation test and carbon transformation test.

Mean nitrogen content (mg NO₃/kg soil d.w.), standard deviation and coefficient of variation as well as the mean nitrogen content/day (mg NO₃/kg soil d.w./day) were calculated for each treatment group and sampling date.

For the evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated (based on the mean nitrogen content/day) for each sampling date.

The cumulative O₂ consumption after 24 hours was calculated. Furthermore, standard deviation and coefficient of variation were calculated for each treatment group and sampling dates.

For evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated for each sampling date.

RESULTS AND DISCUSSION

Results from the Nitrogen transformation test and the Carbon transformation test are summarised in the tables below.

Table 9.9-1: Effects on Nitrogen Transformation in Soil after Treatment with the Test Item

Time Interval (days)	Control		5 mg test item/kg soil dry weight			25 mg test item/kg soil dry weight		
	NO ₃ -N [mg/kg soil d.w.]	NO ₃ -N [mg/kg soil d.w./day]	NO ₃ -N [mg/kg soil d.w.]	NO ₃ -N [mg/kg soil d.w./day]	Deviation from control [%] ¹⁾	NO ₃ -N [mg/kg soil d.w.]	NO ₃ -N [mg/kg soil d.w./day]	Deviation from control [%] ¹⁾
0 - 7	40	1.18	40.8	1.28	-8	41.7	1.23	-4
0 - 14	48.7	1.21	52.8	1.5	-24	50.3	1.23	-2
0 - 28	57.5	0.917	57.6	0.92	0	57.1	0.856	7

The calculations were performed with non-rounded values

1) based on NO₃-nitrogen production; + = inhibition; - = stimulation

No statistically significant differences between the control and the test item treatments were calculated

The mean nitrogen transformation rate has also been calculated by the HSE evaluator for the time intervals 0-7, 7-14 and 14-28 days, to determine whether delayed effects were occurring, whereby lack of early effects masks effects occurring later on. The results are shown in the table below:

Table 9.9-2: Effects on nitrogen transformation rate calculated section-by-section

Treatment (mg formulation/kg soil d.w.)	Mean nitrogen transformation rate (mg NO ₃ -N /kg soil d.w./day) (% deviation from the control)		
	Day 0-7	Day 7-14	Day 14-28
Control	1.18	1.24	0.62
5.00	1.29 (+9.31)	1.71 (+37.93)	0.34 (-45.04)
25.00	1.23 (+4.45)	1.23 (-1.15)	0.42 (-33.21)

+ = inhibition; - = stimulation

Table 9.9-3: Effects on Carbon Transformation in Soil after Treatment with the Test Item

Days after application	Control		5 mg test item/kg soil dry weight			25 mg test item/kg soil dry weight		
	O ₂ -consumption [mg/kg soil d.w./h]	CV [%]	O ₂ -consumption [mg/kg soil d.w./h]	CV [%]	Deviation from control [%] ¹⁾	O ₂ -consumption [mg/kg soil d.w./h]	CV [%]	Deviation from control [%] ¹⁾
0	10.66	4	10.28	2	4	10.7	1	0
7	10.41	2	9.72	5	7	10.54	1	-1

14	8.7	8	8.46	1	3	8.93	0	-3
28	8.2	5	8.12	4	1	8.28	2	-1

The calculations were performed with non-rounded values.

1) based on O₂ consumption; + = inhibition; - = stimulation

* statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

VALIDITY CRITERIA

The validity criteria are listed below:

Criterion	Required	Observed
Coefficient of variation in the control	Must be ≤ 15 %	Nitrogen transformation test: max 4.0 % Carbon transformation test: max 8.0 %

The coefficient of variation in the Nitrogen and Carbon transformation tests were < 15 % (must be ≤ 15 %)

CONCLUSIONS

The effects of the test item on the microbial soil activity (carbon and nitrogen transformation tests) were analysed on the day of treatment (day 0) and subsequently after 7, 14 and 28 days.

The test item Pydiflumetofen EC (A21857B) (tested at 5 mg/kg soil dry weight and 25 mg/kg soil dry weight) caused no adverse effects (deviation from control < 25 %, OECD 216/217) on soil carbon transformation (measured as O₂-consumption) and on soil nitrogen transformation (measured as Nitrate-N-production) at the end of the 28-day incubation period.

(██████████ 2017a)

HSE COMMENTS

This study has been conducted in accordance with GLP and follows OECD 216 and OECD 217. The validity criteria have been met for both the carbon and nitrogen transformation test. A minor deviation from the guidelines is noted; the soil used in both tests was stored at 6 ± 2 °C rather than 4 ± 2 °C. This deviation is considered to be minor and is not thought to have impacted the outcome of either study.

Nitrogen transformation test:

A reference test conducted with cyanoguanidine at 100 mg/kg soil dw resulted in 92 % inhibition after 28 days, demonstrating the sensitivity of the test system. The soil nitrate formation rate has been calculated for intervals 0-7, 0-14 and 0-28 days. When considering the whole test period (0-28 days), the deviations from the control were 0 and 7 % at 5 and 25 mg/kg dry soil respectively. Whilst these results are below the < 25 % threshold, it is possible that delayed effects were occurring i.e. a lack of effects early on masking effects occurring later in the test period. HSE has calculated section-by-section formation rates, as seen in the table 9.9-3, to determine if delayed effects were occurring. For the 14-28 day interval, deviations from the control were 45 and 33 % at 5 and 25 mg/kg dry soil respectively. This is an indication of delayed effects, and because deviations from the control exceeded 25 %, the study duration should have been extended as per OECD 217 guidelines. The results of the soil nitrogen transformation study are therefore not considered to be acceptable.

Carbon transformation test:

The carbon transformation test is considered to be acceptable. < 25 % effects were seen on carbon transformation after 28 days at both test item concentrations. A reference test conducted with Dinoterb at 18.3 mg/kg soil dw resulted in 48 % inhibition after 28 days, demonstrating the sensitivity of the test system.

The endpoints suitable for use in risk assessment are:

- No long-term effect (>25%) on carbon transformation at concentrations up to 25 mg A21857B/kg soil dry weight.

B.9.10. RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION

Three soil micro-organism (nitrogen transformation) studies were submitted for assessment, two conducted using the active substance, and one study conducted with the representative product 'Miravis Plus'. The study conducted using 'Miravis Plus' was found to be unsuitable, as although the deviation from the control nitrogen transformation rate for the whole test period was below the < 25 % threshold, the 14–28 day section-by-section nitrogen transformation rate exceeded this threshold, indicating delayed effects of the test substance. As a result, the study duration should have been extended (See Section B.9.9. for further details). For this reason, only the two submitted active substance studies were used for the purposes of the risk assessment. Table 9.10-1 below displays the available endpoints for the effects of pydiflumetofen and the representative product 'Miravis Plus', on soil nitrogen transformation.

Table 9.10-1 Endpoints used in the risk assessment

Test substance	Study ID	Effect	Reference
Pydiflumetofen	KCA1 8.5-01	No effects on nitrogen transformation rate, greater than or equal to 25 %, were observed by day 28 at up to 2.71 mg a.s./kg dry soil.	K-CA 8.5 (2015)
Pydiflumetofen	KCA1 8.5-02	No effects on nitrogen transformation rate, greater than or equal to 25 % compared to control at 13.5 mg active substance/kg dry soil	K-CA 8.5 (2017)
A21857B (Miravis Plus)	KCP 10.5-01	This study will not be considered further as part of the risk assessment.	K-CP 10.5 (2017a)

Endpoint used in the risk assessment is listed in **bold**.

Exposure

Estimates of the maximum predicted environmental concentrations in soil (PECs) of pydiflumetofen, and the representative formulation 'Miravis Plus', have been established in Section B.8 of this assessment report by the Environmental Fate and Behaviour specialist.

The relevant predicted environmental concentrations (PEC) values considered for toxicity exposure ratio (TER) calculations based on the proposed uses are summarised in Tables 9.8.1-2 and 9.8.1-3 of the earthworm risk assessment section. The maximum PEC_{soil} value of 0.736 mg/kg was used for the risk assessment.

Risk assessment for soil micro-organisms (nitrogen transformation)

According to the SANCO Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2 final), the trigger for acceptable risk is a < 25% difference (increase or decrease) in activity compared to the control treatment. A comparison has been made of the study endpoints and the maximum PEC_{soil} values in the table below:

Table 9.10-2 Risk assessment for pydiflumetofen for soil micro-organisms

Test substance	Species	Endpoint (mg a.s./kg dry soil)	PEC _{soil max} (mg/kg)	Refinement required?
Pydiflumetofen	Soil micro-organisms	2.71	0.736	No

Conclusion

According to SANCO/10329/2002 rev2 final (2002), the outcome of the soil micro-organism test is directly assessed in terms of risk. The decisive parameter is the magnitude of effect compared to the untreated control (be it increase or decrease of activity), and the time-course of recovery. The critical level is $\pm 25\%$ after 100 days. The selected test concentrations in the nitrogen transformation study which was used for risk assessment purposes cover the maximum PEC value. No effects were observed at greater than or equal to a 25 % difference from the control condition at any test concentration, indicating a low risk to soil microorganisms. No further refinement is necessary, as an acceptable risk has been demonstrated. There is some uncertainty regarding the effects of the formulation on nitrogen transformation rate due to the lack of valid study testing the formulation 'Miravis Plus' as effects were still occurring at day 28 and therefore the study should have been extended. However, as the product 'Miravis Plus' only contains one active substance, and a high margin of safety is demonstrated in the risk assessment above, it is considered that the active substance risk assessment is protective of the risk from the product 'Miravis Plus'.

B.9.11. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

B.9.11.1. Summary of screening data

Report	KCP 10.6.1 - [REDACTED], (2017), Pydiflumetofen EC (A21857B) – Phytotoxicity to Non-Target Plants Screening Test, Report Number ACE-16-134. AgroChemex Ltd., Aldhams Farm Research Station, Dead Lane, Lawford, Manningtree, Essex, CO11 2NF, United Kingdom. (VV-467318).
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Guideline(s): The design of the study is generally based on the OECD guideline test No. 208 Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (July 2006), and test No. 227 Terrestrial Plant Test: Vegetative Vigour Test (July 2006)

GLP: Yes

Duplication (if vertebrate study) Not applicable

Materials

Test Material	Pydiflumetofen EC - A21857B
Lot/Batch #:	JEA001-118-001
Actual content of active ingredients:	Pydiflumetofen - 5.62 % w/w corresponding to 61.7 g /L
Description:	Light yellow clear liquid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	End of June 2019
Density:	1097 kg /m ³

Treatments

Test rates:	Nominal concentrations of 200, 400, 800, 1600 and 3200 mL A21857B /ha
Control:	Untreated

Application method:	Mardrive cabinet track sprayer with 8004E TeeJet even flat fan nozzle at ca. 67 cm above the soil surface. The sprayer was calibrated to deliver 200 L /ha \pm 10%.
Test organisms	
Species:	Onion <i>Allium cepa</i> (Monocotyledoneae: Alliaceae) Wheat <i>Triticum aestivum</i> (Monocotyledoneae) Soybean <i>Glycine max</i> (Dicotyledoneae: Fabaceae) Sugar beet <i>Beta vulgaris</i> (Dicotyledoneae: Chenopodiaceae) Oilseed rape <i>Brassica napus</i> (Dicotyledoneae: Brassicaceae) Cucumber <i>Cucumis sativus</i> (Dicotyledoneae: Cucurbitaceae)
Test design	
Vessels:	Non-porous plastic pots (8x8x8 cm)
Test soil:	Sandy loam (72 % sand, 11 % silt, 17 % clay; total organic carbon content 1.4 %)
Replication:	Three pots per treatment with four seeds/plants per pot (six seeds/plants for onion)
Sampling interval:	Seedling emergence: Visual phytotoxicity ratings were recorded 28 days after the application of the test item. Vegetative vigour: Visual phytotoxicity ratings were recorded 21 days after the application of the test item.
Duration of test:	Seedling Emergence: 28 days after application Veg Vigour: 21 days after application
Environmental test conditions	
Temperature:	11.9 – 23.4 °C
Humidity	36.5 – 94.1 %
pH of soil:	7.8
Water content of soil:	Not stated (Sub-irrigation)
Photoperiod:	16 hr light and 8 hr dark, > 2000 lux

Study Design and Methods

Experimental dates: 10 November 2016 to 06 January 2017

Six species of higher plant were treated with 5 test concentrations of test item and a control, prepared in deionised water. For the seedling emergence and growth test, the test plants were sown directly into the pots and covered with soil. Immediately after sowing the surface applications were made by spraying onto the soil. The test duration was 28 days. The seeds of the test plants for the vegetative vigour were sown directly into untreated soil. The seedlings were grown to the 1-3 true leaf stage (BBCH stage 11-13) before treatment. The date of the spray application to the plants was designated as the first day of experiment. The test duration was 28 days after application for the seedling emergence test, and 21 days after application for the vegetative vigour test.

Visual phytotoxicity ratings were recorded at test termination (28 days after application for the seedling emergence test and 21 days after application for the vegetative vigour test). Evaluation of phytotoxicity was done by visual observation and recording inhibition of emergence or plant injury using a rating scale. Plants were rated on a scale from 0 to 10, with 0 representing 'Vigorous healthy plants, indistinguishable from the untreated control', and 10 representing 'Complete destruction of plant parts above ground'. Data given are the average of three replicates rounded to the nearest whole number.

Calibration was carried out by weight of water, using 3 replicates of 5 applications to six petri dishes with an inner diameter of 86 mm. The nozzle output was considered successful if the mean water weight from each replicate

was within ± 10 % of the target value. The sprayer was calibrated within 24 hrs prior to application. The highest concentration of spray solution was prepared by weighing a calculated amount of A21857B and diluting with tap water.

No statistical analysis was carried out, as there were no effects > 50 % observed for any tested species.

Results and Discussion

The evaluation of effects on seedling emergence and vegetative vigour are given in the Tables below.

Table 9.11.1-1: Effect of A21857B on seedling emergence

Test species	Application rate (A21857B L /ha)					
	Control	200	400	800	1600	3200
<i>Allium cepa</i>	0	0	0	0	0	0
<i>Triticum aestivum</i>	0	0	0	0	0	0
<i>Beta vulgaris</i>	0	0	0	0	0	0
<i>Brassica napus</i>	0	0	0	0	0	0
<i>Cucumis sativus</i>	0	0	0	0	0	0
<i>Glycine max</i>	0	0	0	0	0	0

Table 9.11.1-2: Effect of A21857B on vegetative vigour

Test species	Application rate (A21857B L /ha)					
	Control	200	400	800	1600	3200
<i>Allium cepa</i>	0	0	0	0	0	0
<i>Triticum aestivum</i>	0	0	0	0	0	0
<i>Beta vulgaris</i>	0	0	0	0	0	0
<i>Brassica napus</i>	0	0	0	0	0	0
<i>Cucumis sativus</i>	0	0	0	1	2	4
<i>Glycine max</i>	0	0	0	0	0	2

Validity Criteria

The validity criteria for ‘OECD 208 (2006): Seedling emergence and seedling growth test’; and ‘OECD 227 (2006): Vegetative vigour test’ are listed below:

Validity criterion	Required	Obtained
OECD 208 (2006): Seedling emergence and seedling growth test		
Control seedling emergence	Seedling emergence in the controls should be at least 70 %	100 % seedling emergence
Phytotoxic effects in the controls	Control plants should not exhibit visible phytotoxic effects. Plants only exhibit normal variation in growth and morphology.	No adverse effects observed for any tested species.

Survival of emerged control seedlings	Mean plant survival in the control should be at least 90 % for the duration of the study.	100 % control plant survival
Environmental conditions	Environmental conditions for a particular species are identical, and the growing media should contain the same amount of soil/substrate, from the same source.	Environmental conditions for each particular species were identical and growing media contained the same amount of soil matrix, support media or substrate from the same source.
OECD 227 (2006): Vegetative vigour test		
Seedling emergence	Seedling emergence in all conditions should be at least 70 %	See HSE comments
Phytotoxic effects in the controls	Control plants should not exhibit visible phytotoxic effects. Plants only exhibit normal variation in growth and morphology.	No adverse effects observed for any tested species.
Mean control plant survival	Mean plant survival in the control should be at least 90 % for the duration of the study.	100 % control plant survival
Environmental conditions	Environmental conditions for a particular species are identical, and the growing media should contain the same amount of soil/substrate, from the same source.	Environmental conditions for each particular species were identical and growing media contained the same amount of soil matrix, support media or substrate from the same source.

Conclusions

For seedling emergence, Onion, wheat, sugar beet, oilseed rape, cucumber and soybean did not show any phytotoxic effects at rates up to and including 3200 mL A21857B /ha.

For vegetative vigour, the most sensitive species was cucumber showing phytotoxic effects at 800 mL, 1600 mL and 3200 mL A21857B /ha. Soybean showed slight phytotoxic effects at 3200 mL A21857B /ha. Whilst onion, wheat, sugar beet and oilseed rape did not show any phytotoxic effects at rates up to and including 3200 mL A21857B /ha.

(██████, 2017)

HSE COMMENTS

This study was conducted to GLP standards, and was generally based upon two guideline documents: OECD 227 (2006) Terrestrial plant test: Vegetative Vigour Test, and OECD 208 (2006) Terrestrial plant test: Seedling Emergence and Seedling Growth test. The study authors have only reported compliance with two of the four validity criteria from each guideline.

The validity criteria omitted from the study by the applicant were as follows:

From OECD 208, the seedling emergence in the controls should be at least 70 %. This validity criterion was met, the applicant reported that all of the control seedlings emerged in the control condition. Additionally, the mean survival of emerged control seedlings should be at least 90 % for the duration of the study. This is not specifically addressed although it is assumed that this criterion was also met, as there were no adverse effects noted for any tested species, in the control condition or at any tested concentration throughout the duration of the study.

For OECD 227, as with OECD 208, the mean survival of emerged control seedlings should be at least 90 % for the duration of the study. Based on the data provided, this validity criterion was met, as all control plants were rated '0', meaning that they were 'Vigorous healthy plants'. Additionally, the seedling emergence in all conditions should be at least 70 %, however, **it isn't possible to verify that this validity criterion was met** for all conditions in the vegetative vigour part of the study, as no raw phytotoxicity observation data has been provided, and the

plants were not separately rated on their emergence and the occurrence of any phytotoxic effects – these measurements were taken together. The cucumber plants used for the highest tested concentration in the vegetative vigour experiment were rated as ‘4’, which is described as: ‘Less vigorous, with more pronounced discolouration, malformation, or stunting – recovery possible, clear reduction of rate of emergence’. Although the applicant had stated that necrosis was observed in this condition, it is not clear to what extent the rating can be ascribed to the necrosis, or to a reduction in the rate of emergence.

The following deviations from the two guidelines were noted:

Although required by both OECD 208 (2006) and OECD 277 (2006), the study authors did not analytically verify the test concentrations used in the experiment. They state that analytical verification of the test concentrations was not required for this study, however, it is unclear how they came to this conclusion.

The humidity slightly fell below the range specified (70 ± 25 %) in the guidelines on occasions; the lowest recorded humidity was 36.5 %, however the plants were healthy and grew well. This is a minor deviation, which had no impact upon the study results.

Both OECD 208 (2006) and OECD 227 (2006) recommend that in addition to measurements of the seedling emergence and vegetative vigour, measurements of plant biomass, and maybe shoot height should be taken, and compared to the controls. This was not done in the current study, and the only endpoints provided were a combined measurement of phytotoxicity and either the seedling emergence rate, or vegetative vigour.

Both OECD 208 (2006), and OECD 227 (2006) recommend that ‘for each treatment and control group, the number of replicates should be at least four, and the total number of plants should be at least 20.’ However, in the current experiment, only three replicates per condition were used, with four plants /pot (six plants /pot for onion), making a total of 12 plants per condition (18 onion plants). This has implications for the reliability of the findings, as fewer replicates equals less certainty, and less statistical power. This wouldn’t be reason to invalidate the study, as no statistical analysis was carried out due to the lack of effects above 50 % for any tested plant species.

The OECD 227 (2006) Vegetative Vigour guidelines state that plants should be observed for visual phytotoxicity and mortality at least weekly, and if possible, daily. In this study, the plants were only assessed for phytotoxicity and mortality on the day of experimental termination (21 days after application). This means that any potential transient effects may have been missed, and although the minimal effects observed at test termination suggest that this may not be too much of an issue, it is not ideal, given the lack of biomass measurements taken.

Also recommended in both guidelines was the use of a reference substance, or the comparison of control plant biomass data to previous studies conducted at the same site, for use as an intra-laboratory quality control measure. No data was provided for this, or justification for its omission. It may be necessary to contact the applicant to see if there is any positive control data available for the period in which the study was conducted.

There were no observations of phytotoxicity in any of the six tested plants in the seedling emergence test, at rates up to and including 3,200 mL A21857B /ha.

For the vegetative vigour test, onion, wheat, sugar beet, and oilseed rape did not show any phytotoxic effects at rates up to and including 3,200 mL A21857B /ha. The most sensitive species was cucumber, showing phytotoxic effects (necrosis) at 800, 1,600, and 3,200 mL A21857B /ha. Soybean showed slight phytotoxic effects (necrosis) at 3,200 mL A21857B /ha.

B.9.11.2. Testing on non-target plants

Please note that the following study was conducted with the EU representative formulation (A19649B); further details are given in the risk assessment.

Report:	K-CA 8.6.2/01 [REDACTED], [REDACTED], [REDACTED], [REDACTED], (2015) SYN545974 SC (A19649B) - Toxicity Effects on the Vegetative Vigor of Ten Species of Plants. Laboratory Report No: 528P116. Wildlife International Ltd., Easton, MD 21601, USA. Unpublished report. (Syngenta File No. A19649B_10077)
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Guidelines

US EPA Ecological Effects Test Guidelines, OPPTS 850.5400: Terrestrial Plant Toxicity, Tier I (Vegetative Vigour) (1996)

GLP: Yes

Materials

Test material	A19649B
Lot/Batch #:	SMU2JP001
Actual content of active ingredients:	SYN545974: 18.6 % corresponding to 204 g /L
Description:	Off-white suspension
Stability of test compound:	Stable under test conditions.
Reanalysis/expiry date:	End of February 2016

Treatments

Test concentrations:	200 g a.s. /ha (1000 mL A19649B /ha)
Control:	Reverse osmosis purified well water
Nominal spray volume:	200 L /ha
Application method:	DeVries Research Track Spray Booth; spray pressure 1.38 bar; distance, nozzle to target: 41 cm, single application.

Test organisms

Species:	<i>Allium cepa</i> (onion) <i>Lolium perenne</i> (ryegrass) <i>Triticum aestivum</i> (wheat) <i>Zea mays</i> (corn) <i>Beta vulgaris</i> (sugarbeet) <i>Brassica napus</i> (oilseed rape) <i>Brassica oleracea</i> (cabbage) <i>Glycine max</i> (soybean) <i>Lactuca sativa</i> (lettuce) <i>Lycopersicon esculentum</i> (tomato)
Test soil:	Loamy sand soil composed of 87 % sand, 5 % silt, 8 % clay with an organic carbon content of 0.59 % (organic matter content of 1.01 %)

Test design

Plant selection:	After planting, pots were placed in the glasshouse where plants emerged; seedlings of the appropriate size (typically 2 – 4 leaves) were selected one day prior to application
Sampling interval:	Plant condition assessments were made on days 7, 14 and 21. Plant height measurements were taken on days 14 and 21, and shoot dry weight was measured at test termination on day 21.
Replication:	Six replicate experimental units consisting of five plants, with each plant contained in a separate pot
Duration:	21 days

Environmental conditions

Test temperature:	15.10 – 35.68 °C
Humidity:	40.97 – 92.60 % RH

Soil pH:	6.5
Lighting:	16 hour photoperiod. Illumination 11.7 (min.) to 15.8 (max.) photosynthetically active radiation (E/d/m ²)

Study Design and Methods

Experimental dates: 6 August to 22 September 2014

Young plants of four monocot species (*Allium cepa*, *Lolium perenne*, *Triticum aestivum*, *Zea mays*) and six dicot species (*Beta vulgaris*, *Brassica rapa*, *Brassica oleracea*, *Glycine max*, *Lactuca sativa*, *Lycopersicon esculentum*) were sprayed with one test concentration of formulation A19649B. The nominal test concentration used in the definitive test for all test species was 200 g a.s. /ha (equivalent to 1000 mL A19649B /ha). The nominal spray volume was 200 L /ha.

The primary stock solution (used as spray mixtures for the 200 g a.s. /ha application rate) was prepared by diluting 2.6882 g of the test substance to 500 mL with well water purified by reverse osmosis and mixing by swirling and inversion. The well water and soil used in this experiment are periodically screened for pesticides and metals. No analytes were measured at levels that were expected to have an impact on the study.

Seeds were planted in pots and placed in the glasshouse, where emergence and development into seedlings occurred. Seeds were not treated with fungicides, insecticides, or repellents prior to test initiation. Once at the appropriate size for spraying (2 – 4 leaves), the seedlings were randomly assigned to treatment or control groups. Pots containing the test plants were placed into the Track Sprayer booth and applications were made from 41 cm above the plant canopy. Applications were made with the negative control first and then the treatment. The sprayer was calibrated prior to use – a clean, tared piece of absorbent paper was sprayed with water under the same conditions as the application of the test substance. The paper was then re-weighed, and this procedure was repeated twice more with new paper. The mean weight of water collected in the three trials was used to calculate the applied spray volume.

Observations of plant condition and any abnormalities in appearance were taken prior to application, and then 7, 14 and 21 days after application (DAA). Plant condition was described by noting the presence or absence of possible signs of phytotoxicity including recovery, leaf loss and death. Height measurements were taken prior to application, and then at 14 and 21 DAA. Plant height was measured with a ruler to the nearest whole centimetre from the surface of the soil to the apical meristem (*G. max* and *L. esculentum*), or to the tip of the tallest leaf (all other species). Plants with a height of less than 1 cm were assigned a height of 0.25 cm. After test termination, the plants were clipped at soil level, combined by replicate, dried, and weighed. Mean height and total replicate biomass were then determined for each treatment group.

Concentrations/rates of application were confirmed by analytical verification, using HPLC equipped with a variable wavelength detector. The limit of quantitation (LOQ) was defined as 250 µg a.s. /mL. Samples of the 1000 ppm a.s. spray mixtures had a mean concentration and standard deviation of 981 ± 8.08 ppm a.s., with a coefficient of variation of 0.824 %. This value represented 98.1 % of nominal concentrations. As such, analysis and reporting of data was based on the nominal values.

Statistical analysis of the data was used to assess the probability that the treatment group mean height or dry weight was reduced by 25 % relative to the control mean using a Test of Significant Toxicity (TST), a variation of Welch's t-test ($\alpha = 0.05$). Effects of treatment were also evaluated by comparing control and treatment group means with a one-tailed Student's t-test ($\alpha = 0.05$). Statistical analyses also included determination of effect rates (if appropriate) using non-linear regression when reductions in test endpoints were 25 % or more relative to control means. All statistical tests were performed using SAS version 8.2. The limit of quantitation (LOQ) for this study was defined as 250 µg a.s. /mL.

Results and Discussion

Validity Criteria

The validity criteria were met according to OECD 227 (2006):

Table 9.11.2-1: Validity criteria

Validity criterion	Required	Obtained
Seedling emergence	> 70 %	> 70 %
Phytotoxic effects in controls	Control plants should not exhibit any visible phytotoxic effects. Plants should exhibit only normal variation in growth and morphology.	No visible phytotoxic effects. Only normal variation in growth and morphology was observed.
Control plant survival	The mean control plant survival rate should be > 90 % for the duration of the study.	100 %
Control environmental conditions	Environmental conditions and growing medium for each species should be identical for all test groups.	Environmental conditions and growing medium for each species were the same for all test groups.

Samples of the 1000 ppm a.s. spray mixtures had a mean concentration and standard deviation of 981 ± 8.08 ppm a.s., with a coefficient of variation of 0.824 %. This value represented 98.1 % of nominal concentrations.

No significant, adverse treatment related effects were observed in the survival, plant height and dry weight of the ten species tested. The treatment group means of each endpoint for the ten species were not significantly different from the negative control group means. Additionally, no significant signs of phytotoxicity were observed in the study on Day 21. The observations are summarised in Table 9.11.2-2 below:

Table 9.11.2-2: Observations of plant condition in ten terrestrial plant species following post-emergence spray treatment with A19649B

Species		Observations of plant condition		
		Day 7	Day 14	Day 21
<i>Allium cepa</i> (onion)	Control	0	0	0
	200g a.s. /ha	0	(1) 40 [N]	(1x) 100 (N)
<i>Lolium perenne</i> (Ryegrass)	Control	0	0	0
	200g a.s. /ha	0	0	0
<i>Triticum aestivum</i> (wheat)	Control	0	0	0
	200g a.s. /ha	0	0	0
<i>Zea mays</i> (corn)	Control	0	0	0
	200g a.s. /ha	0	0	0
<i>Beta vulgaris</i> (sugarbeet)	Control	0	0	0
	200g a.s. /ha	0	(1) 40 [LC]	0
<i>Brassica napus</i> (oilseed rape)	Control	0	0	0
	200g a.s. /ha	0	0	0
<i>Brassica oleracea</i> (cabbage)	Control	0	0	0
	200g a.s. /ha	0	(1) 10 [ID]	0
<i>Glycine max</i> (soybean)	Control	0	0	0

Species		Observations of plant condition		
		Day 7	Day 14	Day 21
	200g a.s. /ha	0	0	0
<i>Lactuca sativa</i> (lettuce)	Control	0	0	0
	200g a.s. /ha	0	0	0
<i>Lycopersicon esculentum</i> (tomato)	Control	0	0	0
	200g a.s. /ha	0	0	0

N.B.: Plant condition was graded on an incremental scale from 0 to 100. A score of 0 indicates all plants were healthy, while a score of 100 indicates a dead plant. ‘()’ parentheses indicate the number of afflicted plants in each condition, and ‘[]’ parentheses indicate the type of affliction. If only one plant is listed as afflicted, then all other plants in that condition were healthy. There were 6 replicates per condition, with 5 plants in each replicate, for a total of 30 plants per condition.

N = Necrosis

LC = Leaf curl

ID = Insect damage

Table 9.11.2-3: Effects of A19649B on height, survival, and dry weight of ten terrestrial plant species in a 21-day vegetative vigour test

Species	Treatment group (L/ha)	Height (cm) Mean \pm SD (% reduction D 21)	Survival (%) Mean \pm SD (% reduction D 21)	Dry weight (g) Mean \pm SD (% reduction D 21)
<i>Allium cepa</i> (Onion)	Negative control	26.1 \pm 0.48	100.0 \pm 0.00	0.067 \pm 0.0164
	200g a.s./ha	28.2 \pm 3.71 (-8%)	96.7 \pm 8.16 (3%)	0.091 \pm 0.0367 (-36%)
<i>Lolium perenne</i> (Ryegrass)	Negative control	36.5 \pm 2.19	100.0 \pm 0.00	0.94 \pm 0.136
	200g a.s./ha	35.8 \pm 2.65 (2%)	100.0 \pm 0.00 (0%)	0.97 \pm 0.165 (-3%)
<i>Triticum aestivum</i> (Wheat)	Negative control	54.0 \pm 3.10	100.0 \pm 0.00	1.21 \pm 0.158
	200g a.s./ha	56.0 \pm 1.89 (-4%)	100.0 \pm 0.00 (0%)	1.26 \pm 0.082 (-4%)
<i>Zea mays</i> (Corn)	Negative control	124.9 \pm 10.94	100.0 \pm 0.00	6.4 \pm 0.81
	200g a.s./ha	124.6 \pm 11.12 (0%)	100.0 \pm 0.00 (0%)	6.4 \pm 1.11 (0%)
<i>Beta vulgaris</i> (Sugarbeet)	Negative control	31.7 \pm 2.25	100.0 \pm 0.00	2.5 \pm 0.18
	200g a.s./ha	31.6 \pm 1.59 (0%)	100.0 \pm 0.00 (0%)	2.5 \pm 0.34 (0%)
<i>Brassica napus</i> (Oilseed rape)	Negative control	34.9 \pm 1.79	100.0 \pm 0.00	5.07 \pm 0.742
	200g a.s./ha	36.1 \pm 0.91	100.0 \pm 0.00	5.08 \pm 0.630

		(-3%)	(0%)	(0%)
<i>Brassica oleracea</i> (Cabbage)	Negative control	26.5 ± 1.09	100.0 ± 0.00	3.7 ± 0.35
	200g a.s./ha	26.3 ± 1.11 (1%)	100.0 ± 0.00 (0%)	3.8 ± 0.33 (-2%)
<i>Glycine max</i> (Soybean)	Negative control	113.9 ± 12.54	100.0 ± 0.00	6.61 ± 0.383
	200g a.s./ha	118.7 ± 10.06 (-4%)	100.0 ± 0.00 (0%)	6.97 ± 0.652 (-6%)
<i>Lactuca sativa</i> (Lettuce)	Negative control	22.0 ± 1.57	100.0 ± 0.00	4.58 ± 0.395
	200g a.s./ha	221. ± 1.76 (0%)	100.0 ± 0.00 (0%)	4.25 ± 0.550 (7%)
<i>Lycopersicon esculentum</i> (Tomato)	Negative control	59.5 ± 4.92	100.0 ± 0.00	6.3 ± 0.98
	200g a.s./ha	60.0 ± 4.80 (-1%)	100.0 ± 0.00 (0%)	6.3 ± 0.83 (-1%)

Mean ± SD = Mean plus or minus one standard deviation

No treatment group mean was significantly different from control group mean (one-tailed t-test, $p > 0.05$).

The observed difference from control is significantly less than 25% (Test of Significant Toxicity, $p < 0.05$).

¹ Test of Significant Toxicity was not performed on survival data.

Table 9.11.2-3: Effect rates of SYN545974 in ten terrestrial plant species following post-emergence spray treatment with A19649B

Species	Survival, growth, plant condition (g a.s. /ha)		
	ER ₂₅	NOER	Most Sensitive Endpoint
Monocots			
<i>Allium cepa</i> (onion)	> 200	200	⁻¹
<i>Lolium perenne</i> (ryegrass)	> 200	200	⁻¹
<i>Triticum aestivum</i> (wheat)	> 200	200	⁻¹
<i>Zea mays</i> (corn)	> 200	200	⁻¹
Dicots			
<i>Beta vulgaris</i> (sugarbeet)	> 200	200	⁻¹
<i>Brassica napus</i> (oilseed rape)	> 200	200	⁻¹
<i>Brassica oleracea</i> (cabbage)	> 200	200	⁻¹
<i>Glycine max</i> (soybean)	> 200	200	⁻¹
<i>Lactuca sativa</i> (lettuce)	> 200	200	⁻¹
<i>Lycopersicon esculentum</i> (tomato)	> 200	200	⁻¹

⁻¹ Not applicable as there were no significant adverse effects or reductions greater than 25 %

Conclusions

A foliar application of A19649B, at a rate of 200 g a.s. /ha (1000 mL A19649B /ha) resulted in ER₂₅ values of > 200 g a.s. /ha. Ten species (4 monocot, 6 dicot) were exposed to a negative control and one application rate of the test substance. None of the tested species demonstrated sensitivity to the treatment application, thus, the NOER values are 200 g a.i. /ha for each of the 10 species tested.

(██████ et al., 2015)

HSE comments

This study was conducted according to GLP, and the US EPA Ecological Effects Test Guidelines, OPPTS 850.5400: Terrestrial Plant Toxicity, Tier I (Vegetative Vigour) (1996). The study was also assessed against OECD 227 (2006). All validity criteria were met. The following deviations were noted:

The recorded environmental conditions fell outside of those recommended by OECD 227 (2006). The temperature ranged from 15.10 to 35.68 °C during the study, whereas the guidelines recommend 22 ± 10 °C (12 – 22 °C) for greenhouse testing. Although the minimum recorded daily temperature never fell below the minimum recommended temperature of 12 °C, the maximum recorded daily temperature exceeded the maximum recommended temperature of 22 °C on every single day of the experiment. The relative humidity in the glasshouse also ranged from 40.97 to 92.60 %, when the guideline recommends $70 \% \pm 25 \%$ (45 – 95 %). The minimum recorded relative humidity never exceeded the OECD 227 (2006) recommended maximum, however, the recorded relative humidity fell below the minimum recommended value on three separate days. This is unlikely to influence the reliability of the data, as all validity criteria were met, and there were no mortalities or other adverse effects observed in any of the control conditions.

The lighting levels provided to the plants in this study were difficult to compare to the OECD guidelines, as different units were used in the study report, the study summary, and in the OECD guidelines. Illumination is presented as photosynthetically active radiation (E/d/m²) in the study summary, it is also presented as ‘Moles of photosynthetically active radiation’ in the study report, whereas the OECD guidelines state that the acceptable illumination range is 350 ± 50 µE/m²/s.

The study authors state that the validity criterion for seedling emergence was met, but no raw data was provided to back this up. There is, however, no reason to suspect that the seedling emergence did not exceed 70 %, as there was no control mortality in any condition, and the other validity criteria were also met.

The OECD 227 (2006) guideline suggests that ‘A reference substance may be tested at regular intervals, to verify that the performance of the test and the response of the particular test plants and the testing facility have not changed significantly over time. Alternatively, historical biomass or growth measurement of controls could be used to evaluate the performance of the test system in particular laboratories, and can serve as an intra-laboratory quality control measure.’ No evidence of either procedure was provided by the applicant, however, the guideline does not explicitly require it, and all of the validity criteria were met, so this does not represent a significant issue.

Although no signs of phytotoxicity or other adverse effects were observed at the study termination on day 21, it is noted that the *Allium cepa* (onion) test condition showed a 36 % increase in d.w. compared to the control condition.

The OECD 227 (2006) guidelines do not provide a clear explanation of the statistical methods that should be used. The statistics used in the study do seem appropriate for this study type. They are detailed in the methods section above.

The analytical methods have been evaluated by HSE Chemistry specialists in Vol. 3CA Part B5.1.2.6. The following was concluded for this method: “Acceptable method. LOQ: 500 mg/L in test spray solutions”.

No significant adverse effects greater than 25 % were observed, and so only ER₂₅ endpoints were provided by the study authors. Based on nominal test concentrations, the ER₂₅ values were > 200 g a.s. /L for all tested species. For the purposes of the risk assessment, these ER₂₅ values can be assumed to be equivalent to ER₅₀ values. NOER values were determined to be 200 g a.s. /L for all species tested.

Please note that the following study was conducted with the EU representative formulation (A19649B); further details are given in the risk assessment.

Report:	K-CA 8.6.2 [REDACTED], [REDACTED], [REDACTED], [REDACTED] (2015a) SYN545974 SC (A19649B) - Toxicity Effects on the Seedling Emergence of Ten Species of Plants. Laboratory Report No: 528P-115. Wildlife International Ltd., Easton, MD 21601, USA. Unpublished report. (Syngenta File No. A19649B_10105)
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Guidelines

US EPA Ecological Effects Test Guidelines, OCSPP 850.5400: Seedling Emergence and Seedling Growth (2012)

GLP: Yes

Materials

Test material	A19649B
Lot/Batch #:	SMU2JP001
Actual content of active ingredients:	SYN545974: 18.6 % w/w corresponding to 204 g/L
Description:	Off-white suspension
Stability of test compound:	Stable under test conditions.
Reanalysis/expiry date:	End of February 2016
Density:	1096 kg/m ³

Treatments

Test concentrations:	200 g a.s./ha (1000 mL A19649B/ha)
Control:	Water, well water purified by reverse osmosis
Spray volume:	200 L/ha
Application method:	DeVries Research Track Spray Booth; spray pressure 1.38 bar; distance, nozzle to target: 41 cm

Test organisms

Species:	<i>Lolium perenne</i> (ryegrass) <i>Brassica oleracea</i> (cabbage) <i>Lactuca sativa</i> (lettuce) <i>Allium cepa</i> (onion) <i>Triticum aestivum</i> (wheat) <i>Zea mays</i> (corn) <i>Beta vulgaris</i> (sugarbeet) <i>Brassica napus</i> (oilseed rape) <i>Glycine max</i> (soybean) <i>Lycopersicon esculentum</i> (tomato)
Test soil:	Loamy sand soil composed of 87 % sand, 5 % silt, 8 % clay with an organic carbon content of 0.59 % (organic matter content of 1.01 %)

Test design

Test vessels:	Plastic pots (16 cm diameter, 12 cm depth)
Sampling interval:	Seedling emergence in water control documented daily until 50 % emergence was reached; further observations were conducted on days 7 and 14 after 50 % emergence was reached; seedling growth and condition were documented on day 21; height and dry weight assessed on day 21
Replication:	8 replicate pots with 5 seeds per pot
Duration:	21 days (after 50 % emergence was reached in the control)

Environmental conditions

Test temperature:	16.62 to 38.28 °C
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Humidity:	34.80 to 91.40 % RH
Soil pH:	6.5
Lighting:	16-hour photoperiod. Illumination 10.0 (min.) to 13.4 (max.) photosynthetically active radiation

Study Design and Methods

Experimental dates: 6 August to 15 September 2014

Primary stock solution was prepared on the day of application. 2.6882 g of test substance were added to 500 mL well water purified by reverse osmosis. The spray mixture was sampled and analysed to confirm test concentration.

Young plants of four monocot species (*Allium cepa*, *Lolium perenne*, *Triticum aestivum*, *Zea mays*) and six dicot species (*Beta vulgaris*, *Brassica rapa*, *Brassica oleracea*, *Glycine max*, *Lactuca sativa*, *Lycopersicon esculentum*) were sprayed with one test concentration of formulation A19649B. The nominal test concentration used in the definitive test for all test species was 200 g a.s./ha (1000 mL A19649B/ha). Seedlings were initially watered from the top, then sub-irrigated with sub-irrigation trays.

Observations documenting seedling emergence in water control were made daily until 50 % emergence was reached. Further seedling emergence observations were conducted 7 and 14 days after 50 % emergence in the control treatment to document seedling emergence. Additionally, observations were made on Day 21 to document seedling growth and general condition of seedlings. Observations consisted of noting whether emergence had or had not occurred and assessing the condition of each seedling. Emergence was defined as the presence of visible plant tissue at the surface of the soil. Seedling condition was described by noting the presence or absence of possible signs of phytotoxicity such as chlorosis, necrosis or leaf curling.

The growth of emerged seedlings was evaluated on Day 21 by assessing the height and biomass of seedlings. Plant biomass was estimated by measuring the total dry weight of the shoots within each replicate. Seedling height was measured with a ruler to the nearest whole centimetre from the surface of the soil to the apical meristem, or to the tip of the tallest leaf. Dead or non-emerged seedlings were assigned a height of 0.25 cm. Seedlings were then clipped at soil level; the shoots of all living seedlings within a replicate were placed in a labelled paper container, dried in an oven, and weighed as a group. Mean seedling height and replicate biomass were determined for each treatment group containing living seedlings at test termination.

Statistical analyses were used to evaluate effects of test substance application on plant emergence, height, biomass, and survival. The probability of a 25 % reduction relative to the control mean was conducted using a Test of Significant Toxicity, a variation of Welch's t-test ($\alpha = 0.05$). Effects were also evaluated by comparing the treatment and control group means with a one-tailed standard (Student's/Satterthwaite) t-test ($\alpha = 0.05$). All statistical tests were conducted using SAS version 8.2.

Results and Discussion

Table 9.11.2-4 Summary of seedling emergence, survival, dry weight, and height in the ten species compared to the negative control.

Treatment group	Emergence % reduction	Height % reduction	Survival % reduction	Dry weight % reduction
<i>Allium cepa</i>	3	-2	-3	0
<i>Lolium perenne</i>	11 ^a	5	0	17 ^a
<i>Triticum aestivum</i>	-5	-2	0	3
<i>Zea mays</i>	-3	-7	0	-8
<i>Beta vulgaris</i>	-8	2	0	-1
<i>Brassica napus</i>	3	-6	0	7
<i>Brassica oleracea</i>	12 ^a	2	-17	10 ^a
<i>Glycine max</i>	3	-1	-3	5
<i>Lactuca sativa</i>	11 ^a	0	0	14 ^a
<i>Lycopersicon esculentum</i>	0	3	0	4

^aThe observed difference from control is not significantly less than 25 % (Test of Significant Toxicity, $p > 0.05$)
There were adverse, treatment-related effects on three of the ten species. *L. perenne*, *B. oleracea*, and *L. sativa* had a significantly reduced group seedling emergence compared to the negative control group means. According to the Test of Significant Toxicity, there were adverse effects on the survival of the ten species tested. The treatment group plant dry weight means for *L. perenne*, *B. oleracea*, and *L. sativa* were significantly reduced compared to the negative control mean.

Signs of phytotoxicity were recorded qualitatively. One plant was observed with signs of phytotoxicity in the negative control groups of *Z. mays* and *B. vulgaris*. Three plants were observed with signs of phytotoxicity in the treatment group of *G. max*. No further information was presented in the study report. Based on the reported phytotoxicity results the ER₅₀ is protective of phytotoxicity.

The NOER and ER₅₀ for each of the ten test species are presented in tables below:

Table 9.11.2-5: Effect rates of A19649B in ten terrestrial plant species following pre-emergence spray application with A19649B

Species	Effect rates (g a.s./ha)	
	ER ₅₀	NOER
Monocots		
<i>Allium cepa</i> (onion)	> 200	200
<i>Lolium perenne</i> (ryegrass)	> 200	< 200
<i>Triticum aestivum</i> (wheat)	> 200	200
<i>Zea mays</i> (corn)	> 200	200
Dicots		
<i>Beta vulgaris</i> (sugarbeet)	> 200	200
<i>Brassica napus</i> (oilseed rape)	> 200	200
<i>Brassica oleracea</i> (cabbage)	> 200	< 200
<i>Glycine max</i> (soybean)	> 200	200
<i>Lactuca sativa</i> (lettuce)	> 200	< 200
<i>Lycopersicon esculentum</i> (tomato)	> 200	200

Conclusions

A pre-emergent application of A19649B, at a rate of 200 g a.s./ha (1000 mL A19649B/ha) resulted in ER₅₀ values of > 200 g a.s./ha. Ten species were exposed to a negative control and one application rate of the test substance. There was an adverse, treatment-related effect in three species (ryegrass, cabbage, lettuce), indicating a NOER of < 200 g a.s./ha.

(██████ et al., 2015a)

HSE comments

Validity Criteria	Required	Obtained
Mean seedling emergence in the controls.	≥ 70 %	65 % for <i>B. vulgaris</i> > 83 % for all other species
Control seedlings do not exhibit visible phytotoxic effects.	Plants exhibit only normal variation in growth and morphology for that species.	One plant in negative controls of <i>Z. mays</i> and <i>B. vulgaris</i> .
Mean survival of emerged seedlings in controls.	≥ 90 %	97.8 %
Environmental conditions for each species.	Must be identical and growing media contain same amount of soil matrix, support media, or substrate from the same source.	Identical conditions for all tested species.

The study was performed according to US EPA Ecological Effects Test Guidelines, OCSPP 850.5400: Seedling Emergence and Seedling Growth (2012) and checked against OECD 208 (2006). The validity criteria are summarised in the table above. There were some minor deviations to the guidelines.

The maximum recorded temperature was 38.28 °C, which is higher than the recommended temperature range in OECD 208 (2006) of 22 ± 10 °C. Also, the minimum humidity was recorded at 34.80 % RH which is lower than the recommended range of 75 ± 25 % from OECD 208 (2006). Whilst the environmental conditions were not in line with the guidance there were no observed negative effects, so it is not considered that this has had an effect on the validity of the endpoints. It is unclear to HSE whether recommended light levels in OECD 208 were achieved. However, given the relatively high emergence the conditions appear appropriate.

It was noted that there was not a toxic reference substance tested to verify the performance of the test. Additionally, there is no historical data provided for response of the test plants.

Two plants in the negative controls exhibited phytotoxic effects. One plant in *Z. mays* and one plant in *B. vulgaris*. This can be considered to be normal variation in growth and morphology. However, for *B. vulgaris* mean emergence of the seedlings in the control group was 65 %. There is some uncertainty as to the health of the *B. vulgaris* seeds. *B. vulgaris* did have an 8 % increase of seedling emergence in the test substance replicate compared to the control so the endpoints can be considered valid.

As the mean emergence for *B. vulgaris* was 65 %, this is lower than recommended for the validity criteria in OECD 208 (2006). Therefore, the validity criteria for emergence for this study is not met, as the lowest emergence rate did not meet the criteria. However, it is a 5 % difference in emergence for *B. vulgaris*, and all other test species met the validity criteria for emergence.

Due to the study results statistical analysis to determine ER₅₀'s was not possible i.e. for all species the ER₅₀ was greater than the highest test concentration.

The analytical methods have been evaluated by HSE Chemistry specialists in Vol. 3CA Part B5.1.2.6. The following was concluded for this method: "Acceptable method. LOQ: 500 mg/L in test spray solutions".

The agreed endpoint is:

- ER₅₀ = > 200 g a.s./ha based on seedling emergence ^a

^a note uncertainty as the validity criteria were not all met.

Please note that the following study was conducted with the EU representative formulation (A19649B); further details are given in the risk assessment.

Report:	K-CA 8.6.2. [REDACTED], [REDACTED], [REDACTED] & [REDACTED] (2015b) SYN545974 SC (A19649B) - Toxicity Effects on the Seedling Emergence of Ten Species of Plants, Report Number 528P-124, Wildlife International A Division of EAG Inc. 8598 Commerce Drive Easton, MD 21601 USA (Syngenta file No. A19649B_10178)
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Guidelines

- US EPA Ecological Effects Test Guidelines, OCSPP 850.5400: Seedling Emergence and Seedling Growth (2012)
- OECD Guideline 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (2006)

GLP: Yes

Materials

Test material	A19649B
Lot/Batch #:	SMU2JP001

Actual content of active ingredients: SYN545974: 18.6 % w/w corresponding to 204 g/l

Description: Off-white suspension

Stability of test compound: Stable under test conditions.

Reanalysis/expiry date: End of February 2016

Treatments

Test concentrations: 25, 50, 100, 200 and 400 g a.s./ha (nominally equivalent to 125, 250, 500, 1000 and 2000 mL a.s./ha)

Control: Reverse osmosis water

Spray volume: 200 mL /ha

Application method: Laboratory track sprayer. Calibration was performed by water weight (n=3).

Test organisms

Species:

Monocots:
Allium cepa (Onion)
Triticum aestivum (Wheat)
Lolium perenne (Ryegrass)
Zea mays (Corn)

Dicots:
Brassica oleracea (Cabbage)
Beta vulgaris (Sugarbeet)
Glycine max (Soybean)
Lactuca sativa (Lettuce)
Brassica napus (Oilseed Rape)
Lycopersicon esculentum (Tomato)

Test soil: Sandy loam

Test design

Test vessels: plastic pots (16 cm in diameter and 12 cm deep)

Sampling interval: Daily until 50 % emergence in control was reached, day 7, 14, and 21 after 50 % emergence in control.

Replication: Eight replicate pots with five seeds planted in each pot.

Duration: 21 days after 50 % emergence in controls

Environmental conditions

Test temperature: 10.81 to 30.95 °C

Humidity: 12.97 to 89.00 %

Soil pH: 6.3

Lighting: 16 hour photoperiod

Study Design and Methods

Experimental dates: 13 November to 17 December 2014

Planted seeds of four monocot species (*Allium cepa* (onion), *Lolium perenne* (ryegrass), *Triticum aestivum* (wheat) and *Zea mays* (corn)) and six dicot species (*Brassica oleracea* (cabbage), *Brassica napus* (oilseed rape), *Beta vulgaris* (sugarbeet), *Glycine max* (soybean), *Lactuca sativa* (lettuce), and *Lycopersicon esculentum* (tomato)) were sprayed with a series of five test concentrations of formulation A19649B. Nominal test concentrations used in the definitive test for all test species ranged from 25 to 400 g of formulated product per hectare. The number of emerged seedlings, number of surviving seedlings, seedling height and weight were determined at test termination (21-days).

Observations were made 7 and 14 days after 50 % emergence in control plants to document seedling emergence. Observations were made 21 days after 50 % control emergence to document seedling emergence and growth, and to determine the general condition of seedlings. Observations consisted of noting whether emergence had or had not occurred and assessing the condition of each seedling. Emergence was defined as the presence of visible plant tissue at the surface of the soil. Seedling condition was described by noting the presence or absence of possible signs of phytotoxicity such as necrosis, leaf wrinkle, chlorosis, plant lodging or plant stunting.

The growth of emerged seedlings was evaluated on Day 21 by assessing the height and dry weight of seedlings. Plant dry weight was estimated by measuring the total dry weight of the shoots within each replicate. Seedling height was measured with a ruler to the nearest whole centimetre from the surface of the soil to the apical meristem (*G. max* and *L. esculentum*), or to the tip of the tallest leaf (all other species). Dead or non-emerged seedlings were assigned a height of 0 cm, and seedlings less than 1 cm tall were assigned a height of 0.25 cm for calculation of means. Seedlings were then clipped at soil level; the shoots of all living seedlings within a replicate were placed in a labelled paper container, dried, and weighed as a group. Mean seedling height and total replicate dry weight were determined for each treatment group containing living seedlings at test termination.

Results and Discussion

Emergence, survival and growth

A summary of data for 21-day seedling emergence, survival and growth (dry weight and height) are shown in the tables below. Test data were evaluated to determine the lowest-observed-effect-rate (LOER) and no-observed-effect rate (NOER) for plant emergence, survival, dry weight, and height. Dunnett's test was used to establish the LOER and NOER by determining which treatment groups differed significantly ($p > 0.01$ and $p > 0.05$) from the control group. Mean seedling emergence, survival, dry weight, and height of the control and treatment groups were compared using the Dunnett option of the general linear model (GLM) procedure of SAS version 8. Significance was determined at the levels of 0.01 and 0.05.

There were no adverse effects of the test substance on the emergence of the ten species tested (Table 9.11.2-6).

There was also no significant effect of the test substance on seedling survival, apart from a single treatment group for *L. esculentum* at 25 g a.s./ha (Table 9.11.2-6). This is considered to be incidental to treatment due to no significant effects at higher treatment levels. In addition, further analysis with a Jonckheere-Terpstra test indicated that there was no detectable trend of decreasing survival ($p > 0.05$). Therefore, the NOER for *L. esculentum* was confirmed to be 400 g a.s./ha.

No significant, adverse, treatment related effects were observed on the height and dry weight of the ten species tested (Table 9.11.2-7).

Table 9.11.2-6. Summary of 21-day emergence, survival and plant condition data for ten plant species after pre-emergence exposure to A19649B.

Species	Endpoint (%)	Mean effect (%) at each treatment level					
		Control	25 g a.s./ha	50 g a.s./ha	100 g a.s./ha	200 g a.s./ha	400 g a.s./ha
Monocots: <i>Allium cepa</i> (Onion)	Emergence ¹	78	98	90	92	98	96
	Survival ²	100	98	90	93	95	95
	Sublethal ³	12.5	3.1	2.5	3.1	28.1	11.3
<i>Lolium perenne</i> (Ryegrass)	Emergence ¹	78	88	86	82	88	88
	Survival ²	100	98	93	98	98	100
	Sublethal ³	4.2	0.0	0.0	3.1	0.0	0.0
<i>Triticum aestivum</i> (Wheat)	Emergence ¹	98	98	98	95	90	100
	Survival ²	98	100	100	100	100	100
	Sublethal ³	0.0	2.5	0.0	0.0	4.2	25.0
<i>Zea mays</i> (Corn)	Emergence ¹	95	90	100	90	98	93
	Survival ²	100	100	98	100	100	100
	Sublethal ³	2.5	0.0	10.0	8.1	17.5	8.8
Dicots: <i>Beta vulgaris</i> (Sugarbeet)	Emergence ¹	98	98	95	98	93	98
	Survival ²	100	100	100	100	100	100
	Sublethal ³	0.0	7.5	5.0	7.5	6.3	5.0
<i>Brassica napus</i> (Oilseed Rape)	Emergence ¹	93	93	100	95	95	90
	Survival ²	100	100	98	98	100	100
	Sublethal ³	0.0	9.8	0.0	2.5	2.5	6.3
<i>Brassica oleracea</i> (Cabbage)	Emergence ¹	98	100	95	88	93	100
	Survival ²	95	100	100	98	100	100
	Sublethal ³	0.0	10	15	21.7	30.6	17.5
<i>Glycine max</i> (Soybean)	Emergence ¹	90	93	98	95	98	95
	Survival ²	98	98	100	100	100	100
	Sublethal ³	5.6	8.3	5.0	5.0	0.0	7.5
<i>Lactuca sativa</i> (Lettuce)	Emergence ¹	93	93	98	90	95	93
	Survival ²	98	100	100	100	100	100
	Sublethal ³	0.0	2.5	0.0	0.0	0.0	0.0
<i>Lycopersicon esculentum</i> (Tomato)	Emergence ¹	80	76	72	78	86	88
	Survival ²	100	78*	100	89	100	91
	Sublethal ³	10.4	3.1	7.3	7.3	0	15

¹ Emergence: Mean number of emerged seedlings at day 21 per five planted seeds in each pot, expressed as a percentage by HSE using available data.

² Survival: The mean percentage of emerged seedlings surviving at test termination (day 21) in each replicate.

³ Sublethal: The mean percentage of emerged seedlings in each replicate exhibiting any sublethal phytotoxic effects across all severities and types (see Table 9.11.2-8 for a breakdown of phytotoxic effects by severity).

*Treatment group mean is significantly different from the control mean (Dunnett's test, $p \leq 0.05$). However, no significant difference was found using Jonckheere-Terpstra test ($p > 0.05$) indicating that there is no detectable trend of decreasing survival.

Table 9.11.2-7. Effect of A1964B on the weight and height of seedlings at 21-days after pre-emergence exposure.

Species	Endpoint (% reduction compared to control)	Treatment Level				
		25 g a.s./ha	50 g a.s./ha	100 g a.s./ha	200 g a.s./ha	400 g a.s./ha
Monocots:	Weight	-49%	-16%	-10%	-2%	-21%
<i>Allium cepa</i> (Onion)	Height	-22%	-6%	-17%	-1%	-9%
<i>Lolium perenne</i> (Ryegrass)	Weight	-36%	-6%	-24%	-16%	-39%
	Height	-9%	-2%	-2%	0%	-6%
<i>Triticum aestivum</i> (Wheat)	Weight	-2%	-4%	-8%	-6%	-8%
	Height	-2%	-1%	-2%	0%	-2%
<i>Zea mays</i> (Corn)	Weight	-1%	-1%	-10%	-4%	-5%
	Height	-1%	-2%	-2%	-3%	-3%
Dicots:	Weight	-12%	-10%	-19%	-11%	-8%
<i>Beta vulgaris</i> (Sugarbeet)	Height	-2%	0%	-2%	-1%	-3%
<i>Brassica napus</i> (Oilseed Rape)	Weight	-2%	-2%	-2%	-4%	-5%
	Height	-1%	-4%	-3%	-5%	-8%
<i>Brassica oleracea</i> (Cabbage)	Weight	0%	-22%	-18%	-18%	-5%
	Height	-3%	-8%	-6%	-8%	-2%
<i>Glycine max</i> (Soybean)	Weight	-10%	-16%	-14%	-32%	-18%
	Height	-4%	-5%	-11%	-24%	-9%
<i>Lactuca sativa</i> (Lettuce)	Weight	-10%	-4%	-8%	-13%	-17%
	Height	-5%	-1%	-2%	-2%	-3%
<i>Lycopersicon esculentum</i> (Tomato)	Weight	-15%	-23%	-3%	-15%	-10%
	Height	-21%	-1%	-14%	-16%	-13%

Negative percentage indicates an increase in weight or height compared to the control.

*Treatment group mean is significantly different from the control mean (Dunnett's test, $p \leq 0.05$). No significant effects were detected.

Seedling condition

The data for the condition of surviving seedlings (sublethal phytotoxic effects) is summarised below in Table 9.11.2-8. Signs of phytotoxicity observed in the study included chlorosis, necrosis, leaf curl and in one case stem curl. The greatest number of plants exhibiting signs of phytotoxicity appeared in *B. oleracea* (up to 30.6 %) and the fewest appeared in *L. sativa* (0 - 2.5 %). The observed signs of phytotoxicity were not dose-responsive and were considered incidental to treatment.

Table 9.11.2-8 Mean percentage of surviving seedlings showing sublethal phytotoxic effects 21-days after pre-emergence exposure to A19649B.

Species	Severity ²	Mean percentage (%) of surviving seedlings with phytotoxic effects recorded at each treatment level ¹					
		Control	25 g a.s./ha	50 g a.s./ha	100 g a.s./ha	200 g a.s./ha	400 g a.s./ha
Monocots: <i>Allium cepa</i> (Onion)	Slight	8.3 ^N	0	0	0	13.1 ^N	6.3 ^N
	Moderate	4.2 ^N	3.1 ^N	0	0	12.5 ^N	2.5 ^N
	Severe	0	0	2.5 ^N	3.1 ^N	2.5 ^N	2.5 ^N
	Total	12.5	3.1	2.5	3.1	28.1	11.3
<i>Lolium perenne</i> (Ryegrass)	Slight	4.2 ^L	0	0	0	0	0
	Moderate	0	0	0	0	0	0
	Severe	0	0	0	3.1 ^N	0	0
	Total	4.2	0	0	3.1	0	0
<i>Triticum aestivum</i> (Wheat)	Slight	0	2.5 ^C	0	0	4.2 ^{C,N}	2.5 ^C
	Moderate	0	0	0	0	0	0
	Severe	0	0	0	0	0	0
	Total	0	2.5	0	0	4.2	2.5
<i>Zea mays</i> (Corn)	Slight	2.5 ^C	0	10 ^{C,N,L}	8.1 ^{N,L}	12.5 ^{N,C}	5.6 ^C
	Moderate	0	0	0	0	0	3.1 ^{C,N,L}
	Severe	0	0	0	0	0	0
	Total	2.5	0	10	8.1	17.5	8.8
Dicots: <i>Beta vulgaris</i> (Sugarbeet)	Slight	0	7.5 ^{C,L,N}	5 ^L	7.5 ^C	6.3 ^C	5 ^C
	Moderate	0	0	0	0	0	0
	Severe	0	0	0	0	0	0
	Total	0	7.5	5	7.5	6.3	5
<i>Brassica napus</i> (Oilseed Rape)	Slight	0	6.7 ^{C,L}	0	2.5 ^{C,L}	2.5 ^L	6.3 ^{C,L,N}
	Moderate	0	3.1 ^{C,L,N}	0	0	0	0
	Severe	0	0	0	0	0	0
	Total	0	9.8	0	2.5	2.5	6.3
<i>Brassica oleracea</i> (Cabbage)	Slight	0	10 ^L	12.5 ^{C,L}	21.7 ^{C,L}	26.5 ^{C,L}	17.5 ^{C,L}
	Moderate	0	0	2.5 ^{C,N}	0	4.2 ^{C,N}	0
	Severe	0	0	0	0	0	0
	Total	0	10	15	21.7	30.6	17.5
<i>Glycine max</i> (Soybean)	Slight	5.6 ^{L,N}	4.2 ^L	2.5 ^N	2.5 ^{C,L}	0	0
	Moderate	0	0	0	0	0	2.5 ^{C,L,N}
	Severe	0	4.2 ^N	2.5 ^N	2.5 ^N	0	5.0 ^N
	Total	5.6	8.3	5	5	0	7.5
<i>Lactuca sativa</i> (Lettuce)	Slight	0	2.5 ^{C,L}	0	0	0	0
	Moderate	0	0	0	0	0	0
	Severe	0	0	0	0	0	0
	Total	0	2.5	0	0	0	0
<i>Lycopersicon esculentum</i> (Tomato)	Slight	6.3 ^{C,N}	0	3.1 ^{C,L}	0	0	10.8 ^{C,L,S}
	Moderate	0	3.1 ^{C,L,N}	4.2 ^{C,N}	3.1 ^{C,L}	0	4.2 ^{C,L,N}
	Severe	4.2 ^N	0	0	4.2 ^{C,N,L}	0	0
	Total	10.4	3.1	7.3	7.3	0	15

¹ Table assembled and calculated by HSE based on available data from study report: the percentage of surviving seedlings with any type of recorded phytotoxic effect in each severity category was calculated for each replicate, then the mean percentage for each treatment level is presented in the table. Types of phytotoxic effects recorded are indicated in superscript letters and include necrosis (^N), chlorosis (^C), leaf curl (^L) and stem curl (^S). Multiple effect types may be recorded for the same individual plant.

² Severity rating score (adapted from [REDACTED] *et al.*, 1977): Slight, 10-30 (effect barely noticeable to more pronounced, not obviously detrimental); Moderate, 40-60 (moderate effect appearing able to recover, to lasting effect with doubtful recovery); Severe, 70-90 (heavy injury and loss of leaves, to occasional surviving leaves).

Endpoints

In this test, the rate corresponding to the NOER has no statistically significant effect ($p > 0.01$ and $p > 0.05$) within a given exposure period when compared to the control. ER_x estimates were not calculated for plant condition because those data are qualitative and therefore not conducive to statistical analysis. The LOER, NOER, ER_{25} , and ER_{50} for each of the ten test species are presented in the table below:

Table 9.11.2-9: Effect Rates of A19649B on endpoints for all tested plant species.

Endpoints	NOER	LOER	ER_{25}	ER_{50}
21-Day Emergence (g a.s./ha)	400	> 400	> 400	> 400
21-Day Survival (g a.s./ha)				
21-Day Height (g a.s./ha)				
21-Day Dry Weight (g a.s./ha)				

Validity Criteria

The study is considered valid if:

- There was at least 70 % emergence in the control means by day 21.
- The control seedlings did not exhibit any phytotoxic effects and only normal variation in growth and morphology for that particular species.
- The mean survival of the emerged control seedlings was at least 90 %
- The environmental conditions were identical for all the tested species.

In this study the above criteria were met with one exception, as phytotoxicity was observed in control groups for some species. This has been discussed further by HSE in the comments below.

Conclusions

The effects of the formulation, A19649B, were evaluated on the seedling emergence, survival, height, and dry weight of ten non-target plant species. At pre-emergence, application rates of up to 400 g a.s./ha (nominally equivalent to 2000 mL A19649B/ha) resulted in no adverse, treatment-related effects on all ten species tested. Therefore, the NOER values as well as the ER_{50} and ER_{25} values are equivalent to or greater than the highest rate tested (400 g a.s./ha), respectively, for all species.

([REDACTED] *et al.*, 2015b)

HSE Comments

This study was conducted to GLP. This study was assessed using guideline OECD 208 (2006).

The authors verified the test substance in the highest spray mixture test concentration on two occasions and found it to be 93 -99 % of nominal concentration. Therefore, the authors report their results as nominal concentrations. The analytical methods have been evaluated by HSE Chemistry specialists in Vol. 3CA Part B5.1.2.6. The following was concluded for this method: “Acceptable method. LOQ: 500 mg/L in test spray solutions”.

The study does not fully meet the validity criteria, due to phytotoxic effects seen in the controls:

- Five species (*A. cepa*, *L. perenne*, *Z. mays*, *G. max* and *L. esculentum*) exhibited phytotoxic effects in the negative control ranging from 2.5 – 12.5 % of surviving seedlings (Table 9.11.2-8). For reference, seedling emergence was 78 – 95 % with survival of 98 – 100 % in these species (see table 9.11.2-6). The effect severity was ‘slight’ in all cases, with the exception of *A. cepa* and *L. esculentum* where there was also a single plant with ‘moderate’ or ‘severe’ effects, respectively (Table 9.11.2-8).
- The OECD validity criteria states that seedlings in the negative control should “*not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and the plants exhibit only normal variation in growth and morphology for that particular species*”. However, the authors

provide the following justification that the observed phytotoxic effects are expected during natural growth of these species:

- *“One to three plants in the control groups of five species exhibited chlorosis, necrosis and/or leaf curl. Occasional symptoms of phytotoxicity are expected during natural growth in these species. Therefore, the condition of negative control plants was not considered detrimental to the study.”*
- HSE has not invalidated the study. This is because the phytotoxic effects in the control are sporadic across replicates and generally low severity. However, it does generate some uncertainty, particularly in species *L. esculentum* and *A. cepa* where the effects were most prominent.

Some phytotoxic effects were also observed in the treatment levels in individual plants across all species. A summary of the observations is shown in table 9.11.2-8. HSE notes that there was < 50 % phytotoxicity effects at the highest test concentration for all species. Therefore, the proposed ER₅₀ is sufficiently protective of phytotoxicity.

Additional minor points relating to the study in general and noted for reference are:

- In the study data appendices there is a discrepancy between the number of emerged seedlings on day 7 for the negative controls in some species, with two different emergence numbers recorded for the same day. However, day 7 emergence is not used as an endpoint in risk assessment, therefore this does not compromise the study conclusions.
- No test with a reference substance as a positive control is carried out or historical data referred to as mentioned in the guidelines, though this is not a reason to invalidate the study.
- It was unclear whether the soil used was artificial or field soil, but a description of the soil composition was provided (kaolinite clay, industrial quartz sand and peat; 87 % sand 5% silt 8% clay; pH 6.3-6.5). Additionally, the parameters such as organic carbon and organic matter are within the recommended guidelines, therefore no further information is needed.

Statistical analysis of this study has also been considered. The authors used standard statistical techniques, as described below:

- Dunnett's test was used to establish the LOER and NOER values for the data of seedling emergence, survival, dry weight and height. No data transformation prior to analysis is mentioned.
- The authors used a general linear model (GLM) procedure with Dunnett option from SAS software to compare mean seedling emergence, survival, dry weight and heights of the treatment and control groups. No data transformation prior to analysis is mentioned.
- The ER₂₅ and ER₅₀ values are estimates which were not statistically derived due to lack of significant treatment effects.

The agreed endpoint for consideration in risk assessment is:

- ER₅₀: > 400 g a.s./ha (nominal concentration)

B.9.11.3. Extended laboratory studies on non-target plants

None submitted.

B.9.11.4. Semi-field and field tests on non-target plants

None submitted.

B.9.12. RISK ASSESSMENT FOR TERRESTRIAL NON-TARGET HIGHER PLANTS**Tier 1: Screening step**

This risk assessment is based on the ‘Guidance Document on Terrestrial Ecotoxicology’, (SANCO/10329/2002 rev2 final, 2002). It is restricted to off-field situations, as non-target plants are defined as non-crop plants located outside the treated area. Spray drift from the treated areas may lead to deposition of the applied product on plants in adjacent off-crop areas, or else onto ground where non-target plants will shortly germinate and emerge.

No non-target plant active substance studies were submitted for evaluation. One non-target plant screening study was submitted for the representative product ‘Miravis Plus’ (A21857B). This is acceptable, as the active substance is not a herbicide, and does not demonstrate a herbicidal MOA.

The results of the vegetative vigour and seedling emergence screening study after exposure to the representative product for the pydiflumetofen active substance assessment ‘Miravis Plus’ (A21857B) are summarised in section B.9.11, and the endpoints are summarised in Table 9.12.-1 below. Plants were exposed at the following rates: 0, 200, 400, 800, 1,600, and 3200 mL A21857B /ha, which includes the maximum proposed single application rate of 3.2 L A21857B /ha (equivalent to 200 g a.s. /ha).

Table 9.12-1 Summary of screening data for ‘Miravis Plus’ (A21857B)

Test substance	Study type	Assessment type	Test plant		Observed effects	Reference
Miravis Plus (A21857B)	Phytotoxicity to non-target plants - screening test	Seedling emergence	Monocots	<i>Allium cepa</i> (onion)	No effects observed at any test concentration.	[REDACTED] (2017) KCP 10.6.1-01
				<i>Triticum aestivum</i> (wheat)	No effects observed at any test concentration.	
			Dicots	<i>Glycine max</i> (soybean)	No effects observed at any test concentration.	
				<i>Beta vulgaris</i> (sugar beet)	No effects observed at any test concentration.	
				<i>Brassica napus</i> (Oilseed rape)	No effects observed at any test concentration.	
				<i>Cucumis sativus</i> (cucumber)	No effects observed at any test concentration.	
		Vegetative vigour	Monocots	<i>Allium cepa</i> (onion)	No effects observed at any test concentration.	
				<i>Triticum aestivum</i> (wheat)	No effects observed at any test concentration.	
			Dicots	<i>Glycine max</i> (soybean)	Slight phytotoxic effects (necrosis) at 3,200 [2]* mL A21857B /ha	
				<i>Beta vulgaris</i> (sugar beet)	No effects observed at any test concentration.	
				<i>Brassica napus</i> (Oilseed rape)	No effects observed at any test concentration.	
				<i>Cucumis sativus</i> (cucumber)	Phytotoxic effects (necrosis) at 800 [1]*, 1,600 [2]*, and 3,200 [4]* mL A21857B /ha	

* numbers in square brackets [#] represent the numerical score assigned as a rating of phytotoxicity. Plants were rated on a scale from 0 to 10, with 0 representing ‘Vigorous healthy plants, indistinguishable from the untreated control’, and 10 representing ‘Complete destruction of plant parts above ground’.

There were no observations of phytotoxicity in any of the six tested plants in the seedling emergence part of the screening assessment, at rates up to and including 3,200 mL A21857B /ha.

For the vegetative vigour part of the screening assessment, onion, wheat, sugar beet, and oilseed rape did not show any phytotoxic effects at rates up to and including 3,200 mL A21857B /ha. Observations of mild phytotoxic effects were recorded for soybean, and cucumber. The most sensitive species was cucumber, showing phytotoxic effects (necrosis) at 800 (10%), 1,600 (20%), and 3,200 (40%) mL A21857B /ha.

It is stated in the SANCO/10329/2002 guidance document that ‘The risk should be considered acceptable, if there are no data indicating more than 50 % phytotoxic effects at the maximum application rate. If the results show more than 50 % effect for one species, or clear indications of effects on more than one species, data requirements and assessment move to the next tier.’. There is no quantitative measure of what ‘clear indications of effects’ means. Phytotoxicity was observed in two species in the vegetative vigour part of the screening assessment, however this was only mild, and no effects exceeded the trigger of 50 %.

The assertion that an acceptable risk is demonstrated by the screening assessment can be qualitatively supported by conclusions from studies conducted using the EU representative formulation ‘Miravis’ (A19649B). Although a certain level of uncertainty surrounds the extrapolation of these data as the two products have been deemed non-comparable (see Volume 4), both contain the same active substance, which was applied at 200 g a.s. /ha in the respective studies, and so it is likely that any effects resulting from exposure to the active substance would be similar in magnitude. This is further justified for seedling emergence, given that application occurs prior to emergence of the seedling, hence direct exposure to the intact formulation is not expected.

A total of three studies were carried out using ‘Miravis’ (A19649B), including one vegetative vigour study, and two seedling emergence studies. Each study tested ten species of plant, at or exceeding the maximum proposed application rate of ‘Miravis Plus’ (A21857B) from the GAP table. No effects > 50 % were observed for any of the tested plants at any concentration in any of the three studies (See table 9.12-2), supporting the conclusions from the screening assessment.

All of the validity criteria were met for the vegetative vigour study (██████ et al., 2015), and the study was deemed acceptable.


For the first of the two seedling emergence studies, ████████ et al., (2015a), two plants in the negative controls exhibited phytotoxic effects: One *Z. mays* plant and one *B. vulgaris* plant. This can be considered to be normal variation in growth and morphology. However, for *B. vulgaris*, the mean emergence of the seedlings in the control group was 65 %, leading to some uncertainty as to the health of the *B. vulgaris* seeds. *B. vulgaris* did have an 8 % increase of seedling emergence in the test substance replicate compared to the control, so the endpoints can be considered valid.

As the mean emergence for *B. vulgaris* was 65 %, this is lower than the threshold of 70 % required by the validity criteria in OECD 208 (2006). Therefore, **the validity criterion for emergence for this study is not met**. However, the *B. vulgaris* emergence rate is only 5 % below the validity criteria threshold, and all other test species met the validity criteria for emergence.

In the second of the two seedling emergence studies, ████████ et al., (2015b), phytotoxic effects ranging from 2.5 – 12.5 % were observed in the negative controls for five species (*A. cepa*, *L. perenne*, *Z. mays*, *G. max* and *L. esculentum*), meaning that one of the validity criteria was not met. HSE has not invalidated the study. This is because the phytotoxic effects in the control are sporadic across replicates and generally low severity. However, it does generate some uncertainty, particularly in species *L. esculentum* and *A. cepa* where the effects were most prominent.

Table 9.12-2: Summary of non-target plant testing data for ‘Miravis’ (A19649B)

Test substance	Study type	Test plant		Endpoint mL product /ha (g a.s. /ha)	Reference
A19649B	Vegetative vigour of ten species of plant	Monocots	<i>Allium cepa</i> (onion)	ER ₅₀ > 1000 (> 200)	██████ et al., (2015) KCA 8.6.2/01
				NOER 1000 (200)	
			<i>Lolium perenne</i> (ryegrass)	ER ₅₀ > 1000 (> 200)	
				NOER 1000 (200)	
			<i>Triticum aestivum</i> (wheat)	ER ₅₀ > 1000 (> 200)	
				NOER 1000 (200)	
		Dicots	<i>Zea mays</i> (corn)	ER ₅₀ > 1000 (> 200)	
				NOER 1000 (200)	
			<i>Beta vulgaris</i> (sugar beet)	ER ₅₀ > 1000 (> 200)	
				NOER 1000 (200)	
			<i>Brassica napus</i> (oilseed rape)	ER ₅₀ > 1000 (> 200)	
				NOER 1000 (200)	
				ER ₅₀ > 1000 (> 200)	

Test substance	Study type	Test plant	Endpoint mL product /ha (g a.s. /ha)	Reference
A19649B	Seedling Emergence and Seedling Growth	Monocots	<i>Brassica oleracea</i> (cabbage)	NOER 1000 (200)
			<i>Glycine max</i> (soybean)	ER ₅₀ > 1000 (> 200) NOER 1000 (200)
			<i>Lactuca sativa</i> (lettuce)	ER ₅₀ > 1000 (> 200) NOER 1000 (200)
			<i>Lycopersicon esculentum</i> (tomato)	ER ₅₀ > 1000 (> 200) NOER 1000 (200)
			<i>Allium cepa</i> (onion)	ER ₅₀ > 1000 (>200) NOER 1000 (200)
			<i>Lolium perenne</i> (ryegrass)	ER ₅₀ > 1000 (>200) NOER < 1000 (<200)
			<i>Triticum aestivum</i> (wheat)	ER ₅₀ > 1000 (>200) NOER 1000 (200)
			<i>Zea mays</i> (corn)	ER ₅₀ > 1000 (>200) NOER 1000 (200)
		Dicots	<i>Beta vulgaris</i> (sugar beet)	ER ₅₀ > 1000 (>200) NOER 1000 (200)
			<i>Brassica napus</i> (oilseed rape)	ER ₅₀ > 1000 (>200) NOER 1000 (200)
			<i>Brassica oleracea</i> (cabbage)	ER ₅₀ > 1000 (>200) NOER < 1000 (<200)
			<i>Glycine max</i> (soybean)	ER ₅₀ > 1000 (>200) NOER 1000 (200)
			<i>Lactuca sativa</i> (lettuce)	ER ₅₀ > 1000 (>200) NOER < 1000 (<200)
			<i>Lycopersicon esculentum</i> (tomato)	ER ₅₀ > 1000 (>200) NOER 1000 (200)
A19649B	Seedling Emergence and Growth	Monocots: <i>Allium cepa</i> (Onion) <i>Triticum aestivum</i> (Wheat) <i>Lolium perenne</i> (Ryegrass) <i>Zea mays</i> (Corn) Dicots: <i>Brassica oleracea</i> (Cabbage) <i>Beta vulgaris</i> (Sugar beet) <i>Glycine max</i> (Soybean) <i>Lactuca sativa</i> (Lettuce) <i>Brassica napus</i> (Oilseed Rape) <i>Lycopersicon esculentum</i> (Tomato)	ER ₅₀ > 400 g a.s./ha (nominal concentration)	 et al., (2015b) KCA 8.6.2

Conclusion

Concentrations of A21857B were tested up to and including the maximum application rate from the GAP, on 6 species of terrestrial plants. The tested species encompassed both monocotyledonous and dicotyledonous species, and the effects on both seedling emergence and vegetative vigour were considered. No effects of > 50 % were observed for any of the species tested, at any test concentration in the tier 1 screening assessment, this conclusion is qualitatively supported by conclusions from studies conducted using 'Miravis' (A19649B), indicating an acceptable risk to non-target terrestrial plants from the proposed uses.

B.9.13. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No data required or submitted.

B.9.14. RISK ASSESSMENT FOR OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No data required or submitted.

B.9.15. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT

No studies were submitted with the formulation; only tests conducted with the active substance are considered necessary to indicate the potential risk to biological sewage treatment systems.

B.9.16. RISK ASSESSMENT FOR BIOLOGICAL METHODS FOR SEWAGE TREATMENT

Studies are not required for the formulation as only tests conducted with the active substance are considered necessary to assess the potential risk to biological sewage treatment systems.

Table 9.16-1: Endpoints for activated sludge exposed to pydiflumetofen

Test item	Test system	Endpoint (mg a.s./L)	Reference
Pydiflumetofen	Activated sludge respiration inhibition	EC ₅₀ (3h) > 1.5 ¹	██████ (2013)

¹Limit of solubility of Pydiflumetofen in water

Treatment rates up to 1000 mg a.s./L Pydiflumetofen had no effect on the respiration rate of activated sewage sludge and indicate that microbial activity in these systems is at low risk. However due to issues with the solubility of the test item, the EC₅₀ was defined as > than the limit of solubility (1.5 mg a.s./L). The worst-case PEC_{sw} was 0.001847 mg a.s./L which is significantly lower than the EC₅₀ value of > 1.5 mg a.s./L.

B.9.17. 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
KCP 10.2.1	██████	2019	Pydiflumetofen EC (A21857B) - Acute Toxicity to Fish (<i>Rainbow trout</i>), Static, 96 Hours. Report No. ██████████. ██ ██ GLP Unpublished Syngenta File No. VV-619141	Y	Y	Data for first approval	SYN	N

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
KCP 10.2.1	██████	2019a	Pydiflumetofen EC (A21857B) - Toxicity to the Water Flea <i>Daphnia magna</i> Straus under Laboratory Conditions (Acute Immobilization Test – Static) Report No. 160713SF / DAI17425 Noack Laboratorien GmbH, Käthe-Paulus-Straße 1, 31157 Sarstedt Germany GLP Unpublished Syngenta File No. VV-725187	N	Y	Data for first approval	SYN	N
KCP 10.2.1	██████	2019b	Pydiflumetofen EC (A21857B) – Toxicity to <i>Pseudokirchneriella subcapitata</i> in a 96-Hour Algal Growth Inhibition Test. Report No. 160713SF / SPO17425 Noack Laboratorien GmbH, Käthe-Paulus-Straße 1, 31157 Sarstedt, Germany. GLP Unpublished Syngenta File No. VV-619320	N	Y	Data for first approval	SYN	N

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
KCP 10.3.1.1	██████	2016	Pydiflumetofen EC (A21857B) – Acute Oral and Contact Toxicity to the Honey Bee, <i>Apis mellifera</i> L. under Laboratory Conditions Report No. S16-05072 Eurofins Agrosience Services EcoChem GmbH / Eurofins Agrosience Services Ecotox GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany GLP Unpublished Syngenta File No. VV-466570	N	Y	Data for first approval	SYN	N
KCP 10.3.1.1.1	██████	2015	SYN545974 SC (A19649B) - Acute Oral and Contact Toxicity to the Honey Bee, <i>Apis mellifera</i> L. under Laboratory Conditions, Report Number S14-04061. Eurofins Agrosience Services EcoChem GmbH Eutinger Str. 24 75223 GLP Unpublished NiefernÖschelbronn, Germany (Syngenta file No. A19649B_10036).					
KCA 8.3.1.2	██████	2014	A19649B – Chronic Toxicity to the Honeybee <i>Apis mellifera</i> L. in a 10 Day Continous Laboratory Feeding Study Report No. 14 10 48 004 B Document No. VV-411102 , A19649B_10055 Test Facility BioChem agrar GLP Unpublished	N	Y	Data for first approval. <u>Used as supporting information in risk assessment</u>	SYN	N

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
KCA 8.3.1.3	██████	2015	SYN545974 SC (A19649B) – Chronic toxicity to the honeybee larvae <i>Apis mellifera</i> L. under laboratory conditions (in vitro) Report No. 14 10 48 005 B Document No. VV-411273 , A19649B_10076 Test Facility BioChem agrar GLP Unpublished	N	Y	Data for first approval	SYN	N
KCA 8.3.1.3	██████	2015a	SYN545974 SC (A19649B) - A laboratory study to determine the chronic effects on the brood of the honey bee <i>Apis mellifera</i> L. (Hymenoptera: Apidae). Report No. 037SRFR15C07 (Including Amendment 1) Document No. VV-414129 , A19649B_10184 Test Facility SynTech Research France SAS GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.3.1.3	██████	2016	Pydiflumetofen - Statistical re-analysis -A laboratory study to determine the chronic effects of SYN545974 SC (A19649B) on the brood of the honey bee <i>Apis mellifera</i> L. (Hymenoptera: Apidae) Report No. CEA.1831 Document No. VV-134501 , A19649B_10294 Test Facility Cambridge Environmental Assessments Not GLP Unpublished This is CONFIDENTIAL INFORMATION	N	N	N/A	SYN	N

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
KCA1 8.3.1.3	██████████.	2016	SYN545974 - Response to ANSES comments regarding the bee brood toxicity test with honeybees (<i>Apis mellifera</i>) (██████████, 2015a) Report No. N/A Document No. VV-137218 , SYN545974_10462 Test Facility N/A Not GLP Unpublished	N/A	N	N/A	SYN	N
KCA1 8.3.1.3	██████████	2016a	Pydiflumetofen – Statistical re-analysis: SYN545974 SC (A19649B) – Chronic toxicity to the honeybee larvae <i>Apis mellifera</i> L. under laboratory conditions (in vitro) Report No. CEA.1832 Document No. VV-134503 , A19649B_10296 Test Facility Cambridge Environmental Assessments Not GLP Unpublished This is CONFIDENTIAL INFORMATION	N	N	N/A	SYN	N

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
KCP 10.3.1.5	██████	2017	Pydiflumetofen SC (A19649B) - A Semi-Field Study to Evaluate the Side Effects on Honeybees (<i>Apis mellifera</i> L.) in <i>Phacelia tanacetifolia</i> in Germany 2016. Report number S16-000293. Eurofins Agroscience Services EcoChem GmbH/Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 46, 75223 NiefernÖschelbronn, Germany GLP Unpublished (Syngenta file No. A19649B_10312)	N	Y	Data for first approval	SYN	N
KCP 10.3.1.5	██████	2017	Pydiflumetofen SC (A19649B) - A Semi-Field Study to Evaluate the Side Effects on Honeybees (<i>Apis mellifera</i> L.) in <i>Phacelia tanacetifolia</i> in Germany 2016. Report number S16-04919. Eurofins Agroscience Services EcoChem GmbH/Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 46, 75223 Niefern-Öschelbronn, Germany GLP Unpublished (Syngenta File No. A19649B_10314)	N	Y	Data for first approval	SYN	N

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
KCP 10.3.1.5	██████	2018	Pydiflumetofen SC (A19649B) - A semi-field study to evaluate the side effects on the honey bee <i>Apis mellifera</i> L. in Germany in 2017. Report Number 17 48 BTB 0003. BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany GLP Unpublished (Syngenta file no A19649B_10349)	N	Y	Data for first approval	SYN	N
KCP 10.3.2.1	██████	2018	Pydiflumetofen EC (A21857B) – A laboratory bioassay of the effects of fresh residues on the parasitic wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae) Report No. SYN-18-19 Mambo-Tox Ltd. 2 Venture Road, University Science Park, Southampton SO16 7NP, United Kingdom GLP Unpublished Syngenta File No. VV-469963	N	Y	Data for first approval	SYN	N
KCP 10.3.2.1	██████	2018	Pydiflumetofen EC (A21857B) - A laboratory bioassay to determine the effects of fresh residues on the predatory mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae) Report No. SYN-18-20 Mambo-Tox Ltd. 2 Venture Road, University Science Park, Southampton SO16 7NP, United Kingdom GLP Unpublished Syngenta File No. VV-470237	N	Y	Data for first approval	SYN	N

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
KCP 10.3.2.2	██████	2017	Pydiflumetofen EC (A21857B) – A rate-response extended laboratory bioassay of the effects of fresh residues on the parasitic wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae) Report No. SYN-16-45 Mambo-Tox Ltd. 2 Venture Road, University Science Park, Southampton SO16 7NP, United Kingdom GLP Unpublished Syngenta File No. VV-466923	Y	Data for first approval	SYN	N	Y
KCP 10.3.2.2	██████	2017	Pydiflumetofen EC (A21857B) – A rate-response extended laboratory bioassay of the effects of fresh residues on the predatory mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae) Report No. SYN-16-44 Mambo-Tox Ltd. 2 Venture Road, University Science Park, Southampton SO16 7NP, United Kingdom GLP Unpublished Syngenta File No. VV-467035	N	Y	Data for first approval	SYN	N

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
KCP 10.3.2.2	██████	2019	Pydiflumetofen EC (A21857B) – A Rate-Response Extended Laboratory Study to Evaluate the Effects of Fresh Residues on the Green Lacewing, <i>Chrysoperla carnea</i> (Neuroptera, Chrysopidae) Report No. SYN-19-26 Mambo-Tox, A Division of Cawood Scientific Ltd., 2 Venture Road, University Science Park, Southampton SO16 7NP, United Kingdom GLP Unpublished Syngenta File No.VV-732035	N	Y	Data for first approval	SYN	N
KCP 10.4.1.1	██████	2017	Pydiflumetofen EC (A21857B) - Sublethal Toxicity to the Earthworm <i>Eisenia fetida</i> in Artificial Soil with 5 % peat Report No. 160713SF / RBR17425 Noack Laboratorien GmbH, Käthe-Paulus-Straße 1, 31157 Sarstedt, Germany GLP Unpublished Syngenta File No.VV-468032	N	Y	Data for first approval	SYN	N
KCP 10.4.2.1	██████	2017	Pydiflumetofen EC (A21857B) - Effects on the Reproduction of the Collembolan <i>Folsomia candida</i> Report No. 160713SF / ICR17425 Noack Laboratorien GmbH, Käthe-Paulus-Straße 1, 31157 Sarstedt, Germany GLP Unpublished Syngenta File No. VV-467124	N	Y	Data for first approval	SYN	N

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
KCP 10.4.2.1	██████	2017a	Pydiflumetofen EC (A21857B) - Effects on the Reproduction of the Predatory mite (<i>Hypoaspis aculeifer</i>) Report No. 160713SF / IHL17425 Noack Laboratorien GmbH, Käthe-Paulus-Straße 1, 31157 Sarstedt, Germany GLP Unpublished Syngenta File No.VV-467137	N			SYN	
KCP 10.5	██████	2017a	Pydiflumetofen EC (A21857B) - Effects on the Activity of Soil Microflora (Carbon and Nitrogen Transformation Tests) Report No.160713SF /TBM17425 Noack Laboratorien GmbH, Käthe-Paulus-Straße 1, 31157 Sarstedt, Germany GLP Unpublished Syngenta File No. VV-467942	N	Y	Data for first approval <u>Not used in risk assessment</u>	SYN	N
KCP 10.6.1	██████	2017	Pydiflumetofen EC (A21857B) – Phytotoxicity to Non-Target Plants Screening Test Report No. ACE-16-134 AgroChemex Ltd., Aldhams Farm Research Station, Dead Lane, Lawford, Manningtree, Essex, CO11 2NF GLP Unpublished Syngenta File No. VV-467318	N	Y	Data for first approval	SYN	N

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
KCA 8.6.2	[REDACTED]	2015	SYN545974 SC (A19649B) – Toxicity Effects on the Seedling Emergence of Ten Species of Plants Report No. 528P-124 Document No. VV-413402 , A19649B_10178 Test Facility Wildlife International Ltd. GLP Unpublished	N	Y	Data for first approval <u>Used as supporting information in risk assessment</u>	SYN	N
KCA 8.6.2	[REDACTED]	2015a	SYN545974 SC (A19649B) – Toxicity Effects on the Vegetative Vigour of Ten Species of Plants Report No. 528P-116 Document No. VV-411577 , A19649B_10077 Test Facility Wildlife International Ltd. GLP Unpublished	N	Y	Data for first approval <u>Used as supporting information in risk assessment</u>	SYN	N
KCA 8.6.2	[REDACTED]	2015b	SYN545974 SC (A19649B) - Toxicity Effects on the Seedling Emergence of Ten Species of Plants Report No. 528P-115 Document No. VV-412594 , A19649B_10105 Test Facility Wildlife International Ltd. GLP Unpublished	N	Y	Data for first approval <u>Used as supporting information in risk assessment</u>	SYN	N