FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: **CHR/F/PYRA 250 EC**

Product name(s): **Etiuda 250 EC, Fermata 250 EC**

Chemical active substance:

Pyraclostrobin, 250 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Innvigo Sp. z o.o.

Submission date: October 2021

Update: July 2022

MS Finalisation date: 15/12/2022

Version history

When	What
October 2021	dRR submitted by applicant to the Polish Ministry of Agriculture and Rural Development
December 2021	Submission to the evaluation
July 2022	First update of dRR on request of zRMS (marked in yellow)
September 2022	zRMS evaluation of dRR
December 2022	Final version prepared by zRMS after Commenting period

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9 Ecotoxicology (KCP 10)

Review Comments:
This document describes the acceptable use conditions required for registration of CHR/F/PYRA 250 EC, an emulsifiable concentration (EC) formulation containing pyraclostrobin 250 g/L, for use as a fungicide in cereals.
This Part B document only reviews data and additional information that has not previously been considered within the EU review process.
Since this document is based on the information provided by the applicant, all review comments, additions and corrections have been made using commenting boxes or highlighted in grey. Any incorrect data or text not evaluated by the zRMS has been crossed out.

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	15	11	12	13	14	15	16	17	18	19	20	21
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha (f)	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	PL	Winter wheat (TRZAW)	F	*Mycosphaerella graminicola*, *Fusarium avenaceum*, *Pyrenophora tritici*, *Puccinia striiformis*, *Blumeria graminis*, *Phaeosphaeria nodorum*, *Puccinia recondita*	Spray, medium sprayer	Spring BBCH 25-69	a) 2 b) 2	21	a) 1 l/ha b) 2 l/ha	a) 0.25 kg a.s./ha b) 0.5 kg a.s/ha	100-400	35								
2	PL	Winter triticale (TTLWI)	F	*Rhynchosporium secalis*, *Blumeria graminis*, *Pyrenophora tritici-repentis* , *Mycosphaerella graminicola*, *Fusarium avenaceum*	Spray, medium sprayer	Spring BBCH 25-69	a) 2 b) 2	21	a) 1 l/ha b) 2 l/ha	a) 0.25 kg a.s./ha b) 0.5 kg a.s/ha	100-400	35								

1	2	3	4	5	6	7	8	9	15	11	12	13	14	15	16	17	18	19	20	21
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha (f)	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
3	PL	Winter rye (SECCW)	F	*Rhynchosporium secalis, Puccinia recondita, Mycosphaerella graminicola, Blumeria graminis*	Spray, medium sprayer	Spring BBCH 25-69	a) 2 b) 2	21	a) 1 l/ha b) 2 l/ha	a) 0.25 kg a.s/ha b) 0.5 kg a.s/ha	100-400	35								
4	PL	Spring barley (HORVS)	F	*Pyrenophora teres, Blumeria graminis, Rhynchosporium secalis, Cochliobolus sativus, Puccinia hordei*	Spray, medium sprayer	Spring BBCH 25-59	a) 2 b) 2	21	a) 1 l/ha b) 2 l/ha	a) 0.25 kg a.s/ha b) 0.5 kg a.s/ha	100-400	35								
Interzonal uses (use as seed treatment, in greenhouses (or other closed places of plant production), as post-harvest treatment or for treatment of empty storage rooms)																				
1																				
2																				
Minor uses according to Article 51 (zonal uses)																				
1	PL	Spring Rye (SECCS)	F	*Rhynchosporium secalis, Puccinia recondita, Mycosphaerella graminicola , Blumeria graminis, Phaeosphaeria*	Spray, medium sprayer	Spring BBCH 25-69	a) 2 b) 2	21	a) 1 l/ha b) 2 l/ha	a) 0.25 kg a.s/ha b) 0.5 kg a.s/ha	100-400	35								

1	2	3	4	5	6	7	8	9	15	11	12	13	14	15	16	17	18	19	20	21
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha (f)	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
				nodorum																
2																				
Minor uses according to Article 51 (interzonal uses)																				
1																				
2																				

Remarks table heading:

(a) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(b) Catalogue of pesticide formulation types and international coding system CropLife International Technical Monograph n°2, 6th Edition Revised May 2008
(c) g/kg or g/l
(d) Select relevant
(e) Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1
(f) No authorization possible for uses where the line is highlighted in grey, Use should be crossed out when the notifier no longer supports this use.

Remarks columns:

1 Numeration necessary to allow references
2 Use official codes/nomenclatures of EU Member States
3 For crops, the EU and Codex classifications (both) should be used; when relevant, the use situation should be described (e.g. fumigation of a structure)
4 F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
5 Scientific names and EPPO-Codes of target pests/diseases/ weeds or, when relevant, the common names of the pest groups (e.g. biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named.
6 Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated.
7 Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
8 The maximum number of application possible under practical conditions of use must be provided.
9 Minimum interval (in days) between applications of the same product
10 For specific uses other specifications might be possible, e.g.: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products.
11 The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
12 If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under “application: method/kind”.
13 PHI - minimum pre-harvest interval
14 Remarks may include: Extent of use/economic importance/restrictions

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1.

** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application

Column 15: zRMS conclusion.

A	Acceptable
R	Acceptable with further restriction
C	To be confirmed by cMS
N	Not acceptable / evaluation not possible
n.r.	Not relevant for section 3

New and additional information is highlighted in yellow.

9.1.1 Overall conclusions

9.1.1.1 Effects on birds (KCP 10.1.1), Effects on terrestrial vertebrates other than birds (KCP 10.1.2), Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

CHR/F/PYRA 250 EC pose no unacceptable risk to birds and mammals while used according to the label.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

The product CHR/F/PYRA 250 EC is classified as **Aquatic Acute 1, H400** and **Aquatic Chronic 1, H410.**
Based on the predicted rates of CHR/F/PYRA 250 EC, the TER values describing the risk for aquatic species following exposure to CHR/F/PYRA 250 EC according to the GAP of the formulation CHR/F/PYRA 250 EC achieve the acceptability criteria when using 20 m no-spray buffer zone and 20 m vegetative filter strip and 50 % nozzle reduction.

9.1.1.3 Effects on bees (KCP 10.3.1)

CHR/F/PYRA 250 EC pose no unacceptable risk to bees according to the label.

The evaluation of the acute risk for bees 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). Based on the submitted risk assessment it can be concluded that there will be negligible acute risk associated with the exposure of *Apis mellifera* to CHR/F/PYRA 250 EC.

The data requirements in accordance with Commission Regulation (EU) No 284/2013 for the chronic toxicity to adult honeybees and honeybee larvae are fulfilled.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

~~The risk for non-target arthropods while using CHR/F/PYRA 250 EC is acceptable with the use of 75% drift reduction and 1m of no-spray buffer strip or 5m of no-spray buffer strip without drift reduction.~~

Based on the results of the conducted risk assessments it can be concluded that low risk for non-target arthropods is expected from the use of CHR/F/PYRA 250 EC according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats. No mitigation measures are required.

9.1.1.5 Effects on non-target soil meso- and macrofauna (KCP 10.4), Effects on soil microbial activity (KCP 10.5)

CHR/F/PYRA 250 EC pose no unacceptable risk to non-target soil meso- and macrofauna and microbial activity according to the label.

9.1.1.6 Effects on non-target terrestrial plants (KCP 10.6)

CHR/F/PYRA 250 EC pose no unacceptable risk to non-target terrestrial plants according to the label with appropriate buffer zone.

9.1.1.7 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

Not relevant

9.1.2 Grouping of intended uses for risk assessment

The following table documents the grouping of the intended uses to support application of the risk envelope approach (according to *SANCO/11244/2011*).

Table 9.1-2: Critical use pattern of CHR/F/PYRA 250 EC grouped according to soil

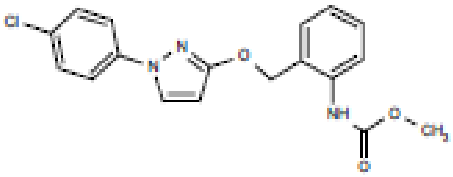
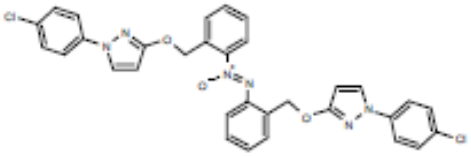
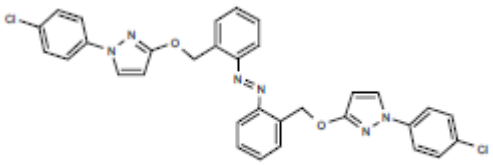
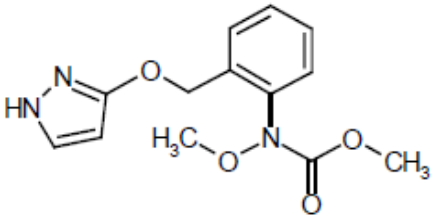
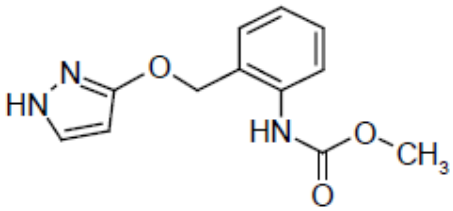
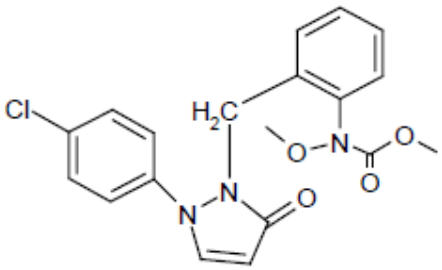
Group	Intended uses	relevant use parameters for grouping	relevant parameter or value
Terrestrial vertebrates (Birds and Mammals; 9.2 and 9.3)	According to GAP	Scenarios according to EFSA Birds and Mammals Guidance (2009) The use in wheat, triticale, barley and rye covered by cereals scenario.	Crop, application rate, timing criterion
Aquatic organisms (**9.5**)	According to GAP	Crops according to FOCUS surface water guidance (2015)[1]	FOCUS modelling**,** for detalis see Part B 8
Bees (**9.6**)	Generic risk envelope covering all product uses	Risk assessments are based on the maximum application rate.	Maximum application rate
Terrestrial non-target arthropods other than bees (**9.7**)	According to GAP In-field	In-field and off-field risk assessments are based on the maximum application rate for cereals.	Application rate and number of uses
	According to GAP Off-field		Crop type (height), application rate and number of uses
Soil meso- and macrofauna / soil microorganisms (**9.8** and **9.9**)	Generic risk envelope covering all product uses	Risk assessments are based on the maximum application rate .	Worst case PECsoil value taken from Section 8 (Environmental Fate)
Non-target terrestrial plants (**9.10**)	According to GAP	Risk assessments are based on the maximum application rate for cereals.	Maximum application rate and worst case drift rate

[1] FOCUS (2015): Generic guidance for FOCUS surface water Scenarios. Version 1.4.

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of CHR/F/PYRA 250 EC is indicated in the table.

Table 9.1-3 Metabolites of Pyraclostrobin

Metabolite	Molar mass	Chemical structure	Maximum observed occurence in compartements	Exposue assessment required due to
BF 500-3 “des-methoxy” 500M07	357		Soil: 95.8 % Water: 2.3 % Sediment: 65.7 %	Sediment
BF 500-6 “azoxy” 500M01	611		Soil: 30.9 % Sediment: 6.5 %	Soil Potential leaching to groundwater Sediment
BF 500-7 “azo” 500M02	596		Soil: 12.5 % Sediment: 6.3 %	Soil Potential leaching to groundwater Sediment
BF 500-11 “M277” 500M60	277		Water: 44.5 % (photolysis study), 11.4 % Sediment: 0.6 %	Surface Water
BF 500-13 “M2427” 500M62	247		Water: 16.8 % (photolysis study), 15.7% Sediment: 2.1 %	Surface Water
BF 500-14 „M387TypeA” 500M76	387		Water: 14.8 % (photolysis study), 11.4% Sediment: 0.7 %	Surface Water

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with Pyraclostrobin. Full details of these studies are provided in the respective EU DAR and related documents.
Effects on birds of CHR/F/PYRA 250 EC were not evaluated as part of the EU assessment of Pyraclostrobin.

Table 9.2-1: Endpoints and effect values relevant for the risk assessment for birds

Species	Substance	Exposure System	Results	Reference
Colinus virginiamus	Pyraclostrobin	Oral 1 d Acute	**LD_{50} >2000 mg a.s./kg bw**	SANCO/1420/2001-Final, 2004
Colinus virginiamus	Pyraclostrobin	Dietary Reproductove toxicity	**NOEL = 105 mg a.s./kg bw/day**	SANCO/1420/2001-Final, 2004
Anas platyrhynchos	Pyraclostrobin	Dietary Reproductive toxicity	NOEL = 128 mg a.s./kg bw/day	SANCO/1420/2001-Final, 2004

9.2.1.1 Justification for new endpoints

No new endpoint is proposed.

9.2.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

9.2.2.1 First-tier assessment (screening/generic focal species)

The results of the acute and reproductive first-tier risk assessments are summarised in the following tables.

Table 9.2-2: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CHR/F/PYRA 250 EC in cereals

Intended use		**Cereals**			
Active substance/product		Pyraclostrobin			
Application rate (g/ha)		2 × 250			
Acute toxicity (mg/kg bw)		LD_{50} = >2000			
TER criterion		10			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening step	Small omnivorous bird	158.8	1.1	43.67	45.8
Reprod. toxicity (mg/kg bw/d)		NOEL = 105			
TER criterion		5			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	~~MAF_{90}~~ **MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening step	Small omnivorous bird	64.8	1.2 x 0.53	10.30	10.2

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

Conclusions

The calculated TER values for Pyraclostrobin are above the trigger value of 10 and 5 at the screening step of the Acute and Reproductive risk assessment.

Effects on metabolites

Risk assessments for terrestrial organisms should also be performed for all major metabolites, i.e. those occurring at levels ≥10% of the parent compound at any one time, found in relevant compartments. The Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) designates 'Plants' as the relevant compartment for considering the relevance of metabolites regarding birds and mammals.

The metabolism of pyraclostrobin in potential food items of wild living birds was investigated in plant metabolism studies. Most metabolites occurred only at trace amounts far below 10% TRR in the potential food items. The only metabolite that occurred at higher levels in potential food items was the BF 500-3. However, this metabolite was also detactable in metabolism in livestock studies. Hence, it can be concluded that the toxicity studies with pyraclostrobin cover this metabolite, and that the dietary risk assessment for pyraclostrobin provided for birds covers the potential risk for BF 500-3.

Review Comments:
According information provided in the Monograph for pyraclostrobin dated 01 August 2001 the potential risk from metabolites to birds and mammals is considered to be covered by the risk assessment for parent compound.

9.2.2.2 Higher-tier risk assessment

No refinement of the first-tier 1 risk assessment is necessary as safe uses for birds were demonstrated.

9.2.2.3 Drinking water exposure

When necessary, the assessment of the risk for birds due to uptake of contaminated drinking water is conducted for a small granivorous bird with a body weight of 15.3 g (*Carduelis cannabina*) and a drinking water uptake rate of 0.46 L/kg bw/d (*cf.* Appendix K of EFSA/2009/1438).

Leaf scenario

Since CHR/F/PYRA EC is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have 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 (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

With a K(f)oc of 9304, Pyraclostrobin belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied (double dose).

Effective application rate (g/ha)	=	~~250~~ 500			
Acute toxicity (mg/kg bw)	=	2000	quotient	=	~~0.125~~ 0.25
Reprod. toxicity (mg/kg bw/d)	=	105	quotient	=	~~2.38~~ 4.76

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of Pyraclostrobin is 3.99 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating birds via secondary poisoning

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

Table 9.2-3: Assessment of the risk for earthworm-eating birds due to exposure to Pyraclostrobin via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals

Parameter	Pyraclostrobin	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	~~0.3697~~ 0.4141	
log P_{ow} / P_{ow}	~~3.99~~ 9772	SANCO/1420/2001-final
Koc	9304	conservative value agreed in PRAPeR 78
foc	0.02	Default
BCF_{worm}	~~0.0394~~ 0.635	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC_{worm}	~~0.0146~~ 0.263	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	~~0.0153~~ 0.276	$DDD = PEC_{worm} \times 1.05$

Parameter	Pyraclostrobin	comments
NOEL (mg/kg bw/d)	105	
TER_{lt}	~~6862.75~~ 380.4	

TER values shown in bold fall below the relevant trigger.

Conclusions

The calculated TER_{lt} value for Pyraclostrobin is above the trigger value of 5 indicating an acceptable risk to earthworm -easting birds via secondary poisoning.

Risk assessment for fish-eating birds via secondary poisoning

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 / is based on the regulatory acceptable concentration for aquatic organisms as a limit value for admissible concentrations of pyraclostrobin in water.

Table 9.2-4: Assessment of the risk for fish-eating birds due to exposure to pyraclostrobin via bioaccumulation in fish (secondary poisoning) for the intended use in sugar beets

Parameter	Pyraclostrobin	comments
$PEC_{sw;max}$ (mg/L)	~~0.00159~~ 0.00218	Step 2 (please refer to B8 section)
BCF_{fish}	736	SANCO/1420/2001-final
BMF	Not relevant	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	~~1.17~~ 1.60	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	~~0.186~~ 0.255	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	105	
TER_{lt}	~~564.52~~ 411.8	

TER values shown in bold fall below the relevant trigger.

Conclusions

The calculated TER_{lt} value for Pyraclostrobin is above the trigger value of 5 indicating an acceptable risk to fish -easting birds via secondary poisoning.

9.2.2.5 Biomagnification in terrestrial food chains

Low potential for accumulation in animal tissue was concluded in the EU review of pyraclostrobin (SANCO/1420/2001-final).

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

Not relevant.

9.2.4 Overall conclusions

Based on the GAP uses intended for CHR/F/PYRA EC, no unacceptable risk from the active substance Pyraclostrobin for birds is expected from acute or long-term exposure to contaminated food. Furthermore, no unacceptable risks are expected arising from residue uptake from drinking water, earthworm-eating and fish-eating birds.

Review Comments:

The acute and chronic risks of CHR/F/PYRA EC to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredient, its metabolites, and maximum residues occurring on food items. No acute toxicity test with the formulation was required.

All TER values exceed the relevant triggers indicating that CHR/F/PYRA EC does not pose an unacceptable risk to birds following applications according to recommended use pattern.

Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. The risk to earthworm- and fish-eating animals from secondary poisoning is low.

9.3 Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with Pyraclostrobin. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of CHR/F/PYRA 250 EC were not evaluated as part of the EU assessment of Pyraclostrobin.

However, the provision of further data on the CHR/F/PYRA 250 EC is not considered essential, because the formulation is comparable with the representative formulations used in the DAR. As in the EU DAR, there is no increased risk from the formulated products (applied diluted as a spray) over that posed from the active substance Pyraclostrobin; the risk assessment for mammals was therefore conducted with Pyraclostrobin endpoints.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.3-1: Endpoints and effect values relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference
Rat	Pyraclostrobin	Oral 1 d Acute	**LD_{50} > 5000 mg a.s./kg b.w**	SANCO/1420/2001-Final, 2004
Rat	Pyraclostrobin	Dietary Reproductive toxcicity Two-generation study	$NOEL_{Reproduction}$ = 32.6 mg a.s./kg b.w./d **$NOEL_{Offspring}$ = 8.2 mg a.s./kg b.w./d** **$NOEL_{Parents}$ = 8.2 mg a.s./kg b.w./d**	SANCO/1420/2001-Final, 2004

Species	Substance	Exposure System	Results	Reference
Rat	Pyraclostrobin	Oral Prenatal Developmental toxicity	$NOEL_{Rel.Maternal}$ = 10 mg a.s./kg b.w./d ~~NOEL~~ $LOAEL_{Rel.Developmental}$ = 50 mg a.s./kg b.w./d	~~SANCO/1420/2001-Final, 2004~~ DAR and Addendu 2, report no. 1999/11511
~~Rat~~ Rabbit	Pyraclostrobin	Oral Prenatal Developmental toxicity	$NOEL_{Rel.Maternal}$ = 3 mg a.s./kg b.w./d $NOEL_{Rel.Developmental}$ = 5 mg a.s./kg b.w./d	SANCO/1420/2001-Final, 2004

9.3.1.1 Justification for new endpoints

No new endpoints were established.

9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Mammals and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).
To achieve a concise risk assessment, the risk envelope approach is applied. The results of the acute and reproductive first-tier risk assessments are summarised in the following tables.

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CHR/F/PYRA 250 EC in cereals

Intended use	**Cereals**				
Active substance/product	Pyraclostrobin				
Application rate (g/ha)	2 × 250				
Acute toxicity (mg/kg bw)	LD_{50} = >5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening Step	Small herbivorous mammal	118.4	1.1	32.56	153.6
Reprod. toxicity (mg/kg bw/d)	NOEL = 3				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening Step	Small herbivorous mammal	48.3	1.2 x 0.53	7.68	**0.39**
Cereals BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods	1.9	-	-	9.9
Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	21.7	-	-	**0.9**
Cereals BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods	2.3	-	-	8.2
Cereals BBCH 10-29	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods	7.8	-	-	**2.4**
Cereals BBCH 30 - 39	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods	3.9	-	-	**4.8**
Cereals Early (shoots)	Large herbivorous mammal “lagomorph” Grass + cereals 100% cereal shoots	22.3	-	-	**0.8**

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

Conclusions

The calculated TER value for Pyraclostrobin is above the trigger value of 10 at the screening step of the Acute risk assessment.

The calculated TER value for Pyraclostrobin exceeds the trigger value of 5 in the reproductive risk assessment, therefore further tier risk assessment is presented.

Effects on metabolites

Risk assessments for terrestrial organisms should also be performed for all major metabolites, i.e. those occurring at levels ≥10% of the parent compound at any one time, found in relevant compartments. The Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) designates 'Plants' as the relevant compartment for considering the relevance of metabolites regarding birds and mammals.

The metabolism of pyraclostrobin in potential food items of wild living mammals was investigated in plant metabolism studies. Most metabolites occurred only at trace amounts far below 10% TRR in the potential food items. The only metabolite that occurred at higher levels in potential food items was the BF 500-3. However, this metabolite was also detactable in metabolism in livestock studies. Hence, it can be concluded that the mammalian toxicity studies with pyraclostrobin cover this metabolite, and that the dietary risk assessment for pyraclostrobin provided for mammals covers the potential risk for BF 500-3.

Review Comments:
According information provided in the Monograph for pyraclostrobin dated 01 August 2001 the potential risk from metabolites to birds and mammals is considered to be covered by the risk assessment for parent compound.

9.3.2.1 Higher-tier risk assessment

According to Document L-CP, Section 1 attached to RAR of Pyraclostrobin 2018 detailed analysis of the prenatal toxicity studies with rabbits was provided. The following main results and arguments are listed below:

The available database comprises one multi-generation study conducted with rats, one prenatal toxicity study conducted with rats, and two prenatal toxicity studies conducted with rabbits. According to the proposal of EFSA 2009/1438 for the first tier risk assessment the lowest toxicity value of these studies, i.e. the NOAEL = 3 mg/kg b.w./d derived from the prenatal toxicity studies conducted with rabbits, is used for TER calculations. For higher tier risk assessment EFSA/2009/1438 recommends a re-examination of the study data to derive an endpoint of biological and/or ecological relevance.
A transient decrease in maternal food consumption during treatment days 1-5 in the rabbit developmental studies was followed by a clear recovery of maternal food consumption till the end of the treatment period. The transient variations in food consumption observed during the first days of treatment did not translate into effects on body weight for animals dosed with up to and including 10 mg/kg bw/d pyraclostrobin.
In respect of overall reproductive outcome up to and including doses of 10 mg/kg bw/d, the number of live, male and female fetuses was not affected. The % of live fetuses per implant was not altered, and no effects were seen on the sex ratio of fetuses. Furthermore, no dead fetuses were reported at any dose rate.
In respect of parameters for dams, there was no statistically significant difference in the number of corpora lutea, the number of implantation sites, the rate of pre-implantation loss, and the number of late resorptions between all dose groups, the concurrent controls, and the set of historical control data.
A slight increase in early resorptions and consequently a higher rate of post-implantation loss was

observed at the high doses. However, up to and including doses of 10 mg/kg bw/d slight differences in early resorptions and post-implantation loss were not of any significance and did not translate into effects on the overall number of live fetuses and the % of live fetuses per implant.
Minor changes on the fetal body weights at doses of 10 mg/kg bw/d and above were not of statistical significance as compared to the concurrent controls at any dose rate.
In conclusion, no effect was seen on the overall reproductive success, population relevant parameters, and potential fitness of the offspring, and hence no impact on size and stability of wild mammal populations is to be expected. Consequently, the ecotoxicologically relevant endpoint from the rabbit prenatal toxicity studies is the NOAEL = 10 mg/kg bw/d.
Further, the different types of toxicity studies (multi-generation, prenatal toxicity) were compared regarding their general relevance for the route and conditions of exposure to wild mammals in the field. The multi-generation study was found to reflect best the route of exposure (dietary), duration (several generations continuously exposed to treated food) and endpoints (development, survival and, most important for the reproduction risk, reproductive endpoints) relevant for small mammals in the wild. The lowest endpoint from the multigeneration study with rats is the NOEL = 8.2. mg/kg b.w./d.
Finally, in order to propose an overall ecologically relevant endpoint for higher tier risk assessment, the specific endpoints from the available studies were compared: The relevant endpoint from the multi-generation study with rats is the NOEL = 8.2 mg/kg b.w./d, and hence well in range with the endpoint from the prenatal toxicity study with rat (NOEL = 10 mg/kg b.w./d). The presented analysis supports furthermore for the prenatal toxicity study in rabbit a NOAEL = 10 mg/kg bw/d as ecotoxicological relevant endpoint.

Conclusion

Based on the detailed analysis of the prenatal toxicity studies with rabbits, considerations on the most relevant study type, and the comparison of the ecotoxicologically relevant endpoints from the different available studies, a conservative value for the refined wild mammal risk assessment for pyraclostrobin the NOAEL=8.2 mg/kg b.w./d from the multi-generation study with rats should be used as a risk refinement.

Review Comments:

There is no ecotoxicological EU agreed endpoint as this section of the Review Report has been left blank. In toxicological part of LoEP several endpoinds concerning reproductive toxicity of pyraclostrobin to mammals were presented. The endpint of 75 ppm was the value selected by the RMS - Germany and was considered acceptable to use for the risk assessment by ECCO 126 Peer Review Meeting (7038/ECCO/PSD/02 18 June 2002). Moreover, the NOAEL of 8.2 mg a.s./kg bw/d (75 ppm) was accepted by most of central zone MS for authorization PPP with pyraclostrobin. In zRMS opinion the NOAEL of 8.2 mg a.s/kg bw/d seems to be reasonable to be used in the higher tier risk assessment.

Table 9.3-3: Higher-tier assessment of the long-term/reproductive risk for ma-mals due to the use of CHR/F/PYRA 250 EC in cereals

Intended use	**Cereals**				
Active substance/product	Pyraclostrobin				
Application rate (g/ha)	2 × 250				
Reprod. toxicity (mg/kg bw/d)	NOEL = **8.2**				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening Step	Small herbivorous mammal	48.3	1.2 x 0.53	7.68	**1.07**
Cereals BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception	1.9	-	-	27.1

	100% ground arthropods				
Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	21.7	-	-	**2.4**
Cereals BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods	2.3	-	-	22.4
Cereals BBCH 10-29	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods	7.8	-	-	6.6
Cereals BBCH 30 - 39	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods	3.9	-	-	13.2
Cereals Early (shoots)	Large herbivorous mammal “lagomorph” Grass + cereals 100% cereal shoots	22.3	-	-	**2.3**

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

The risk for *vole* and *lagomorph* is still unacceptable, therefore dissipation study was provided and described in B7 Section of this dRR. Considering the highest DT_{50} value of 3.71d redefined f_{TWA} value were estimated using following equation:

$f_{twa} = (1 – e^{-kt})/ kt$, where:

f_{twa} – time weighted average factor
n – number of applications
$k = ln(n)/DT_{50}$
t – averaging time (21 days)

f_{twa} =0.25

Studies were fully described in Section B7 of the dRR, but please find below a brief summary of the results originating from the study.
The objective of the studies was the determination of residues of pyraclostrobin and residue degradation time (DT_{50}) in winter wheat after one spray application of CHR/F/PYRA 250 EC.
4 trails was established in :

- Northern France (study number 21SGS39)
- Hungary (study number 21SGS40)
- Germany (study number 21SGS41)
- Poland (study number 21SGS42)

There were one typical for fungicide applications of CHR/F/PYRA 250 EC (batch number 04/2020).The target dose rate of the test item according to study plan was 1,0 l/ha, equivalent to 250 g a. s./ha and target water volume 100-400 l/ha according to Good Agricultural Practice. Applications were performed at BBCH 25 (foliar), only trial 21SGS39 (France) at BBCH 29.

Review Comments:

Normally a DT_{50} of 10 days is assumed in the birds and mammals risk assessment as a default value. For this product CHR/F/PYRA 250 EC however, a lower DT_{50} could be expected based on four plant residue trials that were conducted in Europe (see dRR Part B5 and B7 for a description of these studies).

The residue trials were performed in three Central Zone countries (Germany, Hungary and Poland) as well as in one Southern Zone country (Northern France). As the environmental conditions during the tests duration were comparable in all countries, thus the results from France, in zRMS opinion, can be included in the overall analysis. No rain occurred within a minimum of 3 hours after application (no further data were given).

Due to expected rapid decline of pyraclostrobin, the samplings were carried out at 0, then 2, 4, 8, 12, 24, 48, 72, 96, 120 and 144 hours after the application. The sampling schedule gave 11 data points for each trial, which is sufficient to perform the reliable kinetic analysis.

A kinetic analysis of the dissipation of pyraclostrobin in winter wheat were conducted by SGS Polska Sp. z o. o. and the reports of those studies were submitted to support this refinement of the DT_{50} of pyraclostrobin on monocots (Wańczyk, 2022).

FOCUS (2006, 2014) degradation kinetics guidance was applied to calculate DT_{50} endpoints for pyraclostrobin modelling from residues measured in four plant residue trials in Europe. The data were described reasonably well by either SFO, DFOP, HS kinetics and bi-phasic FOMC kinetics and acceptable endpoints were derived for all studies.

The calculated DT_{50} values and statistics for the decline of pyraclostrobin in cereals are shown in the table below. The final DT_{50} recommended for modelling is the highest DT_{50} value of 3.71 days. This leads to a new TWA of 0.25 and MAF of 1.02.

Kinetic evaluation

On the basis of the obtained pyraclostrobin residues in wheat samples, DT50 value was determined. For this purpose CAKE (Computer Assisted Kinetic Evaluation, version 3.3) program, following single first-order kinetics (SFO) was used. Residue decay is described by:

$$c = c_0 e^{-kt} \qquad \frac{dC}{dt} = -kC$$

, where:

c – *concentration at time t*

c_0 – *initial concentration*

k – *rate constant*

t – *time*

Single first-order kinetics (SFO) is a simple exponential equation with only two parameters. The rate of the change in pesticide concentration (dC/dt) is at any time directly proportional to the actual concentration remaining in the system. For SFO kinetics, the time for a decrease in the concentration by a certain percentage is constant throughout the experiment and independent of the initial concentration of the pesticide.

Determined value of DT_{50}

Location	Study number	Trial number	DT_{50} [h]	DT_{50} [days]	Error [%]
Northern France	21SGS39	21SGS39-01	89.1	3.71	9.91
Hungary	21SGS40	21SGS40-01	59.8	2.49	7.19
Germany	21SGS41	21SGS41-01	45.7	1.90	12.9
Poland	21SGS42	21SGS42-01	78.7	3.28	5.52

Conclusions

The objective of the studies was the determination of residues of pyraclostrobin and residue degradation time (DT_{50}) in winter wheat after one spray application of CHR/F/PYRA 250 EC in four location (Northern France, Hungary, Germany, Poland). Obtained DT50 value was between 45.7 h for Germany and 89.1 h for Northern France.

~~Vole- 100% grass ≥ 40 BBCH~~

~~Food type~~	~~FIR~~	~~PD~~	~~PT~~	~~RUD~~	~~MAF~~	~~21-d twa~~	~~DF~~	~~App. Rate~~	~~DDD~~
~~Grass~~	~~1.33~~	~~1~~	~~1~~	~~54.2~~	~~1.2~~	~~0.25~~	~~0.3~~	~~0.25~~	~~1.62~~
								~~Toxicity endpoint~~	~~8.2~~
								~~TER~~	~~5.06~~

~~Logomorph- 100% cereal shoots- cereals early(schoots)~~

~~Food type~~	~~FIR~~	~~PD~~	~~PT~~	~~RUD~~	~~MAF~~	~~21-d twa~~	~~DF~~	~~App. Rate~~	~~DDD~~
~~Non-grass herbs~~	~~0.41~~	~~1~~	~~1~~	~~54.2~~	~~1.2~~	~~0.25~~	~~1~~	~~0.25~~	~~1.67~~
								~~Toxicity endpoint~~	~~8.2~~
								~~TER~~	~~4.91~~

~~The risk for logomorph is still unacceptable, therefore~~ The redefined MAF value was estimated based on dissipation study was provided and described in B7 Section of this dRR and interval between applications.

$$MAF_m = \frac{1 - e^{-nki}}{1 - e^{-ki}}$$, where:

k = ln(2)/DT50 (rate constant)
n = number of applications
i = application interval (d)
MAF= 1.02

~~Logomorph- 100% cereal shoots- cereals~~

~~early(schoots)~~

~~Food type~~	~~FIR~~	~~PD~~	~~PT~~	~~RUD~~	~~MAF~~	~~21-d twa~~	~~DF~~	~~App. Rate~~	~~DDD~~
~~Non-grass herbs~~	~~0.41~~	~~1~~	~~1~~	~~54.2~~	~~1.02~~	~~0.25~~	~~1~~	~~0.25~~	~~1.42~~
								~~**Toxicity endpoint**~~	~~**8.2**~~
								~~**TER**~~	~~5.77~~

Intended use	**Cereals**
Active substance/product	Pyraclostrobin
Application rate (g/ha)	2 × 250
Reprod. toxicity (mg/kg bw/d)	NOEL = 8.2
TER criterion	5

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	21.7	1.02 x 0.25	1.38	5.9
Cereals Early (shoots)	Large herbivorous mammal "lagomorph" Grass + cereals 100% cereal shoots	22.3	1.02 x 0.25	1.42	5.8

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

~~To establish a new FIR/bw factor the date form "The Impact of the Common Vole on the Vegetation of Agroecosystems" (J. Truszkowski; Acta Theriologica Vol. 27, 23: 305-345, 1982) were taken into account.~~

~~1. The average body weight of a vole from the study was 18.5 g.~~
~~2. For the purpose of the present study the amount of average daily consumption by a vole weighing 18.5 g was taken as 15.0 kcal/individual/day calculated from data contained in the paper by Sawicka-Kapusta et al., (1975).~~
~~3. In relation to the energy value of food consumed, 77.5% is assimilated. The energy value of excreted faeces is 18.7%, urine 3.8% (Drożdż, 1968).~~
~~4. The caloria value of 1 gm of dry mass of lucterne shoots Wasi taken as 4.4 kcal, rape 4.2 kcal, rye 4.1 kcal (after estimates by Sawicka-Kapusta et al., 975; Kukielska). On the basis of these data the average caloricvalue of the mass of upper parts of plants which form the basic food of the voles examined was taken as 4.2 kcal/g of dry mass.~~
~~The latest data about energy content show that voles eat less than 4 g/day (15.0 kcal/individual/day / 4.1 kcal = 3.66).~~

~~According to the "COMMON VOLE (*MICROTUS ARVALIS*) ECOLOGY AND MANAGEMENT: IMPLICATIONS FOR RISK ASSESSMENT OF PLANT PROTECTION PRODUCTS" Jacob, J., Manson, P., Barfknecht, R., Fredricks, T. (2014): "*The selected habitat needs to provide food to satisfy the energy budgets for maintaining essential body functions including reproduction as well as providing shelter from predators. Food uptake is dependent on ambient temperature and can vary between 4.4 g/day/adult vole (at 27°C) and 7.8 g/day/adult vole (at 0°C) 21. For instance, twice as much alfalfa is consumed at 5°C versus 22°C (ca. 5 g versus 2.5 g)*".~~

Based on food uptake indicated in Jacob J., Manson P. (2014) and a bodyweight of 18.5 g (Truszyński (1982)), FIR/bw can conservatively be calculated to 0.42 (7.8 g/day/adult vole / 18.5g = 0.42).

Vole 100% grass ≥ 40 BBCH

Food type	FIR	PD	PT	RUD	MAF	21 d twa	DF	App. Rate	DDD
Grass	0.42	1	1	54.2	1.2	0.53	0.3	0.25	1.09
								Toxicity endpoint	**8.2**
								TER	7.52

Furthermore, although voles are listed as relevant focal species, it is widely acknowledged that voles are not relevant for arable crops and orchards.

- Gurney, *et al.* (1998) reports the feeding habit of field voles (*Microusagrestis*) to be mainly rough, ungrazed grassland, including thick grass ground cover. In a two year study of small mammals on Scottish arable land and set aside (Rodgers 19931) 159 field voles were caught, which were reported to have an almost exclusive preference for rough grassland and were completely absent from the wood and also infrequent in set aside and crops.
- In a three year study of small mammals on an arable farm in Oxfordshire Tew (19941) failed to capture any field voles away from hedgerows around cereal fields. In the Boxworth project, field voles were occasionally caught in the fields but this was restricted to areas with dense ground cover, such as patches infested with blackgrass (Johnson *et al*., 19921).
- No data are available from radio tracking studies for the bank vole or the field vole. Radio tracking has been tried unsuccessfully in both species (Plesner Jensen 1993). Trapping studies have shown that although both species do not use arable fields as main habitat, they are common in hedgerows and woods adjacent to arable fields (Pollard &Relton 1970; Jefferies et al 1973; Green 1979; Loman 1991; Johnson et al. 1992).The preference of the common vole for non cropped areas are discussed in Jacob *et al* (2014), in which it states: "The common vole is primarily a grassland species that is well adapted to steppe habitats. Primary habitats are meadows, set-aside land, flower strips, grassy field verges and alfalfa and clover fields. It prefers to inhabit undisturbed short vegetation and can be found in grass leys in forests after clear cuts and other grassy habitats."

Furthermore, information from DEFRA's research project on "Estimating wildlife exposure to pesticides in crops: additional scenarios and data" (2009) supports the non-relevance of the vole. The aim of this work was to provide further information on use of crops by wildlife by extensive surveying and by review of public literature. The following table taken from this report shows the number of captures of small mammals in the various habitat types.

Table 9.3-4: Captures of small mammals during 11,000 trap events in different agricultural habitats (Table 3 from Report PS2328)

	Captures per 100 trap events						
	Potatoes	**Arable hedge**	**Cereal**	**Sugar beet**	**Other non-crop**	**Orchard hedge**	**Orchard crop**
Field vole	0	0.15	0.08	0	0	1.52	1.31
Pygmy shrew	0.02	0.53	0.23	0	0.34	1.82	0.51
Common shrew	0.38	6.43	1.36	1.00	6.38	3.33	1.85
Bank vole	0.02	6.43	1.44	0	1.55	4.24	0.27
Woodmouse	0.82	8.06	7.04	0.50	2.76	7.88	2.49
Total	1.24	21.6	10.15	1.5	11.03	18.79	6.43
Trap events in this habitat	5020	2630	2570	200	580	330	2970

Results clearly show that wood mice are much more prevalent in arable crops (including potatoes, cereal and sugar beet) than voles. A follow on research project by DEFRA, on "Small mammal activity in soft fruit, cane fruit and top fruit orchards" (2012), focused only on the activity of wood mice as the key focal species. The report stated that "Although a number of species of small mammals occurred in orchards in PS2328 [DEFRA 2009], wood mice were caught in the greatest numbers and are probably at greatest risk from pesticides applied there. They are omnivorous and forage above ground, while shews and voles tend to forage beneath thatch and litter layers".

In contrast to the common vole, the wood mouse is a ubiquitous and euryoecious non-specialist (Tattersall et al., 1997), inhabiting a wide range of landscapes (Montgomery, 1999), and is found in arable habitats throughout the year (e.g. (Tew& Macdonald, 1994); (Loman, 1991); (Green, 1979); (Kikkawa, 1964); (Bergstedt, 1965)). This species has no specific habitat requirements, yet it tends to avoid habitats with a dense herb layer (Braun &Dieterlen, 2005). Tew et al. (2000) suggests that wood mice, due to their bouncing locomotion and granivorous habits, even favour habitats with partly bare ground over which they can easily travel and find fallen seed.

Moreover, EFSA's Bird and Mammal Guidance Document (2009) identifies the European rabbit (*Oryctolagus cuniculus*) as the representative species for large herbivorous mammals. This species is abundant across Europe and may be associated with arable crops. Gurney *et al*. (1998) reports the feeding habitat of the rabbit to be areas of short grass; naturally occurring, dry heaths or closely grazed agricultural pastures with secure refuge nearby. The brown hare (*Lepus europaeus*) is also widespread and abundant across Europe.

Taking all of the above into consideration (high fecundity and population recuperation of the vole; primary source of food outside crops fields for the vole; necessity of population control measures since the vole is considered a crop pest when high population levels are reached; other agricultural techniques being also means of population control), voles are not considered to be a relevant focal species. Therefore, the risk assessment for small herbivorous mammals can be concluded to be acceptable (even with TER lower than the trigger value) if it is acceptable for other small omnivorous mammals (wood mouse and brown hare) which are considered as a relevant focal species.

9.3.2.2 Drinking water exposure

When necessary, the assessment of the risk for mammals due to uptake of contaminated drinking water is conducted for a small omnivorous mammal with a body weight of 21.7 g (*Apodemus sylvaticus*) and a drinking water uptake rate of 0.24 L/kg bw/d (*cf*. Appendix K of EFSA/2009/1438).

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

With a K(f)oc of 9304, Pyraclostrobin belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied (double dose).

Effective application rate (g/ha)	= ~~250~~ 500		
Acute toxicity (mg/kg bw)	= 5000	quotient	= ~~0.05~~ 0.1
Reprod. toxicity (mg/kg bw/d)	= 3	quotient	= ~~83.33~~ 166.7

9.3.2.3 Effects of secondary poisoning

The log P_{ow} of Pyraclostrobin amounts to 3.99 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil (dry soil approach).

Table 9.3-5: Assessment of the risk for earthworm-eating mammals due to exposure to pyraclostrobin via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals

Parameter	Pyraclostrobin	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	~~0.3697~~ 0.4141	
log P_{ow} / P_{ow}	~~0.1506~~ 9772	SANCO/1420/2001-final
Koc	9304	conservative value agreed in PRAPeR 78
foc	0.02	Default
BCF_{worm}	~~0.0394~~ 0.635	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.12 \times P_{ow}) / foc \times Koc$
PEC_{worm}	~~0.015~~ 0.263	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	~~0.0186~~ 0.337	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	3	
$aTER_{lt}$	~~161.29~~ 8.9	

TER values shown in bold fall below the relevant trigger.

Conclusions

The calculated TER_{lt} value for Pyraclostrobin is above the trigger value of 5 indicating an acceptable risk to earthworm -easting birds via secondary poisoning.

Risk assessment for fish-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous mammals is assessed for a mammal of 3000 g body weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water / is based on the regulatory acceptable concentration for aquatic organisms as a limit value for admissible concentrations of pyraclostrobin water.

Table 9.3-3: Assessment of the risk for fish-eating mammals due to exposure to pyraclostrobin via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Pyraclostrobin	comments
$PEC_{sw;max}$ (mg/L)	~~0.00159~~ 0.00218	Step 2 (please refer to B8 section)
BCF_{fish}	736	SANCO/1420/2001-final

BMF	Not relevant	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	~~1.17~~ 1.60	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	~~0.166~~ 0.227	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	3	
TER_{lt}	~~18.072~~ 13.2	

TER values shown in bold fall below the relevant trigger.

Conclusions

The calculated TER_{lt} value for Pyraclostrobin is above the trigger value of 5 indicating an acceptable risk to fish -easting birds via secondary poisoning.

9.3.2.4 Biomagnification in terrestrial food chains

Low potential for accumulation in animal tissue was concluded in the EU review of pyraclostrobin (SANCO/1420/2001-final).

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

Not relevant.

9.3.4 Overall conclusions

Based on the GAP uses intended for CHR/F/PYRA EC, no unacceptable risk from the active substance Pyraclostrobin for mammals is expected from acute or long-term exposure to contaminated food. Furthermore, no unacceptable risks are expected arising from residue uptake from drinking water, earthworm-eating and fish-eating ~~birds~~ mammals.

Review Comments:

The acute and chronic risks of CHR/F/PYRA EC to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredients occurring on food items.

In the screening step all TER_A for pyraclostrobin exceed the trigger value of 10. The TER_{LT} values from the Tier 1 reproductive risk assessment are above the trigger for all scenarios except for the small herbivorous mammal “vole” and for the large herbivorous mammal “lagomorph”. Using a refined reproductive toxicity endpoint and field foliage residue dissipation data (the highest DT_{50} value of 3.71d), the TER_{LT} value for those mammals are above the trigger of 5.

All TER values exceed the relevant triggers indicating that CHR/F/PYRA EC does not pose an unacceptable risk to mammals following applications according to recommended use pattern.

Evaluation of exposing to mammals through the drinking water demonstrated the acceptable risk. The risk to earthworm- and fish-eating animals from secondary poisoning is low.

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

According to the revised data requirements under regulation 1107/2009, the risk to amphibians and

reptiles shall be addressed. Nevertheless, unlike birds and mammals, toxicity tests for amphibian and reptile species are not requested. There is no guidance or validated regulatory protocols yet available: neither on the type of regulatory testing necessary nor how to conduct a risk assessment for amphibian and reptiles. No further test were provided and considered necessary.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with Pyraclostrobin and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of CHR/F/PYRA 250 EC were not evaluated as part of the EU assessment of Pyraclostrobin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Pyraclostrobin and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	Pyraclostrobin	96 h, s	LC_{50} = 0.00616 mg a.s./L $_{mm}$	SANCO/1420/2001-final
Cyprinus carpio	Pyraclostrobin	96 h, s	EC_{50} = 0.0177 mg a.s./L $_{mm}$	SANCO/1420/2001-final
Danio rerio	Pyraclostrobin	96 h, s	LC_{50} = 0.0619 mg a.s./L $_{mm}$	DAR, 2001
Lepomis macrochirus	Pyraclostrobin	96 h, s	LC_{50} = 0.0254 mg a.s./L $_{mm}$	DAR, 2001
Leuciscus idus melanotus	Pyraclostrobin	96 h, s	LC_{50} = 0.0191 mg a.s./L $_{mm}$	DAR, 2001
Oryzias latipes	Pyraclostrobin	96 h, s	LC_{50} = 0.0533 mg a.s./L $_{mm}$	DAR, 2001
Pimephales promelas	Pyraclostrobin	96 h, s	LC_{50} = 0.0161 mg a.s./L $_{mm}$	DAR, 2001
Oncorhynchus mykiss	Pyraclostrobin	28 d, f	NOEC = 0.00464 mg a.s./L $_{mm}$	SANCO/1420/2001-final
Oncorhynchus mykiss	Pyraclostrobin	98 d, f	NOEC = 0.00235 mg a.s./L $_{im}$	SANCO/1420/2001-final
Oncorhynchus mykiss	Pyraclostrobin	97 d, f	NOEC = 0.005 mg a.s./L $_{nom}$	DAR, 2001
L. macrochirus	Pyraclostrobin	BCF	~~379 – 507~~ 675 (chlorophenyl label) 736 (tolyl label)	SANCO/1420/2001-final
Daphnia magna	Pyraclostrobin	48 h, s	EC_{50} = 0.0157 mg a.s./L $_{nom}$	SANCO/1420/2001-final
Daphnia magna	Pyraclostrobin	21 d, ss	NOEC = 0.004 mg a.s./L $_{nom}$	SANCO/1420/2001-final
Chironomus riparius	Pyraclostrobin	28 d, s	NOEC = ~~0.004~~ 0.04 mg a.s./L $_{nom}$	SANCO/1420/2001-final
Pseudokirchneriella subcapitata	Pyraclostrobin	72 h, s	E_rC_{50}> 0.843 mg a.s./L mm E_bC_{50}> 0.148 mg a.s./L mm	SANCO/1420/2001-final
Oncorhynchus mykiss	BF 500-11	96 h, s	LC_{50} > 100 mg/L $_{mm}$	SANCO/1420/2001-final
Oncorhynchus mykiss	BF 500-13	96 h, s	LC_{50} > 50 mg/L $_{nom}$ < 100 mg/L $_{nom}$	SANCO/1420/2001-final
Oncorhynchus mykiss	BF 500-14	96 h, s	LC_{50} > 39.4 mg/L $_{mm}$ < 82.6 mg/L $_{mm}$	SANCO/1420/2001-final
Daphnia magna	BF 500-11	48 h, s	EC_{50}> 100 mg/L $_{nom}$	SANCO/1420/2001-final
Daphnia magna	BF 500-13	48 h, s	EC_{50}> 100 mg/L $_{nom}$	SANCO/1420/2001-final
Daphnia magna	BF 500-14	48 h, s	EC_{50}> 60.9 mg/L $_{nom}$	SANCO/1420/2001-final
Scenedesmus subspicatus	BF 500-11	72 h, s	E_rC_{50}> 100 mg a.s./L $_{nom}$ E_rC_{50}> 100 mg a.s./L $_{nom}$	SANCO/1420/2001-final
Scenedesmus subspicatus	BF 500-13	72 h, s	E_rC_{50}> 100 mg a.s./L $_{nom}$ E_bC_{50}> 66.0 mg a.s./L $_{nom}$	SANCO/1420/2001-final

Species	Substance	Exposure System	Results	Reference
Scenedesmus subspicatus	BF 500-14	72 h, s	E_rC_{50}> 100 mg a.s./L $_{nom}$ E_bC_{50}> 46.6 mg a.s./L $_{nom}$	SANCO/1420/2001-final
Higher-tier studies (micro- or mesocosm studies)				
N/A				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations
Bold figures: endpoints selected for risk assessment

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – CHR/F/PYRA 250 EC

Species	Substance	Exposure System	Results	Reference
Raphidocelis subcapitata	CHR/F/PYRA 250 EC	72 h, s	E_rC_{50} = 2.68 mg/L $_{nom}$ E_yC_{50} = 1.20 mg/L $_{nom}$	Czarnecka, M., 2020 W-24-20, *Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), Growth inhibition test*
Daphnia magna	CHR/F/PYRA 250 EC	48 h, s	EC_{50} = 0.177 mg/L $_{nom}$ NOEC = 0.063 mg/L	Czarnecka, M., 2020 W-23-20, *Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Daphnia magna, Acute Immobilisation Test*
Higher-tier studies (micro- or mesocosm studies)				
No higher tier studies are available				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations

9.5.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).
The relevant global maximum FOCUS Step 1, 2 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.

In the following table, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

The product CHR/F/PYRA 250 EC is classified as **Aquatic Acute 1, H400** and **Aquatic Chronic ~~2~~ 1, H410.**

Table 9.5-3.1: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for pyraclostrobin for each organism group based on FOCUS Steps 1, 2 calculations for the use of CHR/F/PYRA 250 EC

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Chironomus riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	ErC_{50}	NOEC
(µg/L)		6.16	2.35	15.7	4.0	> 843	40
AF		100	10	100	10	10	10
RAC (µg/L)		0.0616	0.235	0.157	0.4	> 84.3	4.0
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	17.03	**276.461**	**72.468**	**108.471**	**42.575**	0.202	**4.258**
Step 2							
N-Europe	2.18	**35.390**	**9.277**	**13.885**	**5.450**	0.026	0.545

Table 9.5-4.1.1: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for pyraclostrobin for *Oncorhynchus mykiss* based on FOCUS Steps 3 calculations for the use of Pyraclostrobin in cereals

Step 3- RAC= 0.0616 µg/L					
Winter cereals					
Scenario FOCUS	Waterbody	Max PEC_{sw} (µg/L)	Dominant entry route	21 d- $PEC_{sw,twa}$ (µg/L)	Max PEC_{sed} (µg/kg)
D1	ditch	**1.391**	drainage	0.487	5.671
D1	stream	**1.169**	drainage	0.014	0.242
D2	ditch	**1.405**	drainage	0.361	5.385
D2	stream	**1.213**	drainage	0.288	3.780
D3	ditch	**1.373**	drainage	0.073	1.356
D4	pond	**0.065**	drainage	0.051	0.710
D4	stream	**1.036**	drainage	0.002	0.048
D5	pond	**0.070**	drainage	0.057	0.703
D5	stream	**1.207**	drainage	0.007	0119
D6	ditch	**1.383**	drainage	0.334	4.090
R1	pond	**0.078**	runoff	0.061	1.001
R1	stream	**0.893**	runoff	0.019	5.652
R3	stream	**1.263**	runoff	0.028	2.676
R4	stream	**0.897**	runoff	0.031	6.648
Spring cereals					
D1	ditch	**1.763**	drainage	0.957	10.21
D1	stream	**1.201**	drainage	0.051	1.031
D3	ditch	**1.373**	drainage	0.075	1.383
D4	pond	**0.070**	drainage	0.056	0.643
D4	stream	**1.152**	drainage	0.010	0.1828
D5	pond	**0.066**	drainage	0.053	0.668
D5	stream	**1.184**	drainage	0.005	0.086
R4	stream	**0.900**	runoff	0.069	7.943

Table 9.5-5.1.2: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for pyraclostrobin for *Oncorhynchus mykiss* based on FOCUS Steps 4 calculations for the use of CHR/F/PYRA 250 EC in cereals

Step 4- RAC= 0.0616 µg/L			
PEC_{sw} (µg/L)	**Scenario**	**Winter cereals**	**Spring cereals**
Nozzle reduction	Vegetative strip (m)	None-20	None-20
	No spray buffer (m)	20	20
None	D1 ditch	**0.09501**	**0.1191**
50 %		0.04749	0.05933
None	D1 stream	**0.1089**	**0.1119**
50 %		0.05442	0.05592
None	D2 ditch	**0.09601**	-
50 %		0.04799	-
None	D2 stream	**0.1130**	-
50 %		0.05649	-
None	D3 ditch	**0.09376**	**0.09378**
50 %		0.04686	0.04687
None	D4 pond	0.02607	0.02820
50 %		0.01300	0.01407
None	D4 stream	**0.09646**	**0.1073**
50 %		0.04821	0.05363
None	D5 pond	0.02812	0.02646
50 %		0.01403	0.01320
None	D5 stream	**0.1124**	**0.1103**
50 %		0.05620	0.05512
None	D6 ditch	**0.09447**	-
50 %		0.04722	-
None	R1 pond	0.02841	Covered by winter cereals.
50 %		0.0156	
None	R1 stream	**0.08320**	Covered by winter cereals.
50 %		0.05981	
None	R3 stream	**0.1177**	-
50 %		0.05884	-
None	R4 stream	**0.1315**	**0.1344**
50 %		**0.1315**	**0.1344**

PEC_{sw} values for pyraclostrobin from STEP 4 are lower than RAC = 0.0616 µg/L (Acute toxicity for fish *O. mykiss*) when using 20 m buffer zone + 20 m vegetative strip and 50 % nozzle reduction beside PEC_{sw} for R4 stream scenario. It is not considered as relevant scenario for Poland, therefore it can be neglected.

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for BF 500-11 for each organism group based on FOCUS Steps 1, 2 calculations for the use of CHR/F/PYRA EC in cereals

Group		**Fish acute**	**Algae**	**Algae**
Test species		*Oncorhynchus mykiss*	*Daphnia magna*	*Scenedesmus subspicatus*
Endpoint (µg/L)		LC_{50} > 100000	EC_{50} > 100000	EC_{50} > 100000
AF		100	100	10
RAC (µg/L)		>1000	>1000	> 10000
FOCUS Scenario	**PEC gl-max (µg/L)**			
Step 1				
	14.71	0.01471	0.01471	0.00147
Step 2				
N-Europe	-	-	-	-

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for BF 500-13 for each organism group based on FOCUS Steps 1, 2 calculations for the use of CHR/F/PYRA EC in cereals

Group		**Fish acute**	**Algae**	**Algae**
Test species		*Oncorhynchus mykiss*	*Daphnia magna*	*Scenedesmus subspicatus*
Endpoint (µg/L)		LC_{50} > 50000 < 100000	EC_{50} > 100000	EC_{50} > 100000
AF		100	100	10
RAC (µg/L)		> 500 < 1000	>1000	> 10000
FOCUS Scenario	**PEC gl-max (µg/L)**			
Step 1				
	19.45	< 0.0389 > 0.01945	0.01945	0.001945
Step 2				
N-Europe	-	-	-	-

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for BF 500-14 for each organism group based on FOCUS Steps 1, 2 calculations for the use of CHR/F/PYRA EC in cereals

Group		**Fish acute**	**Algae**	**Algae**
Test species		*Oncorhynchus mykiss*	*Daphnia magna*	*Scenedesmus subspicatus*
Endpoint (µg/L)		LC_{50} > 39400 < 82600	EC_{50} > 60900	EC_{50} > 100000
AF		100	100	10
RAC (µg/L)		> 394 < 826	>609	> 10000
FOCUS Scenario	**PEC gl-max (µg/L)**			
Step 1				
	20.74	< 0.0494 > 0.0236	0.0319	0.002074
Step 2				
N-Europe	-	-	-	-

The calculated PEC/RAC ratios for the pyraclostrobin metabolites indicate an acceptable risk for all groups of aquatic organism for the intended uses in winter and spring cereals, based on FOCUS Step 1 PEC_{SW} calculations. No further assessment is necessary for the relevant metabolites of pyraclostrobin.

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for CHR/F/PYRA 250 EC for each organism group based on Drift Calculator SWASH MODEL ver 5.3 calculations for the use in winter cereals

Intended use	Winter cereals
Formulation	CHR/F/PYRA 250 EC
Application rate (g[prod]/ha)	2 × 1063.7
Entry into surface water via spraydrift (Drift alculator from SWASH)	
Buffer zone (m)	**PECsw µg prod/L**
1	~~6.4246 (focus ditch)~~ 6.8339
5	1.8524
10	0.9824
Entry into surface water via spray drift (Drift alculator from SWASH)	
Buffer zone (m)	~~**RAC/PEC ratio**~~ **PEC/RAC ratio** **Daphnia magna =EC_{50} ~~1770~~ 177 µg/L** **RAC=~~17.70~~ 1.77 (AF=100)**

1	~~0.3630~~ **3.86**
5	**1.05**
10	0.555
Buffer zone (m)	**RAC/PEC ratio** **Raphidocelis subcapitata ~~=~~ E_rC_{50} = 2680~~0~~ µg/L** **RAC=268~~0~~ (AF=10)**
1	~~0.0024~~ 0.025

9.5.3 Overall conclusions

Based on the predicted rates of CHR/F/PYRA 250 EC, the TER values describing the risk for aquatic species following exposure to CHR/F/PYRA 250 EC according to the GAP of the formulation CHR/F/PYRA 250 EC achieve the acceptability criteria when using 20 m no-spray buffer zone and 20 m vegetative filter strip and 50 % nozzle reduction.

Review Comments:

The relevant predicted environmental concentrations in water (PEC_{sw}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate). The initial risk assessment was based on the worst case PECsw values and the results of laboratory toxicity testing. The PEC_{sw} Step 1-2 (for active substance and its the metabolites) and Step 3 and 4 (for active substance) were used.

CHR/F/PYRA 250 EC applications close to surface water pose acceptable risk to aquatic organisms with appropriate mitigation measures (20 m no-spray buffer zones + 20 m vegetative filter strips + 50% drift reducting technices). The risk assessment for active substance cover the risk from formulation.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with pyraclostrobin. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of CHR/F/PYRA 250 EC were not evaluated as part of the EU assessment of pyraclostrobin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.6-1: Endpoints and effect values relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Pyraclostrobin	Acute oral 48 h	LC_{50} > 73.1 µg a.s./bee	SANCO/1420/2001-final
Apis mellifera	Pyraclostrobin	Acute contact 48 h	LC_{50} > 100.0 µg a.s./bee	SANCO/1420/2001-final

Species	Substance	Exposure System	Results	Reference
Apis mellifera	CHR/F/PYRA 250 EC	Oral	96h LD_{50} = 38.3 µg prod/bee	Stalmach, M., 2020, B-76-20, *Pyraclostrobin 250 EC Honeybees (Apis mellifera L.), Acute Oral Toxicity Test*
Apis mellifera	CHR/F/PYRA 250 EC	Contact	48h LD_{50} > 200.0 µg prod/bee	Stalmach, M., 2020, B-77-20, *Pyraclostrobin 250 EC Honeybees (Apis mellifera L.), Acute Contact Toxicity Test*
Apis mellifera	CHR/F/PYRA 250 EC	Larval 22 d	LD_{10} = 27.9 µg prod/larva bee LD_{20} = 34.1 µg prod/larva bee LD_{50} = 50.1 µg prod/larva bee NOED ≥ 16000 µg prod/larva LC_{10} = 181.1 mg prod/kg of food µg prod/bee LC_{20} = 221.5 mg prod/kg of food µg prod/bee LC_{50} = 325.6 mg prod/kg of food µg prod/bee NOEC ≥ 104000 mg prod/kg of food	Orzechowska, U., 2020, 0038/0012/E *Chronic Toxicity Test for Honey Bee Larvae according to OECD GD 239*
Apis mellifera	CHR/F/PYRA 250 EC	Chronic 10 d	LDD_{10} = 6.8 µg prod/bee/day LDD_{20} = 8.6 µg prod/bee/day LDD_{50} = 13.5 µg prod/bee/day NOEDD = 4.6 µg prod/bee/day LC_{10} = 220.4 mg prod/kg of food µg prod/bee LC_{20} = 275.1 mg prod/kg of food µg prod/bee LC_{50} = 420.4 mg prod/kg of food µg prod/bee NOEC = 171.5 mg prod/kg of food	Orzechowska, U., 2020, 0038/0014/E, *Honey Bee, Chronic Oral Toxicity Test according to OECD 245 Guideline*
Higher-tier studies (tunnel test, field studies)				
N/A				

9.6.2 Risk assessment

The evaluation of the risk for bees 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).

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of CHR/F/PYRA 250 EC in cereals

Intended use	Cereals		
Product	CHR/F/PYRA 250 EC		
Application rate (g/ha)	2 × 1063.7		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	38.3 µg prod/bee	1063.7	27.77
Contact toxicity	> 200.0 µg prod/bee		5.32

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

9.6.2.2 Higher-tier risk assessment for bees (tunnel test, field studies)

Not relevant.

9.6.3 Effects on bumble bees

Not available

9.6.4 Effects on solitary bees

Not available

9.6.5 Overall conclusions

All hazard quotients (HQ) are considerably less than 50, indicating that CHR/F/PYRA 250 EC applied at the maximum use rate in cereals poses low acute oral and contact risk to bees. Calculations were not performed for larvae and chronic toxicity, as there is no guidance explaining how to accomplish such risk assessment at the moment.

Review Comments:

The evaluation of the acute risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002). The submitted risk assessment, based on laboratory studies, has been accepted. It can therefore be concluded that there will be negligible acute risk associated with the exposure of *Apis mellifera* to CHR/F/PYRA 250 EC.

The data requirements in accordance with Commission Regulation (EU) No 284/2013 for the chronic toxicity to adult honeybees and honeybee larvae are fulfilled.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with Pyraclostrobin. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target arthropods of CHR/F/PYRA 250 EC were not evaluated as part of the EU assessment of Pyraclostrobin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods

Species	Substance	Exposure System	Results	Reference
Aphidius rhopalosiphi	CHR/F/PYRA 250 EC	Extended laboratory test barley plants (3D)	LR_{50} > 425 g a.i./ha (>1.7 L/ha) ER_{50} > 425 g a.i./ha (>1.7 L/ha)	Stalmach, M., 2020, B-80-20, *An extended laboratory test for evaluating the effects of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on the parasitic wasp, Aphidius rhopalosiphi (De Stefani-Perez).*
Typhlodromus pyri	CHR/F/PYRA 250 EC	Extended laboratory test disc leaves (2D)	LR_{50} = 132 g a.i./ha (0.528 L/ha) ER_{50} = 100 g a.i./ha (>0.4 L/ha)	Holewik, P., 2020, B-79-20, *An extended laboratory test for evaluating the effects of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on the predatory mite, Typhlodromus pyri (Sch.).*
Coccinella septempunctata	CHR/F/PYRA 250 EC	Extended laboratory test disc leaves (2D)	LR_{50} = 28 g a.i./ha (0.112 L.ha) ER_{50} – > 20 g a.i./ha (>0.08 L.ha) $NOER_{mortality}$ = 20 g a.i./ha (0.08 L.ha)	Knapik, M., 2021, B-78-20, *An extended laboratory test for evaluating effects of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on the ladybird beetle, Coccinella septempunctata (L.)*
Chrysoperla carnea	CHR/F/PYRA 250 EC	Extended laboratory test disc leaves (2D)	LR_{50} = 48 g a.i./ha (0.192 L.ha) ER_{50} – >20 g a.i./ha (>0.08 L.ha) $NOER_{mortality}$ = 1.25 g a.i./ha (0.005 L.ha)	Knapik, M., 2021, B-81-20, *An extended laboratory test for evaluating effects of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on the green lacewing, Chrysoperla carnea (Steph.).*
Higher tier studies- Aged residue study				
Coccinella	CHR/F/PYRA 250 EC	Aged residue	The bioassays evaluating	Vaughan, R., 2021, CHR-21-08,

Species	Substance	Exposure System	Results	Reference
septempunctata		extended laboratory test (2D)	14-day-old and 28-day-old foliar residues, no unacceptable effects were found with respect to the survival, or the subsequent reproductive capacity of the ladybirds when applied at rate 1.0 L product/ha.	*CHR/F/PYRA 250 EC – A series of aged-residue extended laboratory tests to determine effects on the ladybird, Coccinella septempunctata (Coleoptera: Coccinellidae)*

9.7.2 Risk assessment

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)), and in consideration of the recommendations of the guidance document ESCORT 2.

9.7.2.1 Risk assessment for in-field exposure

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 to Table 8.7-6. According to the assessment of environmental fate data, multi-annual accumulation in soil does not need to be considered for Pyraclostrobin.

To achieve a concise risk assessment, the risk envelope approach is applied.

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of CHR/F/PYRA 250 EC

Intended use	**Cereals**		
Active substance/product	**Pyraclostrobin**		
Application rate (g a.s./ha)	250		
MAF	1.7		
Test species Tier II	**LR_{50}/ER_{50} (lab.) (g a.s./ha)**	**$PER_{in\text{-}field}$ (g a.s./ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 1**
Aphidius rhopalosiphi	> 425	425	1.0
Typhlodromus pyri	100		**4.25**
Coccinella septempunctata	20		**21.25**
Chrysoperla carnea	20		**21.25**
Additional study			
N/A			

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DALT: Days after last treatment.
Criteria values shown in bold breach the relevant trigger.

* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

9.7.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied.

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of CHR/F/PYRA 250 EC in cereals

Intended use	**Cereals**
Active substance/product	**Pyraclostrobin**
Application rate (g/ha)	250
MAF	1.7
vdf	10 / 5 (2D) ~~(Tier 1) 0 (Tier 2)~~

Test species Tier II	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off-field}$ (g/ha)**	**CF**	**$HQ_{off-field}$ criterion: HQ ≤ 1**
Aphidius rhopalosiphi	> 425	0.0238	10.115	5	0.119
Typhlodromus pyri	100		1.0115 / 2.023		0.051/0.10
Chrysoperla carnea	20				0.253/0.51
Coccinella septempunctata	20				0.253/0.51

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

9.7.2.3 Additional higher-tier risk assessment

The in-field HQ values for exposure to maximum residues on leaves for *Typhlodromus pyri* and *Coccinella septempunctata* and *Chrysoperla carnea* falls above the ESCORT 2 trigger value, and therefore triggers further assessment. Such assessment was made for CHR/F/PYRA 250 EC with aged residues study for the most sensitive species – the ladybird beetle.

The test item in the study was CHR/F/PYRA 250 EC, an emulsifiable concentrate formulation containing pyraclostrobin (nominally 250 g/L). The aim of this study was to evaluate the effects of both freshly-dried and field-aged foliar residues of the test item on the ladybird beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), under extended laboratory test conditions.

The results of the mortality assessments are summarised below.

Bioassay initiated	Treatment	Test-item rate (L/ha)	% pre-imaginal mortality [a]	Corrected % pre-imaginal mortality [b]
0 DAT	Control	-	0.0	-
	CHR/F/PYRA 250 EC	1.0	97.5 *	97.5
	Toxic reference	-	100 *	100
14 DAT	Control	-	10.0	-
	CHR/F/PYRA 250 EC	1.0	12.5	2.8
28 DAT	Control	-	0.0	-
	CHR/F/PYRA 250 EC	1.0	50.0 *	50.0

a) For each bioassay, pre-imaginal mortality in the test item treatment and the toxic reference treatment was compared to the control using Fisher's exact binomial test (one-sided, > control, $\alpha = 0.05$). An asterisk (*) indicates where differences were significant.

b) Corrected mortalities were calculated using Abbott's formula. A positive value indicates an increase and a negative a decrease.

The results of the reproduction assessments are summarised below.

Bioassay initiated	Treatment	Test-item rate (L/ha)	Mean no. eggs/♀/ day	Mean % egg viability	Mean no. viable eggs/♀/ day
14 DAT	Control	-	28.4	36.8	10.5
	CHR/F/PYRA 250 EC	1.0	27.6	52.9	14.6
28 DAT	Control	-	16.6	56.7	9.4
	CHR/F/PYRA 250 EC	1.0	21.3	29.3	6.3

In the 14 and 28 DAT bioassays, the mean numbers of viable eggs produced in all the treatments evaluated was ≥ 2.0 eggs/female/day. This threshold is currently viewed as being indicative of no harmful treatment effects.

Table 1. Pre-imaginal mortality during larvae of *C. septempunctata* development, in bioassays initiated 0, 14 and 28 days after treatment (DAT).

Bioassay initiated	Treatment	Test item rate (L prod./ha)	% larvae pupating	% pupae emerging as adults	Overall % pre-imaginal mortality [a]	Corrected % pre-imaginal mortality [b]
0 DAT	Control	-	100	100	0.0	-
	CHR/F/PYRA 250 EC	1.0	2.5	2.5	97.5 *	97.5
	Toxic reference	-	0	-	100 *	100
14 DAT	Control	-	90.0	90.0	10.0	-
	CHR/F/PYRA 250 EC	1.0	87.5	87.5	12.5	2.8
28 DAT	Control	-	100	100	0.0	-
	CHR/F/PYRA 250 EC	1.0	50.0	50.0	50.0 *	50.0

a) For each bioassay, pre-imaginal mortality in the test item treatments, and the toxic reference in the 0 DAT bioassay, was compared to the control using Fisher's exact binomial test (one sided, > control, $\alpha = 0.05$). An asterisk (*) indicates where differences were significant.

b) Corrected pre-imaginal mortality calculated using Abbott's formula (Abbott, 1925). A positive value indicates an increase in mortality compared to the control.

The effects of both fresh and aged foliar residues of CHR/F/PYRA 250 EC on the ladybird beetle, *Coccinella septempunctata*, were evaluated under extended laboratory conditions. When applied at a rate equivalent to 1.0 L product/ha, on two occasions with an 8-day interval, fresh residues (0-day-old) of

CHR/F/PYRA 250 EC showed unacceptable effects on the survival of ladybirds. The subsequent bioassays evaluating 14-day-old and 28-day-old foliar residues of CHR/F/PYRA 250 EC, had no unacceptable effects on either the survival, or the subsequent reproductive capacity of the ladybirds.

The off-field HQ values do not exceed the ESCORT 2 trigger value, and therefore not trigger further assessment, which is estimation of risk mitigation measures.

Conclusions

The risk for non-target arthropods while using CHR/F/PYRA 250 EC is acceptable without the use of additional risk mitigation factors.

Review Comments:

Based on the results of the conducted risk assessments it can be concluded that low risk for non-target arthropods is expected from the use of CHR/F/PYRA 250 EC according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats. No mitigation measures are required.

9.8 Effects on non-target soil meso- and macrofauna (KCP 10.4)

9.8.1 Toxicity data

~~Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with Pyraclostrobin containing formulations and relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.~~

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of CHR/F/PYRA 250 EC were not evaluated as part of the EU assessment of Pyraclostrobin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.8-1: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance	Exposure System	Results	Reference
~~*Eisenia fetida*~~	~~Pyraclostrobin~~	~~Mixed into substrate 14 d, acute 10 % peat content~~	~~LC_{50} = 567 mg/kg dry soil $LC_{50,corr}$ = 283* mg/kg dry soil~~	~~SANCO/1420/2001-final~~
~~*Eisenia fetida*~~	~~BF 500-6~~	~~Mixed into substrate 14 d, acute 10 % peat content~~	~~LC_{50} > 1000 mg/kg dry soil $LC_{50,corr}$ > 500* mg/kg dry soil~~	~~SANCO/1420/2001-final~~
~~*Eisenia fetida*~~	~~BF 500-7~~	~~Mixed into substrate 14 d, acute 10 % peat content~~	~~LC_{50} > 1000 mg/kg dry soil $LC_{50,corr}$ > 500* mg/kg dry soil~~	~~SANCO/1420/2001-final~~
Earthworm (*Eisenia andrei)*	CHR/F/PYRA 250 EC	Reproductive toxicity	LC_{50} > 560 mg /kg soil $LC_{50,corr}$ > 280* mg /kg soil	Wróbel, A., 2020, G-32-20, *Pyraclostrobin 250 EC*

Species	Substance	Exposure System	Results	Reference
			EC_{50} = 162.5 mg /kg soil $EC_{50,corr}$ = 81.25* mg/kg soil $NOEC_{rep}$=56 mg/kg dws (13.5 mg a.s./kg dws) $NOEC_{corr}$ = 28* mg/kg dws (6.75 mg a.s./kg dws) EC_{10} = 58.2 mg /kg soil $EC_{10,corr}$ = 29.1* mg/kg soil	*Earthworm Reproduction Test (Eisenia andrei).*
Collembolan (*Folsomia candida*)	CHR/F/PYRA 250 EC	Reproductive toxicity	LC_{50} = 84.5 mg/kg soil $LC_{50,corr}$ = 42.25* mg/kg soil EC_{50} = 64.7 mg /kg soil $EC_{50,corr}$ = 32.35* mg/kg soil $NOEC_{rep}$=32 mg/kg dws $NOEC_{corr}$ = 16* mg/kg dws EC_{10} = 21.8 mg /kg soil (5.3 mg a.s./kg dws) $EC_{10,corr}$ = 10.9* mg/kg soil (2.65 mg a.s./kg dws)	Arendarczyk, A., 2020, G-33-20, *Pyraclostrobin 250 EC Collembolan (Folsomia candida) Reproduction Test.*
Predatory mite *(Hypoaspis (Geolaelaps) aculeifer)*	CHR/F/PYRA 250 EC	Reproductive toxicity	LC_{50} = 350.5 mg /kg soil $LC_{50,corr}$ > 175.25* mg /kg soil EC_{50} = 330.3 mg/kg soil $EC_{50,corr}$ = 165.15* mg/kg soil $NOEC_{rep}$=100 mg /kg dws (24.2 mg a.s./kg dws) $NOEC_{corr}$ = 50* mg/kg dws (12.1 mg a.s./kg dws) EC_{10} = 179.6 mg /kg soil $EC_{10,corr}$ = 89.8* mg/kg soil	Wołany, M., 2020, G-34-20, *Pyraclostrobin 250 EC Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil.*
Field studies				
No field studies are available and necessary				
Litter bag test				
No litter bag studies are available and necessary				

* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

9.8.2 Risk assessment

The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna) 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).

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for pyraclostrobin.

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of CHR/F/PYRA 250 EC in cereals

Intended use			
Acute effects on earthworms			
Product/active substance	**LC_{50} / EC_{50} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_a (criterion TER ≥ 10)**
Pyraclostrobin	$LC_{50,corr}$ = 283* mg/kg dry soil	0.4466	633.68
BF 500-6	$LC_{50,corr}$ > 500* mg/kg dry soil	0.2289	2184.36
BF 500-7	$LC_{50,corr}$ > 500* mg/kg dry soil	0.0902	5543.24
CHR/F/PYRA 250 EC	$LC_{50,corr}$ > 280* mg /kg soil	2.27	123.35
CHR/F/PYRA 250 EC	$EC_{50,corr}$ = 81.25* mg/kg soil	2.27	35.79
Acute effects on collembolan			
Product/active substance	**LC_{50} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_a (criterion TER ≥ 10)**
CHR/F/PYRA 250 EC	$LC_{50,corr}$ = 42.25* mg/kg soil	2.27	18.61
CHR/F/PYRA 250 EC	$EC_{50,corr}$ = 32.35* mg/kg soil	2.27	14.25
Acute effects on Predatory mite			
Product/active substance	**LC_{50} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_a (criterion TER ≥ 10)**
CHR/F/PYRA 250 EC	$LC_{50,corr}$ > 175.25* mg /kg soil	2.27	77.20
CHR/F/PYRA 250 EC	$EC_{50,corr}$ = 165.15* mg/kg soil	2.27	72.75
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
CHR/F/PYRA 250 EC	$NOEC_{corr}$ = 28* mg/kg dws $NOEC_{corr}$ = 6.75* mg a.s./kg dws	2.27 1.135 mg prod/kg dw 0.4713 mg a.s./kg dw	12.33 24.7 14.3
Chronic effects on collembolan			
Product/active substance	**NOEC/ EC_{10} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
CHR/F/PYRA 250 EC	$NOEC_{corr}$ = 16* mg/kg dws $EC_{10,corr}$ = 10.9* mg prod/kg dw $EC_{10,corr}$ = 2.65* mg a.s./kg dw	2.27 1.135 mg prod/kg dw 0.4713 mg a.s./kg dw	7.05 9.6 5.6
Chronic effects on Predatory mite			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**

CHR/F/PYRA 250 EC	$NOEC_{corr}$ = 50* mg/kg dws $NOEC_{corr}$ = 12.1* mg a.s./kg dws	~~2.27~~ 1.135 mg prod/kg dw 0.4713 mg a.s./kg dw	~~22.03~~ 44.1 25.7

TER values shown in bold fall below the relevant trigger.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The acute and long term risk to earthworms and other non-target soil organisms (meso- and macrofauna) was assessed as low for CHR/F/PYRA 250 EC in a first-tier risk assessment.

Review Comments:

The long-term risks of CHR/F/PYRA 250 EC to earthworms, soil meso- and macro-organisms were assessed from toxicity exposure ratios between toxicity endpoints and maximum PEC_{soil}. The relevant predicted environmental concentrations in soil for risk assessments covering the proposed use pattern were taken from Part B Section 8 (Environmental Fate).

Safe use of CHR/F/PYRA 250 EC in cereals was confirmed based on TER_{LT} calculations for formulation.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with pyraclostrobin and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document.

Effects on soil microorganisms of CHR/F/PYRA 250 EC were not evaluated as part of the EU assessment of pyraclostrobin. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.9-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	BF 500-6 BF 500-7	28 d, aerobic loamy sand	Nitrate formation rate BF 500-6: NOEC = 1.0 mg/kg soil dw BF 500-7: NOEC = 0.5 mg/kg soil dw (9.8% effects)	SANCO/1420/2001-final

Endpoint	Substance	Exposure System	Results	Reference
C-mineralisation	BF 500-6 BF 500-7	28 d, aerobic loamy sand	CO_2 formation BF 500-6: NOEC = 1.0 mg/kg soil dw BF 500-7: NOEC = 0.5 mg/kg soil dw (9.8% effects)	SANCO/1420/2001-final
N-mineralisation	CHR/F/PYRA 250 EC	28 d, agricultural soil	NOEC = 11.3 and 56.7 mg prod./kg soil dw NOEC = 2.74 and 13.7 mg a.s./kg soil dw (<25% effects)	Arendarczyk, A., 2020, G-35-20, *Pyraclostrobin 250 EC Soil Microorganisms: Nitrogen Transformation Test.*

9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).
The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of CHR/F/PYRA 250 EC in cereals

Intended use			
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
CHR/F/PYRA 250 EC	NOEC = ~~11.3~~ 56.7 mg prod./kg soil dw NOEC = ~~2.74~~ 13.7 mg a.s./kg soil dw	~~2.27~~ ~~0.4466~~ 1.135 mg prod/kg dw 0.4713 mg a.s./kg dw	Yes
BF 500-6	NOEC = 1.0 mg/kg soil dw	~~0.2289~~ 0.2915	Yes
BF 500-7	NOEC = 0.5 mg/kg soil dw	~~0.0902~~ 0.0865	Yes
C-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
BF 500-6	NOEC = 1.0 mg/kg soil dw	~~0.2289~~ 0.2915	Yes
BF 500-7	NOEC = 0.5 mg/kg soil dw	~~0.0902~~ 0.0865	Yes

9.9.3 Overall conclusions

The Predicted Environmental Concentrations of the formulation CHR/F/PYRA 250 EC, pyraclostrobin and its metabolties in soil are below the concentrations at which no unacceptable effects (< 25%) regarding the soil microbial activity were observed indicating that the proposed use of CHR/F/PYRA 250 EC poses an acceptable risk to soil microorganisms.

Review Comments:

Based on the results of the conducted first tier risk assessment it can be concluded that no risk for soil micro-organisms is expected from use of CHR/F/PYRA 250 EC in cereals.

9.10 Effects on non-target terrestrial plants (KCP 10.6)

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with Pyraclostrobin and the representative EU formulation CHR/F/PYRA 250 EC. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of CHR/F/PYRA 250 EC were not evaluated as part of the EU assessment of Pyraclostrobin. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

Table 9.10-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Species	Substance	Exposure System	Results [g a.s./ha]	Reference
~~Sunflower *(Helianthus annuus)*~~	~~CHR/F/PYRA 250 EC~~	~~21 d Vegetative vigour~~	~~1) ER_{50} emergence > 500.0 g/ha 2) ER_{50} plant weight > 500.0 g/ha 3) ER_{50} plant height > 500.0 g/ha~~	~~Wolany, M., 2020, G-37-20, *Pyraclostrobin 250 EC Terrestial Plant Test: Seedling Emergence and Seedling Growth Test.*~~
~~Cabbage *(Brassica oleracea ver. capitata)*~~	~~CHR/F/PYRA 250 EC~~	~~21 d Vegetative vigour~~	~~1) ER_{50} emergence > 500.0 g/ha 2) ER_{50} plant weight > 500.0 g/ha 3) ER_{50} plant height > 500.0 g/ha~~	~~Wolany, M., 2020, G-37-20, *Pyraclostrobin 250 EC Terrestial Plant Test: Seedling Emergence and Seedling Growth Test.*~~
~~Pea (*Pisum sativum*)~~	~~CHR/F/PYRA 250 EC~~	~~21 d Vegetative vigour~~	~~1) ER_{50} emergence > 500.0 g/ha 2) ER_{50} plant weight > 500.0 g/ha 3) ER_{50} plant height > 500.0 g/ha~~	~~Wolany, M., 2020, G-37-20, *Pyraclostrobin 250 EC Terrestial Plant Test: Seedling Emergence and Seedling Growth Test.*~~

Species	Substance	Exposure System	Results [g a.s./ha]	Reference
Carrot *(Daucus carota)*	CHR/F/PYRA 250 EC	21 d Vegetative vigour	1) ER_{50} emergence > 500.0 g/ha 2) ER_{50} plant weight > 500.0 g/ha 3) ER_{50} plant height > 500.0 g/ha	Wolany, M., 2020, G-37-20, *Pyraclostrobin 250 EC Terrestial Plant Test: Seedling Emergence and Seedling Growth Test.*
Perennial ryegrass (*Lolium perenne*)	CHR/F/PYRA 250 EC	21 d Vegetative vigour	1) ER_{50} emergence > 500.0 g/ha 2) ER_{50} plant weight > 500.0 g/ha 3) ER_{50} plant height > 500.0 g/ha	Wolany, M., 2020, G-37-20, *Pyraclostrobin 250 EC Terrestial Plant Test: Seedling Emergence and Seedling Growth Test.*
Oats *(Avena sativa)*	CHR/F/PYRA 250 EC	21 d Vegetative vigour	1) ER_{50} emergence > 500.0 g/ha 2) ER_{50} plant weight > 500.0 g/ha 3) ER_{50} plant height > 500.0 g/ha	Wolany, M., 2020, G-37-20, *Pyraclostrobin 250 EC Terrestial Plant Test: Seedling Emergence and Seedling Growth Test.*
Sunflower *(Helianthus annuus)*	CHR/F/PYRA 250 EC	14 d Seedling emergence	1) ER_{50} emergence > 500.0 g/ha 2) ER_{50} plant weight > 500.0 g/ha 3) ER_{50} plant height > 500.0 g/ha	Wolany, M., 2020, G-36-20, *Pyraclostrobin 250 EC Terrestial Plant Test: Vegetative Vigour Test.*
Cabbage *(Brassica oleracea ver. capitata)*	CHR/F/PYRA 250 EC	14 d Seedling emergence	1) ER_{50} emergence > 500.0 g/ha 2) ER_{50} plant weight > 500.0 g/ha 3) ER_{50} plant height > 500.0 g/ha	Wolany, M., 2020, G-36-20, *Pyraclostrobin 250 EC Terrestial Plant Test: Vegetative Vigour Test.*
Pea (*Pisum sativum*)	CHR/F/PYRA 250 EC	14 d Seedling emergence	1) ER_{50} emergence > 500.0 g/ha 2) ER_{50} plant weight > 500.0 g/ha 3) ER_{50} plant height > 500.0 g/ha	Wolany, M., 2020, G-36-20, *Pyraclostrobin 250 EC Terrestial Plant Test: Vegetative Vigour Test.*
Carrot *(Daucus carota)*	CHR/F/PYRA 250 EC	14 d Seedling emergence	1) ER_{50} emergence > 500.0 g/ha 2) ER_{50} plant weight > 500.0 g/ha 3) ER_{50} plant height > 500.0 g/ha	Wolany, M., 2020, G-36-20, *Pyraclostrobin 250 EC Terrestial Plant Test: Vegetative Vigour Test.*
Perennial ryegrass (*Lolium perenne*)	CHR/F/PYRA 250 EC	14 d Seedling emergence	1) ER_{50} emergence > 500.0 g/ha 2) ER_{50} plant weight > 500.0 g/ha 3) ER_{50} plant height > 500.0 g/ha	Wolany, M., 2020, G-36-20, *Pyraclostrobin 250 EC Terrestial Plant Test: Vegetative Vigour Test.*
Oats *(Avena sativa)*	CHR/F/PYRA 250 EC	14 d Seedling emergence	1) ER_{50} emergence > 500.0 g/ha 2) ER_{50} plant weight > 500.0 g/ha 3) ER_{50} plant height > 500.0 g/ha	Wolany, M., 2020, G-36-20, *Pyraclostrobin 250 EC Terrestial Plant Test: Vegetative Vigour Test.*

Species	Substance	Exposure System	Results [g a.s./ha]	Reference
Sunflower, cabbage, pea, carrot, perennial ryegrass, oats	CHR/F/PYRA 250 EC	21 d Vegetative vigour	[1] ER_{50} emergence > 513.2 g a.s./ha [2] ER_{50} plant weight > 513.2 g a.s./ha [3] ER_{50} plant height > 513.2 g a.s./ha	Wołany, M., 2020, G-37-20, *Pyraclostrobin 250 EC Terrestial Plant Test: Seedling Emergence and Seedling Growth Test.*
Sunflower, cabbage, pea, carrot, perennial ryegrass, oats	CHR/F/PYRA 250 EC	14 d Seedling emergence	[1] ER_{50} emergence > 513.2 g a.s./ha [2] ER_{50} plant weight > 513.2 g a.s./ha [3] ER_{50} plant height > 513.2 g a.s./ha	Wołany, M., 2020, G-36-20, *Pyraclostrobin 250 EC Terrestial Plant Test: Vegetative Vigour Test.*

m: monocotyledonous; d: dicotyledonous

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

9.10.2.2 Tier-2 risk assessment (based on dose-response data)

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (*SANCO/10329/2002* rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

Table 9.10-2: Assessment of the risk for non-target plants due to the use of CHR/F/PYRA 250 EC

~~Intended use~~ ~~Active substance/product~~ ~~Application rate (g/ha)~~ ~~MAF~~	~~Cereals~~ ~~CHR/F/PYRA 250 EC~~ ~~250~~ ~~1.7~~			
~~Test species~~	~~ER_{50} (g/ha)~~	~~Drift rate~~	~~$PER_{off\text{-}field}$ (g/ha)~~	~~TER criterion: TER ≥ 5~~
~~Sunflower *(Helianthus annuus)* (CHR/F/PYRA 250 EC)~~	~~500~~	~~0.0238~~	~~5.950~~	~~21008.4~~
~~Cabbage *(Brassica oleracea ver. capitata)* (CHR/F/PYRA 250 EC)~~	~~500~~	~~0.0238~~	~~5.950~~	~~21008.4~~
~~Pea (*Pisum sativum*) (CHR/F/PYRA 250 EC)~~	~~500~~	~~0.0238~~	~~5.950~~	~~21008.4~~

~~Carrot (*Daucus carota*) (CHR/F/PYRA 250 EC)~~	~~500~~	~~0.0238~~	~~5.950~~	~~21008.4~~
~~Perennial ryegrass (*Lolium perenne*) (CHR/F/PYRA 250 EC)~~	~~500~~	~~0.0238~~	~~5.950~~	~~21008.4~~
~~Oats (*Avena sativa*) (CHR/F/PYRA 250 EC)~~	~~500~~	~~0.0238~~	~~5.950~~	~~21008.4~~
~~Sunflower (*Helianthus annuus*) (CHR/F/PYRA 250 EC)~~	~~500~~	~~0.0238~~	~~5.950~~	~~21008.4~~
~~Cabbage (*Brassica oleracea ver. capitata*) (CHR/F/PYRA 250 EC)~~	~~500~~	~~0.0238~~	~~5.950~~	~~21008.4~~
~~Pea (*Pisum sativum*) (CHR/F/PYRA 250 EC)~~	~~500~~	~~0.0238~~	~~5.950~~	~~21008.4~~
~~Carrot (*Daucus carota*) (CHR/F/PYRA 250 EC)~~	~~500~~	~~0.0238~~	~~5.950~~	~~21008.4~~
~~Onion (*Allium cepa*) (CHR/F/PYRA 250 EC)~~	~~500~~	~~0.0238~~	~~5.950~~	~~21008.4~~
~~Perennial ryegrass (*Lolium perenne*) (CHR/F/PYRA 250 EC)~~	~~500~~	~~0.0238~~	~~5.950~~	~~21008.4~~

~~MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.~~

Intended use	Cereals			
Active substance/product	CHR/F/PYRA 250 EC			
Application rate (g/ha)	250			
MAF	1.0			
Test species	**ER_{50} (g a.s./ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g a.s./ha)**	**TER criterion: TER ≥ 5**
Sunflower, cabbage, pea, carrot, perennial ryegrass, oats	>513.2	0.0277	6.925	>74.1

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

Not relevant.

9.10.3 Overall conclusions

The TER values describing the risk for non-target plants following exposure to CHR/F/PYRA 250 EC according to the GAP achieve the acceptability criteria TER ≥ 5 without use of any risk mitigations. Default no-spray buffer zone of 1m is sufficient.

9.11 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

No data for effects on any other terrestrial organisms is available and necessary.

9.12 Monitoring data (KCP 10.8)

No data on monitoring is available and necessary.

9.13 Classification and Labelling

Having considered risk to the human health posed by ingredients of the preparation, product is classified as Aquatic Acute 1, H400 and Aquatic Chronic 2, H410.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and 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	Owner
KCP 10.1.2	Wańczyk, K.	2022	*Magnitude of residue and degradation time (DT50) of pyraclostrobin in winter wheat (Raw Agricultural Commodity) after one spray application of CHR/F/PYRA 250 EC in Northern France - 2021,* S. Niewelt, K. Wańczyk, DPL/37/2021, 21SGS39 Amendment 2	N	Chemirol
KCP 10.1.2	Wańczyk, K.	2022	*Magnitude of residue and degradation time (DT50) of pyraclostrobin in winter wheat (Raw Agricultural Commodity) after one spray application of CHR/F/PYRA 250 EC - in Hungary - 2021,* S. Niewelt, K. Wańczyk, DPL/38/2021, 21SGS40 Amendment 2	N	Chemirol
KCP 10.1.2	Wańczyk, K.	2022	*Magnitude of residue and degradation time (DT50) of pyraclostrobin in winter wheat (Raw Agricultural Commodity) after one spray application of CHR/F/PYRA 250 EC - Germany – 2021,* G. Paszek, K. Wańczyk, DPL/39/2021, 21SGS41 Amendment 2	N	Chemirol
KCP 10.1.2	Wańczyk, K.	2022	*Magnitude of residue and degradation time (DT50) of pyraclostrobin in winter wheat (Raw Agricultural Commodity) after one spray application of CHR/F/PYRA 250 EC in Poland - 2021,* M. Jędrusik, K. Wańczyk, DPL/40/2021, 21SGS42 Amendment 2	N	Chemirol
KCP 10.2/01	Czarnecka, M.	2020	*Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), Growth inhibition test* W-24-20 Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Pszczyna, Poland GLP- Yes Unpublished	N	Chemirol
KCP 10.2/02	Czarnecka, M.	2020	*Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Daphnia magna, Acute Immobilisation Test.* W-23-20 Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland	N	Chemirol

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**	**Owner**
			GLP- Yes Unpublished		
KCP 10.3.1/01	Stalmach, M.	2020	*Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Honeybees (Apis mellifera L.), Acute Oral Toxicity Test* B-76-20 Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland GLP- Yes Unpublished	N	Chemirol
KCP 10.3.1/02	Stalmach, M.	2020	*Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Honeybees (Apis mellifera L.), Acute Contact Toxicity Test* B-77-20 Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland GLP- Yes Unpublished	N	Chemirol
KCP 10.3.1/03	Orzechowska, U.	2020	*Chronic Toxicity Test for Honey Bee Larvae according to OECD GD 239.* 0038/0012/E SORBOLAB Research Laboratory LLC, Poznań, Poland GLP- Yes Unpublished	N	Chemirol
KCP 10.3.1/04	Orzechowska, U.	2020	*Chronic Toxicity Test for Honey Bee Larvae according to OECD GD 239.* 0038/0014/E SORBOLAB Research Laboratory LLC, Poznań, Poland GLP- Yes Unpublished	N	Chemirol
KCP 10.3.2/01	Stalmach, M.	2020	*An extended laboratory test for evaluating the effects of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on the parasitic wasp, Aphidius rhopalosiphi (De Stefani-Perez).* B-80-20 Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna,	N	Chemirol

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**	**Owner**
			Poland GLP- Yes Unpublished		
KCP 10.3.2/02	Holewik, P.	2020	*An extended laboratory test for evaluating the effects of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on the predatory mite, Typhlodromus pyri (Sch.).* B-79-20 Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland GLP- Yes Unpublished	N	Chemirol
KCP 10.3.2/03	Knapik, M.	2021	*An extended laboratory test for evaluating effects of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on the ladybird beetle, Coccinella septempunctata (L.)* B-78-20 Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland GLP- Yes Unpublished	N	Chemirol
KCP 10.3.2/04	Knapik, M.	2021	*An extended laboratory test for evaluating effects of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on the green lacewing, Chrysoperla carnea (Steph.).* B-81-20 Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland GLP- Yes Unpublished	N	Chemirol
KCP 10.3.2/05	Vaughan, R.	2021	*CHR/F/PYRA 250 EC – A series of aged-residue extended laboratory tests to determine effects on the ladybird beetle, Coccinella septempunctata (Coleoptera: Coccinellidae)* CHR-21-08 Mambo-Tox, Southampton, UK GLP- Yes	N	Chemirol

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**	**Owner**
			Unpublished		
KCP 10.4/01	Wróbel, A.	2020	*Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Earthworm Reproduction Test (Eisenia andrei).* G-32-20 Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland GLP- Yes Unpublished	N	Chemirol
KCP 10.4/02	Arendarczyk, A.	2020	*Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Collembolan (Folsomia candida) Reproduction Test.* G-33-20 Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland GLP- Yes Unpublished	N	Chemirol
KCP 10.4/03	Wołany, M.	2020	*Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil.* G-34-20 Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland GLP- Yes Unpublished	N	Chemirol
KCP 10.5/01	Arendarczyk, A.	2020	*Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Soil Microorganisms: Nitrogen Transformation Test.* G-35-20 Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland GLP- Yes Unpublished	N	Chemirol
KCP 10.6/01	Wołany, M.	2020	*Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Terrestial Plant Test: Seedling Emergence and Seedling Growth Test.* G-37-20	N	Chemirol

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**	Owner
			Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland GLP- Yes Unpublished		
KCP 10.6/02	Wołany, M.	2020	*Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Terrestial Plant Test: Vegetative Vigour Test.* G-36-20 Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland GLP- Yes Unpublished	N	Chemirol

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

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**	**Owner**
KCP 10.1.1/01	xxxxxxxxxx	1997	*Report BAS 500 F (Reg.No.304 428) – Avian single-dose oral LD50 on the bobwhite quail (Colinus virginianus).* 11W0494/96117 /BAS 97/11136 GLP- Yes Unpublished	Y	BAS
KCP 10.1.1/02	xxxxxxxxx	1998	*Test Report BAS 500 F - Avian dietary LC50 test in chicks of the mallard duck (Anas platyrhynchos L.).* 31W0494/96123/BAS 98/10933 GLP- Yes Unpublished	Y	BAS
KCP 10.1.1/03	xxxxxxxxx	1998	*Test Report BAS 500 F - Avian dietary LC50 test in chicks of the bobwhite quail (Colinus virginianus);.* 31W0494/96126/BAS 98/10932 GLP- Yes Unpublished	Y	BAS
KCP 10.1.1/04	xxxxxxxxxx	1999	*BAS 500 F: A reproduction study with the northern bobwhite.* 145-175 GLP- Yes Unpublished	Y	BAS
KCP 10.1.1/05	xxxxxxxxxx	1999	*BAS 500 F: A reproduction study with the mallard.* 147-176 GLP- Yes Unpublished	Y	BAS
KCP 10.2/01	Dohmen, G. P.	1999	*Effect of BAS 500 F on Daphnia magna Straus in a 48 hours acute toxicity test.* 35806	N	BAS

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**	**Owner**
			GLP- Yes Unpublished		
KCP 10.2/02	xxxxxxxxxxx	1998	*BAS 500 F Acute toxicity study on the common carp (Cyprinus carpio L.) in a static system (96 hours).* 12F0494/965178 GLP- Yes Unpublished	Y	BAS
KCP 10.2/03	xxxxxxxxxxx	1998	*BAS 500 F Acute toxicity study on the bluegill (Lepomis macrochirus Raf.) in a static system (96 hours).* 12F0494/965179 GLP- Yes Unpublished	Y	BAS
KCP 10.2/04	xxxxxxxxxxx	1999	*Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours).* 12F0249/995035 GLP- Yes Unpublished	Y	BAS
KCP 10.2/05	xxxxxxxxxxx	1999	*Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours).* 12F0252/995034 GLP- Yes Unpublished	Y	BAS
KCP 10.2/06	xxxxxxxxxxx	1999	*Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours).* 12F0251/995037 GLP- Yes	Y	BAS

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**	**Owner**
			Unpublished		
KCP 10.2/07	xxxxxxxxxxx	1999	*Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours).* 12F0494/965180 GLP- Yes Unpublished	N	BAS
KCP 10.2/08	xxxxxxxxxxx	1999	*Sublethal toxic effects on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a flow-through system (28 days).* 42F0494/965177 GLP- Yes Unpublished	N	BAS
KCP 10.2/09	xxxxxxxxxxx	2000	*Acute toxicity study on the rainbow trout (Oncorhynchus mykiss)after short time exposure over 0,5, 2 and 8 hours in a flow-through system followed up by a post exposure period.* 12F0494/965190 GLP- Yes Unpublished	N	BAS
KCP 10.2/10	xxxxxxxxxxx	1999	*Early life-stage toxicity test on the rainbow trout (Oncorhynchus mykiss Walbaum 1792).* 52F0494/965141 GLP- Yes Unpublished	N	BAS
KCP 10.2/11	Dohmen, G. P.	1999	*Effects of BAS 500 F on mortality and reproduction of Daphnia magna.* 35811 GLP- Yes Unpublished	N	BAS
KCP 10.2/12	Jatzek	1999	*Determination on the acute effect of BF 500-14 on the swimming ability of the water flea Daphnia magna Straus.*	N	BAS

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**	**Owner**
			99/0519/50/1 GLP- Yes Unpublished		
KCP 10.2/13	Jatzek	1999	*Determination on the acute effect of BF 500-13 on the swimming ability of the water flea Daphnia magna Straus.* 99/0518/50/1 GLP- Yes Unpublished	N	BAS
KCP 10.2/14	Jatzek	1999	*Determination on the acute effect of BF 500-11 on the swimming ability of the water flea Daphnia magna Straus.* 99/0517/50/1 GLP- Yes Unpublished	N	BAS
KCP 10.2/15	Dohmen, G. P.	1999	*Effect of BAS 500 F on the growth of the green algae Pseudokirchneriella subcapitata.* 35803 GLP- Yes Unpublished	N	BAS
KCP 10.2/16	Reuschenbach	1999	*Determination of the inhibitory effect of BF 500-14 on the cell multiplication of unicellular green algae.* 99/0519/60/1 GLP- Yes Unpublished	N	BAS
KCP 10.2/17	Reuschenbach	1999	*Determination of the inhibitory effect of BF 500-13 on the cell multiplication of unicellular green algae.* 99/0518/60/1 GLP- Yes Unpublished	N	BAS

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**	**Owner**
KCP 10.2/18	Reuschenbach	1999	*Determination of the inhibitory effect of BF 500-11 on the cell multiplication of unicellular green algae.* 99/0517/60/1 GLP- Yes Unpublished	N	BAS
KCP 10.2/19	Dohmen, G. P.	2000	*Effects of BAS 500 F on the development of sediment dwelling larvae of Chironomus riparius in a water-sediment system.* 35966 GLP- Yes Unpublished	N	BAS
KCP 10.3	Sack, D.	1999	*Effects of Reg.No. 304 428 on the Honeybee (Apis mellifera L.) in Laboratory Trials.* 11457 GLP- Yes Unpublished	N	BAS
KCP 10.4/01	Ebert, D.	1999	*Investigations on the release of soil-bound residues of 14C-BAS 500 F by earthworms.* 1999/11289 GLP- Yes Unpublished	N	BAS
KCP 10.4/02	Krieg, W.	1999	*Effect of BF 500-7 on the mortality of the earthworm Eisenia foetida.* 1999/11309 GLP- Yes Unpublished	N	BAS
KCP 10.4/03	Krieg, W.	1999	*Effect of BF 500-6 on the mortality of the earthworm Eisenia foetida.* 1999/11308 GLP- Yes Unpublished	N	BAS

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**	**Owner**
KCP 10.4/04	Krieg, W.	1999	*Effect of BAS 500 F on the mortality of the earthworm Eisenia foetida.* 1999/10708 GLP- Yes Unpublished	N	BAS
KCP 10.5	Krieg, W.	1999	*Effect of BF 500-6 and BF 500-7 on the nitrogen turnover in soil.* 1999/11311 GLP- Yes Unpublished	N	BAS

Appendix 2 Detailed evaluation of the new studies

Review Comment:
In order to provide sufficient detail, where appropriate, the following studies summaries have been adapted by the zRMS from the full tests reports provided in the dossier. zRMS text is highlighted in grey. The comments on individual studies are provided in grey comment boxes.

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

No additional studies were performed.

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

No additional studies were performed.

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

No additional studies were performed.

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

No additional studies were performed.

Comments of zRMS:	The calculation of DT_{50}/DT_{90} methodology are considered appropriate. As the error value for all trial is below 15%, the results were considered to be reliable and suitable for the risk assessment. The calculation is considered valid and acceptable for regulatory use.

APPENDIX TO THE AMENDMENT TO FINAL REPORTS 21SGS39,21SGS40,21SGS41,21SGS42

F. SUMMARY DATA FROM TRIALS

Magnitude of residue and degradation time (DT50) of pyraclostrobin in winter wheat (Raw Agricultural Commodity) after one spray application of CHR/F/PYRA 250 EC in Northern France, Hungary, Germany and Poland – 2021

1 STUDY OBJECTIVE

The objective of the studies was the determination of residues of pyraclostrobin and residue degradation time (DT50) in winter wheat after one spray application of CHR/F/PYRA 250 EC.

2 FIED PART

4 trails was established in :

- Northern France (study number 21SGS39)
- Hungary (study number 21SGS40)
- Germany (study number 21SGS41)
- Poland (study number 21SGS42)

Table 1. Identyfication of the field trails

Tral number	Study type	Country (region)	Trail site	Zip code
21SGS39-01	DCS	France (Grand-est)	Bourgogne	51110
21SGS40-01	DCS	Hungry (Borsod-Abaúj-Zemplén)	Monok	H-3905
21SGS41-01	DCS	Germany (Schleswig Holstein)	Fahrdorf	24857
21SGS42-01	DCS	Poland (Kujawsko-Pomorskie)	Cerekwica	88-400

There were one typical for fungicide applications of CHR/F/PYRA 250 EC (batch number 04/2020).The target dose rate of the test item according to study plan was 1,0 l/ha, equivalent

to 250 g a. s./ha and target water volume 100-400 l/ha according to Good Agricultural Practice. Applications were performed at BBCH 25 (foliar), only trial 21SGS39 (France) at BBCH 29.

Table 2. Application data

Trial number	Application date	Actual rate (l/ha)	Actual rate (g a. s./ha)	Deviation (%)	Spray volume applied (l/ha)	Treated area (m²)
21SG39-01	22/03/2021	0,991	247,75	-0,9	247,6	315
21SG40-01	30/03/2021	0,985	246,25	-1,5	216,7	150
		0,982	245,50	-1,8	216,0	150
21SG41-01	25/03/2021	0,958	239,5	-4,2	191,67	360
21SG42-01	30/03/2021	0,999	249,75	-0,1	299,7	360

According to the study plan there were 11 sampling event.

To determinate degradation time 50, RAC specimens for analyses (whole plants without roots, minimum weight of samples was 100 g/ > 24 plants) were collected in intervals:

0, 2,4,8,12,24,48,72,96,120,144 hours after application

RAC specimens were put in deep freezing conditions at a target temperature of ≤ -18° C on the day of sampling, within 15 minutes after sampling on dry ice.

All specimens remained deep frozen during storage at the test site.

3 ANALYTICAL PROCEDURE

The field specimens arrived at the Test Site frozen and were stored at ≤ -18°C before analysis. After removal from the freezer the samples were homogenized with dry ice at Test Site.

Next, there was performed specimen extraction and determination of pyraclostrobin according to the multi-residue QuEChERS method.

Quantification was performed by use of LC-MS/MS detection. The limit of quantification (LOQ) of the analytical method was 0.01 mg/kg.

Details of the analytical procedure are described in Section 6 of the Analytical Phase Reports DPL/37-40/2021.

Table 1. Residue concentrations of pyraclostrobin detected in analyzed field samples (Study No.: 21SGS39, Trial No.: 21SGS39-01 Decline Curve Study), Northern France

No	Timing	Study sample code	Type of commodity	Sample number given by the laboratory	Result [mg/kg]
1	0 DBA	20SGS39-01-1	wheat (whole plant without root)	DPL/37/2021/01U	< LOD
2	0 DAA	20SGS39-01-2	wheat (whole plant without root)	DPL/37/2021/02T	16.10
3	2 HAA	20SGS39-01-3	wheat (whole plant without root)	DPL/37/2021/03T	15.53
4	4 HAA	20SGS39-01-4	wheat (whole plant without root)	DPL/37/2021/04T	14.62
5	8 HAA	20SGS39-01-5	wheat (whole plant without root)	DPL/37/2021/05T	11.95
6	12 HAA	20SGS39-01-6	wheat (whole plant without root)	DPL/37/2021/06T	10.89
7	24 HAA	20SGS39-01-7	wheat (whole plant without root)	DPL/37/2021/07T	10.75
8	48 HAA	20SGS39-01-8	wheat (whole plant without root)	DPL/37/2021/08T	10.64
9	72 HAA	20SGS39-01-9	wheat (whole plant without root)	DPL/37/2021/09T	8.33
10	96 HAA	20SGS39-01-10	wheat (whole plant without root)	DPL/37/2021/10T	5.59
11	120 HAA	20SGS39-01-11	wheat (whole plant without root)	DPL/37/2021/11T	5.68
12	144 HAA	20SGS39-01-12	wheat (whole plant without root)	DPL/37/2021/12U	<LOD
13	144 HAA	20SGS39-01-13	wheat (whole plant without root)	DPL/37/2021/13T	6.28

DBA – Days Before Application, DAA – Days After Application, HAA – Hours After Application
Residues are not corrected for procedural recoveries;
Calculation based on unrounded values, LOD = 0.003 mg/kg, LOQ = 0.01 mg/kg

Table 2. Residue concentrations of pyraclostrobin detected in analyzed field samples (Study No.: 21SGS40, Trial No.: 21SGS40-01 Decline Curve Study), Hungary

No	Timing	Study sample code	Type of commodity	Sample number given by the laboratory	Result [mg/kg]
1	0 DBA	20SGS40-01-1	wheat (whole plant without root)	DPL/38/2021/01U	< LOD
2	0 DAA	20SGS40-01-2	wheat (whole plant without root)	DPL/38/2021/02T	16.45
3	2 HAA	20SGS40-01-3	wheat (whole plant without root)	DPL/38/2021/03T	16.25
4	4 HAA	20SGS40-01-4	wheat (whole plant without root)	DPL/38/2021/04T	16.02
5	8 HAA	20SGS40-01-5	wheat (whole plant without root)	DPL/38/2021/05T	15.93
6	12 HAA	20SGS40-01-6	wheat (whole plant without root)	DPL/38/2021/06T	15.71
7	24 HAA	20SGS40-01-7	wheat (whole plant without root)	DPL/38/2021/07T	14.76
8	48 HAA	20SGS40-01-8	wheat (whole plant without root)	DPL/38/2021/08T	11.68
9	72 HAA	20SGS40-01-9	wheat (whole plant without root)	DPL/38/2021/09T	7.08
10	96 HAA	20SGS40-01-10	wheat (whole plant without root)	DPL/38/2021/10T	3.97
11	120 HAA	20SGS40-01-11	wheat (whole plant without root)	DPL/38/2021/11T	3.67
12	144 HAA	20SGS40-01-12	wheat (whole plant without root)	DPL/38/2021/12U	<LOD
13	144 HAA	20SGS40-01-13	wheat (whole plant without root)	DPL/38/2021/13T	3.47

DBA – Days Before Application, DAA – Days After Application, HAA – Hours After Application
Residues are not corrected for procedural recoveries;
Calculation based on unrounded values, LOD = 0.003 mg/kg, LOQ = 0.01 mg/kg

Table 3. Residue concentrations of pyraclostrobin detected in analyzed field samples (Study No.: 21SGS41, Trial No.: 21SGS41-01 Decline Curve Study), Germany

No	Timing	Study sample code	Type of commodity	Sample number given by the laboratory	Result [mg/kg]
1	0 DBA	20SGS41-01-1	wheat (whole plant without root)	DPL/39/2021/01U	< LOD
2	0 DAA	20SGS41-01-2	wheat (whole plant without root)	DPL/39/2021/02T	19.8
3	2 HAA	20SGS41-01-3	wheat (whole plant without root)	DPL/39/2021/03T	28.1
4	4 HAA	20SGS41-01-4	wheat (whole plant without root)	DPL/39/2021/04T	23.9
5	8 HAA	20SGS41-01-5	wheat (whole plant without root)	DPL/39/2021/05T	23.7
6	12 HAA	20SGS41-01-6	wheat (whole plant without root)	DPL/39/2021/06T	18.0
7	24 HAA	20SGS41-01-7	wheat (whole plant without root)	DPL/39/2021/07T	15.2
8	48 HAA	20SGS41-01-8	wheat (whole plant without root)	DPL/39/2021/08T	10.4
9	72 HAA	20SGS41-01-9	wheat (whole plant without root)	DPL/39/2021/09T	10.1
10	96 HAA	20SGS41-01-10	wheat (whole plant without root)	DPL/39/2021/10T	5.27
11	120 HAA	20SGS41-01-11	wheat (whole plant without root)	DPL/39/2021/11T	4.82
12	144 HAA	20SGS41-01-12	wheat (whole plant without root)	DPL/39/2021/12U	<LOD
13	144 HAA	20SGS41-01-13	wheat (whole plant without root)	DPL/39/2021/13T	2.41

DBA – Days Before Application, DAA – Days After Application, HAA – Hours After Application
Residues are not corrected for procedural recoveries;
Calculation based on unrounded values, **LOD = 0.003 mg/kg, LOQ = 0.01 mg/kg**

Table 4. Residue concentrations of acetamiprid detected in analyzed field samples (Study No.: 21SGS42, Trial No.: 21SGS42-01 Decline Curve Study), Poland

No	Timing	Study sample code	Type of commodity	Sample number given by the laboratory	Result [mg/kg]
1	0 DBA	20SGS42-01-1	wheat (whole plant without root)	DPL/40/2021/01U	< LOD
2	0 DAA	20SGS42-01-2	wheat (whole plant without root)	DPL/40/2021/02T	14.43

3	2 HAA	20SGS42-01-3	wheat (whole plant without root)	DPL/40/2021/03T	13.49
4	4 HAA	20SGS42-01-4	wheat (whole plant without root)	DPL/40/2021/04T	14.69
5	8 HAA	20SGS42-01-5	wheat (whole plant without root)	DPL/40/2021/05T	12.23
6	12 HAA	20SGS42-01-6	wheat (whole plant without root)	DPL/40/2021/06T	12.16
7	24 HAA	20SGS42-01-7	wheat (whole plant without root)	DPL/40/2021/07T	11.31
8	48 HAA	20SGS42-01-8	wheat (whole plant without root)	DPL/40/2021/08T	10.50
9	72 HAA	20SGS42-01-9	wheat (whole plant without root)	DPL/40/2021/09T	6.72
10	96 HAA	20SGS42-01-10	wheat (whole plant without root)	DPL/40/2021/10T	6.10
11	120 HAA	20SGS42-01-11	wheat (whole plant without root)	DPL/40/2021/11T	5.40
12	144 HAA	20SGS42-01-12	wheat (whole plant without root)	DPL/40/2021/12U	<LOD
13	144 HAA	20SGS42-01-13	wheat (whole plant without root)	DPL/40/2021/13T	3.46

DBA – Days Before Application, DAA – Days After Application, HAA – Hours After Application
Residues are not corrected for procedural recoveries;
Calculation based on unrounded values, **LOD = 0.003 mg/kg, LOQ = 0.01 mg/kg**

5 KINETIC EVALUATION

On the basis of the obtained pyraclostrobin residues in wheat samples, DT_{50} value was determined. For this purpose CAKE (Computer Assisted Kinetic Evaluation, version 3.3) program, following single first-order kinetics (SFO) was used. Residue decay is described by:

$$c = c_0 e^{-kt} \qquad \frac{dC}{dt} = -kC$$

$\boldsymbol{c}$ – *concentration at time t*

$\boldsymbol{c_0}$ – *initial concentration*

k – *rate constant*

$\boldsymbol{t}$ – *time*

Single first-order kinetics (SFO) is a simple exponential equation with only two parameters. The rate of the change in pesticide concentration (dC/dt) is at any time directly proportional to the actual concentration remaining in the system. For SFO kinetics, the time for a decrease in the concentration by a certain percentage is constant throughout the experiment and independent of the initial concentration of the pesticide.

The obtained results are summarized in table 5

Table 5. Determined value of DT_{50}

Location	Study number	Trial number	DT_{50} [h]	DT_{50} [days]	Error [%]
Northern France	21SGS39	21SGS39-01	89.1	3.71	9.91
Hungary	21SGS40	21SGS40-01	59.8	2.49	7.19
Germany	21SGS41	21SGS41-01	45.7	1.90	12.9
Poland	21SGS42	21SGS42-01	78.7	3.28	5.52

In accordance with the guidance of Work Group on Degradation Kinetics of FOCUS, Single First-Order (SFO) kinetics is the preferred option to derive degradation endpoints. However, degradation cannot always be described by SFO kinetics. Sometimes a fast initial decrease in pesticide concentrations is followed by a slower decline. This is usually referred to as a bi-phasic pattern of pesticide degradation. In that case, the other model is used, for example FOMC (First-Order Multi-Compartment model) or Hockey-stick model.

For comparison in the attached documents it was presented results obtained using four different kinetic models:

- Single First-Order (SFO)
- First-Order Multi-Compartment (FOMC)
- Double First-Order in Parallel (DFOP)
- Hockey-Stick Model (HS)

6 CONCLUSIONS

The objective of the studies was the determination of residues of pyraclostrobin and residue degradation time (DT50) in winter wheat after one spray application of CHR/F/PYRA 250 EC in four location (Northern France, Hungary, Germany, Poland). Obtained DT50 value was between 45.7 h for Germany and 89.1 h for Northern France.

TRIAL 21SGS39

CAKE Kinetic Evaluation Report

Study: New Study

Study date: poniedziaĹ,ek, 27 czerwca 2022
Report generated: poniedziaĹ,ek, 27 czerwca 2022

Experiment 1 (SFO)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	16.1	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Fit step: Final

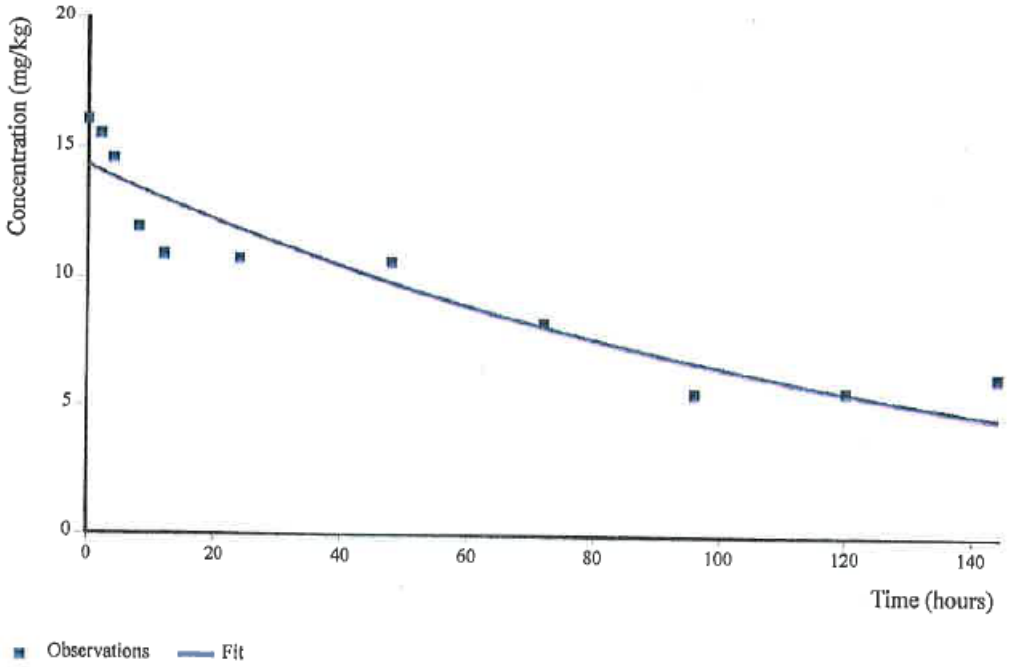
Used Extra Solver: No

Reference Table:

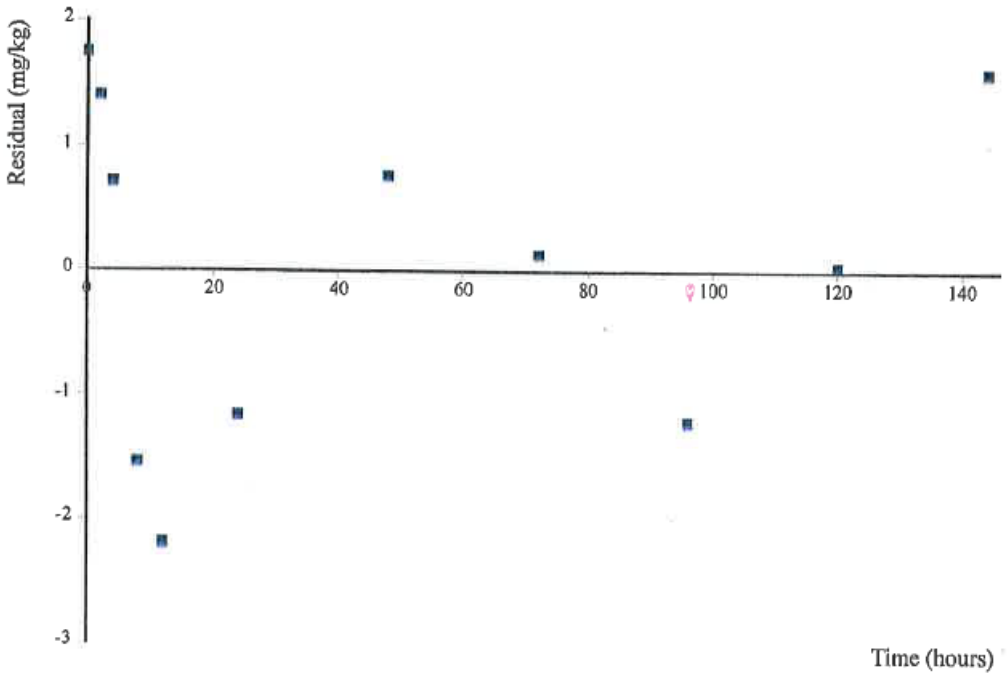
Compartment	Name
Parent	pyraclostrobin

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	16.1	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	14.35	0.6713	N/A	13.12	15.58	12.83	15.87
k_Parent	0.007782	0.001209	6.01E-005	0.005565	0.009998	0.005046	0.011

χ_c

Parameter	Error %	Degrees of Freedom
All data	9.91	9
Parent	9.91	9

Decay Times:

Compartment	DT50 (hours)	DT90 (hours)
Parent	89.1	296

Additional Statistics:

Parameter	r_c (Obs v Pred)	Efficiency
All data	0.8731	0.8724
Parent	0.8731	0.8724

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.552
k_Parent	0.552	1

Observed v. Predicted:

Compartment Parent

Time (hours)	Value (mg/kg)	Predicted Value	Residual
0	16.1	14.35	1.75
2	15.53	14.13	1.402
4	14.62	13.91	0.7099
8	11.95	13.48	-1.534
12	10.89	13.07	-2.181
24	10.75	11.91	-1.155
48	10.64	9.877	0.7629
72	8.33	8.194	0.1356
96	5.59	6.798	-1.208

120	5.68	5.64	0.03974
144	6.28	4.679	1.601

Sequence Creation Information:
Fit generated by CAKE version 3.3 (Release)
running on R version 3.0.0 (2013-04-03)

Experiment 1 (DFOP)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	16.1	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
g_Parent	0.5	0 to 1	No

Fit step: Final

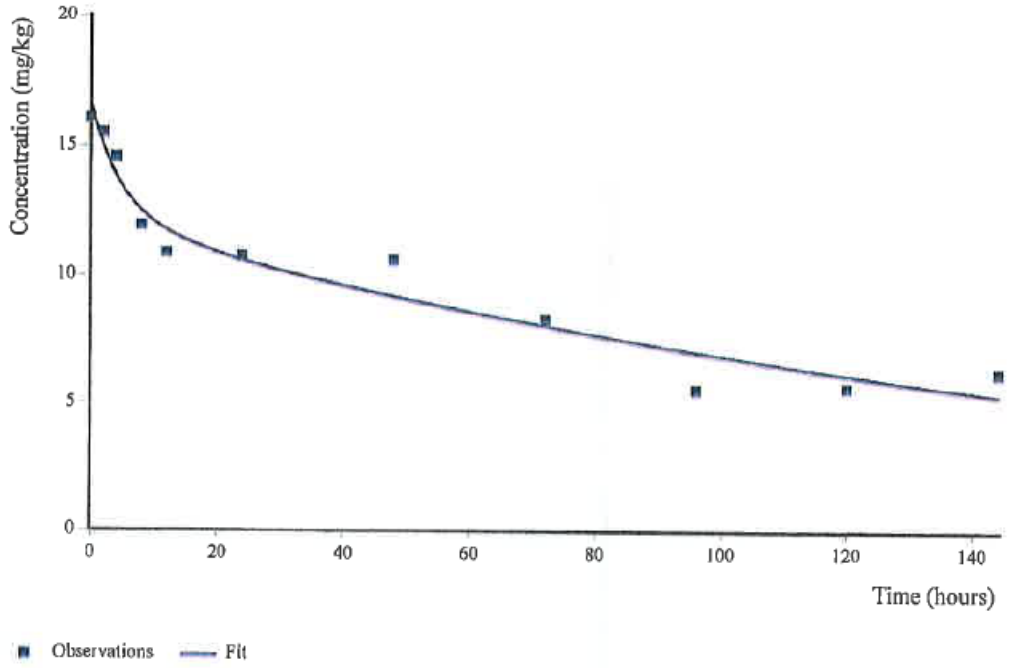
Used Extra Solver: No

Reference Table:

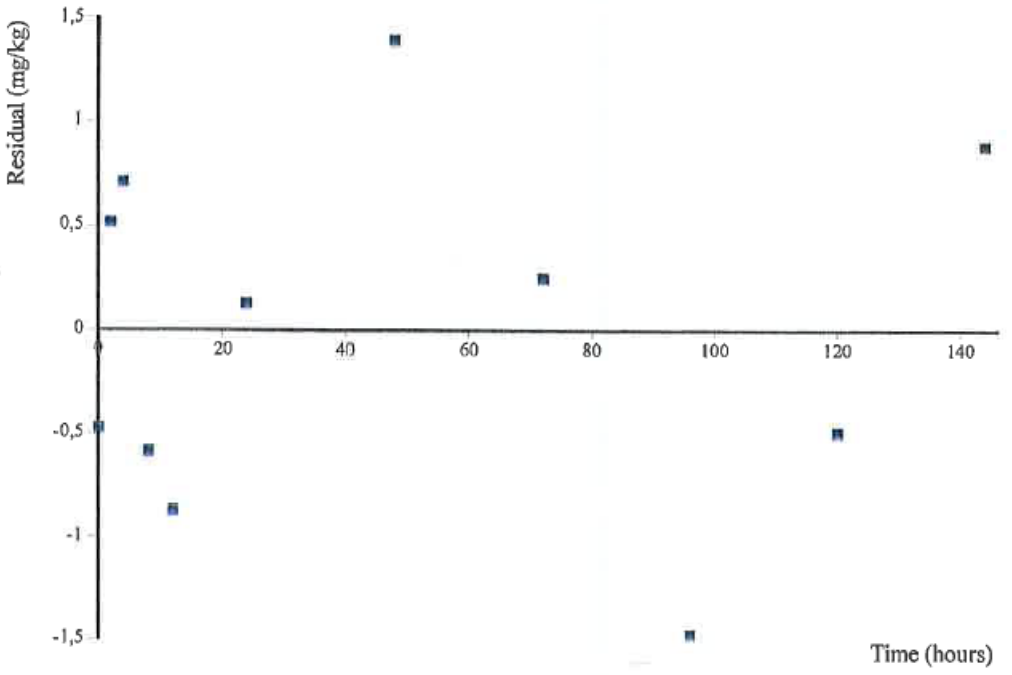
Compartment	Name
Parent	pyraclostrobin

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	16.1	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
g_Parent	0.5	0 to 1	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	16.57	0.9469	N/A	14.78	18.36	14.33	18.81
k1_Parent	0.1911	0.1367	0.1023	-0.0678	0.45	-0.132	0.514
k2_Parent	0.005606	0.001353	0.002163	0.003043	0.008169	0.002408	0.009
g_Parent	0.2701	0.0792	N/A	0.12	0.4201	0.08279	0.457

χ_c

Parameter	Error %	Degrees of Freedom
All data	6.82	7
Parent	6.82	7

Decay Times:

Compartment	DT50 (overall hours)	DT90 (overall hours)	k1 DT50 (hours)	k2 DT50 (hours)
Parent	67.5	355	3.63	124

Additional Statistics:

Parameter	r_c (Obs v Pred)	Efficiency
All data	0.9498	0.9498
Parent	0.9498	0.9498

Parameter Correlation:

	Parent_0	k1_Parent	k2_Parent	g_Parent
Parent_0	1	0.4864	0.1387	0.3591
k1_Parent	0.4864	1	0.6251	-0.4698
k2_Parent	0.1387	0.6251	1	-0.7437
g_Parent	0.3591	-0.4698	-0.7437	1

Observed v. Predicted:

Compartment Parent

Time (hours)	Value (mg/kg)	Predicted Value	Residual
0	16.1	16.57	-0.47
2	15.53	15.01	0.5165
4	14.62	13.91	0.7099

8	11.95	12.53	-0.5844
12	10.89	11.76	-0.8696
24	10.75	10.62	0.1321
48	10.64	9.242	1.398
72	8.33	8.078	0.252
96	5.59	7.061	-1.471
120	5.68	6.172	-0.4921
144	6.28	5.395	0.8849

Sequence Creation Information:
Fit generated by CAKE version 3.3 (Release)
running on R version 3.0.0 (2013-04-03)

Experiment 1 (HS)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	16.1	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
tb_Parent	Automatic	0 to (unbounded)	No

Fit step: Final

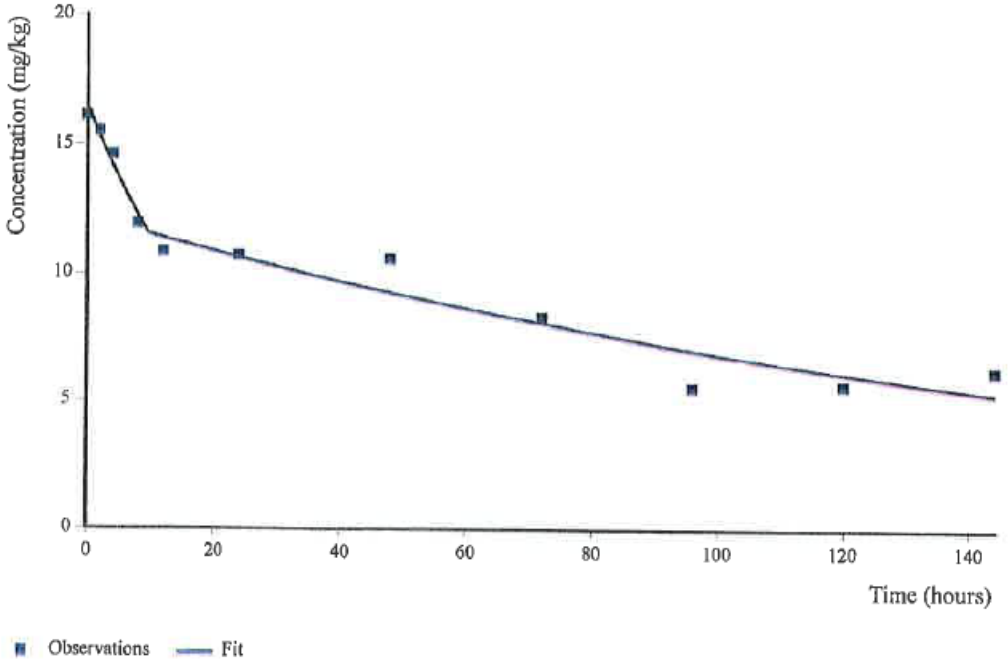
Used Extra Solver: No

Reference Table:

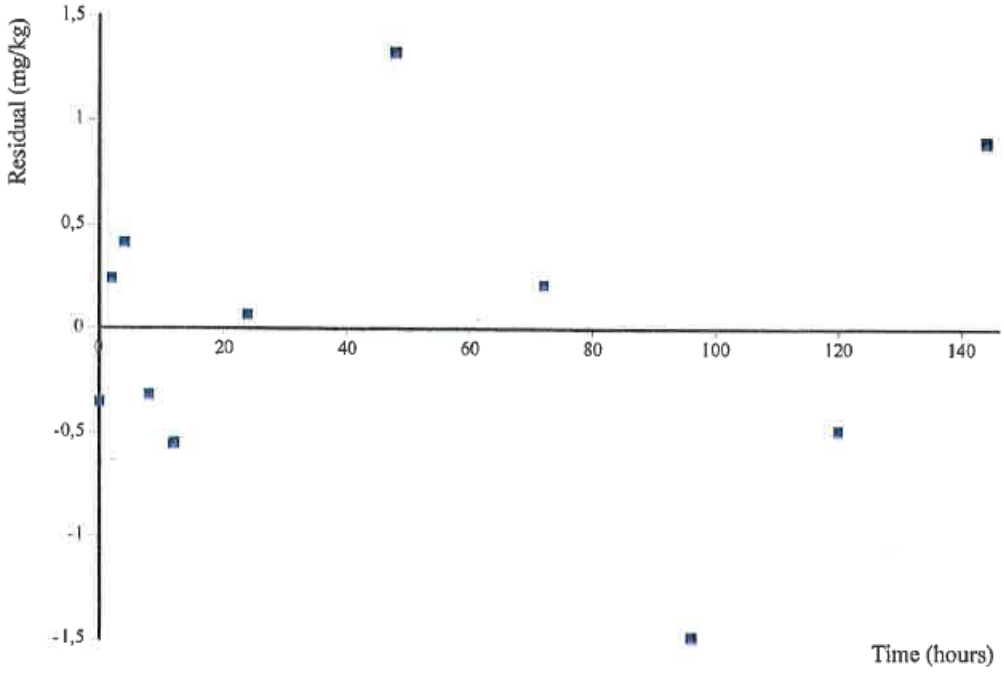
Compartment	Name
Parent	pyraclostrobin

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	16.1	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
tb_Parent	10.5	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	16.45	0.7405	N/A	15.05	17.85	14.7	18.2
k1	0.03668	0.01122	0.006839	0.01543	0.05793	0.01015	0.063
k2	0.005724	9.94E-004	3.45E-004	0.003842	0.007607	0.003375	0.008
tb	9.516	3.213	N/A	3.428	15.6	1.917	17.11

χ_c

Parameter	Error %	Degrees of Freedom
All data	6.09	7
Parent	6.09	7

Decay Times:

Compartment	DT50 (overall hours)	DT90 (overall hours)	k1 DT50 (hours)	k2 DT50 (hours)
Parent	69.6	351	18.9	121

Additional Statistics:

Parameter	r_a (Obs v Pred)	Efficiency
All data	0.96	0.96
Parent	0.96	0.96

Parameter Correlation:

	Parent_0	k1	k2	tb
Parent_0	1	0.721	0.0002988	-0.3211
k1	0.721	1	0.0001415	-0.7468
k2	0.0002988	0.0001415	1	-0.4242
tb	-0.3211	-0.7468	-0.4242	1

Observed v. Predicted:

Compartment Parent

Time (hours)	Value (mg/kg)	Predicted Value	Residual
0	16.1	16.45	-0.3497
2	15.53	15.29	0.2439
4	14.62	14.21	0.4151

8	11.95	12.27	-0.3164
12	10.89	11.44	-0.5493
24	10.75	10.68	0.07007
48	10.64	9.309	1.331
72	8.33	8.114	0.216
96	5.59	7.072	-1.482
120	5.68	6.165	-0.4846
144	6.28	5.373	0.9068

Sequence Creation Information:
Fit generated by CAKE version 3.3 (Release)
running on R version 3.0.0 (2013-04-03)

Experiment 1 (FOMC)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	16.1	0 to (unbounded)	No
alpha_Parent	0.1	0 to (unbounded)	No
beta_Parent	0.01	0 to (unbounded)	No

Fit step: Final

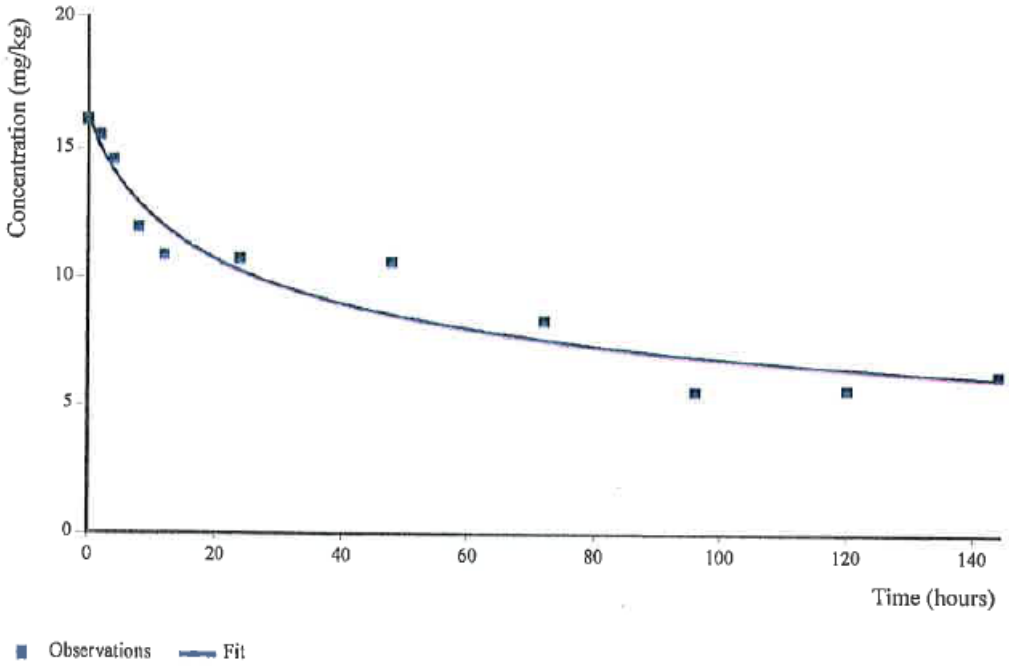
Used Extra Solver: No

Reference Table:

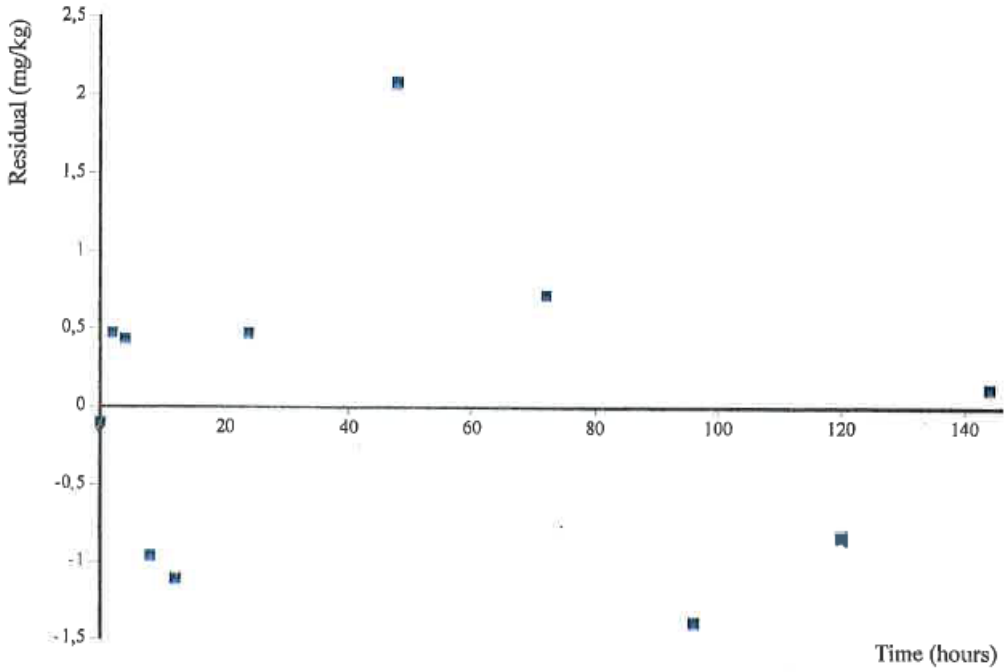
Compartment	Name
Parent	pyraclostrobin

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	16.1	0 to (unbounded)	No
alpha_Parent	0.1	0 to (unbounded)	No
beta_Parent	0.01	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	16.2	0.9621	N/A	14.41	17.99	13.98	18.42
alpha	0.3292	0.1026	N/A	0.1385	0.5199	0.09269	0.566
beta	8.045	6.857	N/A	-4.706	20.8	-7.768	23.86

χ_c

Parameter	Error %	Degrees of Freedom
All data	7.71	8
Parent	7.71	8

Decay Times:

Compartment	DT50 (hours)	DT90 (hours)	DT90 / 3.32 (hours)
Parent	58	8.77E+03	2.64E+03

Additional Statistics:

Parameter	r_c (Obs v Pred)	Efficiency
All data	0.9293	0.9293
Parent	0.9293	0.9293

Parameter Correlation:

	Parent_0	alpha	beta
Parent_0	1	-0.4761	-0.6926
alpha	-0.4761	1	0.9384
beta	-0.6926	0.9384	1

Observed v. Predicted:

Compartment Parent

Time (hours)	Value (mg/kg)	Predicted Value	Residual
0	16.1	16.2	-0.09945
2	15.53	15.06	0.4723
4	14.62	14.18	0.436
8	11.95	12.91	-0.9563
12	10.89	11.99	-1.104
24	10.75	10.28	0.472

48	10.64	8.55	2.09
72	8.33	7.604	0.7261
96	5.59	6.975	-1.385
120	5.68	6.514	-0.8344
144	6.28	6.156	0.1238

Sequence Creation Information:
Fit generated by CAKE version 3.3 (Release)
running on R version 3.0.0 (2013-04-03)

Report Information:
Report generated by CAKE version 3.3 (Release)
CAKE developed by Tessella Ltd, Abingdon, Oxfordshire, UK, sponsored by Syngenta
Running on .NET version 4.0.30319.42000

TRIAL 21SGS40

CAKE Kinetic Evaluation Report

Study: New Study

Study date: poniedziaĹ,ek, 27 czerwca 2022
Report generated: poniedziaĹ,ek, 27 czerwca 2022

Experiment 1 (SFO)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	16.45	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Fit step: Final

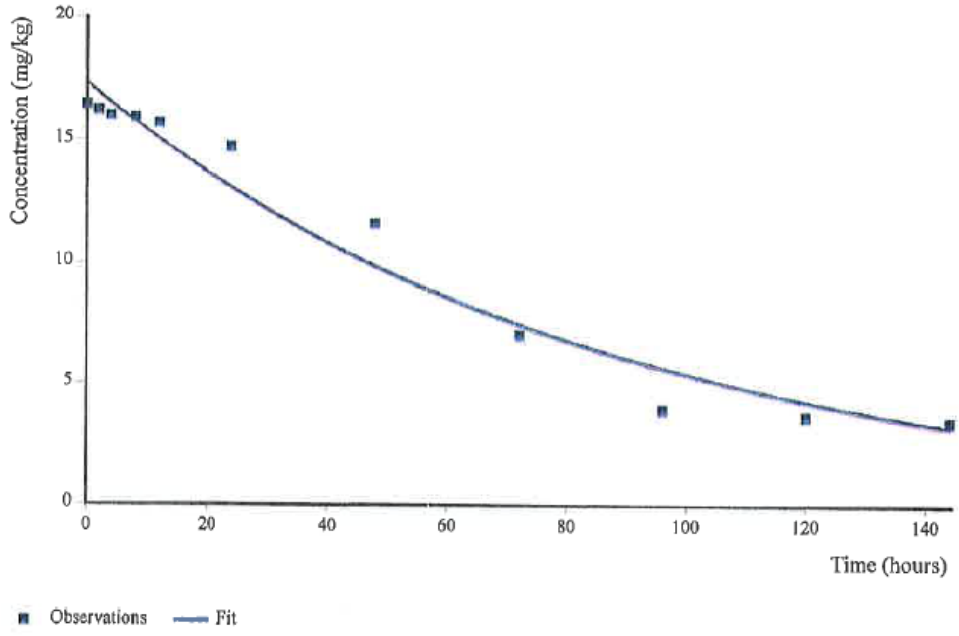
Used Extra Solver: No

Reference Table:

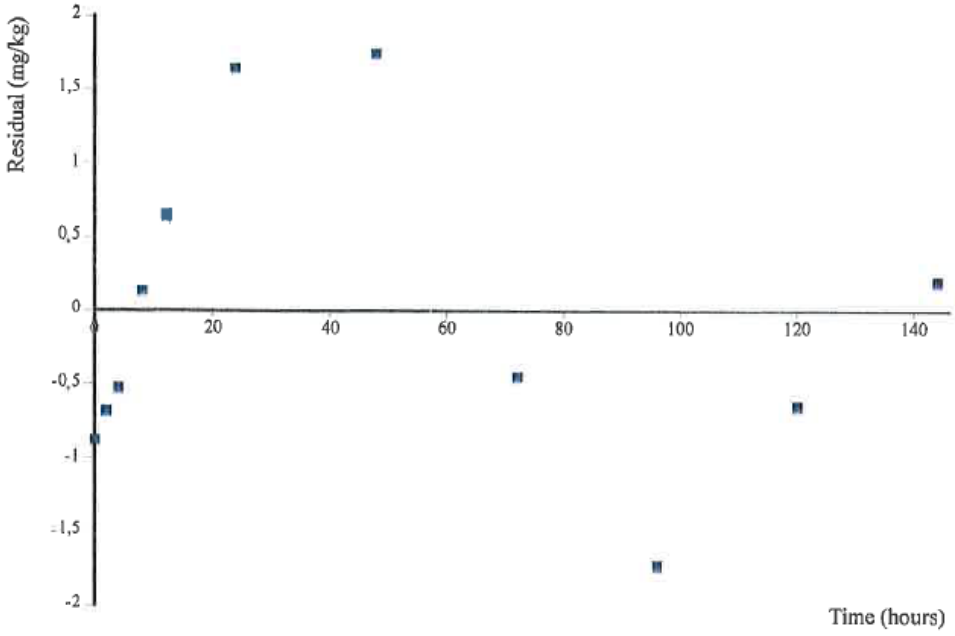
Compartment	Name
Parent	pyraclostrobin

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	16.45	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	17.33	0.5475	N/A	16.32	18.33	16.09	18.56
k_Parent	0.01159	0.001086	1.04E-006	0.009595	0.01358	0.00913	0.014

χ_c

Parameter	Error %	Degrees of Freedom
All data	7.19	9
Parent	7.19	9

Decay Times:

Compartment	DT50 (hours)	DT90 (hours)
Parent	59.8	199

Additional Statistics:

Parameter	r_c (Obs v Pred)	Efficiency
All data	0.9649	0.9644
Parent	0.9649	0.9644

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.5218
k_Parent	0.5218	1

Observed v. Predicted:

Compartment Parent

Time (hours)	Value (mg/kg)	Predicted Value	Residual
0	16.45	17.33	-0.8757
2	16.25	16.93	-0.6788
4	16.02	16.54	-0.5211
8	15.93	15.79	0.138
12	15.71	15.08	0.6331
24	14.76	13.12	1.64
48	11.68	9.935	1.745
72	7.08	7.524	-0.4435
96	3.97	5.697	-1.727

120	3.67	4.314	-0.6443
144	3.47	3.267	0.203

Sequence Creation Information:
Fit generated by CAKE version 3.3 (Release)
running on R version 3.0.0 (2013-04-03)

Experiment 1 (DFOP)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	16.45	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
g_Parent	0.5	0 to 1	No

Fit step: Final

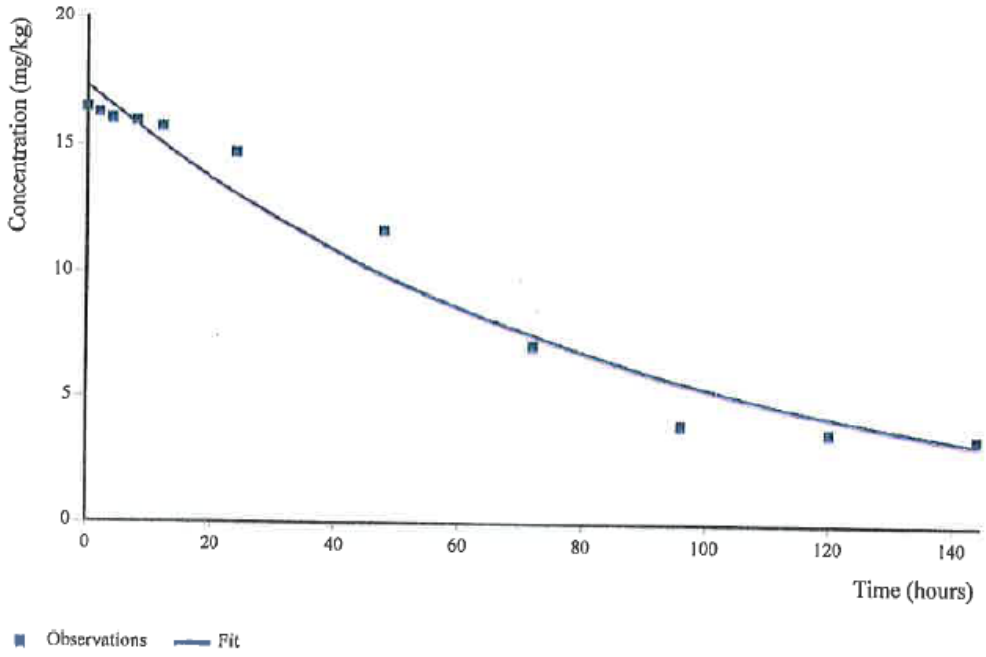
Used Extra Solver: No

Reference Table:

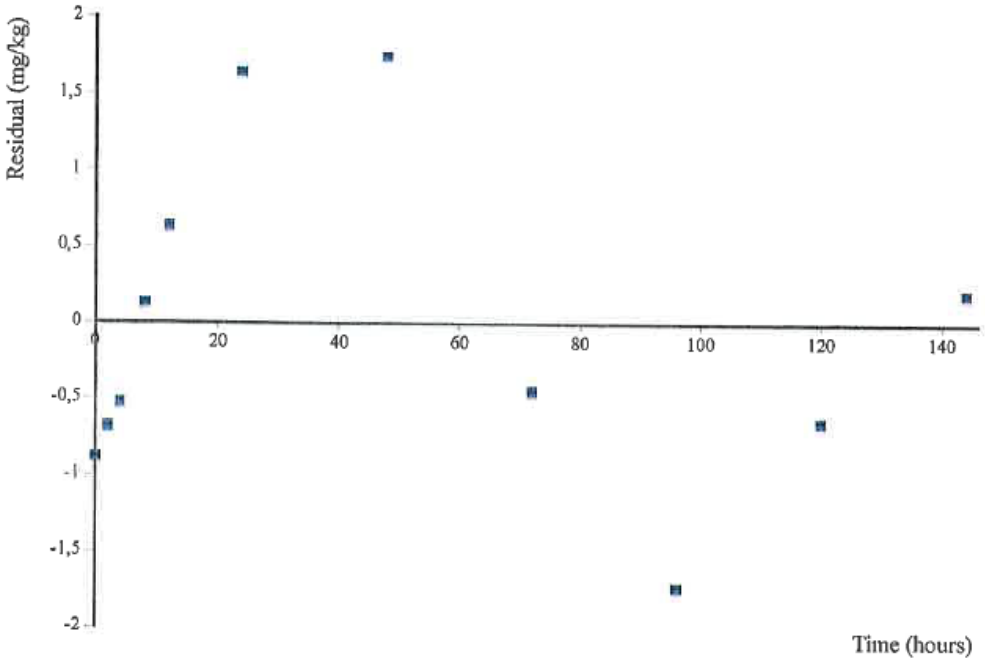
Compartment	Name
Parent	pyraclostrobin

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	16.45	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
g_Parent	0.5	0 to 1	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	17.33	0.7677	N/A	15.87	18.78	15.51	19.14
k1_Parent	0.01159	487.6	0.5	-923.7	923.7	-1153	1.15E+003
k2_Parent	0.01159	93.09	0.5	-176.4	176.4	-220.1	220.1
g_Parent	0.1603	1.63E+003	N/A	-3092	3.09E+003	-3859	3.86E+003

χ_c

Parameter	Error %	Degrees of Freedom
All data	7.88	7
Parent	7.88	7

Decay Times:

Compartment	DT50 (overall hours)	DT90 (overall hours)	k1 DT50 (hours)	k2 DT50 (hours)
Parent	59.8	199	59.8	59.8

Additional Statistics:

Parameter	r_c (Obs v Pred)	Efficiency
All data	0.9649	0.9644
Parent	0.9649	0.9644

Parameter Correlation:

	Parent_0	k1_Parent	k2_Parent	g_Parent
Parent_0	1	-0.3452	0.3452	0.1954
k1_Parent	-0.3452	1	-1	0.5689
k2_Parent	0.3452	-1	1	-0.5689
g_Parent	0.1954	0.5689	-0.5689	1

Observed v. Predicted:

Compartment Parent

Time (hours)	Value (mg/kg)	Predicted Value	Residual
0	16.45	17.33	-0.8757
2	16.25	16.93	-0.6788
4	16.02	16.54	-0.5211

8	15.93	15.79	0.138
12	15.71	15.08	0.6331
24	14.76	13.12	1.64
48	11.68	9.935	1.745
72	7.08	7.523	-0.4435
96	3.97	5.697	-1.727
120	3.67	4.314	-0.6443
144	3.47	3.267	0.203

Sequence Creation Information:
Fit generated by CAKE version 3.3 (Release)
running on R version 3.0.0 (2013-04-03)

Experiment 1 (HS)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	16.45	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
tb_Parent	Automatic	0 to (unbounded)	No

Fit step: Final

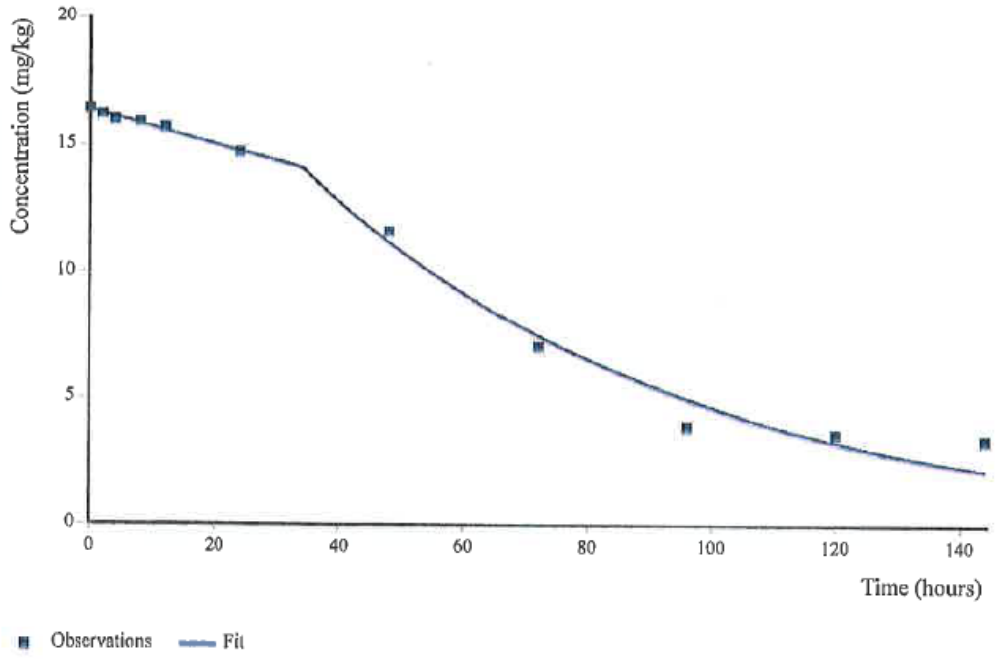
Used Extra Solver: No

Reference Table:

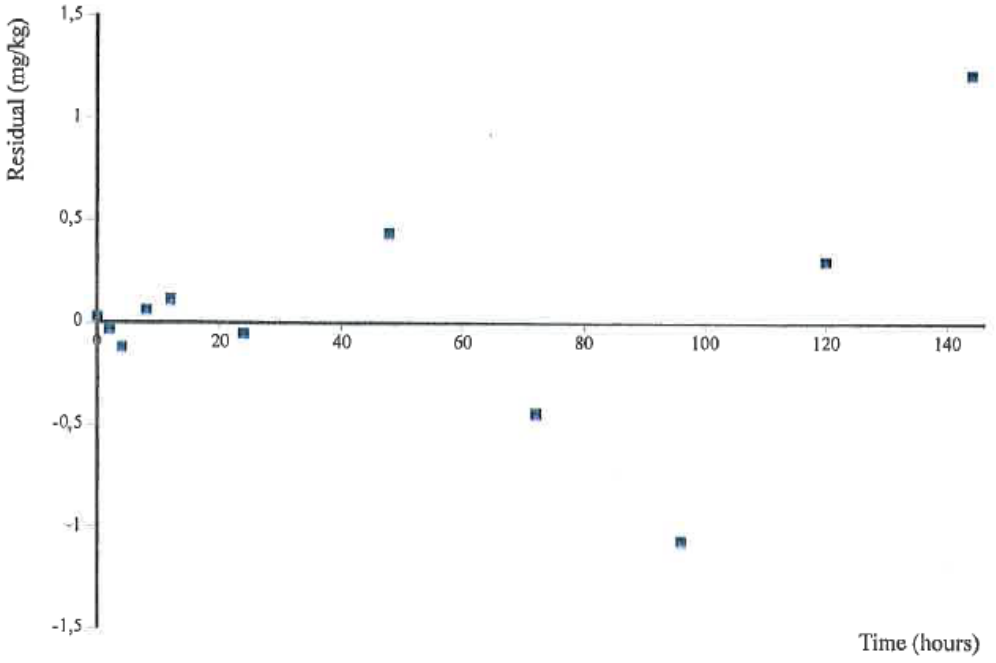
Compartment	Name
Parent	pyraclostrobin

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	16.45	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
tb_Parent	16.31	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	16.42	0.4003	N/A	15.66	17.18	15.47	17.37
k1	0.004289	0.002215	0.04701	9.28E-005	0.008486	-0.0009484	0.01
k2	0.01674	0.001856	2.10E-005	0.01323	0.02026	0.01236	0.021
tb	34.12	7.604	N/A	19.71	48.52	16.14	52.1

χ_c

Parameter	Error %	Degrees of Freedom
All data	4.15	7
Parent	4.15	7

Decay Times:

Compartment	DT50 (overall hours)	DT90 (overall hours)	k1 DT50 (hours)	k2 DT50 (hours)
Parent	66.8	163	162	41.4

Additional Statistics:

Parameter	r, (Obs v Pred)	Efficiency
All data	0.9905	0.9902
Parent	0.9905	0.9902

Parameter Correlation:

	Parent_0	k1	k2	tb
Parent_0	1	0.7086	-1.105E-07	0.3134
k1	0.7086	1	-2.768E-06	0.6156
k2	-1.105E-07	-2.768E-06	1	0.6086
tb	0.3134	0.6156	0.6086	1

Observed v. Predicted:

Compartment Parent

Time (hours)	Value (mg/kg)	Predicted Value	Residual
0	16.45	16.42	0.0294
2	16.25	16.28	-0.03033
4	16.02	16.14	-0.1213

8	15.93	15.87	0.06332
12	15.71	15.6	0.1132
24	14.76	14.81	-0.05435
48	11.68	11.24	0.4373
72	7.08	7.522	-0.4423
96	3.97	5.033	-1.063
120	3.67	3.367	0.3025
144	3.47	2.253	1.217

Sequence Creation Information:
Fit generated by CAKE version 3.3 (Release)
running on R version 3.0.0 (2013-04-03)

Experiment 1 (FOMC)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	16.45	0 to (unbounded)	No
alpha_Parent	0.1	0 to (unbounded)	No
beta_Parent	0.01	0 to (unbounded)	No

Fit step: Final

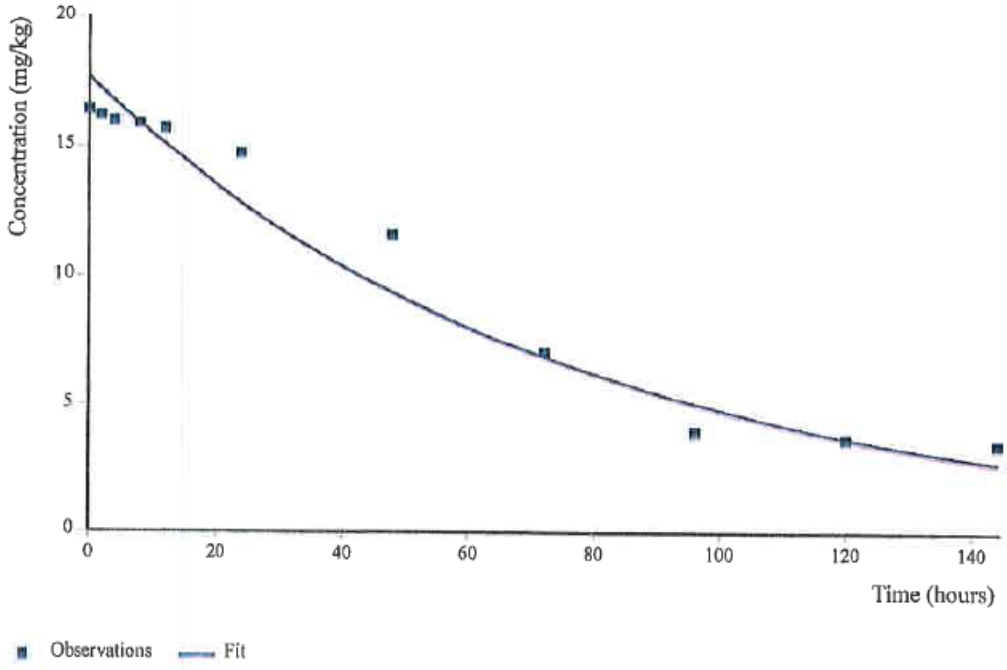
Used Extra Solver: Yes

Reference Table:

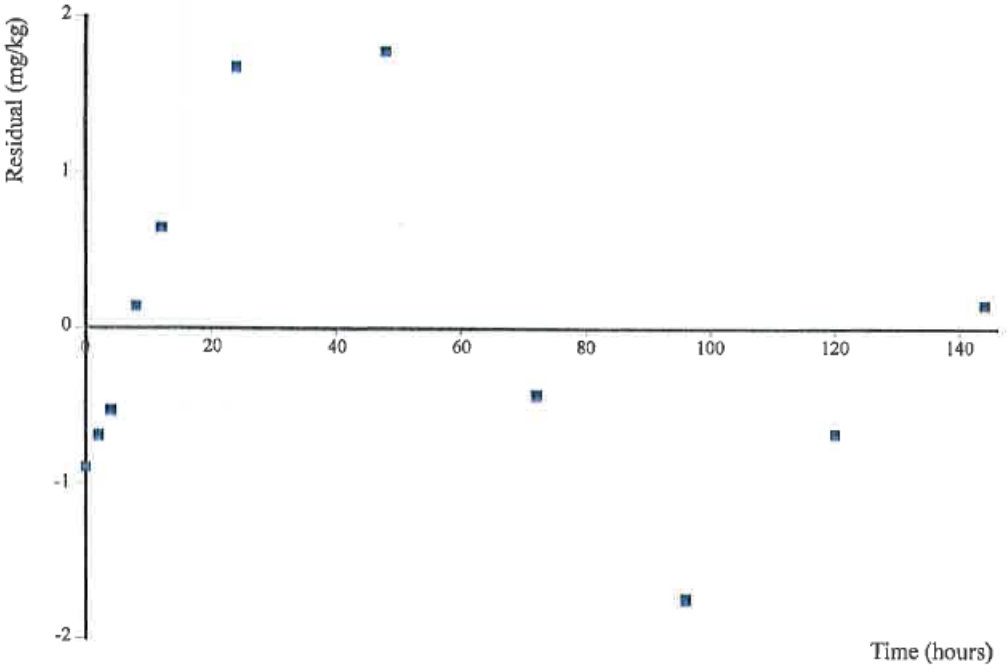
Compartment	Name
Parent	pyraclostrobin

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	16.45	0 to (unbounded)	No
alpha_Parent	0.1	0 to (unbounded)	No
beta_Parent	0.01	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	17.71	0.64	N/A	16.52	18.9	16.23	19.19
alpha	38.97	nd	N/A	nd	nd	nd	nd
beta	2.93E+003	nd	N/A	nd	nd	nd	nd

Note: Errors and T-test values could not be calculated because the covariance matrix could not be created.

χ_4

Parameter	Error %	Degrees of Freedom
All data	7.62	8
Parent	7.62	8

Decay Times:

Compartment	DT50 (hours)	DT90 (hours)
Parent	52.7	179

Additional Statistics:

Parameter	r_4 (Obs v Pred)	Efficiency
All data	0.9639	0.9634
Parent	0.9639	0.9634

Parameter Correlation:

	Parent_0	alpha	beta
Parent_0	1	nd	nd
alpha	nd	1	nd
beta	nd	nd	1

Observed v. Predicted:

Compartment Parent

Time (hours)	Value (mg/kg)	Predicted Value	Residual
0	16.45	17.34	-0.8937
2	16.25	16.94	-0.6903
4	16.02	16.55	-0.5266
8	15.93	15.79	0.1428

12	15.71	15.06	0.6464
24	14.76	13.09	1.669
48	11.68	9.903	1.777
72	7.08	7.508	-0.4283
96	3.97	5.705	-1.735
120	3.67	4.345	-0.6746
144	3.47	3.316	0.1545

Sequence Creation Information:
Fit generated by CAKE version 3.3 (Release)
running on R version 3.0.0 (2013-04-03)

Report Information:
Report generated by CAKE version 3.3 (Release)
CAKE developed by Tessella Ltd, Abingdon, Oxfordshire, UK, sponsored by Syngenta
Running on .NET version 4.0.30319.42000

TRIAL 21SGS41

CAKE Kinetic Evaluation Report

Study: New Study

Study date: poniedziaĹ,ek, 27 czerwca 2022
Report generated: poniedziaĹ,ek, 27 czerwca 2022

Experiment 1 (SFO)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	19.8	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Fit step: Final

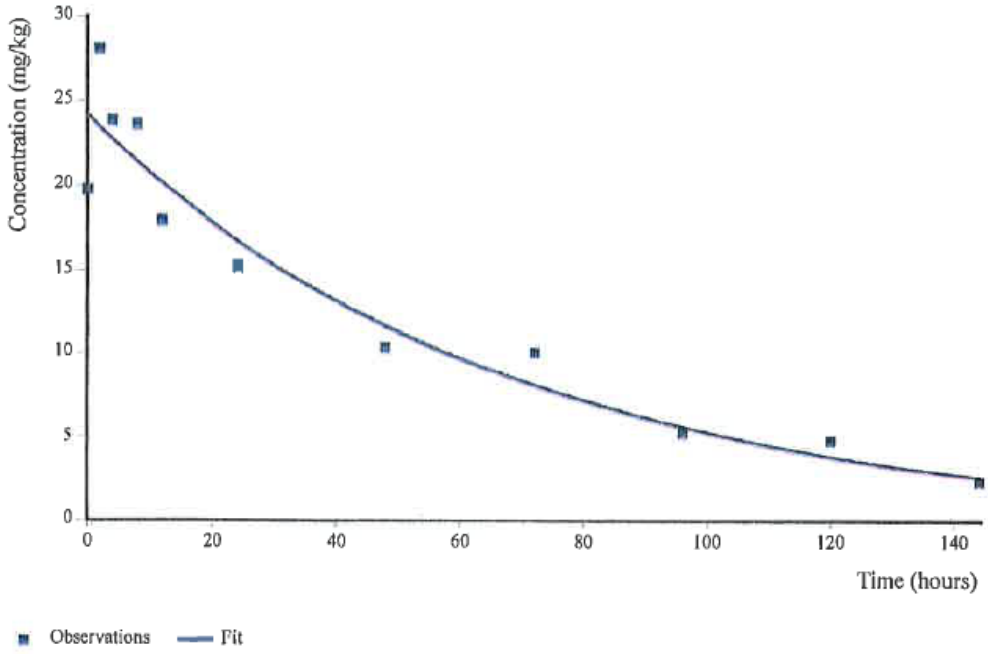
Used Extra Solver: No

Reference Table:

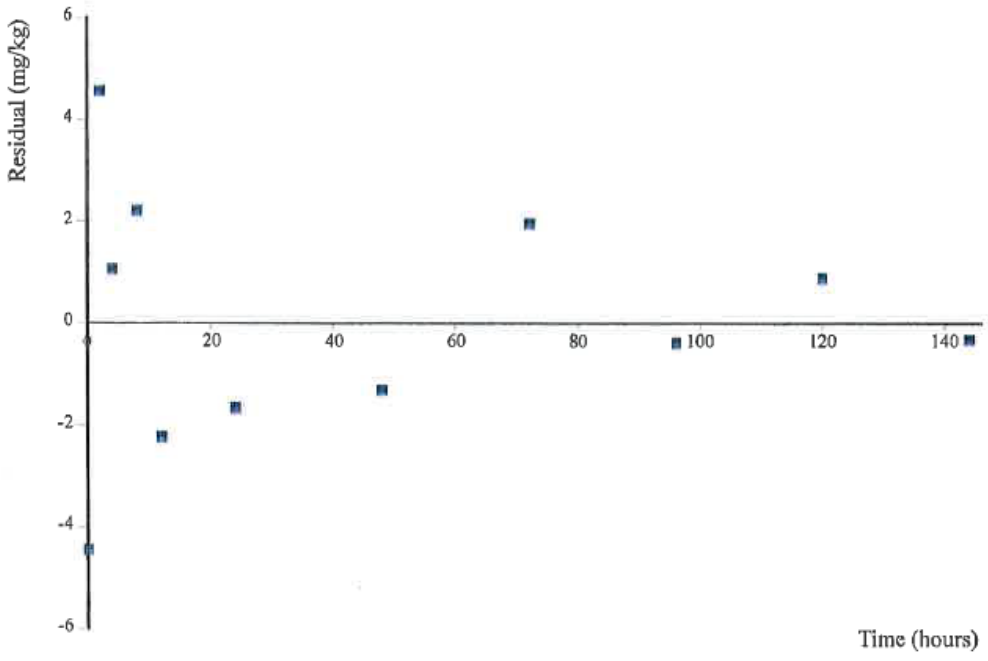
Compartment	Name
Parent	pyraclostrobin

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	19.8	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	24.26	1.328	N/A	21.82	26.69	21.25	27.26
k_Parent	0.01518	0.002407	6.99E-005	0.01077	0.01959	0.009736	0.021

χ_c

Parameter	Error %	Degrees of Freedom
All data	12.9	9
Parent	12.9	9

Decay Times:

Compartment	DT50 (hours)	DT90 (hours)
Parent	45.7	152

Additional Statistics:

Parameter	r_c (Obs v Pred)	Efficiency
All data	0.9197	0.9196
Parent	0.9197	0.9196

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.5136
k_Parent	0.5136	1

Observed v. Predicted:

Compartment Parent

Time (hours)	Value (mg/kg)	Predicted Value	Residual
0	19.8	24.26	-4.458
2	28.1	23.53	4.568
4	23.9	22.83	1.071
8	23.7	21.48	2.216
12	18	20.22	-2.218
24	15.2	16.85	-1.651
48	10.4	11.71	-1.306
72	10.1	8.131	1.969
96	5.27	5.648	-0.3784

120	4.82	3.924	0.8963
144	2.41	2.726	-0.3156

Sequence Creation Information:
Fit generated by CAKE version 3.3 (Release)
running on R version 3.0.0 (2013-04-03)

Experiment 1 (DFOP)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	19.8	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
g_Parent	0.5	0 to 1	No

Fit step: Final

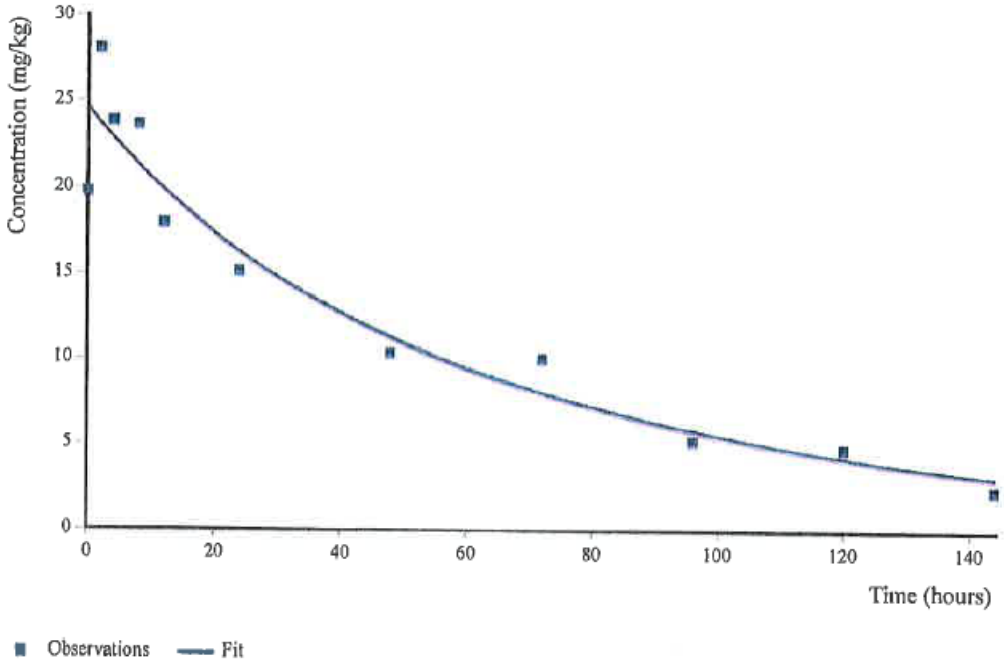
Used Extra Solver: No

Reference Table:

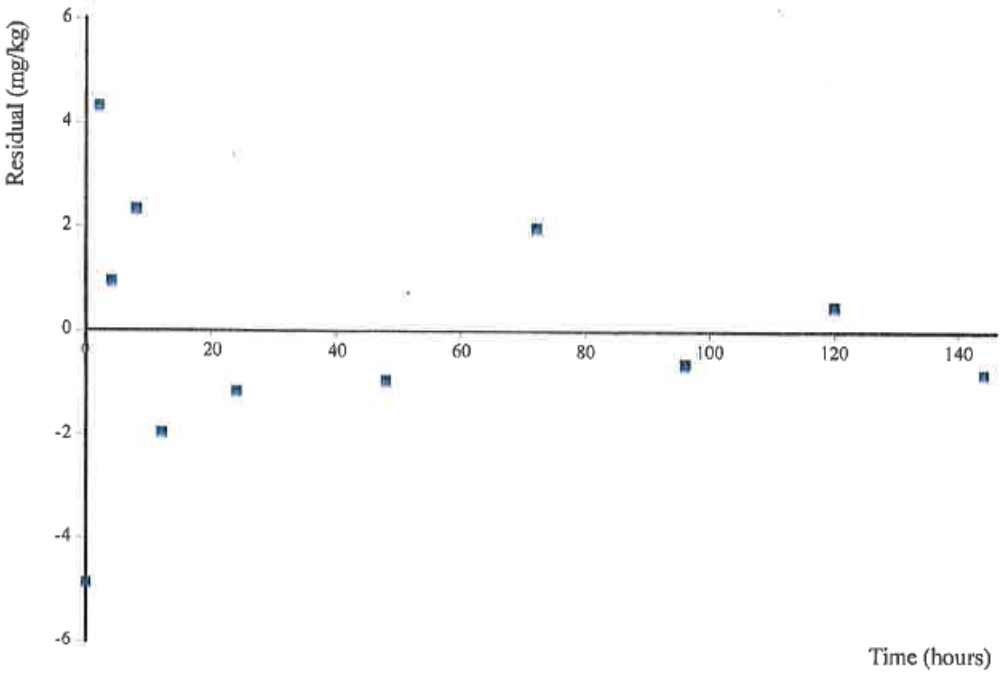
Compartment	Name
Parent	pyraclostrobin

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	19.8	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
g_Parent	0.5	0 to 1	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	24.65	2.094	N/A	20.68	28.62	19.7	29.6
k1_Parent	0.03627	0.2338	0.4406	-0.4068	0.4793	-0.5167	0.589
k2_Parent	0.01225	0.02609	0.3265	-0.03719	0.06169	-0.04946	0.074
g_Parent	0.252	2.713	N/A	-4.888	5.392	-6.163	6.667

χ_c

Parameter	Error %	Degrees of Freedom
All data	14	7
Parent	14	7

Decay Times:

Compartment	DT50 (overall hours)	DT90 (overall hours)	k1 DT50 (hours)	k2 DT50 (hours)
Parent	42.3	165	19.1	56.6

Additional Statistics:

Parameter	r_c (Obs v Pred)	Efficiency
All data	0.9212	0.9212
Parent	0.9212	0.9212

Parameter Correlation:

	Parent_0	k1_Parent	k2_Parent	g_Parent
Parent_0	1	0.4946	0.3535	-0.3738
k1_Parent	0.4946	1	0.9503	-0.977
k2_Parent	0.3535	0.9503	1	-0.991
g_Parent	-0.3738	-0.977	-0.991	1

Observed v. Predicted:

Compartment Parent

Time (hours)	Value (mg/kg)	Predicted Value	Residual
0	19.8	24.65	-4.85
2	28.1	23.77	4.331
4	23.9	22.93	0.9706

8	23.7	21.37	2.336
12	18	19.94	-1.938
24	15.2	16.34	-1.144
48	10.4	11.33	-0.9316
72	10.1	8.09	2.01
96	5.27	5.88	-0.6105
120	4.82	4.32	0.4996
144	2.41	3.194	-0.7839

Sequence Creation Information:
Fit generated by CAKE version 3.3 (Release)
running on R version 3.0.0 (2013-04-03)

Experiment 1 (HS)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	19.8	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
tb_Parent	Automatic	0 to (unbounded)	No

Fit step: Final

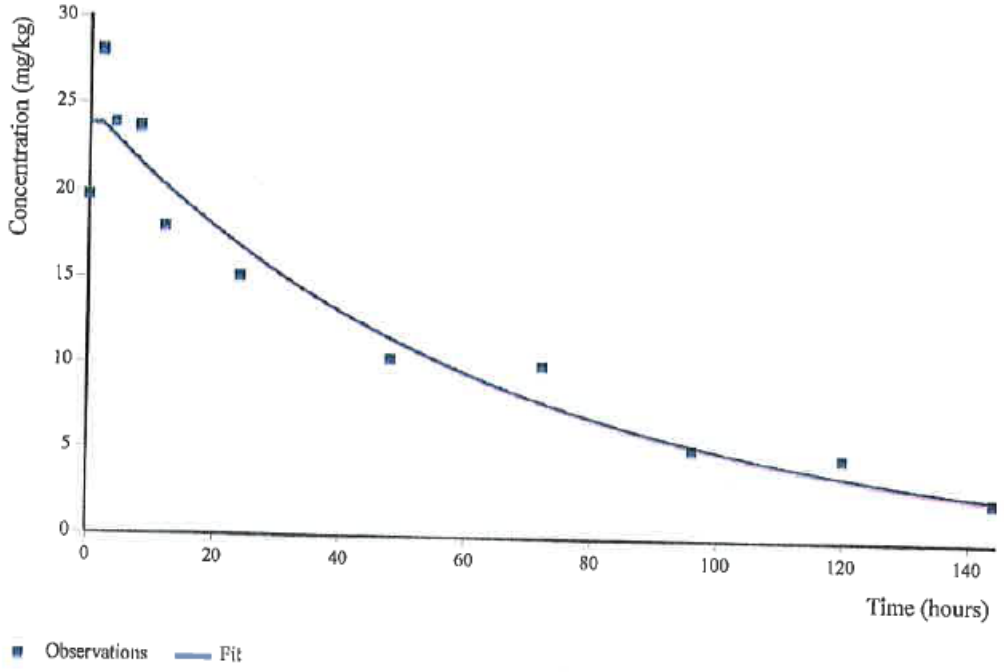
Used Extra Solver: No

Reference Table:

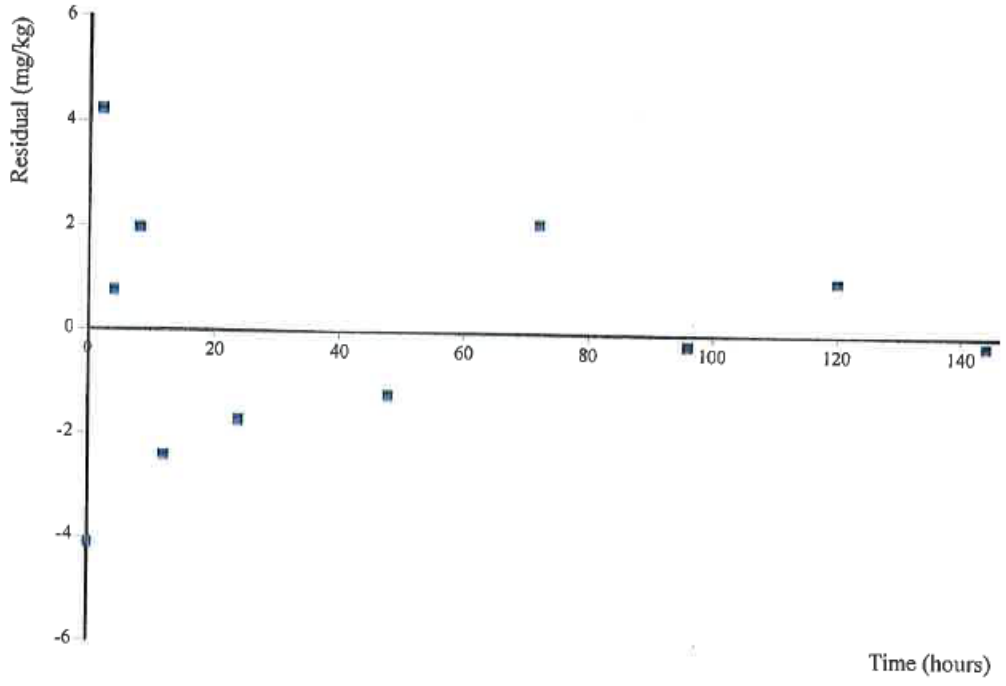
Compartment	Name
Parent	pyraclostrobin

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	19.8	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
tb_Parent	26.4	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	23.9	2.81	N/A	18.58	29.22	17.26	30.54
k1	0.001057	0.08423	0.4952	-0.1585	0.1606	-0.1981	0.2
k2	0.01565	0.002929	5.37E-004	0.0101	0.02119	0.008721	0.023
tb	2.015	9.311	N/A	-15.63	19.65	-20	24.03

χ_c

Parameter	Error %	Degrees of Freedom
All data	13.5	7
Parent	13.5	7

Decay Times:

Compartment	DT50 (overall hours)	DT90 (overall hours)	k1 DT50 (hours)	k2 DT50 (hours)
Parent	46.2	149	656	44.3

Additional Statistics:

Parameter	r_c (Obs v Pred)	Efficiency
All data	0.9276	0.9273
Parent	0.9276	0.9273

Parameter Correlation:

	Parent_0	k1	k2	tb
Parent_0	1	0.6623	-7.792E-05	-0.1836
k1	0.6623	1	0.03212	0.4799
k2	-7.792E-05	0.03212	1	0.3641
tb	-0.1836	0.4799	0.3641	1

Observed v. Predicted:

Compartment Parent

Time (hours)	Value (mg/kg)	Predicted Value	Residual
0	19.8	23.9	-4.101
2	28.1	23.85	4.25
4	23.9	23.12	0.7796

8	23.7	21.72	1.982
12	18	20.4	-2.4
24	15.2	16.91	-1.708
48	10.4	11.62	-1.215
72	10.1	7.979	2.121
96	5.27	5.481	-0.2111
120	4.82	3.765	1.055
144	2.41	2.587	-0.1765

Sequence Creation Information:
Fit generated by CAKE version 3.3 (Release)
running on R version 3.0.0 (2013-04-03)

Experiment 1 (FOMC)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	19.8	0 to (unbounded)	No
alpha_Parent	0.1	0 to (unbounded)	No
beta_Parent	0.01	0 to (unbounded)	No

Fit step: Final

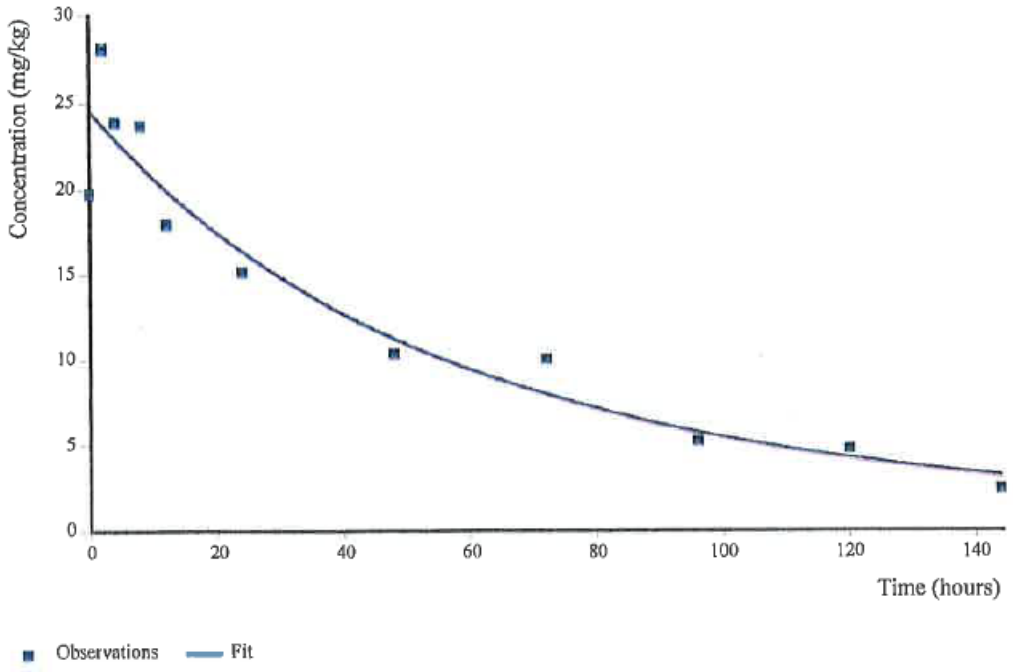
Used Extra Solver: No

Reference Table:

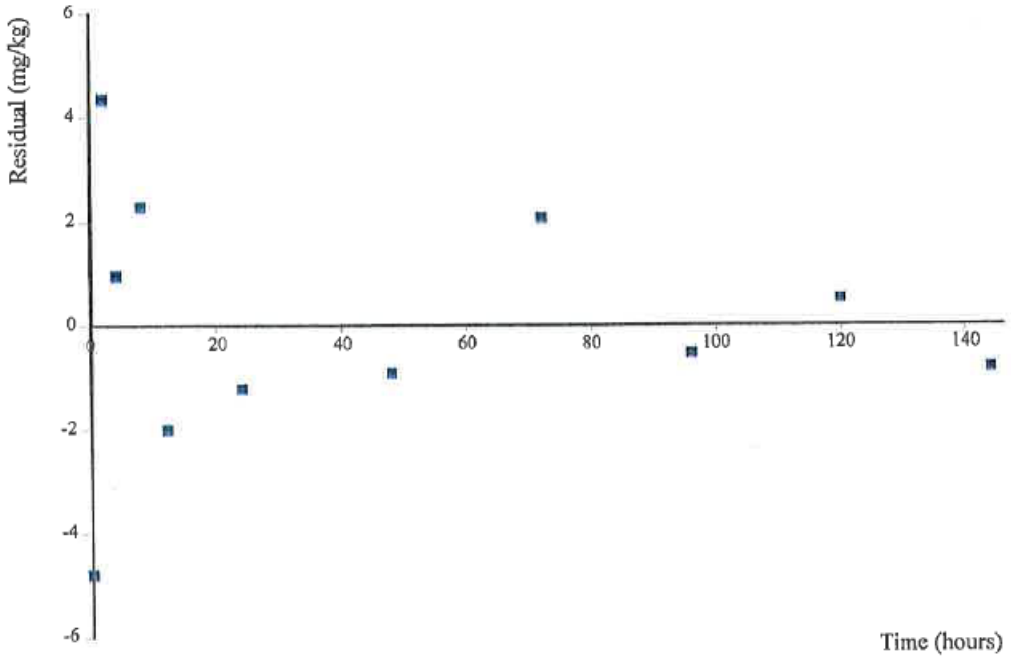
Compartment	Name
Parent	pyraclostrobin

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	19.8	0 to (unbounded)	No
alpha_Parent	0.1	0 to (unbounded)	No
beta_Parent	0.01	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	24.58	1.692	N/A	21.44	27.73	20.68	28.48
alpha	4.72	13.34	N/A	-20.09	29.53	-26.05	35.49
beta	268.9	869.3	N/A	-1348	1.89E+003	-1736	2.27E+003

χ_c

Parameter	Error %	Degrees of Freedom
All data	13.4	8
Parent	13.4	8

Decay Times:

Compartment	DT50 (hours)	DT90 (hours)
Parent	42.5	169

Additional Statistics:

Parameter	r_c (Obs v Pred)	Efficiency
All data	0.9209	0.9209
Parent	0.9209	0.9209

Parameter Correlation:

	Parent_0	alpha	beta
Parent_0	1	-0.5093	-0.5355
alpha	-0.5093	1	0.9984
beta	-0.5355	0.9984	1

Observed v. Predicted:

Compartment Parent

Time (hours)	Value (mg/kg)	Predicted Value	Residual
0	19.8	24.58	-4.781
2	28.1	23.74	4.364
4	23.9	22.93	0.9739
8	23.7	21.4	2.296
12	18	20	-2.003
24	15.2	16.42	-1.219

48	10.4	11.32	-0.921
72	10.1	8.021	2.079
96	5.27	5.818	-0.5477
120	4.82	4.307	0.5131
144	2.41	3.246	-0.8364

Sequence Creation Information:
Fit generated by CAKE version 3.3 (Release)
running on R version 3.0.0 (2013-04-03)

Report Information:
Report generated by CAKE version 3.3 (Release)
CAKE developed by Tessella Ltd, Abingdon, Oxfordshire, UK, sponsored by Syngenta
Running on .NET version 4.0.30319.42000

TRIAL 21SGS42

CAKE Kinetic Evaluation Report

Study: New Study

Study date: poniedziaŁ,ek, 27 czerwca 2022
Report generated: poniedziaŁ,ek, 27 czerwca 2022

Experiment 1 (SFO)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	14.43	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Fit step: Final

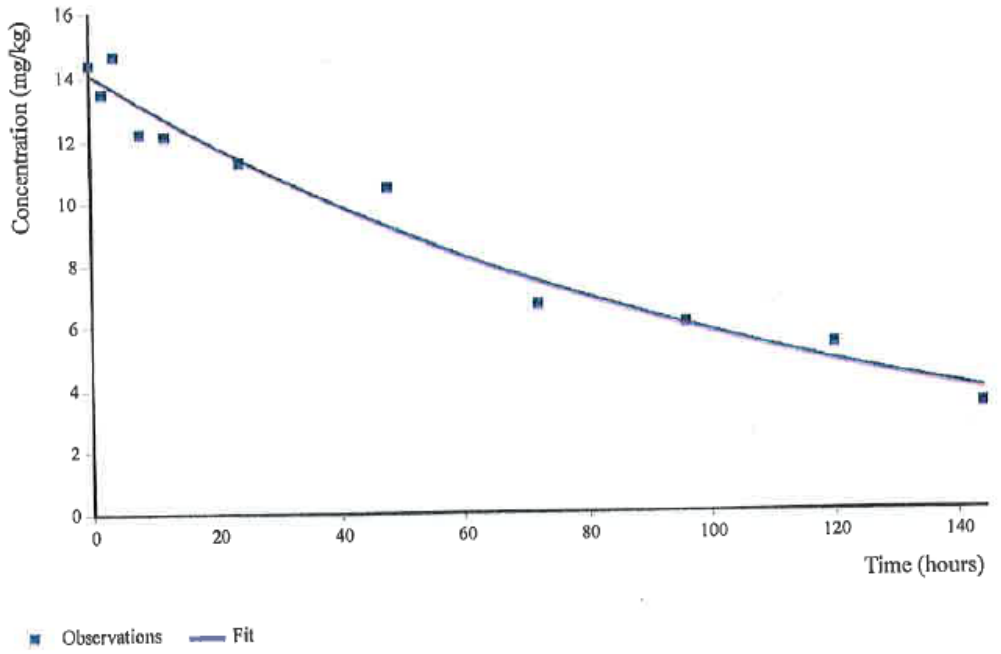
Used Extra Solver: No

Reference Table:

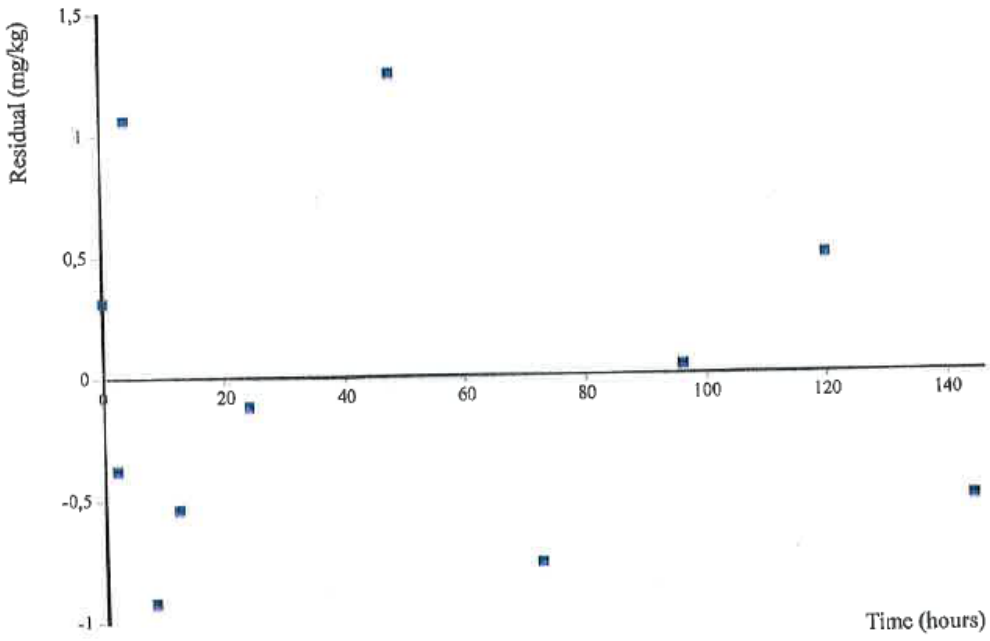
Compartment	Name
Parent	pyraclostrobin

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	14.43	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	14.11	0.3592	N/A	13.45	14.77	13.3	14.93
k_Parent	0.008804	7.11E-004	2.96E-007	0.0075	0.01011	0.007195	0.01

χ_c

Parameter	Error %	Degrees of Freedom
All data	5.52	9
Parent	5.52	9

Decay Times:

Compartment	DT50 (hours)	DT90 (hours)
Parent	78.7	262

Additional Statistics:

Parameter	r_c (Obs v Pred)	Efficiency
All data	0.9666	0.9666
Parent	0.9666	0.9666

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.5414
k_Parent	0.5414	1

Observed v. Predicted:

Compartment Parent

Time (hours)	Value (mg/kg)	Predicted Value	Residual
0	14.43	14.11	0.3179
2	13.49	13.87	-0.3758
4	14.69	13.62	1.066
8	12.23	13.15	-0.9224
12	12.16	12.7	-0.5373
24	11.31	11.42	-0.1143
48	10.5	9.248	1.252
72	6.72	7.487	-0.7669
96	6.1	6.061	0.03909

120	5.4	4.907	0.4935
144	3.46	3.972	-0.512

Sequence Creation Information:
Fit generated by CAKE version 3.3 (Release)
running on R version 3.0.0 (2013-04-03)

Experiment 1 (DFOP)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	14.43	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
g_Parent	0.5	0 to 1	No

Fit step: Final

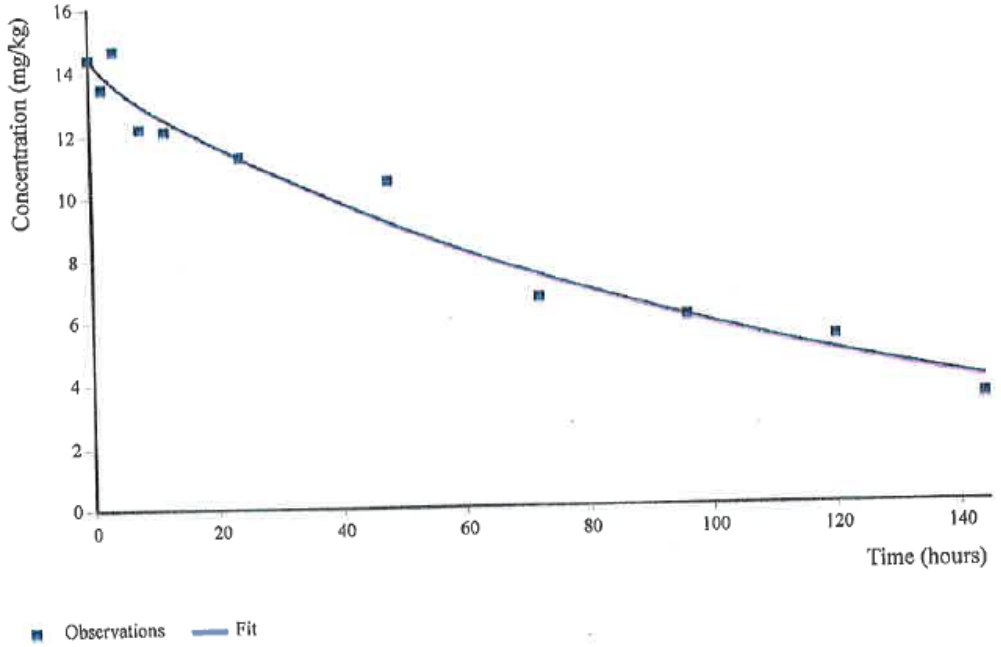
Used Extra Solver: No

Reference Table:

Compartment	Name
Parent	pyraclostrobin

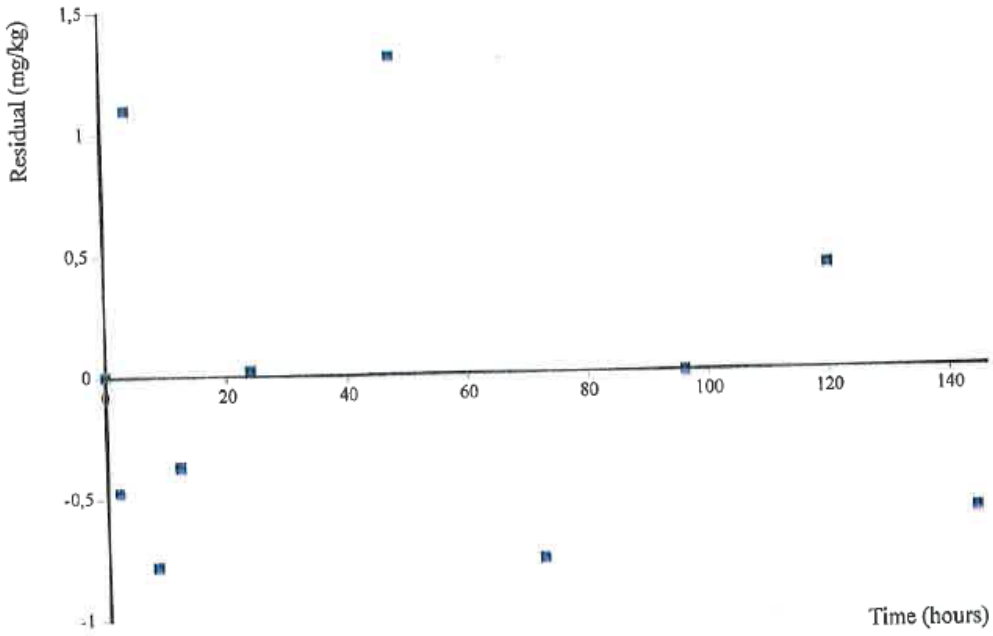
Graphical Summary:

Observations and Fitted Model:



Observations Fit

Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	14.43	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
g_Parent	0.5	0 to 1	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	14.42	0.8125	N/A	12.88	15.96	12.5	16.35
k1_Parent	0.2557	1.192	0.4181	-2.002	2.514	-2.562	3.074
k2_Parent	0.008549	0.001134	6.66E-005	0.0064	0.0107	0.005867	0.011
g_Parent	0.0395	0.07974	N/A	-0.1116	0.1906	-0.1491	0.228

χ_c

Parameter	Error %	Degrees of Freedom
All data	5.94	7
Parent	5.94	7

Decay Times:

Compartment	DT50 (overall hours)	DT90 (overall hours)	k1 DT50 (hours)	k2 DT50 (hours)
Parent	76.4	265	2.71	81.1

Additional Statistics:

Parameter	r_c (Obs v Pred)	Efficiency
All data	0.9678	0.9678
Parent	0.9678	0.9678

Parameter Correlation:

	Parent_0	k1_Parent	k2_Parent	g_Parent
Parent_0	1	0.4496	0.1089	0.5498
k1_Parent	0.4496	1	0.5659	-0.3296
k2_Parent	0.1089	0.5659	1	-0.6103
g_Parent	0.5498	-0.3296	-0.6103	1

Observed v. Predicted:

Compartment Parent

Time (hours)	Value (mg/kg)	Predicted Value	Residual
0	14.43	14.42	0.006269
2	13.49	13.96	-0.4708
4	14.69	13.59	1.097

8	12.23	13.01	-0.7818
12	12.16	12.53	-0.3697
24	11.31	11.29	0.02463
48	10.5	9.191	1.309
72	6.72	7.486	-0.766
96	6.1	6.097	0.00262
120	5.4	4.966	0.4337
144	3.46	4.045	-0.5851

Sequence Creation Information:
Fit generated by CAKE version 3.3 (Release)
running on R version 3.0.0 (2013-04-03)

Experiment 1 (HS)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	14.43	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
tb_Parent	Automatic	0 to (unbounded)	No

Fit step: Final

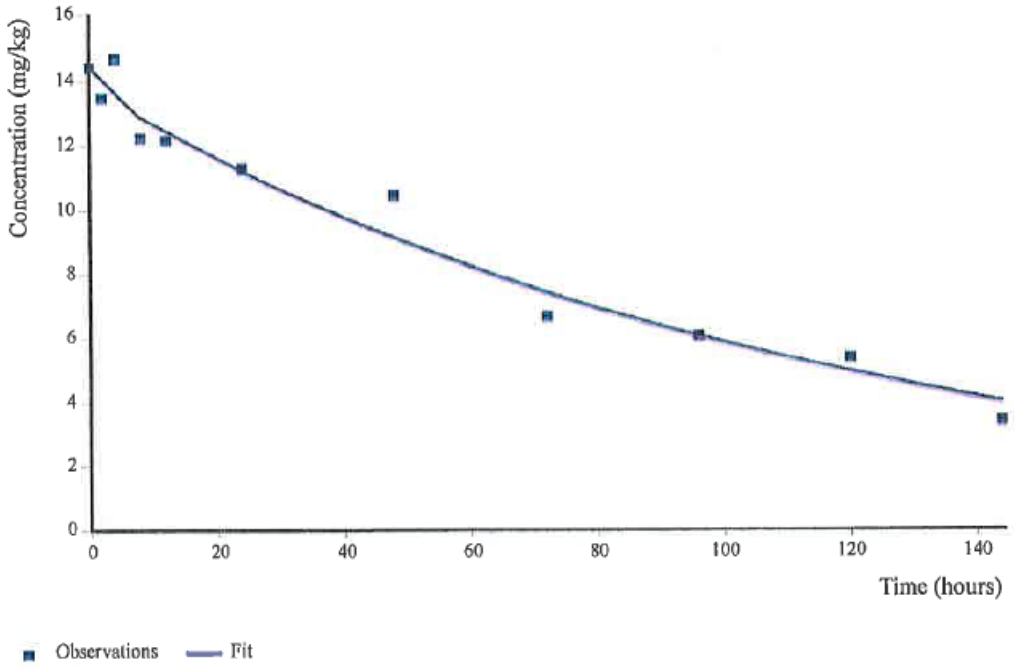
Used Extra Solver: No

Reference Table:

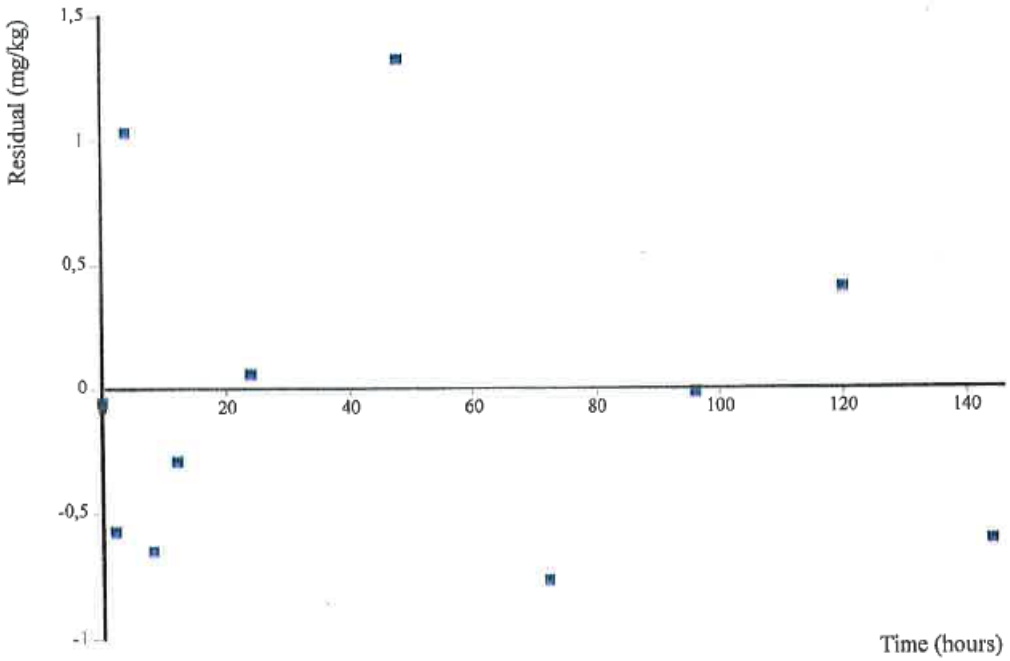
Compartment	Name
Parent	pyraclostrobin

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	14.43	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
tb_Parent	5.7	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	14.48	0.4979	N/A	13.54	15.42	13.3	15.66
k1	0.01466	0.002189	1.39E-004	0.01051	0.0188	0.00948	0.02
k2	0.008468	0.001064	4.72E-005	0.006452	0.01048	0.005952	0.011
tb	8.014	13.36	N/A	-17.29	33.32	-23.57	39.6

χ_c

Parameter	Error %	Degrees of Freedom
All data	5.81	7
Parent	5.81	7

Decay Times:

Compartment	DT50 (overall hours)	DT90 (overall hours)	k1 DT50 (hours)	k2 DT50 (hours)
Parent	76	266	47.3	81.9

Additional Statistics:

Parameter	r_c (Obs v Pred)	Efficiency
All data	0.9692	0.9691
Parent	0.9692	0.9691

Parameter Correlation:

	Parent_0	k1	k2	tb
Parent_0	1	0.4252	-0.137	0.5427
k1	0.4252	1	-0.2159	0.9765
k2	-0.137	-0.2159	1	-0.332
tb	0.5427	0.9765	-0.332	1

Observed v. Predicted:

Compartment Parent

Time (hours)	Value (mg/kg)	Predicted Value	Residual
0	14.43	14.48	-0.05006
2	13.49	14.06	-0.5718
4	14.69	13.66	1.034

8	12.23	12.88	-0.6491
12	12.16	12.45	-0.2882
24	11.31	11.25	0.06468
48	10.5	9.177	1.323
72	6.72	7.489	-0.7692
96	6.1	6.112	-0.01178
120	5.4	4.988	0.4123
144	3.46	4.07	-0.6104

Sequence Creation Information:
Fit generated by CAKE version 3.3 (Release)
running on R version 3.0.0 (2013-04-03)

Experiment 1 (FOMC)

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05)
SANN Max Iterations: 10000
Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	14.43	0 to (unbounded)	No
alpha_Parent	0.1	0 to (unbounded)	No
beta_Parent	0.01	0 to (unbounded)	No

Fit step: Final

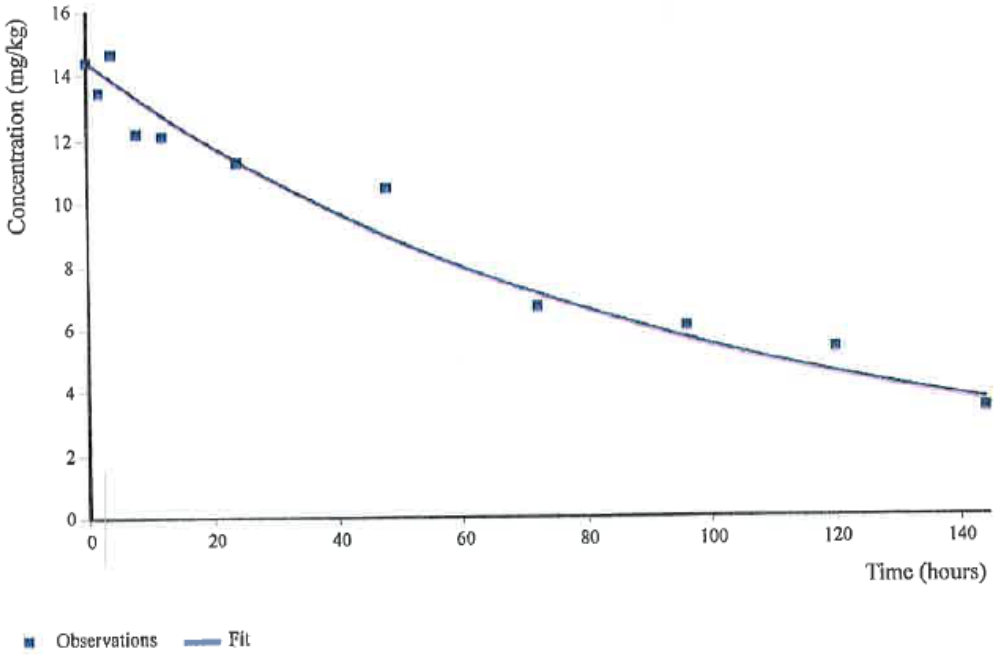
Used Extra Solver: Yes

Reference Table:

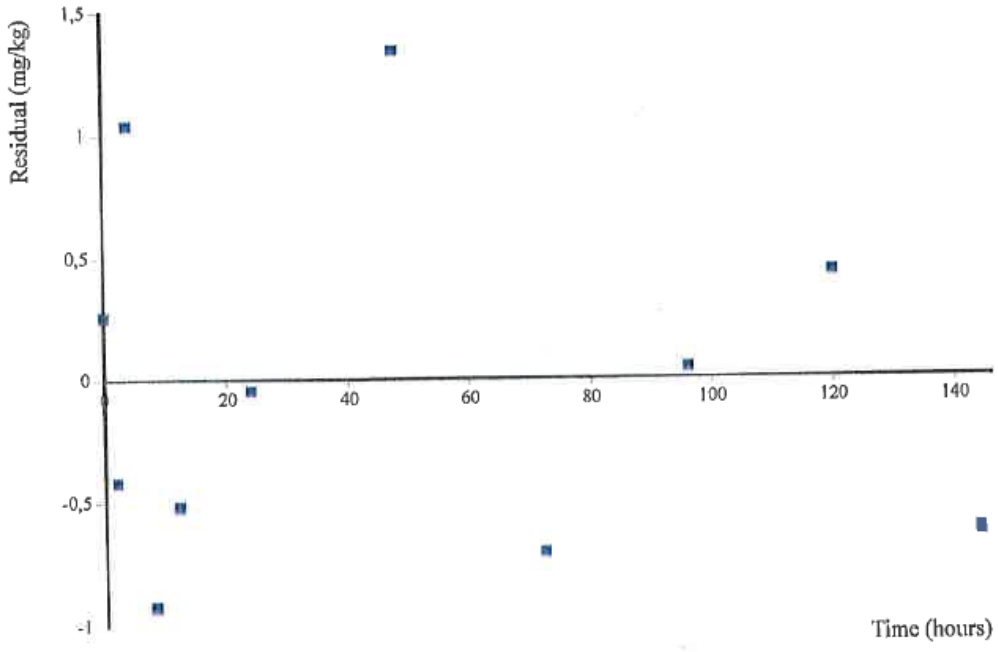
Compartment	Name
Parent	pyraclostrobin

Graphical Summary:

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	14.43	0 to (unbounded)	No
alpha_Parent	0.1	0 to (unbounded)	No
beta_Parent	0.01	0 to (unbounded)	No

Estimated Values:

Parameter	Value	σ	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	14.46	0.4136	N/A	13.69	15.23	13.51	15.41
alpha	8.549	nd	N/A	nd	nd	nd	nd
beta	840	nd	N/A	nd	nd	nd	nd

Note: Errors and T-test values could not be calculated because the covariance matrix could not be created.

χ_c

Parameter	Error %	Degrees of Freedom
All data	5.84	8
Parent	5.84	8

Decay Times:

Compartment	DT50 (hours)	DT90 (hours)
Parent	71	260

Additional Statistics:

Parameter	r_c (Obs v Pred)	Efficiency
All data	0.9657	0.9657
Parent	0.9657	0.9657

Parameter Correlation:

	Parent_0	alpha	beta
Parent_0	1	nd	nd
alpha	nd	1	nd
beta	nd	nd	1

Observed v. Predicted:

Compartment Parent

Time (hours)	Value (mg/kg)	Predicted Value	Residual
0	14.43	14.17	0.2626
2	13.49	13.91	-0.4151
4	14.69	13.65	1.042
8	12.23	13.15	-0.9207

12	12.16	12.67	-0.5134
24	11.31	11.36	-0.04465
48	10.5	9.156	1.344
72	6.72	7.425	-0.7051
96	6.1	6.054	0.04556
120	5.4	4.962	0.4376
144	3.46	4.087	-0.6274

Sequence Creation Information:
Fit generated by CAKE version 3.3 (Release)
running on R version 3.0.0 (2013-04-03)

Report Information:
Report generated by CAKE version 3.3 (Release)
CAKE developed by Tessella Ltd, Abingdon, Oxfordshire, UK, sponsored by Syngenta
Running on .NET version 4.0.30319.42000

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

No additional studies were performed.

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

No additional studies were performed.

A 2.1.3 KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

No additional studies were performed.

A 2.2 KCP 10.2 Effects on aquatic organisms

A 2.2.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

A 2.2.1.1.1 Study 1

Comments of zRMS:	The study was conducted to OECD guideline 201 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment. The results refer to nominal concentrations of formulation.

Reference: KCP 10.2/01

Report *Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), Growth inhibition test*, M. Czarnecka; Institute of Industrial Organic Chemistry Branch

	Pszczyna Department of Ecotoxicology, Pszczyna, Poland; STUDY CODE: W-24-20
Guideline(s):	OECD Guideline No. 201 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC); batch no. 04/2020, the (determined) content of pyraclostrobin: 256.6 g/L; density at 20°C: 1.0623 g/cm^3; manufacturing date: April 23, 2020, expiry date: April 23, 2022.
Test Species:	The unicellular freshwater green algae, Raphidocelis subcapitata (formerly Pseudokirchneriella subcapitata (Korshikov) Hindák, Selenastrum capricornutum Prinz) SAG 61.81 cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Department of Ecotoxicological Studies, Laboratory of Aquatic Organisms Toxicology. The algae were obtained from the Culture Collection of Algae at Göttingen University, Germany.
Test Design:	72 hours of exposure; three replicates per each test item concentration; six replicates per control; a background for each treatment; initial algal cell density: 1 x 10^4 cells/mL..
Endpoints:	ErC50/72 h, EyC50/72 h, NOEC/72 h, LOEC/72 h.
Test Concentration:	10, 4, 1.6, 0.64, 0.256 mg/L plus the control.
Test Conditions:	Temperature: 22.0 – 22.8°C; pH of the control: 7.55 – 8.21; mean light intensity: 6193 – 6400 lux; constant illumination and shaking; medium: AAP.

Results and discussion
The endpoint values based on nominal test item concentrations are given below:
The ErC50/72 h value is 2.68 mg/L (95% confidence interval: 2.45 – 2.92).
The EyC50/72 h value is 1.20 mg/L (95% confidence interval: 0.99 – 1.46).
The LOEC/72 h value for yield is lower than or equal to 0.256 mg/L.
The NOEC/72 h value for yield is lower than 0.256 mg/L.

Preliminary test (non-GLP)
The recorded temperature was in the range of 20.8 – 22.8°C. The mean light intensity was in the range of 6185 – 6220 lux. The pH values were in the ranges of 7.27 – 7.33 at exposure initiation and 7.89 – 8.32 at exposure termination.

Nominal test item concentration [mg/L]	pH values# at exposure initiation	pH values* at exposure termination
Control	7.33	8.32
0.1	7.27	8.10
1.0	7.28	8.02
10	7.27	7.89

The average transmittance values were between 93.9 – 99.9% at exposure initiation and 98.8 – 100.8% at exposure termination when compared with the control [SOP/W/12]. No growth rate inhibition was observed in the test item concentrations of 0.1 mg/L. The growth rate inhibition after 72 hours of exposure was 3.17% and 80.60% in the test item concentrations of 1.0 and 10 mg/L, respectively. No yield inhibition was observed in the test item concentrations of 0.1 mg/L. The yield inhibition after 72 hours of exposure was 14.66% and 98.08% in the test item concentrations of 1.0 and 10 mg/L, respectively. The inhibition of growth rate and yield estimated in comparison to the control after 72 hours of exposure are given.

Nominal test item concentration [mg/L]	% inhibition after 72 h of exposure (growth rate)	% inhibition after 72 h of exposure (yield)
Control	0.0	0.0
0.1	-3.49*	-13.90*
1.0	3.17	14.66
10	80.60	98.08

Definitive test

Morphology observations of the algae cells were performed at exposure termination. In the test item concentrations of 0.256, 0.64, 1.6, and 4 mg/L, no differences in shape, size and colour of algal cells were reported as compared to the algae cells in the control. In the test item concentration of 10 mg/L, deformed cells were observed as compared to the algae cells in the control.

Nominal test item concentration [mg/L]	Observations
Control	Normal colour, shape and size of the algal cells
0.256	No changes
0.64	No changes
1.6	No changes
4	No changes
10	Deformed cells

The average transmittance values were in the range of 93.0 – 100.0% at exposure initiation and in the range of 99.9 – 100.4% at exposure termination when compared with the control.

Nominal test item concentration [mg/L]	Mean transmittance [%]	
	at exposure initiation	at exposure termination
Control	100.0	100.0
0.256	100.0	100.0
0.64	99.5	100.4
1.6	99.4	100.1
4	98.9	100.0
10	93.0	99.9

Hence, the indirect method was adequate to determine the number of algal cells. The average specific growth rates and yield were calculated based on the numbers of cells recalculated from absorbance values measured at 24, 48 and 72 h for the cel number based on the standard curve. Absorbance values recalculated for algal cell density are presented in the table below.

Nominal test item concentration [mg/L]	Average absorbance measured during exposure in each replicate, background values subtracted			Algal cell density [x10^6 cells/mL] recalculated according to the standard curve formula*		
	24 h	48 h	72 h	24 h	48 h	72 h
Control	0.020	0.092	0.421	0.054	0.248	1.133
	0.020	0.094	0.429	0.054	0.253	1.155
	0.018	0.085	0.390	0.048	0.229	1.050
	0.022	0.091	0.422	0.059	0.245	1.136
	0.018	0.070	0.360	0.048	0.188	0.969
	0.019	0.071	0.340	0.051	0.191	0.915
mean	**0.020**	**0.084**	**0.394**	**0.052**	**0.226**	**1.060**
standard deviation	*0.002*	*0.011*	*0.037*	*0.004*	*0.029*	*0.100*
0.256	0.016	0.076	0.360	0.043	0.205	0.969
	0.013	0.063	0.300	0.035	0.170	0.808
	0.014	0.071	0.340	0.038	0.191	0.915
mean	**0.014**	**0.070**	**0.333**	**0.039**	**0.189**	**0.897**
standard deviation	*0.002*	*0.007*	*0.031*	*0.004*	*0.018*	*0.082*
0.64	0.018	0.065	0.294	0.048	0.175	0.791
	0.016	0.059	0.280	0.043	0.159	0.754
	0.015	0.064	0.310	0.040	0.172	0.834
mean	**0.016**	**0.063**	**0.295**	**0.044**	**0.169**	**0.793**
standard deviation	*0.002*	*0.003*	*0.015*	*0.004*	*0.009*	*0.040*
1.6	0.017	0.047	0.206	0.046	0.127	0.555
	0.015	0.042	0.170	0.040	0.113	0.458
	0.010	0.037	0.172	0.027	0.100	0.463
mean	**0.014**	**0.042**	**0.183**	**0.038**	**0.113**	**0.492**
standard deviation	*0.004*	*0.005*	*0.020*	*0.010*	*0.014*	*0.055*
4	0.009	0.007	0.017	0.024	0.019	0.046
	0.007	0.007	0.007	0.019	0.019	0.019
	0.007	0.003	0.010	0.019	0.008	0.027
mean	**0.008**	**0.006**	**0.011**	**0.021**	**0.015**	**0.031**
standard deviation	*0.001*	*0.002*	*0.005*	*0.003*	*0.006*	*0.014*
10	0.004	0.003	0.001	0.011	0.008	0.003
	0.004	0.001	0.002	0.011	0.003	0.005
	0.005	0.001	0.000	0.013	0.003	0.000
mean	**0.004**	**0.002**	**0.001**	**0.012**	**0.005**	**0.003**
standard deviation	*0.001*	*0.001*	*0.001*	*0.001*	*0.003*	*0.003*

The average section-by-section specific growth rates and yield calculated for the whole exposure are provided in the table below.

Nominal test item concentration [mg/L]	Growth rate* [10^6 cells/mL]				Yield** [10^6 cells/mL]
	0-24 h	24-48 h	48-72 h	0-72 h	72 h
Control	1.686	1.524	1.519	1.577	1.123
	1.686	1.544	1.518	1.583	1.145
	1.569	1.563	1.523	1.551	1.040
	1.775	1.424	1.534	1.578	1.126
	1.569	1.365	1.640	1.525	0.959
	1.629	1.320	1.567	1.505	0.905
mean	**1.652**	**1.457**	**1.550**	**1.553**	**1.050**
standard deviation	*0.080*	*0.101*	*0.048*	*0.032*	*0.100*
0.256	1.459	1.562	1.553	1.525	0.959
	1.253	1.580	1.559	1.464	0.798
	1.335	1.615	1.567	1.505	0.905
mean	**1.349**	**1.586**	**1.560**	**1.498**	**0.887**
standard deviation	*0.104*	*0.027*	*0.007*	*0.031*	*0.082*
0.64	1.569	1.294	1.509	1.457	0.781
	1.459	1.308	1.556	1.441	0.744
	1.386	1.459	1.579	1.475	0.824
mean	**1.471**	**1.353**	**1.548**	**1.457**	**0.783**
standard deviation	*0.092*	*0.091*	*0.036*	*0.017*	*0.040*
1.6	1.526	1.016	1.475	1.339	0.545
	1.386	1.039	1.399	1.275	0.448
	0.993	1.309	1.533	1.278	0.453
mean	**1.302**	**1.121**	**1.469**	**1.297**	**0.482**
standard deviation	*0.276*	*0.163*	*0.067*	*0.036*	*0.055*
4	0.875	n.d.	0.884	0.509	0.036
	0.642	0.000	0.000	0.214	0.009
	0.642	n.d.	1.216	0.331	0.017
mean	**0.720**	**n.d.**	**0.700**	**0.351**	**0.021**
standard deviation	*0.135*	*n.d.*	*0.629*	*0.148*	*0.014*
10	0.095	n.d.	n.d.	n.d.	n.d.
	0.095	n.d.	0.511	n.d.	n.d.
	0.262	n.d.	n.d.	n.d.	n.d.
mean	**0.151**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
standard deviation	*0.096*	*n.d.*	*n.d.*	*n.d.*	*n.d.*

The relationship between the inhibition of growth rate and the nominal test item concentrations at 72 h is provided in the table below.

Nominal test item concentration [mg/L]	[%] inhibition after 72 h of exposure (growth rate)	[%] inhibition after 72 h of exposure (yield)
Control	0.0	0.0
0.256	3.5	15.5
0.64	6.2	25.4
1.6	16.5	54.1
4	77.4	98.0
10	130.0*	100.7*

Results of chemical determinations

The concentrations of pyraclostrobin were determined using with the validated high performance liquid chromatographic method with DAD detection [SOP/C/164]. Samples of each treatment were collected at exposure initiation and at exposure termination. At exposure initiation, the determined concentrations of pyraclostrobin were in the range of 99.0 – 116.6% of the nominal concentration. The results confirm that the test item concentrations were prepared correctly. At exposure termination, the determined concentrations of pyraclostrobin were in the range of 82.9 – 92.7% of the nominal concentration. Therefore, the concentrations of pyraclostrobin were stable under test conditions.

Nominal test item concentration [mg/L]	Nominal concentration of pyraclostrobin [mg/L]	Average determined concentration of pyraclostrobin (n=3) in samples collected			
		at exposure initiation [mg/L]	[%] of nominal concentration	at exposure termination [mg/L]	[%] of nominal concentration
Control	-	< LoD	-	< LoD	-
0.256	0.062	0.0637	102.7	0.0572	92.3
0.64	0.155	0.1535	99.0	0.1285	82.9
1.6	0.387	0.4065	105.0	0.3587	92.7
4	0.966	1.045	108.2	0.887	91.8
10	2.416	2.816	116.6	2.125	88.0

Endpoint value

The endpoint values are presented in the tables below.

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_rC_{50}	3.22 (2.36 – 4.43)	2.33 (2.07 – 2.65)	2.68 (2.45 – 2.92)
E_rC_{20}	1.34 (0.67 – 1.90)	1.52 (1.25 – 1.74)	1.70 (1.47 – 1.90)
E_rC_{10}	0.85 (0.32 – 1.33)	1.22 (0.94 – 1.44)	1.34 (1.11 – 1.55)
LOEC	≤0.256	≤0.256	n.d.
NOEC	<0.256	<0.256	n.d.

Endpoint value [mg/L]	Time of exposure:		
	24 h	48 h	72 h
E_yC_{50}	1.70 (0.96 – 3.03)	1.21 (0.97 – 1.50)	1.20 (0.99 – 1.46)
E_yC_{20}	0.44 (0.10– 0.81)	0.49 (0.32 – 0.65)	0.52 (0.36 – 0.67)
E_yC_{10}	0.21 (0.03 – 0.48)	0.31 (0.17 – 0.44)	0.34 (0.20 – 0.46)
LOEC	≤0.256	≤0.256	≤0.256
NOEC	<0.256	<0.256	<0.256

Test validity criteria
In the definitive test, the following validity criteria specified in the OECD Guideline No. 201 (2006) were met:
- the biomass in the control increased by a factor of 106.0 within the 72-hour test period (criterion: at least a 16-fold growth),
- the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 2.1% (criterion: it must not exceed 7%),
- the mean coefficient of variation for the section-by-section growth rate in the control culture was 7.5% (criterion: it must not exceed 35%).

A 2.2.1.1.2 Study 2

Comments of zRMS:	The study was conducted to OECD guideline 202 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment. The results refer to nominal concentrations of formulation.

Reference:	KCP 10.2/02
Report	*Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC), Daphnia magna, Acute Immobilisation Test*, M. Czarnecka; Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland; STUDY CODE: W-23-20
Guideline(s):	OECD Guideline No. 202 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC); batch no. 04/2020, the (determined) content of pyraclostrobin: 256.6 g/L; density at 20°C: 1.0623 g/cm^3; manufacturing date: April 23, 2020, expiry date: April 23, 2022.
Test Species:	Daphnia magna Straus (< 24 h old at exposure initiation); not first brood progeny; neonates collected from a laboratory culture cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna.
Test Design:	Semi-static test (48 h of exposure with renewal after 24h); 4 replicates per each test item concentration and the control; 5 Daphnia magna in each replicate.
Endpoints:	EC50/48 h, NOEC/48 h, and LOEC/48 h.
Test Concentration:	1.0, 0.5, 0.25, 0.125, 0.063, 0.031, 0.016, and 0.008 mg/L plus the control.

Test Conditions: Temperature: 20.5 – 21.2°C; pH of the control: 7.53 – 8.07; dissolved oxygen concentration in the control: 8.5 – 9.4 mg/L; daily cycle 16 h light : 8 h dark; fluorescent light source; no feeding; no aeration; medium: Elendt M7.

Results and discussion

The endpoint values based on nominal test item concentrations are given below:
The EC_{50}/48 h value is 0.177 mg/L (95% confidence interval: 0.149 – 0.210).
The LOEC/48 h is 0.125 mg/L
The NOEC/48 h is 0.063 mg/L

Preliminary test (non–GLP)

In the test, the recorded temperature was in a range of 20.3 – 21.8°C. The pH values measured in the test item concentrations and the control were in the range of 7.42 – 7.51 at exposure initiation and in the range of 7.49 – 7.57 at exposure termination. The measured dissolved oxygen concentrations in the test item concentrations and the control were 8.8 mg/L at exposure initiation and in the range of 8.1 – 8.5 mg/L at exposure termination (Table 4).

Table 4. Immobilisation of *Daphnia magna*, preliminary test (non-GLP)

Nominal test item concentration [mg/L]	Number of *Daphnia magna*	Number of immobilised *Daphnia magna*								Total of immobilised *Daphnia magna* [%]	
		24 h				48 h					
		Replicates									
		A	B	C	D	A	B	C	D	24 h	48 h
Control	20	1	0	0	0	1	0	0	0	5	5
0.01	20	0	0	0	0	0	0	0	0	0	0
0.1	20	0	0	2	0	1	1	2	3	10	35
1.0	20	5	5	5	5	5	5	5	5	100	100

Time of exposure: 28.05.2020 – 30.05.2020

In the preliminary test, in the test item concentration of 0.01 mg/L, no immobilisation of Daphnia magna was observed during exposure. At exposure termination, in the control, the immobilisation of Daphnia magna was 5%, whereas in the test item concentrations of 0.1 and 1.0 mg/L, the immobilisation of Daphnia magna was 35 and 100%, respectively (Table 5).

Table 5. Concentration and stability of pyraclostrobin, preliminary test (non-GLP)

Nominal test item concentration [mg/L]	Average determined concentration of pyraclostrobin (n=3) in samples collected [mg/L]					
	at exposure initiation	% of nominal concentration	after 24 h of exposure	% of nominal concentration	at exposure termination	% of nominal concentration
Control	<LoD	–	<LoD	–	<LoD	–
0.1	0.1045	104.5	0.0941	94.1	0.0843	84.3

Definitive test

The measured pH values were in the ranges of 7.70 – 8.04 in fresh test item concentrations and the control at exposure initiation and during the renewal, and in the range of 7.53 – 8.07 in spent test item concentrations and the control at exposure termination and during the renewal. The measured dissolved oxygen concentrations were in the ranges of 8.9 – 9.5 mg/L in fresh test item concentrations and the

control at exposure initiation and during the renewal, and in the range of 8.3 – 9.0 mg/L in spent test item conce trations and the control at exposure termination and during the renewal (Table 6).

Table 6. pH values and dissolved oxygen concentrations, definitive test

Nominal test item concentration [mg/L]	pH values				Dissolved oxygen concentrations [mg/L]			
	at exposure initiation	after 24 h of exposure		at exposure termination	at exposure initiation	after 24 h of exposure		at exposure termination
	fresh#	spent*	fresh#	spent*	fresh#	spent*	fresh#	spent*
Control	7.71	8.07	8.01	7.53	9.1	8.5	9.4	9.0
0.008	7.73	8.05	8.04	7.61	9.0	8.6	9.3	8.9
0.016	7.70	8.07	8.04	7.64	8.9	8.3	9.3	9.0
0.031	7.71	8.04	8.01	7.66	9.3	8.5	9.3	8.9
0.063	7.72	8.07	8.00	7.66	9.1	8.3	9.5	9.0
0.125	7.73	8.04	8.01	7.66	9.4	8.4	9.3	9.0
0.25	7.77	8.02	7.99	7.69	8.9	8.6	9.4	8.9
0.5	7.77	8.04	7.98	7.66	9.4	8.3	9.4	8.9
1.0	7.80	8.06	–	–	9.1	8.3	–	–

#- pH values and dissolved oxygen concentrations measured in samples before split up into replicates
*- pH values and dissolved oxygen concentrations measured in samples of pooled replicates
– no value

In the control and the test item concentrations of 0.008, 0.016, 0.031, and 0.063 mg/L, no immobilisation of Daphnia magna was observed during exposure. At exposure termination in the test item concentrations of 0.125 and 0.25 mg/L, the immobilisation of Daphnia magna was 15 and 85%, respectively. In the test item concentrations of 0.5 and 1.0 mg/L, the immobilisation of Daphnia magna was 100%. No abnormal behaviour of Daphnia magna was observed during exposure. The immobilisation of Daphnia magna after 24 h and 48 h of exposure is given in Table 7.

Table 7. Immobilisation of *Daphnia magna*, definitive test

Nominal test item concentration [mg/L]	Number of *Daphnia magna*	Number of immobilised *Daphnia magna*								Total of immobilised *Daphnia magna* [%]	
		24 h				48 h					
		Replicates									
		A	B	C	D	A	B	C	D	24 h	48 h
Control	20	0	0	0	0	0	0	0	0	0	0
0.008	20	0	0	0	0	0	0	0	0	0	0
0.016	20	0	0	0	0	0	0	0	0	0	0
0.031	20	0	0	0	0	0	0	0	0	0	0
0.063	20	0	0	0	0	0	0	0	0	0	0
0.125	20	0	0	0	0	1	1	1	0	0	15
0.25	20	0	1	2	1	4	5	4	4	20	85
0.5	20	5	5	4	5	5	5	5	5	95	100
1.0	20	5	5	5	5	5	5	5	5	100	100

Endpoint values
The endpoint values were determined based on the nominal test item concentrations. The endpoint values were calculated with a probit method. The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were estimated on the basis of statistical analysis. To make calculations and to conduct statistical analysis, the ToxRat Professional commercial software was used [SOP/W/68]. The endpoint values are presented in Table 9.

Table 9. Endpoint values based on the nominal test item concentrations, definitive test

Endpoint values [mg/L]	Time of exposure	
	24 h	48 h
EC_{50}	0.316 (0.271 – 0.373)	0.177 (0.149 – 0.210)
EC_{20}	0.250 (0.196 – 0.290)	0.134 (0.102 – 0.158)
EC_{10}	0.222 (0.162 – 0.261)	0.116 (0.081 – 0.139)
LOEC	0.250	0.125
NOEC	0.125	0.063

Calculations were made according to [7], [SOP/W/68].
(-) - 95% confidence interval

The median concentration causing 50% immobilisation of Daphnia magna after 24 h of exposure, i.e. the EC50/24 h value is 0.316 mg/L (95% confidence interval 0.271 – 0.373). The EC20/24 h value is 0.250 mg/L (95% confidence interval 0.196 – 0.290). The EC10/24 h value is 0.222 mg/L (95% confidence interval 0.162 – 0.261). The median concentration causing 50% immobilisation of Daphnia magna after 48 h of exposure, i.e. the EC50/48 h value is 0.177 mg/L (95% confidence interval 0.149 – 0.210). The EC20/48 h value is 0.134 mg/L (95% confidence interval 0.102 – 0.158). The EC10/48 h value is 0.116 mg/L (95% confidence interval 0.081 – 0.139). The data on immobilisation of the Daphnia magna at exposure termination were analysed using Step-down Cochran-Armitage Test Procedure, which showed significant differences between the nominal test item concentrations in the range of 0.125 – 1.0 mg/L and the control. Therefore, the LOEC/48 h value was 0.125 mg/L and the NOEC/48 h value was 0.063 mg/L.

Test validity criteria
In the definitive test, the validity criteria were met according to the OECD Guideline No. 202 (2004) and EU Method C.2.:
- the percentage of immobilisation of Daphnia magna in the control was 0% (criterion: not more than 10%),
- the dissolved oxygen concentrations in the test vessels were within the range of 8.3 – 9.5 mg/L (criterion: not less than 3 mg/L).

A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

No additional studies were performed.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

No additional studies were performed.

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:	The study was conducted to OECD guideline 213 and according to the principles of GLP. No deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.

Reference	KCP 10.3.1/01
Report	*Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC), Honeybees (Apis mellifera L.), Acute Oral Toxicity Test*, M. Stalmach; Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland; STUDY CODE: B-76-20
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 213 (1998) and the EU Method C.16. (2008)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) content: 253.1 g/L of pyraclostrobin batch no.: 04/2020 production date: 23.04.2020 expiry date: 23.04.2022
Test Species:	the honeybee, Apis mellifera L., strain: carnica – age: approximately 3 weeks – source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna,
Test Design:	– the test item: - exposure duration: 96 hours - number of doses: 5 doses and a control - number of replicates: 3 replicates - number of bees: 10 bees/replicate – the reference item:

- exposure duration: 24 hours
- number of doses: 3 doses
- number of replicates: 3 replicates
- number of bees: 10 bees/replicate \

Endpoints: – honeybee mortality after 24, 48, 72 and 96 hours of the exposure,
– the oral LD50 of the test item after 24, 48, 72 and 96 hours of the exposure,
– the oral LD50/24 h of the reference item (dimethoate).

Test Concentration: 12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee and a control (0.0 µg/bee)

Test Conditions: temperature:
24.0 - 27.0°C
– relative air humidity:
58.0 - 64.0%

Preliminary test
Mortality results obtained in the preliminary experiment are presented in Tables 1 and 2. Mortality of the control group after 48 hours of exposure was 0.0%. After 48 hours the percentages of mortality of the bees treated with the test item at the doses of 8.0, 40.0 and 200.0 µg/honeybee were 20.0, 10.0 and 30.0%, respectively. No abnormal behavioural effects were observed during the test.

Definitive test
Mortality of the treated insects is presented in the tables below. They contain raw data which were then converted to percentages in order to calculate the LD50.
After 4 hours of exposure, mortality of the control group was 0.0% and for the treated groups' mortality percentages at the doses 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee, were 3.3, 6.7, 13.3, 3.3 and 13.3%, respectively (Table 3).

Table 3. Honeybee mortality after 4 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality				
		Number of dead bees [no.] replicates			Total	
		I	II	III	[no.]	[%]
0.0 (control)	30	0	0	0	0	0.0
12.5	30	1	0	0	1	3.3
25.0	30	1	0	1	2	6.7
50.0	30	1	2	1	4	13.3
100.0	30	1	0	0	1	3.3
200.0	30	0	2	2	4	13.3

After 24 hours of exposure, mortality of the control group was 0.0% and for the treated groups' mortality percentages at the doses 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee, were 13.3, 10.0, 26.7, 60.0 and 96.7%, respectively (Table 4).

Table 4. Honeybee mortality and the LD_{50} after 24 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality					LD_{50} [µg/bee]
		Number of dead bees [no.] replicates			Total		
		I	II	III	[no.]	[%]	
0.0 (control)	30	0	0	0	0	**0.0**	**67.2*** *(20.9 – 482.7)*
12.5	30	2	2	0	4	**13.3**	
25.0	30	2	0	1	3	**10.0**	
50.0	30	5	2	1	8	**26.7**	
100.0	30	3	6	9	18	**60.0**	
200.0	30	9	10	10	29	**96.7**	

*: oral LD_{50} value (with 95% confidence limits) was estimated with the log-probit method (ToxRat Proffesional 3.3.0 computer software), [9]

After 48 hours of exposure, mortality of the control group was 0.0% and for the treated groups' mortality percentages at the doses 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee, were 20.0, 20.0, 43.3, 66.7 and 100.0%, respectively (Table 5).

Table 5. Honeybee mortality and the LD_{50} after 48 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality					LD_{50} [µg/bee]
		Number of dead bees [no.] replicates			Total		
		I	II	III	[no.]	[%]	
0.0 (control)	30	0	0	0	0	**0.0**	**48.7*** *(16.0 – 142.6)*
12.5	30	2	3	1	6	**20.0**	
25.0	30	4	0	2	6	**20.0**	
50.0	30	5	3	5	13	**43.3**	
100.0	30	5	6	9	20	**66.7**	
200.0	30	10	10	10	30	**100.0**	

*: oral LD_{50} value (with 95% confidence limits) was estimated with the log-probit method (ToxRat Proffesional 3.3.0 computer software), [9]

After 72 hours of exposure, mortality of the control group was 0.0% and for the treated groups' mortality percentages at the doses 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee, were 23.3, 20.0, 43.3, 76.7 and 100.0%, respectively (Table 6).

Table 6. Honeybee mortality and the LD_{50} after 72 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality					LD_{50} [µg/bee]
		Number of dead bees [no.] replicates			Total		
		I	II	III	[no.]	[%]	
0.0 (control)	30	0	0	0	0	**0.0**	**43.9*** *(11.5 – 130.9)*
12.5	30	2	3	2	7	**23.3**	
25.0	30	2	0	4	6	**20.0**	
50.0	30	5	3	5	13	**43.3**	
100.0	30	5	8	10	23	**76.7**	
200.0	30	10	10	10	30	**100.0**	

*: oral LD_{50} value (with 95% confidence limits) was estimated with the log-probit method (ToxRat Proffesional 3.3.0 computer software), [9]

After 96 hours of exposure, mortality of the control group was 3.3% and for the treated groups' mortality percentages at the doses 12.5, 25.0, 50.0, 100.0 and 200.0 µg/bee, after Abbott's correction, were 34.5, 17.2, 51.7, 75.9 and 100.0%, respectively (Table 7).

Table 7. Honeybee mortality and the LD_{50} after 96 hours of exposure – definitive test

Dose [µg/bee]	Number of tested bees [no.]	Mortality						LD_{50} [µg/bee]
		Number of dead bees [no.] replicates			Total			
		I	II	III	[no.]	[%]	[%][c]	
0.0 (control)	30	0	1	0	1	3.3	–	**38.3**
12.5	30	5	4	2	11	36.7	**34.5**	
25.0	30	4	0	2	6	20.0	**17.2**	
50.0	30	7	4	5	16	53.3	**51.7**	
100.0	30	5	8	10	23	76.7	**75.9**	
200.0	30	10	10	10	30	100.0	**100.0**	

The definitive experiment was conducted between 08 – 12.07.2020.

[c]: mortality corrected according formula of Abbott's [6]

The median lethal dose LD50/24 h is 67.2 µg/honeybee (with 95% confidence limits: 20.9 – 482.7 µg/honeybee) of the test item. The median lethal dose LD50/48 h is 48.7 µg/honeybee (with 95% confidence limits: 16.0 – 142.6 µg/honeybee) of the test item. The median lethal dose LD50/72h is 43.9 µg/honeybee (with 95% confidence limits: 11.5 – 130.9 µg/honeybee) of the test item. The median lethal dose LD50/96 h is 38.3 µg/honeybee of the test item.
During the definitive test abnormal behavioural effects as paralysis were observed after 4 hours of exposition at the doses 50.0, 100.0 and 200.0 µg/honeybee (Table 8).

Table 8. Behavioural effects – definitive experiment

Dose [µg/bee]	Exposure / Replicates	4 h	24 h	48 h	72 h	96 h
		Number of bees showing adverse behaviour* / number of living bees				
0.0 (control)	I	0/10	0/10	0/10	0/10	0/10
	II	0/10	0/10	0/10	0/10	0/9
	III	0/10	0/10	0/10	0/10	0/10
12.5	I	0/9	0/8	0/8	0/8	0/5
	II	0/10	0/8	0/7	0/7	0/6
	III	0/10	0/10	0/9	0/8	0/8
25.0	I	0/9	0/8	0/6	0/8	0/6
	II	0/10	0/10	0/10	0/10	0/10
	III	0/9	0/9	0/8	0/8	0/8
50.0	I	2d/9	0/5	0/5	0/5	0/3
	II	0/8	0/8	0/7	0/7	0/6
	III	0/9	0/9	0/5	0/5	0/5
100.0	I	1d/9	0/7	0/5	0/5	0/5
	II	0/10	0/4	0/4	0/4	0/2
	III	4d/10	0/1	0/1	0/0	0/0
200.0	I	8d/10	0/1	0/0	0/0	0/0
	II	2d/8	0/0	0/0	0/0	0/0
	III	2d/8	0/0	0/0	0/0	0/0

*: sublethal toxic effects were:

a- uncoordinated movements
b- increased activity
c- intensive cleaning
d- paralysis

Definitions of the endpoints
The LD50 (median lethal dose) oral is a statistically derived single dose of a test or reference item that can cause death in 50 per cent of biological test systems when administered by the oral route. The LD50 is expressed in µg of the test item per bee or in µg of the active ingredient contained in the reference item per bee. It was calculated with the log-probit method using ToxRat Proffesional software, Version 3.3.0 [SPO/B/67]. Mortality: a honeybee is considered dead if it is completely immobile.

Validity of the study
The following validity criteria were met during the test:
- the average mortality for the total number of controls was 3.3% at the end of the experiment (criterion: it must not exceed 10%).
- the LD50/24 h of the reference item (dimethoate) was 0.28 µg a.i./bee (criterion: 0.10 – 0.35 µg a.i./bee).

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	The study was conducted to OECD guideline 214 and according to the principles of GLP. One deviation to the guideline was noted. The immobilization of the bees to apply the test item instead of anaesthesia with CO2. The mentioned change had not effect on the results of the study. The study is considered to be reliable and suitable for the risk assessment.

Reference: KCP 10.3.1/02

Report	*Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC), Honeybees (Apis mellifera L.), Acute Contact Toxicity Test*, M. Stalmach; Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland; STUDY CODE: B-77-20
Guideline(s):	OECD Guideline for the Testing of Chemicals No. 214 (1998) and the EU Method C.17. (2008)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) content: 253.1 g/L of pyraclostrobin batch no.: 04/2020 production date: 23.04.2020 expiry date: 23.04.2022
Test Species:	Biological test system: the honeybee, Apis mellifera L., strain: carnica – age: approximately 3 weeks – source: an apiary at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna
Test Design:	– the test item: - exposure duration: 48 hours - number of doses: 5 doses and a control - number of replicates: 3 replicates - number of bees: 10 bees/replicate – the reference item: - exposure duration: 24 hours - number of doses: 3 doses - number of replicates: 3 replicates - number of bees: 10 bees/replicate
Endpoints:	– honeybee mortality after 24 and 48 hours of the exposure, – the contact LD50 of the test item after 24 and 48 hours of the exposure, – the contact LD50/24 h of the reference item (dimethoate).
Test Concentration:	12.5, 25.0, 50.0, 100.0 and 200.0 µg test item/bee and a control (0.0 µg/bee)
Test Conditions:	Test conditions: 24.0 - 26.0°C – temperature: 58.0 - 63.0% – relative air humidity: 16 hours light : 8 hours dark

Preliminary test

Mortality results obtained in the preliminary experiment are presented in Tables 1 and 2. Mortality of the control group after 48 hours of exposure was 0.0%. After 24 and 48 hours the percentages of mortality of the bees treated with the test item at the doses of 8.0, 40.0 and 200.0 µg/honeybee were 0.0, 0.0 and 10.0%, respectively. No abnormal behavioural effects were observed during the test.

Definitive test

Mortality of the treated insects is presented in Tables 3 – 5. They contain raw data which were then converted to percentages in order to calculate the LD50. Mortality of the control group after 4, 24 and 48 hours of the test were 0.0%. After 4 and 24 hours the percentages of mortality of the bees treated with the test item at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/honeybee were 0.0%. After 48 hours the percentages of mortality of the bees treated with the test item at the doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg/honeybee were 0.0, 3.3, 3.3, 0.0 and 0.0%, respectively.

The median lethal doses (LD50/24 h and LD50/48 h contact) are higher than the highest dose used in the test, i.e. 200.0 µg/honeybee. No abnormal behavioural effects were observed during the test (Table 6).

Table 6. Behavioural effects – definitive experiment

Dose [µg/bee]	Exposure / Replicates	4 h	24 h	48 h
		Number of bees showing adverse behaviour*/ number of living bees		
0.0 (control)	I	0/10	0/10	0/10
	II	0/10	0/10	0/10
	III	0/10	0/10	0/10
12.5	I	0/10	0/10	0/10
	II	0/10	0/10	0/10
	III	0/10	0/10	0/10
25.0	I	0/10	0/10	0/9
	II	0/10	0/10	0/10
	III	0/10	0/10	0/10
50.0	I	0/10	0/10	0/10
	II	0/10	0/10	0/9
	III	0/10	0/10	0/10
100.0	I	0/10	0/10	0/10
	II	0/10	0/10	0/10
	III	0/10	0/10	0/10
200.0	I	0/10	0/10	0/10
	II	0/10	0/10	0/9
	III	0/10	0/10	0/9

*: sub-lethal toxic effects were:

a- uncoordinated movements
b- increased activity
c- intensive cleaning
d- paralysis

Mortality of the bees treated with the reference item after 4 and 24 hours are presented in the tables below.

The median lethal dose of dimethoate (LD50/24 h) determined with the log-probit method is 0.21 µg/bee (95% confidence limits: 0.18 – 0.24 µg a.i./bee). The LD50/24 h is presented in Table 8.

Table 7. Honeybee mortality after 4 hours of exposure – dimethoate

Dose [µg a.i./bee]	Number of tested bees [no.]	Mortality				
		Number of dead bees [no.] replicates			Total	
		I	II	III	[no.]	[%]
0.0 (control)	30	0	0	0	0	0.0
0.1	30	0	0	0	0	0.0
0.2	30	0	0	0	0	0.0
0.4	30	0	0	0	0	0.0

Table 8. Honeybee mortality and the LD_{50} after 24 hours of exposure – dimethoate

Dose [µg a.i./bee]	Number of tested bees [no.]	Mortality					LD_{50} [µg/bee]
		Number of dead bees [no.] replicates			Total		
		I	II	III	[no.]	[%]	
0.0 (control)	30	0	0	0	0	0.0	0.21** (0.18 – 0.24)
0.1	30	0	1	0	1	3.3	
0.2	30	5	8	0	13	43.3	
0.4	30	9	10	10	29	96.7	

**: contact LD_{50} value (with 95% confidence limits) was estimated with the log-probit method (ToxRat Proffesional 3.3.0 computer software)

Definitions of endpoints

The LD50 (median lethal dose) contact, is a statistically derived single dose of a substance that can cause death in 50 per cent of animals when administered by contact route. The LD50 is expressed in µg test item/bee or µg a.i./bee. It was calculated with the log-probit method.
Mortality: a honeybee is dead if it is completely immobile.

Validity of the study

The following validity criteria were met during the test:
- the average mortality for the total number of controls was 0.0% after 48 h (criterion: it must not exceed 10%),
- the LD50/24 h of the reference item (dimethoate) was 0.21 µg a.i./bee (criterion: 0.10 – 0.30 µg a.i./bee).

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. Some deviations to the OECD GD 239 were noted. During the first 8 days of definitive test, temperature decreased, as an effect average temperature for this period was 32.582°C. In course of the range-finding and definitive test, periodic decreases of temperature (required: 34-35°C) and humidity (required: 50-100%) occurred. It resulted from daily feedings and observations. These drops were short-termed, did not affect the condition of the test system. Nevertheless, those deviations had no effect of the test results. The study is considered to be reliable and suitable for the risk assessment.
Reference	KCP 10.3.1/03
Report	*Chronic Toxicity Test for Honey Bee Larvae according to OECD GD 239*, U. Orzechowska; Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland; STUDY CODE: 0038/0012/E
Guideline(s):	OECD GD 239
Deviations:	No

GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test design	stability test: tested concentrations and control in one replicate range-finding, definitive, reference test: tested concentrations and control in one replicate; 36 larvae per replicate
Test cages	stability test: volumetric flask of 250 mL volume range-finding, definitive, reference test: 48-well breeding plates with queen-cell cups placed in the dissector and placed in incubator; from day 8 dissectors placed in test room; from day 15 of the test – transparent plastic boxes placed in test room
Exposition time	4 days (from day 3 to day 6)
Duration of the test	stability test: 72 hours range-finding, definitive, reference test: 22 days
Tested concentrations	stability test: control, 0.05 g/L corresponding to 0.65 mg/kg of food; 50 g/L corresponding to 650 mg/kg of food range-finding test: control; 0.65 mg/kg; 6.5 mg/kg; 65 mg/kg; 650 mg/kg of food definitive test: control; 16.64 mg/kg; 41.6 mg/kg; 104 mg/kg; 260 mg/kg; 650 mg/kg of food reference test: control; dimethoate 48 mg/kg of food

Test conditions	stability test: average temperature 5.516°C (minimal temperature 4.9°C; maximal temperature 7.0°C); darkness range-finding test: average temperature 33.986°C (minimal temperature 32.2°C; maximal temperature 35.2°C); average humidity: 81.527% (minimal humidity 45.0%; maximal humidity 98.8%); darkness definitive test and reference test: average temperature 33.882°C (minimal temperature 24.7°C; maximal temperature 35.7°C); average humidity: 83.715% (minimal humidity 40.0%; maximal humidity 99.9%); darkness

Final results

In course of the experiment, the test item has shown apitoxic effect in mortality of following developmental stages of bees after 22 days of the test.

Table 19. Larval mortality – definitive test

Concentration [mg/kg of food]	Time [day]											
	3	4		5		6		7		8		
	Introduced larvae [pcs.]	Dead larvae*) [pcs.]	Intoxication signs	Dead larvae*) [pcs.]	Intoxication signs	Dead larvae*) [pcs.]	Intoxication signs	Dead larvae*) [pcs.]	Intoxication signs	Dead larvae*) [pcs.]	Intoxication signs
Control	36	0	none	0	none	0	stun. dev. – 1	0	stun. dev. – 2	0	stun. dev. – 1
16.64	36	0	none	0	none	0	stun. dev. – 2	0	stun. dev. – 1	1	stun. dev. – 1
41.6	36	0	none	0	none	0	stun. dev. – 2	0	stun. dev. – 3	0	stun. dev. – 2
104	36	0	stun. dev. – 2	0	stun. dev. – 3	0	stun. dev. – 4	1	stun. dev. – 4	1	stun. dev. – 5
260	36	0	stun. dev. – 5	1	stun. dev. – 7	5	stun. dev. – 7	7	stun. dev. – 5	9	stun. dev. – 8
650	36	5	stun. dev. – 11	9	stun. dev. – 15	15	stun. dev. – 11	21	stun. dev. – 9	28	stun. dev. – 6

* cumulative amount
stun. dev.- stunted development

Table 20. Final larval mortality results – definitive test

Concentration [mg/kg of food]	4		5		6		7		8	
	Mortality [%]	Statistical significance*)	Mortality [%]	Statistical significance*)	Mortality [%]	Statistical significance*)	Mortality [%]	Statistical significance*)	Mortality [%]	Statistical significance*)
Control	0.0	not applicable	0.0	not applicable	0.0	not applicable	0.0	not applicable	0.0	not applicable
16.64	0.0	not determined	0.0	-	0.0	-	0.0	-	2.8	-
41.6	0.0	not determined	0.0	-	0.0	-	0.0	-	0.0	-
104	0.0	not determined	0.0	-	0.0	-	2.8	-	2.8	-
260	0.0	not determined	2.8	-	13.9	+	19.4	+	25.0	+
650	13.9	+	25.0	+	41.7	+	58.3	+	77.8	+

\- statistically insignificant + statistically significant
*) values calculated using ToxRat Professional using Chi2 2x2 Test after Bonferroni correction and Step-Down Cochran-Armitage Test with significance level p>0.05

Table 21. Pupal mortality – definitive test

Concentration [mg/kg of food]	Time [day]			
	8	15		22
	Alive larvae [pcs.]	Dead pupae [pcs.]	Intoxication signs	Dead pupae [pcs.]
Control	36	7	stunted development – 2	9
16.64	35	6	stunted development – 3	8
41.6	36	4	none	7
104	35	6	stunted development – 4	7
260	27	8	stunted development – 2	9
650	8	5	stunted development – 2	6

Table 22. Final pupal mortality results – definitive test

Concentration [mg/kg of food]	Time [day]			
	15		22	
	Mortality [%]	Statistical significance*)	Mortality [%]	Statistical significance*)
Control	19.4	not applicable	25.0	not applicable
16.64	17.1	-	22.9	-
41.6	11.1	-	19.4	-
104	17.1	-	20.0	-
260	29.6	-	33.3	-
650	62.5	-	75.0	-

\- statistically insignificant
*) values calculated using ToxRat Professional using Chi2 2x2 Table with Bonferroni correction with significance level p>0.05

Table 23. Number of emerged adults – definitive test

Concentration [mg/kg of food]	Time [day]					
	3	22				
	Introduced larvae [pcs.]	Emerged adults [pcs.]	Number of emerged adults [%]	Unemerged adults [pcs.]	Number of unemerged adults [%]	Statistical significance*)
Control	36	27	75.0	9	25.0	not applicable
16.64	36	27	75.0	9	25.0	-
41.6	36	29	80.6	7	19.4	-
104	36	28	77.8	8	22.2	-
260	36	18	50.0	18	50.0	+
650	36	2	5.6	34	94.4	+

\- statistically insignificant
\+ statistically significant
*) values calculated using ToxRat Professional using Step-Down Cochran-Armitage Test with significance level p>0.05

At the end of the study, the concentration and the dose causing 50% mortality of the population in the test (LC50 and LD50 values) were determined, as well as NOEC and NOED values at 22 day.
The final results of the experiment are presented in Table 1.

Parameter	Concentration [mg/kg of food]	Parameter	Dose [µg/larva]
LC_{10}	181.105 (130.399 – 220.934)*	LD_{10}	27.862 (20.061 – 33.990)*
LC_{20}	221.501 (171.528 – 262.797)*	LD_{20}	34.077 (26.389 – 40.430)*
LC_{50}	325.584 (275.892 – 384.725)*	LD_{50}	50.090 (42.445 – 59.189)*
NOEC	≥104.000	NOED	≥16.000

* upper and lower confidence limits (95%) given in the brackets
LC_{10} test item concentration causing reduction by 10%
LC_{20} test item concentration causing reduction by 20%
LC_{50} test item concentration causing reduction by 50%
NOEC the highest test item concentration not causing statistically significant differences in relations to the control
LD_{10} test item dose causing reduction by 10%
LD_{20} test item dose causing reduction by 20%
LD_{50} test item dose causing reduction by 50%
NOED the highest test item dose not causing statistically significant differences in relations to the control

Reference test

Observations of mortality of honey bee in reference test were conducted parallel to the observations of intoxication of test item.
As a result of exposition to reference item, larval mortality on days 4-8 was 69.4%.
The reference item in the course of the present study showed apitoxic effects on the honey bee (Apis mellifera, L.). The results obtained are in accordance with the requirements of the OECD GD 239 Guideline (required ≥50%) and confirm the correct reaction of the test system.
Statistical analysis was performed using statistical software ToxRat Professional.
Results of mortality during the reference test are shown in the table below.

Table 24. Larval mortality – dimethoate – definitive reference test

Concentration [mg/kg of food]	Time [day]														
	4			5			6			7			8		
	Dead larvae* [pcs.]	Intoxication signs	Statistical sign.*)	Dead larvae* [pcs.]	Intoxication signs	Statistical sign. *)	Dead larvae* [pcs.]	Intoxication signs	Statistical sign. *)	Dead larvae* [pcs.]	Intoxication signs	Statistical sign. *)	Dead larvae* [pcs.]	Intoxication signs	Statistical sign. *)
Control	0	none	n.a.	0	none	n.a.	0	none	n.a.	0	none	n.a.	0	none	n.a.
48	6	stunted development – 8	+	11	stunted development – 10	+	17	stunted development – 11	+	20	stunted development – 9	+	25	stunted development – 7	+

* cumulative amount
n.a. not applicable
statistical sign. statistical significance
\+ statistically significant
*) values calculated using ToxRat Professional using Fisher Test with significance level p>0.05

Validity criteria

The test met the validity criteria of the experiment listed in OECD GD 239 Guideline:
- cumulative larval mortality in control on days 4-8 was 0.0% (required: ≤15%),
- the adults emergence rate in control on day 22 was 75.0% (required: ≥70%),
- larval mortality in reference test on days 4-8 was 69.4% (required: ≥50%),

A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. Some deviations to the OECD GD 245 were noted. During the range-finding test, periodic, humidity fluctuations of short duration were recorded as an effect of feedings and observations record. During the definitive test, slight increase of temperature above 35°C was noted (required: 33±2°C). The average air humidity was 78.616% (required: 50-70%). The changes were minor, which did not affect the condition of the research system. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference	KCP 10.3.1/04
Report	*Honey Bee, Chronic Oral Toxicity Test according to OECD 245 Guideline*, U. Orzechowska; Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland; STUDY CODE: 0038/0014/E
Guideline(s):	OECD GD 245
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test design	stability test: tested concentrations and control in one replicate range-finding, definitive and reference test: tested concentrations and control in three replicates, 10 bees per replicate
Test cages	stability test: storage conditions: plastic containers of 100 mL volume test conditions: plastic syringe of 2 mL volume range-finding, definitive and reference test: cages 20 x 20 x 20 cm
Duration time	stability test: storage conditions: 48 hours test conditions: 24 hours range-finding, definitive and reference test: 10 days
Tested concentrations	stability test: control; 2.5 mg/kg; 2500 mg/kg of food range-finding test: control; 2.5 mg/kg; 25 mg/kg; 250 mg/kg; 2500 mg/kg of food definitive test: control; 95.26 mg/kg; 171.47 mg/kg; 308.64 mg/kg; 555.56 mg/kg; 1000 mg/kg of food

	reference test: control; dimethoate: 0.5 mg/kg of food
Study conditions	stability test: storage conditions: average temperature 5.227°C (minimal temperature 3.5°C; maximal temperature 6.3°C); darkness test conditions: average temperature 34.476°C (minimal temperature 33.4°C; maximal temperature 35.1°C); average humidity: 60.740% (minimal humidity 54.7%; maximal humidity 70.0%); darkness range-finding test: average temperature 34.399°C (minimal temperature 33.4°C; maximal temperature 34.5°C); average humidity: 70.292% (minimal humidity 32.7%; maximal humidity 78.2%); darkness definitive test and reference test: average temperature 34.5815°C (minimal temperature 33.6°C; maximal temperature 35.5°C); average humidity: 78.616% (minimal humidity 57.1%; maximal humidity 87.6%); darkness

Final results
In the course of the study, test item has shown apitoxic effect in honey bee mortality after 10 days of the test.

Table 8. Mortality of the bees – definitive test

Concentration of test item [mg/kg of food]	r*	day 1		day 2		day 3		day 4		day 5		day 6		day 7		day 8		day 9		day 10		Mortality at the end of test [%]	Statistical significance **
		Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*		
Control	1	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	3.3	na.
	2	0		0		0		0		0		0		0		0		0		0			
	3	0		0		0		0		1		1		1		1		1		1			
95.26	1	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	3.3	-
	2	0		0		0		0		0		0		0		0		0		1			
	3	0		0		0		0		0		0		0		0		0		0			
171.47	1	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	6.7	-
	2	0		0		0		0		0		0		0		0		0		0			
	3	0		0		0		0		0		0		0		0		2		2			
308.64	1	0	none	0	none	0	none	0	none	0	none	0	none	0	none	1	none	2	2ap	3	2ap 1v	23.3	+
	2	0		0		0		0		0		0		1		1		1		1			
	3	0		0		0		0		0		0		0		2		3		3			
555.56	1	0	none	1	none	1	none	2	none	2	none	2	none	3	2ap	3	2ap	5	3ap	8	3ap	86.7	+
	2	0		0		0		0		0		0		1		4		5		9			
	3	0		0		1		1		2		3		4		5		9		9			
1000	1	0	none	1	none	2	none	4	none	7	none	8	3ap	8	3ap	9	3ap	9	3ap	9	3ap	90.0	+
	2	0		0		1		5		7		7		7		8		9		9			
	3	1		1		4		5		6		7		8		9		9		9			

*r replicate
na. not applicable
- statistical insignificant
+ statistical significant
* abbreviations explained in point 4.2.4.3.
** statistical calculations using Cochran-Armitage Test using ToxRat Professional software

Table 9. Amount of consumed food – definitive test

Concentration of test item [mg/kg of food]	r*	day 1		day 2		day 3		day 4		day 5		day 6		day 7		day 8		day 9		day 10	
		R**	R_{av}***	R**	R_{av}***	R**	R_{av}***	R**	R_{av}***	R**	R_{av}***	R**	R_{av}***	R**	R_{av}***	R**	R_{av}***	R**	R_{av}***	R**	R_{av}***
Control	1	296		353.5		448.5		419.8		266.4		80.5		557.8		214.4		161.7		406.1	
	2	347.6	253.13	521.3	354.80	426.5	361.37	389.4	464.90	331.4	188.87	479.6	290.70	152.7	238.33	393.4	228.83	222.1	259.60	254.8	264.00
	3	226.8		413.2		340.7		651.2		160.6		437.8		142.6		234.1		528.5		259.6	
95.26	1	330.2		260.5		428		505.5		401.1		374.4		200.9		410.3		394.3		306.2	
	2	553.1	369.17	443.9	335.83	511.8	433.93	389.5	414.77	435.2	359.37	295.3	261.93	468.3	303.00	118.4	202.10	375.8	292.50	241.9	307.63
	3	335.2		526.7		493.6		415		433.6		241.9		377.9		233.0		240.9		503.3	
171.47	1	345.9		437.2		523.8		200.3		417.5		217.8		220.5		627.6		77.9		130.6	
	2	382	305.50	514.4	364.40	518.4	460.33	501.7	302.37	286.6	262.90	234.1	172.70	259.3	174.40	271.6	378.70	342.1	136.57	203.4	93.03
	3	299.6		365.2		470.4		270.8		276.4		192.0		181.5		392.3		123.2		73.6	
308.64	1	245.9		670.6		369.9		430.5		493.3		408.6		425.5		417.9		310.0		283.9	
	2	235.3	236.70	542.7	470.60	391.6	313.47	370	421.43	264.1	256.80	205.9	307.73	306.9	321.17	396.5	329.97	106.9	183.57	324.1	245.93
	3	339.9		422.1		310.5		529.5		204.8		434.5		369.2		330.9		267.3		258.3	
555.56	1	330.4		320.3		345		552.1		269.0		425.7		162.8		272.7		167.3		187.8	
	2	362.5	307.03	463.6	394.67	333.3	233.07	313.8	484.87	432.3	269.70	194.7	262.73	290.2	144.70	278.9	191.77	125.6	91.00	50.9	55.13
	3	339.2		623.7		152.5		654.4		299.6		293.6		119.2		179.1		113.6		55.2	
1000	1	187		534.2		267.5		140.1		75.6		73.3		58.1		74.7		50.5		69.1	
	2	214.4	220.83	369.7	435.73	361.3	198.13	336	176.33	464.0	136.60	278.7	141.40	230.9	115.83	138.5	62.37	81.1	6.50	43.6	7.23
	3	372.1		626.9		97.2		118.6		62.0		198.0		196.6		129.3		21.4		37.5	

*r replicate
R** intake of food [mg]
R_{av}*** average intake of food corrected by evaporated food [mg]

Table 10. Data concerning food intake per bee – definitive test

Concentration of test item [mg/kg]	Average daily intake of food [mg/bee/day]	Average daily intake of test item [µg/bee/day]	Average intake of test item [mg/bee]
Control	29.55	0	0.0000
95.26	32.91	3.135	0.0313
171.47	26.67	4.574	0.0457
308.64	32.70	10.092	0.1009
555.56	33.09	18.383	0.1838
1000	33.26	33.259	0.3326

Table 11. Average daily intake of food per bee for each replicate of tested concentration

Concentration of test item [mg/kg of food]	*r	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	Average
		R**	R**	R**	R**	R**	R**	R**	R**	R**	R**	R_{av}***
Control	1	29.60	35.35	44.85	41.98	26.64	8.05	55.78	21.44	16.17	40.61	**32.05**
	2	34.76	52.13	42.65	38.94	33.14	47.96	15.27	39.34	22.21	25.48	**35.19**
	3	22.68	41.32	34.07	65.12	17.84	48.64	15.84	26.01	58.72	28.84	**35.91**
95.26	1	33.02	26.05	42.80	50.55	40.11	37.44	20.09	41.03	39.43	30.62	**36.11**
	2	55.31	44.39	42.80	38.95	43.52	29.53	46.83	11.84	37.58	26.88	**37.76**
	3	33.52	52.67	49.36	41.50	43.36	24.19	37.79	23.30	24.09	50.33	**38.01**
171.47	1	34.59	43.72	52.38	20.03	41.75	21.78	22.05	62.76	7.79	13.06	**31.99**
	2	38.20	51.44	51.84	50.17	28.66	23.41	25.93	27.16	34.21	20.34	**35.14**
	3	29.96	36.52	47.04	27.08	27.64	19.20	18.15	39.23	15.40	9.20	**26.94**
308.64	1	24.59	67.06	36.99	43.05	49.33	40.86	42.55	46.43	38.75	40.56	**43.02**
	2	23.53	54.27	39.16	37.00	26.41	20.59	34.10	44.06	11.88	36.01	**32.70**
	3	33.99	42.21	31.05	52.95	20.48	43.45	36.92	41.36	38.19	36.90	**37.75**
555.56	1	33.04	35.59	38.33	69.01	33.63	53.21	23.26	38.96	33.46	93.90	**45.24**
	2	36.25	46.36	33.33	31.38	43.23	19.47	32.24	46.48	25.12	50.90	**36.48**
	3	33.92	62.37	16.94	72.71	37.45	41.94	19.87	35.82	113.60	55.20	**48.98**
1000	1	18.70	59.36	33.44	23.35	25.20	36.65	29.05	74.70	50.50	69.10	**42.00**
	2	21.44	36.97	40.14	67.20	154.67	92.90	76.97	69.25	81.10	43.60	**68.42**
	3	41.34	69.66	16.20	23.72	15.50	66.00	98.30	129.30	21.40	37.50	**51.89**

*r replicate
R** average intake of food per bee for each day (mg/bee), calculated for number of alive bees
R_{av}*** total average intake of food per bee for each day per replicate during the test (mg/bee/day)

On the basis of data analysis, value LC50=420.3721 mg/kg and LDD50=13.4649 µg/bee/day were determined for mortality. Values NOEC=171.47 mg/kg and NOEDD=4.574 µg/bee/day were also determined.
The final results of the test are presented in Table 1.

Table 1. Final results of the study

Final results calculated using ToxRat Professional software			
Parameter	Concentration [mg/kg of food]	Parameter	Dose [µg/bee/day]
LC_{10}	220.4195 (55.2004 – 325.0999)*	LDD_{10}	6.7746 (4.9108 – 8.3326)*
LC_{20}	275.1060 (101.1989 – 392.8677)*	LDD_{20}	8.5761 (6.6688 – 10.2181)*
LC_{50}	420.3721 (265.1460 – 686.8061)*	LDD_{50}	13.4649 (11.4367 – 15.8061)*
NOEC	171.47	NOEDD	4.574
LOEC	308.64	LOEDD	10.092

LC_{10} test item concentration causing 10% reduction
LC_{20} test item concentration causing 20% reduction
LC_{50} test item concentration causing 50% reduction
NOEC the highest test item concentration not causing statistically significant differences in comparison to the control
LOEC the lowest test item concentration causing statistically significant differences in comparison to the control
LDD_{10} daily dietary test item dose causing 10% reduction
LDD_{20} daily dietary test item dose causing 20% reduction
LDD_{50} daily dietary test item dose causing 50% reduction
NOEDD the highest daily dietary test item dose not causing statistically significant differences in comparison to the control
LOEDD the lowest daily dietary test item dose causing statistically significant differences in comparison to the control

Reference test
During the test, tested concentration of reference item 0.5 mg/kg of food caused mortality 73.3% of population.
The reference item in the course of the study showed apitoxic effects on the honey bee (Apis mellifera, L.). The results obtained are in accordance with the requirements of the OECD Guideline 245 (required: ≥ 50%) and confirm the correct response of the test system.
Results of bee mortality during the reference test are shown Table 12.

Table 12. Mortality of the bees – reference test

Concentration of test item [mg/kg of food]	*r	day 1		day 2		day 3		day 4		day 5		day 6		day 7		day 8		day 9		day 10		Mortality at the end of test [%]	Statistical significance **
		Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*	Number of dead	Signs of intoxication*		
Control	1	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	0	none	3.3	na.
	2	0		0		0		0		0		0		0		0		0		0			
	3	0		0		0		0		1		1		1		1		1		1			
0.5	1	0	none	0	none	0	1ap	0	1ap	0	1ap	0	1ap	0	1ap	2	2ap	3	2ap	4	2ap	73.3	+
	2	0		0		0		0		0		2		5		8		8		9			
	3	0		0		0		2		3		4		4		5		8		9			

*r replicate
na. not applicable
+ statistical significant
* abbreviations explained in point 4.2.4.3.
** statistical calculations using Fisher Test using ToxRat Professional software

Validity criteria
The test met the validity criteria of the experiment:
- bee mortality in control after 10 days was 3.3% (required: ≤15%),

- bee mortality in the reference test after 10 days was 73.3% (required: ≥50%)

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

No additional studies were performed.

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

No additional studies were performed.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

No additional studies were performed.

9.14 KCP 10.3.1.7 Non target arthropods studies

Study 1

Comments of zRMS:	The study was conducted to the guidelines and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.2/01
Report	*An extended laboratory test for evaluating the effects of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on the parasitic wasp, Aphidius rhopalosiphi (De Stefani-Perez),* M. Stalmach, 2020; Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland; STUDY CODE: B-80-20
Guideline(s):	ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Mead-Briggs M.A. et al., 2000; Mead-Briggs M.A. et al., 2010)
Deviations:	Yes
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Name: Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Active substance: 256.6 g/L of pyraclostrobin Batch number: 04/2020 Manufacture date: 23.04.2020 Expiry date: 23.04.2022
Test Species:	the parasitic wasp, Aphidius rhopalosiphi (De Stefani-Perez); Hymenoptera: Braconidae, Aphiidinae – age: adult females (24 – 48 hours after emerging from mummies) – source: the culture was obtained from a commercial breeder (BiasLabts Ltd., London, UK)
Test Design:	5 study groups: • a control group (0.0 L/ha) – 0.425 L/ha – 0.85 L/ha – 1.7 L/ha • Reference item: Bi 58 Top 400 EC at the rate of 5.0 mL/ha mortality assessment: 6 replicates/group; 5 females/replicate fecundity assessment: 15 replicates/group; 1 females/replicate
Endpoints:	– wasp mortality after 48 hours of exposure, – determination of the LR50 and the NOERmortality, – determination of the ER50 and the NOERfecundity. – reduction in fecundity (Pr) of the surviving female wasps exposed to Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC), 12 days after the oviposition pe riod
Test Conditions:	– temperature: 18 – 21°C – relative air humidity: 64 –73% – photoperiod: 16 hours light : 8 hours dark – light intensity: mortality and oviposition assessment: 2696 lx fecundity phase: 5109 lx

Results and discussion

In the definitive test, after 48 hours mortality of the control wasps was 0.0%. Morality, in the groups treated with Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rates of 0.425, 0.85 and 1.7 L/ha were 0.0, 3.3 and 0.0%, respectively.

At the significance level of 0.05, there were no statistically significant differences in mortality between the wasps exposed to the test item at the rates of 0.425, 0.85 and 1.7 L/ha and the control group (Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, $p>0.05$).

Based on the obtained results the LR50 value could not be estimated. It could be assumend that LR50 is higher than 1.7 L/ha. The NOER mortality is higher than or equal to 1.7 L/ha.

Mortality of the wasps exposed to Bi 58 Top 400 EC at the rate of 5.0 mL/ha was 80.0% after 48 hours. Therefore, the validity criterion specified in the Method description was met [6]. The results showed that the test organisms were sensitive to dimethoate.

The fecundity assessment showed that the mean number of mummies per female in the control group was 15.5 (after 12 days after oviposition). As for the wasps treated with Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rates of 0.425, 0.85 and 1.7 L/ha the mean number of mummies per female were 14.4, 11.7 and 8.5, respectively. Fecundity reduction (Pr) in the group treated with the test item at the rates of 0.425, 0.85 and 1.7 L/ha were 6.9, 24.6 and 44.8%, respectively.
At the significance level of 0.05, there were no statistically significant differences in fecundity between the wasps exposed to the test item at the rate of 0.425 L/ha and the control group. There were statistically significant differences in fecundity between the wasps exposed to the test item at the rates of 0.85 and 1.7 L/ha and the control group (Williams Multiple Sequential t-test Procedure, p>0.05).
Based on the obtained fecundity results it could be assumed that the ER50 value is higher than 1.7 L/ha and the NOERfecundity is equal to 0.425 L/ha of the test item.
The effects of the test item, Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on mortality and fecundity of Aphidius rhopalosiphi in the extended laboratory test are summarized below.

Parametr (endpoint)						
Mortality			Fecundity			
Test item [L/ha]	Total [%]	LR_{50} [L/ha]	Test item [L/ha]	Mean no. of mummies/ female	Fecundity reduction Pr [%]	ER_{50} [L/ha]
Control	0.0	>1.7	Control	15.5	–	>1.7
0.425	0.0		0.425	14.4	6.9	
0.85	3.3		0.85*	11.7	24.6	
1.7	0.0		1.7*	8.5	44.8	
$NOER_{mortality}$ ≥ 1.7 [L/ha]			$NOER_{fecundity}$ 0.425 [L/ha]			
Reference item: Bi 58 Top 400 EC						
Reference item [mL/ha]	5.0					
Mortality (after 24 h)						
Total [%]	80.0					

*: statistically significant differences

Repellent effects
The results of the assessments of settling behaviour are presented in Table 2. Three hours after the introduction of the insects into the test units, the mean percentages of wasps settled on the plants were 56.0% in the control group, 38.0, 29.8 and 6.3% in the groups treated with Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at rates of 0.425, 0.85 and 1.7 L/ha, and 23.2% in the reference item group.
Repellent properties of the test item and the reference item were assessed. For the control group and groups treated with the test item at the rates of 0.425, 0.85 and 1.7 L/ha and the reference item group the calculated p-values (0.759, 0.925, 0.468, 0.074 and 0.324) are greater than the significance level of 0.05 (Shapiro-Wilk's test, p > 0.05). At the significance level of 0.05, there were no evidence to reject the null hypothesis assuming a normal data distribution is sufficient. All data distribution of the treated groups is consistent with a normal distribution. Levene's test (p > 0.05) confirmed variances homogenity in all the study groups (p=0.227).
There were statistically significant differences in the mean percentages of wasps settled on the plants between the all test item groups, the group treated with the reference item at the control group (Duncan test procedure, alpha = 0.05).

Mortality

After 48 hours of exposure, there was no dead wasp in the control group. Morality of the wasps after 48

hours in the groups treated with Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rates of 0.068, 0.34 and 1.7 L/ha were 0.0, 0.0 and 10.0%, respectively.
Wasp mortality recorded in the definitive test are presented in Tables 3 – 5.

Table 3. Mortality *of A. rhopalosiphi* after 2 hours – definitive test

Study group [L/ha]	Tested wasps [no.]	Mortality							
		Dead wasps [no.]						Total	
		Replicates							
		I	II	III	IV	V	VI	[no.]	[%]
Control	30	0	0	0	0	0	0	0	0.0
0.425	30	0	0	0	0	0	0	0	0.0
0.85	30	0	0	0	0	0	0	0	0.0
1.7	30	0	0	0	0	0	0	0	0.0
[mL/ha]	Bi 58 Top 400 EC								
5.0	30	0	0	0	0	0	0	0	0.0

Table 4. Mortality of *A. rhopalosiphi* after 24 hours – definitive test

Study group [L/ha]	Tested wasps [no.]	Mortality							
		Dead wasps [no.]						Total	
		Replicates							
		I	II	III	IV	V	VI	[no.]	[%]
Control	30	0	0	0	0	0	0	0	0.0
0.425	30	0	0	0	0	0	0	0	0.0
0.85	30	0	0	0	0	0	0	0	0.0
1.7	30	0	0	0	0	0	0	0	0.0
[mL/ha]	Bi 58 Top 400 EC								
5.0	30	1	0	2	2	3	3	11	36.7

Table 5. Mortality of *A. rhopalosiphi* after 48 hours – definitive test

Study group [L/ha]	Tested wasps [no.]	Mortality							
		Dead wasps [no.]						Total	
		Replicates							
		I	II	III	IV	V	VI	[no.]	[%]
Control	30	0	0	0	0	0	0	0	0.0
0.425	30	0	0	0	0	0	0	0	0.0
0.85	30	0	1	0	0	0	0	1	3.3
1.7	30	0	0	0	0	0	0	0	0.0
$NOER_{mortality}$	≥ 1.7 [L/ha]								
$NOER_{fecundity}$	0.425 [L/ha]								
[mL/ha]	Bi 58 Top 400 EC								
5.0	30	4	3	5	3	5	4	24	80.0

The definitive test was performed between 20.10 – 04.11.2020

After 2, 24 and 48 hours of exposure, there were no dead wasps in the control group. Mortality of the wasps after 48 hours in the groups treated with Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at rates of 0.425, 0.85 and 1.7 L/ha morality there was equal to 0.0, 3.3 and 0.0%, respectively.

At the significance level of 0.05, there were no statistically significant differences in mortality between the wasps exposed to the test item at the rates of 0.425, 0.85 and 1.7 L/ha and the control group Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, p>0.05).
Based on the obtained results the LR50 value could not be estimated. It could be assumend that LR50 is higher than 1.7 L/ha. The NOERmortality is higher than or equal to 1.7 L/ha
Wasp mortality after 48 hours of exposure to Bi 58 Top 400 EC was 80.0%. The relation between Bi 58 Top 400 EC and wasp mortality showed that the insects were sensitive to dimethoate.

Fecundity
All wasps survived the 24-hour oviposition period. The fecundity assessment showed that the mean number of mummies per female in the control group was 15.5. The mean number of mummies per female in the group treated with Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rates of 0.425, 0.85 and 1.7mL/ha were 14.4, 11.7 and 8.5 respectively. Reduction in wasp fecundity (Pr) caused by Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rates previous mentioned was 6.9, 24.6 and 44.8%, respectively.
At the significance level of 0.05, there were no statistically significant differences in fecundity between the wasps exposed to the test item at the rate of 0.425 L/ha and the control group. There were statistically significant differences in fecundity between the wasps exposed to the test item at the rates of 0.85 and 1.7 L/ha and the control group (Williams Multiple Sequential t-test Procedure, p>0.05).
Based on the obtained fecundity results it could be assumed that the ER50 value is higher than 1.7 L/ha and the NOERfecundity is equal to 0.425 L/ha of the test item, [SOP/B/67].

Table 9. Fecundity of *A. rhopalosiphi* – definitive test

Replicates (isolator number)	Mummies per female 12 days after oviposition [no.]			
	Control	Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Study group [L/ha]		
	0.0	0.425	0.85+	1.7+
I	10	16	10	8
II	16	13	11	5
III	13	15	15	6
IV	17	18	13	12
V	12	10	14	15
VI	14	18	17	10
VII	13	11	9	4
VIII	16	14	12	7
IX	15	13	10	5
X	17	11	8	4
XI	20	15	10	15
XII	14	17	12	11
XIII	16	15	10	9
XIV	19	13	10	6
XV	20	17	14	11
Mean number mummies per female ± SD	15.5 ± 2.9	14.4 ± 2.6	11.7 ± 2.5	8.5 ± 3.7
Fecundity reduction relative to the control (Pr) [%]	–	6.9	24.6	44.8
ER_{50}	> 1.7 [L/ha]			
$NOER_{facundity}$	0.425 [L/ha]			

SD: standard deviation
+: statistically significant differences

Test validity criteria
The following validity criteria were met during the study:

– after 48 hours, mortality of the control group was 0.0% (criterion: a maximum of 10.0%),
– after 48 hours, mortality of the group treated with the reference item at the rate of 5.0 mL/ha was 80.0% (criterion: a minimum of 50%),
– all wasps survived the 24-hour oviposition period (criterion: only wasps that survive oviposition can be examined for fecundity),
– the mean number of mummies per female in the control group was 15.5 (criterion: a minimum of 5.0 mummies/female),
– all wasps in the control group gave offspring (criterion: a maximum of 2 females giving no offspring).

Deviations in the study
The experiment was performed according to the ESCORT 1 and the ESCORT 2 guidance documents, the guidelines developed by the IOBC, BART, and EPPO Joint Initiative, the Standard Operating Procedure SOP/B/28: 'An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, Aphidius rhopalosiphi (De Stefani-Perez.)', other references given in section 9 and the SOP's listed in section 10 of the report and the Study Plan.
In the Study Plan B-80-20 there were an editorial errors regarding to the duration of the mortality assessment in the group exposed to the reference item and required mortality range. According to the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Mead-Briggs M.A. et al., 2000; Mead-Briggs M.A. et al., 2010) mortality of the wasp should be assessed within 48 hour and it should not be lower than 50%. In the definitive test mortality of the group exposed to the test item lasted 48 hours and was not lower than 50%. This deviation had no impact on the obtained results.

Study 2

Comments of zRMS:	The study was conducted to the guidelines and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.2/02
Report	*An extended laboratory test for evaluating the effects of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on the predatory mite, Typhlodromus pyri (Sch.).*, P. Holewik, 2020; Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland; STUDY CODE: B-79-20
Guideline(s):	ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M. P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Blümel S. et al., 2000))
Deviations:	Yes
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Name: Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Active substance: 256.6 g/L of pyraclostrobin Batch number: 04/2020 Manufacture date: 23.04.2020 Expiry date: 23.04.2022
Test Species:	Biological test system: the predatory mite, Typhlodromus pyri (Sch.) (Acari: Phytoseiidae) – age: 24-hour-old protonymphs – source: a laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was augmented from a commercial breeder
Test Design:	7 study groups: - a control group (0.0 L/ha) - 0.026 L/ha - 0.064 L/ha - 0.16 L/ha - 0.4 L/ha - 1.0 L/ha - reference item: Bi 58 Top 400 EC at the rate of 9.0 mL/ha - number of replicates: 3 number of mites in each replicate: 20
Endpoints:	– mite mortality after 7 days of the treatment – LR50 and NOERmortality – reproduction reduction (Pr) after 14 days of the treatment – ER50 and NOERreproduction
Test Conditions:	temperature: 23 – 25°C – relative air humidity: 61 – 78% – photoperiod: 16 h light : 8 h dark – light intensity: 809 lux

Results and discussion

In the definitive test, mortality of the control group after 7 days of exposure was 0.0%. After 7 days of exposure to Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at rates of 0.026, 0.064, 0.16, 0.4 and 1.0 L/ha, the percentages of T. pyri, mortality were 0.0, 1.7, 6.7, 31.7 and 81.7%, respectively.

There were no statistically significant differences in mortality between group treated with the test item at the rates of 0.026 and 0.064 L/ha and the control group. There were statistically significant differences in mortality between group treated with the test item at the rates of 0.16, 0.4 and 1.0 L/ha and the control group (Step-down Cochran-Armitage Test Procedure, $p(trend)>\alpha$).

The LR50 value is equal to 0.528 L/ha of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) (95% confidence limits: 0.444 – 0.640). NOERmortality is 0.064 L/ha of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC).

After 7 days of exposure to Bi 58 Top 400 EC at the rate of 9.0 mL/ha mortality was 81.7%. Therefore, the validity criterion specified in the Method description was met. The results obtained in the reference item group showed that the test organisms were sensitive to dimethoate.

Reproduction of the surviving mites from the control group and the groups treated with Pyraclostrobin

250 EC (CHR/F/PYRA 250 EC) at the rates of 0.026, 0.064, 0.16 and 0.4 L/ha, was assessed since mortality of these groups was < 50.0%.
The mean reproduction rate (Rr) in the control group was 7.0 eggs/female. The mean Rr after 14 days of exposure to Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rates of 0.026, 0.064, 0.16 and 0.4 L/ha were 6.5, 5.5, 5.6 and 4.7 eggs/female, respectively. The percentages of reproduction reduction (Pr) caused by test item at the rates of 0.026, 0.064, 0.16 and 0.4 L/ha were 7.0, 22.0, 19.5 and 32.2 %, respectively.
There were no statistically significant differences in reproduction between group treated with the test item at the rates of 0.026, 0.064 and 0.16 L/ha and the control group. There were statistically significant differences in reproduction between group treated with the test item at the rate of 0.4 L/ha and the control group (Williams Multiple Sequential t-test Procedure,|t|>|t*|).
On the basis of the obtained mortality results, it could be assumend that the ER50 value is above 0.4 L/ha of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC). NOERreproduction is equal to 0.16 L/ha of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC).
The effects of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on mortality and reproduction of Typhlodromus pyri in the definitive test are summarized in the table.

Test item rate [L/ha]	Parameter (endpoint)					
	Mortality		Reproduction			
	Total [%]	LR_{50} [L/ha]	Test item rate [L/ha]	Mean number of eggs/ female (Rr) [no.]	Repro-duction reduction Pr [%]	ER_{50} [L/ha]
Control (0.0)	0.0	0.528 *(0.444 – 0.640)**	Control (0.0)	7.0	–	> 0.4
0.026	0.0		0.026	6.5	7.0	
0.064	1.7		0.064	5.5	22.0	
0.16⁺	6.7		0.16	5.6	19.5	
0.4⁺	31.7		0.4⁺	4.7	32.2	
1.0⁺	81.7					
$NOER_{mortality}$ 0.064 [L/ha]			$NOER_{reproduction}$ 0.160 [L/ha]			
Reference item: Bi 58 Top 400 EC						
Reference item [mL/ha]	9.0					
Mortality						
Total [%]	81.7					

ᶜ: mortality corrected according formula of Abbott [1]
⁺: statistically significant differences between control and groups exposed to test item; ToxRat Professional 3.3.0. software [12], [SOP/B/67]
*: 95%-confidence limits

Mortality
In the preliminary test, mortality of the control group after 7 days of exposure was 0.0%. After 7 days of exposure the mortality percentages, of the groups treated with Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rates of 0.068, 0.34 and 1.0 L/ha, were 30.0, 57.5 and 100.0%, respectively.

Table 1. Mortality of *T. pyri* after 7 days of exposure –preliminary range-finding test (non-GLP)

Study group [L/ha]	Number of tested mites [no.]	Mortality			
		Number of dead & escaped[e] mites [no.]		Total	
		Replicates			
		I	II	[no.]	[%]
Control	40	0	0	0	0.0
0.068	40	4	8	12	30.0
0.34	40	18	5	23	57.5
1.7	40	20	20	40	100.0

There were no escaped mites in group exposed to the test item and the control group in preliminary test.
In the definitive test, mortality of the control group after 7 days of exposure was 0.0%. After 7 days of exposure to Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rates of 0.026, 0.064, 0.016, 0.4 and 1.0 L/ha, the percentages of T. pyri, mortality, 0.0, 1.7, 6.7, 31.7 and 81.7%, respectively.

Table 2. Mortality of *T. pyri* after 7 days of exposure – definitive test

Study group [L/ha]	Number of tested mites [no.]	Mortality				
		Number of dead & escaped[e] mites [no.]			Total	
		Replicates				
		I	II	III	[no.]	[%]
Control	60	0	0	0	0	0.0
0.026	60	0	0	0	0	0.0
0.064	60	0	1	0	1	1.7
0.16+	60	0	2	2	4	6.7
0.4+	60	6	6	7	19	31.7
1.0+	60	18	15	16	49	81.7
LR_{50}		0.528 [L/ha] *(0.444 – 0.640)**				
$NOER_{mortality}$		0.064 [L/ha]				
[mL/ha]	Bi 58 Top 400 EC					
9.0	60	17	13	19	49	81.7

The definitive test was performed between 05 – 19.11.2020
+: statistically significant differences
*: 95%-confidence limits

There were no statistically significant differences in mortality between group treated with the test item at the rates of 0.026 and 0.064 L/ha and the control group. There were statistically significant differences in mortality between group treated with the test item at the rates of 0.16, 0.4 and 1.0 L/ha and the control group (Step-down Cochran-Armitage Test Procedure, p(trend)>α).
On the basis of the obtained mortality results, the LR50 value with 95% confidence limits was calculated. It was 0.528 L/ha of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) (95% confidence limits: 0.444 – 0.640). NOERmortality was 0.064 L/ha of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC), [SPO/B/67].
After 7 days of exposure to Bi 58 Top 400 EC at the rate of 9.0 mL/ha, mortality was 81.7%. Therefore, the validity criterion specified in the Method description was met. The results obtained in the reference item group showed that the test organisms were sensitive to dimethoate.

Reproduction
The sex ratio after 7 days of exposure is presented in Table 3 and the values met the criteria.

Table 3. Sex ratio after 7 days of exposure – definitive test

Study group [L/ha]	No. of tested mites	Males and females [no.]								
		Replicates								
		I			II			III		
		♀	♂	SR*	♀	♂	SR*	♀	♂	SR*
Control 0.0	**60**	15	5	**0.75**	14	6	**0.70**	14	6	**0.70**
0.026	**60**	13	7	**0.65**	15	5	**0.75**	12	8	**0.60**
0.064	**59**	14	6	**0.70**	13	6	**0.68**	12	8	**0.60**
0.16	**56**	13	7	**0.65**	10	8	**0.56**	12	6	**0.67**
0.4	**41**	10	4	**0.71**	9	5	**0.64**	7	6	**0.54**

SR*: sex ratio – the number of females divided by the total number of females and males per replicate after 7 days of exposure

Table 4. Reproduction of *T. pyri* – definitive test

Study group [L/ha]	Replicates (X)	Developmental stages of the mites	Observation period			RrX	Rr	Pr [%]
			DAT 8	DAT 11	DAT 14			
Control 0.0	I	Eggs	13	36	38	6.2	7.0	–
		Larvae	0	4	1			
		Males	5	5	4			
		Females	15	15	14			
	II	Eggs	17	40	37	7.1		
		Larvae	0	2	3			
		Males	5	5	5			
		Females	14	14	14			
	III	Eggs	19	45	42	7.7		
		Larvae	0	0	0			
		Males	6	6	6			
		Females	14	14	13			
0.026	I	Eggs	11	41	35	7.2	6.5	7.0
		Larvae	0	0	0			
		Males	7	6	6			
		Females	13	12	11			
	II	Eggs	6	34	33	5.0		
		Larvae	0	2	0			
		Males	5	5	5			
		Females	15	15	15			
	III	Eggs	18	40	30	7.3		
		Larvae	0	0	0			
		Males	8	7	6			
		Females	12	12	12			
0.064	I	Eggs	11	35	29	6.0	5.5	22.0
		Larvae	0	1	3			
		Males	6	6	6			
		Females	14	13	12			
	II	Eggs	5	27	24	5.0		
		Larvae	0	2	0			
		Males	5	5	5			
		Female	12	12	10			
	III	Eggs	7	24	26	5.4		
		Larvae	0	2	2			
		Males	8	6	6			
		Females	12	11	11			

continued on next page

Table 4. cont. Reproduction of *T. pyri* – definitive test.

Study group [L/ha]	Replicates (X)	Developmental stages of the mites	Observation period			RrX	Rr	Pr [%]
			DAT 8	DAT 11	DAT 14			
0.16	I	Eggs	18	40	28	7.2	5.6	19.5
		Larvae	0	1	1			
		Males	6	5	5			
		Females	13	12	11			
	II	Eggs	5	20	24	5.6		
		Larvae	0	0	0			
		Males	7	6	5			
		Females	9	9	8			
	III	Eggs	5	19	17	4.1		
		Larvae	0	1	2			
		Males	6	6	6			
		Females	11	11	9			
0.4+	I	Eggs	6	21	20	5.3	4.7	32.2
		Larvae	0	1	0			
		Males	3	3	3			
		Females	9	9	9			
	II	Eggs	4	19	13	4.3		
		Larvae	0	2	1			
		Males	5	5	4			
		Females	9	9	9			
	III	Eggs	4	12	10	4.6		
		Larvae	0	2	0			
		Males	4	4	3			
		Females	6	6	6			
ER_{50}		**> 0.4 [L/ha]**						
$NOER_{reproduction}$		**0.16 [L/ha]**						

DAT: days after treatment
RrX: the reproduction rate for each replicate (X) of a given study group after 14 days, calculated according to equation no. 1 (section. 5.2. Reproduction)
Rr: the mean reproduction rate in a given study group after 14 days
Pr: the percentage of reproduction reduction calculated according to equation no. 2 (section. 5.2. Reproduction)
+: statistically significant differences between control and groups exposed to test item; ToxRat Professional 3.3.0. software [12], [SOP/B/67]

Reproduction of the surviving mites from the control group and the groups treated with Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rates of 0.026, 0.064, 0.16 and 0.4 L/ha, was assessed since mortality of these groups was < 50.0%. Reproduction of the surviving mites from groups treated with Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rate of 1.0 L/ha were not assessed due to mortality higher than 50% [SOP/B/36].

The mean reproduction rate (Rr) in the control group was 7.0 eggs/female. The mean Rr after 14 days of exposure to Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at rates 0.026, 0.064, 0.16 and 0.4 L/ha a were, 6.5, 5.5, 5.6 and 4.7 eggs/female, respectively. The percentages of reproduction reduction (Pr) caused by at the rates of 0.026, 0.064, 0.16 and 0.4 L/ha were 7.0, 22.0, 19.5 and 32.2%, respectively.

There were no statistically significant differences in reproduction between group treated with the test item at the rates of 0.026, 0.064 and 0.16 L/ha and the control group. There were statistically significant differences in reproduction between group treated with the test item at the rate of 0.4 L/ha and the control group (Williams Multiple Sequential t-test Procedure,|t|>|t*|) [12], [SPO/B/67].

Test validity criteria

The following validity criteria were met during the study:
- mortality of the control group was 0.0% on day 7 of exposure (criterion: a maximum of 20%),
- mortality of the mites exposed to the reference item at the rate of 9.0 mL/ha was 81.7% on day 7 of exposure (criterion: from 50 to 100%),
- the mean number of eggs per female in the control group was 7.0 (required: ≥ 4 eggs per female).

Deviations in the study

The experiment was performed according to the ESCORT 1 and the ESCORT 2 guidance documents, the guidelines developed by the IOBC, BART, and EPPO Joint Initiative, the Standard Operating Procedure SOP/B/36: 'An extended laboratory test for evaluating the effects of plant protection products on the predatory mite, Typhlodromus pyri (Sch.)', other references given in section 9 and the SOP's listed in section 10 of the report and the Study Plan. According to the guideline developed by the IOBC, BART, EPPO Joint Initiative, as a food source only pollen was used. However, in the experiment additional food in the form of the two-spotted spider mite (T. urticae) eggs, was used. Another food source prevents the mites from escaping from discs.

Study 3

Comments of zRMS:	The study was conducted to the guidelines and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.2/03
Report	*An extended laboratory test for evaluating effects of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on the ladybird beetle, Coccinella septempunctata (L.)*, M. Knapik, 2021; Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland; STUDY CODE: B-78-20
Guideline(s):	ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Schmuck et al., 2000)
Deviations:	Yes
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) content: 256.6 g/L of pyraclostrobin batch no.: 04/2020 production date: 23.04.2020 expiry date: 23.04.2022
Test Species:	Biological test system: the ladybird beetle, C. septempunctata L. (Arthropoda: Coccinellidae) – age: 4-day-old larvae – source: Beetles was obtained from commercial breeder (BiasLabs Ltd., London UK)
Test Design:	7 study groups: − a control group (0.0 L/ha) − Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rates of: - 0.013 L/ha - 0.032 L/ha - 0.08 L/ha - 0.2 L/ha - 0.5 L/ha − Bi 58 Top 400 EC at the rate of 8.0 mL/ha number of replicates: 40 replicates/group number of larvae: 1 larva of Coccinella septempunctata /replicate
Endpoints:	– preimaginal mortality of the ladybird beetles – LR50 – NOERmortality

– reproductive performance of the moulted beetles over a period of 14 days (the mean number of fertile eggs/female/day) reproduction reduction (Pr)
– ER50

Test Conditions:
– temperature:
23.0 – 27.3°C
– relative air humidity:
60.0 − 79.2%
– photoperiod:
16 hours light : 8 hours dark
– light intensity
1701 lx
Statistical analysis:
Probit analysis using linear max. likelihood regression,
Step-down Cochran-Armitage Test Procedure

Results and discussion

The effects of the test item, Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on mortality and reproductive capacity of the ladybird beetle, Coccinella septempunctata L. in the laboratory test are summarized below.

Study group	Parameters (endpoints)						
	Mortality			Reproduction			
Test item [L/ha]	[%]	[%][a]	LR_{50} [L/ha]	Mean no. of eggs/female/day	Mean no. of fertile eggs/female/day	Reproduction reduction Pr [%]	ER_{50} [L/ha]
Control (0.0)	20.0	–	0.112 (0.01 - 115.514*)	5.0	4.0	–	> 0.08
0.013	35.0	18.8		5.6	4.6	-15.0	
0.032	35.0	18.8		4.6	4.1	-2.5	
0.08	37.5	21.9		5.7	4.5	-12.5	
0.2	70.0	62.5		-	-	-	
0.5	95.0	93.8		-	-	-	
$NOER_{mortality}$	0.08 [L/ha]						
Bi 58 Top 400 EC							
Reference item [mL/ha]	87.5	84.4	–				
8.0							

[a]: mortality was corrected according Abbott's equation [1]
*: statistically significant differences
* - confidence limits

The validity criterion concerning mortality was met, because mortality of the ladybird beetle, Coccinella septempunctata L. in the control group was equal to 20.0% (≤ 30.0%). The corrected mortality of the ladybird beetles exposed to the test item at the rates of 0.013, 0.032, 0.08, 0.2 and 0.5 L/ha, after Abbott's correction, were 18.8, 18.8, 21.9, 62.5 and 93.8%, respectively.
At the significance level of 0.05, there were statistically significant differences in mortality between the ladybirds exposed to the test item at the rates of 0.2 and 0.5 L/ha of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) and the control group (Step-down Cochran-Armitage Test Procedure, (α=0.05)).

The LR50 value is equal to 0.112 L/ha (confidence limits: 0.01 – 115.514) of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC). The NOERmortality is equal to 0.08 L/ha of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC).
The corrected mortality of the ladybird beetles exposed to the reference item at the rate of 8.0 mL of Bi 58 Top 400 EC/ha, after Abbott's correction, was equal to 84.4%. Therefore, the validity criterion was met. The results showed that the insects were sensitive to dimethoate.
The mean number of fertile eggs/female/day in the control group was 4.0 (criterion: ≥ 2 eggs/female/day). The mean numbers of fertile eggs/female/day in the group treated with the of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rates of 0.013, 0.032 and 0.08 L/ha were equal to 4.6, 4.1 and 4.5 it refers to -15.0, -2.5 and -12.5% reproduction reduction. The negative values means that in the tested rates there were higher mean numbers of fertile eggs per viable female per day than in the control group.
It can be concluded that Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rates of 0.013, 0.032 and 0.08 L/ha had no adverse effect on the reproduction capacity of the ladybird beetle. The ER50 value is above 0.08 L/ha of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC).

Mortality
In the preliminary non-GLP range finding test, mortality of the control group was 0.0% and the groups treated with the test item at all the rates i.e. 0.106, 0.425 and 1.7 L/ha, were 20.0, 100.0, 100.0%, respectively.

Table 1. Mortality of *C. septempunctata* – non-GLP preliminary test

Study group [L/ha]	Number of tested beetles [no.]	Mortality				
		Number of dead individuals [no.]			Total	
		larvae	pupae	adults	[no.]	[%]
Control	10	0	0	0	0	0.0
0.106	10	2	0	0	2	20.0
0.425	10	9	1	0	10	100.0
1.7	10	10	0	0	10	100.0

In the definitive test mortality in the control group was 20.0%. The corrected mortality of Coccinella septempunctata exposed to the test item at the rates of 0.013, 0.032, 0.08, 0.2 and 0.5 L/ha, after Abbott's correction, were 18.8, 18.8, 21.9, 62.5 and 93.8%, respectively.

Table 2. Mortality of *C. septempunctata* – definitive test

Study group [L/ha]	Number of tested beetles [no.]	Mortality					
		Number of dead individuals [no.]			Total		
		larvae	pupae	adults	[no.]	[%]	[%][a]
Control	40	8	0	0	8	20.0	–
0.013	40	11	3	0	14	35.0	18.8
0.032	40	9	5	0	14	35.0	18.8
0.08	40	15	0	0	15	37.5	21.9
0.2	40	25	3	0	28	70.0	62.5*
0.5	40	37	1	0	38	95.0	93.8*
LR_{50}		0.112 [L/ha] (0.01 – 115.514)					
$NOER_{mortality}$		0.08 [L/ha]					
[mL/ha]	Bi 58 Top 400 EC						
8.0	40	35	0	0	35	87.5	84.4

At the significance level of 0.05, there were no statistically significant differences in mortality between the ladybirds exposed to the test item at the rates of 0.013, 0.032 and 0.08 L/ha and the control (Step-down Cochran-Armitage Test Procedure).
At the significance level of 0.05, there were statistically significant differences in mortality between the ladybirds exposed to the test item at the rates of 0.2 and 0.5 L/ha and the control (Step-down Cochran-Armitage Test Procedure).
Based on the obtained mortality results, it could be assumed that the LR50 value (the application rate at which 50% mortality of the test system is observed) is equal to 0.112 L/ha (confidence limits: 0.01 – 115.514) of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC). The NOERmortality is equal to 0.08 L/ha of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC).
The percentage of mortality of C. septempunctata (L.) exposed to Bi 58 Top 400 EC at rate of 8.0 mL/ha, after Abbott's correction, was 84.4%. The results obtained in the reference item group indicated that the biological test system was sensitive to dimethoate.
At the rates of 0.2 and 0.5 L/ha the development of ladybirds were delayed compared to the control group and group exposed to the test item at the rates of 0.08, 0.2 and 0.5 L/ha. Additionally, in each group exposed to the test item there were observed pupae that did not stick to the substrate.

Reproduction
Sex of the beetles at the pre-oviposition period is presented in Table 4, whereas the detailed results of the reproduction test are presented in Table 5.

Table 4. Sex of the *C. septempunctata* (L.) – pre-oviposition period

Study group [L/ha]	Number of males and females [no.]		Number of tested insects [no.]
	♀	♂	
Control	21	11	32
0.013	15	9	24
0.032	15	11	26
0.08	12	13	25

Table 5. Reproduction of *C. septempunctata*

DAT	Laid eggs [no.]	Viable females [no.]	Eggs/female /day	Hatched larvae [no.]	Hatching rate [%]	Fertile eggs/female /day
Control [0.0 L/ha]						
30	235	21	11.2	116	49.4	5.5
34	30	21	1.4	25	83.3	1.2
35	0	21	0.0	0	0.0	0.0
36	67	19	3.5	52	77.6	2.7
37	84	18	4.7	77	91.7	4.3
38	138	18	7.7	127	92.0	7.1
39	45	18	2.5	42	93.3	2.3
40	110	18	6.1	102	92.7	5.7
42	148	18	8.2	128	86.5	7.1
MEAN			5.0	74.3	74.1	**4.0**
0.013 [L/ha]						
30	245	15	16.3	166	67.8	11.1
34	24	15	1.6	24	100.0	1.6
35	81	15	5.4	72	88.9	4.8
36	34	14	2.4	25	73.5	1.8
37	101	14	7.2	91	90.1	6.5
38	0	14	0.0	0	0.0	0.0
39	40	14	2.9	35	87.5	2.5
40	89	14	6.4	87	97.8	6.2
42	111	14	7.9	101	91.0	7.2
MEAN			5.6	66.8	77.4	**4.6**

DAT	Laid eggs [no.]	Viable females [no.]	Eggs/female /day	Hatched larvae [no.]	Hatching rate [%]	Fertile eggs/female /day
0.032 [L/ha]						
30	98	15	6.5	79	80.6	5.3
34	122	15	8.1	110	90.2	7.3
35	0	15	0.0	0	0.0	0.0
36	24	15	1.6	22	91.7	1.5
37	46	15	3.1	31	67.4	2.1
38	35	15	2.3	34	97.1	2.3
39	134	15	8.9	129	96.3	8.6
40	78	15	5.2	76	97.4	5.1
42	85	15	5.7	78	91.8	5.2
MEAN			4.6	62.1	79.2	**4.1**
0.08 [L/ha]						
30	210	12	17.5	133	63.3	11.1
34	0	12	0.0	0	0.0	0.0
35	0	12	0.0	0	0.0	0.0
36	47	11	4.3	32	68.1	2.9
37	103	11	9.4	73	70.9	6.6
38	33	11	3.0	33	100.0	3.0
39	66	11	6.0	65	98.5	5.9
40	89	11	8.1	86	96.6	7.8
42	38	11	3.5	31	81.6	2.8
MEAN			5.7	50.3	64.3	**4.5**

In the definitive test reproduction of the ladybird beetles from the control group and the groups treated with the test item at the rates of 0.013, 0.032 and 0.08 L/ha were assessed, since the mortality were < 50% after Abbott's correction. However, the reproduction in the group treated with the test item at the rates of 0.2 and 0.5 L/ha were not assessed due to mortality > 50% after Abbott's correction.
At the rate of 0.013 L/ha there were two adult beetles with deformations that could not make identification of their sex.
The mean number of fertile eggs/female/day in the control group was 4.0 (criterion: ≥ 2 eggs/female/day)
The mean numbers of fertile eggs/female/day in the groups treated with the Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rates of 0.013, 0.032 and 0.08 L/ha were equal to 4.6, 4.1 and 4.5 and it refers to -15.0, -2.5 and -12.5% reproduction reduction, respectively (Table 5 and 6). The negative values means that in the tested rates there were higher mean numbers of fertile eggs per viable female per day than in the control group
The mean number of fertile eggs per viable female per day were above 2 in the groups treated with the test item at the rates of 0.013, 0.032 and 0.08 L/ha, therefore there is no treatment related effect on the reproductive performance in mentioned rates. It can be concluded that Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rates of 0.013, 0.032 and 0.08 L/ha has no adverse effect on the reproduction capacity of the ladybird beetle The ER50 value is above 0.08 L/ha of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC).
Other information on reproductive performance of Coccinella septempunctata (mean number of eggs/female/day and mean hatching rate [%]) are presented in Table 6.

Table 6. Effect of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on *C. septempunctata* reproduction

Study group [L/ha]	Mean no. of eggs/female /day*	Mean hatching rate [%]*	Mean no. of fertile eggs/ female/day **	Reproduction reduction Pr [%]***	ER_{50} [L/ha]
Control	5.0	74.1	4.0	–	> 0.08
0.013	5.6	77.4	4.6	-15.0	
0.032	4.6	79.2	4.1	-2.5	
0.08	5.7	64.3	4.5	-12.5	

*: mean value of 9 assessment days (details are presented in Table 5)
**: mean number of fertile eggs/female/day was calculated according to equation no. 2 (5.2. Reproduction performance)
***: The percentage of reproduction reduction (Pr) was calculated according to equation no. 3 (5.2. Reproduction performance). The negative values means that in the tested rates there were higher mean numbers of fertile eggs per viable female per day than in the control group

Test validity criteria
The following validity criteria were met during the study:
− pre-imaginal mortality of the control group was 20.0% (criterion: a maximum of 30.0%),
− mean corrected mortality of the reference item group was 84.4% (criterion: a minimum of 40%),
− fertility (the mean number of fertile eggs/female/day) in the control group was 4.0 (criterion: ≥ 2 fertile eggs/female).

Deviations in the study
The experimental part of the study was conducted according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART and EPPO Joint Initiative (Schmuck V. et al., 2000), SOP/B/63 and other procedures related with the study and the Study Plan.
In the experimental part of the study a deviation from the guidelines developed by the IOBC, BART and EPPO Joint initiative (Schmuck V., et al., 2000) occurred. This deviation is to use leaf discs as a surface instead of plastic discs. This method was described in the Study Plan and the SOP/B/63.

During the definitive test there were short term deviations (below one hour) in the temperature, which was above range from SOP/B/63 and the guideline developed by IOBC, BART and EPPO Joint Initiative (Schmuck V., et al. 2000).
According to the Study Plan the source of organisms was from a laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, however the definitive study was performed on the beetles obtained from commercial breeder (BiasLabs Ltd., London UK).
Mentioned deviations had no effect on the obtained results.

Study 4

Comments of zRMS:	The study was conducted to the guidelines and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.2/04
Report	*An extended laboratory test for evaluating effects of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on the green lacewing, Chrysoperla carnea (Steph.),* M. Knapik, 2020; Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland; STUDY CODE: B-81-20
Guideline(s):	ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Vogt H. et al., 2000)
Deviations:	Yes
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) content: 256.6 g/L of pyraclostrobin batch no.: 04/2020 production date: 23.04.2020 expiry date: 23.04.2022
Test Species:	Biological test system: the green lacewing, Chrysoperla carnea (Steph.), Neuroptera: Chrysopidae – age: first instars' larvae (3 days old) – source: a laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna; the culture was augmented by commercial breeder
Test Design:	7 study groups: − a control group (0.0 L/ha) − Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rates of - 0.005 L/ha - 0.013 L/ha - 0.032 L/ha

- 0.08 L/ha
- 0.2 L/ha
− Bi 58 Top 400 EC at the rate of 38.0 mL/ha
number of replicates: 30 replicates/group
number of larvae: 1 larva of Chrysoperla carnea /replicate

Endpoints:
– cumulative mortality of larvae, pupae, and adults after emergence
– LR50 value
– reproduction of the lacewings:
− fecundity (mean number of eggs/female/day)
− fertility (mean hatching rate)
− ER50

Test Conditions:
– temperature:
22.6 − 27.3°C
– relative air humidity:
60.2 − 79.2%
– photoperiod:
16 hours light : 8 hours dark
– light intensity
1793 lux
Statistical analysis:
Probit analysis using linear max. likelihood regression, Step-down Cochran-Armitage Test Procedure

Results and discussion
The effects of the test item, Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on mortality and reproductive capacity the green lacewings, Chrysoperla carnea (Steph.) in the laboratory test are summarized below.

Study group [application rate]	Parameter (endpoints)				
	Mortality		Reproduction		
Test item [L/ha]	[%]	LR_{50} [L/ha]	Mean number of eggs/female /day [no.]	Mean hatching rate [%]	ER_{50} [L/ha]
Control (0.0)	0.0	0.192 (0.101 – 0.753*)	40.6	90.8	> 0.08
0.005	6.7		34.8	79.6	
0.013+	16.7		25.8	71.2	
0.032+	16.7		35.5	78.7	
0.08+	30.0		28.9	67.5	
0.2+	56.7				
$NOER_{mortality}$	0.005 [L/ha]				
Reference item [mL/ha]	Bi 58 Top 400 EC				
38.0	66.7	–			

+: statistically significant differences
* - confidence limits

The validity criterion concerning mortality was met, because mortality of the green lacewings, Chrysoperla carnea (Steph.) in the control group was 0.0%. The mortality of the green lacewings exposed to the test item at the rates of 0.005, 0.013, 0.032, 0.08 and 0.2 L/ha of Pyraclostrobin 250 EC

(CHR/F/PYRA 250 EC) was 6.7, 16.7, 16.7, 30.0 and 56.7%, respectively.
There were statistically significant differences in mortality of the green lacewings in the groups treated with the test item at the rates of 0.013, 0.032, 0.8 and 0.2 L/ha in comparison to the control group. There were no statistically significant differences in mortality of the green lacewings in the groups treated with the test item at the rate of 0.005 L/ha in comparison to the control group (Step-down Cochran-Armitage Test Procedure, p(trend)> α, (α=0.05)).
The LR50 value is equal to 0.192 L/ha (confidence limits: 0.101 – 0.753). The NOERmortality value is equal to 0.005 L/ha.
The percentage of mortality of Ch. carnea (Steph.) exposed to Bi 58 Top 400 EC at rate of 38.0 mL/ha, was 66.7%. The results obtained in the reference item group indicated that the biological test system was sensitive to dimethoate.
The reproduction in the group treated with the test item at the rate of 0.2 L/ha were not assessed due to mortality higher than 50%.
The mean number of fertile eggs/female/day in the control group was equal to 40.6 (criterion: ≥ 15.0). The mean numbers of fertile eggs/female/day in the groups treated with Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rates of 0.005, 0.013, 0.032 and 0.08 L/ha were equal to 39.0, 35.8, 35.5 and 28.9, respectively. The mean hatching rate in the control group was 90.8% (criterion: ≥ 70%). The mean hatching rate in the groups treated with the test item at the rates of 0.005, 0.013, 0.032 and 0.08 L/ha were 79.6, 71.2, 78.7 and 67.5%, respectively.
Fecundity reduction (Pr) in the group treated with the test item at the rates of 0.005, 0.013, 0.032 and 0.08 L/ha were 12.3, 21.6, 13.3 and 25.7%, respectively.
Based on the results, it can be presumed that Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rate of 0.08 L/ha had an adverse effect on the reproductive performance of the lacewings and the ER50 value is above 0.08 L/ha.

Mortality
In the preliminary range-finding test, mortality of the control group was 0.0% and the groups treated with the test item at all the rates i.e. 0.106, 0.425 and 1.7 L/ha, were 90.0, 90.0 and 100.0%, respectively (Table 1).

Table 1. Mortality of *Chrysoperla carnea* – preliminary test

Study group [L/ha]	Number of tested green lacewings [no.]	Mortality				
		Number of dead individuals [no.]			Total	
		larvae	pupae	adults	[no.]	[%]
Control	10	0	0	0	0	0.0
0.106	10	9	0	0	9	90.0
0.425	10	9	0	0	9	90.0
1.7	10	10	0	0	10	100.0

Mortality of the lacewings recorded in the definitive test is presented in Table 2. In the definitive test mortality of in the control group was 0.0%. The mortality of Chrysoperla carnea (Steph.) exposed to the test item at the rates of 0.005, 0.13, 0.32, 0.08 and 0.2 L/ha of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) was 6.7, 16.7, 16.7, 30.0 and 56.7%, respectively (Table 2).
There were statistically significant differences in mortality of the green lacewings in the groups treated with the test item at the rates of 0.013, 0.032, 0.8 and 0.2 L/ha in comparison to the control group. There were no statistically significant differences in mortality of the green lacewings in the groups treated with the test item at the rate of 0.005 L/ha in comparison to the control group (Step-down Cochran-Armitage Test Procedure, p(trend)> α, (α=0.05)).
The LR50 is equal to 0.192 L/ha (confidence limits: 0.101 – 0.753). The NOERmortality value is equal to 0.005 L/ha (Table 2).
The percentage of mortality of Ch. carnea (Steph.) exposed to Bi 58 Top 400 EC at rate of 38.0 mL/ha,

was 66.7%. The results obtained in the reference item group indicated that the biological test system was sensitive to dimethoate (Table 2).

Table 2. Mortality of *Chrysoperla carnea* – definitive test

Study group [L/ha]	Number of tested green lacewings [no.]	Mortality				
		Number of dead individuals [no.]			Total	
		larvae	pupae	adults	[no.]	[%]
Control	30	0	0	0	0	0
0.005	30	2	0	0	2	6.7
0.013+	30	5	0	0	5	16.7
0.032+	30	5	0	0	5	16.7
0.08+	30	8	1	0	9	30.0
0.2+	30	17	0	0	17	56.7
LR_{50}		0.192 [L/ha] (0.101 – 0.753*)				
$NOER_{mortality}$		0.005 [L/ha]				
[mL/ha]	Bi 58 Top 400 EC					
38.0	30	17	3	0	20	66.7

Reproductive

Reproduction of the lacewings from the control group and the groups treated with the test item at the rates of 0.005, 0.013, 0.032 and 0.08 L/ha was assessed, since the mortality were < 50%. However, the reproduction in the group treated with the test item at the rate of 0.2 L/ha were not assessed due to mortality higher than 50%.

Sex of the lacewings at the pre-oviposition period is presented in Table 4, whereas the results of the reproduction test are presented in Table 5. The mean number of fertile eggs/female/day in the control group was equal to 40.6 (criterion: ≥ 15.0). The mean numbers of fertile eggs/female/day in the groups treated with Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the rates of 0.005, 0.013, 0.032 and 0.08 L/ha were 39.0, 35.8, 35.5 and 28.9 respectively. The mean hatching rate in the control group was 90.8% (criterion: ≥ 70%). The mean hatching rate in the groups treated with the test item at the rates of rate of 0.005, 0.013, 0.032 and 0.08 L/ha were 79.6, 71.2, 78.7 and 67.5% (Table 5).

Fecundity reduction (Pr) in the group treated with the test item at the rates of 0.005, 0.013, 0.032 and 0.08 L/ha were 12.3, 21.6, 13.3 and 25.7%, respectively (Table 5).

Based on the obtained results, it can be presumed that the Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) had an adverse effect on the reproductive performance of the green lacewing, Chrysoperla carnea (Steph.) at rate of 0.08 L/ha. At the rates of 0.005, 0.013 and 0.032 L/ha Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) had no adverse effect on the reproductive performance of the green lacewing, Chrysoperla carnea (Steph.) and the ER50 value is above 0.08 L/ha.

Table 4. Sex of the green lacewings *Chrysoperla carnea* – pre-oviposition period

Study group [L/ha]	Number of males and females [no.]		Number of tested insects [no.]
	♀	♂	
Control	16	14	30
0.005	13	15	28
0.013	14	11	25
0.032	12	13	25
0.08	11	10	21

Table 5. Reproduction of *Chrysoperla carnea*

Study group / application rate [L/ha]	DAT	Number of [no.]							Mean hatching rate (Fertility) [%]	Fecundity reduction relative to the control (Pr) [%]
		Eggs (total)	Viability females ♀	Eggs/ female /day	Mean	Eggs used for hatching assessment	Hatched larvae	Hatching rate [%]		
Control	29	634	15	42.3	40.6	491	467	95.1	90.8	-
	33	584	15	38.9		281	243	86.5		
0.005	29	624	13	48.0	39.0	350	280	80.0	79.6	12.3
	33	359	12	29.9		279	221	79.2		
0.013	29	610	14	43.6	35.8	550	376	68.4	71.2	21.6
	33	392	14	28.0		254	188	74.0		
0.032	29	430	11	39.1	35.5	351	300	85.5	78.7	13.3
	33	351	11	31.9		284	204	71.8		
0.08	29	337	11	30.6	28.9	276	173	62.7	67.5	25.7
	33	271	10	27.1		250	181	72.4		
ER_{50}	> 0.08 L/ha									

DAT: day after the treatment

Test validity criteria

The following validity criteria were met during the study [5]:
– pre-imaginal mortality of the control group was 0.0% (criterion: a maximum of 20.0%),
– mean mortality of the reference item group was 66.7% (criterion: a minimum of 50%),
– the mean number of eggs per female per day in the control group (fecundity) was 40.6 (criterion: ≥ 15.0),
– the mean hatching rate in the control group (fertility) was 90.8 (criterion: ≥ 70%).

Deviations

The study was conducted according to the ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART and EPPO Joint Initiative (Vogt H. et al., 2000), SOP/B/62 and other procedures related to the study and the Study Plan.

In the experimental part of the study a deviation from the guidelines developed by the IOBC, BART and EPPO Joint initiative (Vogt H. et al., 2000) occurred. This deviation is to use leaf discs as a surface instead of plastic discs. This method was described in the Study Plan and the SOP/B/62.

During the definitive test there were short term deviations (below one hour) in the temperature, which

was out of range from SOP/B/63 and the guideline developed by IOBC, BART and EPPO Joint Initiative (Schmuck V., et al. 2000).

Study 5

Comments of zRMS:	The study was conducted to the guidelines and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.2/05
Report	*CHR/F/PYRA 250 EC – A series of aged-residue extended laboratory tests to determine effects on the ladybird beetle, Coccinella septempunctata (Coleoptera: Coccinellidae), Vaughan, R.*, 2021; Mambo-Tox, Southampton, UK; STUDY CODE: CHR-21-08
Guideline(s):	Schmuck et al. (2000). A laboratory test system for assessing effects of plant protection products on the plant-dwelling insect Coccinella septempunctata L. (Coleoptera: Coccinellidae).
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) content: 253.1 g/L of pyraclostrobin batch no.: 04/2020 production date: 23.04.2020 expiry date: 23.04.2022
Test Species:	For each bioassay, a cohort of eggs of the ladybird beetle, C. septempunctata, were obtained from an in-house culture maintained at the Test Facility (originally obtained from a commercial source - Katz Biotech AG, Baruth, Germany). They were stored in 9-cm-diameter plastic Petri dishes and, once larvae had emerged, pea aphids (Acyrthosiphon pisum (Harris)) were provided ad libitum for food. Ladybird larvae hatching over a 24-h period were set aside for each bioassay. The test commenced when these were 3-5 days old (0 DAT, 14 DAT and 28 DAT bioassays).
Test Design:	A single treatment rate of the test item, which was applied on two separate occasions with an 8-day interval, was evaluated and compared to a water-treated control and, for the initial bioassay, a toxic reference treatment. All treatments were applied to sufficient pots of dwarf French bean plants to provide leaves for bioassays initiated 0 14, and 28 days after treatment (DAT).
Endpoints:	The endpoints of the individual bioassays were an assessment of pre-imaginal mortality and the subsequent assessment of the reproductive capacity of adult ladybirds that survived their initial exposure. The fecundity assessments were only made for the control and test-item treatment in bioassays where the latter had resulted in ≤ 50% corrected mortality.

Test Conditions:

Bioassay	Temperature	Lighting
0 DAT	23.8-25.0°C	16 h photoperiod (2700-3200 lux)
14 DAT	24.0-25.5°C	16 h photoperiod (2300-2500 lux)
28 DAT	24.2-25.4°C	16 h photoperiod (1700-3000 lux)

Introduction

The test item in this study was CHR/F/PYRA 250 EC an emulsifiable concentrate formulation containing pyraclostrobin (nominally 250 g/L).

The aim of the study was to determine under extended laboratory test conditions the effects of both freshly-dried and field-aged foliar residues of CHR/F/PYRA 250 EC on the seven-spot ladybird beetle, Coccinella septempunctata L. (Coleoptera: Coccinellidae). C. septempunctata is considered to be a suitable species for determining the effects of pesticides on non-target species of foliar-dwelling arthropods in the agricultural ecosystem (Barrett et al., 1994).

The test method was based on the laboratory test guideline of Schmuck et al. (2000), but with leaves of the French bean (Phaseolus vulgaris L.) being used as a test substrate, rather than glass. In addition to the main objective to identify any harmful effects of the test item, a check was also made for sub-lethal treatment effects on the reproductive performance of the surviving insects. The test design was in line with current European guidance documents (Barrett et al., 1994 and Candolfi et al., 2001), which themselves meet the relevant requirements of Commission Regulations (EU) No. 283/2013 and 284/2013 (EU, 2013).

CHR/F/PYRA 250 EC was evaluated at a single application rate, equivalent to 1.0 L product/ha. The treatment was applied to the test plants on two occasions with an 8-day spray-interval.

Results and discussion

The intention of the bioassay programme was to demonstrate that residues of the test item did not result in unacceptable effects in two consecutive bioassays (i.e. demonstrating that corrected pre-imaginal mortality was ≤ 50% and that certain fecundity and fertility criteria were met).

The results of the mortality assessments are summarised below.

Bioassay initiated	Treatment	Test-item rate (L/ha)	% pre-imaginal mortality [a)]	Corrected % pre-imaginal mortality [b)]
0 DAT	Control	-	0.0	-
	CHR/F/PYRA 250 EC	1.0	97.5 *	97.5
	Toxic reference	-	100 *	100
14 DAT	Control	-	10.0	-
	CHR/F/PYRA 250 EC	1.0	12.5	2.8
28 DAT	Control	-	0.0	-
	CHR/F/PYRA 250 EC	1.0	50.0 *	50.0

a) For each bioassay, pre-imaginal mortality in the test item treatment and the toxic reference treatment was compared to the control using Fisher's exact binomial test (one-sided, > control, $\alpha = 0.05$). An asterisk (*) indicates where differences were significant.

b) Corrected mortalities were calculated using Abbott's formula. A positive value indicates an increase and a negative a decrease.

The results of the reproduction assessments are summarised below.

Bioassay initiated	Treatment	Test-item rate (L/ha)	Mean no. eggs/♀/ day	Mean % egg viability	Mean no. viable eggs/♀/ day
14 DAT	Control	-	28.4	36.8	10.5
	CHR/F/PYRA 250 EC	1.0	27.6	52.9	14.6
28 DAT	Control	-	16.6	56.7	9.4
	CHR/F/PYRA 250 EC	1.0	21.3	29.3	6.3

In the 14 and 28 DAT bioassays, the mean numbers of viable eggs produced in all the treatments evaluated was ≥ 2.0 eggs/female/day. This threshold is currently viewed as being indicative of no harmful treatment effects.

Validity criteria
According to the guideline of Schmuck et al. (2000), for the test to be considered valid:
a) Pre-imaginal mortality (this includes dead larvae, pupae and adults dying during emergence from their pupae) in the control treatment should not exceed 30%.
b) The level of mortality in the toxic reference treatment should be ≥ 50%.
c) Mean egg production should be > 2 viable eggs/female/day in the control treatment.
All of these criteria were met throughout the study, where applicable.

Conclusions
The effects of both fresh and aged foliar residues of CHR/F/PYRA 250 EC on the ladybird beetle, Coccinella septempunctata, were evaluated under extended laboratory conditions. When applied at a rate equivalent to 1.0 L product/ha, on two occasions with an 8-day interval, fresh (0-day-old) foliar residues of CHR/F/PYRA 250 EC showed unacceptable effects on the survival of ladybirds. The subsequent bioassays evaluating 14-day-old and 28-day-old foliar residues, no unacceptable effects were found with respect to the survival, or the subsequent reproductive capacity of the ladybirds.

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Study 1

Comments of zRMS:	The study was conducted to the OECD guideline 222 and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.4/01
Report	*Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Earthworm Reproduction Test (Eisenia andrei)*; A. Wróbel; Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland; STUDY CODE: G-32-20
Guideline(s):	OECD Guideline No. 222 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) batch no.: 04/2020 Active substance: pyraclostrobin: 256.6 g/L
Artificial soil:	10% sphagnum peat, 20% kaolin clay, 70% air-dried quartz sand
Test Species:	the earthworm, Eisenia andrei obtained from a standard laboratory culture cultivated at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicological Studies, Laboratory of Soil Toxicology
Test Design:	test duration: 8 weeks; number of replicates: 4 replicates/concentration + 8 replicates/control; number of earthworms: 10 earthworms/replicate
Endpoints:	EC10, EC20, EC50, NOEC, LOEC (reproduction), LC50, NOEC, LOEC (survival)
Concentrations:	control, 3.2, 5.6, 10.0, 18.0, 32.0, 56.0, 100.0, 180.0, 320.0 and 560.0 mg/kg dry weight of the artificial soil
Test Conditions:	temperature: 20.0 – 22.0°C; pH at the beginning of the experiment: 5.44 – 5.73; pH at the end of the experiment: 5.52 – 5.77; soil moisture content at the beginning of the experiment: 22.4 – 28.1% (42.7 – 53.6% of the maximum water holding capacity); soil moisture content at the end of the experiment: 22.8– 26.4% (43.5 – 50.3% of the maximum water holding capacity); light-dark cycle: 16h : 8h; light intensity at the beginning of the experiment: 568 – 625 lux light intensity at the end of the experiment: 584 – 607 lux

Mortality
The impact of the test item on mortality of the earthworms is presented in table below.

Table 1. The preparation of the water emulsions.

Concentration of the test item [mg/kg of dry artificial soil]	Quantity of the test item [mg/ 3000 g dry artificial soil]	Concentration of water emulsion [mg/mL]	Volume of water emulsion [mL]	Volume of deionized water [mL]	Total volume of emulsion introduced to the sample weight of artificial soil [mL]
0.0 (control)	-	-	-	848.00	-
3.2	9.6	16	0.60	508.40	509
5.6	16.8	16	1.05	507.95	509
10	30.0	16	1.88	507.12	509
18	54.0	16	3.38	505.62	509
32	96.0	16	6.00	503.00	509
56	168.0	16	10.50	498.50	509
100	300.0	16	18.75	490.25	509
180	540.0	16	33.75	475.25	509
320	960.0	16	60.00	449.00	509
560	1680.0	16	105.00	404.00	509

After 4 weeks of the experiment, at the control group mortality of adult earthworms was not observed. At concentrations ranging from 3.2 to 560 mg of the test item/kg dry weight of artificial soil, after 4 weeks of exposure to the test item, mortality of the adult earthworms was between 0.0 – 30.0%.
The concentration of the test item causing 50% mortality of the adult earthworms (LC50) is above 560 mg of the test item/kg dry weight of artificial soil (135.3 mg of pyraclostrobin/kg dry weight of artificial soil).

Observations of the earthworms
The results of the observations of the earthworms for changes in behaviour and in morphology are presented in table below.

Table 6. Results of the observations of the adult earthworms (*Eisenia andrei*) for changes in behavior and in morphology.

Concentration of the test item [mg/kg dry weight of the artificial soil]	Replicate	Number of tested earthworms [no.]	Changes in behaviour and in morphology
0.0 (control)	1	10	10 Nc
	2	10	10 Nc
	3	10	10 Nc
	4	10	10 Nc
	5	10	10 Nc
	6	10	10 Nc
	7	10	10 Nc
	8	10	10 Nc
3.2	1	10	10 Nc
	2	9	1 D, 9 Nc
	3	10	10 Nc
	4	10	10 Nc
5.6	1	10	10 Nc
	2	10	10 Nc
	3	10	10 Nc
	4	10	10 Nc
10	1	10	10 Nc
	2	10	10 Nc
	3	10	10 Nc
	4	10	10 Nc
18	1	10	10 Nc
	2	10	10 Nc
	3	10	10 Nc
	4	10	10 Nc
32	1	10	10 Nc
	2	10	10 Nc
	3	10	10 Nc
	4	10	10 Nc
56	1	10	10 Nc
	2	10	10 Nc
	3	10	10 Nc
	4	10	10 Nc
100	1	10	10 Nc
	2	10	10 Nc
	3	10	10 Nc
	4	10	10 Nc
180	1	10	10 Nc
	2	10	10 Nc
	3	10	10 Nc
	4	10	10 Nc
320	1	10	10 Nc
	2	10	10 Nc
	3	8	2 D, 8 Nc
	4	10	10 Nc
560	1	5	5 D, 5 Nc
	2	8	2 D, 8 Nc
	3	9	1 D, 9 Nc
	4	6	4 D, 6 Nc

After 4 weeks of the experiment, the treated alive earthworms did not exhibit any changes in appearance and behaviour.

Body weights of the living adult earthworms

Table 7 illustrates body weights of the living adult earthworms, whereas Table 8 presents the average body weight and body weight increase recorded after 4 weeks of the experiment.

After the application of the test item at the concentrations ranging from 3.2 to 560 mg/kg dry weight of artificial soil, the body weight increase was between 19.6 and 32.7%. As for the control group, the body weight increase was equal to 23.9%.

Table 7. Body weights of the living adult earthworms (*Eisenia andrei*).

Concentration of the test item [mg/kg dry weight of the artificial soil]	Replicate	Weight of living earthworms at the beginning of the experiment [mg]	Weight of living earthworms after 4 weeks of the experiment [mg]
0 (control)	1	3110	3650
	2	3300	4120
	3	3100	4160
	4	3090	3800
	5	3000	3530
	6	3170	4020
	7	3100	3870
	8	3060	3740
3.2	1	3170	3560
	2	2930	3060
	3	2880	3810
	4	2980	3970
5.6	1	3090	3770
	2	3100	3780
	3	2700	3790
	4	2710	3340
10	1	2940	3580
	2	3030	3620
	3	2800	3910
	4	2730	3620
18	1	2920	3630
	2	2960	3790
	3	2860	3690
	4	2710	3480
32	1	3000	3780
	2	3030	3530
	3	2800	3710
	4	2620	3600
56	1	3140	4270
	2	3130	4180
	3	2830	3660
	4	2670	3520
100	1	3040	3570
	2	3000	3950
	3	2900	3720
	4	2680	3840
180	1	2780	3380
	2	2880	3860
	3	2920	3650
	4	2530	3050
320	1	2640	3080
	2	3080	3690
	3	3000	2960
	4	2900	3440

Concentration of the test item [mg/kg dry weight of the artificial soil]	Replicate	Weight of living earthworms at the beginning of the experiment [mg]	Weight of living earthworms after 4 weeks of the experiment [mg]
560	1	2700	1800
	2	3090	3100
	3	2900	3270
	4	3060	2280

Table 8. Body weight increase in the adult earthworms (*Eisenia andrei*).

Concentration of the test item [mg/kg dry weight of the artificial soil]	Replicate	Mean weight of 1 earthworm at the beginning of the experiment [mg]	Mean weight of 1 earthworm after 4 weeks of the experiment [mg]	Body weight increase		Mean body weight increase	
				mg	%	mg	%
0 (control)	1	311	365	54	17.4	74.5	23.9
	2	330	412	82	24.8		
	3	310	416	106	34.2		
	4	309	380	71	23.0		
	5	300	353	53	17.7		
	6	317	402	85	26.8		
	7	310	387	77	24.8		
	8	306	374	68	22.2		
3.2	1	317	356	39	12.3	69.5	23.5
	2	293	340	47	16.0		
	3	288	381	93	32.3		
	4	298	397	99	33.2		
5.6	1	309	377	68	22.0	77.0	26.9
	2	310	378	68	21.9		
	3	270	379	109	40.4		
	4	271	334	63	23.2		
10	1	294	358	64	21.8	80.8	28.4
	2	303	362	59	19.5		
	3	280	391	111	39.6		
	4	273	362	89	32.6		
18	1	292	363	71	24.3	78.5	27.4
	2	296	379	83	28.0		
	3	286	369	83	29.0		
	4	271	348	77	28.4		
32	1	300	378	78	26.0	79.3	28.1
	2	303	353	50	16.5		
	3	280	371	91	32.5		
	4	262	360	98	37.4		
56	1	314	427	113	36.0	96.5	32.7
	2	313	418	105	33.5		
	3	283	366	83	29.3		
	4	267	352	85	31.8		
100	1	304	357	53	17.4	86.5	30.2
	2	300	395	95	31.7		
	3	290	372	82	28.3		
	4	268	384	116	43.3		
180	1	278	338	60	21.6	70.8	25.3
	2	288	386	98	34.0		
	3	292	365	73	25.0		
	4	253	305	52	20.6		
320	1	264	308	44	16.7	57.3	19.6
	2	308	369	61	19.8		
	3	300	370	70	23.3		
	4	290	344	54	18.6		

Concentration of the test item [mg/kg dry weight of the artificial soil]	Replicate	Mean weight of 1 earthworm at the beginning of the experiment [mg]	Mean weight of 1 earthworm after 4 weeks of the experiment [mg]	Body weight increase		Mean body weight increase	
				mg	%	mg	%
560	1	270	360	90	33.3	79.0	27.1
	2	309	388	79	25.4		
	3	290	363	73	25.3		
	4	306	380	74	24.2		

Impact of the test item on reproduction of the earthworms

The number of juveniles at the end of the experiment is presented in Table 9, whereas the results concerning the impact of the test item on reproduction are shown in Table 11.

Table 9. Number of juvenile earthworms (*Eisenia andrei*) after 8 weeks of the experiment.

Concentration of the test item [mg/kg dry weight of the artificial soil]	Replicate	Number of juveniles [no.]	Mean ±SD	Comparison to the control [%]	CV* [%]
0.0 (control)	1 2 3 4 5 6 7 8	74 69 68 85 63 54 73 44	66 ± 13	-	19.1
3.2	1 2 3 4	79 68 58 70	69 ± 9	**103.8**	12.5
5.6	1 2 3 4	77 63 55 66	65 ± 9	**98.5**	14.0
10	1 2 3 4	60 68 60 84	68 ± 11	**102.6**	16.6
18	1 2 3 4	82 63 56 52	63 ± 13	**95.5**	21.0
32	1 2 3 4	59 76 59 57	63 ± 9	**94.7**	14.2
56	1 2 3 4	63 72 53 58	62 ± 8	**92.8**	13.2
100	1 2 3 4	54 68 52 39	53^{+} ± 12	**80.4**	22.3
180	1 2 3 4	23 19 12 34	22^{+} ± 9	**33.2**	41.8
320	1 2 3 4	12 10 11 32	16^{+} ± 11	**24.5**	64.8
560	1 2 3 4	1 15 13 2	8^{+} ± 7	**11.7**	93.9

Table 11. Endpoint values determined during the earthworm reproduction test (*Eisenia andrei*).

Parameter	Value [mg test item/kg dry weight of artificial soil]	Value [mg of pyraclostrobin/kg dry weight of artificial soil]
EC_{10}	58.2 (34.9 – 77.7)	14.0 (8.4 – 18.8)
EC_{20}	82.8 (57.2 – 103.6)	20.0 (13.8 – 25.0)
EC_{50}	162.5 (135.0 – 195.5)	39.2 (32.6 – 47.2)
NOEC (reproduction)	56	13.5
LOEC (reproduction)	100	24.2
LC_{50}	>560	>135.3
NOEC (survival)	320	77.3
LOEC (survival)	560	135.3

After the application of the test item at the concentrations ranging from 3.2 to 560 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 8 and 69 per replicate. The mean number of juveniles in the control group was equal to 66 per replicate.
After 8 weeks of the experiment, it was concluded that Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) had a statistically significant impact on reproduction of the earthworms at the concentrations ranging from 100 to 560 mg/kg dry weight of artificial soil.
The concentration of the test item causing a 10% reduction in the number of juveniles produced within the exposure period (EC10) is equal to 58.2 mg/kg dry weight of the artificial soil (14.0 mg of pyraclostrobin/kg dry weight of artificial soil).
The concentration of the test item causing a 20% reduction in the number of juveniles produced within the exposure period (EC20) is equal to 82.8 mg/kg dry weight of the artificial soil (20.0 mg of pyraclostrobin/kg dry weight of artificial soil).
The concentration of the test item causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is equal to 162.5 mg/kg dry weight of the artificial soil (39.2 mg of pyraclostrobin/kg dry weight of artificial soil).
The highest concentration at which the test item is observed to have no statistically significant effects on reproduction (NOEC) is equal to 56 mg/kg dry weight of the artificial soil (13.5 mg of pyraclostrobin/kg dry weight of artificial soil).
The lowest concentration at which the test item is observed to have a statistically significant effect on reproduction (LOEC) is equal to 100 mg/kg dry weight of the artificial soil (24.2 mg of pyraclostrobin/kg dry weight of artificial soil).

Observations of the juveniles of earthworms
After 8 weeks of the experiment, the juveniles of earthworms did not exhibit any changes in appearance and behaviour.

Results of the reference test

Table 12 shows the number of juveniles hatched from the cocoons during the reference test, the NOEC, and the LOEC.

According to the OECD Guideline No. 222, the LOEC should be between 1 – 5 mg/kg dry weight of the artificial soil; hence, it may be concluded that the sensitivity of the test organisms was proper.

Table 12. Reference substance – carbendazim. Number of juvenile earthworms (*Eisenia andrei*).

Concentration [mg/kg dry soil]	Replicate	Number of juveniles [no.]	Mean ± SD	Comparison to the control [%]	CV [%]
0.0 (control with acetone)	1 2 3 4 5 6 7 8	147 143 127 136 134 149 174 123	141.6 ± 16.0	-	11.3
0.0 (control)	1 2 3 4 5 6 7 8	138 166 160 154 163 172 144 127	153.0 ± 15.4	108.0	10.1
1.0	1 2 3 4	148 151 136 121	139.0 ± 13.6	98.1	9.8
1.5	1 2 3 4	135 120 146 139	135.0 ± 11.0	95.3	8.1
2.25	1 2 3 4	102 109 123 132	116.5⁺ ± 13.5	82.3	11.6
3.37	1 2 3 4	88 130 91 60	92.3⁺ ± 28.8	65.1	31.2
5.0	1 2 3 4	39 31 18 27	28.8⁺ ± 8.7	20.3	30.4
NOEC	mg/kg dry weight of the artificial soil	1.50			
LOEC		2.25			

Validity criteria

The results are considered valid because the following criteria were satisfied in the controls:

- each replicate produced from 44 to 85 juveniles (66.3 mean) at the end of the experiment (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 19.1% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 0.0% (criterion: ≤ 10%).

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

No additional studies were performed.

A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

Study 2

Comments of zRMS:	The study was conducted to the OECD guideline 232 and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.4/02
Report	*Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Collembolan (Folsomia candida) Reproduction Test*; A. Arendarczyk; Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland; STUDY CODE: G-33-20
Guideline(s):	OECD Guideline No. 232 (2016)
Deviations:	Yes
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) batch no.: 04/2020 Active substance: pyraclostrobin: 256.6 g/L
Artificial soil:	5% sphagnum peat, 20% kaolin clay, and 75% air-dried industrial sand
Test Species:	the collembolan, Folsomia candida obtained from a standard laboratory culture at the Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna, Laboratory of Soil Toxicology. The collembolans used in the study were 9 – 11 days old.
Test Design:	test duration: 28 days number of replicates: 4 replicates / concentration + 8 replicates / control; number of collembolans: 10 / replicate
Endpoints:	EC10, EC20, EC50, NOEC LC10, LC20, LC50, NOEC
Concentrations:	a control, 5.6, 10, 18, 32; 56; 100; 180; 320; 560; 1000 mg of the test item/kg of dry weight of the artificial soil
Test Conditions:	temperature: 19.0 – 21.0°C; pH at the beginning of the test: 5.72 – 5.98; pH at the end of the test: 5.46 – 5.73;

soil moisture content at the beginning of the test: 16.1 – 16.8% (45.7 – 47.8% of the maximum water holding capacity);
soil moisture content at the end of the test: 14.9 – 15.9% (42.2 – 45.2% of the maximum water holding capacity);
lighting: 16 h light and 8h dark;
light intensity at the beginning of the experiment: 572 – 614 lux;
light intensity at the end of the experiment: 616 – 671 lux

Mortality

Mortality of the adults after 28 days of the experiment is presented in Table 5, whereas the results showing the impact of the test item on mortality are shown in Table 6.

At the concentrations ranging from 5.6 to 100 mg/kg dry weight of the artificial soil, the mortality of adults was between 15.0 and 40.0%. At the concentrations ranging from 180 to 1000 mg/kg dry weight of the artificial soil mortality of adults was between 97.5 and 100.0%. As for the control group, it was equal to 13.8% (Table 5).

The concentration of the test item causing a 50% mortality of adults within the exposure period (LC50) is equal to 84.5 mg/kg dry weight of the artificial soil (20.4 mg of pyraclostrobin/kg dry weight of the artificial), (Table 6).

Table 5. Mortality of adult collembolans *(Folsomia candida)* after 28 days of the experiment.

Concentration [mg/kg dry weight of the artificial soil]	Replicate	Number of tested collembolans	Number of living collembolans after 28 days [no.]	Total mortality	
				No.	%
0 (control)	1 2 3 4 5 6 7 8	10 10 10 10 10 10 10 10	10 10 9 7 7 10 9 7	11	13.8
5.6	1 2 3 4	10 10 10 10	9 7 8 10	6	15.0
10	1 2 3 4	10 10 10 10	9 9 7 9	6	15.0
18	1 2 3 4	10 10 10 10	9 8 9 7	7	17.5
32	1 2 3 4	10 10 10 10	8 7 8 9	8	20.0
56	1 2 3 4	10 10 10 10	5 6 6 7	16+	40.0
100	1 2 3 4	10 10 10 10	5 6 6 7	16+	40.0
180	1 2 3 4	10 10 10 10	0 1 0 0	39+	97.5
320	1 2 3 4	10 10 10 10	0 0 0 0	40+	100.0
560	1 2 3 4	10 10 10 10	0 0 0 0	40+	100.0
1000	1 2 3 4	10 10 10 10	0 0 0 0	40+	100.0

Table 6. Endpoint values - the impact of the test item on the mortality of adult collembolans *(Folsomia candida)*.

Endpoint	Value [mg test item /kg dry weight of the artificial soil]	Value [mg of pyraclostrobin/kg dry weight of the artificial soil]
LC_{10}	31.8 (5.2 – 56.8)	7.7 (1.3 – 13.7)
LC_{20}	44.5 (11.7 – 75.6)	10.7 (2.8 – 18.3)
LC_{50}	84.5 (43.3 – 165.8)	20.4 (10.5 – 40.0)
NOEC	32.0	7.7

Impact on reproduction

The number of juveniles at the end of the test is presented in Table 7, whereas the results showing the impact of the test item on reproduction are shown in Table 8.

Table 7. Number of juvenile collembolans *(Folsomia candida)* after 28 days of the experiment.

Concentration [mg/kg dry weight of the artificial soil]	Replicate	Number of juveniles	Mean ±SD	Comparison to the control [%]	CV* [%]
0.0 (control)	1 2 3 4 5 6 7 8	674 774 600 701 590 632 515 659	643.1 ± 78.3	-	12.2
5.6	1 2 3 4	572 544 651 709	619.0 ± 75.2	96.2	12.1
10	1 2 3 4	704 595 525 594	604.5 ± 74.0	94.0	12.2
18	1 2 3 4	604 750 544 510	602.0 ± 106.0	93.6	17.6
32	1 2 3 4	472 439 486 625	505.5 ± 82.1	78.6	16.2
56	1 2 3 4	362 320 318 368	342.0^{+} ± 26.7	53.2	7.8
100	1 2 3 4	335 271 168 324	274. 5^{+}± 76.3	42.7	27.8
180	1 2 3 4	1 0 0 0	0.3^{+} ± 0.5	0.0	200.0
320	1 2 3 4	1 0 0 0	0.3^{+} ± 0.5	0.0	200.0
560	1 2 3 4	0 0 0 0	0.0^{+}	0.0	-
1000	1 2 3 4	0 0 0 0	0.0^{+}	0.0	-

Table 8. Endpoint values - the impact of the test item on reproduction of collembolans *(Folsomia candida)*.

Endpoint	Value [mg test item /kg dry weight of the artificial soil]	Value [mg of pyraclostrobin/kg dry weight of the artificial soil]
EC_{10}	21.8 (12.2 – 30.0)	5.3 (2.9 – 7.2)
EC_{20}	31.7 (20.8 – 40.6)	7.6 (5.0 – 9.8)
EC_{50}	64.7 (52.6 – 79.8)	15.6 (12.7 – 19.3)
NOEC	32.0	7.7

After the exposure of collembolans to the test item at the concentrations ranging from 5.6 to 100 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 274.5 and 619.0 per replicate. After the exposure of collembolans to the test item at the concentrations ranging from 180 to 1000 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 0.0 and 0.3 per replicate. As for the control group, the number of juveniles was equal to 643.1 per replicate.
The obtained results led to the following conclusions:
- The concentration of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) causing a 10% reduction in the number of juveniles produced within the exposure period (EC10) is equal to 21.8 mg/kg dry weight of the artificial soil (i.e. 5.3 mg of pyraclostrobin/kg dry weight of the artificial).
- The concentration of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) causing a 20% reduction in the number of juveniles produced within the exposure period (EC20) is equal to 31.7 mg/kg dry weight of the artificial soil (i.e. 7.6 mg of pyraclostrobin/kg dry weight of the artificial).
- The concentration of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is equal to 64.7 mg/kg dry weight of the artificial soil (i.e. 15.6 mg of pyraclostrobin/kg dry weight of the artificial).
- The highest concentration at which the test item is observed to have no statistically significant effects on collembolan reproduction (NOEC) is equal to 32 mg/kg dry weight of the artificial soil (i.e. 7.7 mg of pyraclostrobin/kg dry weight of the artificial).

Results of the reference test
The number of juveniles at the end of the experiment and the endpoints are presented in Table 9. The concentration of boric acid causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is 102.3 mg/kg dry weight of the artificial soil.
According to the OECD Guideline No. 232, the EC50 should be about 100 mg/kg dry weight of the artificial soil; hence, it may be concluded that the sensitivity of the test organisms was proper. The test was conducted 20.11.2019 – 20.12.2019.

Table 9. Reference substance – boric acid. Number of juvenile collembolans *(Folsomia candida)* after 28 days of the experiment.

Concentration [mg/kg dry artificial soil]	Replicate	Number of juveniles	Mean ±SD	Comparison to the control [%]	CV* [%]
0 (control)	1 2 3 4 5 6	654 615 730 618 768 656	673.5 ± 62.15	-	9.2
15	1 2	687 655	671.0 ± 22.63	99.6	3.4
22	1 2	613 692	652.5 ± 55.86	96.9	8.6
32	1 2	625 603	614.0 ± 15.56	91.2	2.5
46	1 2	539 573	556.0⁺ ± 24.04	82.6	4.3
68	1 2	458 538	498.0⁺ ± 56.57	73.9	11.4
100	1 2	351 326	338.5⁺ ± 17.68	50.3	5.2
150	1 2	191 259	225.0⁺ ± 48.08	33.4	21.4
220	1 2	103 78	90.5⁺ ± 17.68	13.4	19.5
320	1 2	51 23	37.0⁺ ± 19.80	5.5	53.5
460	1 2	14 0	7.0⁺ ± 9.90	1.0	141.4
680	1 2	0 0	0.0⁺	0.0	-
1000	1 2	0 0	0.0⁺	0.0	-
EC_{50} mg/kg dry soil	102.3 (97.5 – 107.3)				

Validity criteria
The results are considered valid because the following criteria were satisfied in the controls:
- mean adult mortality: 13.8% (criterion: ≤ 20%),
- the mean number of juveniles per vessel at the end of the test: 643.1 (criterion: ≥100 juveniles at the end of the test),

Deviations
No deviations from the study plan and the SOPs related to the study occurred.
Deviations from the OECD Guideline No. 232 (2016):
- culturing of collembolans takes place in plastic containers containing an artificial substrate consisting of plaster and charcoal in ratio 9:1 and not 10:1 or 8:1 as is mentioned in OECD Guideline No. 232 (2016) (3.3),
- at the end of the test the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels as it is mentioned in OECD Guideline No. 232 (2016) (3.6.6),
- physiological or pathological symptoms or distinct changes in behavior were not described (3.6.7).
The deviations did not affect the results of the study.

Study 3

Comments of zRMS:	The study was conducted to the OECD guideline 226 and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.4/03
Report	*Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil*; M. Wołany; Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland; STUDY CODE: G-34-20
Guideline(s):	OECD Guideline No. 226 (2016)
Deviations:	Yes
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) batch no.: 04/2020 Active substance: pyraclostrobin: 256.6 g/L
Artificial soil:	5% sphagnum peat, 20% kaolin clay, and 75% air-dried industrial sand
Test Species:	the predatory mites, Hypoaspis (Geolaelaps) aculeifer (adult female mites from a synchronized culture) obtained from a standard laboratory culture at the Łukasiewicz Research Network - Institute of Industrial Organic Chemistry Branch Pszczyna, Department of Ecotoxicological Studies, Laboratory of Soil Organisms Toxicology. The mites were introduced 7 – 14 days after becoming adult.
Test Design:	test duration: 14 days number of replicates: 4 replicates / concentration + 8 replicates / control; number of mites: 10 mites / replicate
Endpoints:	EC10, EC20, EC50, NOEC LC10, LC20, LC50, NOEC
Concentrations:	a control, 3.2, 5.6, 10, 18, 32, 56, 100, 180, 320, 560 and 1000 mg test item/kg dry weight of the artificial soil.
Test Conditions:	temperature: 20.0 – 21.0°C pH at the beginning of the test: 5.70 – 5.78 pH at the end of the test: 5.61 – 5.73 soil moisture content at the beginning of the test: 14.0 – 15.2% (44.9 – 48.8% of the maximum water holding capacity) soil moisture content in the middle of the test: 14.1 – 15.1% (45.3 – 48.5% of the maximum water holding capacity) soil moisture content at the end of the test: 13.3 – 14.8% (42.7 – 47.5% of the

maximum water holding capacity)
light-dark cycle: 16 h light and 8 h dark
light intensity at the beginning of the test: 597– 648 lux
light intensity at end of the test: 572 – 614 lux

Results

Mortality of the predatory mites exposed to the test item at the concentrations ranging from 3.2 to 1000 mg/kg dry weight of the artificial soil was between 0% and 100%. In the control group mortality of predatory mites was not observed.

After the application of the test item at the concentrations ranging from 3.2 to 1000 mg/kg dry weight of the artificial soil the mean number of juveniles was between 0.0 – 90.8 per replicate. The mean number of juveniles in the control group was equal to 92.8 per replicate.

Concentration [mg/kg dry weight of the artificial soil]	Adult mites		Number of juveniles (mean)
	Number of tested mites	Number of dead mites after 14 days	
Control	80	0	92.8
3.2	40	0	90.0
5.6	40	0	90.8
10	40	0	83.8
18	40	0	89.8
32	40	0	89.0
56	40	1	86.0
100	40	1	88.0
180	40	0	78.5
320	40	11	53.8
560	40	38	7.8
1000	40	40	0.0

Endpoint values – the impact of the test item on reproduction and on mortality of the predatory mites *(Hypoaspis aculeifer)*.

Endpoint	Value [mg/kg dry weight of the artificial soil]	Value [mg of pyraclostrobin / kg dry weight of the artificial soil]
EC_{10}	**179.6** (120.7 – 218.9)	**43.4** (29.2 – 52.9)
EC_{20}	**221.4** (166.3 – 258.4)	**53.5** (40.2 – 62.4)
EC_{50}	**330.3** (289.0 – 377.0)	**79.8** (69.8 – 91.1)
NOEC (reproduction)	**100.0**	**24.2**
LC_{10}	**190.3**	**46.0**
LC_{20}	**234.7**	**56.7**
LC_{50}	**350.5**	**84.7**
NOEC (survival)	**180.0**	**43.5**

Mortality

Mortality of adult mites after 14 days of the experiment is presented in Table 6. The endpoint values are given in Table 7.

Mortality of the predatory mites exposed to the test item at the concentrations ranging from 3.2 to 1000 mg/kg dry weight of the artificial soil was between 0% and 100%. In the control group mortality of predatory mites was not observed.

The concentration of the test item causing a 50% mortality of adults within the exposure period (LC50) is equal to 350.5 mg/kg dry weight of the artificial soil (equal to 84.7 mg of pyraclostrobin / kg dry weight of the artificial soil).

Table 6. Mortality of adult mites (*Hypoaspis aculeifer*) after 14 days of the experiment.

Concentration [mg/kg dry weight of the artificial soil]	Replicate	Number of tested mites	Number of alive mites after 14 days [no.]	Mortality	
				no.	%
	1	10	10		
	2	10	10		
	3	10	10		
	4	10	10		
	5	10	10	0	0.0
	6	10	10		
	7	10	10		
	8	10	10		
3.2	1	10	10		
	2	10	10		
	3	10	10	0	0.0
	4	10	10		
5.6	1	10	10		
	2	10	10		
	3	10	10	0	0.0
	4	10	10		
10	1	10	10		
	2	10	10		
	3	10	10	0	0.0
	4	10	10		
18	1	10	10		
	2	10	10		
	3	10	10	0	0.0
	4	10	10		
32	1	10	10		
	2	10	10		
	3	10	10	0	0.0
	4	10	10		
56	1	10	10		
	2	10	10		
	3	10	10	1	2.5
	4	10	9		
100	1	10	10		
	2	10	10		
	3	10	10	1	2.5
	4	10	9		
180	1	10	10		
	2	10	10		
	3	10	10	0	0.0
	4	10	10		
320	1	10	5		
	2	10	9		
	3	10	8	11+	27.5
	4	10	7		
560	1	10	0		
	2	10	1		
	3	10	1	38+	95.0
	4	10	0		
1000	1	10	0		
	2	10	0		
	3	10	0	40+	100.0
	4	10	0		

Table 7. Endpoint values – the impact of the test item on survival of adult females.

Endpoint	Value [mg/kg dry weight of the artificial soil]	Value [mg of pyraclostrobin/ kg dry weight of the artificial soil]
LC_{10}	190.3	46.0
LC_{20}	234.7	56.7
LC_{50}	350.5	84.7
NOEC	180.0	43.5

Reproduction

The number of juveniles at the end of the test is presented in Table 8, whereas the endpoints showing the impact of the test item on reproduction are given in Table 9.

Table 8. Number of juvenile mites (*Hypoasis aculeifer*) after 14 days of the experiment.

Concentration [mg/kg dry weight of soil]	Replicate	Number of juvenile mites	Mean ±SD	Comparison to the control [%]	CV* [%]
0 (control)	1 2 3 4 5 6 7 8	96 112 78 92 90 87 100 87	92.8 ± 10.2	-	11.0
3.2	1 2 3 4	80 102 93 85	90.0 ± 9.6	97.0	10.7
5.6	1 2 3 4	88 93 77 105	90.8 ± 11.6	97.8	12.8
10	1 2 3 4	98 79 81 77	83.8 ± 9.6	90.3	11.5
18	1 2 3 4	85 76 93 105	89.8 ± 12.3	96.8	13.7
32	1 2 3 4	109 72 95 80	89.0 ± 16.4	96.0	18.4
56	1 2 3 4	78 88 99 79	86.0 ± 9.8	92.7	11.4
100	1 2 3 4	92 89 96 75	88.0 ± 9.1	94.9	10.4

Concentration [mg/kg dry weight of soil]	Replicate	Number of juvenile mites	Mean ±SD	Comparison to the control [%]	CV* [%]
180	1 2 3 4	65 74 84 91	78.5 ± 11.4	84.6+	14.5
320	1 2 3 4	10 81 65 59	53.8 ± 30.6	58.0+	56.9
560	1 2 3 4	6 9 11 5	7.8 ± 2.8	8.4+	35.5
1000	1 2 3 4	0 0 0 0	0.0 ± 0.0	0.0+	-

Table 9. Endpoint values - the impact of the test item on reproduction of the predatory mites (*Hypoaspis aculeifer*).

Endpoint	Value [mg/kg dry weight of the artificial soil]	Value [mg of pyraclostrobin/ kg dry weight of the artificial soil]
EC_{10}	179.6 (120.7 – 218.9)	43.4 (29.2 – 52.9)
EC_{20}	221.4 (166.3 – 258.4)	53.5 (40.2 – 62.4)
EC_{50}	330.3 (289.0 – 377.0)	79.8 (69.8 – 91.1)
NOEC	100.0	24.2

After the application of the test item at the concentrations ranging from 3.2 to 1000 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 0.0 – 90.8 per replicate. The mean number of juveniles in the control group was equal to 92.8 per replicate.
The obtained results led to the following conclusions:
• The concentration of the test item causing a 10% reduction in the number of mites produced within the exposure period (EC10) is equal to 179.6 mg/kg dry weight of the artificial soil (equal to 43.4 mg of pyraclostrobin / kg dry weight of the artificial soil).
• The concentration of the test item causing a 20% reduction in the number of mites produced within the exposure period (EC20) is equal to 221.4 mg/kg dry weight of the artificial soil (equal to 53.5 mg of pyraclostrobin / kg dry weight of the artificial soil).
• The concentration of the test item causing a 50% reduction in the number of mites produced within the exposure period (EC50) is equal to 330.3 mg/kg dry weight of the artificial soil (equal to 79.8 mg of pyraclostrobin / kg dry weight of the artificial soil).
• The highest concentration at which the test item is observed to have no statistically significant effects on mite reproduction (NOEC) is equal to 100 mg/kg dry weight of the artificial soil (equal to 24.2 mg of pyraclostrobin / kg dry weight of the artificial soil).

Results of the reference test
The number of juveniles at the end of the experiment and the endpoints are presented in Table 10. The concentration of boric acid causing a 50% reduction in the number of juveniles produced within the exposure period (EC50) is 246.439 mg/kg dry weight of the artificial soil.
According to the OECD Guideline No. 226, the EC50 should be between 100 and 500 mg/kg dry weight of the artificial soil; hence, it may be concluded that the sensitivity of the test organisms was proper.

Validity criteria
The results are considered valid because the following criteria were satisfied in the control:
• mean adult mortality: 0% (criterion: ≤ 20%),
• the mean number of juveniles per vessel at the end of the test: 92.8 (criterion: ≥ 50 juveniles at the end of the test,
• the coefficient of variation for the number of juveniles: 11.0 % (criterion: ≤ 30%).

Deviations
The study was performed according to OECD Guideline No. 226 (2016), Study Plan and the SOPs mentioned in chapter 8.
There are three deviations from the OECD Guideline No. 226 (2016), however they did not affect the results:
1. According to the OECD Guideline No. 226 (2016) the water content of the soil substrate should be maintained throughout the test by weighing and if needed re-watering the vessels periodically. In the study to maintain proper moisture content, a small sample of soil was drying at 105°C and re-weighing at the beginning, after 7 days of the test and at the end of the test (Chapter 3.4.7).
2. Due to the use of the temperature extraction method, there was no need for euthanasia of the extracted organisms since the mites are fixed in a 70% ethanol solution (Chapter 3.4.8).
3. Due to the use of the temperature extraction method, it was not possible to record the symptoms with behavioral and morphology changes of the extracted predatory mites (Chapter 3.4.8).

A 2.4.2.1 KCP 10.4.2.1 Species level testing

No additional studies were performed.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

No additional studies were performed.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study was conducted to the OECD guideline 216 and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.5
Report	*Soil Microorganisms: Nitrogen Transformation Test according to the OECD Guideline No. 216 (2000)/EU Method C.21*; A. Arendarczyk ; Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland; STUDY CODE: G-35-20
Guideline(s):	OECD Guideline No. 216 (2000) / EU Method C.21.
Deviations:	Yes

GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) batch no.: 04/2020 pyraclostrobin: 256.6 g/L
Artificial soil:	Agricultural soil collected from a place belonging to the Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna.
Test Design:	Three portions of soil (3 x 1500 g), i.e. one control group and two treated groups. Every portion was divided into three replicates (3 x 500 g). The soil was enriched with the organic substrate, i.e. lucerne at dose of 5 g/kg dry weight of soil. Test duration: 28 days.
Endpoints:	The concentration of nitrate [mg/kg dry soil] after 0, 7, 14 and 28 days of incubation The nitrate formation rate [mg/kg dry weight of soil/day] for selected time intervals of soil incubation, i.e. 0 – 7, 0 – 14, 0 – 28 days. Percent deviation from the control in nitrate formation rate calculated for selected time intervals i.e. 0 – 7, 0 – 14, 0 – 28 days.
Concentrations:	control, PEC: 11.3 mg of the test item / kg dry weight of soil (2.74 mg of pyraclostrobin/kg dry weight of soil), 5xPEC: 56.7 mg of the test item / kg dry weight of soil (13.7 mg of pyraclostrobin/kg dry weight of soil)
Test Conditions:	temperature: 20.6 – 22.0°C soil moisture: 46.5 – 52.5% of the maximum water holding capacity, incubation in darkness

Results and discussion

The difference in the nitrate formation rate between the control soil and the one treated with the test item at the concentrations corresponding to the PEC: 11.3 mg of the test item / kg dry weight of soil (2.74 mg of pyraclostrobin/kg dry weight of soil) and 5xPEC: 56.7 mg of the test item / kg dry weight of soil (13.7 mg of pyraclostrobin/kg dry weight of soil) did not exceed 25% on 28 day of analysis.
On the basis of the results, it was concluded that Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the concentrations corresponding to the PEC: 11.3 mg of the test item / kg dry weight of soil (2.74 mg of pyraclostrobin/kg dry weight of soil) and 5xPEC: 56.7 mg of the test item / kg dry weight of soil (13.7 mg of pyraclostrobin/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

Results

The nitrate ions concentrations on 0, 7, 14 and 28 day of incubation are shown in Tables 5 – 8.

Table 5. Concentration of the nitrate ions on day 0 of incubation.

Concentration	Control			PEC			5 x PEC		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	12.393	12.363	12.623	15.493	16.693	14.943	18.063	16.603	17.583
Nitrate ion concentration [mg/kg of dry soil]	61.97	61.82	63.12	77.47	83.47	74.72	90.32	83.02	87.92
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	62.30 ± 0.71			78.55+ ± 4.47			87.08+ ± 3.72		
CV	1.1			5.7			4.3		

* - values adjusted for the value of the blank sample

+ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, significance level = 0.05, two sided)

Table 6. Concentration of the nitrate ions on day 7 of incubation.

Concentration	Control			PEC			5 x PEC		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	41.308	40.288	39.848	39.628	40.668	39.828	41.418	42.248	40.418
Nitrate ion concentration [mg/kg of dry soil]	206.54	201.44	199.24	198.14	203.34	199.14	207.09	211.24	202.09
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	202.41 ± 3.74			200.21 ± 2.76			206.81 ± 4.58		
CV	1.9			1.4			2.2		

* - values adjusted for the value of the blank sample

Table 7. Concentration of the nitrate ions on day 14 of incubation.

Concentration	Control			PEC			5 x PEC		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	28.788	28.108	33.608	53.598	54.698	49.218	52.578	56.808	55.388
Nitrate ion concentration [mg/kg of dry soil]	143.94	140.54	168.04	267.99	273.49	246.09	262.89	284.04	276.94
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	150.84 ± 14.99			262.52+ ± 14.49			274.62+ ± 10.76		
CV	9.9			5.5			3.9		

* - values adjusted for the value of the blank sample

+ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, significance level = 0.05, two sided)

Table 8. Concentration of the nitrate ions on day 28 of incubation.

Concentration	Control			PEC			5 x PEC		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	65.837	66.857	69.947	74.177	75.837	77.067	77.507	77.717	76.567
Nitrate ion concentration [mg/kg of dry soil]	329.19	334.29	349.74	370.89	379.19	385.34	387.54	388.59	382.84
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	337.74 ± 10.70			378.47+ ± 7.25			386.32+ ± 3.06		
CV	3.2			1.9			0.8		

* - values adjusted for the value of the blank sample

+ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, significance level = 0.05, two sided)

Nitrate formation rates [mg nitrate/kg dry weight soil/day] for selected time intervals, i.e. 0 – 7, 0 – 14 and 0 – 28 days are given in Table 9. Deviations from the control based on nitrogen ions formation rates are shown in Table 10.

Table 9. Nitrate formation rate* [mg nitrate/kg dry weight of soil/day] for selected time intervals.

Time interval [d]	Control				PEC				5 x PEC			
	Replicate			Mean ± SD	Replicate			Mean ± SD	Replicate			Mean ± SD
	I	II	III		I	II	III		I	II	III	
0 – 7	20.606	19.877	19.563	20.015 ± 0.53	17.085	17.827	17.227	17.380+ ± 0.39	17.144	17.737	16.430	17.104+ ± 0.65
0 – 14	5.832	5.589	7.553	6.324 ± 1.07	13.532	13.924	11.967	13.141+ ± 1.04	12.558	14.068	13.561	13.396+ ± 0.77
0 – 28	9.532	9.714	10.266	9.837 ± 0.38	10.441	10.737	10.957	10.711+ ± 0.26	10.730	10.768	10.563	10.687+ ± 0.11

* - Rate of nitrate ions formation per a day = [(mg nitrate / kg of soil dry weight on sampling day 'a') - (mg nitrate / kg of soil dry weight on day 0)]/ 'a' day; 'a' = 7. 14 and 28 day

+ - statistically significant difference between the control and the treatment group (Williams Multiple Sequential t-test Procedure, significance level = 0.05, two sided)

Table 10. Deviations from the control based on nitrate formation rate for selected time intervals [%].

Time interval [d]	PEC	5 x PEC
0 – 7	13.2	14.6
0 – 14	-107.8	-111.8
0 – 28	-8.9	-8.6

"-" – nitrate formation rate in the treatment group was higher than in the control group

Values obtained using ToxRat 2.10. computer software.

After 0, 14 and 28 days of incubation the statistically significant differences in nitrate concentration between the control and both group treated with the test item, i.e. PEC and 5xPEC were noticed (Tables 5, 7, 8) [SPO/G/80].
After 7 days of incubation the statistically significant differences in nitrate concentration between the control and the both groups treated with the test item, i.e. PEC and 5xPEC, were not observed (Table 6) [SPO/G/80].
At the time intervals: 0 – 7, 0 – 14, 0 – 28 statistically significant differences in nitrate formation rate between the control and both groups treated with test item, i.e. PEC and 5xPEC were noticed (Table 9) [SPO/G/80].
The difference in the nitrate formation rate between the control soil and the one treated with the test item at the concentrations corresponding to the PEC: 11.3 mg of the test item/kg dry weight of soil (2.74 mg of pyraclostrobin/kg dry weight of soil) and 5xPEC: 56.7 mg of the test item/kg dry weight of soil (13.7 mg of pyraclostrobin/kg dry weight of soil) did not exceed 25% on 28 day of analysis. (Table 10) [SPO/G/80].
When the difference in the nitrates formation rate between the lower treatment (PEC) and a control is equal to or less than 25% at any sampling day after day 28, the product can be evaluated as having no long-term influence on nitrogen transformation in soil.
On the basis of the results, it was concluded that Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) at the concentrations corresponding to the PEC: 11.3 mg of the test item / kg dry weight of soil (2.74 mg of pyraclostrobin/kg dry weight of soil) and 5xPEC: 56.7 mg of the test item / kg dry weight of soil (13.7 mg of pyraclostrobin/kg dry weight of soil) did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

Validity criterion
The coefficients of variation (CV) in the control group were 1.1, 1.9, 9.9 and 3.2%, after 0, 7, 14 and 28 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than ± 15%.

Deviations
Deviation from the OECD Guideline No. 216 (2000), the EU Method C.21:
According the Guideline, the soil extraction should be conducted at 150 rpm for 60 min. However, in this study, the extraction was performed at 90 rpm for 24 hours. The modification resulted from the optimization of the nitrate extraction which showed that the extraction was more effective when the shaking rate was lower and the extraction lasted longer.
The predicted environmental concentration (PEC) is calculated assuming 1 cm of the soil depth. Thus, the applied soil depth is a deviation from OECD Guideline No. 216 (2000) and EU Method C.21, where the PEC is calculated by using 5 cm of the soil depth.

A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

No additional studies were performed.

A 2.6.1 KCP 10.6.1 Summary of screening data

No additional studies were performed.

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1.1 Study 1

Comments of zRMS:	The study was conducted to the OECD guideline 208 and according to the principles of GLP. All validity criterions were met. During the experiment the plant damages were not observed. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.6/01
Report	*Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Terrestial Plant Test: Seedling Emergence and Seedling Growth Test*; M. Wołany; Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland; STUDY CODE: G-37-20
Guideline(s):	OECD Guideline No. 208 (2006)
Deviations:	Yes
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) batch number: 04/2020 active substances: pyraclostrobin: 256.6 g/L
Artificial soil:	sandy loam
Test Design:	number of rates: 5 + control; number of replicates/rate: 4 (carrot, perennial ryegrass, oats) or 7 (sunflower, cabbage, pea). The total number of seeds per application rate – 20 (carrot, perennial ryegrass, oats) or 21 (sunflower, cabbage, pea). test termination: 14 days after the emergence of 50% of the control seedlings
Test species:	*sunflower (Helianthus annuus), cabbage (Brassica oleracea var. capitata), pea (Pisum sativum), carrot (Daucus carota), perennial ryegrass (Lolium perenne), oats (Avena sativa).*
Endpoints:	ER25, ER50, NOER.
Application rates:	- a control, - 24.7 mL of the test item/ha (6.3 g of pyraclostrobin/ha), - 74.1 mL of the test item/ha (19.0 g of pyraclostrobin/ha), - 222.2 mL of the test item/ha (57.0 g of pyraclostrobin/ha), - 666.7 mL of the test item/ha (171.1 g of pyraclostrobin/ha), - 2000.0 mL of the test item/ha (513.2 g of pyraclostrobin/ha).
Test Conditions:	temperature: 19.1 – 28.0°C, humidity: 47.7 – 88.2%, lighting: 16 h light : 8 h dark; light intensity: 53.4 – 141.1 μE/m2/s; carbon dioxide concentration: 337 – 363 ppm

Results and discussion

The ER50 and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as mL of the test item/ha for all test species are given below.

	Sunflower *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	>2000.0	>2000.0	>2000.0	>2000.0	>2000.0	>2000.0
NOER	≥2000.0	≥2000.0	≥2000.0	≥2000.0	>2000.0	≥2000.0
Shoot length (plants without roots)						
ER_{50}	>2000.0	>2000.0	>2000.0	>2000.0	>2000.0	>2000.0
NOER	≥2000.0	≥2000.0	≥2000.0	≥2000.0	≥2000.0	≥2000.0
Plant dry weight (plants without roots)						
ER_{50}	>2000.0	>2000.0	>2000.0	>2000.0	>2000.0	>2000.0
NOER	≥2000.0	≥2000.0	≥2000.0	≥2000.0	≥2000.0	≥2000.0

The ER50 and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as g of pyraclostrobin/ha for all test species are given below.

	Sunflower *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Pea** *Pisum sativum*	**Carrot** *Daucus carota*	**Perennial ryegrass** *Lolium perenne*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	>513.2	>513.2	>513.2	>513.2	>513.2	>513.2
NOER	≥513.2	≥513.2	≥513.2	>513.2	≥513.2	≥513.2
Shoot length (plants without roots)						
ER_{50}	>513.2	>513.2	>513.2	>513.2	>513.2	>513.2
NOER	≥513.2	≥513.2	≥513.2	≥513.2	≥513.2	≥513.2
Plant dry weight (plants without roots)						
ER_{50}	>513.2	>513.2	>513.2	>513.2	>513.2	>513.2
NOER	≥513.2	≥513.2	≥513.2	≥513.2	≥513.2	≥513.2

The test item i.e. Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) had no impact on the growth and seedling emergence of the tested plant species.
On the basis of ER25, ER50 and NOER values determined from final number of plants it was proved that the test item did not inhibit the seedling emergence of all tested plant species.
On the basis of ER25, ER50 and NOER values determined from the shoot length and dry shoot weight it was proved that the test item had no impact on the process of growth of all tested plant species.
During the experiment the plant damages were not observed.

Conclusions
The test item i.e. Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) had no impact on the growth and seedling emergence of the tested plant species.

On the basis of ER25, ER50 and NOER values determined from final number of plants it was proved that the test item did not inhibit the seedling emergence of all tested plant species.
On the basis of ER25, ER50 and NOER values determined from the shoot length and dry shoot weight it was proved that the test item had no impact on the process of growth of all tested plant species.
During the experiment the plant damages were not observed.

Validity criteria
On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on seedling emergence and seedling growth of terrestrial plants were met:
- the seedling emergence in the control (validity criterion: at least 70%) was as follows:
90.5% – sunflower,
100.0% – cabbage,
90.5% – pea,
90.0% – carrot,
100.0% – perennial ryegrass,
85.0% – oats,
- the mean survival of the emerged control seedlings was 100% for sunflower, cabbage, pea, carrot, perennial ryegrass and oats (validity criterion: at least 90%);
- the control seedlings did not exhibit any visible phytotoxic effects;
- environmental conditions for all plants of the same species were identical.

Deviations
Deviations from OECD Guideline No. 208:
According to OECD Guideline No. 208 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 53.4 and 141.1 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing.
This deviation did not affect results of the experiment.

Study 2

Comments of zRMS:	The study was conducted to the OECD guideline 227 and according to the principles of GLP. All validity criterions were met. The phytotoxic symptoms in cultivation of sunflower, pea, cabbage, carrot, perennial ryegrass and oats were not observed. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.6/02
Report	*Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) Terrestial Plant Test: Vegetative Vigour Test*; M. Wołany; Institute of Industrial Organic Chemistry Branch Pszczyna Department of Ecotoxicology, Pszczyna, Poland; STUDY CODE: G-36-20
Guideline(s):	OECD Guideline No. 227 (2006)
Deviations:	Yes
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test Item:	Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) batch number: 04/2020 active substances: pyraclostrobin: 256.6 g/L
Artificial soil:	sandy loam
Test Design:	number of rates: 5 + control; number of replicates/rate: 4 (carrot, perennial ryegrass, oats) or 7 (sunflower, cabbage, pea). The total number of plants per application rate – 20 (carrot, perennial ryegrass, oats) or 21 (sunflower, cabbage, pea,) test termination: 21 days after the spraying
Test species:	sunflower (Helianthus annuus), cabbage (Brassica oleracea var. capitata), pea (Pisum sativum), carrot (Daucus carota), perennial ryegrass (Lolium perenne), oats (Avena sativa)
Endpoints:	ER25, ER50, NOER.
Concentrations:	- a control, - 24.7 mL of the test item/ha (6.3 g of pyraclostrobin/ha), - 74.1 mL of the test item/ha (19.0 g of pyraclostrobin/ha), - 222.2 mL of the test item/ha (57.0 g of pyraclostrobin/ha), - 666.7 mL of the test item/ha (171.1 g of pyraclostrobin/ha), - 2000.0 mL of the test item/ha (513.2 g of pyraclostrobin/ha). volume of deionized water used to prepare the highest rate corresponded to 300 L water/ha
Test Conditions:	temperature: 19.1 – 28.0°C, humidity: 47.7 – 88.2%, lighting: 16 h light : 8 h dark; light intensity: 98.7 – 151.2 µE/m2/s; carbon dioxide concentration: 324 – 374 ppm

Results and discussion

The ER50 and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as mL of the test item/ha for all test species are given below.

	Sunflower *Helianthus annuus*	Cabbage *Brassica oleracea var. capitata*	Pea *Pisum sativum*	Carrot *Daucus carota*	Perennial ryegrass *Lolium perenne*	Oats *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	>2000.0	>2000.0	>2000.0	>2000.0	>2000.0	>2000.0
NOER	>2000.0	>2000.0	>2000.0	>2000.0	>2000.0	>2000.0
Shoot length (plants without roots)						
ER_{50}	>2000.0	>2000.0	>2000.0	>2000.0	>2000.0	>2000.0
NOER	≥2000.0	≥2000.0	≥2000.0	≥2000.0	≥2000.0	≥2000.0
Plant dry weight (plants without roots)						
ER_{50}	>2000.0	>2000.0	>2000.0	>2000.0	>2000.0	>2000.0
NOER	≥2000.0	≥2000.0	≥2000.0	≥2000.0	≥2000.0	≥2000.0

The ER50 and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as g of pyraclostrobin/ha for all test species are given below.

	Sunflower *Helianthus annuus*	Cabbage *Brassica oleracea var. capitata*	Pea *Pisum sativum*	Carrot *Daucus carota*	Perennial ryegrass *Lolium perenne*	Oats *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	>513.2	>513.2	>513.2	>513.2	>513.2	>513.2
NOER	>513.2	>513.2	>513.2	>513.2	>513.2	>513.2
Shoot length (plants without roots)						
ER_{50}	>513.2	>513.2	>513.2	>513.2	>513.2	>513.2
NOER	≥513.2	≥513.2	≥513.2	≥513.2	≥513.2	≥513.2
Plant dry weight (plants without roots)						
ER_{50}	>513.2	>513.2	>513.2	>513.2	>513.2	>513.2
NOER	≥513.2	≥513.2	≥513.2	≥513.2	≥513.2	≥513.2

On the basis of the obtained results it was proved that the test item i.e. Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) had no influence on the plant number, shoot length and shoot dry weight of the tested plant species at the end of the experiment.
The phytotoxic symptoms in cultivation of sunflower, cabbage, pea, carrot, perennial ryegrass and oats were not observed.

Conclusions
On the basis of the obtained results it was proved that the test item i.e. Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) had no influence on the plant number, shoot length and shoot dry weight of the all tested plant species at the end of the experiment.
The phytotoxic symptoms in cultivation of sunflower, pea, cabbage, carrot, perennial ryegrass and oats

were not observed.

Validity criteria
On the basis of the obtained results, it was stated that the following validity criteria of the study aimed at evaluating the impact of Pyraclostrobin 250 EC (CHR/F/PYRA 250 EC) on vegetative vigour of terrestrial plants were met:
- the seedling emergence of plants (validity criterion: at least 70%) was as follows:
83.3 – 90.5– sunflower,
85.7 – 90.5 – cabbage,
88.1 – 92.9 – pea,
87.5 – 92.5 – carrot,
80.0 – 95.0 – perennial ryegrass,
82.5 – 95.0 – oats,
- the mean plant survival of the control was 100% for all tested species (validity criterion: at least 90%),
- the control plants did not exhibit any visible phytotoxic symptoms,
- environmental conditions for all plants belonging to the same species were identical.

Deviations
Deviation from OECD Guideline No. 227:
According to OECD Guideline No. 227 (2006), the light intensity should be 350 ± 50µE/m2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 98.7 and 151.2 µE/m2/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing.
This deviation did not affect results of the experiment.

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

No additional studies were performed.

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

No additional studies were performed.

A 2.8 KCP 10.8 Monitoring data

No additional studies were performed.