Document Title

Summary of the toxicological studies for Fluopicolide + Fluoxastrobin FS 350 (200+150 g/L)

Data Requirement(s)

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

Document MCP

Section 7: Toxicological studies

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

Date

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Author(s)

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on behalf of

Bayer AG

Crop Science Division

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Version history

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

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4, 'How to revise an Assessment Report'.

Table of Contents

CP 7 TOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

Fluopicolide was included in Annex I to Council Directive 91/414/EEC in 2010 (Commission Directive 2010/15/EU, Entry into Force on June 1, 2010). The expiration of approval of fluopicolide is May 31, 2023 (Commission Implementing Regulation (EU) 2017/1527). The Supplementary Dossier contains only data which were not submitted at the time of the Annex I inclusion of fluopicolide under Council Directive 91/414/EEC and which were therefore not evaluated during the first EU review. All data which were already submitted by Bayer AG (former Bayer CropScience) for the Annex I inclusion under Council Directive 91/414/EEC are contained in the Draft Assessment Report (DAR) and its Addenda, and are included in the Baseline Dossier provided by Bayer AG.

The formulation Fluopicolide + Fluoxastrobin FS 350 (200+150 g/L), abbreviation FLC + FXA FS 350, is a flowable concentrate for seed treatment formulation (FS) containing 200 g/L of fluopicolide. This formulation is registered in Europe under the trade name Scenic Gold. FLC + FXA FS 350 was not a representative formulation of Bayer AG for the Annex I inclusion of fluopicolide under Council Directive 91/414/EEC.

Fluopicolide (AE C638206) is a fungicidal active substance developed by Bayer. It is the only active substance in Europe representing a class of chemistry (pyridinylmethyl-benzamides) with a unique mode of action via delocalization of a spectrin-like protein in the Oomycetes fungi.

Fluopicolide is active against a wide range of Oomycete fungi low dose rates against a wide range of Oomycete (Phycomycetes) diseases including downy mildews (Pseudoperonospora, Peronospora, Bremia), late blight (Phytophthora). It is also effective against downy mildews and some Pythium species causing damping-off at emergence time.

Fluopicolide is redistributed via the xylem and effective disease control can be achieved from foliar and seed applications. Fluopicolide is used in mixture in a range of foliar formulations in potatoes, horticultural crops and industrial crops such as oilseed.

Fluopicolide has a long track record of safe use in a large number of targeted crops within industrial crops.

Fluopicolide can be formulated with other active ingredients in different types of formulations to optimise and complete its activity.

The development of resistances of Oomycetes against existing, well-established fungicide groups represent a threat for European farmers by increasing the complexity of their plant protection programs leading to severe economic impacts. With Fluopicolide, farmers in EU-27 have access to a modern tool for their integrated crop protection programs, contributing to effective and sustainable management of resistance development and preserving high level of protection against Oomycete diseases.

By reducing the Oomycete damages, applications of Fluopicolide + Fluoxastrobin FS 350 on target crops contribute to the achievement of optimum emergence ensuring yield and quality, thus securing sufficient supply of high-quality oilseed for European consumer destinations and markets abroad, for the processing industry.

CP 7.1 Acute toxicity

Fluopicolide + Fluoxastrobin FS 350 (200+150 g/L) is not acutely toxic via the oral route (LD_{50} >2000), the dermal route (LD_{50} >2000) or the inhalation route (LC_{50} >3.72 mg/L). FLC+FXA FS 350 is not irritating to the eyes or skin, and a mouse LLNA revealed that there was no skin sensitising potential. Overall, no classification for acute toxicity is required for FLC+FXA FS 350.

Table 7.1- 1: Acute toxicity studies with FLC + FXA FS 350

Study Type	Species	Study Results	Classification by calculation	Reference
Acute oral toxicity	Rat	LD_{50} >2000 mg/kg bw	Not classified	[redacted] 2015; M-531437-01-1
Acute dermal toxicity	Rat	LD_{50} >2000 mg/kg bw	Not classified	[redacted] 2015; M-531440-01-1
Acute inhalation toxicity	Rat	4-h LC_{50} >3.72 mg/L MAC	Not classified	[redacted] 2015; M-537527-01-1
Skin irritation	Rabbit	Non-irritant	Not classified	[redacted] 2015; M-526850-01-1
In vitro eye irritation, ICET	Chicken eye	Non-irritant	Not classified	[redacted] 2015; M-521709-01-1
Eye irritation	Rabbit	Non-irritant	Not classified	[redacted] 2015; M-523295-01-1
Skin sensitisation, LLNA	Mouse	Non-sensitising	Not classified	[redacted] 2015; M-526851-01-1

CP 7.1.1 Oral toxicity

Data Point:	KCP 7.1.1/01
Report Author:	[redacted]
Report Year:	2015
Report Title:	Fluopicolide + fluoxastrobin FS 350 (200+150 g/L) - Acute oral toxicity study in the rat (Up and down procedure)
Report No:	15/049-001P
Document No:	M-531437-01-1
Guideline(s) followed in study:	OECD 425 (2008); Commission Regulation (EC) No. 440/2008 B.1.TRIS (2008); US-EPA 712-C-02-190, OPPTS 870.1100 (2002)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In an acute oral toxicity test, following the up and down procedure, 5 fasted female Wistar rats, were given a single oral dose (gavage) of FLC + FXA FS 350 (specification 102000028578 containing 198.7 g/L fluopicolide and 148.4 g/L Fluoxastrobin) mixed with a distilled water vehicle at the limit dose of 2000 mg/kg bw, and were observed for 14 days. There were no mortalities. Decreased activity and vocalisation was observed in 4/5 animals, hunched back position was noted in all animals, piloerection was noted in 1/5 animal. All animals recovered within approximately 24 hours post treatment.

There were no treatment-related effects on body weight, and no gross pathological findings at necropsy.

In this well-conducted study FLC + FXA FS 350 was found to be of a low order of acute oral toxicity in female rats.

In female rats Oral LD_{50} > 2000 mg/kg bw

The study follows current OECD test guidelines and was conducted to GLP.

I. Materials and Methods

A. Materials

1. Test material

Test substance:	Fluopicolide + Fluoxastrobin FS 350 (200+150 g/L)
Purity:	Fluopicolide (AE C638206) – 17.0 % w/w (198.7 g/L) Fluoxastrobin (HEC 5725 E-iso) – 13.1 % w/w (148.4 g/L)
Batch no.:	2014-014396, specification 102000028578

2. Vehicle and/or positive control

Vehicle:	Distilled water

3. Test animals

Species:	Rats, female
Strain:	CRL:(WI)
Age:	8-10 weeks
Weight at dosing:	192 – 204 g
Source:	[redacted].
Acclimatisation period:	At least 5 days
Diet:	Ssniff® SM R/M "Autoclavable complete diet for rats and mice – breeding and maintenance" produced by Ssniff Spezialdiäten
Water:	Water quality analysis performed every 3 months
Housing:	Type II polypropylene/polycarbonate
Temperature:	19.1 – 23.4 °C
Humidity:	41 – 64 %
Air changes:	15-20 air exchanges/hour
Photoperiod:	12 hours daily, from 6.00 a.m. to 6.00 p.m.

B. Study design

1. In life dates: 03 to 31 March 2015

2. Animal assignment and treatment

No. of animals (group size)	5 females
Dose(s)	2000 mg/kg bw
Exposure	Once *via* gavage
Post exposure observation period	14 days

FLC + FXA FS 350 was tested for acute oral toxicity by dosing 5 female rats with a single oral dose of 2000 mg/kg mixed in distilled water, stirred with a magnetic stirrer up to the finishing of the treatment. The dose volume was 10 mL/kg bw. The animals were fasted overnight prior to treatment. Water was still available ad libitum overnight and food was returned 3 hours after treatment.

C. Methods

1. Observations

Clinical examination of the behaviour and general condition all rats were monitored regularly in the 6 hours after treatment and then checked daily during the 14-day observation period after dosing. All animals were weighed before treatment and on days 7 and 14.

2. Necropsy

Macroscopic examinations were carried out on all animals at sacrifice. After examination of the external appearance, the cranial, thoracic and the abdominal cavities were opened, and the organs and the tissues were observed.

II. Results and Discussion

A. Results

1. Dose-response table (LD_{50})

The results of the study for acute oral toxicity in the fasted rat, are summarized in Table 7.1.1- 1 below.

Table 7.1.1- 1: FLC + FXA FS 350 - Acute oral toxicity study in rats – mortality and clinical signs

Sex	Dose (mg/kg bw)	Number animals*	Duration of signs	LD50 (mg/kg bw) (14 days)
Females	2000	0/5/5	~24 h	>2000
* Number of animals which died/number of animals with clinical signs/number of animals used.				

2. Clinical signs

There were no mortalities. Decreased activity was observed in 4/5 animals, vocalisation was noted in 4/5 animals, hunched back position was noted in 5/5 animals, piloerection was noted in 1/5 animal. All animals recovered within approximately 24 hours post treatment.

3. Body weights

Body weight and body weight gain of the surviving animals was normal, as expected in this age and strain of rats.

4. Necropsy findings

No macroscopic findings were noted.

III. Conclusions

Under these test conditions the oral LD_{50} of FLC + FXA FS 350 in female rats was >2000 mg/kg/bw. FLC + FXA FS 350 is concluded to be of a low order of acute oral toxicity.

Assessment and conclusion by applicant:

This study was conducted to GLP and follows current OECD test guidelines.

The oral LD_{50} of FLC + FXA FS 350 g/L was to be greater than 2000 mg/kg bw in female Wistar rats. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

Using the calculation method also gives no classification for acute oral toxicity.

CP 7.1.2 Dermal toxicity

Data Point:	KCP 7.1.2/01
Report Author:	[redacted]
Report Year:	2015
Report Title:	Fluopicolide + fluoxastrobin FS 350 (200+150 g/L) - Acute dermal toxicity study in rats
Report No:	15/049-002P
Document No:	M-531440-01-1
Guideline(s) followed in study:	OECD 402 (1987); US-EPA 712-C-98-192, OPPTS 870.1200 (1998); Commission Regulation (EC) 440/2008 (2008)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary:

In an acute dermal toxicity test, groups of Wistar rats 5/sex were given a single dermal dose of FLC + FXA FS 350 (specification 102000028578 containing 198.7 g/L fluopicolide and 148.4 g/L fluoxastrobin) 2000 mg/kg bw, was applied undiluted to shaven unbroken skin in an area of 5 x 5 cm. The skin was covered in a semi-occlusive dressing. The test item was removed by washing 24 hours after dose administration and the animals were observed for 14 days.

There were no mortalities, no clinical signs of toxicity, no local dermal signs, no effects on body weight and no findings at necropsy.

In conclusion FLC + FXA FS 350 was found to be of a low order of acute dermal toxicity following exposure in rats.

In rats Dermal LD_{50} > 2000 mg/kg bw in both sexes

The study was conducted to GLP and follows the current OECD test guidelines (the use of more animals than currently recommended, and no dose range study has no impact on the validity of the test).

I. Materials and Methods

A. Materials

1. Test material

Test substance:	fluopicolide + fluoxastrobin FS 350 (200+150 g/L)
Purity:	Fluopicolide (AE C638206) – 17.0 % w/w (198.7 g/L) Fluoxastrobin (HEC 5725 E-iso) – 13.1 % w/w (148.4 g/L)
Batch no.:	2014-014396, specification 102000028578

2. Vehicle and/or positive control

Vehicle:	None

3. Test animals

Species:	Rat
Strain:	CRL:(WI)
Age:	Young adult
Weight at start:	211 – 240 g
Source:	[redacted]
Acclimation period:	5 days
Identification:	Cage cards and individual markings
Diet:	Ssniff® SM R/M "Autoclavable complete diet for rats and mice – breeding and maintenance" produced by Ssniff Spezialdiäten
Water:	Available *ad libitum*
Housing:	Type II polypropylene/polycarbonate
Temperature:	20.1 – 24.4 °C
Humidity:	28 – 58 %
Air changes:	15-20 air exchanges/hour
Photoperiod:	12-hours

B. Study design

1. In-life dates: 31 March to 14 April 2015

2. Animal assignment and treatment

No. of animals (group size)	5/sex
Dose(s)	2000 mg/kg bw
Exposure	24 hours, dermal
Post exposure observation period	14 days

FLC + FXA FS 350 was tested for acute dermal toxicity by dosing 5 male and 5 female Wistar rats with a single dermal dose of 2000 mg/kg undiluted test substance. The back of the animals was shorn (approximately 10% area of the total body surface) approximately 24 hours prior to the treatment. Only animals without injury or irritation on the skin were used in the test. The test substance was applied to an area of approximately 5 x 5 cm by use of a gauze pad which remained in contact with the skin during the 24-hour exposure period by a patch attached with adhesive hypoallergenic plaster. During this time, the entire trunk of each animal was wrapped in semi occlusive plastic wrap. At the end of the exposure period the test item was washed off with water.

C. Methods

1. Observations

Clinical examinations were performed on the day of treatment, at 1 and 5 hours after the application of the test item, and once each day for 14 days thereafter. Observations included the skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic, and central nervous system, and somatomotor activity and behaviour patterns. Particular attention was directed to the observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, and coma. Adverse skin reactions at the site of application were recorded following the removal of the dressing. Body weight was recorded on day 0, day 7 and day 14.

2. Necropsy

Animals were sacrificed at the end of the study and subject to a macroscopic examination including cranial, thoracic, and abdominal cavities were opened and the appearance of the tissues and organs were observed. Any gross macroscopic findings were recorded.

II. Results and Discussion

A. Results

Table 7.1.2- 1: FLC + FXA FS 350 - acute dermal toxicity study in rats – mortality and clinical signs

Dose (mg/kg bw)	Toxicological results *	Duration of signs	Time of death	LD50 (mg/kg bw) (14 days)
Male rats				
2000	0/0/5	N/A	N/A	> 2000
Female rats				
2000	0/0/5	N/A	N/A	> 2000
* Number of animals which died/number of animals with clinical signs/number of animals used.				

2. Clinical signs

There were no deaths or systemic clinical signs noted in any animals throughout the study.

3. Body weights

There were effects on body weight in any animal.

4. Necropsy findings

Gross necropsy did not reveal any treatment-related changes.

III. Conclusions

Under these test conditions the dermal LD_{50} of FLC + FXA FS 350 in male and female rats was >2000 mg/kg/bw. FLC + FXA FS 350 is concluded to be of a low order of acute dermal toxicity.

Assessment and conclusion by applicant:

This study was conducted to GLP and follows OECD test guidelines (any minor deviations have no impact on the validity of the test). The dermal LD_{50} of FLC + FXA FS 350 g/L was to be greater than 2000 mg/kg bw in male and female rats. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

Using the calculation method also gives no classification for acute dermal toxicity.

CP 7.1.3 Inhalation toxicity

Data Point:	KCP 7.1.3/01
Report Author:	[redacted]
Report Year:	2015
Report Title:	Acute inhalation toxicity study (nose-only) in the rat with fluopicolide + fluoxastrobin FS 350 (200+150 g/L)
Report No:	15/049-004P
Document No:	M-537527-01-1
Guideline(s) followed in study:	OECD 403 (2009); US-EPA OPPTS 870.1300 (1998); EC 440/2008, Annex Part B, B.2 (2008)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:.	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary:

In an acute inhalation toxicity study, groups of Wistar rats (5/sex), were exposed by the inhalation route to FLC + FXA FS 350 (specification 102000028578 containing 198.7 g/L fluopicolide and 148.4 g/L fluoxastrobin) in air for 4 hours to nose only at the maximum attainable concentration of 3.72 mg/L. The test item was administered as a 60% w/w water formulation and was administered as an aerosol achieving a MMAD of 3.89 µm with a GSD of 1.98. Animals were observed for 14 days following exposure.

One male died on day 12 of the study. All other animals survived to study termination. Clinical signs during exposure were laboured breathing in all animals, which had resolved by the end of day 1. Wet fur, ruffled fur and red-brown staining resolved by day 3 and considered to be related to the restraint and exposure conditions but not toxicologically relevant. Body weight loss was seen in both sexes during the first week of the study, but all animals gained weight by the end of the study (including the animal that died on day 12). No gross findings were reported at necropsy with the exception of the animal which died which had an enlarged uneven spleen, discolouration of the lungs, and red liquid material in the perianal fur. The cause of death could not be determined.

In conclusion FLC + FXA FS 350 was found to be of a low order of acute inhalation toxicity following 4 hours exposure in rats.

Inhalation LC_{50} > 3.72 mg/L

The study was conducted to GLP and follows OECD test guidelines.

I. Materials and Methods

1. Test material

Test substance:	fluopicolide + fluoxastrobin FS 350 (200+150 g/L)
Purity:	Fluopicolide (AE C638206) – 17.0 % w/w (198.7 g/L) Fluoxastrobin (HEC 5725 E-iso) – 13.1 % w/w (148.4 g/L)
Batch no.:	2014-014396, specification 102000028578

2. Vehicle and/or positive control

Vehicle:	Distilled water

3. Test animals

Species:	Rat
Strain:	CRL:(WI)
Age:	11 weeks
Weight at start:	Sighting study: 438g males/240g females Main study: 357-447 g (males) and 254-271 g (females).
Acclimation period:	29 days for sighting study, 33 days for main study
Diet:	Ssniff® SM R/M "Autoclavable complete diet for rats and mice – breeding and maintenance" produced by Ssniff Spezialdiäten
Water:	Water quality analysis performed every 3 months
Housing:	Type II polypropylene/polycarbonate
Temperature:	17.9 – 26.4 °C
Humidity:	44 – 67 %
Air changes:	At least 15 air changes/hour
Photoperiod:	12 hours daily, from 6.00 a.m. to 6.00 p.m.

B. Study design

1. In-life dates: 1. In life dates: 01 to 29 July 2015

2. Animal assignment and treatment

No. of animals (group size)	5/sex
Dose(s)	198.7 g/L fluopicolide and 148.4 g/L fluoxastrobin
Exposure	4 hours, nose-only
Post exposure observation period	14 days

The animals were exposed to an atmosphere of the test item for a single, continuous four-hour period, generated according to the system and flow rates determined during the technical trials. One male and female were used for a preliminary sighting study, followed by 5 males/females used in

the main test. The test item was prepared by homogenization in distilled water and used as a 60% w/w water formulation. The non-volatile component of the test material was determined and found to be 45.80%. Preliminary trials with the test substance were used to achieve the required aerosol concentration of particles with a mass median aerodynamic diameter (MMAD) of 1 to 4 µm, with a geometric standard deviation (GSD) in the range of 1.5 to 3. Particle size was measured from the animals' breathing zone using a cascade impactor (determined 3 times during the exposure period). The animals were exposed to the test substance under positive pressure and held in polycarbonate restraint tubes which allowed only the animals nostrils to enter the exposure port. The test item was aerosolized using a stainless-steel concentric jet nebulizer (TSE Systems GmbH, Bad Homburg, Germany), with the flow controlled by a syringe pump. Airflows and pressures were constantly monitored and controlled by a computer system to ensure uniform distribution. The test atmosphere was sampled at regular intervals (every 10 to 20 minutes) during the exposure period to determine the test atmosphere concentration.

C. Methods

1. Observations

Animals were checked hourly during exposure for clinical signs and morbidity. During the 14-day post-treatment period animals were checked for morbidity twice daily, and once daily for clinical signs. Body weights were recorded prior to treatment and on days 1,3,7 and 14.

2. Necropsy

At the end of the study the animals were sacrificed and subjected to a gross pathology examination of the abdominal and thoracic cavities.

II. Results and Discussion

A. Test Atmosphere Concentration

The achieved test atmosphere is shown in the table below and meets the acceptability requirements of the OECD test guideline.

Table 7.1.3- 1: FLC + FXA FS 350 - acute inhalation study - exposure conditions

Parameters	Sighting Study	Main Study
Flow rate (L/min)	at least 0.5L/min	
Actual concentration (mg/L) ± standard deviation	4.08 ± 0.24	3.72 ± 0.24
Particle size (µm) MAAD[1]	3.90	3.89
Geometric standard Deviation (GSD)	2.05	1.98
Inhalable Fraction (% < 4µm)	51.3	51.6

[1] Mass median aerodynamic diameter

B. Mortality

One male in the main study died on day 12 (see table below). There were no other deaths.

Table 7.1.3- 2: FLC + FXA FS 350 - acute inhalation study in rats – mortality and clinical signs

Sex	Mean Achieved Concentration (mg/L)	Toxicological results *	Onset and Duration of signs (day)	Time of death (day)
Sighting study				
Male	4.08	0/1/1	0-8	-
Female	4.08	0/1/1	0-1	-
Main study				
Male	3.72	1/5/5	0-3	12
Female	3.72	0/5/5	0-1	-

* Number of animals which died/number of animals with clinical signs/number of animals used

C. Clinical Observations

In the sighting study laboured respiration (slight to moderate), noisy respiration (slight), and decreased activity (slight) were recorded in the male animal during and after the exposure, while laboured respiration (slight) was observed in the female animal during exposure. From Day 2 to Day 8 fur loss on the left cheek was recorded in the male animal.

In the main study laboured respiration (slight to moderate) was recorded in all animals, while noisy respiration (slight) was recorded in two males during the exposure. During the observation period, no clinical signs were detected except two males having laboured respiration (slight) in Day 1.

Wet fur, ruffled fur or red-brown staining (as chromodacryorrhea) occurred elsewhere in study animals from Day 0 up to Day 7 which were considered to be related to the restraint and exposure procedures or discomfort of the animals but not to be toxicologically significant.

D. Bodyweight

In the sighting study no body weight effects were seen in the female. In the male moderate body weight loss (8.2%) was recorded in Day 1 and body weight gain was observed from Day 7.

In the main study minimal body weight loss (0.8-6.7 %) was recorded during the first week of the observation period in both males and females, and all animals had body weight gain by the end of the observation period except the animal found dead on Day 12, but the body weight of this animal also exceeded its initial body weight at the time of its death.

E. Necropsy

No macroscopic findings attributable to the test item were seen in the animals that survived to termination. In the animal that died on day 12 a specific cause of death could not be determined. Macroscopic findings in the dead male were dark/red discolouration of the non-collapsed lungs, enlargement of the spleen with uneven surface and red liquid material at the perianal fur.

III. Conclusions

In this well conducted GLP study and guideline compliant study, acute inhalation LC_{50} of FLC + FXA FS 350 g/L in the Wistar rats was greater than the maximum achievable concentration of 3.72 mg/L (4-hour nose only exposure).

Assessment and conclusion by applicant:

This study was conducted to GLP and follows current OECD test guidelines.

The inhalation IC_{50} of FLC + FXA FS 350 g/L was > 3.72 mg/L in male and female Wistar rats (four hours nose-only exposure to liquid aerosol), the highest attainable concentration.

Thus, no classification is required according to Regulation (EC) No. 1272/2008.

Using the calculation method also gives no classification for acute inhalation toxicity.

CP 7.1.4 Skin irritation

Data Point:	KCP 7.1.4/01
Report Author:	[redacted]
Report Year:	2015
Report Title:	Fluopicolide + fluoxastrobin FS 350 (200+150 g/L) - Acute skin irritation study in rabbits
Report No:	15/049-006N
Document No:	M-526850-01-1
Guideline(s) followed in study:	OECD 404 (2002); Commission Regulation (EC) No 440/2008, B.4 (2008); US-EPA 712-C-98-196, OPPTS 870.2500 (1998)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary:

In a primary skin irritation study, 3 male New Zealand rabbits were exposed via the dermal route to 0.1mL of undiluted FLC + FXA FS 350 (specification 102000028578 containing 198.7 g/L fluopicolide and 148.4 g/L fluoxastrobin) per animal. The test material was applied to an area of 10 x 10 cm of clipped skin by use of a gauze pad held in place in a plastic wrap. 4 hours later the dressing was removed, and the test item washed off with water. Local signs of irritation were scored 1, 24, 48 and 72 hours after treatment using the Draize scheme. There were no signs of erythema or oedema at the application sites in any of the rabbits at any of the time points.

In this study, FLC + FXA FS 350 was not irritating to the skin in rabbits according to the Draize classification system.

The study was conducted to GLP with no significant deviations from OECD test guidelines.

I. Materials and Methods

A. Materials

1. Test material

Test substance:	fluopicolide + fluoxastrobin FS 350 (200+150 g/L)
Purity:	Fluopicolide (AE C638206) – 17.0 % w/w (198.7 g/L) Fluoxastrobin (HEC 5725 E-iso) – 13.1 % w/w (148.4 g/L)
Batch no.:	2014-014396, specification 102000028578

2. Vehicle and/or positive control

Vehicle:	None

3. Test animals

Species:	Rabbit (3 males)
Strain:	New Zealand White
Age:	17 weeks
Weight at start:	3.6 to 3.9 kg
Source:	[redacted]
Acclimation period:	Not stated
Diet:	UNI diet for rabbits produced by Cargill Takarmány Zrt., H-5300 Karcag, Madarasi road 0399, Hungary, ad libitum.
Water:	Municipal tap water, as for human consumption, ad libitum
Housing:	Individually housed in AAALAC approved metal wire rabbit cages.
Temperature:	20 ± 3 °C
Humidity:	22 - 58%
Air changes:	At least 15 air exchanges/hour
Photoperiod:	12 hours daily, from 6.00 a.m. to 6.00 p.m.

B. Study design

1. In-life dates: 22 to 25 April 2015

2. Animal assignment and treatment

No. of animals (group size)	3 males
Dose(s)	0.5 mL
Exposure	4 hours, semi-occlusive
Post exposure observation period	4 days

The hair was removed from back and flanks of the rabbits with an electric clipper. 24 hours later 0.5 mL of the test item was applied undiluted by applying to a 10 x 10 cm gauze pad applied to the clipped skin and attached using adhesive hypoallergenic plaster. The entire trunk of each animal was then wrapped with plastic wrap held in place with an elastic stocking. 4 hours later the dressing was removed, and the test item was removed with water. Initially only one animal was treated. As no irritant effect was observed after 1 hour the test was completed with the remaining 2 animals.

C. Methods

1. Observations

Local signs of irritation were recorded 1, 24, 48 and 72 hours after treatment and scored for erythema and oedema using the Draize scoring system. The irritation was classified according to the following criteria:

P.I.I. = 0 Non-Irritant
0 < P.I.I. ≤ 2 Mild irritant
2 < P.I.I. ≤ 5 Moderate Irritant
5 < P.I.I. Severe Irritant

II. Results and Discussion

1. Dermal reactions

The observed dermal reactions for each animal, and the mean scores for 24, 48 and 72 hours for each animal are provided in the Table 7.1.4- 1 below:

Table 7.1.4- 1: Dermal irritation scores

Animals Identification	Observations after patch removal	1h	24h	48h	72h	Mean scores (12, 24, 72 hr)
Animal 1 (1864)	Erythema (redness) and eschar formation	0	0	0	0	0.00
	Oedema formation	0	0	0	0	0.00
Animal 2 (1854)	Erythema (redness) and eschar formation	0	0	0	0	0.00
	Oedema formation	0	0	0	0	0.00
Animal 3 (1844)	Erythema (redness) and eschar formation	0	0	0	0	0.00
	Oedema formation	0	0	0	0	0.00

Erythema criteria for classification ≥ 2.3
Oedema criteria for classification ≥ 2.3
Score for erythema and oedema: 0 = no irritation; 1 = questionable; 2 = slight; 3 = pronounced; 4 = severe

III. Conclusions

Under these test conditions FLC + FXA FS 350 was not irritating to the skin in a guideline compliant and GLP test conducted in rabbits.

Assessment and conclusion by applicant:

This study was conducted to GLP, with no significant deviations from current OECD test guidelines.

FLC + FXA FS 350 g/L was not a skin irritant in the rabbit. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

Using the calculation method also gives no classification for skin irritancy.

CP 7.1.5 Eye irritation

Data Point:	KCP 7.1.5/01
Report Author:	[redacted]
Report Year:	2015
Report Title:	Fluopicolide + fluoxastrobin FS 350 (200 + 150 g/L) - In vitro eye irritation test in isolated chicken eyes
Report No:	15/049-038CS
Document No:	M-521709-01-1
Guideline(s) followed in study:	OECD 438 (2013); EU Commission Regulation (EC) No 1272/2008 (2008); EU Commission Regulation (EC) No 152/2010 (2010) , Method B 48; US-EPA 712-C-98-195: OPPTS 870.2400 (1998)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary:

In an *in vitro* eye irritation study, 30 µL of undiluted FLC + FXA FS 350 (specification 102000028578 containing 198.7 g/L fluopicolide and 148.4 g/L fluoxastrobin) was applied onto the surface of the cornea of 3 isolated chicken eyes for a period of 10 seconds then washed off with saline. The eyes were evaluated for corneal opacity and corneal thickness prior to treatment and at 30, 75, 120, 180 and 240 minutes after the post-treatment rinse. Fluorescein retention was measured prior to treatment and 30 minutes after the post-treatment rinse. A further 3 eyes treated with physiological saline and 3 eyes treated with a positive control underwent the same treatment and evaluation procedure to confirm the validity of the test.

In this study, FLC + FXA FS 350 was not corrosive in isolated chicken eyes.

The study was conducted to GLP with no significant deviations from OECD test guidelines. The positive response in the positive control and negative response in the negative control confirmed the validity of the test.

I. Materials and Methods

A. Materials

1. Test material

Test substance:	fluopicolide + fluoxastrobin FS 350 (200+150 g/L)
Purity:	Fluopicolide (AE C638206) – 17.0 % w/w (198.7 g/L) Fluoxastrobin (HEC 5725 E-iso) – 13.1 % w/w (148.4 g/L)
Batch no:	2014-014396, specification 102000028578

2. Vehicle and/or controls

Negative control:	Physiological saline (0.9% w/v NaCl)
Positive control:	Benzalkonium chloride solution 50% in water (mixed with distilled water to achieve final concentration of 5% (w/v)
Fluorescein:	10% (w/v) solution mixed with physiological saline to achieve 2% solution.
Solubility:	The test item formed a suspension in physiological saline

3. Test eye collection

Species:	Chicken (used for human consumption)
Strain:	COBB 500
Age:	7 weeks old
Weight:	Not stated
Source:	TARAVIS Ltd. (Address: H-9600 Sárvár, Rábasomjéni út. 129., Hungary)
Storage and transport:	Chicken heads wrapped in tissue moistened with saline and stored in plastic box (4-5 heads per box)
Preparation of eyes:	Heads were processed within 2 hours after collection.

B. Study Design

1. In life dates: 17 March 2015

2. Animal assignment and treatment

Preparation of the eyes

The cornea integrity of each eye was checked by applying a small drop of 2% (w/v) fluorescein solution onto the cornea for a few seconds then rinsed off with 20 mL physiological saline. The fluorescein-treated cornea was examined with a hand-held slit lamp or slit-lamp microscope, to ensure the cornea was not damaged. Only eyes with undamaged corneas were used. The eye-ball was carefully removed from the orbit and placed in a steel clamp and transferred to a chamber of the superfusion apparatus, supplied with physiological saline solution dripping from a stainless steel tube, at a rate of approximately 3-4 drops/minute or 0.1-0.15 mL/minute. Eyes were examined again with the slit lamp microscope and eyes with a high baseline fluorescein staining or corneal opacity score were rejected. The cornea thickness was measured using the depth measuring device on the slit-lamp microscope and any with a thickness deviating more than 10% form the mean value for all eyes, or any eye showing other signs of damage were rejected. The selected eyes were acclimatized for 45 to 60 minutes. The chambers of the superfusion apparatus were at controlled temperature (32±1.5°C) during the acclimatization and treatment periods.

Test procedure

At the end of the acclimatization period, a zero-reference measurement was recorded for cornea thickness and opacity to serve as a base line (t=0) for each individual eye. The cornea thickness of the eyes should not change by more than 5% between the -45 min and the zero time. No corneal thickness changes (0.0%) were observed in the eyes. Following the equilibration period, the fluorescein retention was measured. Base line values were required to evaluate any potential test item-related effect after treatment. All eyes were considered to be suitable for the assay. Three eyes were allocated to the treated group, three eyes to the positive control group and one eye to the negative control group.

The undiluted test item was applied in a volume of 30 µL onto the entire surface of each cornea in the treated group. 30 µL of the positive and negative control solution were applied in a similar manner to the corneas in the positive and negative control groups respectively.

After an exposure period of 10 seconds all the corneas were rinsed with 20 mL of physiological saline.

Evaluation

The control and test eyes were evaluated pre-treatment and at approximately 30, 75, 120, 180 and 240 minutes after the post-treatment rinse. Corneal thickness and corneal opacity were measured at all time points. Fluorescein retention was measured on two occasions, at base line (t=0) and approximately 30 minutes after the post-treatment rinse. Haag-Streit Bern 900 slit-lamp microscope was used for the measurements. At the end of the procedures, the corneas were carefully removed from the eyes and placed individually into labelled containers of preservative fluid (10% neutral buffered formalin) for potential histopathology and stored at room temperature.

II. Results and Discussion

A. Findings

The mean values of the treated eyes for maximum corneal thickness change, corneal opacity change and fluorescein retention change are given in the table below.

Table 7.1.5-1: FLC + FXA FS 350 – *in vitro* eye irritation scores

Treatment	Observations									
	Corneal Swelling				Corneal Opacity		Flourescein Retention		Other Observations	Overall ICE Class
	75 Mins		240 Mins							
	Mean Max. Swelling (%)	ICE Class	Mean Max. Swelling (%)	ICE class	Mean Max. Corneal Opacity	ICE Class	Mean Flourescein Retention	ICE Class		
Negative Control (saline)	0.0	I	0.0	I	0.0	I	0.0	I	None	3 x I
Positive Control (5% w/v benzalkonium chloride)	12.2	III	31.2	III	3.83	IV	2.83	IV	Loosening of epithelium in 2/3 eyes at 180 and 240 mins post treatment rinse	1 x III 2 x IV
Test Item (FLC + FXA FS 350)	0.0	I	0.0	I	0.0	I	0.17	I	None	3 x I

III. Conclusions

Under these test conditions FLC + FXA FS 350 was not irritating to the eye in a guideline compliant and GLP test conducted in isolated chicken eyes.

Assessment and conclusion by applicant:

This study was conducted to GLP and follows current OECD test guidelines.

Under the experimental conditions FLC + FXA FS 350 g/L is not an eye irritant in a test conducted on isolated chicken eyes. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

Using the calculation method also gives no classification for eye irritancy.

Data Point:	KCP 7.1.5/02
Report Author:	[redacted]
Report Year:	2015
Report Title:	Fluopicolide + fluoxastrobin FS 350 (200+150 g/L) - Acute eye irritation study in rabbits
Report No:	15/049-005N
Document No:	M-523295-01-1
Guideline(s) followed in study:	OECD 405 (2012); US-EPA 712-C-98-195; OPPTS 870.2400 (1998); Commission Regulation (EC) No 440/2008, B.5 (2008)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In a primary eye irritation study 0.1g of undiluted FLC + FXA FS 350 (specification 102000028578 containing 198.7 g/L fluopicolide and 148.4 g/L fluoxastrobin) was instilled into the conjunctival sac of the left eye of 3 male New Zealand White rabbits. The eyes were examined 1, 24, 48 and 72 hours after test substance administration. Irritation was scored using the Draize scheme. There were minimal signs of redness and discharge in the first hour in all animals but this had resolved by 24 hours.

In this study, FLC + FXA FS 350 was not irritating to the eyes in rabbits according to the Draize classification system.

The study was conducted to GLP with no significant deviations from OECD test guidelines.

I. Materials and Methods

A. Materials

1. Test material

Test substance:	Fluopicolide + fluoxastrobin FS 350 (200+150 g/L)
Purity:	Fluopicolide (AE C638206) – 17.0 % w/w (198.7 g/L) Fluoxastrobin (HEC 5725 E-iso) – 13.1 % w/w (148.4 g/L)
Batch no.:	2014-014396, specification 10200028578

2. Vehicle and/or positive control

Vehicle:	None

3. Test animals

Species:	Rabbit (3 males)
Strain:	New Zealand White
Age:	14 weeks
Weight at start:	3.5 to 3.7 kg
Source:	[redacted]
Acclimation period:	At least 27 days
Diet:	UNI diet for rabbits produced by Cargill Takarmány Zrt., H-5300 Karcag, Madarasi road 0399, Hungary, *ad libitum*.
Water:	Municipal tap water, as for human consumption, *ad libitum*
Housing:	Individually housed in AAALAC approved metal wire rabbit cages.
Temperature:	20 ± 3 °C
Humidity:	27 - 60%
Air changes:	At least 15 air exchanges/hour
Photoperiod:	12 hours daily

B. Study design

1. In-life dates: 28 April to 3 May 2015

2. Animal assignment and treatment

The pH was found to be 6.5, so the test item was permitted for use in animal studies. The eyes of the animals were examined 24 hours before the start of the study by instillation of fluorescein solution and subsequent examination with a hand slit lamp. Only those animals were accepted to the trial that did not display any changes. Initially only one animal was treated. As the effects were non-irritant at 24 hours, two further rabbits were treated with the test item.

0.1ml of the test substance was placed in the conjunctival sac of the left eye of each animal. The untreated eye acted as a control.

Sixty minutes (60 ±10 min) prior to test substance application, a systemic opiate analgesic was administered subcutaneous injection. Five minutes (5 ±1.5 min) prior to test substance application, a topical ocular anaesthetic was applied to each eye (including the control eye to ensure direct comparison

of any ocular observations). Eight hours (8 to 9 hr) after test substance application, a systemic opiate analgesic, and a nonsteroidal anti-inflammatory drug (NSAID) were administered by subcutaneous injection.

The eyes were examined 1, 24, 48 and 72 hours after application with fluorescein staining and scored according to the Draize system. Any clinical signs were also recorded. Body weight was recorded on the day of treatment and before sacrifice.

II. Results And Discussion

1. Ocular reactions

No clinical signs of toxicity. Eye irritation scores are shown in the table below. Any signs seen one hour after dosing were reversible 24 hours after treatment.

Table 7.1.5-2: FLC + FXA FS 350 – Eye irritation scores in rabbits

Animal	Time after treatment:	1h	24h	48h	72h	Mean scores 24-72 hours	Response	Reversible (day)
Animal 1 (19)	Corneal opacity	0	0	0	0	0.00	--	n.a.
	Iritis	0	0	0	0	0.00	--	n.a.
	Redness conjunctivae	2	0	0	0	0.00	--	1*
	Chemosis conjunctivae	0	0	0	0	0.00	--	n.a.
	Discharge	1	0	0	0	0.00	--	1*
Animal 2 (28)	Corneal opacity	0	0	0	0	0.00	--	n.a.
	Iritis	0	0	0	0	0.00	--	n.a.
	Redness conjunctivae	2	0	0	0	0.00	--	1*
	Chemosis conjunctivae	0	0	0	0	0.00	--	n.a.
	Discharge	0	0	0	0	0.00	--	1*
Animal 3 (40)	Corneal opacity	0	0	0	0	0.00	--	n.a.
	Iritis	0	0	0	0	0.00	--	n.a.
	Redness conjunctivae	2	0	0	0	0.00	--	1*
	Chemosis conjunctivae	0	0	0	0	0.00	--	n.a.
	Discharge	1	0	0	0	0.00	--	1*

n.a. = not applicable;
* in respect of the result 1 hr post application

Negative: --

CO <1	IR <1	R <2	OE <2

Regulation (EC) No 1272/2008 and GHS

Mild irritant: (+)

CO ≥1 - <3	IR ≥1 - <2	R ≥2	OE ≥2

GHS category 2B (effects reversible within 7 days)

Irritant: +

CO ≥1 - <3	IR ≥1 - <2	R ≥2	OE ≥2	GHS category 2B (effects reversible within 7 days)
CO ≥1 - <3	IR ≥1 - <2	R ≥2	OE ≥2	Regulation (EC) No 1272/2008 (GHS) category 2

Irreversible effect/serious damage: ++

CO ≥3	IR ≥2	R -	OE -	Regulation (EC) No 1272/2008 and GHS category 1

2. Bodyweight

Within normal range.

III. Conclusions

Under these test conditions FLC + FXA FS 350 was not irritating to the eye in a guideline compliant and GLP test conducted in rabbits.

Assessment and conclusion by applicant:

The study was conducted to GLP, with no significant deviations from current OECD test guidelines.

Under the experimental conditions FLC + FXA FS 350 g/L was not an eye irritant in the rabbit.

Thus, no classification is required according to Regulation (EC) No. 1272/2008.

Using the calculation method also gives not classification for eye irritancy.

CP 7.1.6 Skin sensitization

Data Point:	KCP 7.1.6/01
Report Author:	[redacted]
Report Year:	2015
Report Title:	Fluopicolide + fluoxastrobin FS 350 (200 + 150 g/L) - Local lymph node assay in the mouse
Report No:	15/049037E
Document No:	M-526851-01-1
Guideline(s) followed in study:	OECD 429 (2010), Commission Regulation (EC) No 440/2008, B.42 (2008); US-EPA 712-C-03-197, OCSPP 870.2600 (2003)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary:

In a local lymph node assay, FLC + FXA FS 350 (specification 102000028578 containing 198.7 g/L fluopicolide and 148.4 g/L fluoxastrobin) was applied at concentrations of 0, 25, 50 and 100% (w/v) in a volume of 25 µL to the ears of female CBA/CaOlaHsd mice (5 per dose group). A positive control group was treated with 25% (w/v) α-Hexylcinnamaldehyde. The treatment was applied daily for 3

consecutive days. On day 6 the cell proliferation in the local lymph nodes was measured by incorporation of tritiated methyl thymidine (3HTdR) and the values obtained were used to calculate stimulation indices (SI).

The observed stimulation index values were 2.0, 0.8 and 1.2 at concentrations of 100 % (undiluted), 50 and 25 % (w/v), respectively. As the stimulation index is below the threshold value of 3, the test item is not a skin sensitizer. A stimulation index in the positive control of 8.4 confirms the validity of the assay. Therefore, FLC+FXA FS 350 was not a skin sensitiser under the conditions of this local lymph node assay.

I. Materials and Methods

A. Materials

1. Test material

Test substance:	fluopicolide + fluoxastrobin FS 350 (200+150 g/L)
Purity:	Fluopicolide (AE C638206) – 17.0 % w/w (198.7 g/L) Fluoxastrobin (HEC 5725 E-iso) – 13.1 % w/w (148.4 g/L)
Batch no.:	2014-014396, specification 102000028578

2. Vehicle and/or positive control

Vehicle:	1% aqueous Pluronic PE9200

3. Test animals

Species:	SPF female mice, 5 per dose group
Strain:	CBA/CaOlaHsd
Age:	9 weeks
Weight at start:	18.6 to 23.1 g
Source:	[redacted]
Acclimation period:	14 days
Diet:	Ssniff® SM Rat/Mouse – Breeding and Maintenance, "autoclavable Complete diet for rats/mice – Breeding and Maintenance"by ssniff Spezialdiäten GmbH, *ad libitum*.
Water:	Municipal tap water as for human consumption, *ad libitum*
Housing:	Individually housed in Type II. polypropylene/ polycarbonate cages
Temperature:	19.4 – 25.7°C
Humidity:	31 - 69%
Air changes:	At least 15 air exchanges/hour
Photoperiod:	12 hours daily, from 6.00 a.m. to 6.00 p.m.

B. Study design

1. In-life dates: 06 to 12 May 2015

2. Animal assignment and treatment

Preliminary irritation test

The Preliminary Irritation/Toxicity Test used two doses (2 animals / dose) at test item concentrations of 100 % (undiluted) and 50 % (w/v) in 1% Pluronic. The preliminary experiment was conducted in a similar experimental manner to the main study, but it was terminated on Day 6 and the radioactive proliferation assay was not performed.

Based on the observation of the solubility test, the maximum achievable concentration was 100 % (undiluted).

All mice were observed daily for any clinical signs of systemic toxicity or local irritation at the application site. Both ears of each mouse were scored for erythema. Ear thickness was also measured using a thickness gauge on Day 1 (pre-dose), Day 3 (before treatment, approximately 48 hours after the first dose) and Day 6. Additional quantification of the ear thickness was performed by ear punch weight determination after the euthanasia of the experimental animals.

No mortality or clinical signs of systemic toxicity were observed. Test item precipitate / minimal amount of test item precipitate was observed on the ears of the animals in the 100 % (undiluted) group on Days 1-5 and in the 50 % (w/v) dose group on Days 1-3. No marked body weight loss (>5%) was observed.

The ear punch weights were within the historical control range. There were no indications of any irritancy at the site of application.

The draining auricular lymph nodes of the animals were visually examined: they were considered normal in both dose groups (subjective judgement by analogy with observations of former experiments).

<u>Main test</u>

Based on the results of the preliminary irritation test, the 100 % (undiluted) dose was selected as top dose for the main test. Treatments in the main assay were performed as follows:

Table 7.1.6-1: FLC + FXA FS 350 – Local lymph node assay - Groups and Treatments

<table>
<tr><th>Groups</th><th>Test item concentration (% w/v)</th><th>No. of animals</th></tr>
<tr><td>Negative (vehicle) control (1% Pluronic)</td><td>-</td><td rowspan="5">5</td></tr>
<tr><td>Positive control (25% HCA in 1% Pluronic)</td><td>-</td></tr>
<tr><td rowspan="3">FLC + FXA FS 350</td><td>100</td></tr>
<tr><td>50</td></tr>
<tr><td>25</td></tr>
</table>

Each mouse was topically dosed on the dorsal surface of each ear with 25 µL of the appropriate formulation applied using a pipette. Each animal was dosed once a day for three consecutive days (Days 1, 2 and 3). There was no treatment on Days 4, 5 and 6. On Day 6 each mouse was intravenously injected via the tail vein with 250 µL of sterile PBS (phosphate buffered saline) containing approximately 20 µCi of 3HTdR using a gauge 25G x 1" hypodermic needle with 1 mL sterile syringe. Once injected, the mice were left for 5 hours (± 30 minutes) prior to sacrifice. The draining auricular lymph nodes were excised and processed individually to prepare single cell suspensions of lymph node cells. Cells were mixed with scintillation fluid and ^{3}HTdR incorporation was measured using a β-scintillation counter (Tri-Carb 2810 Liquid Scintillation Analyzer by Perkin Elmer), expressed as number of radioactive disintegrations per minute (DPM). Background level DPM was also measured in duplicates by adding 1 mL of 5 % (w/v) TCA solution into a scintillation vial filled with 10 mL of scintillation liquid.

3. Statistics

The results are expressed as disintegrations per node (DPN = DPM divided by the number of lymph nodes) for each animal. Stimulation index (SI = mean DPN of treated group divided by mean DPN of the appropriate control group).

The statistical analysis was performed using the SPSS/PC+ (4.0.1) software package. The heterogeneity of variance between groups was checked by Bartlett's test for the measured DPM values.

Where no significant heterogeneity was detected, a one-way analysis of variance was carried out. If the result was positive, then Duncan's Multiple Range test was used to assess the significance of inter-group differences. Where significant heterogeneity was found, the normal distribution of data was examined by Kolmogorow-Smirnow test. In the case of not normal distribution, the non-parametric method of Kruskal-Wallis One-Way analysis of variance was applied. If a positive result was detected, the inter-group comparisons were performed using Mann-Whitney U-test.

II. Results and Discussion

A. Results

1. Clinical signs and body weight

There were no deaths. No systemic toxicity was observed. Slight alopecia was observed on some of the experimental animals in the 100 % (undiluted) dose group on Days 4-6. Test item precipitate / minimal amount of test item precipitate was observed on the ears of the animals in the 100 % (undiluted) dose group on Days 1-5, and in the 50 % (w/v) groups on Days 1 and 3. In the 100 % (undiluted) dose group three out of five animals had more than 5 % body weight loss. The mean change of this group was -5.2 %. No other signs referring to systemic toxicity were observed in this group; also, no marked body weight loss was detected at this dose in the preliminary experiment using two animals. Therefore, the dose levels used in this study are considered to be acceptable for the evaluation. No treatment-related effects were observed on the mean body weight changes of the other groups in the experiment. However marked body weight loss was also detected for one animal in the 50 % (w/v) group and for one animal in the positive control group.

2. Proliferation Assay

The results of the proliferation assay are summarized in the table below. The appearance of the lymph nodes was normal in the negative control group and in all the test item treated dose groups. Larger than normal lymph nodes were observed in the positive control group.

Table 7.1.6-2: FLC + FXA FS 350 – Local lymph node assay - DPM, DPN and Stimulation Index

Test Group	Mean DPN	Stimulation Index
Background (5% w/v TCA)	-	-
Negative control (1% Pluronic)	164.9	1.0
FLC + FXA FS 350 100% (w/v)	321.7	2.0*
FLC + FXA FS 350 50% (w/v)	137.1	0.8
FLC + FXA FS 350 25% (w/v)	204.2	1.2
Positive control (25% w/v in 1% Pluronic)	1386.7	8.4**

* = Significant ($p<0.05$, Mann-Whitney U-test versus negative control)
** = Significant ($p<0.01$, Mann-Whitney U-test versus negative control)

The test item was a suspension, which was used undiluted or formulated in 1% Pluronic. Since there were no confounding effects of irritation or systemic toxicity at the applied concentrations, the proliferation values obtained are considered to reflect the real potential of the test item to cause lymphoproliferation in the Local Lymph Node Assay.

The positive control group animals showed a significant lymphoproliferative response (stimulation index value of 8.4) in this experiment. The results of the positive control group demonstrated the appropriate performance of the assay.

The observed mean DPN values for the negative and positive control were within the historical control range.

III. Conclusions

Under these test conditions FLC + FXA FS 350 was not a skin sensitizer in the local lymph node assay.

Assessment and conclusion by applicant:
The study was conducted to GLP, with no significant deviations from current OECD test guidelines. Under the experimental conditions FLC + FXA FS 350 g/L was not a skin sensitizer in the local lymph node assay. Thus, no classification is required according to Regulation (EC) No. 1272/2008. Using the calculation method also gives no classification for skin sensitization.

The product contains 1,2-Benzisothiazol-3(2H)-one and 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H isothiazol-3-one both of which are classified as skin sensitisers (H317) but at levels below the specific concentration limits for classification. These both trigger that the product label should contain the phrase EUH208 ‘contains 1,2-Benzisothiazol-3(2H)-one and 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H isothiazol-3-one, may produce an allergic reaction’.

CP 7.1.7 Supplementary studies on the plant protection product

No such studies are necessary since there are no concerns arising, e.g., from potential synergistic or additive effects exerted by the active substance(s) or other components in FLC + FXA FS 350 that would require further investigations.

CP 7.1.8 Supplementary studies for combinations of plant protection products

No such studies are necessary since FLC + FXA FS 350 is not intended for use in combination with other plant protection products.

CP 7.2 Data on exposure

The safe use FLX + FXA FS 350 to operators during treatment and sowing of seeds has been demonstrated when appropriate PPE is worn. An assessment of worker, resident and bystander exposure is not required as no such exposure is expected during normal use of the product. A combined exposure assessment of both active substances also demonstrated safe use for operators at the first tier and therefore further investigations into combined exposure were not required. A summary of the exposure assessments is presented below, and more detailed calculations are provided in appendix 2.

The product information and toxicological reference values used in the assessment are presented in table 7.2-1 below.

Table 7.2-1: Product information and toxicological reference values for the exposure assessment

Product name and code	FLC+FXA FS 350	
Formulation type	FS	
Category	Fungicide applied as seed treatment	
Active substance(s) (incl. content)	**Fluopicolide** 200 g/L	**Fluoxastrobin** 150 g/L
AOEL systemic	0.07 mg/kg bw/d	0.03 mg/kg bw/d
Inhalation absorption	100 %	100 %
Oral absorption	100 %	100 %
Dermal absorption	Concentrate: 0.21 % Dilution: 2.9% (50 g a.s./L)) (Based on product (formulation))	Concentrate: 0.07 % Dilution: 0.73% (37.5 g a.s./L) (Based on product (formulation))

CP 7.2.1 Operator exposure

CP 7.2.1.1 Estimation of operator exposure

A summary of the exposure models used for estimation of operator exposure to the active substance during application of FLC+FXA FS 350 according to the critical use(s) is presented in table 7.2.1-1. The GAP is presented in appendix 1.

Table 7.2.1-1: Exposure models for intended uses

Critical use(s)	Oilseed rape (max. 1 L product/100 kg seed)
Model(s)	Seed TROPEX

As stated on the label, FLC+FXA FS 350 is available in pack sizes of 5 to 1000 L. Therefore, the exposure assessment for operators has been conducted with reference to selected pack sizes of 200 and 25 L.

200 L pack size

The input parameters for the estimation of operator exposure (200 L pack size) are presented in table 7.2.1-2 for fluopicolide and table 7.2.1-3 for fluoxastrobin.

Table 7.2.1-2: Input parameters considered for the estimation of operator exposure for fluopicolide (200 L packaging)

Formulation type:	FS		**Application technique:**	Commercial seed treatment Loading /sowing	
Application rate (AR):	0.2	kg a.s./dt seed			
Seed treated per day:	40	Tonnes/day	**Product container size**	200	L (for whole day treatment large containers are assumed)
Amount product handled	400	L/day	**Number of loading operations**	2	op/day
Dilution rate:	1	Undiluted (worst case)	**Number of calibration operations**	1	op/day
Dermal absorption (DA):	0.21	% (concentr.)	**Duration of bagging**	8	hours/day
	3.9	% (dilution)	**Number of cleaning operations**	1	op/day
Inhalation absorption (IA):	100	%			
Body weight (BW):	60	kg/person			
AOEL	0.07	mg/kg bw/d	**Duration of loading/ sowing**	10	hours/day

Table 7.2.1-3: Input parameters considered for the estimation of operator exposure for fluoxastrobin (200L pack size)

Formulation type:	FS		**Application technique:**	Commercial seed treatment Loading /sowing	
Application rate (AR):	0.15	kg a.s./dt seed			
Amount product handled	400	L/day	**Product container size**	200	L (for whole day treatment large containers are assumed)
Seed treated per day:	40	tonnes	**Number of loading operations**	2	op/day
Dilution rate:	1	Undiluted (worst case)	**Number of calibration operations**	1	op/day
Dermal absorption (DA):	0.07	% (concentr.)	**Duration of bagging**	8	hours/day
	0.73	% (dilution)	**Number of cleaning operations**	1	op/day
Inhalation absorption (IA):	100	%			
Body weight (BW):	60	kg/person	**Duration of loading/ sowing**	10	hours/day
AOEL	0.03	mg/kg bw/d			

The outcome of the exposure assessment for the 200 L pack size is presented in table 7.2.1-4 below, detailed calculations are presented in appendix 2.

Table 7.2.1-4: Estimated operator exposure 200 L packaging

		Fluopicolide		Fluoxastrobin	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**
Commercial seed treatment Application rate: 1 L product/100 kg seed (200 g fluopicolide/100 kg seed, 150 g fluoxastrobin/100 kg seed)					
Seed TROPEX Body weight: 60 kg	Standard clothing*	0.0725	103.52	0.0467	155.59
	with RPE: mask during cleaning	0.0245	34.95	0.0107	35.59
Seed sowing Application rate: : 1 L product/100 kg seed (200 g fluopicolide/100 kg seed, 150 g fluoxastrobin/100 kg seed)					
Seed TROPEX Body weight: 60 kg	Standard clothing**	0.0035899	5.13	0.0034189	11.40

* Operator wearing one layer of work clothing and additional chemical protective gloves during all tasks except bagging and RPE during cleaning of the equipment

** Operator wearing one layer of work clothing

For the 200 L pack size, the acceptable operator exposure level (AOEL) is not exceeded when normal workwear is worn, and additional appropriate PPE is worn during cleaning tasks.

25 L pack size

The input parameters used for the exposure assessments for fluopicolide and fluoxastrobin with a 25 L pack size are presented in tables 7.2.1-5 and 7.2.1-6 below.

Table 7.2.1-5: Input parameters considered for the estimation of operator exposure for fluopicolide (25 L packaging)

Formulation type:	FS		**Application technique:**	Commercial seed treatment Loading /sowing	
Application rate (AR):	0.2	kg a.s./dt seed			
Seed treated per day:	40	Tonnes/day	**Product container size**	25	L
Amount product handled	400	L/day	**Number of loading operations**	16	op/day
Dilution rate:	1	Undiluted (worst case)	**Number of calibration operations**	1	op/day
Dermal absorption (DA):	0.21	% (concent.)	**Duration of bagging**	8	hours/day
	3.9	% (dilution)	**Number of cleaning operations**	1	op/day
Inhalation absorption (IA):	100	%			
Body weight (BW):	60	kg/person			
AOEL	0.07	mg/kg bw/d	**Duration of loading/ sowing**	10	hours/day

Table 7.2.1-6: Input parameters considered for the estimation of operator exposure for fluoxastrobin (25 L pack size)

Formulation type:	FS		**Application technique:**	Commercial seed treatment Loading /sowing	
Application rate (AR):	0.15	kg a.s./dt seed			
Amount product handled	400	L/day	**Product container size**	25	L (for whole day treatment large containers are assumed)
Seed treated per day:	40	tonnes	**Number of loading operations**	16	op/day
Dilution rate:	1	Undiluted (worst case)	**Number of calibration operations**	1	op/day
Dermal absorption (DA):	0.07	% (concentr.)	**Duration of bagging**	8	hours/day
	0.73	% (dilution)	**Number of cleaning operations**	1	op/day
Inhalation absorption (IA):	100	%			
Body weight (BW):	60	kg/person	**Duration of loading/ sowing**	10	hours/day
AOEL	0.03	mg/kg bw/d			

The outcome of the exposure assessment with a 25 L pack size is presented in table 7.2.1-7 below. Detailed calculations are presented in appendix 2.

Table 7.2.1-7: Estimated operator exposure 25 L packaging

		Fluopicolide		Fluoxastrobin	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**
Commercial seed treatment Application rate: 1 L product/100 kg seed (200 g fluopicolide/100 kg seed, 150 g fluoxastrobin/100 kg seed)					
Seed TROPEX Body weight: 60 kg	Standard clothing*	0.0789	112.78	**0.0513**	**170.95**
	with RPE: mask during cleaning	0.0309	44.21	0.0153	50.95
Seed sowing Application rate: : 1 L product/100 kg seed (200 g fluopicolide/100 kg seed, 150 g fluoxastrobin/100 kg seed)					
Seed TROPEX Body weight: 60 kg	Standard clothing**	0.0035899	5.13	0.0034189	11.40

* Operator wearing one layer of work clothing and additional chemical protective gloves during all tasks except bagging and RPE during cleaning of the equipment

** Operator wearing one layer of work clothing

For the 25 L pack size, the acceptable operator exposure level (AOEL) is not exceeded when normal workwear is worn, and additional appropriate PPE is worn during cleaning tasks.

CP 7.2.1.2 Measurement of operator exposure

Not required as assessments demonstrated safe use using the accepted models.

CP 7.2.2 Bystander and resident exposure

CP 7.2.2.1 Estimation of bystander and resident exposure

The following definitions and assumptions for bystanders and residents may be applied.

Bystanders and residents are not involved in application or handling plant protection products or the professional handling of treated crops. The question arises whether it is necessary to distinguish between bystanders and residents in terms of the potential for exposure and health risks. However, because the circumstances of this exposure could differ with respect to amount, frequency, and duration, this seems to be reasonable.

Bystanders may inadvertently be present within or directly adjacent to an area for a short period of time, typically a matter of minutes, where application of a plant protection product is in progress or has recently taken place. They may be exposed to plant protection products mainly via the dermal route from spray drift and by inhalation of drifting spray droplets. Handheld application is considered to be worse case compared to field crop sprayer.

Residents may live or work near areas of the application of plant protection products (e.g. standing, working, or sitting in a garden in the vicinity of the application). They may be exposed to plant protection products mainly via the dermal route from spray drift deposits and by inhalation of vapour drift (depending on the vapour pressure of the active substance). For infants and toddler's exposure might also occur orally (e.g. through hand-to-mouth transfer and/or object-to-mouth transfer).

Treatment of seeds with FLC + FXA FS 350 is performed in professional plants where no residents or bystanders are present. Further, no other (uninvolved) persons are allowed to enter the plant. Therefore, bystander exposure to FLC + FXA FS 350 during seed treatment is not relevant. A detailed calculation resident or bystander exposure is considered to be not necessary and has thus not been conducted.

CP 7.2.2.2 Measurement of bystander and resident exposure

Since no exposure of residents of bystanders is expected, a study to provide a measure of bystander exposure was not necessary and was therefore not carried out.

CP 7.2.3 Worker exposure

The only intended use of FLC + FXA FS 350 is the treatment of seeds prior to sowing. During sowing, the seeds are immediately covered by soil. Consequently, no re-entry tasks are required that could result in exposure to the worker. Therefore, a worker exposure assessment for FLC + FXA FS 350 is not required and has not been conducted.

CP 7.2.3.1 Estimation of worker exposure

Not considered to be necessary as no worker exposure is expected.

CP 7.2.3.2 Measurement of worker exposure

Not considered to be necessary as no worker exposure is expected.

Combined exposure

As the product is a mixture of 2 active substances a combined exposure assessment is required. At the first tier, combined exposure is calculated as the sum of the component exposures without regard to the mode of action or mechanism/target of toxicity. Initially, the individual Hazard Quotients (HQ) are calculated for all active substances in the PPP by assessing the exposure according to appropriate models and dividing the individual exposure levels by the respective systemic AOEL. This is equivalent to the predicted exposure as % of systemic AOEL from tables 7.2.1-4 and 7.2.1-7 converted to decimal. The Hazard Index (HI) is the sum of the individual HQs.

Table 7.2.3-1: Acute risk assessment from combined exposure

Application scenario	Active Ingredient	Estimated exposure / AOEL (HQ)
Operators – Seed treatment Standard clothing* 200 L packaging 2 loading operations per day	Fluopicolide	1.0352
	Fluoxastrobin	1.5559
	Cumulative risk Operators (HI)	**2.5911**
Operators – Seed treatment with RPE: mask during cleaning 200 L packaging 2 loading operations per day	Fluopicolide	0.3495
	Fluoxastrobin	0.3559
	Cumulative risk Operators (HI)	**0.7054**
Operators – Seed sowing Standard clothing**	Fluopicolide	0.513
	Fluoxastrobin	0.114
	Cumulative risk Operators (HI)	**0.627**
Operators – Seed treatment Standard clothing* 25 L packaging 16 loading operations per day	Fluopicolide	1.1278
	Fluoxastrobin	1.7095
	Cumulative risk Operators (HI)	**2.8373**
Operators – Seed treatment with RPE: mask during cleaning 25 L packaging 16 loading operations per day	Fluopicolide	0.4421
	Fluoxastrobin	0.5095
	Cumulative risk Operators (HI)	**0.9516**
Operators – Seed sowing Standard clothing**	Fluopicolide	0.513
	Fluoxastrobin	0.114
	Cumulative risk Operators (HI)	**0.627**

* Operator wearing one layer of work clothing and additional chemical protective gloves during all tasks except bagging

** Operator wearing one layer of work clothing

The Hazard Index is < 1, if the operator uses the appropriate PPE during seed treatment and during sowing of treated seeds. I.e. During seed treatment the operator is wearing one layer of work clothing and additional chemical protective gloves during all tasks except bagging and RPE during cleaning of the treating equipment. During sowing of treated seeds the operator is wearing one layer of work clothing. Thus, combined exposure to all active substances in FLC + FXA FS 350 is not expected to present a risk for protected operators, workers, bystanders, and residents. No further refinement of the assessment is required.

CP 7.3 Dermal adsorption

A summary of the dermal absorption rates for fluopicolide in the fluopicolide + fluoxastrobin FS 350 (200+150 g/L) (also named FLC+FXA FS 350) formulation is presented in the following table.

Table 7.3-1: Dermal absorption rates for fluopicolide in FLC+FXA FS 350

	fluopicolide
	Value (% of dose applied)
Concentrate	0.21%
Dilution (dilution factor)	3.9% @ 50 g/L

Justification for proposed values – Fluopicolide

The proposed dermal absorption rates for fluopicolide are based on an *in vitro* human skin dermal absorption study using the FLC+FXA FS 350 formulation. The study results are summarized in the following table. A full summary of the study is described in detail below.

Table 7.3-2: Summary of the results of submitted dermal absorption studies for Fluopicolide

Test	Concentrate	Spray dilution (dilution factor)	Formulation in study	Justification provided on representativity of study formulation for current product	Reference
In vitro (Human)	0.21%	3.9% (1 in 4)	FLC+FXA FS 350	Not required	[redacted] 2015; M-537120-01-1

Comparative dermal absorption, *in vitro* using rat and human skin

Data Point:	KCP 7.3/01
Report Author:	[redacted]
Report Year:	2015
Report Title:	FLC+FXA FS 200+150: [14C]-fluopicolide - In vitro dermal absorption study using human skin
Report No:	SA 15160
Document No:	M-537120-01-1
Guideline(s) followed in study:	OECD 428 2004); OECD Environmental Health and Safety Publications Series on testing and Assessment N° 28 (2004). EFSA Panel on Plant Protection Products and their Residues (PPR), EFSA Journal 2012
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Material and methods

Human skin: Source: Xenometrix, Hégenheim, France.
Number and sex: 5 donors, female.
Anatomical region: Abdomen.
Thickness: 366.2 to 477.0 µm.

Test Material:

Non-radiolabelled: Batch: MOY 4627.
Purity = 99.2% w/w.

Radiolabelled: [phenyl-U-^{14}C]-fluopicolide
Batch: KML 9969.
Specific activity: 5.50 MBq/mg.
Radiopurity of the formulation: 98.5%.

Formulation: The formulation used in this experiment was the Fluopicolide + Fluoxastrobin FS 200+150 formulation (specification N° 102000028578) containing fluopicolide at a concentration of 200 g/L. It was used at two nominal concentrations of fluopicolide: neat, 200 g/L and 50 g/L.

Test system: A flow-through diffusion cell system (Franz's cell modified, Gallas, France) was used to study the absorption of the test substance (exposure area of 1 cm^2 skin). A diffusion cell consisted of a donor chamber and a receptor chamber between which the skin was positioned. The receptor fluid was Eagle's medium supplemented with 5% bovine serum albumin and gentamycin (50 mg/L) at a pH of approximately 7.4. The receptor chamber was warmed by a constant circulation of warm water which maintained the receptor fluid at 32 ± 2°C (close to the normal skin temperature). The receptor fluid was pumped through the receptor chamber at a rate of 1.5 mL/h and stirred continuously whilst in the receptor chamber by means of a magnetic bar.

Skin integrity: Before dose application, the integrity of the skin samples was assessed by measuring the trans-epidermal water loss (TEWL) from the stratum corneum. An evaporimeter

probe (Tewameter TM300) was placed securely on the top of the donor chamber and the amount of water diffusing through the skin was measured. Human and rat skin with a TEWL of greater than 15 g/hm² were considered potentially damaged and were not used. These samples were replaced by new skin fragments which were also tested for integrity before use in the study.

Treatment: The dose preparation was applied to the split-thickness skin sample with a pipette at the rate of approximately 10 µL.cm² exposed skin. The dose preparations were assayed for radioactivity content (by LSC) by using dose checks (surrogate dose) taken before, during and after the dosing process.

Sampling: The receptor fluid passing through the receptor chamber was collected in glass vials held in a fraction collector. The fraction collector was started after dose application. Samples were then collected hourly for the duration of the experiment (24 hours). At 8 hours post-application, the skin was swabbed with freshly prepared 1% v/v Tween 80 in PBS (phosphate buffer saline) using precision wipes (Kimtech Sciences from Kimberley-Clark professional), in order to remove and retain the non-absorbed dose, until no radioactivity was detected with a Geiger-Müller monitor. At the end of the study (24 hours after application), the treated skin and the skin adjacent to the treatment site (surrounding swabs) were swabbed. Each skin sample was tape-stripped to remove the stratum corneum. This involved the application of Monaderm adhesive tape (Monaderm, Monaco) for 5 seconds before the tape was carefully removed against the direction of hair growth. This procedure was continued until a 'shiny' appearance of the epidermis was evident, which indicated that the stratum corneum had been removed. The tape-strips were collected into scintillation vials for analysis. The skin surrounding the application site (surrounding skin) was separated from the treated skin. Both surrounding skin and tape-stripped treated skin were retained for analysis.

Radioassay: The amounts of radioactivity in the various samples were determined by liquid scintillation counting (LSC). Samples were counted for 10 minutes or for 2 sigma % in an appropriate scintillation cocktail using a Packard 1900 TR counter with on-line computing facilities. Quenching effects were determined using an external standard and spectral quench parameter (tSIE) method. Efficiency correlation curves were prepared for each scintillation cocktail and were regularly checked by the use of [^{14}C-n-hexadecane standards. The scintillation counter was recalibrated when a deviation of greater than 2% was observed when counting quality control standards. The limit of detection was taken to be twice the background values for blank samples in appropriate scintillation cocktails.

Findings:

Fluopicolide was demonstrated to be sufficiently soluble in the receptor fluid to avoid any risk of back diffusion.

Measurements of the homogeneity of the three concentrations of formulation applied indicated that it was acceptable.

The study results are presented in the following Table.

Table 7.3-3: **Distribution of radioactivity at 24 hours after dose application of [^{14}C]- fluopicolide in a FS 350 formulation at the rates of 200 g/L to human skin samples (All cells).**

	Distribution of radioactivity (% dose applied)						Group Human ID N= 6 K N° = 1	
Donor N°	**X2015/4-4**	**X2014/8-28**	**X2015/2-13**	**X2014/7-7**	**X2015/7-19**	**X2014/11-12**		
Sex	**Female**	**Female**	**Female**	**Female**	**Female**	**Female**		
Cell N°	**H01**	**H02**	**H03**	**H04**	**H05**	**H06**	**MEAN**	**SD**
Skin wash 8h	98.05	97.86	88.40	98.51	99.46	98.05	**96.72**	**4.12**
Skin wash 24h	0.0144	0.0029	0.0434	0.0532	0.0473	0.1844	0.06	0.07
Surrounding swabs 24 h	0.0060	0.0039	0.2494	0.0043	0.0114	0.0018	0.05	0.10
Total swabs	**98.07**	**97.86**	**88.69**	**98.57**	**99.52**	**98.24**	**96.82**	**4.03**
SC 1	0.0589	0.0092	0.2389	0.0488	0.0761	0.0493	0.08	0.08
SC 2	0.0048	0.0031	0.0806	0.0081	0.0228	0.0078	0.02	0.03
Total SC 1 + SC 2	**0.0637**	**0.0123**	**0.3195**	**0.0569**	**0.0989**	**0.0571**	**0.10**	**0.11**
Donor chamber	ND	ND	0.5772	0.0266	0.0397	ND	0.21	0.31
TOTAL NON-ABSORBED	**98.13**	**97.87**	**89.59**	**98.65**	**99.66**	**98.30**	**97.03**	**3.70**
Skin	0.013	0.006	0.278	0.025	0.024	0.017	0.06	0.11
Surrounding skin	0.004	0.004	1.545	0.018	0.026	0.004	0.27	0.63
Total skin	**0.017**	**0.010**	**1.823**	**0.043**	**0.050**	**0.021**	**0.33**	**0.73**
SC3	0.002	0.002	0.050	0.007	0.020	0.011	0.015	0.018
SC4	0.002	0.001	0.084	0.004	0.009	0.005	0.018	0.033
SC5	0.001	n.d	0.087	0.003	0.011	0.003	0.018	0.034
SC6	n.d	n.d	0.035	0.002	0.009	0.005	0.008	0.013
SC7	0.003	n.d	0.040	0.006	0.009	0.001	0.010	0.015
SC8	0.008	n.d	0.081	0.004	0.014	0.002	0.018	0.031
SC9	n.s.	n.d	0.030	0.002	0.006	0.003	0.007	0.012
SC10	n.s.	n.s	0.369	0.002	0.010	0.002	0.064	0.149
SC11	n.s.	n.s	n.s	n.s	0.006	0.002	0.001	0.002
SC12	n.s.	n.s	n.s	n.s	0.010	0.004	0.002	0.004
SC13	n.s.	n.s	n.s	n.s	0.002	0.002	0.001	0.001
SC14	n.s.	n.s	n.s	n.s	0.003	0.001	0.001	0.001
SC15	n.s.	n.s	n.s	n.s	0.004	0.002	0.001	0.002
TOTAL SC3+ [a]	**0.017**	**0.003**	**0.774**	**0.029**	**0.111**	**0.044**	**0.16**	**0.30**
TOTAL DOSE SITE	**0.033**	**0.013**	**2.597**	**0.072**	**0.161**	**0.064**	**0.49**	**1.03**
Receptor fluid (0 - 12h)	**0.029**	**0.036**	**0.197**	**0.043**	**0.043**	**0.027**	**0.06**	**0.07**
Receptor fluid (0 - 24h)	**0.052**	**0.060**	**0.268**	**0.079**	**0.073**	**0.050**	**0.10**	**0.08**

	Distribution of radioactivity (% dose applied)						Group Human HD N= 6 K N° = 1	
Donor N°	**X2015/4-4**	**X2014/8-28**	**X2015/2-13**	**X2014/7-7**	**X2015/7-19**	**X2014/11-12**		
Sex	**Female**	**Female**	**Female**	**Female**	**Female**	**Female**		
Cell N°	**H01**	**H02**	**H03**	**H04**	**H05**	**H06**	**MEAN**	**SD**
%Ratio receptor 12h/24h	54	61	74	54	59	53	**59**	**8**
Residual Rec Fluid	n.d	n.d	0.0085	n.d	n.d	n.d	0.00	0.00
Receptor chamber	n.d	n.d	0.3192	n.d	n.d	n.d	0.05	0.13
TOTAL DIRECT	**0.0523**	**0.0598**	**0.5960**	**0.0792**	**0.0726**	**0.0503**	**0.16**	**0.22**
POTENTIAL (dose site+ receptor)	**0.0856**	**0.0727**	**3.1931**	**0.1515**	**0.2335**	**0.1145**	**0.64**	**1.25**
POTENTIAL (skin+ receptor)	0.0689	0.0706	2.4189	0.1224	0.1229	0.0709	0.48	0.95
TOTAL RECOVERY	**98.22**	**97.95**	**92.78**	**98.80**	**99.89**	**98.41**	**97.7**	**2.5**
Evaluation according to EFSA Guidance								
Absorption >75% within half of study duration?						No (include SC values except SC1 & 2)		
Recovery >95%?						No correction needed		
Total % Potentially Absorbable adjusted according to EFSA (2017)						**Mean (%dose site +%receptor) + (SD*1) = 1.9%**		

[a]: tape-strips excluding numbers 1 & 2 which are considered to be non-absorbed dose.

SD: standard deviation

n.d.: below limit of detection; n.s.: no sample; n.a: not applicable.

In the above table, the presented means do not always calculate exactly from the presented individual data. This is due to rounding-up differences resulting from the use of the spreadsheet program.

In the study report the Cell H03 was excluded from the reported cells due to “low recovery”. Futhermore, looking at the cumulative absorption profile of all the cells it shows that cell H03 can be considered as an outlier compared to other cells as shown in the graphs below.

Figure 7.3-1: Cumulative Absorption Profile after dose application of [^{14}C]-fluopicolide in an FS 350 formulation at the nominal rate of 5 g/L to human skin (All cells)

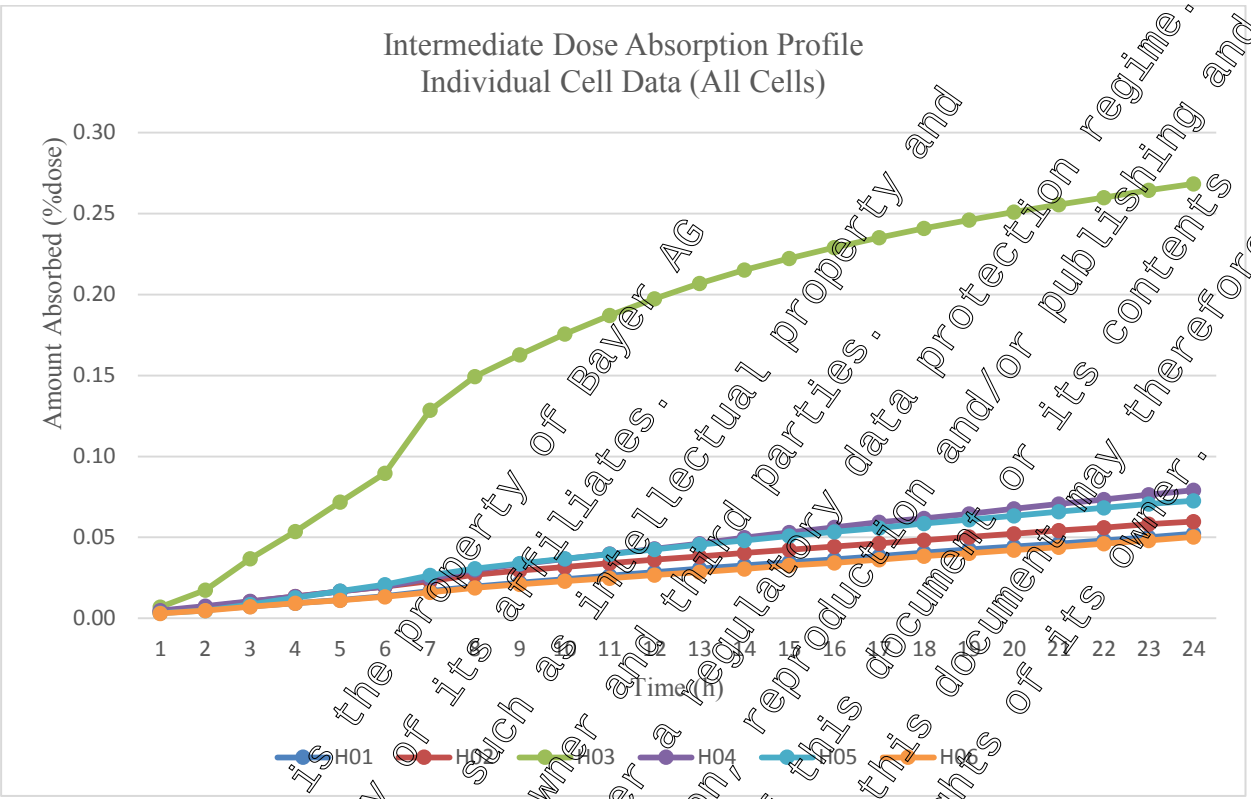


Figure 7.3-2: Cumulative Absorption Profile after dose application of [^{14}C]-fluopicolide in an FS 350 formulation at the nominal rate of 5 g/L to human skin (Reported cells)

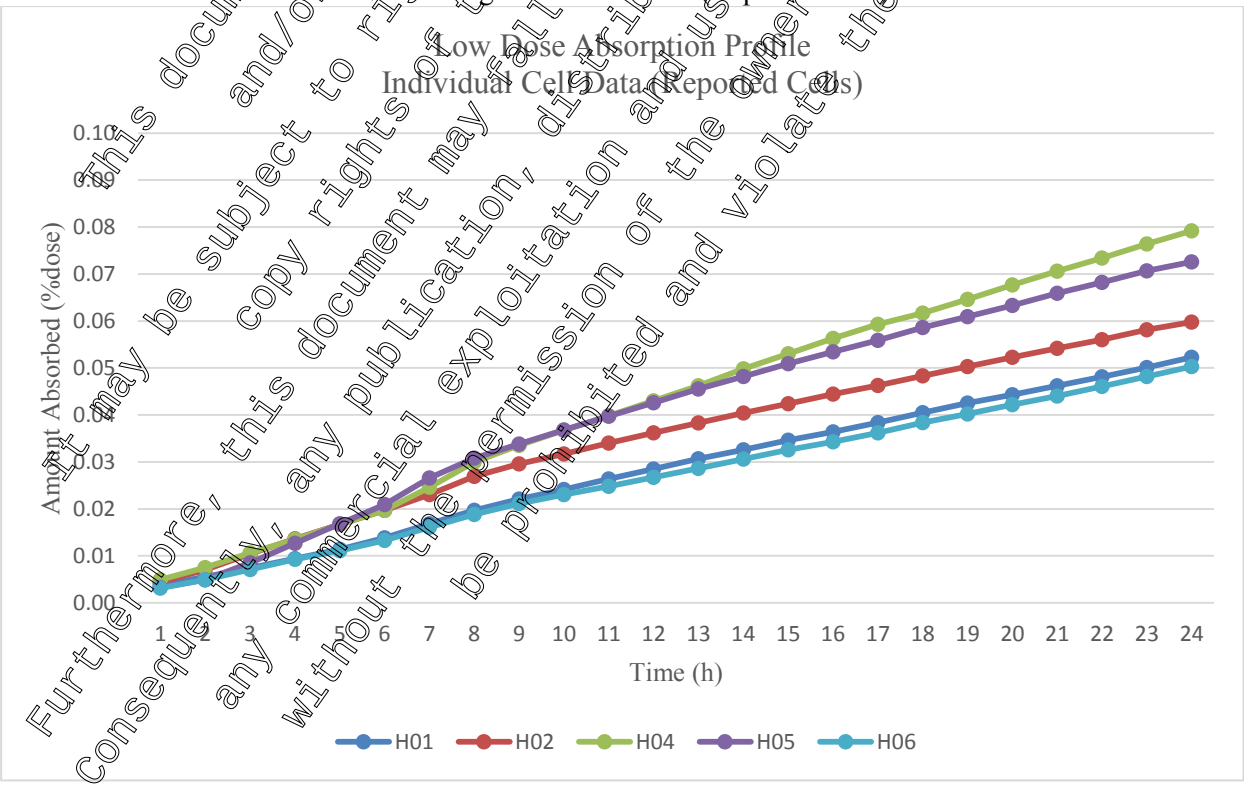


Table 7.3-4: **Distribution of radioactivity at 24 hours after dose application of [^{14}C]- fluopicolide in a FS 350 formulation at the rates of 200 g/L to human skin samples (Reported cells).**

	Distribution of radioactivity (% dose applied)						
Donor N°	**X2015/4-4**	**X2014/8-28**	**X2014/7-7**	**X2015/7-19**	**X2014/11-12**	**Group Human HD N= 5 K N° = 1.2**	
Sex	**Female**	**Female**	**Female**	**Female**	**Female**		
Cell N°	**H01**	**H02**	**H04**	**H05**	**H06**	**MEAN**	**SD**
Skin wash 8h	98.05	97.86	98.51	99.46	98.05	**98.39**	**0.65**
Skin wash 24h	0.0144	0.0029	0.0532	0.0473	0.1844	0.06	0.07
Surrounding swabs 24 h	0.0060	0.0039	0.0043	0.0114	0.0018	0.01	0.00
Total swabs	**98.0700**	**97.8618**	**98.5673**	**99.5172**	**98.2407**	**98.45**	**0.65**
SC 1	0.0589	0.0092	0.0488	0.0761	0.0493	0.05	0.02
SC 2	0.0048	0.0031	0.0081	0.0228	0.0078	0.01	0.01
Total SC 1 + SC 2	**0.0637**	**0.0123**	**0.0569**	**0.0989**	**0.0571**	**0.06**	**0.03**
Donor chamber	ND	ND	0.0266	0.0397	ND	0.03	0.01
TOTAL NON-ABSORBED	**98.13**	**97.87**	**98.65**	**99.66**	**98.30**	**98.52**	**0.69**
Skin	0.013	0.006	0.028	0.024	0.017	0.02	0.01
Surrounding skin	0.004	0.004	0.018	0.026	0.004	0.01	0.01
Total skin	**0.017**	**0.010**	**0.043**	**0.050**	**0.021**	**0.03**	**0.02**
SC3	0.002	0.002	0.007	0.020	0.011	0.008	0.007
SC4	0.002	0.001	0.004	0.009	0.005	0.004	0.003
SC5	0.001	n.d	0.003	0.011	0.003	0.004	0.004
SC6	n.d	n.d	0.002	0.009	0.005	0.003	0.004
SC7	0.003	n.d	0.006	0.009	0.001	0.004	0.004
SC8	0.008	n.d	0.004	0.014	0.002	0.006	0.006
SC9	n.s.	n.d	0.002	0.006	0.003	0.002	0.002
SC10	n.s.	n.s	0.002	0.010	0.002	0.003	0.004
SC11	n.s.	n.s	n.s	0.006	0.002	0.001	0.002
SC12	n.s.	n.s	n.s	0.010	0.004	0.00	0.00
SC13	n.s.	n.s	n.s	0.002	0.002	0.00	0.00
SC14	n.s.	n.s	n.s	0.003	0.001	0.00	0.00
SC15	n.s.	n.s	n.s	0.004	0.002	0.00	0.00
TOTAL SC 3-15	**0.017**	**0.003**	**0.029**	**0.111**	**0.044**	**0.04**	**0.04**
TOTAL DOSE SITE	**0.033**	**0.013**	**0.072**	**0.161**	**0.064**	**0.07**	**0.06**
Receptor fluid (0 - 12h)	**0.029**	**0.036**	**0.043**	**0.043**	**0.027**	**0.04**	**0.01**
Receptor fluid (0 - 24h)	**0.052**	**0.060**	**0.079**	**0.073**	**0.050**	**0.06**	**0.01**

	Distribution of radioactivity (% dose applied)					Group Human HD N= 5 K N° = 1.2	
Donor N°	**X2015/4-4**	**X2014/8-28**	**X2014/7-7**	**X2015/7-19**	**X2014/11-12**		
Sex	**Female**	**Female**	**Female**	**Female**	**Female**		
Cell N°	**H01**	**H02**	**H04**	**H05**	**H06**	**MEAN**	**SD**
%Ratio receptor 12h/24h	54	61	54	59	53	**56**	**3**
Residual Rec Fluid	n.d	n.d	n.d	n.d	n.d	n.d.	n.a.
Receptor chamber	n.d	n.d	n.d	n.d	n.d	n.d	n.a..
TOTAL DIRECT	**0.0523**	**0.0598**	**0.0792**	**0.0726**	**0.0503**	**0.06**	**0.01**
POTENTIAL (dose site+ receptor)	**0.0856**	**0.0727**	**0.1515**	**0.2335**	**0.1145**	**0.132**	**0.065**
POTENTIAL (skin+ receptor)	0.0689	0.0700	0.1224	0.1229	0.0709	0.09	0.03
TOTAL RECOVERY	**98.22**	**97.95**	**98.80**	**99.89**	**98.41**	**98.65**	**0.76**
Evaluation according to EFSA Guidance							
Absorption >75% within half of study duration?					No. (include SC values)		
Recovery <95%?					No correction needed		
Total % Potentially Absorbable adjusted according to EFSA (2017)					**Mean (%dose site +%receptor) + (SD*1.2) = 0.21%**		

[a]: tape-strips excluding numbers 1 & 2 which are considered to be non-absorbed dose.

SD: standard deviation

n.d.: below limit of detection, n.s.: no sample, n.a: not applicable.

In the above table, the presented means do not always calculate exactly from the presented individual data. This is due to rounding-up differences resulting from the use of the spreadsheet program.

Table 7.3.5: **Distribution of radioactivity at 24 hours after dose application of [^{14}C]- fluopicolide in a FS 350 formulation at the rates of 50 g/L to human skin samples (All cells).**

	Distribution of radioactivity (% dose applied)						Group Human HD N = 6 K N° = 1	
Sex	**Female**	**Female**	**Female**	**Female**	**Female**	**Female**		
Donor N°	**X2015/4-3**	**X2014/11-2**	**X2015/1-12**	**X2014/8-1**	**X2015/8-7**	**X2014/7-8**		
Cell N°	**H07**	**H08**	**H09**	**H010**	**H11**	**H12**	**MEAN**	**SD**
Skin wash 8h	91.36	102.93	132.20	101.50	92.67	90.72	101.90	91.36
Skin wash 24h	0.01	0.10	0.01	0.00	0.11	0.14	0.06	0.01
Surrounding swabs 24 h	0.0010	0.0012	0.0040	0.0027	0.0085	0.0049	0.00	0.001
Total swabs	**91.37**	**103.02**	**132.21**	**101.50**	**92.79**	**90.86**	**101.96**	**91.37**
SC1	0.104	1.293	0.088	0.115	0.761	0.452	0.47	0.504
SC2	0.099	0.357	0.020	0.007	0.167	0.084	0.12	0.099
Total SC1 + SC2	**0.20**	**1.65**	**0.11**	**0.12**	**0.93**	**0.54**	**0.59**	**0.20**
Donor chamber	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.
TOTAL NON-ABSORBED	**91.57**	**104.67**	**132.32**	**101.62**	**93.72**	**91.40**	**102.55**	**15.59**
Skin	0.11	0.48	0.08	0.04	0.36	0.42	0.20	0.16
Surrounding skin	0.004	0.008	0.016	0.003	0.010	0.023	0.01	0.01
Total skin	**0.12**	**0.49**	**0.10**	**0.04**	**0.37**	**0.44**	**0.21**	**0.16**
SC3	0.010	0.308	0.009	0.005	0.080	0.062	0.079	0.116
SC4	0.006	0.123	0.018	0.007	0.078	0.031	0.044	0.047
SC5	n.s.	0.067	0.010	n.s.	0.060	0.023	0.027	0.030
SC6	n.s.	0.187	0.005	n.s.	0.055	0.052	0.050	0.072
SC7	n.s.	0.019	0.005	n.s.	0.040	n.s.	0.011	0.016
SC8	n.s.	0.017	0.004	n.s.	0.016	n.s.	0.006	0.008
SC9	n.s.	0.017	0.006	n.s.	0.015	n.s.	0.006	0.008
SC10	n.s.	0.037	0.008	n.s.	0.010	n.s.	0.009	0.014
SC11	n.s.	n.s.	0.004	n.s.	0.135	n.s.	0.023	0.055
SC12	n.s.	n.s.	0.006	n.s.	n.s.	n.s.	0.001	0.003
Total SC3+	**0.02**	**0.77**	**0.07**	**0.01**	**0.49**	**0.17**	**0.26**	**0.31**
TOTAL DOSE SITE	**0.13**	**0.96**	**0.17**	**0.05**	**0.86**	**0.61**	**0.46**	**0.40**
Receptor fluid (0 - 12h)	0.275	0.233	0.071	0.167	0.492	0.667	0.32	0.22
Receptor fluid (0 - 24h)	0.281	0.298	0.128	0.173	0.616	0.796	0.38	0.27
%Ratio receptor 12h/24h	98	78	55	96	80	84	**82**	**15**

	Distribution of radioactivity (% dose applied)						Group Human HD N= 6 K N° = 1	
Sex	**Female**	**Female**	**Female**	**Female**	**Female**	**Female**		
Donor N°	**X2015/4-3**	**X2014/11-2**	**X2015/1-12**	**X2014/8-1**	**X2015/8-7**	**X2014/7-8**		
Cell N°	**H07**	**H08**	**H09**	**H010**	**H11**	**H12**	**MEAN**	**SD**
Residual Rec Fluid	0.012	0.009	0.017	0.007	0.023	0.020	0.012	0.009
Receptor chamber	0.135	4.927	n.d.	0.293	0.722	1.650	0.135	4.927
TOTAL DIRECT	**0.43**	**5.23**	**0.14**	**0.47**	**1.36**	**2.47**	**1.68**	**1.94**
POTENTIAL (dose site+ receptor)	0.56	6.19	0.32	0.52	2.22	3.07	2.15	2.27
POTENTIAL (skin+ receptor)	**0.55**	**5.42**	**0.24**	**0.51**	**1.74**	**2.91**	**1.89**	**2.00**
TOTAL RECOVERY	**92.1**	**110.9**	**132.6**	**102.1**	**95.9**	**94.5**	**104.7**	**15.3**
Evaluation according to EFSA Guidance (2017)								
Absorption >75% within half of study duration?					Yes (exclude SC values)			
Mean Recovery <95%?					No correction needed			
Total % Potentially Absorbable adjusted according to EFSA (2017)					Mean (%skin +%receptor) + (SD*1) = 3.9%			

SD: standard deviation; N: number of skin cells used for calculation

n.d.: not detected (below the limit of detection); n.a.: not applicable

In the above table, the presented means do not always calculate exactly from the presented individual data. This is due to rounding-up differences resulting from the use of the spreadsheet program.

Conclusion:

The dermal penetration through human dermatomed skin of [^{14}C]-fluopicolide in the fluopicolide FS 350 formulation was investigated at three nominal concentrations corresponding to the neat product (200 g /L) and to two representative spray dilutions of 50 g/L.

<u>Concentrate</u>

The mean percentage of fluopicolide in the FLC+FXA FS 350 formulation that was considered to be potentially absorbable for the neat formulation applying the EFSA guidance (2017) to the study data was 0.21%.

<u>Intermediate Dose level</u>

The mean percentage of fluopicolide in the FLC+FXA FS 350 formulation that was considered to be potentially absorbable for the neat formulation applying the EFSA guidance (2017) to the study data was 3.9%.

Therefore, the following dermal absorption values can be proposed for use in the non-dietary risk assessments for fluopicolide in the FLC+FXA FS 350 formulation:

- 0.21% for the neat formulation (200 g/L)
- 3.9% for the intermediate dose (50 g/L)

Assessment and conclusion by applicant:
An acceptable study yielding valid conclusions.

A summary of the dermal absorption rates for fluoxastrobin in the fluopicolide + fluoxastrobin FS 200 + 150 (FLC+FXA FS 350) formulation is presented in the following table.

Table 7.3-6: Dermal absorption rates for fluoxastrobin in FLC+FXA FS 350

	Fluoxastrobin
	Value (% of dose applied)
Concentrate	0.07%
Dilution (dilution factor)	0.73% @ 37.5 g/L

Justification for proposed values – Fluoxastrobin

The proposed dermal absorption rates for fluoxastrobin are based on an *in vitro* human skin dermal absorption study using the FLC+FXA FS 350 formulation. The study results are summarized in the following table. A full summary of the study is described in detail below.

Table 7.3-7: Summary of the results of submitted dermal absorption studies for Fluoxastrobin

Test	Concentrate	Spray dilution (dilution factor)	Formulation in study	Justification provided on representativity of study formulation for current product	Reference
In vitro (Human)	0.07%	0.73% (1 in 4)	FLC+FXA FS 350	Not required	[redacted] 2016; M-548487-01-1

Comparative dermal absorption, *in vitro* using rat and human skin

Data Point:	KCP 7.3.02
Report Author:	[redacted]
Report Year:	2016
Report Title:	FLC + FXA FS 200 + 150 formulation: [14C]-fluoxastrobin - In vitro dermal absorption study using human skin
Report No.:	SA 15202
Document No:	M-548487-01-1
Guideline(s) followed in study:	OECD 428 2004; OECD Environmental Health and Safety Publication Series on testing and Assessment No 28 (2004); EFSA Panel on Plant Protection Products and their Residues (PPR), EFSA Journal (2012)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Material and methods

Human skin: Source: Xenometrix, Hégenheim, France.

Number and sex: minimum of 6 donors, female.
Anatomical region: Abdomen.
Thickness: 357.2 to 496.0 µm.

Test Material:

Non-radiolabelled: Batch: EDCI000205.
Purity = 99.5% w/w.

Radiolabelled: [chlorophenyl-UL-^{14}C]-fluoxastrobin
Batch: KML 10000.
Specific activity: 3.77 MBq/mg.
Radiopurity of the formulation: >99%.

Formulation: The formulation used in this experiment was the Fluopicolide + Fluoxastrobin FS 350 (200 + 150) formulation (specification N° 10200008578) containing fluoxastrobin at a concentration of 150 g/L. It was used at two nominal concentrations of fluoxastrobin: neat, 150 g/L and 37.5 g/L.

Test system: A flow-through diffusion cell system (Franz's cell-modified, Gallas, France) was used to study the absorption of the test substance (exposure area of 1 cm² skin). A diffusion cell consisted of a donor chamber and a receptor chamber between which the skin was positioned. The receptor fluid was Eagle's medium supplemented with 5% bovine serum albumin and gentamycin (50 mg/L) at a pH of 7.43 to 7.45. The receptor chamber was warmed by a constant circulation of warm water which maintained the receptor fluid at 32 ± 2°C (close to the normal skin temperature). The receptor fluid was pumped through the receptor chamber at a rate of 1.5 mL/h and stirred continuously whilst in the receptor chamber by means of a magnetic bar.

Skin integrity: Before dose application, the integrity of the skin samples was assessed by measuring the trans-epidermal water loss (TEWL) from the stratum corneum. An evaporimeter probe (Tewameter TM300) was placed securely on the top of the donor chamber and the amount of water diffusing through the skin was measured. Human and rat skin with a TEWL of greater than 15 g/hm² were considered potentially damaged and were not used. These samples were replaced by new skin fragments which were also tested for integrity before use in the study.

Treatment: The dose preparation was applied to the split-thickness skin sample with a pipette at the rate of approximately 10 µL/cm² exposed skin. The dose preparations were assayed for radioactivity content (by LSC) by using dose checks (surrogate dose) taken before, during and after the dosing process.

Sampling: The receptor fluid passing through the receptor chamber was collected in glass vials held in a fraction collector. The fraction collector was started after dose application. Samples were then collected hourly for the duration of the experiment (24 hours). At 8 hours post-application, the skin was swabbed with freshly prepared 1% v/v Tween 80 in PBS (phosphate buffer saline) using natural sponge swabs, in order to remove and retain the non-absorbed dose, until no radioactivity was detected with a Geiger-Müller monitor. At the end of the study (24 hours after application), the treated skin and the skin adjacent to the treatment site (surrounding swabs) were swabbed. Each skin sample was tape-stripped to remove the stratum corneum. This involved the

application of Monaderm adhesive tape (Monaderm, Monaco) for 5 seconds before the tape was carefully removed against the direction of hair growth. This procedure was continued until a 'shiny' appearance of the epidermis was evident, which indicated that the stratum corneum had been removed. The tape-strips were collected into scintillation vials for analysis. The skin surrounding the application site (surrounding skin) was separated from the treated skin. Both surrounding skin and tape-stripped treated skin were retained for analysis.

Radioassay: The amounts of radioactivity in the various samples were determined by liquid scintillation counting (LSC). Samples were counted for 10 minutes or for 2 sigma % in an appropriate scintillation cocktail using a Packard 1900 TR counter with on-line computing facilities. Quenching effects were determined using an external standard and spectral quench parameter (tSIE) method. Efficiency correlation curves were prepared for each scintillation cocktail and were regularly checked by the use of [^{14}C-n-hexadecane standards. The scintillation counter was recalibrated when a deviation of greater than 2% was observed when counting quality control standards. The limit of detection was taken to be twice the background values for blank samples in appropriate scintillation cocktails.

Findings:

Fluoxastrobin was demonstrated to be sufficiently soluble in the receptor fluid to avoid any risk of back diffusion.

Measurements of the homogeneity of the two concentrations of formulation applied indicated that it was acceptable.

The study results are presented in the following Tables.

Table 7.3-8: **Distribution of radioactivity at 24 hours after dose application of [14C]- fluoxastrobin in a FS 350 formulation at the rate of 150 g/kg to human skin samples (All cells).**

	Distribution of radioactivity (% dose applied)						Group Human HD N=6 KN° = 1	
Donor N°	***X2015/4-7***	**X2014/11-10**	**X2015/5-1**	**X2015/8-10**	**X2014/8-23**	**X2014/7-15**		
Sex	***Female***	**Female**	**Female**	**Female**	**Female**	**Female**		
Cell N°	***H01***	**H02**	**H03**	**H04**	**H05**	**H06**	**MEAN**	**SD**
Skin wash 8h	*94.19*	99.27	94.98	100.42	89.18	90.97	**94.84**	**4.43**
Skin wash 24h	*0.2287*	0.0036	0.0050	0.0012	0.8218	0.1828	0.21	0.32
Surrounding swabs 24h	*0.1601*	n.d.	n.d.	0.0024	0.0074	n.d.	0.03	0.06
Total swabs	***94.58***	**99.27**	**94.98**	**100.43**	**90.01**	**91.15**	**95.07**	**4.18**
SC 1	*0.9662*	0.1172	0.0053	0.0088	0.0151	0.0092	0.19	0.38
SC 2	*0.0647*	0.0055	0.0024	0.0024	0.0062	n.d.	0.01	0.03
Total SC 1 + SC 2	***1.03***	**0.12**	**0.01**	**0.01**	**0.02**	**0.01**	**0.20**	**0.41**
Donor chamber	*0.3284*	0.0296	n.d.	0.0711	0.2835	0.2900	0.17	0.15
TOTAL NON-ABSORBED	***95.94***	**99.42**	**94.99**	**100.51**	**90.32**	**91.45**	**95.44**	**4.10**
Skin	*0.4934*	0.0112	0.0025	0.0098	0.0086	0.0020	0.09	0.20
Surrounding skin	*0.8380*	0.0198	0.0023	0.0058	0.0374	0.0034	0.15	0.34
Total skin	***1.33***	**0.03**	**0.00**	**0.02**	**0.05**	**0.01**	**0.24**	**0.54**
SC3	*0.1250*	0.0099	n.d.	0.0022	0.0014	n.d.	0.0231	0.0501
SC4	*0.0983*	0.0018	n.d.	0.0014	0.0041	n.d.	0.0176	0.0396
SC5	*0.0396*	0.0017	n.d.	0.0013	0.0015	n.s.	0.0088	0.0172
SC6	*n.s.*	0.0059	0.0032	0.0015	n.s.	n.s.	0.0035	0.0022
SC7	*n.s.*	0.0014	n.d.	n.s.	n.s.	n.s.	0.0007	0.0000
SC8	*n.s.*	n.d.	n.d.	n.s.	n.s.	n.s.	0.0000	0.0000
SC9	*n.s.*	0.0010	n.s.	n.s.	n.s.	n.s.	0.0010	0.0000
SC10	*n.s.*	0.0024	n.s.	n.s.	n.s.	n.s.	0.0024	0.0000
SC11	*n.s.*	n.d.	n.s.	n.s.	n.s.	n.s.	0.0000	0.0000
SC12	*n.s.*	0.0017	n.s.	n.s.	n.s.	n.s.	0.0017	0.0000
SC13	*n.s.*	0.0011	n.s.	n.s.	n.s.	n.s.	0.0000	0.0000
TOTAL SC 3+	***0.263***	**0.027**	**0.003**	**0.006**	**0.007**	**n.d.**	**0.05**	**0.10**
TOTAL DOSE SITE	***1.59***	**0.06**	**0.01**	**0.02**	**0.05**	**0.01**	**0.29**	**0.64**
Receptor fluid (0 - 12h)	*0.0224*	n.d.	n.d.	n.d.	n.d.	n.d.	0.0037	0.0091
Receptor fluid (0 - 24h)	*0.0679*	n.d.	n.d.	n.d.	0.0147	n.d.	0.0138	0.0272
%Ratio receptor 12h/24h	***33.0***	**100.0**	**100.0**	**100.0**	**0.0**	**100.0**	**72**	**44**

	Distribution of radioactivity (% dose applied)						Group Human HD N= 6 K N° = 1	
Donor N°	*X2015/4-7*	X2014/11-10	X2015/5-1	X2015/8-10	X2014/8-23	X2014/7-15		
Sex	*Female*	Female	Female	Female	Female	Female		
Cell N°	*H01*	H02	H03	H04	H05	H06	**MEAN**	**SD**
TOTAL DIRECT	***2.49***	**0.00**	**0.00**	**0.00**	**0.02**	**0.01**	**0.42**	**1.01**
POTENTIAL (dose site+ receptor)	***4.09***	**0.06**	**0.01**	**0.02**	**0.07**	**0.02**	**0.71**	**1.65**
POTENTIAL (skin+ receptor)	*3.82*	0.03	0.00	0.02	0.07	0.02	0.66	1.55
TOTAL RECOVERY	***100.0***	**99.5**	**95.0**	**100.5**	**90.4**	**91.5**	**96.15**	**4.51**
Evaluation according to EFSA Guidance								
Absorption >75% within half of study duration?					No. (include SC values except SC1 & 2)			
Recovery <95%?					No correction needed			
Total % Potentially Absorbable adjusted according to EFSA (2017)					Mean (%dose site +%receptor) + (SD*1) = **2.4%**			

SD: standard deviation

n.d.: below limit of detection; n.s.: no sample; n.a.: not applicable.
In the above table, the presented means do not always calculate exactly from the presented individual data. This is due to rounding-up differences resulting from the use of the spreadsheet program.

Cell H01 was excluded as the values for the stratum, corneum, skin and direct absorption were clear outliers compared to the other cells as measured by the modified Z-score test.

In general, finding the "Outliers" in a data set can be done by calculating the deviation for each number, expressed as either a "Z-score" or "modified Z-score" and testing it against certain predefined threshold. Z-score typically refers to number of standard deviation relative to the statistical average. The Modified Z-score applies the median computation technique to measure the deviation. Mathematically the Modified Z-score can be written as:

$$Mi=0.6745 * (Xi - Median(Xi)) / MAD,$$

where MAD stands for Median Absolute Deviation. Any number in a data set with the absolute value of a modified Z-score exceeding 3.5 is considered an "Outlier"

Table 7.3-9: Modified Z-score result for the stratum corneum (SC3+), skin and direct absorption results for FXA following application of [^{14}C]-fluoxastrobin in the FLC+FXA FS 350 formulation at the rate of 150 g/L to human skin samples (All cells).

Cell N°	SC3+ (%dose)		mean-value	mod Z score
H01	0.263	OUTLIER	-0.2505	34.6
H02	0.027	NORMAL	0.0205	2.8
H03	0.003	NORMAL	0.0035	-0.5
H04	0.006	NORMAL	0.0005	-0.1
H05	0.007	NORMAL	-0.0005	0.1
H06	0	NORMAL	0.0065	-0.9
	Skin (%dose)		mean-value	mod Z score
H01	1.33	OUTLIER	-1.305	**44.0**
H02	0.03	NORMAL	-0.005	0.2
H03	0	NORMAL	0.025	-0.8
H04	0.02	NORMAL	0.005	-0.2
H05	0.05	NORMAL	-0.025	0.8
H06	0.01	NORMAL	0.015	-0.5
	Direct (%dose)		mean-value	mod Z score
H01	2.49	OUTLIER	-2.485	**335.2**
H02	0	NORMAL	0.005	-0.7
H03	0	NORMAL	0.005	-0.7
H04	0	NORMAL	0.005	-0.7
H05	0.02	NORMAL	-0.015	2.0
H06	0.01	NORMAL	-0.005	0.7

Furthermore, the value for the receptor chamber was anomalously high suggesting an experimental error such as contamination of the sample.

Table 7.3-10: **Distribution of radioactivity at 24 hours after dose application of [^{14}C]- fluoxastrobin in a FS 350 formulation at the rate of 150 g/kg to human skin samples (Reported cells).**

	Distribution of radioactivity (% dose applied)					Group Human HD N= 5 K.S° = 1.2	
Donor N°	**X2014/11-10**	**X2015/5-1**	**X2015/8-10**	**X2014/8-23**	**X2014/7-15**		
Sex	**Female**	**Female**	**Female**	**Female**	**Female**		
Cell N°	**H02**	**H03**	**H04**	**H05**	**H06**	**MEAN**	**SD**
Skin wash 8h	99.27	94.98	100.42	89.18	90.97	**94.96**	**4.94**
Skin wash 24h	0.0036	0.0050	0.0012	0.8218	0.1828	0.20	0.35
Surrounding swabs 24h	n.d.	n.d.	0.0024	0.0074	n.d.	0.002	0.003
Total swabs	**99.27**	**94.98**	**100.43**	**90.01**	**91.15**	**95.17**	**4.67**
SC 1	0.1172	0.0053	0.0088	0.0151	0.0092	0.03	0.05
SC 2	0.0055	0.0024	0.0024	0.0062	n.d.	0.003	0.003
Total SC 1 + SC 2	**0.12**	**0.01**	**0.01**	**0.02**	**0.01**	**0.03**	**0.05**
Donor chamber	0.0296	n.d.	0.0711	0.2835	0.2900	0.13	0.14
TOTAL NON ABSORBED	**99.42**	**94.99**	**100.51**	**90.32**	**91.45**	**95.34**	**4.58**
Skin	0.0112	0.0025	0.0098	0.0086	0.0029	0.007	0.004
Surrounding skin	0.0198	0.0023	0.0058	0.0374	0.0034	0.014	0.015
Total skin	**0.03**	**0.00**	**0.02**	**0.05**	**0.01**	**0.02**	**0.02**
SC3	0.0099	n.d.	0.0022	0.0014	n.d.	0.003	0.004
SC4	0.0018	n.d.	0.0014	0.0041	n.d.	0.001	0.002
SC5	0.0017	n.d.	0.0013	0.0015	n.s.	0.001	0.001
SC6	0.0059	0.0032	0.0015	n.s.	n.s.	0.004	0.002
SC7	0.0014	n.d.	n.s.	n.s.	n.s.	0.001	0.000
SC8	n.d.	n.d.	n.s.	n.s.	n.s.	n.d.	n.a.
SC9	0.0010	n.s.	n.s.	n.s.	n.s.	0.001	0.000
SC10	0.0024	n.s.	n.s.	n.s.	n.s.	0.002	0.000
SC11	n.d.	n.s.	n.s.	n.s.	n.s.	n.d.	n.a.
SC12	0.0017	n.s.	n.s.	n.s.	n.s.	0.002	0.000
SC13	0.0011	n.s.	n.s.	n.s.	n.s.	0.000	0.000
TOTAL SC 3+	**0.027**	**0.003**	**0.006**	**0.007**	**n.d.**	**0.01**	**0.01**
TOTAL DOSE SITE	**0.06**	**0.01**	**0.02**	**0.05**	**0.01**	**0.03**	**0.02**
Receptor fluid (0 - 12h)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.
Receptor fluid (0 - 24h)	n.d.	n.d.	n.d.	0.0147	n.d.	**0.003**	**0.007**

	Distribution of radioactivity (% dose applied)					Group Human HD N= 5 K N° = 1.2	
Donor N°	**X2014/11-10**	**X2015/5-1**	**X2015/8-10**	**X2014/8-23**	**X2014/7-15**		
Sex	**Female**	**Female**	**Female**	**Female**	**Female**		
Cell N°	**H02**	**H03**	**H04**	**H05**	**H06**	**MEAN**	**SD**
%Ratio receptor 12h/24h	**100.0**	**100.0**	**100.0**	**0.0**	**100.0**	**80**	**45**
TOTAL DIRECT	**0.00**	**0.00**	**0.00**	**0.02**	**0.01**	**0.01**	**0.01**
POTENTIAL (dose site+ receptor)	**0.06**	**0.01**	**0.02**	**0.07**	**0.02**	**0.04**	**0.03**
POTENTIAL (skin+ receptor)	0.03	0.00	0.02	0.07	0.02	**0.03**	**0.02**
TOTAL RECOVERY	**99.5**	**95.0**	**100.5**	**90.4**	**91.5**	**95.37**	**4.57**
Evaluation according to EFSA Guidance							
Absorption >75% within half of study duration?						No. (include SC values except SC1 & 2)	
Recovery <95%?						No correction needed	
Total % Potentially Absorbable adjusted according to EFSA (2017)						Mean (%dose site +%receptor) + (SD*1.2) = **0.07%**	

SD: standard deviation

n.d.: below limit of detection; n.s.: no sample; n.a.: not applicable.
In the above table, the presented means do not always calculate exactly from the presented individual data. This is due to rounding-up differences resulting from the use of the spreadsheet program.

Table 7.3-11: **Distribution of radioactivity at 24 hours after dose application of [^{14}C]- fluoxastrobin in a FS 350 formulation at the rate of 37.5 g/L to human skin samples (All cells).**

	Distribution of radioactivity (% dose applied)						Group Human LD N=6 K◦N° = 1	
Donor N°	**X2014/8-14**	***X2014/7-18***	**X2015/2-11**	**X2015/3-1**	**X2015/8-8**	**X2014/11-3**		
Sex	**Female**	**Female**	**Female**	**Female**	**Female**	**Female**		
Cell N°	**H07**	**H08**	**H09**	**H10**	**H11**	**H12**	**MEAN**	**SD**
Skin wash 8h	102.62	*27.54*	101.17	105.49	105.85	107.50	91.69	31.71
Skin wash 24h	0.03	*5.85*	0.02	0.01	0.02	0.26	1.03	2.36
Surrounding swabs 24 h	0.0044	*0.06*	0.07	0.0028	0.02	0.02	0.03	0.03
Total swabs	**102.65**	***33.45***	**101.26**	**105.50**	**105.88**	**107.78**	**92.75**	**29.15**
SC 1	0.04	*0.12*	0.01	0.02	0.005	0.03	0.04	0.04
SC 2	0.00	*0.37*	0.06	0.01	0.02	0.01	0.08	0.15
Total SC 1 + SC 2	**0.04**	***0.49***	**0.06**	**0.02**	**0.02**	**0.04**	**0.11**	**0.19**
Donor chamber	0.08	*0.25*	0.09	0.59	0.00	0.33	0.37	0.37
TOTAL NON-ABSORBED	**102.76**	***34.20***	**102.31**	**106.11**	**105.90**	**108.14**	**93.24**	**29.01**
Skin	0.02	*76.14*	0.16	0.02	0.01	0.10	12.74	31.06
Surrounding skin	0.01	*1.36*	0.54	0.01	0.01	0.08	0.34	0.54
Total skin	**0.03**	***77.50***	**0.70**	**0.03**	**0.02**	**0.18**	**13.08**	**31.56**
SC3	n.d.	*0.1409*	0.0134	0.0024	0.0042	0.0099	0.028	0.055
SC4	n.d.	*0.2351*	0.0029	0.0014	0.0012	0.0108	0.042	0.095
SC5	n.s.	*0.0637*	n.s.	0.0016	0.0013	0.0040	0.018	0.031
SC6	n.s.	*0.1068*	n.s.	0.0023	n.s.	0.0090	0.037	0.055
SC7	n.s.	*0.0510*	n.s.	0.0013	n.s.	0.0070	0.020	0.027
SC8	n.s.	n.s.	n.s.	0.0031	n.s.	0.0052	0.004	0.001
SC9	n.s.	n.s.	n.s.	n.d.	n.s.	0.0053	0.003	0.000
SC10	n.s.	n.s.	n.s.	n.d.	n.s.	0.0050	0.003	0.000
TOTAL SC 3+	n.d.	***0.5915***	**0.0163**	**0.0121**	**0.0067**	**0.0562**	**0.114**	**0.235**
TOTAL DOSE SITE	**0.03**	***78.09***	**0.72**	**0.04**	**0.02**	**0.24**	**13.19**	**31.80**
Receptor fluid (0 - 12h)	0.0030	*0.1906*	0.0824	n.d.	0.0896	0.0025	0.0614	0.0756
Receptor fluid (0 - 24h)	0.0030	*0.3666*	0.1088	n.d.	0.1297	0.0025	0.1018	0.1421
%Ratio receptor 12h/24h	100	*52*	76	100	69	100	**83**	**20**

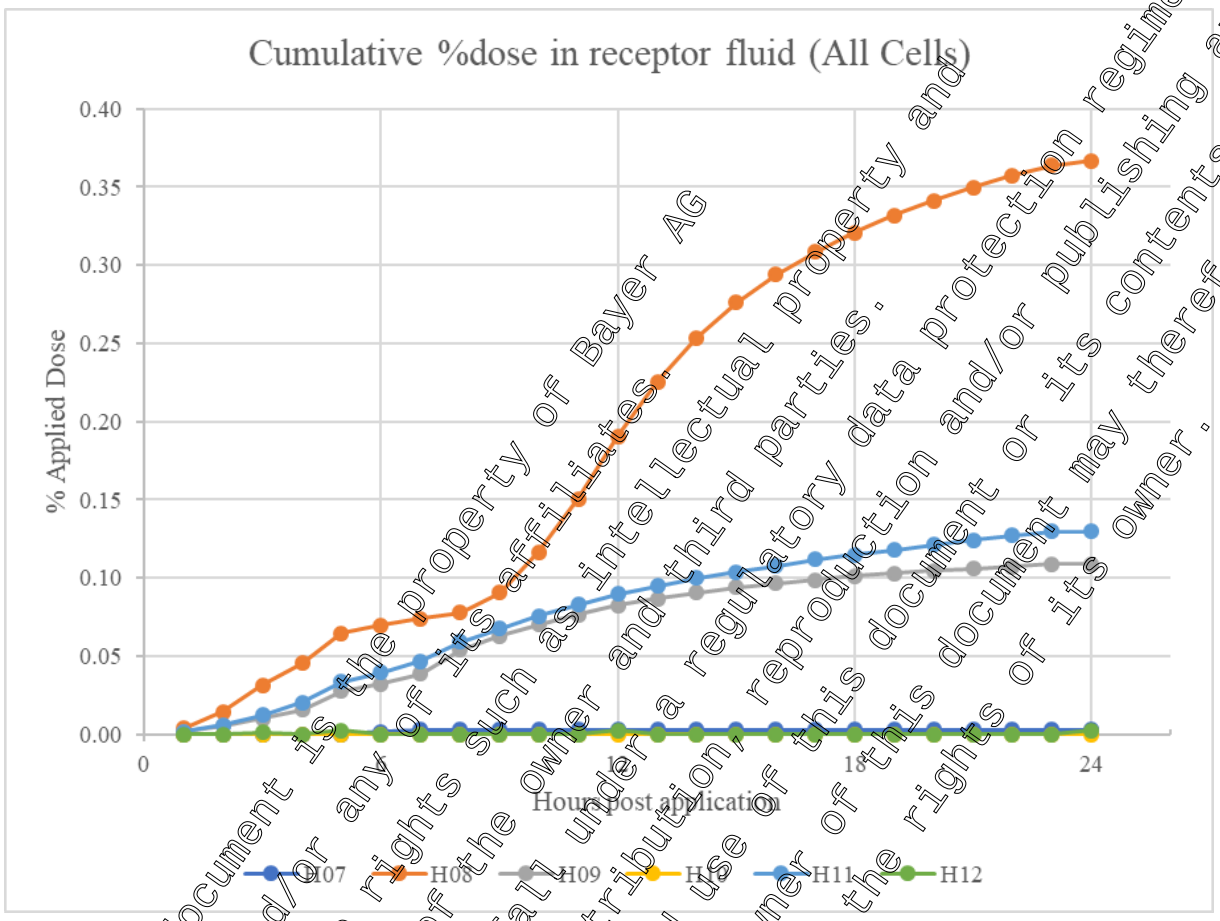
	Distribution of radioactivity (% dose applied)						Group Human LD N= 6 K N° = 1	
Donor N°	**X2014/8-14**	***X2014/7-18***	**X2015/2-11**	**X2015/3-1**	**X2015/8-8**	**X2014/11-3**		
Sex	**Female**	**Female**	**Female**	**Female**	**Female**	**Female**		
Cell N°	**H07**	**H08**	**H09**	**H10**	**H11**	**H12**	**MEAN**	**SD**
Residual Rec Fluid	n.d.	*0.0209*	n.d.	n.d.	n.d.	n.d.	0.0035	0.0085
Receptor Chamber	n.d.	*0.2340*	n.d.	n.d.	n.d.	0.3678	0.1003	0.1610
TOTAL DIRECT	**0.00**	***0.62***	**0.11**	**0.00**	**0.13**	**0.37**	**0.21**	**0.24**
POTENTIAL (dose site+ receptor)	**0.03**	***78.71***	**0.82**	**0.04**	**0.15**	**0.61**	**13.40**	**32.00**
POTENTIAL (skin+ receptor)	0.03	*78.71*	0.81	0.03	0.15	0.55	13.28	31.77
TOTAL RECOVERY	**102.8**	***112.9***	**103.1**	**106.2**	**106.1**	**108.7**	**106.63**	**3.78**
Evaluation according to EFSA Guidance								
Absorption >75% within half of study duration?							No due to variability and mean lower limit confidence value of 62%. (include SC values except SC1 & 2)	
Recovery >95%?							No correction needed	
Total % Potentially Absorbable adjusted according to EFSA (2017)							Mean (%dose site +%receptor) + (SD*1) **=45%**	

SD: standard deviation

n.d.: below limit of detection; n.s.: no sample; n.a.: not applicable.
In the above table, the presented means do not always calculate exactly from the presented individual data. This is due to rounding up differences resulting from the use of the spreadsheet program.

Cell H08 was excluded as the values for the swabbing and the skin were clear outliers compared to the other cells. Furthermore, the absorption profile was also clearly different to the remaining cells as shown in the following figure:

Figure 7.3-3: Cumulative Absorption Profile showing the different absorption profile of cell H08



The results excluding cell H08 are presented in the following table.

Table 7.3-12: Distribution of radioactivity at 24 hours after dose application of [^{14}C]- fluoxastrobin in a FS 350 formulation at the rate of 37.5 g/L to human skin samples (Reported cells).

	Distribution of radioactivity (% dose applied)						
Donor N°	**X2014/8-14**	**X2015/2-11**	**X2015/3-1**	**X2015/8-8**	**X2014/11-3**	**Group Human LD N= 5**	
Sex	**Female**	**Female**	**Female**	**Female**	**Female**	**K N° = 1.2**	
Cell N°	**H07**	**H09**	**H10**	**H11**	**H12**	**MEAN**	**SD**
Skin wash 8h	102.62	101.17	105.49	105.85	107.50	104.52	2.57
Skin wash 24h	0.03	0.02	0.01	0.02	0.26	0.07	0.11
Surrounding swabs 24 h	0.0044	0.07	0.0028	0.02	0.02	0.02	0.03
Total swabs	**102.65**	**101.26**	**105.50**	**105.88**	**107.78**	**104.61**	**2.62**
SC 1	0.04	0.01	0.02	0.005	0.03	0.02	0.01
SC 2	0.00	0.06	0.01	0.02	0.01	0.02	0.02
Total SC 1 + SC 2	**0.04**	**0.06**	**0.02**	**0.02**	**0.04**	**0.04**	**0.02**

Donor chamber	0.08	0.99	0.59	0.00	0.33	0.39	0.40
TOTAL NON ABSORBED	**102.76**	**102.31**	**106.11**	**105.90**	**108.14**	**105.05**	**2.46**
Skin	0.02	0.16	0.02	0.01	0.10	0.06	0.06
Surrounding skin	0.01	0.54	0.01	0.01	0.08	0.13	0.23
Total skin	**0.03**	**0.70**	**0.03**	**0.02**	**0.18**	**0.19**	**0.29**
SC3	n.d.	0.0134	0.0024	0.0042	0.0099	0.006	0.006
SC4	n.d.	0.0029	0.0014	0.0012	0.0108	0.003	0.004
SC5	n.s.	n.s.	0.0016	0.0013	0.0040	0.002	0.001
SC6	n.s.	n.s.	0.0023	n.s.	0.0090	0.006	0.005
SC7	n.s.	n.s.	0.0013	n.s.	0.0070	0.004	0.004
SC8	n.s.	n.s.	0.0031	n.s.	0.0052	0.004	0.001
SC9	n.s.	n.s.	n.d.	n.s.	0.0053	0.003	0.004
SC10	n.s.	n.s.	n.d.	n.s.	0.0050	0.003	0.004
TOTAL SC 3+	**n.d.**	**0.0163**	**0.0121**	**0.0067**	**0.0562**	**0.018**	**0.022**
TOTAL DOSE SITE	**0.03**	**0.72**	**0.04**	**0.02**	**0.24**	**0.21**	**0.30**
Receptor fluid (0 - 12h)	0.0030	0.0824	n.d.	0.0896	0.0025	0.04	0.05
Receptor fluid (0 - 24h)	0.0030	0.1088	n.d.	0.1297	0.0025	0.05	0.06
%Ratio receptor 12h/24h	100	76	100	69	100	88.96	15.29
Residual Rec Fluid	n.d.	n.d.	n.d.	n.d.	n.d.	0.00	0.00
Receptor Chamber	n.d.	n.d.	n.d.	n.d.	0.3678	0.07	0.16
TOTAL DIRECT	**0.00**	**0.11**	**0.00**	**0.13**	**0.37**	**0.12**	**0.15**
POTENTIAL (dose site+ receptor)	0.03	0.82	0.04	0.15	0.61	0.33	0.36
POTENTIAL (skin+ receptor)	**0.03**	**0.81**	**0.03**	**0.15**	**0.55**	**0.31**	**0.35**
TOTAL RECOVERY	**102.8**	**103.1**	**106.2**	**106.1**	**108.7**	**105.38**	**2.46**

Evaluation according to EFSA Guidance	
Absorption >75% within half of study duration?	Yes (exclude SC values)
Recovery <95%?	No correction needed
Total % Potentially Absorbable adjusted according to EFSA (2017)	Mean (%skin +%receptor) + (SD*1.2) = **0.73%**

SD: standard deviation

n.d.: below limit of detection; n.s.: no sample; n.a.: not applicable.
In the above table, the presented means do not always calculate exactly from the presented individual data. This is due to rounding-up differences resulting from the use of the spreadsheet program.

Conclusion:

The dermal penetration through human dermatomed skin of [^{14}C]-fluoxastrobin in the FLC+FXA FS 350 formulation was investigated at two nominal concentrations corresponding to the neat product (150 g /L) and to one representative dilution of 37.5 g/L.

Concentrate

The mean percentage of fluoxastrobin in the FS 350 formulation that was considered to be potentially absorbable for the neat formulation applying the EFSA guidance (2017) to the study data was 0.07%.

Low Dose level (Spray dilution)

The mean percentage of fluoxastrobin in the FS 350 formulation that was considered to be potentially absorbable for the representative dilution applying the EFSA guidance (2017) to the study data was 0.73%.

Therefore, the following dermal absorption values can be proposed for use in the non-dietary risk assessments for [^{14}C]-fluoxastrobin in the FLC+FXA FS 350 formulation:

- 0.07% for the neat formulation (150 g/L).
- 0.73% for the low dose (37.5 g/L).

Assessment and conclusion by applicant:
An acceptable study yielding valid conclusions

CP 7.4 Available toxicological data relating to co-formulants

CONFIDENTIAL information – data provided separately (Document JCP).

Appendix 1 Critical GAP for this assessment

Table of supported uses for this renewal.

Crop and/ or situation	Country	F G or I	Pests or Group of pests controlled	Formulation		Application				Application rate per treatment			PHI	Remarks:
				Type	Conc. of a.s.	Method / kind	Timing / Growth stage of crop & season	Number min - max	Interval between applications min	g a.s./100 kg seeds	kg seeds/ha	g a.s./ha	Days	
(a)		(b)	(c)	(d-f)	(i)	(f-h)	(j)	(k)		min - max	min - max	min - max	(l)	(m)
Rape, winter	EU	F	*Plenodomus lingam* (LEPTMA), *Peronospora parasitica* (PEROPA), *Alternaria brassicae* (ALTEBA), *Rhizoctonia spp.* (RHIZSP)	FS	FLC: 200 g/L FXA: 150 g/L	Seed treatment	BBCH 00	1 - 1	n.a.	FLC: 200 FXA: 150	2.5 - 6	FLC: 5 - 12 FXA: 3.75 - 9	n.a.	

FLC: Fluopicolide
FLX: Fluoxastrobin
n.a: not applicable

Appendix 2 Spreadsheets for exposure calculations

2.1 Estimation of operator exposure during seed treatment, fluopicolide 200 L packaging, standard clothing

TASK	Total Potential Dermal Exposure (mg/op)*	Estimated Actual Dermal Exposure (mg/op)*	Inhalation Exposure (mg/op)*	*Frequency of operation ** / day*	*PPE# Use of additional RPE yes/no*	Total Potential Dermal Exposure (mg/day)	Estimated Actual Dermal Exposure (mg/day)	Inhalation Exposure (mg/day)	Inhalation Exposure Worst case (mg/day)
Calibration	6.51	2.85	0.276	*1*	*no*	6.5115	2.8456	0.2762	
Mixing / Loading	1.0384	1.038	0.026	*2*	*no*	2.0769	2.0769	0.0512	
Bagging (mg/hr)	1.84	0.698	0.0054	*8*	*no*	14.7200	5.5840	0.0432	0.4320
Cleaning	174	16.67	3.2	*1*	*no*	174.3514	16.6728	3.2000	
Dermal absorption/Inhaltion absorption									
			Calibration			n/a	3.90%	100%	0%
			Mixing/loading			n/a	0.21%	100%	0%
			Bagging			n/a	0.21%	100%	0%
			Cleaning			n/a	3.90%	100%	0%
Task specific absorbed dose (mg/kg bw/day)									
			Calibration				**0.00185**	**0.00460**	
			Mixing/loading				0.00007	0.00085	
			Bagging				**0.00020**	**0.00072**	
			Cleaning				**0.01084**	**0.05333**	
Total absorbed dose (mg/kg bw/day)							**0.0725**		0.0002

standard clothing of the operators is one layer of work clothing during all tasks and in addition protective gloves except for bagging
* exposure during bagging mg/hour
** frequency during bagging in hours/day

% of AOEL	103.52	*0.07*	mg/kg bw/day
MoS	88	6.4	mg/kg bw/day

Enter variables in red colour

SCENARIO VARIABLES		
Given a.i. concentration =	*200*	g/l
Given dilution factor =	*1*	
Given average bodyweight =	*60*	kg
Given Dermal absorption		
Concentrate	*0.21*	%
Dilution	*3.9*	%
Given Inhalation absorption =	*100*	%

2.2 Estimation of operator exposure during seed treatment, fluopicolide 200 L packaging, standard clothing + RPE during cleaning

TASK	Total Potential Dermal Exposure (mg/op)*	Estimated Actual Dermal Exposure (mg/op)*	Inhalation Exposure (mg/op)*	*Frequency of operation ** / day*	*PPE# Use of additional RPE yes/no*	Total Potential Dermal Exposure (mg/day)	Estimated Actual Dermal Exposure (mg/day)	Inhalation Exposure (mg/day)	Inhalation Exposure Worst case (mg/day)
Calibration	6.51	2.85	0.276	*1*	*no*	6.5115	2.8456	0.2762	
Mixing / Loading	1.0384	1.038	0.026	*2*	*no*	2.0769	2.0769	0.0512	
Bagging (mg/hr)	1.84	0.698	0.0054	*8*	*no*	14.7200	5.5840	0.0432	0.4320
Cleaning	174	16.67	3.2	*1*	*yes*	174.3514	16.6728	0.3200	
Dermal absorption/Inhaltion absorption									
			Calibration			n/a	3.90%	100%	0%
			Mixing/loading			n/a	0.21%	100%	0%
			Bagging			n/a	0.21%	100%	0%
			Cleaning			n/a	3.90%	100%	0%
Task specific absorbed dose (mg/kg bw/day)									
			Calibration				**0.00185**	**0.00460**	
			Mixing/loading				0.00007	0.00085	
			Bagging				**0.00020**	**0.00072**	
			Cleaning				**0.01084**	**0.00533**	
Total absorbed dose (mg/kg bw/day)							**0.0245**		0.0002

standard clothing of the operators is one layer of work clothing during all tasks and in addition protective gloves except for bagging
* exposure during bagging mg/hour
** frequency during bagging in hours/day

% of AOEL	34.95	*0.07*	mg/kg bw/day
MoS	262	6.4	mg/kg bw/day

Enter variables in red colour

SCENARIO VARIABLES		
Given a.i. concentration =	*200*	g/l
Given dilution factor =	*1*	
Given average bodyweight =	*60*	kg
Given Dermal absorption		
Concentrate	*0.21*	%
Dilution	*3.9*	%
Given Inhalation absorption =	*100*	%

2.3 Estimation of operator exposure during loading/sowing of treated seeds, fluopicolide

SOWING SEED			
	Total Potential Dermal exposure (mg/day)	Estimated Actual Dermal exposure (mg/day)	Inhalation exposure (mg/day)
Loading / Sowing (x10 hrs)	14.79	7.33	0.200
Total exposure (mg/kg bw/day)	**0.25**	**0.122**	**0.003**

	Dermal	Inhalation	Total
Systemic Exposure (mg/kg bw/day)	0.00025658	0.003	0.0035899

%AOEL	**5.13**

Route of exposure	**Specific exposure** [mg a.s./hour]		**Working duration per day**		**Exposure result** [mg/person/day]	**Exposure result** [mg/kg bw/day]	**Absorbed dose** [mg/kg bw/day]
Total Dermal	1.479	x	10 hours	=	14.79	0.25	
Actual Dermal	0.733	x	10 hours	=	7.33	0.122	0.00025658
Total Inhalation	0.02	x	10 hours	=	0.200	0.003	0.003
Total systemic exposure							**0.0035899**
% AOEL							**5.13**

2.4 Estimation of operator exposure during seed treatment, Fluoxastrobin 200 L packaging, , standard clothing

TASK	Total Potential Dermal Exposure (mg/op)*	Estimated Actual Dermal Exposure (mg/op)*	Inhalation Exposure (mg/op)*	*Frequency of operation ** / day*	*PPE# Use of additional RPE yes/no*	Total Potential Dermal Exposure (mg/day)	Estimated Actual Dermal Exposure (mg/day)	Inhalation Exposure (mg/day)	Inhalation Exposure Worst case (mg/day)
Calibration	4.88	2.13	0.207	*1*	*no*	4.8836	2.1342	0.2071	
Mixing / Loading	0.7788	0.779	0.019	*2*	*no*	1.5576	1.5576	0.0384	
Bagging (mg/hr)	1.84	0.698	0.0054	*8*	*no*	14.7200	5.5840	0.0432	0.4320
Cleaning	131	12.50	2.4	*1*	*no*	130.7635	12.5046	2.4000	
Dermal absorption/Inhaltion absorption									
			Calibration			n/a	0.73%	100%	0%
			Mixing/loading			n/a	0.07%	100%	0%
			Bagging			n/a	0.07%	100%	0%
			Cleaning			n/a	0.73%	100%	0%
Task specific absorbed dose (mg/kg bw/day)									
			Calibration				**0.00026**	**0.00345**	
			Mixing/loading				0.00002	0.00064	
			Bagging				**0.00007**	**0.00072**	
			Cleaning				**0.00152**	**0.04000**	
Total absorbed dose (mg/kg bw/day)							**0.0467**		0.0001

standard clothing of the operators is one layer of work clothing during all tasks and in addition protective gloves except for bagging
* exposure during bagging mg/hour
** frequency during bagging in hours/day

% of AOEL	155.59	*0.03*	mg/kg bw/day
MoS	137	6.4	mg/kg bw/day

SCENARIO VARIABLES

Given a.i. concentration =	*150*	g/l
Given dilution factor =	*1*	
Given average bodyweight =	*60*	kg
Given Dermal absorption		
Concentrate	*0.07*	%
Dilution	*0.73*	%
Given Inhalation absorption =	*100*	%

Enter variables in red colour

2.5 Estimation of operator exposure during seed treatment, Fluoxastrobin 200 L packaging, , standard clothing + RPE during cleaning

TASK	Total Potential Dermal Exposure (mg/op)*	Estimated Actual Dermal Exposure (mg/op)*	Inhalation Exposure (mg/op)*	*Frequency of operation ** / day*	*PPE# Use of additional RPE yes/no*	Total Potential Dermal Exposure (mg/day)	Estimated Actual Dermal Exposure (mg/day)	Inhalation Exposure (mg/day)	Inhalation Exposure Worst case (mg/day)
Calibration	4.88	2.13	0.207	*1*	*no*	4.8836	2.1342	0.2071	
Mixing / Loading	0.7788	0.779	0.019	*2*	*no*	1.5576	1.5576	0.0384	
Bagging (mg/hr)	1.84	0.698	0.0054	*8*	*no*	14.7200	5.5840	0.0432	0.4320
Cleaning	131	12.50	2.4	*1*	*yes*	130.7635	12.5046	0.2400	
Dermal absorption/Inhaltion absorption									
			Calibration			n/a	0.73%	100%	0%
			Mixing/loading			n/a	0.07%	100%	0%
			Bagging			n/a	0.07%	100%	0%
			Cleaning			n/a	0.73%	100%	0%
Task specific absorbed dose (mg/kg bw/day)									
			Calibration				**0.00026**	**0.00345**	
			Mixing/loading				0.00002	0.00064	
			Bagging				**0.00007**	**0.00072**	
			Cleaning				**0.00152**	**0.00400**	
Total absorbed dose (mg/kg bw/day)							**0.0107**		0.0001

standard clothing of the operators is one layer of work clothing during all tasks and in addition protective gloves except for bagging
* exposure during bagging mg/hour
** frequency during bagging in hours/day

% of AOEL	35.59	*0.03*	mg/kg bw/day
MoS	599	6.4	mg/kg bw/day

SCENARIO VARIABLES

Given a.i. concentration =	*150*	g/l
Given dilution factor =	*1*	
Given average bodyweight =	*60*	kg
Given Dermal absorption		
Concentrate	*0.07*	%
Dilution	*0.73*	%
Given Inhalation absorption =	*100*	%

Enter variables in red colour

2.5 Estimation of operator exposure during loading/sowing of treated seeds, fluopicolide, 200 L packaging

SOWING SEED			
	Total Potential Dermal exposure (mg/day)	Estimated Actual Dermal exposure (mg/day)	Inhalation exposure (mg/day)
Loading / Sowing (x10 hrs)	14.79	7.33	0.200
Total exposure (mg/kg bw/day)	**0.25**	**0.122**	**0.003**

	Dermal	Inhalation	Total
Systemic Exposure (mg/kg bw/day)	0.00008553	0.003	0.0034189

%AOEL	**11.40**

Route of exposure	**Specific exposure** [mg a.s./hour]		**Working duration per day**		**Exposure result** [mg/person/day]	**Exposure result** [mg/kg bw/day]	**Absorbed dose** [mg/kg bw/day]
Total Dermal	1.479	x	10 hours	=	14.79	0.25	
Actual Dermal	0.733	x	10 hours	=	7.33	0.122	0.00008553
Total Inhalation	0.02	x	10 hours	=	0.200	0.003	0.003
Total systemic exposure							**0.0034189**
% AOEL							**11.40**

2.6 Estimation of operator exposure during seed treatment, fluopicolide 25 L packaging, standard clothing

TASK	Total Potential Dermal Exposure (mg/op)*	Estimated Actual Dermal Exposure (mg/op)*	Inhalation Exposure (mg/op)*	*Frequency of operation ** / day*	*PPE# Use of additional RPE yes/no*	Total Potential Dermal Exposure (mg/day)	Estimated Actual Dermal Exposure (mg/day)	Inhalation Exposure (mg/day)	Inhalation Exposure Worst case (mg/day)
Calibration	6.51	2.85	0.276	*1*	*no*	6.5115	2.8456	0.2762	
Mixing / Loading	1.0384	1.038	0.026	*16*	*no*	16.6149	16.6149	0.4096	
Bagging (mg/hr)	1.84	0.698	0.0054	*8*	*no*	14.7200	5.5840	0.0432	0.4320
Cleaning	174	16.67	3.2	*1*	*no*	174.3514	16.6728	3.2000	

Dermal absorption/Inhaltion absorption

Calibration	n/a	3.90%	100%	0%
Mixing/loading	n/a	0.21%	100%	0%
Bagging	n/a	0.21%	100%	0%
Cleaning	n/a	3.90%	100%	0%

Task specific absorbed dose (mg/kg bw/day)

Calibration	**0.00185**	**0.00460**	
Mixing/loading	0.00058	0.00685	
Bagging	**0.00020**	**0.00072**	
Cleaning	**0.01084**	**0.05333**	
Total absorbed dose (mg/kg bw/day)	**0.0789**		0.0002

standard clothing of the operators is one layer of work clothing during all tasks and in addition protective gloves except for bagging

* exposure during bagging mg/hour

** frequency during bagging in hours/day

% of AOEL	112.78	*0.07*	mg/kg bw/day
MoS	81	6.4	mg/kg bw/day

SCENARIO VARIABLES		
Given a.i. concentration =	*200*	g/l
Given dilution factor =	*1*	
Given average bodyweight =	*60*	kg
Given Dermal absorption		
Concentrate	*0.21*	%
Dilution	*3.9*	%
Given Inhalation absorption =	*100*	%

Enter variables in red colour

2.7 Estimation of operator exposure during seed treatment, fluopicolide 25 L packaging, standard clothing + RPE during cleaning

TASK	Total Potential Dermal Exposure (mg/op)*	Estimated Actual Dermal Exposure (mg/op)*	Inhalation Exposure (mg/op)*	*Frequency of operation ** / day*	*PPE# Use of additional RPE yes/no*	Total Potential Dermal Exposure (mg/day)	Estimated Actual Dermal Exposure (mg/day)	Inhalation Exposure (mg/day)	Inhalation Exposure Worst case (mg/day)
Calibration	6.51	2.85	0.276	*1*	*no*	6.5115	2.8456	0.2762	
Mixing / Loading	1.0384	1.038	0.026	*16*	*no*	16.6149	16.6149	0.4096	
Bagging (mg/hr)	1.84	0.698	0.0054	*8*	*no*	14.7200	5.5840	0.0432	0.4320
Cleaning	174	16.67	3.2	*1*	*yes*	174.6514	16.6728	0.3200	
Dermal absorption/Inhaltion absorption									
				Calibration		n/a	3.90%	100%	0%
				Mixing/loading		n/a	0.21%	100%	0%
				Bagging		n/a	0.21%	100%	0%
				Cleaning		n/a	3.90%	100%	0%
Task specific absorbed dose (mg/kg bw/day)									
				Calibration			**0.00185**	**0.00460**	
				Mixing/loading			0.00058	0.00683	
				Bagging			**0.00020**	**0.00072**	
				Cleaning			**0.01084**	**0.00533**	
Total absorbed dose (mg/kg bw/day)							**0.0309**		0.0002

standard clothing of the operators is one layer of work clothing during all tasks and in addition protective gloves except for bagging

* exposure during bagging mg/hour

** frequency during bagging in hours/day

% of AOEL	44.21	*207*	mg/kg bw/day
MoS	20	6.4	mg/kg bw/day

SCENARIO VARIABLES		
Given a.i. concentration =	*200*	g/L
Given dilution factor =	*1*	
Given average bodyweight =	*60*	kg
Given Dermal absorption		
Concentrate	*0.21*	%
Dilution	*3.9*	%
Given Inhalation absorption =	*100*	%

Enter variables in red colour

2.8 Estimation of operator exposure during seed treatment, fluoxastrobin, 25 L packaging, standard clothing

TASK	Total Potential Dermal Exposure (mg/op)*	Estimated Actual Dermal Exposure (mg/op)*	Inhalation Exposure (mg/op)*	*Frequency of operation ** / day*	*PPE# Use of additional RPE yes/no*	Total Potential Dermal Exposure (mg/day)	Estimated Actual Dermal Exposure (mg/day)	Inhalation Exposure (mg/day)	Inhalation Exposure Worst case (mg/day)
Calibration	4.88	2.13	0.207	*1*	*no*	4.8836	2.1342	0.2071	
Mixing / Loading	0.7788	0.779	0.019	*16*	*no*	12.4612	12.4612	0.3072	
Bagging (mg/hr)	1.84	0.698	0.0054	*8*	*no*	14.7200	5.5840	0.0432	0.4320
Cleaning	131	12.50	2.4	*1*	*no*	130.7635	12.5046	2.4000	

Dermal absorption/Inhaltion absorption				
	Calibration	n/a	0.73%	100%	0%
	Mixing/loading	n/a	0.07%	100%	0%
	Bagging	n/a	0.07%	100%	0%
	Cleaning	n/a	0.73%	100%	0%
Task specific absorbed dose (mg/kg bw/day)					
	Calibration		**0.00026**	**0.00345**	
	Mixing/loading		0.00015	0.00512	
	Bagging		**0.00007**	**0.00072**	
	Cleaning		**0.00152**	**0.04000**	
Total absorbed dose (mg/kg bw/day)			**0.0513**		0.0001

standard clothing of the operators is one layer of work clothing during all works and in addition protective gloves except for bagging

* exposure during bagging mg/hour	% of AOEL	170.95	*0.03* mg/kg bw/day
** frequency during bagging in hours/day	MoS	125	6.4 mg/kg bw/day

SCENARIO VARIABLES		
Given a.i. concentration =	*150*	g/l
Given dilution factor =	*1*	
Given average bodyweight =	*60*	kg
Given Dermal absorption		
Concentrate	*0.07*	%
Dilution	*0.73*	%
Given Inhalation absorption =	*100*	%

Enter variables in red colour

2.9 Estimation of operator exposure during seed treatment, fluoxastrobin, 25 L packaging, standard clothing + RPE during cleaning

TASK	Total Potential Dermal Exposure (mg/op)*	Estimated Actual Dermal Exposure (mg/op)*	Inhalation Exposure (mg/op)*	*Frequency of operation ** / day*	*PPE# Use of additional RPE yes/no*	Total Potential Dermal Exposure (mg/day)	Estimated Actual Dermal Exposure (mg/day)	Inhalation Exposure (mg/day)	Inhalation Exposure Worst case (mg/day)
Calibration	4.88	2.13	0.207	*1*	*no*	4.8836	2.1342	0.2071	
Mixing / Loading	0.7788	0.779	0.019	*16*	*no*	12.4612	12.4612	0.3072	
Bagging (mg/hr)	1.84	0.698	0.0054	*8*	*no*	14.7200	5.5840	0.0432	0.4320
Cleaning	131	12.50	2.4	*1*	*yes*	130.7635	12.5046	0.2400	

Dermal absorption/Inhaltion absorption				
	Calibration	n/a	0.73%	100%	0%
	Mixing/loading	n/a	0.07%	100%	0%
	Bagging	n/a	0.07%	100%	0%
	Cleaning	n/a	0.73%	100%	0%
Task specific absorbed dose (mg/kg bw/day)					
	Calibration			**0.00026**	**0.00345**
	Mixing/loading			0.00015	0.00512
	Bagging			**0.00005**	**0.00072**
	Cleaning			**0.00152**	**0.00400**
Total absorbed dose (mg/kg bw/day)				**0.0153**	0.0001

standard clothing of the operators is one layer of work clothing during all works and in addition protective gloves except for bagging

* exposure during bagging mg/hour	% of AOEL	50.95	*0.03* mg/kg bw/day
** frequency during bagging in hours/day	MoS	449	6.4 mg/kg bw/day

SCENARIO VARIABLES		
Given a.i. concentration =	*150*	g/l
Given dilution factor =	*1*	
Given average bodyweight =	*60*	kg
Given Dermal absorption		
Concentrate	*0.07*	%
Dilution	*0.73*	%
Given Inhalation absorption =	*100*	%

Enter variables in red colour