Document Title

Summary of the toxicological studies for Aclonifen + Diflufenican SC 600 (500+100 g/L)

Data Requirement(s)

Regulation (EC) No 1107/2009 & Regulation (EC) 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)

[redacted]

Battelle UK Ltd.

OWNERSHIP STATEMENT

Version history

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

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

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CP 7 TOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

Aclonifen was included in Annex I to Council Directive 91/414/EEC in 2008 (Directive 2008/116/EC, Entry into Force on 01 August 2009).

Diflufenican was included on Annex I of Directive 91/414/EEC on 1 January 2009 under Inclusion Directive 2008/66/EC and implemented under Regulation (EU) No 540/2011. The Annex I Inclusion Directives for Diflufenican (2008/66/EC) provide specific provisions under Part B which need to be considered by the applicant in the preparation of their submission and by the MS prior to granting an authorisation. For the implementation of the uniform principles of Annex VI the conclusions of the review report on Diflufenican and in particular Appendices I and II thereof, as finalised in the Standing Committee on the Food Chain and Animal Health on 14/03/2008 and on 16/06/2000, respectively, shall be taken into account.

The formulation Aclonifen + diflufenican SC 600 (500+100 g/L) (or ACL+DFF SC 600 (500 + 100) G), is a suspension concentrate formulation containing 500 g/L of aclonifen and 100 g/L of diflufenican. This formulation is registered throughout Europe under trade names such as Mateno Duo SC 600 (Product code specification #102000029998). This formulation was not a representative product under the previous dossier submitted for Annex I inclusion.

This present dossier in support of approval renewal includes all the data submitted at the time of the Annex I inclusion, in summaries updated and re-evaluated as necessary to take account of current validity criteria and data requirements.

CP 7.1 Acute toxicity

Aclonifen + diflufenican SC 600 (500+100 g/L) is a Suspension Concentrate (SC) formulation containing 500 g/L of aclonifen and 100 g/L of diflufenican. The *in vivo* studies submitted have been evaluated for registered products throughout Europe. A summary of these the acute toxicity studies including irritancy and skin sensitisation can be found in Table below and the individual study summaries are provided in the subsections CP 7.1 to 7.6.

Summary of Acute toxicity studies with Aclonifen + diflufenican SC 600 (500+100 g/L).

Endpoint	Species (Sex)	Results	Acceptability	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
Acute oral toxicity	Rat (M & F)	LD_{50} > 2000 mg/kg bw	Yes	None	KCP 7.1.1/01 M-557590-01-1 [redacted], 2016
Acute dermal toxicity	Rat (M & F)	LD_{50} > 2000 mg/kg bw	Yes	None	KCP 7.1.2/01 M-555502-01-1 Tarcai, 2016
Acute inhalation toxicity	Rat (M & F)	LC_{50} > 2.44 mg/L air	Yes	None	KCP 7.1.3/01 M-563930-01-1 [redacted], 2016
Acute skin irritation	*In vitro* assay-human skin	Not irritant	Yes	None	KCP 7.1.4/01 M-556102-01-1 [redacted], 2016

Acute eye irritation	*In vitro* assay-chicken eyes	Not irritant	Yes	None	KCP 7.1.5/01 M-554098-01-1 [redacted], 2016
Skin sensitization	Mouse (F) Local Lymph Node Assay	Not sensitising	No	Skin Sens. 1A, H317	KCP 7.1.6/01 M-558669-01-1 [redacted], 2016

Overall, Aclonifen + Diflufenican SC 600 (500+100 g/L) was tested in standard *in vivo* studies for oral, dermal and inhalation toxicity. The LD_{50} was greater than 2000 mg/kg bw for oral and dermal toxicity. For inhalation toxicity the LC_{50} was greater than 2.44 mg/L. (The target concentration of 5 mg/L was not tested in the acute inhalation toxicity study.) The product is not classified based on the result from the acute inhalation toxicity study. Skin and eye irritation were tested in *in vitro* assays. The assays can provide evidence on no classification and as they were negative for skin and eye irritation, respectively, the product is not considered to have irritant properties. Skin sensitisation was tested in a LLNA. Due to the limited reliability of the skin sensitisation study an alternative approach under Regulation (EC) No 1272/2008 is applied. The product contains a.s. aclonifen in amounts (40.65% w/w) significantly exceeding the generic concentration limit (≥ 0.1%) of triggering classification of a mixture as skin sensitiser.

The active substance aclonifen has a harmonised classified with Skin Sens. 1A, H317 and Carc 2., H351. This classification is transferred to the product.

CP 7.1.1 Oral toxicity

Data Point:	KCP 7.1.1/01
Report Author:	[redacted]
Report Year:	2016
Report Title:	Aclonifen + diflufenican SC 600,000 (500,000+100,000 g/L): Acute oral toxicity study in male and female rats (up and down procedure)
Report No:	16/056-001P
Document No:	M-557590-01-1
Guideline(s) followed in study:	OECD Guidelines for testing of chemicals (no.: 425, adopted October 2008) Commission Regulation (EC) no 440/2008 of 30 May 2008, B.1.TRIS EPA Health Effects Test Guidelines (OPPTS 870.1100), United States, EPA 712-C-02-190, December 2002)
Deviations from current test guideline:	Current Guideline: OECD 425, 2008 No significant deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

An acute oral toxicity study was conducted on rats using the Up and down Procedure. The product ACL+DFF SC 600,000 g/L was administered as aqueous suspension by oral gavage to CRL: (WI) rats. Doses were calculated according to the OECD Test Guideline 425. A preliminary dose started at 550 mg/kg bw (one animal per sex) and as both animals survived, a limit dose of 2000 mg/kg bw was then performed (3 animals/sex). All doses were followed by a 14-day observation period.

All animals were observed individually after dosing at 30 min, 1, 2, 3, 4 and 6 hours post-treatment and once daily for 14 days thereafter. Body weight was measured on Day-1 (prior removal of the food), Day 0 (prior administration) and weekly thereafter. All animals were examined macroscopically at the end of the observation period.

No mortality was observed at any dose levels, up to and including 2000 mg/kg bw. No effects were observed on body weights or body weights gains in any animal during the study.

Based on the above results, the median lethal dose (LD_{50}) and the oral LD50 of ACL+DFF SC 6000,000 g/L was found to be greater than 2000 mg/kg bw in male and female rats. Therefore, this product is not classified for oral toxicity according to Regulation (EC) 1272/2008.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test materials: ACL+DFF SC 600,000 g/L
specification 102000029998

Description:	Yellow suspension
Lot/Batch:	Batch No. 2016-000027
Purity:	Aclonifen (AE F068300) 40.7% w/w Diflufenican (AE F088657) 8.59% w/w
Stability of test compound:	Shown to be stable (see MCP2)

2. Vehicle: Distilled water

3. Test animals:

Species:	Rat
Strain:	Wistar (CRL:WI)
Age:	Young adult rats, 8-11 weeks old
Weight at dosing:	198-344 g
Source:	[redacted]
Acclimatisation period:	One week (at least)
Diet:	Diet for rats and mice, ssniff®, SM R/M, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individual caging, material type II. Polypropylene/polycarbonate. Housed with deep wood sawdust bedding to allow digging and other normal rodent activities

4. Environmental conditions:

Temperature:	19.0°C ± 22.4°C
Humidity:	30-66 % relative humidity
Air changes:	12-15 times/hour

Photoperiod: Day-night rhythm 12 hours (7 a.m.- 7 p.m.)

B. STUDY DESIGN AND METHODS

1. In life dates: 16 March 2016 to 21 June 2016

2. Animal assignment and treatment

A single oral (gavage) administration was followed by a 14-day observation period. The animals were fasted overnight prior to treatment. Water was still available ad libitum overnight. Animals were weighed before dosing and food was returned 3 hours after treatment.

Initially one male animal was dosed at the study limit dose. Single animals were dosed sequentially following a surviving interval of at least approximately 48 hours. When the outcome for each animal was established, then the next individual animal was treated at the next appropriate dose, higher or lower than the previous dose depending on outcome, or at the limit dose of 2000 mg/kg bw.

Initially dose level of females was the limit dose of the study. All animals were observed for clinical signs of toxicity immediately after treatment and once daily in the early morning up to the end of the 2 weeks observation period.

All animals were observed individually after dosing at 30 minutes, then 1, 2, 3, 4, and 6 hours and once daily for 14 days thereafter. Individual observations were performed on the skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behavior pattern.

Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. The body weights of the animals were recorded on Day -1 and Days 0 (before treatment), 7 and 14.

3. Statistics

Group means, and standard deviations of bodyweights were calculated.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred as given in the Table below.

Table 7.1.1-1 ACL+DFF SC 600,000 g/L - Acute oral toxicity study in rats – mortality and clinical signs

	Dose (mg/kg bw)	Number animals*	Duration of signals	LD50 (mg/kg bw) (14 days)
Males	550	0/1/1	N/A	> 500
Females	550	0/1/1	N/A	
Males	2000	0/3/3	N/A	> 2000
Females	2000		N/A	

		0/3/3		
* Number of animals which died/number of animals with clinical signs/number of animals used.				

B. CLINICAL OBSERVATIONS

No overt signs of clinical toxicity were observed.

C. BODYWEIGHT

Body weight gain was unaffected by treatment (see table below).

Table 7.1.1-2 ACL+DFF SC 600,000 g/L - Acute oral toxicity study in rats – body weight

Dosage [g/kg bw]	Sex	Body weight (g) Days				Body Weight Gain (g)			
		-1	0	7	14	-1-0	0-7	7-14	-1-14
550	M	337	317	385	430	-20	68	45	93
	F	211	198	236	241	-13	38	5	30
2000	M	352	321	381	420	-31	60	39	68
	M	368	344	395	428	-24	51	33	60
	M	352	324	363	397	-28	39	34	45
	Mean± SD	**357.3 ± 9.2**	**329.7 ± 12.5**	**379.7 ±16.0**	**415.0 ±16.1**	**-27.7 ±3.5**	**50.0 ±10.5**	**35.3 ±3.2**	**57.7 ±11.7**
	F	221	209	233	246	-12	24	13	25
	F	223	215	235	250	-8	20	15	27
	F	218	209	233	245	-9	24	12	27
	Mean± SD	**220.7± 2.5**	**211.0± 3.5**	**233.7 ± 1.2**	**247.0± 2.6**	**-9.7 ± 2.1**	**22.7 ± 2.3**	**13.3± 1.5**	**26.3± 1.2**

D. NECROPSY

No adverse findings.

E. DEFICIENCIES

None.

III. CONCLUSIONS

The oral LD_{50} of ACL+DFF SC 600,000 g/L was to be greater than 2000 mg/kg bw in male and female CRL: (WI) rats. ACL+DFF SC 6000,000 g/L is therefore not classified as harmful by ingestion according to Regulation (EC) 1272/2008.

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

The oral LD50 of ACL+DFF SC 6000,000 g/L was to be greater than 2000 mg/kg bw in male and female CRL: (WI) rats.

Assessment and conclusion by RMS:

CP 7.1.2 Dermal toxicity

Data Point:	KCP 7.1.2/01
Report Author:	[redacted]
Report Year:	2016
Report Title:	Aclonifen + diflufenican SC 600,000 (500,000+100,000 g/L) - Acute dermal toxicity study in rats
Report No:	16056-002P
Document No:	M-555502-01-1
Guideline(s) followed in study:	OECD 402 (1987); EPA OPPTS 870.1200 (1998); EC 440/2008 (2008)
Deviations from current test guideline:	Current guideline: OECD 402, 2017 No deviation
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 5 male and 5 female CRL:(WI) rats were exposed by the dermal route to CL+DFF SC 600,000 (500,000+100,000) g/L at a limit dose of 2000 mg/kg bw. The animals were systematically observed daily during the 14 days observed period.

No mortality occurred. Overt clinical signs of deleterious effects related to treatment were not observed. Only the skin of the application area showed colourisation due to the colour of the test substance. No treatment-related changes were observed at autopsy and no effects were observed on body weights or body weight gains in any animal during the study.

Based on the above results, the median lethal dose (LD_{50}) of CL+DFF SC 600,000 (500,000+100,000) g/L after a single dermal application was > 2000 mg/kg bw in male and female rats. CL+DFF SC 600,000 (500,000+100,000) is therefore not classified for dermal toxicity under Regulation (EC) 1272/2008.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test materials: ACL+DFF SC 600,000 g/L
specification 102000029998

Description:	Yellow suspension
Lot/Batch:	Batch No. 2016-000027
Purity:	Aclonifen (AE F068300) 40.7% w/w
	Diflufenican (AE F088657) 8.59% w/w
Stability of test compound:	Shown to be stable (see MCP2)

2. Vehicle: None

3. Test animals:

Species:	Rat
Strain:	Wistar (CRL:WI)
Age:	Data not provided
Weight at dosing:	221 g – 273 g
Source:	[redacted]
Acclimatisation period:	One week (at least)
Diet:	Diet for rats and mice, ssniff®, SM R/M, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individual caging, material type II. Polypropylene/polycarbonate. Housed with deep wood sawdust bedding to allow digging and other normal rodent activities

4. Environmental conditions:

Temperature:	20°C ± 2/3°C
Humidity:	28-58% relative humidity
Air changes:	15-20 times/hour
Photoperiod:	Day-night rhythm 12 hours (7 a.m.- 7 p.m.)

B. STUDY DESIGN AND METHODS

1. In life dates: 16 March 2016 to 13 May 2016

2. Animal assignment and treatment

A single administration was performed by the dermal route and was followed by a 14-day observation period. The test item was applied as supplied. A limit dose of 2000 mg/kg bw was chosen by the Sponsor in consultation with the Study Director.

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.

On test day 0, the test item was applied as a single dose of 2000 mg/kg bw, applied uniformly over the skin by use of a gauze pad (ca. 5 cm x 5 cm), and remained on the skin throughout a 24-hour exposure period. Sterile gauze pads were placed on the skin of rats at the site of application. These gauze pads were kept in contact with the skin by a patch with adhesive hypoallergenic plaster. The entire trunk of the animal was then wrapped with semi occlusive plastic wrap for 24 hours. At the end of the exposure period, residual test item was removed, using water at body temperature.

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 respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behavior pattern. Particular attention was directed to the observation of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.

Adverse skin reactions at the site of application were recorded daily following the removal of the dressing according to the scheme shown below. The body weight of all animals was recorded on Day 0 (beginning of the experiment) and on Days 7 and 14.

Grading of Skin Reactions

ERYTHEMA AND ESCHAR FORMATION	
No erythema	0
Very slight erythema	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) or eschar formation (injuries in depth preventing erythema) reading	4
OEDEMA FORMATION	
No oedema ..	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)..	2
Moderate oedema (edges raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond the area of exposure) .	4

3. Statistics

Group means, and standard deviations of bodyweights were calculated.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred during the study.

B. CLINICAL OBSERVATIONS

No clinical signs related to the treatment were observed. Only the test item coloured the skin in all animals which could be observed up to 3 days. The colourisation did not influence the observations.

Table 7.1.2-1 ACL+DFF SC 600,000 g/L - 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.				

C. BODYWEIGHT

The body weight gain has not been influenced by the treatment. The changes in the body weight in the main study is given in the Table below.

Table 7.1.2-2 ACL+DFF SC 600,000 g/L – acute dermal toxicity in rats – body weight

Dosage [g/kg bw]	Sex	Initial weight [g]	Weight after		Weight change [g] over 14 days
			One week [g]	Two weeks [g]	
2000	M	257.6 ± 12.5	295 ± 12.9	332.6 ± 15.6	75.0 ± 8.7
	F	230.4 ± 11.0	248.4 ±7.9	262.4 ± 13.6	32.0 ± 6.0
expressed as mean ± standard deviation					

D. NECROPSY

No test-item related observations at a dose level of 2000 mg/kg bw were seen at necropsy.

E. DEFICIENCIES

None.

III. CONCLUSIONS

The acute dermal LD_{50} of ACL+DFF SC 600,000 g/L was determined to be greater than 2000 mg/kg bw. ACL+DFF SC 600,00 is therefore not classified for dermal toxicity under Regulation (EC) 1272/2008.

> Assessment and conclusion by applicant:
>
> All validity criteria were satisfied and therefore this study can be considered to be valid.
>
> The acute dermal LD50 of CL+DFF SC 600,000 g/L was determined to be greater than 2000 mg/kg bw.

> Assessment and conclusion by RMS:

CP 7.1.3 Inhalation toxicity

Data Point:	KCP 7.1.3/01
Report Author:	[redacted]
Report Year:	2016
Report Title:	Amended final report no. 1 - Aclonifen + diflufenican SC 600,000 (500,000+100,000 g/L) - Acute inhalation toxicity study (Nose-only) in the rat
Report No:	16/056-004P
Document No:	M-563930-01-1
Guideline(s) followed in study:	OECD Test Guideline 403 (2009) EPA OPPTS 870.1300 (1998) EC 440/2008, Annex Part B, B.2 (2008)
Deviations from current test guideline:	Current guideline: OECD 203 2009 No deviation
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, Wistar Crl:WI rats were exposed to a test atmosphere of ACL+DFF SC 600,000 (500,000+100,000) g/L at the maximum feasible concentration. The study was performed in two steps. A sighting exposure was performed first, where a test atmosphere at 2.53 mg/L concentration was tested on single animals of both sexes (Group 0.1). No lethality was observed at this concentration. Thereafter, the main study group of 5 male and 5 female CRL: (WI) Wistar strain rats were exposed to an aerosol atmosphere of ACL+DFF SC 600,000 (500,000+100,000) g/L at the maximum feasible concentration (2.44 mg/L). The MMAD was 3.94 and 50.4% particles were less than 4µm (inhalable fraction).

In all study phases, the animals were exposed to the test atmosphere for 4 hours using a nose-only exposure system. Aerosol concentration was measured gravimetrically 17 times during each 4-hour exposure in both parts of the study and the particle size distribution of the test aerosol was determined 3 times. The day of exposure was designated as Day 0 followed by a 14-day observation period.

No mortality occurred in Group 1 (main study) when exposed to a test atmosphere concentration of 2.44 mg/L for 4 hours. The acute inhalation median lethal concentration (LC50) of ACL+DFF SC 600,000 (500,000+100,000) g/L in Wistar Crl:WI rats was therefore considered to be above 2.44 mg/L. The product is therefore not classified as harmful by inhalation according to Regulation (EC) 1272/2008.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test materials: ACL+DFF SC 600,000 g/L
specification 10200029998

Description:	Yellow suspension
Lot/Batch:	Batch No. 2016-000027
Purity:	Aclonifen (AE F068300) 40.7% w/w
	Diflufenican (AE F088657) 8.59% w/w
Stability of test compound:	Shown to be stable (see MCP2)

2. Vehicle: Distilled water

3. Test animals:

Species:	Rat
Strain:	Wistar (CRL:WI)
Age:	8-9 weeks old
Weight at dosing:	327-355 g (male), 205-217g (female)
Source:	[redacted]
Acclimatisation period:	One week (at least)
Diet:	Diet for rats and mice, ssniff®, SM R/M, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Grouping caging (5 animals/sex/cage), material type I and III. With stainless steel mesh lids

4. Environmental conditions:

Temperature:	19.2°C ± 25.9°C
Humidity:	34-76% relative humidity
Air changes:	At least 15 times/hour

Photoperiod: Day-night rhythm 12 hours (7 a.m.- 7 p.m.)

B. STUDY DESIGN AND METHODS

1. In life dates: 04 May 2016 to 17 August 2016

2. Animal assignment and treatment

Due to the viscosity of the test item as supplied, suitable aerosol atmospheres of the undiluted test item could not be produced therefore the formulation was diluted in distilled water (70:30 test item:water).

Prior to atmosphere generation, the non-volatile component of the test material was determined by adding a known amount of the material to glass fiber filters (Type GF/C, Whatman™, GE Healthcare UK Ltd., Cat No: 1822-025). The filters were then dried, at atmospheric pressure, in a dessicator at room temperature for approximately 24 hours and weighed again. The difference in the two weights was taken as the volatile content of the test material and the non-volatile component was calculated as a percentage. The mean non-volatile content of the batch used for the animals' exposure was found to be 62.42% (n = 10) with a standard deviation of 0.38 %.

The test material atmospheres were generated within the exposure chamber. During these technical trials, air-flow settings, test material input rates and test item formulation concentrations (50 and 70%) were varied to achieve the required aerosol concentration of particles with a mass median aerodynamic diameter (MMAD) between 1 to 4 µm and a geometric standard deviation (GSD) in the range of 1.5 to 3.0. Measurements of aerodynamic particle size were performed from the animal's breathing zone using a cascade impactor.

The animals were exposed, nose-only, to an atmosphere of the test item using a TSE Rodent Exposure System ([redacted]). Each rat was individually held in a tapered, polycarbonate restraining tube fitted onto a single tier of the exposure chamber. Only the nose of each animal was exposed to the test atmosphere. Following an equilibration period of at least the theoretical chamber equilibration time (T99), each group of rats was exposed to an atmosphere of the test material for a period of 4 hours. The flow of air was at least 0.5 L/min. This flow rate was considered adequate to minimize re-breathing of the test atmosphere as it is approximately twice the respiratory minute volume of a rat. No control animals were used in the study.

The particle size of the test atmosphere was determined three times during the exposure period using a 7-stage impactor of Mercer style ([redacted]. Animals were checked hourly during exposure, 1 hour after exposure and twice daily (early and late in the working day) during the 14-day observation period for morbidity and/or mortality. All animals were observed for clinical signs at hourly intervals during exposure whilst the animals were still restrained. Following exposure, clinical observations were performed twice on the day of exposure (following removal from the restrainer and approximately one hour after completion of the exposure) and subsequently once daily for 14 days.

Observations included changes in the skin and fur, eyes and mucous membranes and respiratory, circulatory, autonomic and central nervous system, somato-motor activity and behaviour pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Individual body weights were recorded prior to treatment on the day of exposure (on Day 0) and on Days 1, 3, 7 and 14. At the end of the 14-day observation period, the surviving animals were sacrificed.

II. RESULTS AND DISCUSSION

Table 7.1.3-01 ACL+DFF SC 600,000 g/L – acute inhalation study - exposure conditions

Target conc. (mg/L air)	Nominal conc. (mg/L air)	Actual conc. (mg/L air)	MMAD * (µm)	GSD ** (µm)
Maximum feasible	89.71	2.44	3.95	2.04

* MMAD = Mass Median Aerodynamic Diameter
** GSD = Geometric Standard Deviation

A. MORTALITY

No deaths occurred during the study.

B. CLINICAL OBSERVATIONS

On Day 1, all males and females showed signs of slight laboured respiration and sneezing was recorded in 2 males. No further clinical signs were recorded. Also, wet fur or fur staining (as chromodacryorrhea) was commonly recorded on the day of the exposure and several days after exposure which were considered to be related to the restraint and exposure procedures but not to be toxicologically significant.

Table 7.1.3-02 ACL+DFF SC 600,000 g/L - acute inhalation 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

C. BODYWEIGHT

Slight bodyweight loss (2.6 and 1.4 %) was noted in one male and one female animal on Day 0-3, respectively and slight bodyweight loss (1.4%) in one female animal on Day 0-7. The bodyweight gain returned to the normal range, thereafter. The changes in the body weight in the main study is given in the Table below.

Table 7.1.3-03 ACL+DFF SC 600,000 g/L - acute inhalation study in rats - body weight

Dosage	Sex		Weight after	

[mg/L]		Initial weight [g]	One week [g]	Two weeks[g]	Weight gain[g] over 14 days
2.44	M	341.0 ± 11.4	362.0 ± 11.2	398.6 ± 13.9	57.6 ± 7.2
	F	211.8 ± 5.0	223.6 ± 9.1	236.2 ± 7.6	24.4 ± 8.1
Mean ± standard deviation					

D. NECROPSY

No adverse effects were seen at necropsy.

E. DEFICIENCIES

The temperature and the relative humidity were out of the target range of 20 Â± 3Â°C and 30-70%. The actual ranges of humidity and temperature were between 19.2 -25.9°C and 34-76 %, respectively. The draft report was issued later than it was indicated in the study plan. The target concentration of 5 mg/L was not tested in the acute inhalation toxicity study. The maximal attainable concentration was 2,44 mg/L in the Acute inhalation toxicity study. Those deviations had no effect on the purpose and integrity of the study.

III. CONCLUSIONS

Under the experimental conditions, the inhalation LC50 of ACL+DFF SC 600,000 g/L is higher than 2.44 mg/L air in rats. Based upon the minimal clinical signs at the maximal attainable concentration, it is considered that there is no data to support an MLC of less than 5 mg/L. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid. None of the active substances are classified for inhalation toxicity. A single co-formulant is classified H332 and another co-formulant is classified H330, both co-formulants are present in an amount that does not contribute to classification of the product. No classification warranted.

Under the experimental conditions, the inhalation LC50 of ACL+DFF SC 600,000 g/L is higher than 2.44 mg/L air in rats.

Assessment and conclusion by RMS:

CP 7.1.4 Skin irritation

Data Point:	KCP 7.1.4/01
Report Author:	[redacted]
Report Year:	2016
Report Title:	Aclonifen + diflufenican SC 600,000 (500,000 + 100,000 g/L) - In vitro skin irritation test in the Episkin (SM) model
Report No:	16/056-043B
Document No:	M-556102-01-1
Guideline(s) followed in study:	OECD Guidelines for Testing of Chemicals No. 439 (28 July 2015) Commission Regulation (EC) No 761/2009, ANNEX III, B.46. EpiSkin SOP, Version 1.8 (February 2009)
Deviations from current test guideline:	Current guideline: OECD 439, 2019 No deviation
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

An *in vitro* skin irritation test of Aclonifen + Diflufenican SC 600,000 (500,000 + 100,000 g/L) test item was performed in a reconstructed human epidermis model. EPISKIN™(SM) is designed to predict and classify the irritation potential of chemicals by measuring its cytotoxic effect as reflected in the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. 20 µL of test item was applied evenly to the epidermal surface. Exposure was terminated by rinsing with Phosphate Buffered Saline (PBS). The epidermis units were then incubated at 37°C for 42 hours in an incubator with 5% CO2. The viability of each disk was assessed by incubating the tissues for 3 hours with MTT solution at 37°C in an incubator with 5% CO2 protected from light. The precipitated formazan crystals were then extracted using acidified isopropanol and quantified spectrophotometrically. Negative and positive controls were also conducted.

Following exposure with Aclonifen + Diflufenican SC 600,000 (500,000 + 100,000 g/L), the mean relative viability was 117.0% compared to the negative control value. This is above the threshold of 50%, therefore the test item was considered as being non-irritant to skin. The experiment met the validity criteria, therefore the study was considered to be valid.

In conclusion, in this in vitro EPISKIN model test with Aclonifen + Diflufenican SC 600,000 (500,000 + 100,000 g/L), the results indicate that the test item is non-irritant to skin. Therefore, Aclonifen + Diflufenican SC 600,000 (500,000 + 100,000 g/L) is not classified as a skin irritant under Regulation (EC) 1272/2008.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test materials: ACL+DFF SC 600,000 g/L

specification 102000029998

Description: Yellow suspension

Lot/Batch:	Batch No. 2016-000027
Purity:	Considered as 100% aclonifen (AE F068300) 40.7 % w/w – 501.9 g/L diflufenican (AE F088657) 8.59 % w/w – 105.8 g/L
Stability of test compound:	Shown to be stable (see MCP2)
2. Vehicle:	Distilled water (for the preparation of the positive control)
3. Test skin:	
Test system:	EPISKIN™ (SM) – a three-dimensional human epidermis model derived from human epidermal keratinocytes The commercial kit contains: -12 reconstructed epidermis units, 12-well assay plate, EPISKIN™ (SM) biopsy punch for easy sampling of epidermis, a flask of sterile "Maintenance Medium"
Model Test Species:	Human epidermis
Source	[redacted]
4. Testing conditions:	
Temperature:	37°C
CO_2	5%
5. Quality control	MTT cell viability assay conducted at SkinEthic laboratories

B. STUDY DESIGN AND METHODS

1. In life dates: 30 March 2016 to 31 May 2016

2. Test assignment and treatment

Disks of EPISKINTM(SM) (three units) were treated with the test item and incubated for 15 minutes at room temperature. Exposure of the test item was terminated by rinsing with Phosphate Buffered Saline (PBS). The epidermis units were then incubated at 37°C for 42 hours in an incubator with 5% CO2. The viability of each disk was assessed by incubating the tissues for 3 hours with MTT solution at 37°C in an incubator with 5% CO2 protected from light. The precipitated formazan crystals were then extracted using acidified isopropanol and quantified spectrophotometrically. PBS and 5% (w/v) Sodium Dodecyl Sulphate (SDS) solution treated epidermis were used as negative and positive controls, respectively (three units / control). Two additional disks were used to provide an estimate of colour contribution from the test item. For each treated tissue, the viability was expressed as a % relative to the negative control. If the mean relative viability after 15 minutes exposure and 42 hours post incubation is less or equal (≤) to 50% of the negative control, the test item is considered to be irritant to skin.

The OD (optical density or absorbance) of the samples was measured using a plate reader at 570 nm. The mean of 6 wells of acidified isopropanol solution (200 µL/well) was used as blank. The proper status of the instrument was verified by measuring a Verification plate (Manufacturer: Thermo Fisher Scientific, Catalogue Number: 240 72800, Serial Number: 0920-14, Date of calibration: 02

September 2014, calibration is valid until September 2016) at the required wavelength on each day before use.

Validity of the Test

The mean OD value of the three negative control tissues should be between 0.6 and 1.5, and the standard deviation value (SD) of the % viability values should be ≤ 18.
The acceptable mean percentage viability range for positive controls is 0-40% and the standard deviation value (SD) of the % viability values should be ≤ 18.
The SD calculated from individual % tissue viability values of the three-test item treated replicates should be <18.

Interpretation of results

The irritation potential of test substances can be classified according to the United Nations Globally Harmonized System of Classification and Labelling of Chemicals and the similar CLP system. In the present study, the irritancy potential of the test substance is predicted by the mean tissue viability of tissues exposed to the test item. The test item is classed irritant to skin (Category 2), if the mean relative viability after 15 minutes exposure and 42 hours post incubation is less or equal equal (≤) to 50% of the negative control.

The mean OD value of the blank samples (acidified isopropanol) should be <0.1.

The prediction model (PM) is described below:

Criteria for in vitro interpretation	UN GHS classification
% mean tissue viability ≤ 50 %	Cat 2 or Cat 1
% mean tissue viability > 50 %	Non- Irritant

*Note: If there is clear evidence that the test item is not corrosive, then it can be determined as No Category according to the UN GHS. It is plausible that some weaker corrosives could be classified as non-irritant in this *in vitro* assay.

II. RESULTS AND DISCUSSION

A. FINDINGS

To test the validity of the test kit the two indicators of the delivered kit were checked. Based on the observed colours, the epidermis units were in proper conditions. The mean OD value of the three negative control tissues was in the recommended range (0.750). Standard deviation of the viability results for negative control samples was 6.1.

As no colour change was observed after 3 hours incubation in MTT the test material did not react with MTT. Therefore an estimation of viability was not required. As the test item is coloured two additional test item treated tissues were used for the non-specific OD evaluation. The OD at 570 nm was 0.011. non-specific % colour was calculated as 1.5%. This value was below 5% therefore additional data calculation was not necessary.

The results of the optical density (OD) measured at 570 nm of each sample and the calculated relative viability % values are presented in table below. The mean OD values for the test item treated skin samples showed 117.0% relative viability.

The positive control showed 4.9% viability demonstrating proper performance of the assay. The standard deviation of the viability results for positive control samples was 0.9. The standard deviation of viability values of the three test item-treated tissue samples in the MTT assay was 17.8. The mean OD value of the blank samples (acidified isopropanol) was 0.048. All these parameters met the acceptability criteria, therefore the study was considered to be valid.

Table 7.1.4-01 ACL+DFF SC 600,000 g/L – skin irritation as measured by optical density (OD) and percent relative viability

Substance		Measured	Blank corrected	Viability% RV
Negative control (phosphate buffered saline)	1	0.834	0.786	104.7
	2	0.747	0.699	93.2
	3	0.815	0.767	102.2
	mean		**0.750**	**100.0**
Positive control (5% w/v SDS solution)	1	0.079	0.031	4.1
	2	0.083	0.035	4.7
	3	0.092	0.044	5.9
	mean		**0.037**	**4.9**
Test item (ACL + DFF SC 600,000)	1	1.021	0.973	129.6
	2	0.984	0.936	124.7
	3	0.773	0.725	96.6
	mean		**0.878**	**117.0**

Notes:
1. Mean blank value was 0.048.
2. Optical density means the mean value of the duplicate wells for each sample (rounded to three decimal places).

C. DEFICIENCIES

Due to the unscheduled delay of reporting, the Draft Report was issued later than indicated in the Study Plan. However, this fact was considered not to adversely affect the results or integrity of the study.

III. CONCLUSIONS

Following exposure with Aclonifen + Diflufenican SC 600,000 (500,000 + 100,000 g/L), the mean relative viability was 117.0% compared to the negative control value. This is above the threshold of 50%, therefore the test item was considered as being non-irritant to skin. The experiment met the validity criteria and therefore the study was considered to be valid. Therefore, Aclonifen + Diflufenican SC 600,000 (500,000 + 100,000 g/L) is not classified as a skin irritant under Regulation (EC) 1272/2008.

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid. The study is appropriate to provide evidence of no classification. The viability was not diminished to less than 50% and as such the product is not a skin irritant. The product is outside category. No classification is warranted.

Assessment and conclusion by RMS:

CP 7.1.5 Eye irritation

Data Point:	KCP 7.1.5/01
Report Author:	[redacted]
Report Year:	2016
Report Title:	Aclonifen+diflufenican SC 600,000 (500,000+100,000 g/L) In vitro eye irritation test in isolated chicken eyes
Report No:	16/056-038CS
Document No:	M-554098-01-1
Guideline(s) followed in study:	OECD Guidelines for Testing of Chemicals 438 (26 July 2013); EU Commission Regulation (EC) No 1272/2008 (16 December 2008); EU Commission Regulation (EC) No 1152/2010 (08 December 2010) amending Regulation (EC) No 440/2008 Method B 48
Deviations from current test guideline:	Current guideline: OECD 438, 2018 No deviation
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The Enucleated Eye Test with isolated eyes of chickens is a well validated and accepted *in vitro* test system. It has been recognised as a valuable alternative to the Draize eye irritation test, because it represents a test system nearest to the *in vivo* test, without the need to use live animals.

The test item Aclonifen+Diflufenican SC 600,000 (500,000+100,000 g/L) was tested in isolated chicken's eyes. Three eyes were treated with 30 µL test item. The three positive control eyes were treated in a similar way with 30 µL benzalkonium chloride solution 5 % (w/v). The negative control eye was treated with 30 µL of physiological saline (0.9% NaCl solution). Corneal thickness, corneal opacity and fluorescein retention were measured and any morphological effects (e.g. pitting or loosening of the epithelium) evaluated.

No significant corneal swelling was observed during the four-hour observation period on all test item treated eyes. Very slight corneal opacity change (severity 0.5) was noted on all three eyes. No fluorescein retention change was noted on three eyes. No other corneal effect was observed.

Based on this *in vitro* eye irritation test in isolated chicken eyes, the test item Aclonifen+Diflufenican SC 600,000 (500,000+100,000 g/L) is non-irritant.

In conclusion, Aclonifen+Diflufenican SC 600,000 (500,000+100,000 g/L) is not classified as an eye irritant under Regulation (EC) 1272/2008.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test materials: ACL+DFF SC 600,000 g/L
specification 102000029998

Description:	Yellow Suspension
Lot/Batch:	2016-000027
Purity:	Considered as 100%
	aclonifen (AE F068300) 40.7% w/w – 504.9 g/L
	diflufenican (AE F088657) 8.59% w/w- 105.8 g/L
Stability of test compound:	Shown to be stable (see MCP2)
Positive control:	Benzalkonium chloride solution 50% in water
Negative control:	Physiological saline (0.9% w/v NaCl)

2. Vehicle: Distilled water (for the preparation of the positive control)

3. Test animals:

Species:	Chicken
Strain:	COBB 500
Age:	7 weeks old
Weight at dosing:	2.77 kg ± 3.9 %
Source:	[redacted]
Acclimatisation period:	2 hours
Diet:	n/a
Water:	Tap water, *ad libitum*
Housing:	Plastic box (4-5 heads per box)

4. Environmental conditions:

Temperature:	18°C ± 2°C
Humidity:	50% relative humidity
Air changes:	12-15 times/hour
Photoperiod:	Day-night rhythm 12 hours (7 a.m.- 7 p.m.)

B. STUDY DESIGN AND METHODS

1. In life dates: 07 March 2016 to 21 April 2016

2. Animal assignment and treatment

Preparation of the eyes

The eyeball was carefully removed from the orbit avoiding pressure on the eyeball, in order to prevent distortion of the cornea and subsequent corneal opacity. The nictitating membrane was cut away with other connective tissue. The prepared eyes were then placed in a steel clamp with the cornea positioned vertically and transferred to a chamber of the superfusion apparatus. The clamp holding the eye was positioned in such a way that the entire cornea was 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. The door of the chamber was closed except for manipulations and examinations, to maintain temperature and humidity. The eyes were examined to ensure they were in good condition.

Eyes with a high baseline fluorescein staining (i.e., > 0.3) or corneal opacity score (i.e., > 0.5) were rejected. The cornea thickness was also measured at the corneal apex using the depth measuring device on the slit-lamp microscope. Any eye with cornea thickness deviating more than 10% from the mean value for all eyes, or any eye that showed other signs of damage were rejected. If the selected eyes were appropriate for the test, acclimatization started and conducted for approximately 45 to 60 minutes. The chambers of the superfusion apparatus were at controlled temperature (32±1.5°C) during the acclimatization and treatment periods.

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 changes in thickness (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 suitable for the assay.

Test procedure

After the zero reference measurements, the eye in its retainer was taken out of the chamber and placed on a layer of tissue with the cornea facing upwards. The eye was held in horizontal position, while the test item was applied onto the centre of the cornea. For treatment, 30 µL of the test item was applied onto the entire surface of the cornea attempting to cover the cornea surface uniformly with the test item, taking care not to damage or touch the cornea.

The positive control eyes were treated in a similar way with 30 µL of benzalkonium chloride solution 5 % (w/v). The negative control eye was treated with 30 µL of physiological saline.

Three test item treated eyes, three positive control treated eyes and one negative control eye were examined during the study. The time of application was noted, then after an exposure period of 10 seconds the cornea surface was rinsed with 20 mL physiological saline solution. The control and test eyes were evaluated pre-treatment and at approximately 30, 75, 120, 180 and 240 minutes after the post-treatment rinse. Minor variations within approximately ±5 minutes were considered acceptable. 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.

Evaluation

Corneal swelling:

$$CS\ at\ time\ t = \frac{CT\ at\ time\ t - CT\ at\ t{=}0}{CT\ at\ t{=}0} \times 100$$

$$Mean\ CS\ at\ time\ t = \frac{FECS_{(at\ time\ t)} + SECS_{(at\ time\ t)} + TECS_{(at\ time\ t)}}{3}$$

Remark:
CS = cornea swelling
CT = cornea thickness
$FECS_{(at\ time\ t)}$ = first eye cornea swelling at a given time-point
$SECS_{(at\ time\ t)}$ = second eye cornea swelling at a given time-point
$TECS_{(at\ time\ t)}$ = third eye cornea swelling at a given time-point

Corneal opacity:

$$\Delta CO\ at\ time\ t = CO\ at\ time\ t - CO\ at\ t{=}0$$

$$Mean\ \Delta CO_{max} = \frac{FECO_{max(30min\ to\ 240min)} + SECO_{max(30min\ to\ 240min)} + TECO_{max(30min\ to\ 240min)}}{3}$$

Remark:
CO at time t = cornea opacity at a given timepoint (30, 75, 120, 180 and 240 minutes) minutes after the post-treatment rinse
CO at t=0 = base line cornea opacity
ΔCO at time t = difference between cornea opacity at t time and cornea opacity base line
FECO = first eye cornea opacity
SECO = second eye cornea opacity
TECO= third eye cornea opacity
$_{max(30min\ to\ 240min)}$ = maximum opacity of the individual eye at 30 to 240 minutes minus base line cornea opacity of the individual eye.

Fluorescein retention:

$$\Delta FR\ at\ time\ t = FR\ at\ time\ t - FR\ at\ t{=}0$$

$$Mean\ \Delta FR = \frac{FEFR_{(30min)} + SEFR_{(30min)} + TEFR_{(30min)}}{3}$$

Remark:
FR at time t = fluorescein retention at 30 minutes after the post-treatment rinse
FR at t=0 = baseline fluorescein retention
ΔFR at time t = difference between fluorescein retention at t time and fluorescein retention base line
FEFR = first eye fluorescein retention at 30 minutes after the post-treatment rinse minus base line fluorescein retention
SEFR = second eye fluorescein retention at 30 minutes after the post-treatment rinse minus base line fluorescein retention
TEFR = third eye fluorescein retention at 30 minutes after the post-treatment rinse minus base line fluorescein retention

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. The mean maximum corneal swelling up to 240 min, the mean maximum corneal opacity and the mean fluorescein retention ICE classes are used for EC and GHS classification.

The results from all eyes met the quality control standards. The experiment was considered to be valid.

Table 7.1.5-01 ACL+DFF SC 600,000 g/L – eye irritation

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)	9.0	II	26.6	III	4.0	IV	3.0	IV	Loosening of epithelium in 1/3 eyes at 240 mins post treatment rinse	1 x III 2 x IV
Test Item (ACL+DFF SC 600)	0.0	I	1.1	I	0.5	I	0.0	I	None	3 x I

Based on this in vitro eye irritation test in isolated chicken eyes, the test item Aclonifen+Diflufenican SC 600,000 (500,000+100,000 g/L) is non-irritant.

Based on these observations, the positive control substance (benzalkonium chloride solution 5 % (w/v)) was classified as severe irritant according to the EU regulations. GHS Classification: Category 1.

B. DEFICIENCIES

None.

III. CONCLUSIONS

Under the experimental conditions, ACL+DFF SC 600,000 g/L is not an eye irritant. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

Under the experimental conditions, ACL+DFF SC 600,000 g/L is not an eye irritant. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

Assessment and conclusion by RMS:

CP 7.1.6 Skin sensitization

Data Point:	KCP 7.1.6/01
Report Author:	[redacted]
Report Year:	2016
Report Title:	Aclonifen+diflufenican SC 600,000 (500,000+100,000 g/L) - Local lymph node assay in the mouse
Report No:	16/056-037E
Document No:	M-558669-01-1
Guideline(s) followed in study:	OECD Guidelines for Testing of Chemicals No. 429, Skin Sensitisation: Local Lymph Node Assay adopted: 22 July 2010; Commission Regulation (EC) No 440/2008 of 30 May 2008, B.42., Skin Sensitisation: Local Lymph Node Assay (Official Journal L142, 31/05/2008) amended by Commission Regulation (EU) No 640/2012 of 6 July 2012
Deviations from current test guideline:	Current guideline: OECD 429, 2010 No deviation
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Now is no longer acceptable

Executive Summary

The potential of ACL+DFF SC 600,000 (500,000+100,000) g/L formulation to cause delayed contact hypersensitivity was investigated in female CBA/CaOlaHsd mice (4 per group) according to the Local Lymph Node Assay.

A preliminary irritation/toxicity test was performed with CBA/CaOlaHsd mice using two doses (test item concentrations of 100 % (undiluted) and 50% (w/v) in 1% Pluronic). The applicability and biocompatibility of the test item on the ears of animals at 50 % (w/v) concentration of test item was considered to be acceptable.

In the main study, twenty female CBA/CaOlaHsd mice were allocated to five groups of four animals each: i) three groups received the appropriate formulation at concentrations of 50%, 25% and 10% (w/v) in 1% Pluronic; ii) the negative control received 1% Pluronic and iii) the positive control group received 25% (w/v) α-Hexylcinnamaldehyde (HCA) in 1% Pluronic.

No clinical signs and no deaths related to treatment were observed throughout the main study. The body weight gain was not influenced by the treatment. Test item precipitate or minimal amount of

test item precipitate was observed on the ears of the animals in the 50 % (w/v) dose group on Days 1-4, in the 25 % (w/v) group on Days 2-3.

The calculated stimulation index values were 2.0, 0.9 and 0.7 at concentrations of 50%, 25% and 10 % (w/v) ACL+DFF SC 600,000 (500,000+100,000) g/L, respectively.

It was concluded that ACL+DFF SC 600,000 (500,000+100,000) g/L formulation does not exhibit a skin sensitisation potential in the Local Lymph Node Assay.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test materials: Aclonifen + Diflufenican SC 600,000 g/L
specification 102000029998

Description:	Yellow suspension
Lot/Batch:	2016-000027
Purity:	Considered as 100%
	aclonifen (AE F068300) – 40.7 % w/w 501.9 g/L
	diflufenican (AE F088657) – 8.59 % w/w 105.8 g/L
Stability of test compound:	Shown to be stable (see MCP2)
Negative control:	1% aqueous Pluronic PE9200
Positive control:	α-Hexylcinnamaldehyde

2. Vehicle: 1% Pluronic

3. Test animals:

Species:	Mouse
Strain:	CBA/CaOlaHsd
Age:	9 weeks old (females)
Weight at dosing:	19.1-21.2 g (females)
Source:	[redacted]
Acclimatisation period:	13 days
Diet:	Ssniff® SM Rat/Mouse – “Breeding & Maintenance, 15 mm, autoclavable Complete diet for rats/mice (Batch number: 540 5117, Expiry date: 31 July 2016), *ad libitum*
Water:	Municipal supply water, *ad libitum*
Housing:	Group caging, Type II polypropylene/polycarbonate

4. Environmental conditions:

Temperature:	20.9°C ± 25.9°C
Humidity:	24 to 79% relative humidity
Air changes:	15 – 20 times/hour

Photoperiod: Day-night rhythm 12 hours (7 a.m.- 7 p.m.)

B. STUDY DESIGN AND METHODS

1. In life dates: 25 March 2016 to 05 July 2016

2. Animal assignment and treatment

Preliminary irritation test

The Preliminary Irritation/Toxicity Test was started on CBA/CaOlaHsd mice using 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 was terminated on Day 6 and the radioactive proliferation assay was not performed. The maximum concentration of test item in an acceptable solvent was established according to OECD guideline 429. Based on the observation of the solubility test, the maximum achievable concentration was 100 % (undiluted).

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. All mice were observed daily for clinical signs of systemic toxicity or local irritation at the application site. Both ears of each mouse were observed for erythema and scored using according to OECD Guidelines for Testing of Chemicals No. 404. Ear thickness was measured using a thickness gauge on Day 1 (pre-dose), Day 3 and Day 6. Additional quantification of the ear thickness was performed by ear punch weight determination after the euthanasia. Individual body weights were recorded on Day 1 and on Day 6.

No mortality or signs of systemic toxicity were observed. Test item precipitate or minimal amount of test item precipitate was observed on the ears of the animals for both animals of the 100 % (undiluted) and for one animal of the 50 % (w/v) dose groups on Days 1-6 and for one animal of the 50 % (w/v) dose group on Days 1-4. The ear punch weights were within the acceptable range. The detected ear thickness values clearly indicated excessive local irritation for both animals of the 100% (undiluted) dose group on Day 3. The ear thickness values of 50% (w/v) dose group were within the acceptable range. Alopecia around the ears was observed for both animals of the 100% (undiluted) dose group on Days 3-6 or Days 4-6. The draining auricular lymph nodes of the animals were visually examined: they were normal in both dose groups (subjective judgement by analogy with observations of former experiments).

Based on the results of the preliminary irritation study, 50 % (w/v) dose was selected as top dose for the main test.

Main test

During the assay, 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. All mice were observed daily for clinical signs of systemic toxicity or local irritation at the application site. Individual body weights were recorded on Day 1 and on Day 6.

The radioactive proliferation assay was conducted 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, then left for 5 hours (± 30 minutes) before being euthanized. The auricular lymph nodes were excised.

For each treatment group a single cell suspension was prepared from pooled lymph nodes. DPM was measured for each pooled group of nodes. The measured DPM values were corrected with the background DPM value ("DPM"). The average of the two measured DPM values of 5 % (w/v) TCA solutions was used as background DPM value.

The cell proliferation in the local lymph nodes was measured by incorporation of tritiated methyl thymidine (3HTdR) and the values obtained by Liquid Scintillation Analyzer were used to calculate stimulation indices (SI). The SI must be at least equal or greater than three for a test substance to classify as a potential skin sensitizer.

Treatments in the main assay were performed as follows:

Table 7.1.6-1 Aclonifen + Diflufenican SC 600,000 g/L – Local lymph node assay - Groups and Treatments.

Groups	Test item concentration (% w/v)	No. of animals
Negative (vehicle) control (1% Pluronic)	-	4
Positive control (25% HCA in 1% Pluronic)	-	
ACL+ DFF SC 600,000 (500,000+100,000) g/L	50	
	25	
	10	

The degree of dermal reaction to treatment was scored on a 4-point scale.

Table: Erythema Scoring

No response	**0**
Very slight erythema (barely perceptible)	**1**
Well-defined erythema	**2**
Moderate to severe erythema	**3**
Severe erythema (beet redness) t slight eschar formation (injuries in depth)	**4**

Note: Excessive local skin irritation is indicated by an erythema score ≥ 3 and/or an increase in ear thickness of ≥ 25 % on any day of measurement.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred.

B. CLINICAL OBSERVATIONS

No signs of systemic toxicity were observed during the main test. Test item precipitate or minimal amount of test item precipitate was observed on the ears of the animals in the 50 % (w/v) dose group on Days 1-4, in the 25 % (w/v) group on Days 2-3.

C. BODY WEIGHT CHANGES

No treatment related effects were observed on the body weight changes of experimental animals. The mean body weights are given in Table below.

Table 7.1.6-2: Aclonifen + Diflufenican SC 600,000 g/L – Local lymph node assay - body weights

Test Group	Initial Body Weight (g)	Terminal Body Weight (g)	Change (%)
Negative control (1% Pluronic)	19.8	19.6	-1.1
Positive control (25% w/v in 1% Pluronic)	19.8	19.5	-1.5
ACL+ DFF SC 600,000 (500,000+100,000) g/L 50% (w/v)	19.8	19.7	-0.9
ACL+ DFF SC 600,000 (500,000+100,000) g/L 25% (w/v)	19.8	19.5	-1.6
ACL+ DFF SC 600,000 (500,000+100,000) g/L 10% (w/v)	20.0	20.1	0.4

D. PROLIFERATION ASSAY

The results of the proliferation assay are summarized in Table below. The appearance of the lymph nodes was normal in the negative control group and in the 50%, 25% and 10% (w/v) dose groups. Larger than normal lymph nodes were observed in the positive control group. The calculated stimulation index values were 2.0, 0.9 and 0.7 at concentrations of 50%, 25% and 10 % (w/v) ACL+DFF SC 600,000 (500,000+100,000) g/L, respectively.

Table 7.1.6-3: Aclonifen + Diflufenican SC 600,000 g/L – Local lymph node assay - DPM, DPN and Stimulation Index

Test Group	DPM/group (measured)	Number of pooled lymph nodes	DPN	Stimulation Index
Background (5% w/v TCA)	31 32	-	--	
Negative control (1% Pluronic)	2006	8	246.8	1.0
Positive control (25% w/v in 1% Pluronic)	8531	8	1062.4	4.3
ACL+ DFF SC 600,000 50% (w/v)	3908	8	484.6	2.0
ACL+ DFF SC 600,000 (500,000+100,000) g/L 25% (w/v)	1789	8	219.7	0.9
ACL+ DFF SC 600,000 (500,000+100,000) g/L 10% (w/v)	1435	8	175.4	0.7

The test item was a suspension, 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 resulted stimulation indices observed under these exaggerated test conditions was considered to be good evidence that ACL+DFF SC 600,000 (500,000+100,000) g/L is a non-sensitizer (Figure below).

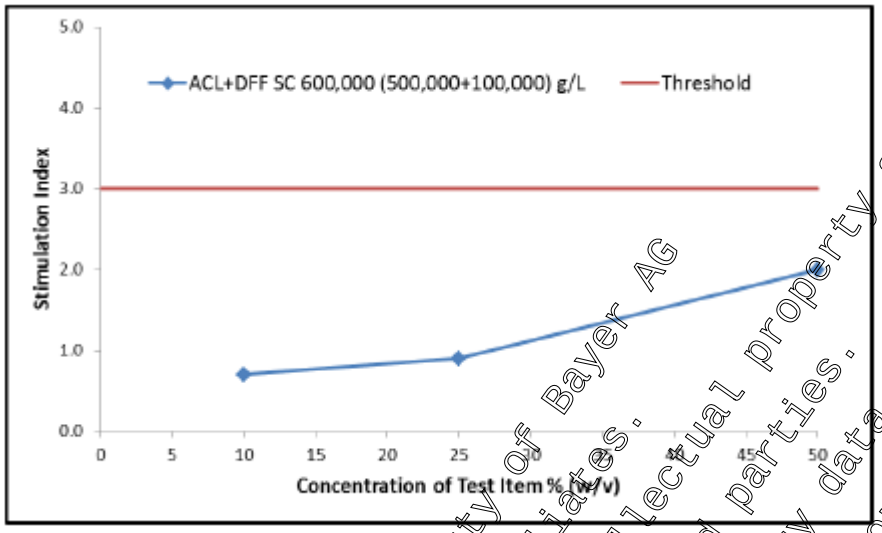


Figure 7.1.6-01 Aclonifen + Diflufenican SC 600,000 g/L – Local lymph node assay - Test Item Stimulation Index Values

E. RELIABILITY OF THE TEST

The positive control group animals were treated with 25% (w/v) HCA solution in a relevant vehicle (1% Pluronic) concurrent to the test item groups had a stimulation index value of 4.3. 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 data. Historical control data for the positive and negative control substances.

F. DEFICIENCIES

Due to technical reasons, the actual relative humidity range was 24-79 % instead of 30-70 % and the actual maximum temperature was 25.9 °C instead of 22 ± 3 °C as it was indicated in the Study Plan. Due to a typing error, incorrect purity data of the HCA was showed in the Study Plan. These deviations are considered not to adversely affect the results or integrity of the study.

III. CONCLUSIONS

Under current evaluation criteria, ACL+DFF SC 600,000 (500,000+100,000) g/L was considered not to be a skin sensitiser in the Local Lymph Node Assay and therefore is not classified as a potential dermal sensitiser according to Regulation (EC) 1272/2008.

Assessment and conclusion by applicant:
At the tested concentrations (10%, 25% and 50% w/v), the test substance was not found to be sensitised. However, it seems like a higher concentration should have been tested (about 75%) in order to get a reliable result as dose-related effect was noticed. The highest dose (50%) selected for

the main study was based on an increase in ear thickness of ≥25% for both animals of the 100% dose on Day 3 only during the Preliminary Test: neither mortality nor signs of systemic toxicity nor indications of erythema were observed and the ear punch weights were within the acceptable range. Overall, the validity criteria were not satisfied and therefore this study cannot be considered to be valid.
Based on the concentration of the active substance aclonifen the product Aclonifen+ diflufenican SC 600,000 (500,000+100,000 g/L) is classified as Skin Sens. 1A, H317 according to the criteria in Reg. 1272/2008.

Assessment and conclusion by RMS:

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 aclonifen or other components in ACL+DFF SC600 (500+100) G that would require further investigations.

CP 7.1.8 Supplementary studies for combinations of plant protection products

No such studies are necessary since ACL+DFF SC600 (500+100) G is not intended for use in combination with other plant protection products.

CP 7.2 Data on exposure

Evaluations of the exposure of operators, bystanders, residents and re-entry workers to aclonifen when used in the ACL+DFF SC600 (500+100) G are provided in the following sections.

Acute non-dietary risk assessment is not included in this submission because an AAOEL is not relevant for aclonifen (CAS No 74070-46-5) or diflufenican (CAS No 83164-33-4).

The Plant Protection Product ACL+DFF SC600 (500+100) G containing 500 g/L of aclonifen and 100 g/L of diflufenican and is intended to be used a foliar spray herbicide on cereals (winter and spring wheat, and spring barley) in Europe. Usage information pertinent to the assessment of exposure is summarised below.

Table 7.2-01 Summary of critical uses patterns (i.e. worst case).

Crop (indoor / field)	Application rate (kg as/ha per application)		Spray dilution (L/ha)	Application equipment	Number applications
	Aclonifen	Diflufenican			
Cereals: Triticale/Wheat	0.350	0.070	100-300	Spraying (foliar)	1

These critical use patterns have been defined following the evaluation of the individual GAPs for the mentioned crop in each relevant Member State.
The estimations of human dermal penetration of aclonifen and diflufenican which are the active substances in the mixed formulation ACL+DFF SC 600 (500+100) G were obtained from two *in vitro*

dermal absorption studies using human skin conducted by [redacted], 2016 and [redacted], 2017, respectively. The proposed values including the AOEL values are summarised below.

Table: 7.2-02 Proposed values for EU endpoints used on the non-dietary human risk assessment.

Aclonifen:

Endpoints used in risk assessment	Result
Dermal penetration Concentrate (%) Spray dilution (%)	**0.19** **21***
AOEL (mg/kg body weight/day)	**0.07**

*Pro-rata calculation for the highest in-use dilution from a value of 0.58 g/L (tested dilution)

Note Dermal penetration data derived from the results of [redacted] (2017), M-569676-01-1. Please refer to section M-CP 7.3 for further detail. Pro-rata calculation for the highest in-use dilution from a value of 1.5 g Aclofinen/L (tested dilution) was conducted. The AOEL value was derived from NOAEL from 2-year rat supported by the multigeneration study and sub-chronic studies in the rat and applying a safety factor of 100. Please refer to Doc N1for further detail.

Diflufenican:

Endpoints used in risk assessment	Result
Dermal penetration Concentrate (%) Spray dilution (%)	**0.11** **26***
AOEL (mg/kg body weight/day)	**0.11**

*Pro-rata calculation for the highest in-use dilution from a value of 0.12 g/L (tested dilution)

Note Dermal penetration data derived from the results of [redacted] (2017), M-583709-01-1. Please refer to section M-CP 7.3 for further detail. Pro-rata calculation for the highest in-use dilution from a value of 1.5g Aclofinen/L (tested dilution) was conducted. The AOEL value was derived from NOAEL from rat study, 13 weeks, corrected for 58% oral absorption and applying a safety factor of 100 EFSA Conclusion/EFSA Scientific Report (2007), 122 1-84 and SANCO/3782/08).

CP 7.2.1 Operator exposure

Operator exposure to ACL+DFF SC 600 (500+100) G was not evaluated as part of the EU review of the both active substance as aclonifen and diflufenican. Therefore, all relevant data and risk assessments are provided here and are considered adequate.

The current EFSA modelling tool on the assessment of exposure of operators, workers, residents, and bystanders, was used to estimate the respective exposures from the application of ACL+DFF SC 600 (500+100) G on cereals. The AOEM calculator released on 30 March 2015 supports the EFSA guidance document[1] that was last updated on 24 April 2015.

[1] Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014;12(10):3874)

CP 7.2.1.1 Estimation of operator exposure

Table 7.2.1-01: Input parameters considered for the estimation of operator exposure for cereals.

Product name and code	**ACL+DFF SC 600 (500+100) G**	
Formulation type	SC soluble or suspension concentrate	
Category	Herbicide	
Crop type	Cereals	
Application method	Downward spraying	
Application equipment	Vehicle-mounted	
Indoor/outdoor	Outdoor	
Active substance(s) (incl. content)	**Aclonifen (ACL)** 500 g/L	**Diflufenican (DFF)** 100 g/L
AOEL systemic	0.07 mg/kg bw/d	0.11 mg/kg bw/d
AAOEL	none	none
Inhalation absorption	100%	100%
Oral absorption	100%	58%
Dermal absorption	Concentrate: 0.19% Dilution: 21% *For more information please refer to chapter 7.5*	Concentrate: 0.11% Dilution: 26% *For more information please refer to chapter 7.5*

The input parameter "RVNAS" (Reference value non-acutely toxic active substance) is equivalent to the AOEL values (= 0.07 mg/kg body weight/day – aclonifen and 0.11 mg/kg body weight/day diflufenican). The "RVAAS" (Reference value acutely toxic active substance) was not applied. The scenario of a tractor mounted sprayer in low crops was assessed ad the defaults settings of the EFSA calculator was used with no refinements. The vapour pressures of both actives are below $5*10^{-3}$ Pa. The following sections show the summary results from the calculator. An attached appendix depicts the related full output pages from the calculator.

AAOEL values have not been set for aclonifen or diflufenican. Therefore, estimates of acute exposure to operators have not been conducted.

Table 7.2.1-02 Estimated longer-term operator exposure, Aclonifen, Cereals.

		Active: aclonifen	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg bw/day)**	**% systemic AOEL**
Spraying (downward spraying), cereals Application rate: 1x 0.350 kg a.s. /ha, 100 L/ha			
Downward spray (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.0169	24.13
	Work wear M/L and A	0.0111	15.87
	Work wear M/L and A + gloves M/L	0.0097	13.90

Table 7.2.1-03 Estimated longer-term operator exposure, Diflufenican, Cereals.

		Active: diflufenican	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg bw/day)**	**% systemic AOEL**
Spraying (downward spraying), cereals Application rate: 1x 0.070 kg a.s. /ha, 100 L/ha			
Downward spray (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.0017	1.52
	Work wear M/L and A	0.0013	1.21
	Work wear M/L and A + gloves M/L	0.0011	0.99

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

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. Hand-held 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 toddlers exposure might also occur orally (e.g. through hand-to-mouth transfer and/or object-to-mouth transfer).

CP 7.2.2.1 Estimation of bystander and resident exposure

Bystanders

Because no AAOEL value has been set and therefore bystanders are assumed to be protected by the resident risk assessment.

Residents

The resident exposure assessment was conducted following the EFSA calculator. The common parameters used for resident exposure risk assessment are presented in the Table below.

Table 7.2.2.1-1 Default input parameters considered for the estimation of resident exposure.

Intended use(s)	Cereals (outdoor)		Drift percentage mean (DR)	4.10 (highest)	%
Application rate (AR)	0.350 (ACL) 0.070 (DFF)	kg a.s./ha	Transfer coefficient surface deposits (TC)	7300	cm^2/h (adult)
				2600	cm^2/h (child)
Minimum water volume (V)	100	L/ha	Drift on surface (D) - 75th perc.		
Buffer strip	2-3	m	Drift on surface (D) - mean		
Number of applications (NA)	1		Turf Transferable Residues (TTR)		
Interval between applications	365	days	Exposure duration dermal (H_D)		
The half-life of active substance	2	days	Exposure duration inhal. (H_I)		
Multiple application factor (MAF)			Exposure duration entry into treated crops (H_E)		
Body weight (BW)	60	kg/person (adults)	Airborne Concentration of Vapour (VC)		
	10	kg/person (children)			
Dermal absorption (DA)	21 or 26	% ('worst case')	Dislodgeable foliar residue (DFR) from	1.05 (highest)	µg/cm^2/kg a.s.

			model		
Inhalation absorption (IA)	100	%	Light clothing adjustment factor (CF)	18	%
Oral absorption (OA)	100 (ACL) 58 (DFF)	%	Saliva Extraction Factor (SE)	50	%
AOEL	0.07 (ACL) 0.11 (DFF)	mg/kg bw/d	Surface Area of Hands (SA)	20	cm^2
Spray drift dermal (SD) - 75th perc.	0.47	mL spray dilution (adult)	Frequency of Hand to Mouth (fFreq)	9.5	events/h
	0.327	mL spray dilution (child)			
Spray drift inhal. (SI) - 75th perc.	0.00010	mL spray dilution (adult)	Dislodgeable residues object to mouth (DR_{OM})	20	%
	0.00022	mL spray dilution (child)			
Spray drift dermal (SD) - mean	0.22318	mL spray dilution (adult)	Ingestion Rate for Mouthing of Grass (IgR)	25	cm^2/d
	0.18	mL spray dilution (child)			
Spray drift inhal. (SD) - mean	0.00009	mL spray dilution (adult)	TC entry into treated crops - 75th perc.	7500	cm^2/h (adult)
	0.00017	mL spray dilution (child)		2250	cm^2/h (child)
Inhalation rate (IR)	0.23	m^3/d/kg (adult)	TC entry into treated crops - mean	5980	cm^2/h (adult)
	1.07	m^3/d / kg (child)		1794	cm^2/h (child)

Based on the above parameters, the total systemic exposure for residents is shown in the Tables below.

Table 7.2.2.1-2 Estimation of resident exposure from the use of ACL+DFF SC 600 (500+100) G in cereals (outdoor uses).

Model data	Level of PPE	Active: aclonifen	
		Total absorbed dose (mg/kg bw/day)	% systemic AOEL
Spraying (downward spraying), cereals Drift reduction technology: No (default values DT_{50} = 30 days and Initial DFR: 3 µg/cm^2/kg a.s./ha) Interval between treatments: 365 days Buffer strip: 2-3 meters			
Number of applications and application rate:		1 x 0.350 kg a.s./ha	
Resident child	Drift (75th perc.)	0.0132	18.81

Body weight: 10 Kg	Vapour (75th perc.)	0.0011	1.53
	Deposits (75th perc.)	0.0006	0.79
	Re-entry (75th perc.)	0.0124	17.72
	Sum (mean)	**0.0187**	**26.68**
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0024	3.42
	Vapour (75th perc.)	0.0002	0.33
	Deposits (75th perc.)	0.0002	0.29
	Re-entry (75th perc.)	0.0069	9.84
	Sum (mean)	**0.0071**	**10.28**

Model data	**Level of PPE**	**Active: diflufenican**	
		Total absorbed dose (mg/kg bw/day)	**% systemic AOEL**
Spraying (downward spraying), cereals Drift reduction technology: No (default values DT_{50}= 30 days and Initial DFR: 3 µg/cm²/kg a.s./ha) Interval between treatments:1 day Buffer strip: 2-3 meters			
Number of applications and application rate:		1 x 0.070 Kg a.s./ha	
Resident child Body weight: 10 Kg	Drift (75th perc.)	0.0016	1.48
	Vapour (75th perc.)	0.0011	0.97
	Deposits (75th perc.)	0.0001	0.06
	Re-entry (75th perc.)	0.0031	2.79
	Sum (mean)	**0.0045**	**4.06**
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0003	0.27
	Vapour (75th perc.)	0.0002	0.21
	Deposits (75th perc.)	0.0000	0.02
	Re-entry (75th perc.)	00017	1.55
	Sum (mean)	**0.0018**	**1.60**

CP 7.2.2.2 Measurement of bystander and resident exposure

Since the exposure estimate carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under practical conditions of use, 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 worker re-entry exposure has been calculated for both active substances following application of ACL+DFF SC 600 (500+100) G formulation for the representative use on crop. The estimation is provided in the following sections.

CP 7.2.3.1 Estimation of worker exposure

For cereals, the main re-entry activity after treatment is related to maintenance (i.e. inspection and irrigation). The common parameters used for worker exposure risk assessment are presented in the Table below. The indicative transfer coefficient values from EFSA calculator for dermal exposure, are presented in the table below and are considered for the worker exposure risk assessment (first-tier assessment) following the use of the product in cereal crops.

Table 7.2.3.1.1-01 Common parameter considered for the estimation of worker exposure from the use of ACL+DFF SC 600 (500+100) G.

Intended use(s)	**Cereals**		Dislodgeable foliar residue (DFR)	3	µg/cm²/kg a.s./ha
Application rate (AR)	0.350 (ACL) 0.070 (DFF)	kg a.s./ha	Dermal absorption (DA)	21 or 26	% (worst case)
Number of applications (NA)	1		Inhalation absorption (IA)	100	%
Interval between applications	365	days	Work rate per day (WR)	8	h/d
Half-life of active substance	30	days	TC dermal (potential)	12500	cm^2/h
Multiple application factor (MAF)	1		TC dermal (work wear)	1400	cm^2/h
Body weight (BW)	60	kg/person	TC dermal (work wear + gloves)	-	cm^2/h
AOEL	0.07 (ACL) 0.11 (DFF)	mg/kg bw/d	Task specific factor inhalation (TSF)	n/a	ha/h x 10^{-3}

Aclonifen and diflufenican show a low vapour pressure of 1.5 x 10^{-4} Pa. Therefore, contamination of workers through inhalation of both substances in open field activities was considered negligible and consequently not used in the calculations.

An AAOEL was not allocated during the peer review for the renewal of approval of both active substances. Therefore, estimates of acute exposure to workers have not been conducted.

Table 7.2.3.1.1-01 Estimation of longer-term worker exposure from the use of ACL+DFF SC 600 (500+100) G in cereals.

		Active: aclonifen	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg bw/day)**	**% systemic AOEL**

Task: reaching and picking, peas MAF: 1.0 Work rate: 8 hours/day (default values DT_{50}= 30 days and Initial DFR: 3 µg/cm²/kg a.s./ha) Interval between treatments: 365 days			
Number of applications and application rate:		1 x 0.350 Kg a.s./ha, 100 L/ha	
Body weight: 60 kg	Workwear (arms, body, and legs covered) TC: 12500 cm²/person/h	0.0919	131.25
	Workwear (hands, arms, body, and legs covered) TC: 1400 cm²/person/h	0.0103	14.70

		Active: diflufenican	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg bw/day)**	**% systemic AOEL**
Task: reaching and picking, peas MAF: 1.0 Work rate: 8 hours/day (default values DT_{50}= 30 days and Initial DFR: 3 µg/cm²/kg a.s./ha) Interval between treatments: 365 days			
Number of applications and application rate:		1 x 0.070 Kg a.s./ha, 100 L/ha	
Body weight: 60 kg	Workwear (arms, body, and legs covered) TC: 12500 cm²/person/h	0.0228	20.68
	Workwear (hands, arms, body, and legs covered) TC: 1400 cm²/person/h	0.0025	2.32

CP 7.2.3.2 Measurement of worker exposure

Not considered to be necessary as a safe use was predicted in the previous section.

CP 7.2.4 Combined exposure

The product is a mixture of two active substances. Therefore, a combined exposure assessment is provided.

CP 7.2.4.1 Exposure Assessment of the actives sunstances (Aclonifen , Diflufenican) in ACL+DFF SC 600 (500+100) G

Note: The combined toxicological effect of these active substances has not been investigated with regard to repeated dose toxicity.

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/RVNAS. This is equivalent to the predicted exposure as % of systemic AOEL/RVNAS to decimal. The Hazard Index (HI) is the sum of the individual HQs.

Table 7.2.4.1-1 Long-term risk assessment from combined exposure

Scenario	Active Substance	Estimated exposure / AOEL (RVNAS) (HQ)[3]
Combined formulation: ACL+DFF SC 600 (500+100) G *Crop: cereals* *Application rate: 0.350 Kg/ha ACL + 0.070kg/ha DFF (worst-case scenario)*		
Operators, with PPE (gloves M&L). For details please refer to 7.2.1. Only the worst-case scenario *Cereals* is presented	Aclonifen	0.1390
	Diflufenican	0.0099
	Cumulative risk Operators (HI)[2]	**0.1489**
Workers, with Workwear *F*or details please refer to 7.2.3. Only the worst-case scenario *Cereals* is presented	Aclonifen	0.1470
	Diflufenican	0.0232
	Cumulative risk Workers (HI)[2]	**0.1702**
Resident – Adult For details please refer to 7.2.2. Only the worst case scenario *Cereals* is presented	Aclonifen	0.1018
	Diflufenican	0.0160
	Cumulative risk Resident-Adult (HI)[2]	**0.1178**
Resident – Child[1] For details please refer to 7.2.2. Only the worst-case scenario *Cereals* is presented	Aclonifen	0.2668
	Diflufenican	0.0406
	Cumulative risk Resident-Child (HI)[2]	**0.3074**

[1] The higher exposure value either from the 75th percentile of each of the four pathways (spray drift, vapour, surface deposits, entry into treated crops) or the sum of the mean exposure values is taken into consideration
[2] HI =Hazard Index
[3] HQ = Hazard Quotient

The Hazard Index is < 1. Therefore, the combined exposure to all active substances in ACL+DFF SC 600 (500+100) G is not expected to present a risk for operators, workers, bystanders and residents. No further refinement of the assessment is required.

CP 7.3 Dermal absorption

Summary of dermal absorption

Two dermal absorption studies were available, comprising two *in vitro* dermal absorption study using human skin, each one conducted with aclonifen or diflufenican. The EFSA guidance on dermal absorption 2017 (section 3.11) allows for the provision to base the dermal absorption value on the results of one well conducted *in vitro* study through human skin; therefore both studies has been used to calculate the final dermal absorption values for the concentrate and the aqueous dilution of each active substance. Both studies were found to be well conducted, of enough quality and conforms to the requirements of the EFSA 2017 guidance.

Summary of dermal absorption values (according to 2017 EFSA guidance)

Active	Study	Concentrate dermal absorption	Tested dilution dermal absorption	Pro-rata adjustment to in-use dilution dermal absorption
Aclonifen	[redacted], 2016. M-569676-01-1	500g/L: 0.19%	1.5 g/L: 8.1%	0.58 g/L: 21%
Diflufenican	[redacted] 2016. M-583709-01-1	100 g/L: 0.11%	0.3 g/L: 10%	0.12 g/L: 26%

Aclonifen

The necessary adjustments have been made to the data evaluation in this summary to comply with the 2017 EFSA guidance. Overall, the estimated amount of aclonifen considered to be absorbed from the concentrate and aqueous spray dilution was 0.19 % and 8.1% of the total applied dose, respectively.

Pro-rata adjustment

For spray dilutions lower than 1.5g/L aclonifen a pro-rata adjustment should be made in accordance with the 2017 EFSA guidance on dermal absorption.

The highest dilution rate for use in barley and rye is a 1 in 857 dilution (0.58g/L aclonifen) (assuming a maximum application rate of 0.175 kg/ha aclonifen in a maximum water volume of 300L/ha). The tested dilution was a 1 in 333 dilution (1.5 g/L aclonifen).

Pro-rata adjustment calculation from a 1 to 333 dilution to a 1 in 857 dilution = 8.1 x 857/333 = 20.8% (rounded to 21%).

The dermal absorption of aclonifen in a 0.58g/L dilution is 21%. This value is used as the most conservative value for the operator exposure calculations for the spray dilutions.

Difenican

The necessary adjustments have been made to the data evaluation in this summary to comply with the 2017 EFSA guidance. Overall, the estimated amount of diflufenican considered to be absorbed from the concentrate and aqueous spray dilution was 0.11 % and 10% of the total applied dose, respectively.

Pro-rata adjustment

For spray dilutions lower than 0.3g/L diflufenican a pro-rata adjustment should be made in accordance with the 2017 EFSA guidance on dermal absorption.

The highest dilution rate for use in barley and rye is a 1 in 857 dilution (0.12 g/L diflufenican) (assuming a maximum application rate of 0.035 kg/ha diflufenican in a maximum water volume of 300L/ha). The tested dilution was a 1 in 333 dilution (0.3 g/L diflufenican).

Pro-rata adjustment calculation from a 1 to 333 dilution to a 1 in 857 dilution = 10 x 857/333 = 25.7% (rounded to 26%).

The dermal absorption of diflufenican in a 0.12g/L dilution is 26%. This value is used as the most conservative value for the operator exposure calculations for the spray dilutions.

Data Point:	KCP 7.3/01
Report Author:	[redacted]
Report Year:	2016
Report Title:	ACL + DFF SC 500+100: [14C]-aclonifen in vitro dermal absorption study using human skin
Report No:	SA 16138
Document No:	M-569676-01-1
Guideline(s) followed in study:	OECD Guideline for the testing of Chemicals Skin Absorption In Vitro Method Guideline 428 (April 2004). OECD Environmental Health and Safety Publications Series on testing and Assessment N° 28, Guidance Document for the Conduct of Skin Absorption Studies (March 2004). EFSA Panel on Plant Protection Products and their Residues (PPR): Guidance on Dermal Absorption, EFSA Journal 2012; 10(4): 2665
Deviations from current test guideline:	Current guideline: OECD 428, 2004 No significant deviations. EFSA dermal absorption guideline 2017 - study evaluated to the 2012 EFSA guidance on dermal absorption so needs to be re-evaluated to the current guidance.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

This report describes the *in vitro* dermal penetration of aclonifen by the use of [14C]-aclonifen in the ACL + DFF SC 500+100 formulation (specification number 102000029998) following single dermal application to excised human dermatomed skin mounted in flow-through diffusion cells. This application was performed at two concentrations corresponding to the neat product (500 g aclonifen/L) and one spray dilution (1.5 g aclonifen/L) with six replicates per concentration.

Dermatomed skin membranes were maintained in flow-through cells. The integrity of the membranes was first tested by the Trans Epidermal Water Loss (TEWL) method. The two formulations were applied at a rate of 10 µL/cm². Receptor fluid samples were collected at hourly intervals for the duration of the study (24 hours). The solubility of the aclonifen in the receptor fluid was demonstrated to be sufficient.

Eight hours post-application, the material was washed off the skin. At the end of the study (24 hours) the skin samples were swabbed again and were tape-stripped to remove residual surface dose and the stratum corneum. The skin samples were removed from the diffusion cells and taken for analysis with liquid scintillation counting.

Good recovery data were obtained for the neat formulation and the spray dilutions, with mean total recoveries of radioactivity exceeding 95%.

For both dose levels tested, the majority of radioactivity was found to be non-absorbed with >99% for the neat formulation and 91% for the low dose formulation. Also, less than 75% of the radioactivity was considered to be directly absorbed during the first half of the study (12 hours).

Therefore, according to the EFSA guidance document (2017), the radioactivity found in the stratum corneum was included in the potentially absorbable fraction. For the dilution one of the replicates was excluded as an outlier from the dermal absorption calculations as the absorption profile was clearly different from the other cells.

The necessary adjustments have been made to the data evaluation in this summary to comply with the 2017 EFSA guidance. Overall, the estimated amount of aclonifen considered to be absorbed from the concentrate and aqueous spray dilution was 0.19 % and 8.1% of the total applied dose, respectively.

I. MATERIALS AND METHODS

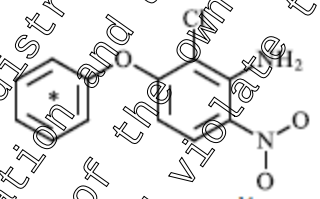
A. MATERIALS

1. Test material (non-radiolabelled):

The neat product formulation contains aclonifen (ACL: 500 g/L) and diflufenican (DFF: 100 g/L) forumulated as ACL + DFF SC 500+100, specification number 10200029998. In the current study, all concentrations are referring to aclonifen only: Aclonifen (AE F068300) 2-amino-3-chloro-4-phenoxy-nitrobenzene.

Description:	Aclonifen (AE F068300)
Lot/Batch:	BES1572
Purity:	99.6% w/w
Stability of test compound:	April 02, 2018. Non-radiolabeled formulation shown to be stable (see MCP2)

2. Test material (labelled):



*: denotes the position of the [^{14}C] radiolabel

[phenoxy-UL-^{14}C] aclonifen

Lot/Batch:	KML 10149
Concentration:	High dose (measured)=3.86 MBq/mL, low dose (measured) = 2.06 Mbq/mL
Specific activity:	6.79 MBq/mg
Purity:	100%

4. Test skin:

Species:	Human skin
Sex and number of donors:	Female (HD=6, LD= 6)
Site of skin samples:	Abdomen, split thickness 372 to 500 µm.

Source: [redacted]

B. STUDY DESIGN AND METHODS

1. In life dates: 01 July 2016 to 09 August 2016

2. Animal assignment and treatment

Six human skin samples were studied simultaneously for each dose level. The flow-through diffusion cell system (Franz's cell modified, Gallas, France) was used to study the absorption of the test item (exposure area of 1 cm^2 skin). The receptor fluid was Eagle's medium supplemented with 5% bovine serum albumin and gentamycin (50 mg/L) at a pH measured between 7.41 and 7.43.

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.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 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 for each group was complete. Receptor fluid samples 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 a minimum of 15 tissue wipes (Kimtech Sciences from Kimberly 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. 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.

3. 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. The limit of detection was taken to be twice the background values for blank samples in appropriate scintillation cocktails.

II. RESULTS AND DISCUSSION

A. FINDINGS

Aclonifen was demonstrated to be soluble in the receptor fluid at the concentration of 1.9 mg/mL of receptor fluid and deemed to be sufficient to reduce any risk of back diffusion. During the study, the maximal concentration per hour of [^{14}C]-aclonifen in the receptor fluid was 0.22 µg/mL. Good

recovery data were obtained for the neat formulation and the spray dilution, with mean total recoveries of radioactivity in the range of 98.5% to 95.7% of the applied dose.

The following table presents the distribution of radioactivity for the human dermatomed skin following a single topical application of the high and low dose formulations of [^{14}C]-aclonifen in the ACL+DFF SC 500+100 formulation.

For the spray dilution one of the replicates was excluded from the calculations as the absorption profile was clearly different from the other cells and it was clearly an outlier. The surrounding skin was contamination and this is thought to be caused by the donor chamber not properly fixed on the skin.

Table 7.3-1: Dermal absorption of [^{14}C]-aclonifen - distribution of radioactivity 24 hours after dose application in an SC formulation at the rates of 500 g/L and 1.5 g/L to human skin samples

Results expressed in terms of percentage of applied radioactivity.

Dose Levels	Distribution of radioactivity (% dose)			
	Neat formulation: High dose (500 g/L)		Dilution: Low dose (1.5 g/L)	
Species	Human (n=6)		Human (n=5)	
SURFACE COMPARTMENT				
	Mean	SD	Mean	SD
Skin swabs (8h)	98.08	1.58	89.46	3.05
Skin swabs (24h)[a]	0.10	0.04	0.59	0.36
Total in skin swabs	98.18	1.57	90.05	2.94
Surface Dose (1st two tape-strips)	0.13	0.07	0.69	0.49
Donor chamber	0.02	0.02	0.29	0.51
Total % non-absorbed	**98.33**	**1.57**	**91.03**	**2.26**
SKIN COMPARTMENT				
Skin [b]	0.08	0.04	0.65	0.65
Stratum corneum [c]	0.03	0.03	0.08	0.07
Total % at dose site	***0.11***	***0.06***	***0.73***	***0.71***
RECEPTOR COMPARTMENT				
Receptor fluid (0-24h)	0.06	0.01	3.55	2.26
Receptor fluid terminal	0.01	0.00	0.18	0.12
Receptor chamber	n.d.	n.a.	0.23	0.51
Total % directly absorbed [d]	***0.07***	***0.01***	***3.96***	***2.51***
STUDY				
Total % Potentially Absorbable	0.18	0.06	4.69	2.89
TOTAL % RECOVERY	**98.52**	**1.54**	**95.72**	**1.11**
Evaluation according to EFSA Guidance				
absorption >75% within half of study duration	No		No	
standard deviation >25%	Yes		Yes	
recovery <95%	No		No	

adjusted: **Total % Potentially Absorbable [f]**	**0.2**	**8**
[b]: sum of radioactivity found in skin after tape-stripping procedure and in surrounding skin.		
[c]: tape-strips excluding numbers 1 & 2 which are considered to be non-absorbed dose.		
[d]: sum of radioactivity found in receptor fluid (0-24h), receptor fluid terminal and receptor chamber.		
[e]: total % directly absorbed + total % at dose site		
[f]: values considered for the adjusted Total % Potentially Absorbable according to EFSA are in ***bold Italics.***		
SD: standard deviation		
n.d.: not detected (below the limit of detection)		
n.a. : not applicable		
n: number of skin cells used for calculation		
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.		

The overall amount of [^{14}C]-aclonifen considered to be directly absorbed was represented by the radioactivity present in the receptor fluid, receptor fluid at termination time and receptor chamber. This accounted for means of 0.07% (±0.01%) of the dose applied for the neat formulation and for mean of 3.96% (±2.51%) of the dose applied for the low dose formulation. The radioactivity found in the skin compartment (skin, surrounding skin and stratum corneum) could be considered as potentially absorbable fraction and the amount of radioactivity found in the stratum corneum was also included in the potentially absorbable fraction as described below.

Table 7.3-2 Dermal absorption of [14C]-aclonifen - Distribution of radioactivity during the test

Distribution of radioactivity (mean % dose ± standard deviation)		
Dose level	Neat formulation (DER1615)	Spray dilution (DER1616)
Number of skin cells used for calculation	n=6	n=5
Receptor fluid (0 - 12h)	0.02	2.22
Total % directly absorbed	0.07	3.96
Receptor fluid at 12 hours/day directly absorbed ration (%)	29%	56%
Total potentially absorbable	0.18 ± 0.06	4.69 ± 2.89

B. RECALCULATIONS

Originally, this study was not evaluated according to the 2017 EFSA Guidance on dermal absorption (2017). The following table summarises the data as prepared in accordance with the EFSA guidance (2017) (using the accompanying spreadsheet). The calculations consider the main requirements of the guidance, including whether or not absorption is complete, tape stripping procedures and rounding of values. Please see Appendix 2 for the original calculations.

Table 7.3-3 Dermal absorption of [^{14}C]-aclonifen - summary of total amount absorbed (% applied dose) from the concentrate and the aqueous spray dilution after 24 hours- according to the BfR template.

	Concentrate		Dilution 1		Dilution 2*	
Dilution	N/A		(1:333)		(1:333)	
Number of replicates	6		6		5	
Target concentration [mg/mL]	500		1.5		1.5	
Target dose [µg/cm^2]	5000		15		15	
Mean actual applied dose [µg/cm^2]	4520		14.47		14.47	
Recovery [%]	Mean	SD	Mean	SD	Mean	SD
Dislodgeable dose						
Skin wash (total after 8 hours, and 24 hours combined)	98.18	1.57	87.15	7.57	90.04	2.94
Donor chamber wash	0.03	0.02	0.58	0.53	0.73	0.66
Skin associated dose						
Tape strips 1-2	0.13	0.07	1.19	1.27	0.70	0.49
Tape strips 3-x	0.04	0.03	0.11	0.11	0.08	0.07
Skin preparation	0.08	0.04	1.96	1.15	0.65	0.65
Absorbed dose						
Receptor fluid	0.02	0.01	5.42	4.65	3.73	2.36
Receptor chamber wash	N/A	N/A	1.04	0.16	1.15	N/A
Total recovery	**98.45**	**1.53**	**95.56**	**1.06**	**95.72**	**1.11**
LLC of t_0.5 absorption	0.00	0.00	55.01	8.72	51.51	11.05
Absorption complete?	No		No		No	
Measured absorption, if LLC of t_0.5<=75%	0.12	0.07	6.93	6.08	4.69	2.88
Measured absorption, if LLC of t_0.5>75%	N/A	N/A	N/A	N/A	N/A	N/A
Measured absorption corrected	0.12	0.07	6.93	6.08	4.69	2.88
Relevant absorption estimate	0.191		13.011		8.143	
Final estimate (rounded)	**0.19**		13		**8.1**	

III. CONCLUSIONS

In this well-conducted GLP and guideline compliant *in vitro* study, using the formulation ACL + DFF SC 500+100, evaluated according to the 2017 EFSA guidance on dermal absorption, the dermal absorption of aclonifen through human skin was 0.19 % in the concentrate (500g/L aclonifen) and 8.1 % in the low dilution spray dilution (1.5g/L aclonifen).

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid. The necessary adjustments have been made to the data evaluation in this summary to comply with the 2017 EFSA guidance.

Overall, the estimated amount of aclonifen considered to be absorbed from the concentrate (500 g/L aclonifen) and aqueous spray dilution (1.5g/L aclonifen) was 0.19% and 8.1 % of the total applied dose, respectively.

The highest dilution rate for use in barley and rye is a 1 in 857 dilution (0.58g/L aclonifen) (assuming a maximum application rate of 0.175 kg/ha aclonifen in a maximum water volume of 300L/ha).

The dermal absorption of aclonifen in a 0.58 g/L dilution is 21%. This value is used as the most conservative value for the operator exposure calculations for the spray dilutions.

Assessment and conclusion by RMS:

Data Point:	KCP 7.3/02
Report Author:	[redacted]
Report Year:	2017
Report Title:	ACL + DFF SC 600 (500+100): [14C]-diflufenican in vitro dermal absorption study using human skin
Report No:	SA 16191
Document No:	M-583709-01-1
Guideline(s) followed in study:	OECD Guideline for the testing of Chemicals Skin Absorption In Vitro Method Guideline 428 (April 2004). OECD Environmental Health and Safety Publications Series on testing and Assessment N° 28, Guidance Document for the Conduct of Skin Absorption Studies (March 2004). EFSA Panel on Plant Protection Products and their Residues (PPR): Guidance on Dermal Absorption, EFSA Journal 2012; 10(4): 2665
Deviations from current test guideline:	Current guideline: OECD 428, 2004 No significant deviations. EFSA dermal absorption guideline 2017 - study evaluated to the 2012 EFSA guidance on dermal absorption so needs to be re-evaluated to the current guidance.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

This report describes the *in vitro* dermal penetration of diflufenican by the use of [14C]-diflufenican in the ACL + DFF SC 500+100 formulation (specification number 102000029998) following single dermal application to excised human dermatomed skin mounted in flow-through diffusion cells. This

application was performed at two concentrations corresponding to the neat product (100 g diflufenican) and one spray dilution (0.3 g) with six replicates per concentration.

Dermatomed skin membranes were maintained in flow-through cells. The integrity of the membranes was first tested by the Trans Epidermal Water Loss (TEWL) method. The two formulations were applied at a rate of 10 µL/cm^2. Receptor fluid samples were collected at hourly intervals for the duration of the study (24 hours). The solubility of the diflufenican in the receptor fluid was demonstrated to be sufficient.

Eight hours post-application, the remaining dose material was washed off the skin. At the end of the study (24 hours) the skin samples were swabbed again and were tape-stripped to remove residual surface dose and the stratum corneum. The skin samples were removed from the diffusion cells and taken for analysis with liquid scintillation counting.

Good recovery data were obtained for the neat formulation with a mean total recovery of radioactivity exceeding 95%. For the low dose mean recovery was slightly below 95%. In addition, in the low dose one replicate was considered to be an outlier due to recovery below 90% this replicate was excluded from the mean calculations.

In the current study, as less than 75% of the radioactivity considered to be directly absorbed was absorbed during the first half of the study (12 hours), the amount of radioactivity found in the stratum corneum has to be included in the potentially absorbable fraction for the spray dilution (based on EFSA Guidance Document, 2017). For the neat formulation, diflufenican was not detected in the receptor compartment; therefore the amount of radioactivity found in the stratum corneum was included in the potentially absorbable fraction.

The necessary adjustments have been made to the data evaluation in this summary to comply with the 2017 EFSA guidance. Overall, the estimated amount of aclonifen considered to be absorbed from the concentrate and aqueous spray dilution was 0.11% and 10% of the total applied dose, respectively.

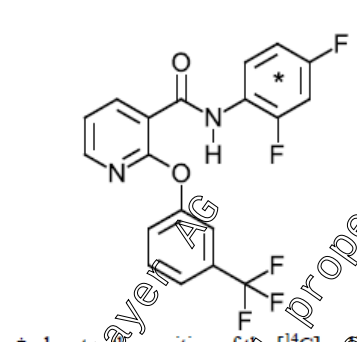
I. MATERIALS AND METHODS

A. MATERIALS

1. Test material (non-radiolabelled): The neat product formulation contains aclonifen (ACL: 500 g/L) and diflufenican (DFF: 100 g/L) forumulated as ACL + DFF SC 500+100, specification number 102000029998. In the current study, all concentrations are referring to diflufenican only: Diflufenican (AE F088657).

Description:	Diflufenican (AE F0888657)
Lot/Batch:	LOP5168 (origin)
Purity:	99.8% w/w
Stability of test compound:	April 21, 2024. Non-radiolabeled formulation shown to be stable (see MCP2)

2. Test material (labelled): [difluorophenyl-UL-^{14}C] Diflufenican



*: denotes the position of the [^{14}C] radiolabel

Lot/Batch:	DER1623
Concentration:	High dose (measured)=3.82 MBq/mL, low dose (measured) = 0.93 Mbq/mL
Specific activity:	3.07 MBq/mg
Purity:	100%

3. Test skin:

Species:	Human skin
Sex and sex of donors:	Female (HD=6, LD= 6)
Site of skin samples:	Abdomen; Thickness: 379 to 464 µm.
Source:	[redacted]

B. STUDY DESIGN AND METHODS

1. In life dates: 26 Sep 2016 to 29 November 2016

2. Animal assignment and treatment

Six human skin samples were studied simultaneously for each dose level. The flow-through diffusion cell system (Franz's cell modified, Gallas, France) was used to study the absorption of the test item (exposure area of 1 cm^2 skin). The receptor fluid was Eagle's medium supplemented with 5% bovine serum albumin and gentamycin (50 mg/L) at a pH measured between 7.40 and 7.43.

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. The dose preparation was applied to the split-thickness skin sample with a pipette at the rate of approximately 10 µL/cm^2 exposed skin.

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 for each group was complete. Receptor fluid samples were 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 a minimum of 15 tissue wipes (Kimtech Sciences from Kimberly 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. 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.

3. 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. The limit of detection was taken to be twice the background values for blank samples in appropriate scintillation cocktails.

II. RESULTS AND DISCUSSION

A. FINDINGS

Diflufenican was demonstrated to be soluble in the receptor fluid at the concentration of 0.7 mg/mL of receptor fluid. During the study, the maximal concentration per hour of [^{14}C]-diflufenican in the receptor fluid was 2.6 ng/mL. The achieved concentrations were at least 10 times lower than the determined solubility concentration, thus the solubility in the receptor fluid was deemed to be sufficient to reduce any risk of back diffusion.

Good recovery data was obtained for the neat formulation, with mean total recovery of radioactivity of 100% of the applied dose.

For the spray dilution one of the replicates was excluded from the calculations as the recovery was below 90%. The absorption profile of this replicate indicates that the amount washed off the skin at 8 hours was considerably lower than the other replicates suggesting the missing material may be in the non-absorbed dose because other compartments were within the range of other replicates with the exception that there was an abnormally the high level of radioactivity in the 1st tape strip.

The following table presents the distribution of radioactivity for the human dermatomed skin following a single topical application of the high and low dose formulations of [^{14}C]-diflufenican in the ACL+DFF SC 600 (500+100): formulation.

Table 7.3-4: Dermal absorption of [^{14}C]-diflufenican - distribution of radioactivity at 24 hours after dose application of in an SC 600 formulation at the rates of 100 g/L and 0.3 g/L to human skin samples

Results expressed in terms of percentage of applied radioactivity.

	Distribution of radioactivity (% dose)	
Dose Levels	Neat formulation: High dose (100 g/L)	Dilution: Low dose (0.3 g/L) (normalised values)
Species	Human (n=6)	Human (n=5)

SURFACE COMPARTMENT				
	Mean	SD	Mean	SD
Skin swabs (8h)	99.76	4.24	92.34 (97.80)	1.52 (1.61)
Skin swabs (24h)[a]	0.28	0.29	0.45 (0.48)	0.57 (0.60)
Total in skin swabs	100.04	4.10	92.80 (98.39)	1.64 (1.74)
Surface Dose (1st two tape-strips)	0.36	0.24	0.63 (0.67)	0.81 (0.81)
Donor chamber	0.04	0.04	0.06 (0.06)	0.13 (0.14)
Total % non-absorbed	**100.4**	**4.13**	**93.48 (99.11)**	**2.34 (2.48)**
SKIN COMPARTMENT				
Skin [b]	0.03	0.03	0.67 (0.71)	0.15 (0.16)
Stratum corneum [c]	0.01	0.01	0.05 (0.05)	0.09 (0.10)
Total % at dose site	***0.04***	***0.04***	***0.72 (0.76)***	***1.14 (1.21)***
RECEPTOR COMPARTMENT				
Receptor fluid (0-24h)	n.d.	n.a.	0.11 (0.12)	0.09 (0.10)
Receptor fluid terminal	n.d.	n.a.	n.d.	n.a.
Receptor chamber	n.d.	n.a.	n.d.	n.a.
Total % directly absorbed [d]	***n.d.***	***n.a.***	***0.11 (0.12)***	***0.09 (0.10)***
STUDY				
Total % Potentially Absorbable [e]	0.04	0.04	0.83 (0.88)	1.09 (1.16)
TOTAL % RECOVERY	**100.5**	**4.12**	**94.32 (100)**	**1.96 (2.08)**
Evaluation according to EFSA Guidance				
absorption >75% within half of study duration	No		No	
standard deviation >25%	Yes		Yes	
recovery >95%	No		Yes	
adjusted: **Total % Potentially Absorbable [f]**	**0.1**		**2**	

[a]: sum of radioactivity found in swabs at termination and in surrounding swabs.

[b]: sum of radioactivity found in skin after tape-stripping procedure and in surrounding skin.

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

[d]: sum of radioactivity found in receptor fluid (0-24h), receptor fluid terminal and receptor chamber.

[e]: total % directly absorbed + total % at dose site

[f]: values considered for the adjusted Total % Potentially Absorbable according to EFSA are in ***bold Italics.***

SD: standard deviation

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

n.a. : not applicable

n: number of skin cells used for calculation

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.

The overall amount of [^{14}C]-diflufenican considered to be directly absorbed was represented by the radioactivity present in the receptor fluid, receptor fluid at termination time and receptor chamber. No radioactivity was detected in the receptor compartment. This accounted for mean of 0.12% of the dose applied for the low dose formulation. According to the following results:
- As less than 75% of the radioactivity considered to be directly absorbed was absorbed during the first half of the study (12 hours), the amount of radioactivity found in the stratum corneum has to be included in the potentially absorbable fraction for the spray dilution

- For the neat formulation, diflufenican was not detected in the receptor compartment, it was decided to include the amount of radioactivity found in the stratum corneum in the potentially absorbable fraction as a conservative measure.

Table 7.3-5 Dermal absorption of [14C]-diflufenican - Distribution of radioactivity during the test

Distribution of radioactivity mean % dose ± standard deviation (normalized values for low dose formulation)		
Dose levels	Neat formulation (DER1624: 100 g/L)	Spray dilution (DER1625: 0.3 g/L)
Number of skin cells used for calculation	n=6	n=5
Receptor fluid (0 - 12h)	ND	0.04
Total % directly absorbed	ND	0.11
Receptor fluid at 12-hour/day directly absorbed ration (%)	NA	36%
Total % potentially absorbable*	*0.07 ± 0.04*	*0.83 ± 1.09* *(0.88± 1.16)*

*: sum of radioactivity in Total % directly absorbed and Total % of dose site

B. RECALCULATIONS

Originally, this study was not evaluated according to the 2017 EFSA Guidance on dermal absorption (2017). The following table summarises the data as prepared in accordance with the EFSA guidance (2017) (using the accompanying spreadsheet). The calculations consider the main requirements of the guidance, including whether or not absorption is complete, tape stripping procedures and rounding of values. Please see Appendix 2 for the original calculations.

Table 7.3-6 Dermal absorption of [14C]-diflufenican - summary of the total amount of aclonifen absorbed (% applied dose) from the concentrate and the aqueous spray dilution after 24 hours- according to the BfR template.

	Concentrate		Dilution 1		Dilution 2*	
Dilution	N/A		(1:333)		(1:333)	
Number of replicates	6		6		5	
Target concentration [mg/mL]	100		0.3		0.3	
Target dose [µg/cm^2]	1000		3		3	
Mean actual applied dose [µg/cm^2]	880		2.97		2.97	
Recovery [%]	Mean	SD	Mean	SD	Mean	SD

Dislodgeable dose						
Skin wash (total after 8 hours, and 24 hours combined)	100.04	4.10	91.24	4.09	92.80	1.64
Donor chamber wash	0.07	0.02	0.26	0.04	0.29	N/A
Skin associated dose						
Tape strips 1-2	0.36	0.24	1.26	1.70	0.63	0.81
Tape strips 3-x	0.02	0.01	0.06	0.09	0.06	0.10
Skin preparation	0.08	0.04	0.58	1.05	0.67	1.15
Absorbed dose						
Receptor fluid	0.00	0.00	0.10	0.10	0.11	0.10
Receptor chamber wash	0.00	0.00	0.00	0.00	0.00	0.00
Total recovery	**100.53**	**4.13**	**93.31**	**3.03**	**94.32**	**1.97**
LLC of t_0.5 absorption	100.00	0.00	-4.63	36.72	-6.01	44.52
Absorption complete?	Yes		No		No	
Measured absorption, if LLC of t_0.5<=75%	N/A	N/A	0.73	1.00	0.83	1.08
Measured absorption, if LLC of t_0.5>75%	0.08	0.04	N/A	N/A	N/A	N/A
Measured absorption corrected	0.08	0.04	6.18	4.71	5.03	4.23
Relevant absorption estimate	0.114		10.888		10.105	
Final estimate (rounded)	**0.11**		11		**10**	

Remarks

* Dilution 2 is dilution 1 excluding outlier

N/A = not applicable
SD = standard deviation

III. CONCLUSIONS

In this well-conducted GLP and guideline compliant *in vitro* study, using the formulation ACL + DFF SC 500+100, evaluated according to the 2017 EFSA guidance on dermal absorption, the dermal absorption of diflufenican through human skin was 0.11 % in the concentrate (100g/L diflufenican) and 10% in the low dilution spray dilution (0.3 g/L diflufenican).

<u>Assessment and conclusion by applicant</u>:

All validity criteria were satisfied and therefore this study can be considered to be valid. The necessary adjustments have been made to the data evaluation in this summary to comply with the 2017 EFSA guidance.

Overall, the estimated amount of diflufenican considered to be absorbed from the concentrate (containing 100g/L diflufenican) and aqueous spray dilution (containing 0.3 g/L diflufenican) was 0.11 % and 10% of the total applied dose, respectively.

The highest dilution rate for use in barley and rye is a 1 in 857 dilution (0.12g/L diflufenican) (assuming a maximum application rate of 0.035 kg/ha diflufenican in a maximum water volume of 300L/ha).

The dermal absorption of diflufenican in a 0.12 g/L dilution is 26%. This value is used as the most conservative value for the operator exposure calculations for the spray dilutions.

Assessment and conclusion by RMS:

CP 7.4 Available toxicological data relating to co-formulants

CONFIDENTIAL information – data provided separately (Document JCP).

Appendix 1 Exposure calculator output

Appendix 1.1 Operator exposure for ACL+DFF SC 600 (500+100) G outdoor spray application in cereal crops.

Aclonifen

Operator exposure for outdoor spray applications

Application rate of active substance	0.35	kg a.s./ha	*i_AppRate*
Assumed area treated	50	ha/day	*d_AreaTreated*
Amount of active substance applied	17.5	kg a.s./day	*i_AmountAS*
Dermal absorption of the product	0.19%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	21.00%		*i_AbsorInuse*
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.		
Indoor or Outdoor application	Outdoor		
Application method	Downward spraying		
Application equipment	Vehicle-mounted		
Season	not relevant		

	Exposure values	µg exposure/day mixed and loaded		Reference	Comment
		75th centile	95th centile		
Mixing and loading	Hands	43983	1652[illegible]	AOEM	
	Body	26675	165429	AOEM	
	Head	908	4980	AOEM	
	Protected hands (gloves)	222	3466	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	300	2559	AOEM	
	Protected head (hood and face shield)	15	282	AOEM	
	Inhalation	9	31	AOEM	
	Protective Equipment		Select for inclusion	Penetration factor	Inhalation Protection factor
	Gloves		Yes	Incl. in AOEM model	
	Clothing		Work wear - arms, body and legs covered	Incl. in AOEM model	
	Head and respiratory PPE		None	1	1
	Water soluble bag		No	1	

	Exposure values	µg exposure/day applied		Reference	Comment
		75th centile	95th centile		
Application	Hands	2596	18645	AOEM	
	Body	1451	7482	AOEM	
	Head	69	207	AOEM	
	Protected hands (gloves)	201	4654	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	40	98	AOEM	
	Inhalation	4	15	AOEM	
	Protective Equipment		Select for inclusion	Penetration factor	Inhalation Protection factor
	Gloves		No		
	Clothing		Work wear - arms, body and legs covered	Incl. in AOEM model	
	Head and respiratory PPE		None	1	1
	Closed cab		Yes	vehicle mounted upward spraying only	

1. Total

	Without RPE/PPE	With RPE/PPE
Longer term		
Total systemic exposure from mixing, loading and application (mg a.s./day)	1.0133	0.5836
Total systemic exposure from mixing, loading and application per kg body weight (mg/kg bw/day)	0.0169	0.0097
% of RVNAS	24.13%	13.90%

Diflufenican

Operator exposure for outdoor spray applications

Application rate of active substance	0.07	kg a.s./ha	*i_AppRate*
Assumed area treated	50	ha/day	*d_AreaTreated*
Amount of active substance applied	3.5	kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	0.11%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	26.00%		*i_AbsorInuse*
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.		
Indoor or Outdoor application	Outdoor		
Application method	Downward spraying		
Application equipment	Vehicle-mounted-Drift Reduction		
Season	not relevant		

	Exposure values	µg exposure/day mixed and loaded		Reference	Comment
		75th centile	95th centile		
Mixing and loading	Hands	12741	47203	AOEM	
	Body	8605	16644	AOEM	
	Head	182	996	AOEM	
	Protected hands (gloves)	78	693	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	72	212	AOEM	
	Protected head (hood and face shield)	3	56	AOEM	
	Inhalation	3	10	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	Yes		Incl. in AOEM model	
	Clothing	Work wear, arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

	Exposure values	µg exposure/day applied		Reference	Comment
		75th centile	95th centile		
Application	Hands	220	1404	AOEM	
	Body	46	46	AOEM	
	Head	2		AOEM	
	Protected hands (gloves)	7	62	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	2	2	AOEM	
	Inhalation	2	3	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear, arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Closed cab	Yes		vehicle mounted upward spraying only	

1. Total

	Without RPE/PPE	With RPE/PPE
Longer term		
Total systemic exposure from mixing, loading and application (mg a.s./day)	0.1003	0.0656
Total systemic exposure from mixing, loading and application per kg body weight (mg/kg bw/day)	0.0017	0.0011
% of NOAEL	1.52%	0.99%

Appendix 1.2 **Worker exposure for ACL+DFF SC 600 (500+100) G outdoor spray application in cereal crops.**

Aclonifen

Worker exposure from residues on foliage for		
Crop type	Cereals	
Indoor or outdoor	Outdoor	
Application method	Downward spraying	
Application equipment	Vehicle-mounted	
Worker's task	Inspection, irrigation	
Main body parts in contact with foliage	Hand and body	
Application rate of active substance	0.35	kg a.s./ha
Number of applications	1	
Interval between multiple applications	365	days
Half-life of active substance	30	days
Multiple application factor	1.0	
Dermal absorption of the product	0.1%	
Dermal absorption of the in-use dilution	21.0%	
Dislodgeable foliar residue (i_AppRate*i_DFR)	1.05	µg a.s./cm²
Working hours	2	hr
Dermal transfer coefficient - Total potential exposure	1250	cm²/hr
Dermal transfer coefficient - arms, body and legs covered	1400	cm²/hr
Dermal transfer coefficient - hands, arms, body and legs covered	no TC available for this assessment	cm²/hr
Inhalation transfer coefficient for automated applications	NA	ha/hr*10^(-3)
Inhalation transfer coefficient for cutting ornamentals	NA	ha/hr*10^(-3)
Inhalation transfer coefficient for sorting / bundling ornamentals	NA	ha/hr*10^(-3)

1. Total

	Potential exposure	Work wear - arms, body and legs covered	Working wear and gloves
Total systemic exposure (mg a.s./day)	5.5125	0.6174	no TC available for this assessment
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0919	0.0103	
% of RVNAS	131.25%	14.70%	

Diflufenican

Worker exposure from residues on foliage for		
Crop type	Cereals	
Indoor or outdoor	Outdoor	
Application method	Downward spraying	
Application equipment	Vehicle-mounted-Drift Reduction	
Worker's task	Inspection, irrigation	
Main body parts in contact with foliage	Hand and body	
Application rate of active substance	0.07	kg a.s./ha
Number of applications	1	
Interval between multiple applications	365	days
Half-life of active substance	30	days
Multiple application factor	1.0	
Dermal absorption of the product	0.11%	
Dermal absorption of the in-use dilution	26.00%	
Dislodgeable foliar residue (i_AppRate*i_DFR)	0.21	µg a.s./cm²
Working hours	2	hr
Dermal transfer coefficient - Total potential exposure	12500	cm²/hr
Dermal transfer coefficient - arms, body and legs covered	1400	cm²/hr
Dermal transfer coefficient - hands, arms, body and legs covered	no TC available for this assessment	cm²/hr
Inhalation transfer coefficient for automated applications	NA	ha/hr*10^(-3)
Inhalation transfer coefficient for cutting ornamentals	NA	ha/hr*10^(-3)
Inhalation transfer coefficient for sorting / bundling ornamentals	NA	ha/hr*10^(-3)

1. Total

	Potential exposure	Work wear - arms, body and legs covered	Working wear and gloves
Total systemic exposure (mg a.s./day)	1.3650	0.1529	no TC available for this assessment
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0228	0.0025	
% of RVNAS	10.68%	2.32%	

Appendix 1.3 Resident exposure for ACL+DFF SC 600 (500+100) G outdoor spray application in cereal crops.

Aclonifen

Resident exposure for		
Croptype	Cereals	
Application method	Downward spraying	
Application equipment	Vehicle-mounted	
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.	
Buffer strip	5	m
Application rate of the product	0.35	kg a.s./ha
Concentration of active substance (in-use dilution for liquid applications)	3.5	g a.s./l
Dermal absorption of product	0.19%	
Dermal absorption of in-use dilution	11.08%	
Oral absorption	100.00%	
Dislodgeable foliar residue (i_AppRate*i_DFR)	1.05	µg a.s./cm²
Vapour pressure of in-use dilution	low volatile substances having a vapour pressure of <5*10-3 Pa	Pa
Concentration in air	0.001	mg/m³
Resident dermal spray drift exposure 75th percentile - adult	0.23798	ml spray dilution/person
Resident dermal spray drift exposure 75th percentile - child	0.2125	ml spray dilution/person
Resident inhal. spray drift exposure 75th percentile - adult	0.00007	ml spray dilution/person
Resident inhal. spray drift exposure 75th percentile - child	0.00017	ml spray dilution/person
Resident dermal spray drift exposure mean - adult	0.12278	ml spray dilution/person
Resident dermal spray drift exposure mean - child	0.12	ml spray dilution/person
Resident inhal. spray drift exposure mean - adult	0.00008	ml spray dilution/person
Resident inhal. spray drift exposure mean - child	0.00004	ml spray dilution/person
Exposure duration dermal	2	hours
Exposure duration inhalation	24	hours
Exposure duration entry into treated crops	0.25	hours
Light clothing adjustment factor	18.8%	
Breathing rate adult	0.23	m³/day/kg
Breathing rate child (1-3 year old)	1.07	m³/day/kg
Drift percentage on surface (75th percentile)	2.30%	
Drift percentage on surface (mean)	1.80%	
Turf transferable residues percentage	5.00%	
Transfer coeff. of surface deposits-adult	300	cm²/hour
Transfer coeff. of surface deposits-child (1-3 year old)	2000	cm²/hour
Saliva extraction percentage	50.00%	
Surface area of hands mouthed	20	cm²
Frequency of hand to mouth activity	9.5	events/hour
Ingestion rate for mouthing of grass per day	25	cm²
Dislodgeable residues percentage transferability for object to mouth	25.00%	
Transfer coefficient for entry into treated crops (75th percentile) - ad	7500	cm²/h
Transfer coefficient for entry into treated crops (75th percentile) - chi	2250	cm²/h
Transfer coefficient for entry into treated crops (mean) - adult	5980	cm²/h
Transfer coefficient for entry into treated crops (mean) - child	1794	cm²/h

Resident - child	Spray drift (75th percentile) mg/kg bw/day	0.0132	% of RVNAS	18.81%
	Vapour (75th percentile) mg/kg bw/day	0.0011	% of RVNAS	1.53%
	Surface deposits (75th percentile) mg/kg bw/day	0.0006	% of RVNAS	0.79%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0124	% of RVNAS	17.72%
	All pathways (mean) mg/kg bw/day	0.0187	% of RVNAS	26.68%
Resident - adult	Spray drift (75th percentile) mg/kg bw/day	0.0024	% of RVNAS	3.42%
	Vapour (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	0.33%
	Surface deposits (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	0.29%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0069	% of RVNAS	9.84%
	All pathways (mean) mg/kg bw/day	0.0071	% of RVNAS	10.18%

Diflufenican

Resident exposure for		
Croptype	Cereals	
Application method	Downward spraying	
Application equipment	Vehicle-mounted-Drift Reduction	
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.	
Buffer strip	5	m
Application rate of the product	0.07	kg a.s./ha
Concentration of active substance (in-use dilution for liquid applications)	0.7	g a.s./l
Dermal absorption of product	0.11%	
Dermal absorption of in-use dilution	26.00%	
Oral absorption	100.00%	
Dislodgeable foliar residue (i_AppRate*i_DFR)	0.21	µg a.s./cm²
Vapour pressure of in-use dilution	low volatile substances having a vapour pressure of <5*10-3Pa	Pa
Concentration in air	0.001	mg/m³
Resident dermal spray drift exposure 75th percentile - adult	0.23798	ml spray dilution/person
Resident dermal spray drift exposure 75th percentile - child	0.2175	ml spray dilution/person
Resident inhal. spray drift exposure 75th percentile - adult	0.00007	ml spray dilution/person
Resident inhal. spray drift exposure 75th percentile - child	0.00017	ml spray dilution/person
Resident dermal spray drift exposure mean - adult	0.12278	ml spray dilution/person
Resident dermal spray drift exposure mean - child	0.12	ml spray dilution/person
Resident inhal. spray drift exposure mean - adult	0.00008	ml spray dilution/person
Resident inhal. spray drift exposure mean - child	0.00014	ml spray dilution/person
Exposure duration dermal	6	hours
Exposure duration inhalation	24	hours
Exposure duration entry into treated crops	0.25	hours
Light clothing adjustment factor	18.0%	
Breathing rate adult	0.29	m³/day/kg
Breathing rate child (1-3 year old)	0.67	m³/day/kg
Drift percentage on surface (75th percentile)	2.30%	
Drift percentage on surface (mean)	1.80%	
Turf transferable residues percentage	5.00%	
Transfer coeff. of surface deposits-adult	7500	cm²/hour
Transfer coeff. of surface deposits-child (1-3 year old)	4600	cm²/hour
Saliva extraction percentage	50.00%	
Surface area of hands mouthed	20	cm²
Frequency of hand to mouth activity	9.5	events/hour
Ingestion rate for mouthing of grass per day	25	cm²
Dislodgeable residues percentage transferability for object to mouth	25.00%	
Transfer coefficient for entry into treated crops (75th percentile) - ad	7500	cm²/h
Transfer coefficient for entry into treated crops (75th percentile) - chi	2250	cm²/h
Transfer coefficient for entry into treated crops (mean) - adult	5980	cm²/h
Transfer coefficient for entry into treated crops (mean) - child	1794	cm²/h

Resident - child	Spray drift (75th percentile) mg/kg bw/day	0.0016	% of RVNAS	1.48%
	Vapour (75th percentile) mg/kg bw/day	0.0011	% of RVNAS	0.97%
	Surface deposits (75th percentile) mg/kg bw/day	0.0001	% of RVNAS	0.06%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0031	% of RVNAS	2.79%
	All pathways (mean) mg/kg bw/day	0.0045	% of RVNAS	4.06%
Resident - adult	Spray drift (75th percentile) mg/kg bw/day	0.0003	% of RVNAS	0.27%
	Vapour (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	0.21%
	Surface deposits (75th percentile) mg/kg bw/day	0.0000	% of RVNAS	0.02%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0017	% of RVNAS	1.55%
	All pathways (mean) mg/kg bw/day	0.0018	% of RVNAS	1.60%

Appendix 2 Accompanying spreadsheet with dermal absorption data from BfR template (from EFSA dermal absorption guidance, 2017)

ACL + DFF SC 500+100: [14C]-aclonifen in vitro dermal absorption study using human skin.

M-569676-01-1. [redacted] 2016.

For some data with values below 0% a low number > zero has been added to aid in the excel formulas which give an error if 0 is used.

Tested doses

	Concentrate	Dilution 1	Dilution 2
Target concentration [mg/mL]	500	1.5	1.5
Surface area dose [µg/cm²]	5000	15	15
Total dose [µg/cell]	5000	15	15
Specific activity [kBq/mL]	3860	2060	2060
No. of donors	6	6	6
No. of replicates used/valid replicates*	6	6	5
Surrounding skin contaminated: donor chamber probably not fixed correctly on the skin and the receptor chamber to avoid contamination. Absorption profile clearly different from the other cells.			

Materials and methods

General information			*
	Species	Human	
	Method	In vitro	
Test material			
Active substance	Name (Lot/Batch No.)	[phenoxy-UL-14C] aclonifen	
	Test preparation	pre-formulated	
	Radiochemical purity	100	%
Product	Name (Lot/Batch No.)	ACL+DFF SC 600 (500+100)	
	Company code	102000029998	
	Concentration a.s.	500	g/L or g/kg
	Type of formulation	SC	

	Vehicle used (if any)		
Blank product	Name (Lot/Batch No.)		
	Concentration a.s.		g/L or g/kg
	Type of formulation		
Test system			
Diffusion cell	Type of diffusion cell	Flow-through	
	(If dynamic) Flow rate	1.5	mL/h
	Exposed skin area	1	cm²
	Cover		
Skin sample	Skin type	Dermatomed	
	Skin thickness range	372-500	µm
	Skin donor age	not stated	years
	Skin donor sex	Female	
	Site	Abdomen	
	Source	Unknown	
	Integrity test	Yes	
Receptor	Receptor medium	Eagle's medium supplemented with 5% bovine serum albumin	
	Solubility in receptor medium	acceptable- found to be 1.9 mg/mL compared to max concentration of aclonifen in receptor fluid of 0.22µg/mL	
Sampling	Exposure time	8	hours
	Sampling duration	24	hours
	Sample intervals	1	hours
	Skin wash/Swabbing	after 8 hours using 1% v/v Tween 80 in PBS (phosphate buffer saline) using a minimum of 15 tissue wipes. Further swabbing at 24 hrs	
Tape strips	Tape stripping	Yes	
	Type of tape strips used	Monaderm adhesive tape	
	TS 1-2 analysed seperately?	Yes	
Remarks			

Aclonifen Concentrate:

Replicate	1	2	3	4	5	6	n	MEAN	SD	MAX				
T0.5 <= 75 %												**Variation**	**DA corrected**	**DA rounded**
Absorbed dose	0.01	0.02	0.01	0.01	0.04	0.01		0.02	0.01	0.04				
Tape strips 3-x	0.03	0.05	0	0	0.08	0.01		0.04	0.03	0.08				
Skin preparation	0.11	0.12	0.06	0.02	0.08	0.08		0.08	0.04	0.12				
Sum	0.15	0.19	0.07	0.03	0.2	0.1		0.12	0.07	0.2				
Relevant data normalised	0.15	0.19	0.07	0.03	0.2	0.1		0.12	0.07	0.2				
Relevant data added	0.15	0.19	0.07	0.03	0.2	0.1		0.12	0.07	0.2				
Relevant data	0.15	0.19	0.07	0.03	0.2	0.1	6	**0.12**	**0.07**	**0.2**		**55.15**	**0.191**	**0.19**
T0.5 > 75 %														
Absorbed dose	0.01	0.02	0.01	0.01	0.04	0.01		0.02	0.01	0.04				
Skin preparation	0.11	0.12	0.06	0.02	0.08	0.08		0.08	0.04	0.12				
Sum	0.12	0.14	0.07	0.03	0.12	0.09		0.10	0.04	0.14				
Relevant data normalised	0.12	0.14	0.07	0.03	0.12	0.09		0.10	0.04	0.14				
Relevant data added	0.12	0.14	0.07	0.03	0.12	0.09		0.10	0.04	0.14				
Relevant data	0.12	0.14	0.07	0.03	0.12	0.09	6	**0.10**	**0.04**	**0.14**		**42.50**	**0.135**	**0.14**
Non-absorbed dose	98.25	97.1	101.04	97.89	96.65	99.05		98.33	1.57	101.04				
Total Recovery	**98.4**	**97.29**	**101.11**	**97.92**	**96.85**	**99.15**		**98.45**	**1.53**	**101.11**				
T0.5	0.001	0.0005	0.001	0.001	0.00025	0.001	6	0.00	0.00	0.001				
								Mean lower limit of confidence		**0.00**				
								k*SD		**0.0003323**				

Replicate	1	2	3	4	5	6		MEAN	SD	MAX
Donor ID	A2016/1	X2016/10	A2016/3	X2016/1	A2016/2	X2016/8				
Receptor fluid	0.01	0.02	0.01	0.01	0.04	0.01		0.02	0.01	0.04
Receptor compartment wash	0	0	0	0	0	0		0.00	0.00	0
Donor compartment wash	0	0	0.06	0.02	0.02	0.03		0.02	0.02	0.06
	98.09	96.99	100.82	97.83	96.39	98.94		98.18	1.57	100.82
	0.16	0.11	0.16	0.04	0.24	0.08		0.13	0.07	0.24
	0.03	0.01	0	0	0.08	0.01		0.03	0.03	0.08
Stripped skin	0.11	0.12	0.06	0.02	0.08	0.08		0.08	0.04	0.12
Receptor fluid after 12 hours	0.0000001	1E-07	1E-07	1E-07	1E-07	1E-07		0.00	0.00	1E-07
Receptor fluid after 24 hours	0.01	0.02	0.01	0.01	0.04	0.01		0.02	0.01	0.04

Aclonifen dilution:

Replicate	1	2	3	4	5	6	n	MEAN	SD	MAX		Variation	DA corrected	DA rounded
T0.5 <= 75 %														
Absorbed dose	1.69	2.59	2.37	5.61	7.54	14.79		5.77	4.96	14.79				
Tape strips 3-x	0.03	0.02	0.12	0.17	0.04	0.3		0.11	0.11	0.3				
Skin preparation	0.39	0.07	0.52	1.77	0.5	3.08		1.06	1.15	3.08				
Sum	2.11	2.68	3.01	7.55	8.08	18.17		6.93	6.08	18.17				
Relevant data normalised	2.11	2.68	3.01	7.55	8.08	18.17		6.93	6.08	18.17				
Relevant data added	2.11	2.68	3.01	7.55	8.08	18.17		6.93	6.08	18.17				
Relevant data	2.11	2.68	3.01	7.55	8.08	18.17	6	**6.93**	**6.08**	**18.17**		**87.66**	**13.011**	**13**
T0.5 > 75 %														
Absorbed dose	1.69	2.59	2.37	5.61	7.54	14.79		5.77	4.96	14.79				
Skin preparation	0.39	0.07	0.52	1.77	0.5	3.08		1.06	1.15	3.08				
Sum	2.08	2.66	2.89	7.38	8.04	17.87		6.82	5.99	17.87				
Relevant data normalised	2.08	2.66	2.89	7.38	8.04	17.87		6.82	5.99	17.87				
Relevant data added	2.08	2.66	2.89	7.38	8.04	17.87		6.82	5.99	17.87				
Relevant data	2.08	2.66	2.89	7.38	8.04	17.87	6	**6.82**	**5.99**	**17.87**		**87.76**	**12.805**	**13**
Non-absorbed dose	91.84	92.97	93.1	88.35	88.89	76.58		88.62	6.24	93.1				
Total Recovery	**93.95**	**95.65**	**96.11**	**95.9**	**96.97**	**94.75**		**95.56**	**1.06**	**96.97**				
T0.5	63.9053	76.0618	58.228	63.677	50.9284	69.575	6	63.73	8.72	76.0618				
								Mean lower limit of confidence	**55.01**					
								1*SD	**8.722625**					

Replicate	1	2	3	4	5	6	MEAN	SD	MAX
Donor ID	X2016/5	A2016/3	X2016/8	A2016/	X2016/10	A2016/			
Receptor fluid	1.69	2.59	2.37	4.46	7.54	13.87	5.42	4.65	13.87
Receptor compartment wash	0	0	0	1.15	0	0.92	0.35	0.54	1.15
Donor compartment wash	0	0	0.26	1.19	0	0.3	0.29	0.46	1.19
	91.49	92.81	91.88	85.78	88.26	72.65	87.15	7.57	92.81
1	0.35	0.13	0.96	1.38	0.63	3.63	1.19	1.27	3.63
2	0.03	0.02	0.12	0.17	0.04	0.3	0.11	0.11	0.3
Stripped skin	0.39	0.07	0.52	1.77	0.5	3.08	1.06	1.15	3.08
Receptor fluid after 12 hours	1.08	1.97	1.38	2.84	2.84	9.65	3.46	3.20	9.65
Receptor fluid after 24	1.69	2.59	2.37	4.46	7.54	13.87	5.42	4.65	13.87

Aclonifen Dilution excluding outlier:

Replicate	1	2	3	4	5	n	MEAN	SD	MAX		Variation	DA corrected	DA rounded
T0.5 <= 75 %													
Absorbed dose	1.69	2.59	2.37	5.61	7.54		3.96	2.50	7.54				
Tape strips 3-x	0.03	0.02	0.12	0.17	0.04		0.08	0.07	0.17				
Skin preparation	0.39	0.07	0.52	1.77	0.5		0.65	0.65	1.77				
Sum	2.11	2.68	3.01	7.55	8.08		4.69	2.88	8.08				
Relevant data	2.11	2.68	3.01	7.55	8.08		4.69	2.88	8.08				
Relevant data added	2.11	2.68	3.01	7.55	8.08		4.69	2.88	8.08				
Relevant data	2.11	2.68	3.01	7.55	8.08	5	**4.69**	**2.88**	**8.08**		**61.47**	**8.143**	**8.1**
T0.5 > 75 %													
Absorbed dose	1.69	2.59	2.37	5.61	7.54		3.96	2.50	7.54				
Skin preparation	0.39	0.07	0.52	1.77	0.5		0.65	0.65	1.77				
Sum	2.08	2.66	2.89	7.38	8.04		4.61	2.85	8.04				
Relevant data	2.08	2.66	2.89	7.38	8.04		4.61	2.85	8.04				
Relevant data added	2.08	2.66	2.89	7.38	8.04		4.61	2.85	8.04				
Relevant data	2.08	2.66	2.89	7.38	8.04	5	**4.61**	**2.85**	**8.04**		**61.93**	**8.036**	**8**
Non-absorbed dose	91.84	92.97	93.1	88.35	88.89		91.03	2.26	93.1				
Total Recovery	**93.95**	**95.65**	**96.11**	**95.9**	**96.97**		**95.72**	**1.11**	**96.97**				
T0.5	63.9053	76.06178	58.2278	63.6771	50.9284	5	62.56	9.21	76.062				
						Mean lower limit of confidence	**51.51**						
						k*SD	**11.05398**						

Replicate	1	2	3	4	5	MEAN	SD	MAX
Donor ID	X2016/5	A2016/3	X2016/8	A2016/3	X2016/10			
Receptor fluid	1.69	2.59	2.37	4.46	7.54	3.73	2.36	7.54
Receptor	0	0	0	1.15	0	0.23	0.51	1.15
Donor compartment	0	0	0.26	1.19	0	0.29	0.52	1.19
	91.49	92.81	91.88	85.78	88.26	90.04	2.94	92.81
1	0.35	0.16	0.96	1.38	0.63	0.70	0.49	1.38
2	0.03	0.02	0.12	0.17	0.04	0.08	0.07	0.17
Stripped skin	0.39	0.07	0.52	1.77	0.5	0.65	0.65	1.77
								0
Receptor fluid after 12	1.08	1.97	1.38	2.84	3.84	2.22	1.13	3.84
Receptor fluid after 24	1.69	2.59	2.37	4.46	7.54	3.73	2.36	7.54

ACL + DFF SC 600 (500+100: [14C]-diflufenican in vitro dermal absorption study using human skin. M-583709-01-1.

[redacted] **2017.**

For some data with values below 0% a low number > zero has been added to aid in the excel formulas which give an error if 0 is used.

Tested doses

	Concentrate	Dilution 1	Dilution 2
Target concentration [mg/mL]	100	0.3	0.3
Surface area dose [µg/cm²]	1000	3	3
Total dose [µg/cell]	1000	3	3
Specific activity [kBq/mL]	3820	932.3	932.3
No. of donors	6	6	6
No. of replicates used/valid replicates*	6	6	5

Dilution 1 had one replicate with recovery of 88.29% the dilution was recalculated without this replicate (in Dilution 2).

Materials and methods

General information			
	Species	Human	
	Method	In vitro	
Test material			
Active substance	Name (Lot/Batch No.)	[difluorophenyl-UL-14C] Diflufenican	
	Test preparation	pre-formulated	
	Radiochemical purity	100	%
Product	Name (Lot/Batch No.)	ACL+DFF SC 600 (500+100)	
	Company code	102000029998	
	Concentration a.s.	100	g/L or g/kg
	Type of formulation	SC	
	Vehicle used (if any)		
Blank product	Name (Lot/Batch No.)		
	Concentration a.s.		g/L or g/kg
	Type of formulation		
Test system			
Diffusion cell	Type of diffusion cell	Flow-through	
	(If dynamic) Flow rate	1.5	mL/h
	Exposed skin area	1	cm^2
	Cover		
Skin sample	Skin type	Dermatomed	
	Skin thickness range	379-464	µm
	Skin donor age	not specified	years
	Skin donor sex	Female	
	Site	Abdomen	
	Source	Unknown	
	Integrity test	yes	
Receptor	Receptor medium	Eagle's medium supplemented with 5% bovine serum albumin	
	Solubility in receptor medium	acceptable- found to be 0.7 mg/mL compared to max concentration of diflufenican in receptor fluid of 2.6ng/mL	
Sampling	Exposure time	8	hours
	Sampling duration	24	hours
	Sample intervals	1	hours

	Skin wash/Swabbing	after 8 hours using 1% v/v Tween 80 in PBS (phosphate buffer saline) using a minimum of 15 tissue wipes. Further swabbing at 24 hrs
Tape strips	Tape stripping	Yes
	Type of tape strips used	Monaderm adhesive tape
	TS 1-2 analysed seperately?	Yes

Diflufenican Concentrate:

Replicate	1	2	3	4	5	6	n	MEAN	SD	MAX			
T0.5 <= 75 %											**Variation**	**DA corrected**	**DA rounded**
Absorbed dose	0.0000002	0.0000002	0.0000002	0.0000002	0.0000002	0.0000002		0.00	0.00	0.0000002			
Tape strips 3-x	0.02	0	0	0.01	0.02			0.02	0.01	0.02			
Skin preparation	0.11	0.12	0.06	0.02	0.08	0.08		0.08	0.04	0.12			
Sum	0.1300002	0.1200002	0.0600002	0.0300002	0.1000002	0.0800002		0.09	0.04	0.1300002			
Relevant data normalised	0.1300002	0.1200002	0.0600002	0.0300002	0.1000002	0.0800002		0.09	0.04	0.1300002			
Relevant data added	0.1300002	0.1200002	0.0600002	0.0300002	0.1000002	0.0800002		0.09	0.04	0.1300002			
Relevant data	0.1300002	0.1200002	0.0600002	0.0300002	0.1000002	0.0800002	6	**0.09**	**0.04**	**0.1300002**	**43.58**	**0.124**	**0.12**
T0.5 > 75 %													
Absorbed dose	0.0000002	0.0000002	0.0000002	0.0000002	0.0000002	0.0000002		0.00	0.00	0.0000002			
Skin preparation	0.11	0.12	0.06	0.02	0.08	0.08		0.08	0.04	0.12			
Sum	0.1100002	0.1200002	0.0600002	0.0200002	0.0800002	0.0800002		0.08	0.04	0.1200002			
Relevant data normalised	0.1100002	0.1200002	0.0600002	0.0200002	0.0800002	0.0800002		0.08	0.04	0.1200002			
Relevant data added	0.1100002	0.1200002	0.0600002	0.0200002	0.0800002	0.0800002		0.08	0.04	0.1200002			
Relevant data	0.1100002	0.1200002	0.0600002	0.0200002	0.0800002	0.0800002		**0.08**	**0.04**	**0.1200002**	**45.97**	**0.114**	**0.11**
Non-absorbed dose	103.2	99.61	107.39	96.74	98.69	97.02		100.44	4.12	107.39			
Total Recovery	**103.31**	**99.7300002**	**107.45**	**96.7700002**	**98.7900002**	**97.1000002**		**100.53**	**4.13**	**107.45**			
T0.5	100	100	100	100	100	100	6	100.00	0.00	100			
								Mean lower limit of confidence		100.00			
								k*SD		0			

Replicate	1	2		4		6		MEAN	SD	MAX
Donor ID	X2016/4	X2016/3	X2016/5	X2016/1	X2016/9	X2016/10				
Receptor fluid	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001		0.00	0.00	0.0000001
Receptor compartment wash	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001		0.00	0.00	0.0000001
Donor compartment wash	0.05	0	0.06	0.07	0.04	0		0.04	0.04	0.09
	103.41	99.5	107.05	96.08	98.3	96.87		100.04	4.10	107.06
	0.7	0.11	0.22	0.59	0.35	0.15		0.36	0.24	0.7
	0.02	0	0	0.01	0.02			0.01	0.01	0.02
Stripped skin	0.11	0.12	0.06	0.02	0.08	0.08		0.08	0.04	0.12
										0
Receptor fluid after 12 hours	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001		0.00	0.00	0.0000001
Receptor fluid after 24 hours	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001		0.00	0.00	0.0000001

Diflufenican Dilution:

Replicate	1	2	3	4	5	6	n	MEAN	SD	MAX			
T0.5 <= 75 %											**Variation**	**DA corrected**	**DA rounded**
Absorbed dose	0.0000002	0.0100001	0.1600001	0.0100001	0.2200001	0.1800001		0.10	0.10	0.2200001			
Tape strips 3-x	0	0.02	0.01	0.04	0.01	0.21		0.06	0.09	0.21			
Skin preparation	2.69	0.03	0.08	0.15	0.06	0.48		0.58	1.05	2.69			
Sum	2.6900002	0.0600001	0.2500001	0.2000001	0.2900001	0.8700001		0.73	1.00	2.6900002			
Relevant data normalised	2.88781556	0.06519624	0.2500001	0.22652633	0.3090697	0.8700001		0.77	1.07	2.88781556			
Relevant data added	9.54	8.03	0.2500001	11.91	6.46	0.8700001		6.18	4.71	11.91			
Relevant data	**9.54**	**8.03**	0.2500001	**11.91**	**6.46**	0.8700001	**6**	**6.18**	**4.71**	**11.91**	**76.28**	**10.888**	**11**
T0.5 > 75 %													
Absorbed dose	0.0000002	0.0100001	0.1600001	0.0100001	0.2200001	0.1800001		0.10	0.10	0.2200001			
Skin preparation	2.69	0.03	0.08	0.15	0.06	0.48		0.58	1.05	2.69			
Sum	2.6900002	0.0400001	0.2400001	0.1600001	0.2800001	0.6600001		0.68	1.01	2.6900002			
Relevant data normalised	2.88781556	0.0434642	0.2400001	0.18122109	0.29841213	0.6600001		0.72	1.08	2.88781556			
Relevant data added	9.54	8.01	0.2400001	11.87	6.45	0.6600001		6.13	4.75	11.87			
Relevant data	**9.54**	**8.01**	0.2400001	**11.87**	**6.45**	0.6600001	6	**6.13**	**4.75**	**11.87**	**77.51**	**10.879**	**11**
Non-absorbed dose	90.46	91.97	95.34	88.09	93.54	96.11		92.59	3.03	96.11			
Total Recovery	**93.1500002**	**92.0300001**	**95.5900001**	**88.2900001**	**93.8300001**	**96.9800001**		**93.31**	**3.03**	**96.9800001**			
T0.5	100	0.001	37.5	0.001	27.2727273	27.7777778	6	32.09	36.72	100			
							Mean lower limit of confidence	**-4.63**					
							k*SD	**36.7212347**					

Replicate	1	2	3	4	5	6	MEAN	SD	MAX
Donor ID	X2016/3	X2016/2	X2016/8	X2016/7	X2016/10	X2016/9			
Receptor fluid	0.0000001	0.01	0.16	0.01	0.22	0.18	0.10	0.10	0.22
Receptor compartment wash	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.00	0.00	0.0000001
Donor compartment wash	0	0	0	0.23	0	0.29	0.09	0.14	0.29
	90.44	91.86	94.52	83.45	92.3	93.86	91.84	4.08	94.52
1	0.02	0.11	0.82	0.41	0.24	1.96	0.26	0.70	1.96
2	0	0.02	0.01	0.06	0.01	0.21	0.05	0.08	0.21
Stripped skin	2.69	0.03	0.08	0.15	0.06	0.48	0.58	1.05	2.69
									0
Receptor fluid after 12 hours	0.0000001	0.0000001	0.06	0.0000001	0.06	0.05	0.03	0.03	0.06
Receptor fluid after 24 hours	0.0000001	0.01	0.16	0.01	0.22	0.18	0.10	0.10	0.22
	Replicant 4 has low recovery and has been excluded in the next spreadsheet								

Diflufenican Dilution excluding outlier:

Replicate	1	2	3	4	5	n	MEAN	SD	MAX			
T0.5 <= 75 %										**Variation**	**DA corrected**	**DA rounded**
Absorbed dose	0.0000002	0.0100001	0.1600001	0.2200001	0.1800001		0.11	0.10	0.2200001			
Tape strips 3-x	0	0.02	0.01	0.01	0.21		0.06	0.10	0.21			
Skin preparation	2.69	0.03	0.08	0.06	0.48		0.67	1.15	2.69			
Sum	2.6900002	0.0600001	0.2500001	0.2900001	0.8700001		0.83	1.08	2.6900002			
Relevant data normalised	2.88781556	0.06519624	0.2500001	0.3090697	0.8700001		0.88	1.16	2.8878156			
Relevant data added	9.54	8.03	0.2500001	6.46	0.8700001		5.03	4.23	9.54			
Relevant data	**9.54**	**8.03**	0.2500001	**6.46**	0.8700001	**5**	**5.03**	**4.23**	**9.54**	**84.08**	**10.105**	**10**
T0.5 > 75 %												
Absorbed dose	0.0000002	0.0100001	0.1600001	0.2200001	0.1800001		0.11	0.10	0.2200001			
Skin preparation	2.69	0.03	0.08	0.06	0.48		0.67	1.15	2.69			
Sum	2.6900002	0.0400001	0.2400001	0.2800001	0.6600001		0.78	1.09	2.6900002			
Relevant data normalised	2.88781556	0.0434642	0.2400001	0.29841213	0.6600001		0.83	1.17	2.8878156			
Relevant data added	9.54	8.01	0.2400001	6.45	0.6600001		4.98	4.28	9.54			
Relevant data	**9.54**	**8.01**	0.2400001	**6.45**	0.6600001	5	**4.98**	**4.28**	**9.54**	**85.94**	**10.116**	**10**
Non-absorbed dose	90.46	91.97	95.34	93.54	96.11		93.48	2.33	96.11			
Total Recovery	**93.1500002**	**92.0300001**	**95.5900001**	**93.8300001**	**96.9800001**		**94.32**	**1.97**	**96.98**			
T0.5	100	0.001	37.5	27.2727273	27.7777778	5	38.51	37.10	100			
						Mean lower limit of confidence	**-6.01**					
						k*SD	**44.5232**					

Replicate	1	2	3	4	5	MEAN	SD	MAX
Donor ID	X2016/3	X2016/2	X2016/8	X2016/10	X2016/9			
Receptor fluid	0.0000001	0.01	0.16	0.22	0.18	0.11	0.10	0.22
Receptor compartment wash	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.00	0.00	0.0000001
Donor compartment wash	0	0	0	0	0.29	0.06	0.13	0.29
	90.44	91.86	94.52	93.3	93.86	92.80	1.64	94.52
	0.02	0.11	0.82	0.24	1.96	0.63	0.81	1.96
	0	0.02	0.01	0.01	0.21	0.05	0.09	0.21
Stripped skin	2.69	0.03	0.08	0.06	0.48	0.67	1.15	2.69
								0
Receptor fluid after 12	0.0000001	0.0000001	0.06	0.06	0.05	0.03	0.03	0.06
Receptor fluid after 24	0.0000001	0.01	0.16	0.22	0.18	0.11	0.10	0.22
								0