Bayer CropScience

Document Title

Summary of the toxicological studies Diflufenican+Flufenacet SC600 (200+400)G

Data Requirements

EU Regulation 1107/2009 & EU Regulation 284/2013

Document MCP

Section 7: Toxicological studies

According to the guidance document, SANCO 10181/2013, for preparing dossiers for the approval of a chemical active substance

Date

2014-03-14

Author(s)

[redacted]

Bayer CropScience

OWNERSHIP STATEMENT

This document, the data contained in it and copyright therein are owned by Bayer CropScience. No part of the document or any information contained therein may be disclosed to any third party without the prior written authorisation of Bayer CropScience.

The summaries and evaluations contained in this document are based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority. Other registration authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this document unless they have received the data on which the summaries and evaluation are based, either:

- **From Bayer CropScience; or**
- **From other applicants once the period of data protection has expired.**

Version history

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

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

Table of Contents

CP 7 TOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

INTRODUCTION

This document summarises the information related to the toxicological studies for the plant protection product DFF+FFA SC 200+400 which contains the active substances flufenacet and diflufenican.

Flufenacet was included into Annex I of Directive 91/414 in 2003 (Directive 2003/84/EC).
Diflufenican was included into Annex I of Directive 91/414 in 2008 (Directive 2008/66/EC).

This product was the representative formulation for the inclusion of diflufenican into Annex I of Directive 91/414/EEC and has thus been evaluated according to Uniform Principles.

The Review Report for flufenacet (7469/VI/98-Final – 3rd July 2003) is considered to provide the relevant scientific information for the review of the product.

The following table summarises the flufenacet EU endpoints and where different those used in the evaluation.

End-Point	**Flufenacet 'EU end-points'** (7469/VI/98-Final – 3rd July 2003)	**Flufenacet end-points used when different from EU end-point**
AOEL	0.017 mg/kg bw/d (90 day and 1 year dog study with a safety factor of 100)	
Dermal penetration*	Concentrate: 10% Spray dilutions: 60% (*In vitro* human skin performed with FOE 5043 60WG))	Concentrate: 0.2% Spray dilutions: 4.7% (*In vitro* human/rat skin study performed with DFF+FFA SC 200+400)

*Since the inclusion of flufenacet into Annex I a study has been performed to assess the dermal absorption of flufenacet in the formulation DFF+FFA SC 200+400.

CP 7.1 Acute toxicity

Summary of acute toxicity

The formulation assessed in this dossier was the representative formulation for the inclusion of diflufenican into Annex I of Directive 91/414/EEC.

All the acute toxicity studies were evaluated by the Member States as part of the European review and were considered to be acceptable. No new studies have been performed.

The toxicological studies were performed with the formulated product DFF+FFA SC 200+400 which is in accordance to Specification 102000007948. The specification of the product has not changed significantly since the EU review of diflufenican and therefore all the studies are considered to be valid for this submission. Full details of the formulation specification and related bridging statements can be found in the confidential part of this submission (Document J of the product dossier).

The table below summarises the results from the acute toxicological studies conducted with the formulated product DFF+FFA SC 200+400.

At the time of study conduct the test substance was named FOE 5043 400 SC & DFF 200.

Study	Result	Reference
Acute oral rat	LD_{50}: >500 <2000 mg/kg bw	[redacted], F., 2002 CP 7.1.1/01, [M-055334-01-1]
Acute dermal rat	LD_{50}: >4000 mg/kg bw	[redacted], F., 2002 CP 7.1.2/01, [M-055277-01-1]
Acute inhalation rat	LC_{50}: >2078 mg/m³ (max. techn. attainable concentration)	[redacted], J., 2002 CP 7.1.3/01, [M-036417-02-1]
Skin irritation rabbit	Not irritating	[redacted], J., 2001 CP 7.1.4/01, [M-083086-02-1]
Eye irritation rabbit	Not irritating	[redacted], J., 2001 CP 7.1.5/01, [M-083083-01-1]
Skin sensitization guinea pig (maximisation test)	Sensitising	[redacted], H. W., 2002 CP 7.1.6/01, [M-071813-01-1]

The test item is moderately toxic after acute oral administration and non-toxic by dermal and inhalation routes of exposure in rats. It is not irritating when applied to the skin and eyes of rabbits. The test item is positive for skin sensitization using the maximisation test.

The following classification/labelling is triggered:

- EU directive 1999/45/EC (as amended): Xn (harmful)
  R22 (harmful if swallowed)
  R43 (may cause sensitisation by skin contact)
  R48/22 (harmful: danger of serious damage to health by prolonged exposure if swallowed; derived from the classification of flufenacet)

- Regulation (EC) No 1272/2008 (CLP): Acute Tox Cat. 4, H302 (harmful if swallowed)
  Skin sensitisation Cat. 1, H317 (may cause an allergic skin reaction)
  STOT Re 2, H373 (may cause damage to organs through prolonged repeated exposure; derived from the classification of flufenacet)

CP 7.1.1 Oral toxicity

Report:	**CP 7.1.1/01, [redacted], F., 2002**
Title:	FOE 5043 400 SC & DFF 200 (c.n.: Flufenacet & Diflufenican) - Study for acute oral toxicity in rats
Document No:	31921 [M-055334-01-1]
Guidelines:	OECD 423; Directive 67/548/EEC, Annex IV B, Part B, B.1 tris; US-EPA OPPTS 870.1100; Deviation(s): The test substance is a commercial product known to be stable and homogenous in both undiluted and in ready-to-use dilution with water. Therefore, analytical determinations of stability and homogeneity of the aqueous formulations were not performed.
GLP	Yes

I. Materials and methods

A. Materials

1. Test material: FOE 5043 SC 400 & DFF 200

Development no.: 3000248463
Description: beige white suspension
Lot/Batch no: 07205024 (0006)
Content: flufenacet: 406.52 g/L, diflufenican: 205.76 g/L
Stability of test compound: guaranteed for study duration; expiry date: 2002-03-05

2. Vehicle: demineralised water

3. Test animals

Species: Wistar rat
Strain: HsdCpb:Wu
Age: males: approx. 8 - 9 weeks; females: approx. 8 weeks
Weight at dosing: males: 210 g - 224g; females: 154g - 163g
Source: [redacted], Germany
Acclimatisation period: at least 5 days

Diet:	"NAFAG® No. 9441 W 10" (Eberle Nafag AG, Gossau, Switzerland)
Water:	tap water
Housing:	group caged conventionally in polycarbonate cages; bedding: low-dust wood granules type BK 8/15 (Ssniff, Spezialdiaeten GmbH, Soest, Germany)

B. Study design and methods

1. Animal assignment and treatment

Dose:	500 - 2000 mg/kg bw
Application route:	oral
Application volume:	10 mL/kg bw
Fasting time:	before administration: approx. 17 ± 1 hours after administration: approx. 2 hours
Group size:	3 rats/sex/group
Post-treatment observation period:	14 days
Observations:	mortality, clinical signs, body weight, gross necropsy

II. Results and discussion

A. Mortality

Table 7.1.1-1 Doses, mortality / animals treated

Dose [mg/kg bw]	Toxicological result*	Duration of signs	Time of death	Mortality [%]
Male rats				
500	0/3/3	45 – 3d	--	0
Female rats				
2000	3/3/3	1h – 5h	2h – 5h	100
500	0/3/3	1h – 4d	--	0
LD_{50}: >500 mg/kg bw				

* 1st number = number of dead animals, 2nd number = number of animals with toxic signs, 3rd number = number of animals used

B. Clinical observations

At 500 mg/kg bw gait was uncoordinated, and breathing laboured in both sexes. Additionally, in males motility was decreased, and in females gait high legged.

At 2000 mg/kg in females motility and reactivity were decreased, gait uncoordinated and spastic, position abdominal, breathing laboured, and in one female atony and in one animal increased salivation was observed.

The signs observed started 45 minutes after administration and lasted up to day 4.

C. Body weight

There were no toxicological effects on body weights or on body weight development in males and females.

D. Necropsy

In animals that died during the observation period the following changes were detected:
Pale discoloration of the liver and the spleen and partly dark-red spotted discoloration of the slightly collapsed lung.
No gross pathologic changes were observed in animals sacrificed at the end of the study period.

III. Conclusion

The test item is moderately toxic to fasted male and female rats after acute oral exposure.
The study result triggers the following classification/labelling:
- EU directive 1999/45/EC (as amended): Xn (harmful)
  R22 (harmful if swallowed)
- Regulation (EC) No 1272/2008 (CLP): acute Tox Cat. 4
  H302 (harmful if swallowed)

CP 7.1.2 Dermal toxicity

Report:	**CP 7.1.2/01, [redacted], F., 2002**
Title:	FOE 5043 400 SC & DFF 200 (c.n.: Flufenacet & Diflufenican) – Study for acute dermal toxicity in rats
Document No:	31920 [M-055277-01-1]
Guidelines:	OECD 402; US-EPA 712-C-98-192, OPPTS 870.1200; Directive 67/548/EEC, Annex V, Part B.3.; Deviation(s): none
GLP	Yes

I. Materials and methods

A. Materials

1. Test material: FOE 5043 400 SC & DFF 200

Development no.:	300248463
Description:	beige white suspension
Lot/Batch no.:	07205/0024 (0006)
Content:	flufenacet 406.52 g/L, diflufenican: 205.76 g/L
Stability of test compound:	guaranteed for study duration; expiry date: 2002-03-05

2. Vehicle: none

3. Test animals

Species:	Wistar rat
Strain:	HsdCpb:WU
Age:	males: approx. 9 weeks; females: approx. 12 weeks
Weight at dosing:	males: 233 g - 258 g; females: 207 g - 224 g
Source:	[redacted], Germany
Acclimatisation period:	at least 5 days
Diet:	"NAFAG® No. 9441 W 10" (Eberle Nafag AG, Gossau, Switzerland)
Water:	tap water

Housing:	individually in polycarbonate cages; bedding: low-dust wood granules type BK 8/15 (Ssniff, Spezialdiaeten GmbH, Soest, Germany)			

B. Study design and methods

1. Animal assignment and treatment

Dose:		Dose (mg/kg bw)	Surface area (cm²)	Range (mg/cm²)
	males	4000	20.0	46.6 - 51.6
	females	4000	20.0	52.6 - 56.9

Application route:	dermal, semi-occlusive dressing
Exposure:	24 hours
Group size:	5 rats/sex/group
Post-treatment observation period:	14 days
Observations:	mortality, clinical signs, skin effects, body weight, gross necropsy

II. Results and discussion

A. Mortality

Table 7.1.2-1 Doses, mortality / animals treated

Dose (mg/kg bw)	Toxicological results*			Occurrence of signs	Time of death	Mortality [%]
Male rats						
4000	0	2	5	2d	--	--
Female rats						
4000	0	2	5	2d	--	--
LD$_{50}$: >4000 mg/kg bw						

* 1st number = number of dead animals, 2nd number = number of animals with signs, 3rd number = number of animals in the group

B. Clinical observations

At 4000 mg/kg in two males and two females gait was uncoordinated on day 2. This effect is considered as most probably due to the occlusive dressing.

Locally, the treatment area was yellowish discolored. The discoloration started on day 2 and lasted up to day 15.

C. Body weight

Body weight and body weight gain of males were not affected by treatment.

In one female a transient body weight decrease occurred (day 8), probably due to the stress caused by occlusive dressing. At the end of the recovery period, in one female the body weight was decreased. This difference to the previous week is regarded as not toxicological significant since the animals attained their adult weight and the observed body weight change is in the biological range.

D. Necropsy

The gross pathology investigations performed at the end of the post-treatment observation period did not afford any treatment-related findings.

III. Conclusion

The test item is non-toxic after acute dermal exposure.

The study result triggers the following classification/labelling:
- EU directive 1999/45/EC (as amended): none
- Regulation (EC) No 1272/2008 (CLP): none

CP 7.1.3 Inhalation toxicity

Report:	**CP 7.1.3/01, [redacted], J., 2002**
Title:	1st revised version of report no. 31766 as of 2002-02-13 – FOE 5043 400 SC & DFF 200 (c.n.: Flufenacet, Diflufenican) - Study on acute inhalation toxicity in rats according to OECD No. 403
Document No:	32133 [M-036417-02-1]
Guidelines:	OECD 403; Directive 92/69/EEC; US- EPA-712C-98-193; OPPTS 870.1300; Deviation(s): none
GLP	Yes

I. Materials and methods

A. Materials

1. Test material: FOE 5043 400 SC & DFF 200

Development no.:	3000248462
Description:	beige white suspension
Lot/Batch no.:	07205/0024 (0006)
Content:	flufenacet: 406.52 g/L, diflufenican: 205.76 g/L
Stability of test compound:	guaranteed for study duration, expiry date: 2002-03-05

2. Vehicle: deionised water

3. Test animals

Species:	Wistar rat
Strain:	Hsd Cpb:WU
Age:	approx. 2 months
Weight at dosing:	males: 188 g - 214 g; females: 164 g - 176 g
Source:	[redacted], Germany
Acclimatisation period:	at least 5 days
Diet:	standard fixed-formula diet (NAFAG No. 9441 W10 pellets maintenance diet for rats and mice)
Water:	tap water
Housing:	singly in conventional Makrolon® Type II cages; bedding: type BK8/15 low-dust wood granulate (Ssniff, Soest, Germany)

B. Study design and methods

1. Animal assignment and treatment

Dose:	0 - 2078 mg/m³ air (max. techn. attainable concentration)
Application route:	inhalation, nose-only

Exposure:	4 hours
Group size:	5 rats/sex/group
Post-treatment observation period:	2 weeks
Observations:	mortality, clinical signs, body weights, body temperature, reflex measurements, gross necropsy

2. Generation of the test atmosphere / chamber description

Generation and characterization of chamber atmosphere

	Group 1	Group 2
Target concentration (mg/m³)	control (water)	5000
Nominal concentration (mg/m³)	--	16162
Gravimetric concentration (mg/m³)[1)]	--	1463
Actual concentration (mg/m³)	--	2078
Temperature (mean, °C)	21.6	21.6
Relative humidity (mean, %)	>95	~95
MMAD (µm)	--	3.58
GSD	-	2.14
Aerosol mass < 3 µm (%)	--	40.9
Mass recovered (mg/m³)	--	1392

MMAD = Mass Median Aerodynamic Diameter, GSD = Geometric Standard Deviation, - = not applicable, [1)] Conversion to test substance: filter mass x 100/70.4, [2)] Relative fraction of actual concentration to nominal concentration.

II. Results and discussion

A. Mortality

Table 7.1.3-1 Doses, mortality / animals treated

Actual concentration (mg/m³)	Toxicological result*			Occurrence of signs	Time of death	Rectal temperature (°C)
Male rats						
0	0	0	5	--	--	38.2
2078	0	0	5	--	--	33.0 **
Female rats						
0	0	0	5	--	--	38.3
2078	0	0	5	--	--	34.6 **
LC_{50}: >2078 mg/m³ (maximum technically attainable concentration)						

* 1st number = number of dead animals, 2nd number = number of animals with signs after cessation of exposure, 3rd number = number of animals exposed

B. Clinical observations

All rats tolerated the exposure without specific signs.
In a battery of reflex measurements made on the first post-exposure day, none of the rats exposed to the test substance group experienced any abnormal reflexes in comparison to the rats of the control group.
Statistical comparisons of rectal temperatures between control animals with those in the exposure group revealed a statistically significant decrease in body temperature.

C. Body weight

Comparisons between the control and exposure group did not reveal any remarkable effect on body weight gains.

D. Necropsy

Animals sacrificed at the end of the observation period;
Macroscopic findings were not observed.

III. Conclusion

The test item (liquid aerosol) proves to have essentially no acute inhalation toxicity to rats.

The study result triggers the following classification/labelling:
- EU directive 1999/45/EC (as amended): none
- Regulation (EC) No 1272/2008 (CLP): none

CP 7.1.4 Skin irritation

Report:	**CP 7.1.4/01, [redacted], J., 2001**
Title:	Acute skin irritation test (patch test) of FOE 5043 400 SC & DFF 200 in rabbits - revised version of report no. 8085 from October 23rd, 2001 -
Document No:	R8100 [M-083086-02-1]
Guidelines:	OECD 404; EC guideline B.4.; Deviation(s): none
GLP	yes

I. Materials and methods

A. Materials

1. Test material: FOE 5043 400 SC & DFF 200
- Development no.: 3000248463
- Description: beige white suspension
- Lot/Batch no.: 07205/0024 (0006)
- Content: flufenacet: 406.52 g/L, diflufenican: 205.76 g/L
- Stability of test compound: guaranteed for study duration; expiry date: 2002-03-05

2. Vehicle: none

3. Test animals
- Species: rabbit
- Strain: Himalayan
- Age: approx. 4.5 months
- Weight at dosing: 2.4 kg - 2.7 kg

Source: [redacted],

Acclimatisation period: at least 20 days

Diet: Altromin 2023 (ALTROMIN GmbH, Lage, Germany)

Water: tap water

Housing: during exposure: singly in special restrainers which allowed free movement of the head but prevented a complete body turn: before/ after exposure: kept separately in cages with dimensions of 425 mm x 600 mm x 380 mm (Dipl. Ing. W. EHRET GmbH, Schoenwalde, Germany)

B. Study design and methods

1. Animal assignment and treatment

Dose: 0.5 mL/patch

Application route: dermal

Exposure: 4 hours

Group size: 3 males

Observations: clinical signs, skin effects, body weight (at beginning of study)

II. Results and discussion

A. Findings

There were no systemic intolerance reactions.

Table 7.1.4-1 Summary of irritant effects (Score)

Animal	Observation (after patch removal)	24h	48h	72h	Mean scores	Response	Reversible (days)
1	Erythema (redness) and eschar formation	0	0	0	0.0	--	na
	Oedema formation	0	0	0	0.0	--	na
2	Erythema (redness) and eschar formation	0	0	0	0.0	--	na
	Oedema formation	0	0	0	0.0	--	na
3	Erythema (redness) and eschar formation	0	0	0	0.0	--	na
	Oedema formation	0	0	0	0.0	--	na

na = not applicable

Response: -- = negative for mean scores

+ = irritant for mean scores

<2	(Directive 1999/45/EC as amended)
<2.3	(Regulation (EC) No 1272/2008)
≥2	(Directive 1999/45/EC as amended)
≥2.3	(Regulation (EC) No 1272/2008 category 2)

III. Conclusion

The test item is not irritating to the skin of rabbits.

The study result triggers the following classification/labelling:

- EU directive 1999/45/EC (as amended): none
- Regulation (EC) No 1272/2008 (CLP): none

CP 7.1.5 Eye irritation

Report:	**CP 7.1.5/01, [redacted], J. , 2001**
Title:	Acute eye irritation study of FOE 5043 400 SC & DFF 200 by instillation into the conjunctival sac of rabbits
Document No:	R8086 [M-083083-01-1]
Guidelines:	OECD 405; EC guideline B.5.; Deviation(s): none
GLP	yes

I. Materials and methods

A. Materials

1. Test material: FOE 5043 400 SC & DFF 200

Development no.: 3000248463
Description: beige white suspension
Lot/Batch no: 07205/0024 (0006)
Content: flufenacet: 406.52 g/L, diflufenican: 205.76 g/L
Stability of test compound: guaranteed for study duration; expiry date: 2002-03-05

2. Vehicle: none

3. Test animals

Species: rabbit
Strain: Himalayan
Age: approx. 4.5 months
Weight at dosing: 2.5 kg - 2.8 kg
Source: [redacted], Germany
Acclimatisation period: at least 20 days
Diet: Altromin 2023 (ALTROMIN GmbH, Lage, Germany)
Water: tap water
Housing: for 8 hours following application: singly in special restrainers which allowed free movement of the head but prevented a complete body turn and wiping of the eyes; acclimatization/after the 8-hour period: separately in cages with dimensions of 425 mm x 600 mm x 380 mm (Dipl. Ing. W. EHRET GmbH, Schoenwalde, Germany)

B. Study design and methods

1. Animal assignment and treatment

Dose: 0.1 mL/animal
Application route: instillation into the conjunctival sac
Rinsing: no
Group size: 3 males

Observations: clinical signs, eye effects, body weight (at beginning of study)

II. Results and discussion

A. Findings

Conjunctival redness (grade 1) was observed in all animals 1 hour after instillation. The cornea and the iris were not affected by instillation of the test compound.

There were no systemic intolerance reactions.

Table 7.1.4-1 Summary of irritant effects (Score)

Animal	Effects	24 h	48 h	72 h	Mean scores	Response	Reversible (days)
1	Corneal opacity	0	0	0	0.0	--	na
	Iritis	0	0	0	0.0	--	na
	Redness conjunctivae	0	0	0	0.0	--	1*
	Chemosis conjunctivae	0	0	0	0.0	--	na
2	Corneal opacity	0	0	0	0.0	--	na
	Iritis	0	0	0	0.0	--	na
	Redness conjunctivae	0	0	0	0.0	--	1*
	Chemosis conjunctivae	0	0	0	0.0	--	na
3	Corneal opacity	0	0	0	0.0	--	na
	Iritis	0	0	0	0.0	--	na
	Redness conjunctivae	0	0	0	0.0	--	1*
	Chemosis conjunctivae	0	0	0	0.0	--	na

Response for mean scores:		Corneal opacity	Iritis	Conjunctival redness	Conjunctival oedema	
--	= negative	<1	<1	<2	<2	(Regulation (EC) No. 1272/2008)
		<2	<1	<2.5		(Directive 1999/45/EC as amended)
+	= irritant	≥1 - <3	≥1 - <1.5	≥2	≥2	(Regulation (EC) No. 1272/2008 category 2)
		≥2 - <3	≥1 - <2	≥2 - <2.5	≥2	(Directive 1999/45/EC as amended)
++	= irreversible effects/ serious damage	≥3	≥1.5			(Regulation (EC) No. 1272/2008 category 1)
		≥3	≥2			(Directive 1999/45/EC as amended)

na : not applicable, *: in respect of the result 1 h post application

III. Conclusion

The test item is not irritating to the eyes of rabbits.

The study result triggers the following classification/labelling:

- EU directive 1999/45/EC (as amended): none
- Regulation (EC) No.1272/2008 (CLP): none

CP 7.1.6 Skin sensitization

Report:	**CP 7.1.6/01, [redacted], H. W., 2002**
Title:	FOE 5043 400 SC & DFF 200 - Study for the skin sensitization effect in guinea pigs (guinea pig maximization test according to Magnusson and Kligman)
Document No:	32190 [M-071813-01-1]
Guidelines:	OECD 406; Guideline 96/54/EC, Method B.6.; US-EPA 712-C-98-197, OPPTS 870.2600; Deviation(s): The test item contains commercial products known to be stable and homogenous both undiluted and in ready-to-use dilution with water. Therefore, analytical determinations of the stability and homogeneity of the formulations in physiological saline solution for administration were not performed. This deviation did not limit the assessment of the results.
GLP	yes

I. Materials and methods

A. Materials

1. Test material: FOE 5043 400 SC & DFF 200

Development no.: 3000248463
Description: beige white suspension
Lot/Batch no: 07205/0024 (0006)
Content: flufenacet: 406.52 g/L; diflufenican: 205.76 g/L
Stability of test compound: guaranteed for study duration; expiry date: 2002-03-05

2. Vehicle: physiological saline solution

3. Test animals

Species: guinea pig
Strain: Hsd Poc:DH
Age: 5 weeks
Weight at dosing: 231 g - 417 g
Source: [redacted] Germany
Acclimatisation period: at least 5 days
Diet: "PROVIMI KLIBA 3420 - Maintenance Diet for Guinea Pigs" (PROVOMI KLIBA AG)
Water: tap water
Housing: conventionally in type IV Makrolon® cages; adaptation: in groups of five, period: in groups of two or three per cage; bedding: low-dust wood shavings (Ssniff Spezialdiaeten GmbH, Soest, Germany

B. Study design and methods

1. Animal assignment and treatment

Dose
Intradermal induction: 2.5% (10 mg test item/animal)
Topical induction: 100% (500 mg test item/animal)

1st Challenge:	100% (500 mg test item/animal)
2nd Challenge:	50% (250 mg test item/animal)
Application route:	intradermal, dermal
Application volume:	intradermal: 0.1 mL/injection, topical: 0.5 mL/patch
Exposure:	topical induction: 48 hours, challenge: 24 hours
Group size:	37 animals (control: 10, test item: 20, range-finding: 7)
Observations:	mortality, clinical signs, skin effects, body weight (at beginning and termination of study)

II. Results and discussion

A. Findings

48 hours after the intradermal induction (1st induction):

- control group showed red wheals
- test item group showed red wheals and encrustations.

7 days after the 1st induction the following effects were recorded at the injection sites:

- control group showed wheals and encrustations
- test item group showed wheals and encrustations

Second induction (topical) from day 10 to the end of the study:

- test item group showed encrustation on the treatment area.

The 1st challenge with the 100% test item concentration led to skin effects (grade 1-3) in all test item group animals and in 6 animals (60%) of the control group (grade 1).
The 2nd challenge with the 50% test item formulation led to skin effects (grade 1-3) in 18 of 20 animals (90%) in the test item group and to no skin effects in the control group.

Appearance and behaviour of the test item group were not different from the control group.
At the end of the study, the mean body weight of the treatment group animals was in the same range than that of the control group animals.

Table 7.1.6-1 Number of animals exhibiting skin effects

	Test item group (20 animals)					Control group (10 animals)				
	Test item patch			Control patch		Test item patch			Control patch	
Hours	48	75	Total	48	72	48	72	Total	48	72
1st Challenge 100%	20	20	20	0	0	6	0	6	0	0
2nd Challenge 50%	18	17	18	0	0	0	0	0	0	0

III. Conclusion

Under the conditions of the Maximization Test and with respect to the evaluation criteria the test item exhibits a skin-sensitisation potential.

The following classification/labelling is triggered:

- EU directive 1999/45/EC (as amended): Xi
  R43 (may cause sensitization by skin contact)
- Regulation (EC) No 1272/2008 (CLP): Skin sensitisation Cat. 1;
  H317 (may cause an allergic skin reaction)

CP 7.1.7 Supplementary studies on the plant protection product

No supplementary studies were performed.

CP 7.1.8 Supplementary studies for combinations of plant protection products

No supplementary studies were performed since this plant protection product is not recommended to be combined with other plant protection products.

CP 7.2 Data on exposure

CP 7.2.1 Operator exposure

Diflufenican+Flufenacet SC 600 (200+400) is a herbicide with a broad spectrum of activity for the control of Alopecurus myosuroides, Apera spica-venti, Poa annua and annual dicot weeds in winter wheat, winter barley and winter rye. The product is formulated as a suspension concentrate (SC) containing 200 g/L diflufenican and 400 g/L flufenacet as active substances. Applications of Diflufenican+Flufenacet SC 600 (200+400) will be conducted via field crop sprayers during the growth stage "post-emergence" (BBCH 10-25). Water will be the diluent/carrier in all situations.

A summary of the proposed use and a selection of the critical GAP (cGAP) used for operator risk assessment is presented in Table CP 7.2.1-1.

Table CP 7.2.1-1 Summary of proposed uses

Application technique	Crop(s)	F G*	Maximum application rate			Min. Spray volume (L/ha)
			(L product/ha)	(kg a.s./ha)		
				Diflufenican	Flufenacet	
Tractor mounted boom sprayer	Winter wheat, Winter barley, Winter rye	F	0.6	0.12	0.24	100

*F = Field use, G = Greenhouse use

Operator exposure to Diflufenican + Flufenacet SC 600 (200+400) was not evaluated as part of the EU review of diflufenican or flufenacet. Therefore, all relevant data and risk assessments are provided here and are considered adequate.

As this submission is intended for Annex 1 renewal (AIR) of Flufenacet, the present risk assessment only considers the exposure to flufenacet, not to diflufenican. Additional exposure assessments to diflufenican will be conducted in post-AIR process dossier for Diflufenican + Flufenacet SC 600 (200+400).

- Consideration on AOEL

An Acceptable Operator Exposure Level (AOEL) of 0.017 mg/kg bw/day is set for flufenacet by the EU (Flufenacet, 7469/VI/98-Final, 3 July 2003). It is based on a NOAEL of 1.67 mg/kg bw/day established in 90-day dog study and an assessment factor of 100.

- Consideration on dermal absorption

The following dermal absorption values for flufenacet will be used in the present risk assessment:

0.2% for the concentrate
4.7% for the in-use dilution

For further information please refer to CP 7.3 of this document.

- Summary of operator exposure

Operator exposure to Diflufenican + Flufenacet SC 600 (200+400) is estimated using the German model[1] and the UK-POEM[2] with the relevant scenario "Tractor-mounted/trailed boom sprayer: hydraulic nozzles". Details are given in CP 7.2.1-2 and in Tables CP 7.2.1.1-1 and 7.2.1.1-2.

Results of the exposure calculations are summarized in Table 7.2.1-2.

Table CP 7.2.1-2: Predicted systemic exposure as a proportion of the AOEL

Substance	PPE	Total systemic exposure (mg/kg bw/day)	% of AOEL[#]
	German model		
Flufenacet	No PPE [1)]	0.00211	12
	With PPE [2)]	0.000576	3
	UK-POEM		
Flufenacet	No PPE [1)]	0.0809	476
	With PPE [2)]	0.0145	85

[#] Flufenacet: AOEL = 0.017 mg/kg bw/day

1) One layer of typical work wear (e.g. trousers and a long sleeved shirt) as well as sturdy foot wear
2) In addition to typical work wear (see 1) protective gloves are worn during mixing and loading as well as during application.

Assessment

[1] [redacted], J.R.; [redacted], D.; [redacted], H.; [redacted], B.; [redacted], S.; [redacted], W.; [redacted], E.-D. (1992): Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products (Uniform Principles for Operator Protections); Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, no 277, 1 - 112 (1992); (M-001230-02-1)

[2] Scientific Subcommittee on Pesticides and British Agrochemicals Joint Medical Panel., Estimation of Exposure and Absorption of Pesticides by Spray Operators (UK MAFF) 1986 and the Predictive Operator Exposure Model (POEM) – A User's Guide (UK MAFF); 1992, revised model 2007

The results of the calculations reveal that the situation regarding operator exposure is favourable for the intended use of Diflufenican + Flufenacet SC 600 (200+400).

German Model
For flufenacet, predicted systemic operator exposure accounts for 12% of the systemic AOEL (0.017 mg/kg bw/day) without PPE and to 3% when gloves are worn during mixing/loading and application.

UK-POEM
For flufenacet, predicted systemic operator exposure accounts for 476% of the proposed systemic AOEL if no PPE is considered. Assuming that in addition to the typical work wear protective gloves are worn when handling the concentrate and during the application the corresponding exposure estimate for flufenacet accounts for 85% of the respective systemic AOEL.

Based on these favourable exposure estimates there is no unacceptable risk anticipated for the operator with the intended use of Diflufenican + Flufenacet SC 600 (200+400) if adequate work clothing is worn and, in addition, protective gloves during mixing/loading and application.

CP 7.2.1.1 Estimation of operator exposure

Operator exposure to Diflufenican + Flufenacet SC 600 (200+400) is estimated using the German Model, as well as the UK-POEM for Tractor-mounted/trailed boom sprayer: hydraulic nozzles.
In the following the assumptions used for the calculations are summarised.

German Model

Treated area:	20 ha/day
Max. dose rate:	0.6 L product/ha, i.e.
- Flufenacet:	0.24 kg a.s./ha
Operator body weight:	70 kg

UK-POEM

Treated area:	20 ha/day
Max. dose rate:	0.6 L product/ha, i.e.
- Flufenacet:	0.24 kg a.s./ha
Min. spray volume:	100 L/ha
Max. spray concentration:	
- Flufenacet:	2.4 mg/mL
Work duration:	6 hours/day
Operator body weight:	60 kg

For both models:

Clothing:	One layer of typical work wear (e.g. trousers and a long sleeved shirt) and sturdy foot wear
Dermal absorption:	
- Flufenacet:	0.2% for the concentrate and 4.7% for the in-use dilution

Personal protective equipment (PPE):

No PPE: No additional PPE is worn during mixing/loading and application
With PPE: Gloves are worn during mixing/loading and during the application

It should be noted that this selection of protective measures is not intended to be a recommendation for the minimum PPE necessary when handling Diflufenican + Flufenacet SC 600 (200+400). It does not consider specific requirements which may exist in individual member states. Additional PPE can be used to further reduce the exposure of the operator.

Taking into account the relevant model parameters exposure estimates are presented in Table CP 7.2.1.1-1 CP 7.2.1.1-2.

Table CP 7.2.1.1-1. Predicted systemic exposure to Flufenacet according to the German model no PPE and with PPE

Operator exposure estimate: German model: Tractor-mounted/trailed boom sprayer: hydraulic nozzles

Product:	Dilfufenican + Flufenacet SC 600 G				
Active substance:	Flufenacet	a.s. concentration:	400	[g/l or kg]	
Formulation:	Liquid	PPE during mix/loading:	Respiration:	None	
Dose [l or kg/ha]:	0.6		Hands:	Gloves	
Work rate [ha/day]:	20	PPE during application:	Respiration:	None	
Body weight [kg]:	70		Hands:	Gloves	
Inhalation absorption [%]	100		Head:	None	
Dermal absorption [%]	0.2 (concentrate)		Body:	Standard protective coverall	
	4.7 (dilution)				

Calculation of route exposure:

Route	Specific exposure [mg/kg a.s.]	a.s. handled [kg/day]	Estimated exposure [mg/kg bw/day] No PPE	Reduction factor	with PPE	
						I = Inhalation
I_M =	0.0006	4.8	0.000041	1.0	0.000041	D = Dermal
$D_{M(H)}$ =	2.4	4.8	0.1646	0.01	0.001646	M = Mix/Loading
I_A =	0.001	4.8	0.000069	1.0	0.000069	A = Application
$D_{A(C)}$ =	0.06	4.8	0.0041	1.0	0.004114	H = Hands
$D_{A(H)}$ =	0.38	4.8	0.0261	0.01	0.000261	C = Head
$D_{A(B)}$ =	1.6	4.8	0.0055	0.05	0.005486	B = Body

Absorbed dose:

Route		Absorption [%]	No PPE: Estimated route exposure [mg/kg bw/day]	No PPE: Systemic exposure [mg/kg bw/day]	With PPE: Estimated route exposure [mg/kg bw/day]	With PPE: Systemic exposure [mg/kg bw/day]
Dermal:	Mix/Loading	0.2	0.164571	0.000329	0.001646	0.000003
	Application	4.7	0.035657	0.001676	0.009861	0.000463
Inhalation:	Mix/Loading	100	0.000041	0.000041	0.000041	0.000041
	Application	100	0.000069	0.000069	0.000069	0.000069
		Total =		**0.00211**		**0.000576**

Table CP 7.2.1.1-2. Predicted systemic exposure to Flufenacet according to the UK-POEM no PPE and with PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles		
Product	**Diflufenican+Flufenacet SC 600**	Active substance	**Flufenacet**
Formulation type	water-based	a.s. concentration	**400** mg/ml
Dermal absorption from product	**0.2** %	Dermal absorption from spray	**4.7** %
Container	5 litres 45 or 63 mm closure		
PPE during mix/loading	Gloves	PPE during application	Gloves
Dose	**0.6** l/ha	Work rate/day	**20** ha
Application volume	**100** l/ha	Duration of spraying	**6** h

EXPOSURE DURING MIXING AND LOADING

Container size	5	litres
Hand contamination/operation	0.01	ml
Application dose	0.6	litres product/ha
Work rate	20	ha/day
Number of operations	3	/day
Hand contamination	0.03	ml/day
Protective clothing	None	Gloves
Transmission to skin	100 %	5 %
Dermal exposure to formulation	0.030 ml/day	0.0015 ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles					
Application volume	100 spray/ha					
Volume of surface contamination	10 ml/h					
Distribution	Hands	Trunk	Legs			
	65 %	10 %	25 %			
Clothing	None	Permeable	Permeable	Gloves	Permeable	Permeable
Penetration	100 %	1 %	15 %	10	5	15 %
Dermal exposure	65	0.05	0.375 ml/h	0.65	0.05	0.375 ml/h
Duration of exposure	6 h			6 h		
Total dermal exposure to spray	41.550 ml/day			6.450 ml/day		

ABSORBED DERMAL DOSE

	Mix/load	Application	Mix/load	Application
Dermal exposure	0.030	41.550 ml/day	0.002	6.450 ml/day
Concen. of a.s. product or spray	400	2.4 mg/ml	400	2.4 mg/ml
Dermal exposure to a.s.	12.000	99.720 mg/day	0.600	15.480 mg/day
Percent absorbed	0.2	4.7 %	0.2	4.7 %
Absorbed dose	0.024	4.687 mg/day	0.001	0.728 mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.01	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	2.4	mg/ml
Inhalation exposure to a.s.	0.144	mg/day
Percent absorbed	100	%
Absorbed dose	0.144	mg/day

PREDICTED EXPOSURE	No PPE	With PPE
Total absorbed dose	4.8548 mg/day	0.8728 mg/day
Operator body weight	60 kg	60 kg
Operator exposure	0.0809 mg/kg bw/day	0.0145 mg/kg bw/day

CP 7.2.1.2 Measurement of operator exposure

Since the risk assessment carried out indicated that the acceptable operator exposure level (AOEL) for flufenacet will not be exceeded under practical conditions of use, a study to provide a measure of operator exposure under field conditions was not necessary and was therefore not carried out.

CP 7.2.2 Bystander and resident exposure

Plant protection products are applied in agriculture in areas that may be accessible to the public. Individuals might therefore be exposed, who are not actively involved in the application of these products. The individual may be temporarily located in the vicinity of the application (the so-called 'bystander') or working or living in the vicinity of the application (the so-called 'resident'). Exposure scenarios associated with the product application are evaluated for bystanders and for residents (including children). Calculations are performed according to the German guideline published in 2008 ([redacted] *et al*., 2008)[3].

- Selection and justification of the critical bystander GAP

Table CP 7.2.2-1: Critical bystander/resident GAP

Crop	Application technique	Max. dose rate (kg a.s./ha)	Max no. of appl.	Drift scenario	% Drift (1 appl.,90th percentile)
Winter wheat, Winter barley, Winter rye (low crops)	Field crop sprayer	DFF: 0.12 FFA: 0.24	1	Field crops	0.29

DFF = Diflufenican, FFA=Flufenacet

Since the maximum number of application is limited by one per season exposure is calculated using the spray drift value from a single application (90th perc.) in 10 m distance for bystanders and for residents.

A summary of the exposure calculations and risk assessment is presented in the following table. Detailed calculations are presented in CP 7.2.2.1.

[3] S. [redacted], D. [redacted], M. [redacted], F. [redacted], C. [redacted], F. [redacted], H. [redacted] and G. [redacted] (2008): Guidance for Exposure and Risk Evaluation for Bystanders and Residents exposed to Plant Protection Products during and after Application, J. Verbr. Lebensm. .3, 272-281.,.

Table CP 7.2.2-2: Predicted systemic bystander and resident exposure as a proportion of the AOEL

Active substance	Crop	Target group	Adult		Child	
			Absorbed dose (mg/kg bw/day)*	% of AOEL**	Absorbed dose (mg/kg bw/day)*	% of AOEL**
Flufenacet	Winter wheat, Winter barley, Winter rye	Bystander	0.000056	<1	0.000045	<1
		Resident	0.000004	<1	0.000016	<1

* Assumes a 60 kg adult and a 16.15 kg child,
For flufenacet a dermal absorption of 4.7% for the diluted spray, 100% absorption via the inhalation route and 100% oral absorption.
**Flufenacet AOEL: 0.017 mg/kg bw/day;

- **Assessment**

Calculations demonstrate that bystander and resident exposure is very low. Absorbed doses are all well below the systemic AOEL of flufenacet.

It is concluded that an unacceptable risk is not anticipated for both child or adult bystanders and residents.

CP 7.2.2.1 Estimation of bystander and resident exposure

The following definitions and assumptions for bystanders may be applied.

Bystanders are persons:

- who are located within or directly adjacent to the area where pesticide application or treatment is in process or has taken place
- whose presence is quite incidental and unrelated to work involving pesticides but whose position may put them at risk of exposure
- who take no action to avoid or control exposure
- that are not wearing protective clothing and/or are wearing light clothing e.g. short sleeved shirt and short trousers

Residents may possibly 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 - the so-called mouthing and/or pica behaviour[4]).

Exposure is calculated for adult and child bystanders as well as adult and child residents. The German guidance for bystander/resident exposure is used.

[4] Pica is typically defined as eating non-nutritive substances. Mouthing is typically defined as putting objects (e.g. hands) into the mouth. Pica and mouthing behaviour are normal parts of development for young children.

a) Bystander exposure assessment

Input parameters considered for the estimation of bystander exposure:

Intended use(s):			**Drift (D):**	0.29	% (FCTM, 10m)
Application rate (AR):	0.24	kg a.s./ha	**Exposed Body Surface Area (BSA):**	1	m² (adults)
				0.21	m² (children)
Body weight (BW):	60	kg/person (adults)	**Specific Inhalation Exposure (I^*_A):**	0.001	mg/kg a.s. (6 hours, adults)
	16.15	kg/person (children)		0.00057	mg/kg a.s. (6 hours, children)
Dermal absorption (DA):	4.70	% ('worst case')	**Area Treated (A):**	20	ha/d (based on Field Crops, Tractor Mounted)
Inhalation absorption (IA):	100	%	**Exposure duration (T):**	5	min
AOEL:	0.017	mg/kg bw/d			

Bystander exposure towards Flufenacet					
Adults			**Children**		
Bystander: Dermal exposure after application in (via spray drift):					
SDE_B = (AR x D x BSA x DA) / BW			SDE_B = (AR x D x BSA x DA) / BW		
(24 x 0.29% x 1 x 4.7%) / 60			(24 x 0.29% x 0.21 x 4.7%) / 16.15		
External exposure	0.0696	mg/person	External exposure	0.014616	mg/person
External exposure	0.00116	mg/kg bw/d	External exposure	0.00090502	mg/kg bw/d
Absorbed dose:	**0.0000545**	**mg/kg bw/d**	**Absorbed dose:**	**0.0000425**	**mg/kg bw/d**
Bystander: Inhalation exposure after application in					
SIE_B = (I^*_A x AR x A x T x IA) / BW			SIE_B = (I^*_A x AR x A x T x IA) / BW		
(0,000 / 360 x 0.24 x 20 x 5 x 100%) / 60			(0,000 / 360 x 0.24 x 20 x 5 x 100%) / 16.15		
External exposure	6.6667E-05	mg/person	External exposure	3.8314E-05	mg/person
External exposure	1.1111E-06	mg/kg bw/d	External exposure	2.3724E-06	mg/kg bw/d
Absorbed dose:	**0.0000011**	**mg/kg bw/d**	**Absorbed dose:**	**0.0000024**	**mg/kg bw/d**
Total systemic exposure: SE_B = SDE_B + SIE_B			Total systemic exposure: SE_B = SDE_B + SIE_B		
Total systemic exposure (absorbed dose)	0.00033787	mg/person	Total systemic exposure (absorbed dose)	0.00072527	mg/person
Total systemic exposure (absorbed dose)	**0.0000556**	**mg/kg bw/d**	**Total systemic exposure** (absorbed dose)	**0.0000449**	**mg/kg bw/d**
% of AOEL:	**0.33**	**%**	**% of AOEL:**	**0.26**	**%**

b) Resident exposure assessments

Input parameters considered for the estimation of resident exposure:

Intended use(s):			**Drift (D):**	0.29	% (FCTM, 10 m)
Application rate (AR):	0.24	kg a.s./ha	**Transfer coefficient (TC):**	7300	cm^2/h (adults)
				2600	cm^2/h (children)
Number of applications (NA):	1		**Turf Transferable Residues (TTR):**	5	%
Body weight (BW):	60	kg/person (adults)	**Exposure Duration (H):**	2	h
	16.15	kg/person (children)	**Airborne Concentration of Vapour (ACV):**	none	
Dermal absorption (DA):	4.70	% ('worst case')	**Inhalation Rate (IR):**	16.57	m^3/d (adults);
Inhalation absorption (IA):	100	%		8.31	m^3/d (children)
Oral absorption (OA)	100	%	**Saliva Extraction Factor (SE):**	50	%
AOEL	0.017	mg/kg bw/d	**Surface Area of Hands (SA):**	20	cm^2
			Frequency of Hand to Mouth (Freq):	20	events/h
			Dislodgeable foliar residues (DFR):	20	%
			Ingestion Rate for Mouthing of Grass/Day (IgR):	25	cm/d

Resident exposure towards Flufenacet					
Adults			**Children**		
Residents: Dermal exposure after application in (via deposits caused by spray drift)					
SDE_R = (AR x NA x D x TTR x TC x H x DA) / BW			SDE_R = (AR x NA x D x TTR x TC x H x DA) / BW		
(0.0024 x 1 x 0.29% x 5% x 7300 x 2 x 4.7%) / 60			(0.0024 x 1 x 0.29% x 5% x 2600 x 2 x 4.7%) / 16.15		
External exposure	0.0050808	mg/person	External exposure	0.0018096	mg/person
External exposure	0.00008468	mg/kg bw/d	External exposure	0.00011205	mg/kg bw/d
Absorbed dose:	**0.0000040**	**mg/kg bw/d**	**Absorbed dose:**	**0.0000053**	**mg/kg bw/d**
Residents: Inhalation exposure to vapour					
SIE_R = (ACV x IR x IA) / BW			SIE_R = (ACV x IR x IA) / BW		
(none x 16.57 x 100%) / 60			(none x 8.31 x 100%) / 16.15		
External exposure		mg/person	External exposure		mg/person
External exposure		mg/kg bw/d	External exposure		mg/kg bw/d
Absorbed dose:		**none**	**Absorbed dose:**		**none**
			Residents: Oral exposure (hand-to-mouth transfer)		
			SOE_H = (AR x NA x D x TTR x SE x SA x Freq x H x OA) /		
			(0.0024 x 1 x 0.29% x 5% x 50% x 20 x 20 x 2 x 100%) / 16.15		
			External exposure	0.0001392	mg/person
			External exposure	8.6192E-06	mg/kg bw/d
			Absorbed dose	**0.0000086**	**mg/kg bw/d**
			Residents: Oral exposure (object-to-mouth transfer)		
			SOE_O = (AR x NA x D x DFR x IgR x OA) / BW		
			(0.0024 x 1 x 0.29% x 20% x 25 x 100%) / 16.15		
			External exposure	0.0000348	mg/person
			External exposure	2.1548E-06	mg/kg bw/d
			Absorbed dose	**0.0000022**	**mg/kg bw/d**
Total systemic exposure: $SE_R = SDE_R + SIE_R$			Total systemic exposure: $SE_R = SDE_R + SIE_R + SOE_H + SOE_O$		
Total systemic exposure (absorbed dose)	0.0002388	mg/person	Total systemic exposure (absorbed dose)	0.00025905	mg/person
Total systemic exposure (absorbed dose)	**0.0000040**	**mg/kg bw/d**	**Total systemic exposure** (absorbed dose)	**0.0000160**	**mg/kg bw/d**
% of AOEL:	**0.02**	**%**	**% of AOEL:**	**0.09**	**%**

CP 7.2.2.2 Measurement of bystander and resident exposure

Since the exposure estimate carried out indicated that 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

According to the application parameters of Diflufenican+Flufenacet SC 600 (200+400) the only intended use is spray application in winter wheat, winter barley and winter rye in an growth stage BBCH 10-25. In this growth stage only few leaves of the plants are unfolded and re-entry activities are not necessary immediately after application of Diflufenican+Flufenacet SC 600 (200+400). However, in the present risk assessment scouting activities in winter cereals after the intended use will be estimated.

The determination of the cGAP for worker re-entry is based on the recommendation provided in the EUROPOEM II report[5] for worker exposure for four different harvesting scenarios with bare hands:

Crop group	Transfer Coefficient (cm^2/h)
Fruits (from trees):	4500
Vegetables:	2500
Ornamentals:	5000
Strawberries:	3000

Exposure of workers is estimated for activities that involve significant contact with treated crops. This will mainly occur when manual work is necessary.

The critical GAP for worker exposure to Diflufenican+Flufenacet SC 600 (200+400) is presented in the following table.

Table CP 7.2.3-1: Critical GAPs for worker exposure

Crop(s)	Max. dose rate		Growth stage of crop	No. of appl.	Re-entry activity	Duration (h/day)	TC (cm2/hr)	PHI (days)
	(L/ha product)	(kg a.s./ha)						
Winter cereals	0.6	DFF: 0.12 FFA: 0.24	BBCH 10-25	1	Scouting	2	2500*	-

DFF = Diflufenican, FFA=Flufenacet
*Transfer coefficient for vegetables serves as a surrogate for winter cereals

A summary of the exposure calculations and risk assessment is presented in the following table. Detailed calculations are presented in CP 7.2.3.1.

[5] EUROPOEM II project FAIR3-CT96-1406; Post Application Exposure of Workers to Pesticides in Agriculture, Report of the Re-entry Working Group; December 2002

Table CP 7.2.3-1: Predicted worker exposure and proportions of the AOEL

Crop grouping	Re-entry task	Systemic exposure* (mg/kg bw/day)	% of AOEL**
Winter cereals	Scouting	0.00282	17

* Assumes a 60 kg adult
For flufenacet a dermal absorption of 4.7% for the diluted spray
**Flufenacet AOEL: 0.017 mg/kg bw/day

- **Assessment**

Exposure of operators entering treated crops is within acceptable levels. Calculations reflect standard work clothing worn by adult workers (shoes, socks, long-legged pants, and long sleeves) working with bare hands. No personal protective equipment is considered to mitigate the exposure.

- **Overall conclusion**

An unacceptable risk is not anticipated for workers with the intended use of Diflufenican+Flufenacet SC 600 (200+400).

CP 7.2.3.1 Estimation of worker exposure

A worst case estimate of the risk of workers entering a newly treated crop is calculated using the worker re-entry model published by Hoernicke E. *et al* (1998)[6].

The following assumptions are made:

-Re-entry exposure is predominantly via the dermal route (contact with the foliage)
-Residues on the foliage depend on:
  - application rate
  - extent of remaining residues from previous applications
  - the Leaf Area Index (LAI) [total size of foliage compared to surface area]
-Transfer of residues from foliage to the clothes or skin of workers depends mainly on the intensity of contact with the foliage
-Activities with a similar pattern can be grouped and a generic Transfer Coefficient (TC) applied.
-Dislodgeable Foliar Residue (DFR) is calculated using a default value of 3 µg as/cm² per kg as/ha as proposed by EUROPOEM II.

Calculations are made for the critical re-entry scenarios in winter cereals (scouting).

- Considerations on Transfer Coefficients

In a general approach, Hoernicke *et al.* (1998) propose that a Transfer Coefficient (TC) of 30,000 (cm²/person/h) is used. This value is considered to represent a worst case for worker

[6] Hoernicke E *et al.* (1998): Details in the instructions for use on the protection of persons carrying out successive work with crops which have been treated with plant protection products. Nachrichtenbl. Deut. Pflanzenschutzd. 50, 267-268

exposure, being derived from tasks requiring intensive contact with foliage and representing an unprotected worker. However, where it is considered that less intensive contact with the foliage will occur, the risk assessment may be refined by the use of alternative Transfer Coefficients (TC).

A TC for winter cereals is not proposed in the EUROPOEM II report. As a surrogate, the TC for re-entry in vegetables is used (**2500** cm²/hr).

- Calculations:

Calculations are performed according to the following equation:

D = DFR x TC x WR x AR x P

where		
	D	= *Dermal exposure*
	DFR	= *Dislodgeable foliar residues (µg as/cm²)*
	TC	= *Transfer Coefficient (cm²/person/h)*
	WR	= *Work rate (hours/day)*
	AR	= *Application rate (kg as/ha)*
	P	= *Protection factor for PPE (1, no PPE)*

Predicted exposures are calculated with the maximum application rate, 2 hours work rate, a body weight of 60 kg and dermal absorption value of 4.7% for flufenacet.

Re-entry in winter cereals, exposure to flufenacet:

D	=	DFR	x	TC	x	WR	x	AR	x	P
		µg/cm²		cm²/hr		hrs/day		kg a.s./ha		
D	=	3	x	2500	x	2	x	0.24	x	1
D	=	3600 µg a.s./person/day								
	=	3.6 mg a.s./person/day								
D/BW	=	0.06 mg/kg bw/day (60 kg person)								
Systemic exposure (4.7% dermal absorption):										
S	=	0.06 x 0.047								
	=	**0.00282 mg/kg bw/day**								

CP 7.2.3.2 Measurement of worker exposure

Since the exposure estimate carried out indicated that the AOEL will not be exceeded under practical conditions of use, a study to provide a measure of worker exposure was not necessary and was therefore not carried out.

CP 7.3 Dermal adsorption

Summary and conclusion on dermal absorption

Flufenacet

The extent of dermal absorption of flufenacet formulated as an SC 600 formulation was investigated *in vitro* using human and rat skin. A summary of the study is given in the following section. A conclusion and recommendation regarding the dermal absorption of flufenacet formulated as an SC 600 is given below.

The *in vitro* study indicated that the mean percentage of [^{14}C]-flufenacet considered to be potentially absorbable over a period of 24 hours for the neat formulation was 0.17% and 6.59% for the human and rat skin, respectively. The mean percentage of [^{14}C]-flufenacet considered to be potentially absorbable at the intermediate dose was 1.82% and 19.98% for the human and rat skin respectively. The mean percentage of [^{14}C]-flufenacet considered to be potentially absorbable at the low dose was 3.84% and 17.76% for the human and rat skin respectively.

In the absence of an appropriate *in vivo* rat study the *in vitro* human skin values were used alone.

According to the new EFSA guidance[7] there is the provision that when the sampling period is 24 hours (which is the case for this study) and over 75% of the total absorption (material in the receptor fluid at the end of the study) occurred within half of the duration (12 hours) of the total sampling period that the absorption will be taken as the sum of receptor fluid, receptor chamber washes and the skin sample excluding all tape strips. These criteria were met for the intermediate and low dose groups in this study. There is also the provision that a standard deviation equal to or larger than 25% of the mean of the absorption requires the use of an alternative value or rejection of the study. The guidance prefers the approach of adding the standard deviation to the mean to cover the upper 84th percentile value of the results. Albeit that the notifier considers that the value of 25% for the standard deviation limit to be too conservative, the application of the guidance results in the following values for [^{14}C]-flufenacet in the SC 600 formulation. For details see table CP 7.3-1:

- 0.2% for the neat formulation (400 g/L)
- 2.6% for the intermediate dose (3 g/L)
- 4.7% for the low dose (0.3 g/L).

Dermal absorption of flufenacet, *in vivo*
No study available.

Dermal absorption of flufenacet, *in vitro*

[7] EFSA Panel on Plant Protection Products and their Residues (PPR); Guidance on Dermal Absorption. EFSA Journal 2012;10(4):2665. [30 pp.] doi:10.2903/j.efsa.2012.2665.

Report:	**KCP 7.3 [redacted], M., (2009)**
Title:	Herold SC600: [Phenyl-UL-^{14}C]-flufenacet: Comparative *in vitro* dermal absorption study using human and rat skin.
Document N°:	M-358525-01-1
Guidelines:	O.E.C.D. guideline for the testing of chemicals, skin absorption: *in vitro* Method 428 (April 2004), O.E.C.D. Environmental health and safety publications series on testing and assessment N°28, Guidance document for the conduct of skin absorption studies (March 2004), European Commission guidance document on dermal absorption-Sanco/222/2000 rev. 7 (March 2004).
GLP	Yes

Material and methods

Rat skin:

Species, strain:	Rat, Wistar Rj: WI(IOPS HAN).
Source:	[redacted] (France).
Sex:	Male.
Number:	14
Anatomical site:	Dorsal
Rat Skin Preparation:	Each animal was killed by cervical dislocation. After sacrifice the skin was clipped and removed for use in the study. The dorsal skin was dermatomed by use of a mini-dermatome to obtain samples of *ca* 430 to 530 µm in thickness.

Human skin: Source: [redacted], France.
Number and sex: 10 donors, female.
Anatomical region: Abdomen.
Thickness: 447 to 602 µm.

Test Material:

Non-radiolabelled:	Batch: K664072. Purity = 97.8%.
Radiolabelled:	[phenyl-UL-^{14}C]-flufenacet Batch: KATH 6299 Specific activity: 6.11 MBq/mg. Radiopurity of the formulation: >99%.

Formulation: The formulation used in this experiment was the Herold SC 600 formulation (specification number 102000007948) containing flufenacet (400 g/L) and diflufenican (200 g/L). It was used at three nominal concentrations of flufenacet: neat, 400 g flufenacet/L, 3 g flufenacet/L and 0.3 g flufenacet/L.

Test system: A flow-through diffusion cell system (Franz's cell modified, Gallas, France) was used to study the absorption of the test substance (exposure area of 1 cm^2 skin). A diffusion cell consisted of a donor chamber and a receptor chamber between which the skin was positioned. The receptor fluid was Eagle's medium supplemented with

5% bovine serum albumin and gentamycin (50 mg/L) at a pH of 7.4. The receptor chamber was warmed by a constant circulation of warm water which maintained the receptor fluid at 32 ± 2°C (close to the normal skin temperature). The receptor fluid was pumped through the receptor chamber at a rate of 1.5 mL/h and stirred continuously whilst in the receptor chamber by means of a magnetic bar.

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

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

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

Radioassay: The amounts of radioactivity in the various samples were determined by liquid scintillation counting (LSC). Samples were counted for 10 minutes or for 2 sigma % in an appropriate scintillation cocktail using a Packard 1900 TR counter with on-line computing facilities. Quenching

effects were determined using an external standard and spectral quench parameter (tSIE) method. Efficiency correlation curves were prepared for each scintillation cocktail and were regularly checked by the use of [^{14}C-n-hexadecane standards. The scintillation counter was recalibrated when a deviation of greater than 2% was observed when counting quality control standards. The limit of detection was taken to be twice the background values for blank samples in appropriate scintillation cocktails.

Findings:
Flufenacet was demonstrated to be soluble in the receptor fluid at the concentration of 0.6 mg/mL of receptor fluid (procedure explained in §1.4 Materials & Methods). During the study, the maximal concentration per hour of flufenacet in the receptor fluid was 20 µg/mL. The achieved concentrations in the study were thus at least 30 times lower than the determined solubility concentration, thus the solubility in the receptor fluid was deemed to be sufficient to avoid any risk of back diffusion.
Measurements of the homogeneity of the three concentrations of formulation applied indicated that it was acceptable.

Good recovery data were obtained, with mean total recoveries of radioactivity in the range of 92.8% to 98.1% of the applied dose.

These study results are presented in Table CP 7.3-1.

Table CP 7.3-1: Mean distribution of radioactivity at 24 hours after dose application of [^{14}C]- flufenacet in an SC 600 formulation at the rates of 400 g/L, 3 g/L and 0.3 g/L to human and rat skin samples.

Results expressed in terms of percentage of applied radioactivity.

	Distribution of radioactivity (% dose)											
Dose Levels	Neat formulation: High dose (SYP13418, 400 g/L)				Dilution: Intermediate dose (SYP13421, 3 g/L)				Dilution: Low dose (SYP13423, 0.3 g/L)			
Species	Human (n=6)		Rat (n=6)		Human (n=5)		Rat (n=9)		Human (n=4)		Rat (n=5)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
SURFACE COMPARTMENT												
Skin swabs (8h)	95.28	1.80	89.60	**2.34**	88.87	5.71	58.63	14.99	87.77	3.84	63.41	6.00
Skin swabs (24h)[a]	0.05	0.04	0.27	**0.17**	1.71	2.53	3.57	3.28	1.39	1.26	5.42	2.65
Surface Dose (tape-strips 1&2)	0.05	0.02	1.37	**0.43**	0.44	0.62	10.66	8.09	0.46	0.44	6.08	5.45
Donor chamber	0.06	0.02	0.21	**0.20**	0.05	0.05	0.21	0.14	0.13	0.16	0.12	0.05
Total % non-absorbed	***95.44***	*1.77*	***91.46***	***2.36***	***91.07***	*3.02*	***73.07***	*9.96*	***89.77***	*2.34*	***75.03***	*5.97*
SKIN COMPARTMENT												
Skin [b]	0.05	0.05	0.93	**0.94**	0.56	0.68	3.04	2.95	0.29	0.18	1.59	2.29
Stratum corneum [c]	0.04	0.02	4.64	**2.25**	0.33	0.36	9.47	8.80	0.40	0.30	6.84	4.02
Total % at dose site	***0.10***	*0.06*	***5.57***	***2.16***	***0.89***	*1.04*	***12.50***	*9.69*	***0.69***	*0.45*	***8.43***	*5.94*
RECEPTOR COMPARTMENT												
Receptor fluid (0-24h)	0.03	0.01	0.25	**0.24**	0.92	0.62	6.49	3.54	3.13	0.90	8.70	3.87
Receptor fluid terminal	0.03	0.03	0.69	**0.43**	0.02	0.01	0.75	0.58	0.02	0.03	0.63	0.33
Receptor chamber	0.01	0.04	0.09	**0.08**	n.d.	n.a.	0.24	0.35	n.d.	n.a.	n.d.	n.a.
Total % directly absorbed[d]	***0.08***	*0.03*	***1.03***	***0.39***	***0.93***	*0.64*	***7.48***	*4.23*	***3.15***	*0.93*	***9.33***	*3.96*
Total % Potentially Absorbable	***0.17***	*0.05*	***6.59***	***2.33***	***1.82***	*1.67*	***19.98***	*8.76*	***3.84***	*1.34*	***17.76***	*6.42*
TOTAL % RECOVERY	**95.62**	1.75	**98.05**	**0.83**	**92.90**	2.52	**93.05**	2.08	**93.61**	1.88	**92.79**	0.96

[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
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.

Conclusion:
The dermal penetration of [^{14}C]-flufenacet through human and rat dermatomed skin from the SC 600 formulation was investigated at three concentrations corresponding to the neat product (400 g /L) and to two representative dilutions (3 and 0.3 g/L), respectively.

Overall, the dermal penetration of [^{14}C]-flufenacet in the Herold SC 600 formulation through human skin was low at all concentrations used. In addition, the absorption was lower in human skin compared to rat skin at all concentrations used.

The mean percentage of flufenacet in the SC 600 formulation that was considered to be potentially absorbable *(directly absorbed plus total remaining at dose site)* over a period of 24 hours for the neat formulation was 0.17% and 6.59% for the human and rat skin, respectively.

The mean percentage of flufenacet in the SC 600 formulation that was considered to be potentially absorbable *(directly absorbed plus total remaining at dose site)* over a period of 24 hours for the intermediate dose rate was 1.82% and 19.98% for the human and rat skin respectively.

The mean percentage of flufenacet in the SC 600 formulation that was considered to be potentially absorbable *(directly absorbed plus total remaining at dose site)* over a period of 24 hours for the low dose rate was 3.84% and 17.76% for the human and rat skin respectively.

According to the new EFSA guidance[8] there is the provision that when the sampling period is 24 hours (which is the case for this study) and over 75% of the total absorption (material in the receptor fluid at the end of the study) occurred within half of the duration (12 hours) of the total sampling period that the absorption will be taken as the sum of receptor fluid, receptor chamber washes and the skin sample excluding all tape strips. These criteria were met for the intermediate and low dose groups in this study. There is also the provision that a standard deviation equal to or larger than 25% of the mean of the absorption requires the use of an alternative value or rejection of the study. The guidance prefers the approach of adding the standard deviation to the mean to cover the upper 84th percentile value of the results. Albeit that the notifier considers that the value of 25% for the standard deviation limit to be too conservative, the application of the guidance results in the following values for [^{14}C]-flufenacet in the SC 600 formulation. For details see table CP 7.3-1:

- 0.2% for the neat formulation (400 g/L)
- 2.6% for the intermediate dose (3 g/L)
- 4.7% for the low dose (0.3 g/L).

CP 7.4 Available toxicological data relating to co-formulants

CONFIDENTIAL information - data provided separately (Document J)

[8] EFSA Panel on Plant Protection Products and their Residues (PPR); Guidance on Dermal Absorption. EFSA Journal 2012;10(4):2665. [30 pp.] doi:10.2903/j.efsa.2012.2665.