A8207M

Fludioxonil 25 g/L FS

NOTIFICATION OF AN ACTIVE SUBSTANCE UNDER COMMISSION REGULATION (EU) 844/2012

DOCUMENT M-CP, Section 7

TOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
12/1/17	In response to questions from the RMS: CP 7.2.1 The risk assessment for operators based on the French Seed-TROPEX model have been added. CP 7.3 Dermal Absorption. Correction of second paragraph in conclusion sections. Original text reads that tape strips 1-2 were included; this contradicted the rest of the study summary and what actually had been done. So the new text reads that tape strips 1-2 have been excluded. All amendments are highlighted in yellow.	A8207M_10365 5 April 2016 updated 12/1/17

[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 PRODUCTS

Introduction

This document supports the application for renewal of the regulatory approval of fludioxonil under Commission Implementing Regulation (EU) 844/2012 of 18 September 2012. This document reviews the toxicological studies for the product A8207M containing:

- 25 g/L fludioxonil

A8207M is a flowable concentrate for seed treatment (FS), containing 25 g/L fludioxonil, for the treatment of wheat and oat seeds to control a wide range of seed and soil-borne diseases. A8207I an older variant of A8207M was together with A9219B (containing 250 g/kg fludioxonil and 350 g/kg cyprodinil) the representative formulation in the EU review of fludioxonil.

Fludioxonil was included into Annex I of Council Directive 91/414/EEC (Commission Directive 2007/76/EC; 20 December 2007). This active substance is an approved active substance under Regulation (EC) 1107/2009 (repealing Commission Directive 91/414/EEC) as specified in Commission Implementing Regulation (EU) No. 540/2011 of 25 May 2011.

In accordance with Commission Implementing Regulation (EU) 844/2012, this document summarises new information which are relevant for the renewal of the approval of fludioxonil under Regulation (EC) 1107/2009. Where appropriate this document refers to the Commission Implementing Regulation (EU) No. 540/2011 for fludioxonil and to the EFSA report for fludioxonil (**EFSA Scientific Report (2007) 110, 1-85**), and in particular the endpoints provided in Appendix I.

This document covers data and risk assessments which were not part of the original dossier and which are necessary to reflect changes:

- In requirements under Commission Regulation (EU) No 284/2013, and the associated Annex, which repeals Commission Regulation (EU) No 545/2011 which, under Regulation (EC) 1107/2009, replaced the requirements of Annex III to Directive 91/414/EEC
- In scientific and technical knowledge since the approval or last renewal of the approval
- To representative uses

The representative use pattern proposed for EU review of fludioxonil is included in Document D1 and summarised below (Table 7.2.1-1).

Each section of this document provides the agreed EU endpoints and if relevant proposals for amended endpoints.

Where new guidance documents have been introduced since the EU review of fludioxonil, an updated evaluation of fludioxonil and A8207M has been included. To adequately assess fludioxonil to the new guidance documents, it may have been necessary to provide new data, if so these are also included.

Information on the detailed composition of A8207M can be found in the confidential dossier of this submission (Document J).

Details of all relevant data from the scientific peer reviewed open literature on the active substance, metabolites and breakdown or reaction products and plant protection products containing the active substance have been provided in the **Document M-CA Section 9** and are discussed within the relevant data point of the associated dossier for the active substance, fludioxonil. If the published literature is also relevant to A8207M, it has been discussed within the relevant data point in this document.

CP 7.1 Acute Toxicity

Summary of acute toxicity

A8207M containing 25 g/L fludioxonil has a low toxicity in respect to acute oral and dermal toxicity and is not irritating to the rabbit skin or eye and is not a skin sensitiser. As this formulation variant was not the representative formulation in the original EU review acute toxicity data are presented here. The classification according to Regulation (EC) No 1272/2008 as amended, is given in the table below.

Table 7.1-1: Summary of acute toxicological data obtained with A8207M

Parameter [Reference]	Species	Result	Classification according to Regulation (EC) No 1272/2008 as amended (GHS)
Acute oral LD50 (Kuhn J, 2005)	Rat	>5000 mg/kg	None
Acute dermal LD50 (Kuhn J, 2005a)	Rat	>5050 mg/kg	None
Acute inhalation	Rat	Not required*	None
Acute skin irritation (Kuhn J, 2005b)	Rabbit	Slightly irritating	None
Acute eye irritation (Kuhn J, 2005c)	Rabbit	Not-irritating	None
Skin sensitisation (Kuhn J, 2005d)	Guinea Pigs	Non-sensitising	None

*The criteria for the need for an acute inhalation study are not met for A8207M according to Commission Regulation (EU) No 545/2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for plant protection products

CP 7.1.1 Oral toxicity

Report:	K-CP 7.1.1/01 Kuhn J. (2005). Fludioxonil 025FS (A8207M): Acute Oral Toxicity In Rats. Stillmeadow, Inc., 12852 Park One Drive, Sugar Land, TX 77478, USA. Unpublished Report No. 9062-05. 23 June 2005. (Syngenta File No. CGA173506/6444)

Guidelines: Acute Oral (rat) OECD 425 (2001): OPPTS 870.1100 (2002)

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

GLP: Signed and dated GLP and Quality Assurance statements were provided.

EXECUTIVE SUMMARY

A limit test with 3 female Sprague Dawley rats was conducted. These animals were dosed by gavage with fludioxonil 025FS (A8207M) as supplied, at a limit dose of 5000 mg/kg body weight.

All animals were examined for mortality and clinical signs frequently during the first day (day 0) and once daily for 14 days. Body weights were recorded just prior to dosing on day 0 and on days 7 and 14. On day 14, all animals were subjected to a gross necropsy and all abnormalities recorded.

All animals survived until the end of the study period. Clinical signs included activity decrease, diarrhoea, gasping, hunched posture, piloerection, polyuria, red faeces, staining at muzzle and tail and sensitivity to touch. All animals exhibited signs through to day 14. Body weight gain was unaffected by administration of the test substance, with the exception of one animal which initially lost weight between days 0 and 7 but had exceeded its starting bodyweight by the end of the study.

Gross necropsy revealed no observable abnormalities.

The acute oral LD 50 for female rats was determined to be greater than 5000 mg/kg. The acute oral toxicity was greater than 2000 mg/kg therefore no classification is required for acute oral toxicity of A8207M according to Regulation (EC) No 1272/2008 as amended.

MATERIALS AND METHODS

Materials

Test Material:	Fludioxonil 025FS (A8207M)
Description:	Formulation, red liquid
Lot/Batch #:	SMU4JP003
Purity:	CGA173506 2.46% w/w, corresponds to 25.8 g/L;
Stability of test compound:	Stable under storage conditions <30°C, light protected

Vehicle and/or positive control: None

Test Animals:	
Species	Rat
Strain	Sprague-Dawley
Age/weight at dosing	8-10 weeks / 176-177 g
Source	Texas Animal Specialties, Humble, TX
Housing	Individually in stainless steel, wire bottomed cages
Acclimatisation period	At least 5 days
Diet	PMI Feeds Inc. Formulab #5008; available ad libitum except for approximately 16hrs before dosing
Water	Municiple water ad libitum
Environmental conditions	Temperature: 22±3°C Humidity: 30-70% Air changes: 10-12 per hour Photoperiod: 12 hours dark / 12 hours light

Study Design and Methods

In-life dates: Start: 5 May 2005 End: 24 May 2005

Animal assignment and treatment: A limit test with 3 female Sprague Dawley rats was conducted. These animals were dosed by gavage with fludioxonil 025FS (A8207M) at a limit dose of 5000 mg/kg body weight. The test item was dosed undiluted using a dose volume of 5.26 mL/kg.

Table 7.1.1-1: Dosing regime and outcome

Animal number	Sex dosed	Dose level (mg/kg)	Dose Volume (mL/kg bodyweight)	Outcome
151	Female	5000	5.26	Survived
152	Female	5000	5.26	Survived
153	Female	5000	5.26	Survived

All animals were examined for mortality and clinical signs frequently during the first day (day 0) and once daily for 14 days. Body weights were recorded just prior to dosing on day 0 and on days 7 and 14. On day 14, all animals were subjected to a gross necropsy and all abnormalities recorded.

Statistics: The oral LD50 was estimated (limit test, no mortalities).

Results and Discussion

Mortality: All animals survived until the end of the study period.

Clinical observations: All animals survived until the end of the study period. Clinical signs included activity decrease, diarrhoea, gasping, hunched posture, piloerection, polyuria, red faeces, staining at muzzle and tail and sensitivity to touch. All animals exhibited signs to day 14.

Body Weight: Body weight gain was unaffected by administration of the test substance, with the exception of one animal which initially lost weight between days 0 and 7 but had exceeded its starting bodyweight by the end of the study.

Necropsy: Gross necropsy revealed no observable abnormalities.

CONCLUSION: The acute oral LD50 of A8207M after a single oral administration to female rats is greater than 5000 mg/kg. The acute oral toxicity was greater than 2000 mg/kg therefore no classification is required for acute oral toxicity of A8207M according to Regulation (EC) No 1272/2008 as amended.

(Kuhn J, 2005)

CP 7.1.2 Dermal toxicity

Report:	K-CP 7.1.2/01 Kuhn J. (2005a). Fludioxonil 025FS (A8207M): Acute Dermal Toxicity Study In Rats. Stillmeadow, Inc., 12852 Park One Drive, Sugar Land, TX 77478, USA. Unpublished Report No. 9063-05. 23 June 2005. (Syngenta File No. CGA173506/6445).

Guidelines: Acute Dermal Toxicity (rat) OECD 402 (1987): OPPTS 870.1200 (1998)

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

GLP: Signed and dated GLP and Quality Assurance statements were provided.

EXECUTIVE SUMMARY

Five male and five female Sprague Dawley rats were treated with fludioxonil 025FS (A8207M) at 5050 mg/kg by dermal application. It was applied undiluted at a volume of 5.31 mL/kg. The test substance was applied uniformly to the shaved back and covered with a semi-occlusive dressing which was wrapped with vet wrap and secured with non-irritating adhesive tape. After 24 hours the dressing was removed and the test sites washed with room temperature water and a clean cloth to remove as much residual test substance as possible. The animals were examined several times on the day of dosing (day 0) and at least once daily thereafter for 14 days. Body weights were recorded just prior to dosing on day 0 and on days 7 and 14. On day 14, all animals were subjected to gross necropsy and all abnormalities were recorded.

No deaths occurred during the study. All animals appeared normal for the duration of the study. There were no signs of skin irritation in any animals at any time during the study; however, test substance staining at the application site was observed in all animals on days 1 and 4, in three males on day 7 and in two males on day 11.

Bodyweight gain was unaffected by administration of the test substance.

The gross necropsy conducted at termination revealed no observable abnormalities.

The acute dermal LD 50 for male and female rats is greater than 5050 mg/kg. The acute dermal toxicity was greater than 2000 mg/kg therefore no classification is required for acute oral toxicity of A8207M according to Regulation (EC) No 1272/2008 as amended.

MATERIALS AND METHODS

Materials

Test Material:	Fludioxonil 025FS (A8207M)
Description:	Formulation, red liquid
Lot/Batch #:	SMU4JP003
Purity:	CGA173506 2.46% w/w, corresponds to 25.8 g/L;
Stability of test compound:	Stable under storage conditions <30°C, light protected

Vehicle and/or positive control: None

Test Animals:	
Species	Rat
Strain	Sprague Dawley
Age/weight at dosing	Males 8 weeks / 233-275 g; females 8 weeks 184-206 g
Source	Texas Animal Specialties, Humble, TX
Housing	Stainless steel wire bottomed cages
Acclimatisation period	At least 5 days
Diet	Purina Formulab Chow #5008; available ad libitum
Water	Municiple water ad libitum
Environmental conditions	Temperature: 22±3°C Humidity: 30-70% Air changes: 10-12 per hour Photoperiod: 12 hours dark / 12 hours light

Study Design and Methods

In-life dates: Start: 12 May 2005 End: 25 May 2005

Animal assignment and treatment: Five male and five female Sprague Dawley rats were treated with fludioxonil 025FS (A8207M) at 2000 mg/kg by dermal application. It was applied undiluted at a volume of 5.31 mL/kg. The test substance was applied uniformly to the shaved back and covered with a semi-occlusive dressing which was wrapped with vet wrap and secured with non-irritating adhesive tape. After 24 hours the dressing was removed and the test sites washed with room temperature water and a clean cloth to remove as much residual test substance as possible.

The animals were examined several times on the day of dosing (day 0) and at least once daily thereafter for 14 days. Body weights were recorded just prior to dosing on day 0 and on days 7 and 14. On day 14, all animals were subjected to gross necropsy and all abnormalities were recorded.

Statistics: The dermal LD50 was estimated (limit test, no mortalities).

Results and Discussion

Mortality: All animals survived until the end of the study period.

Clinical observations: All animals appeared normal for the duration of the study. There were no signs of skin irritation in any animals at any time during the study; however, test substance staining at the application site was observed in all animals on days 1 and 4, in three males on day 7 and in two males on day 11.

Body Weight: Bodyweight gain was unaffected by administration of the test substance.

Necropsy: The gross necropsy conducted at termination revealed no observable abnormalities.

CONCLUSION: The acute dermal LD50 of fludioxonil 025FS (A8207M) after a single dermal application to male and female rats was greater than 5050 mg/kg. The acute dermal toxicity was greater than 2000 mg/kg therefore no classification is required for acute oral toxicity of A8207M according Regulation (EC) No 1272/2008 as amended.

(Kuhn J, 2005a)

CP 7.1.3 Inhalation toxicity

According to Commission Directive 284/2013, the placing of plant protection products on the market, an acute inhalation test must be carried out where the plant protection product:

- is a gas or liquified gas;
- is a smoke generating plant protection product or fumigant;
- is used with fogging/misting equipment;
- is supplied in an aerosol dispenser;
- the active substance has a vapour pressure > 1 × 10 –2 Pa at 20 °C;
- the active substance is a powder containing a significant proportion of particles of a diameter < 50 µm (> 1 % on weight basis);
- to be applied from aircraft in cases where inhalation exposure is relevant;

- is to be applied by spraying.

The active substance, fludioxonil (CGA173506) has a vapour pressure of 3.9·10-7 at 25°C (trigger value: 1·10-2 Pa). The formulation is a liquid. The application technique for treatment of seed (continuous flow/closed system) will not lead to the formation of inhalable particles. Handling the treated seed (as sowing) may lead to the generation of dust. However, model calculations as well as exposure studies show that the inhalative exposure route is of minor relevance only (see CP 7.2).

As the use of fludioxonil 025FS (A8207M) will not result in any significant inhalative exposure, no acute inhalative toxicity testing is required. Considering the favourable acute toxicity profile, the inhalative hazard of the product is expected to be low.

CP 7.1.4 Skin irritation

Report:	K-CP 7.1.4/01 Kuhn J. (2005b). Fludioxonil 025FS (A8207M): Acute Dermal Irritation Study In Rabbits. Stillmeadow, Inc., 12852 Park One Drive, Sugar Land, TX 77478, USA. Unpublished Report No. 9065-05. 23 June 2005. (Syngenta File No. CGA173506/6450).

Guidelines: Primary dermal Irritation – rabbit; OECD 404 (2002): OPPTS 870.2500 (1998)

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

GLP: Signed and dated GLP and Quality Assurance statements were provided.

EXECUTIVE SUMMARY

The primary skin irritation potential of fludioxonil 025FS (A8207M) was investigated. The test item was applied by topical semi-occlusive application of 0.5 ml to the intact flank of each of three young adult New Zealand White rabbits. The duration of treatment was four hours. The scoring of skin reactions was performed according to the Draize scoring system at, 1, 24, 48 and 72 hours after removal of the dressing.

Very slight erythema and oedema was present at each observation through 48 hours. No other signs of irritation were observed; however, test substance staining of the site was observed in all animals.

The mean irritation scores 24 to 72 hours after application were less than the thresholds defined in in Regulation (EC) No 1272/2008 as amended. Therefore, according to in Regulation (EC) No 1272/2008, no classification is required for skin irritating properties of A8207M.

MATERIALS AND METHODS

Materials

Test Material:	Fludioxonil 025FS (A8207M)
Description:	Formulation, red liquid
Lot/Batch #:	SMU4JP003
Purity:	CGA173506 2.46% w/w, corresponds to 25.8 g/L;
Stability of test compound:	Stable under storage conditions <30°C, light protected

Vehicle and/or positive control: None

Test Animals:	
Species	Rabbit
Strain	New Zealand White albino rabbits
Age/weight at dosing	14 weeks / 2800 g Male; 3100 – 3300 g Females
Source	Nichols Rabbitry Inc. ; Lumberton, TX
Housing	Individually in stainless steel wire bottomed cages.
Acclimatisation period	At least 5 days
Diet	PMI Feeds Rabbit Diet #5321; in measured amounts
Water	Municiple water ad libitum
Environmental conditions	Temperature: 20 ± 3°C Humidity: 30-70% Air changes: 10-12 changes per hour Photoperiod: 12 hours light and 12 hours dark

Study Design and Methods

In-life dates: Start: 3 May 2005 End: 6 May 2005

Animal assignment and treatment: Fludioxonil 025FS (A8207M) was applied by topical application of 0.5 mL of undiluted test substance to each clipped test site and covered with a surgical gauze patch to each of three young adult New Zealand White rabbits. The patch was secured in place with a strip of non-irritating adhesive tape. The entire trunk of each animal was loosely wrapped with a semi-permeable dressing (orthopaedic stockinette) and secured on both edges with strips of tape to retard evaporation of volatile substances and to prevent possible ingestion of the test substance. After 4 hours, the patches and wrappings were removed. The test sites were gently washed with room temperature tap water and a clean cloth to remove as much residual test substance as possible. The test sites were observed for erythema and oedema formation (in accordance with the Draize scoring system) and any other dermal defects or irritation at 1, 24, 48 and 72 hours after removal of the dressing.

Results and Discussion

Very slight erythema and oedema was present at each observation through 48 hours. No other signs of irritation were observed; however, test substance staining of the site was observed in all animals.

Table 7.1.4-1: Individual and mean skin irritation scores of Fludioxonil 025FS (A8207M) according to the Draize scheme

Time	Erythema			Oedema		
Animal number	**8626M**	**8625F**	**8627F**	**8626M**	**8625F**	**8627F**
after 1 hour	1	1	1	1	1	1
after 24 hours	0	1	0	0	1	0
after 48 hours	0	1	0	0	1	0
after 72 hours	0	0	0	0	0	0
mean score 24-72 h	0	0.7	0	0	0.7	0
M= male, F = female						

CONCLUSION: The mean irritation scores 24 to 72 hours after application were less than the thresholds defined in in Regulation (EC) No 1272/2008 as amended. Therefore, according to in Regulation (EC) No 1272/2008, no classification is required for skin irritating properties of A8207M.

(Kuhn J, 2005b)

CP 7.1.5 Eye irritation

Report:	K-CP 7.1.5/01 Kuhn J. (2005c). Fludioxonil 025FS (A8207M): Acute Eye Irritation Study In Rabbits. Stillmeadow, Inc., 12852 Park One Drive, Sugar Land, TX 77478, USA. Unpublished Report No. 9064-05 23 June 2005. (Syngenta File No. CGA173506/6449).

Guidelines: Primary Eye Irritation – Rabbit; OECD 405 (2002): OPPTS 870.2400 (1998)

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

GLP: Signed and dated GLP and Quality Assurance statements were provided.

EXECUTIVE SUMMARY

The primary eye irritation potential of fludioxonil 025FS (A8207M) was investigated according to OECD and US OPPTS guidelines. The test substance was applied by instillation of 0.1 ml into the right eye of each of three young adult New Zealand White rabbits. All treated eyes were washed with room temperature deionised water for one minute immediately after recording the 24 hour observation. Scoring of irritation effects was performed approximately 1, 24, 48 and 72 hours, as well as 4 days after test item instillation.

The instillation of fludioxonil 025FS (A8207M) into the eye resulted in mild, early-onset and transient ocular changes, such as reddening of the conjunctivae, discharge and chemosis. These effects were reversible and were no longer evident 4 days after treatment, the end of the observation period in all animals. No abnormal findings were observed in the cornea or iris of any animal at any of the examinations.

Thus A8207M did not induce significant or irreversible damage to the rabbit eye.

The test substance did not produce significant or irreversible damage to the rabbit eye. The mean irritation scores 24 to 72 hours after application were less than the thresholds defined in Regulation (EC) No 1272/2008 as amended. Therefore, according to Regulation (EC) No 1272/2008, no classification is required for eye irritating properties of A8207M.

MATERIALS AND METHODS

Materials

Test Material:	Fludioxonil 025FS (A8207M)
Description:	Formulation, red liquid
Lot/Batch #:	SMU4JP003
Purity:	CGA173506 2.46% w/w, corresponds to 25.8 g/L;
Stability of test compound:	Stable under storage conditions <30°C, light protected

Vehicle and/or positive control: None

Test Animals:	
Species	Rabbit
Strain	New Zealand White Albino rabbits
Age/weight at dosing	15 weeks / Males 2250 g - 2900 g; Female 2600 g
Source	Nichols Rabbitry., Lumberton, TX

Test Animals:	
Housing	Individually in stainless steel wire bottomed cages.
Acclimatisation period	At least days
Diet	PMI Feeds, Inc. Lab Rabbit Diet #5321 in measured amounts
Water	Municipal water *ad libitum*
Environmental conditions	Temperature: 20±3°C Humidity: 30-70% Air changes: 10-12 changes per hour Photoperiod: 12 hours light and 12 hours dark

Study Design and Methods

In-life dates: Start: 2 May 2005 End: 6 May 2005

Animal assignment and treatment: On day 0, 0.1 mL of fludioxonil 025FS (A8207M) was placed into the conjunctival sac of the right eye of each of three young adult New Zealand White rabbits. The eye lids were then gently held together for about one second to prevent loss of the test substance. The left eye remained untreated and served as the reference control. All treated eyes were washed with room temperature deionised water for one minute immediately after recording the 24 hour observation.

The ocular reaction was assessed according to the Draize numerical scoring system, at approximately 1, 24, 48 and 72 hours, as well as 4 days after instillation of the test substance.

Results and Discussion

The instillation of fludioxonil 025FS (A8207M) into the eye resulted in mild, early-onset and transient ocular changes, such as reddening of the conjunctivae, discharge and chemosis. Slight reddening of the conjunctivae was noted in all animals for up to 3 days and slight chemosis & discharge up to day 1. These effects were reversible and were no longer evident 4 days after treatment, the end of the observation period in all animals. No abnormal findings were observed in the cornea or iris of any animal at any of the examinations.

Table 7.1.5-1: Eye irritation scores of fludioxonil 025FS (A8207M)

Time		Cornea			Iris			Conjunctiva					
								Redness			Chemosis		
	Animal number	8628 M	8630 M	8629 F	8628 M	8630 M	8629F	8628 M	8630 M	8629F	8628 M	8630 M	8629 F
after 1 hour		+	+	+	0	0	0	1	1	1	1	1	1
after 24 hours		+	+	0	0	0	0	1	1	0	1	1	1
after 48 hours		0	0	0	0	0	0	1	1	0	0	0	0
after 72 hours		0	0	0	0	0	0	0	1	0	0	0	0
mean scores 24-72h		0	0	0	0	0	0	0.7	1.0	0	0.3	0.3	0.3
after 4 days		0	0	0	0	0	0	0	0	0	0	0	0

M= male, F = female, + slight dulling of normal luster

CONCLUSION: The test substance did not produce significant or irreversible damage to the rabbit eye. The mean irritation scores 24 to 72 hours after application were less than the thresholds defined in Regulation (EC) No 1272/2008 as amended. Therefore, according to Regulation (EC) No 1272/2008, no classification is required for eye irritating properties of A8207M.

(Kuhn J, 2005c)

CP 7.1.6 Skin sensitization

Report:	K-CP 7.1.6/01 Kuhn J. (2005d). Fludioxonil 025FS (A8207M): Skin Sensitisation Study in Guinea Pigs. Stillmeadow, Inc., 12852 Park One Drive, Sugar Land, TX 77478, USA. Unpublished Report No. 9066-05. 17 August 2005. (Syngenta File No. CGA173506/6562).

Guidelines: Dermal sensitisation (guinea pig) OECD 406 (1992): OPPTS 870.2600 (2003)

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

GLP: Signed and dated GLP and Quality Assurance statements were provided.

EXECUTIVE SUMMARY

The sensitisation potential of fludioxonil 025FS (A8207M) was assessed using a nine-induction method based on that described by *Ritz and Buehler (1980)*. Groups of 15 male and 15 female albino Hartley guinea pigs were used for the main study (20 test and 10 controls). Two main procedures were involved; (a) the potential induction of an immune response; (b) a challenge of that response.

A positive control study was conducted using essentially the same methodology and alpha-hexylcinnamaldehyde as the test substance.

Following challenge with the undiluted fludioxonil 025FS (A8207M), there were no skin reactions in any of the test or control animals.

In the contemporary positive control study, 3 of the test animals were observed with positive skin reactions after a first challenge with undiluted Alpha-hexylcinnamaldehyde. Thus the sensitivity of the test system was confirmed.

In this study, none of the previously-induced test or control group animals had a positive sensitisation response following challenge with undiluted A8207M. The sensitisation rate of 0% is less than the threshold of significance (30%) set in Regulation (EC) No 1272/2008 as amended. Therefore, according Regulation (EC) No 1272/2008, no classification is required for skin sensitisation properties of A8207M.

MATERIALS AND METHODS

Materials

Test Material:	Fludioxonil 025FS (A8207M)
Description:	Formulation, red liquid
Lot/Batch #:	SMU4JP003
Purity:	CGA173506 2.46% w/w, corresponds to 25.8 g/l;
Stability of test compound:	Stable under storage conditions <30°C, light protected

Vehicle and/or positive control: Purified water

Test Animals:	
Species	Guinea pig
Strain	Albino Hartley

Test Animals:	
Age/weight at dosing	10 weeks /Males 317-374g; Females 315-382g
Source	Charles River Laboratories., Wilmington, MA
Housing	1-4 per cages, sexes separately in stainless steel wire bottomed cages.
Acclimatisation period	At least 5 days
Diet	PMI Feeds, Inc. Guinea Pig Diet 5025, available *ad libitum*
Water	Municipal water *ad libitum*
Environmental conditions	Temperature: 20±3°C Humidity: 30-70% Air changes: 10-12 per hour Photoperiod: 12 hours dark / 12 hours light

Study Design and Methods

In-life dates: Start: 4 May 2005 End: 8 June 2005

Animal assignment and treatment: The sensitisation potential of fludioxonil 025FS (A8207M) was assessed using a method based on that described by *Ritz and Buehler (1980)*. Groups of 15 male and 15 female albino Hartley guinea pigs were used for the main study (20 test and 10 control). Two main procedures were involved; (a) the potential induction of an immune response; (b) a challenge of that response.

Induction: In test animals, the induction phase involved the topical application of 0.4 ml of the undiluted test substance, underneath a 4-ply, 2.5 x 2.5cm surgical gauze patch. Each patch was placed laterally from the midline of the back of the left front quadrant of the exposure area and secured with a strip of non-irritating adhesive tape. A strip of clear polyethylene film was placed over the patch and securely taped. Each animal was then placed in a restrainer for approximately six hours at the end of the exposure period, the animals were removed from the restrainers, the wrappings and patches were removed and the animals returned to their cages. Test animals were treated nine times during a three week period with 0.4ml of undiluted test substance using the same regimen. Control animals remained untreated but were clipped and wrapped the same as test animals during the induction phase of the study.

Challenge: After a two week rest period, all animals (test and control) were each challenged at a virgin test site with an application of 0.4ml of undiluted test substance. The challenge treatment was on day 36. The dose was applied in a manner identical to the induction treatments, except the test site was placed laterally on the right rear quadrant of the exposure area with the edge of the gauze pad adjacent to the midline of the back.

Skin reactions were evaluated 24 and 48 hours following the challenge applications. Erythema and oedema were scored on a scale of 0-3, where a score ≥ 1 is considered to be a positive response.

Positive controls: A positive control study was conducted using essentially the same methodology. Undiluted Alpha-hexylcinnamaldehyde was the test substance was applied in the induction phase and challenge phase.

Results and Discussion

Induction reactions and duration: No skin reactions were observed during the induction period.

Challenge reactions and duration: Following challenge with undiluted fludioxonil 025FS (A8207M), there were no skin reactions in any of the test or control animals.

Positive control: In the contemporary positive control study, 3 of the test animals were observed with positive skin reactions after a first challenge with undiluted Alpha-hexylcinnamaldehyde. Thus the sensitivity of the test system was confirmed.

Table 7.1.6-1: Buehler test: Number of animals with positive erythema scores

	Test flank	
Scored after:	24 hours	48 hours
Naïve control group 25%	0/10	0/10
Test item group 25%	0/20	0/20
Positive control group (vehicle controls)	3/10 (0/10)	0/10 (0/10)

CONCLUSION: In this study, none of the previously-induced test or control group animals had a positive sensitisation response following challenge with undiluted A8207M. The sensitisation rate of 0% is less than the threshold of significance (30%) set in Regulation (EC) No 1272/2008 as amended. Therefore, according Regulation (EC) No 1272/2008, no classification is required for skin sensitisation properties of A8207M.

Reference:

Ritz HL and Bühler EV, Current Concepts Cutaneous Toxicity, ed. Drill VA and Lazr T (Academic Press, 1980) pp. 25-40: Planning, Conduct and Interpretation of Guinea Pig Sensitisation Patch Tests.

(Kuhn J, 2005d)

CP 7.1.7 Supplementary studies on the plant protection product

No additional studies have been conducted.

CP 7.1.8 Supplementary studies for combinations of plant protection product

This product does not contain recommendations for combinations of plant protection products therefore supplementary studies are not required.

CP 7.2 Data on Exposure

Table 7.2-1: Toxicological endpoints of fludioxonil required for evaluation of operator, worker, bystander and residential risk

Endpoint	EU agreed endpoint (Fludioxonil; EFSA Scientific Report (2007) 110, 1-85)	Proposed endpoint*
AOEL (mg/kg bw day)	0.59	0.59
Dermal absorption of concentrate	1.7%**	0.4%
Oral absorption	80%**	80%

* for dermal absorption refer to Point CP 7.3.
** conclusion for the variant of A8207I

Syngenta does not consider an AOEL value of 0.1 mg/kg bw/day (suggested by the RMS during evaluation) to be appropriate. Please refer to M-CA 5.6.2 for a detailed discussion on the derivation of the AOEL. Therefore, exposure estimates have been compared to the existing EU endpoint for the AOEL of 0.59 mg/kg bw/day.

CP 7.2.1 Operator exposure

Risk assessment for operators based on Seed-TROPEX model

Operator exposure is modelled using the Seed-TROPEX model and assessed against the AOEL of fludioxonil using dermal absorption data for A8207M.

According to the model calculations, it can be concluded that the risk for the operator using A8207M for the proposed uses is acceptable with the use of personal protective equipment.

Suitable protective clothing (coveralls, gloves) should be worn when handling the product or treated seed. When cleaning contaminated equipment the additional use of either a face-shield for protection against splashes or a particle filtering half mask for protection against elevated levels of dust is recommended.

Evaluation

The formulation is a flowable concentrate for seed treatment containing 25 g/L fludioxonil. Estimations of operator exposure have been undertaken for A8207M using the critical uses (Table 7.2.1-1).

Table 7.2.1-1: Summary of critical use patterns (i.e. worst case) for use of A8207M

Seed type	Product application rate (L/100 kg seed)	Active Substance	a.s. content (g/L)	Application rate (g a.s./100 kg seed)	Dilution factor
Cereals	0.2	Fludioxonil	25	5	Not applicable: concentrate is used as worst-case

This critical use pattern has been defined following evaluation of the GAP for each crop.

A 20 L container has been selected as the worst-case scenario for a medium-sized industrial cereal treatment facility treating 75 tonnes of cereals per day.

Estimations of potential operator exposure have been undertaken for fludioxonil using the critical uses (Table 7.2.1-1) and the SeedTROPEX predictive model.

Operator exposure is estimated using the “Seed-TReatment OPerator EXposure” data (Seed-TROPEX). Seed-TROPEX is an exposure data base submitted to UK-PSD in 1996 for national registrations by an Industry Task Force and contains results from studies performed in the UK and France.

The Seed-TROPEX data base submitted in 1996 consists of two parts: Exposure values for operators involved in seed treatment activities and exposure values for operators loading and sowing treated seed.

Seed treatment

In order to estimate the likely exposure to operators involved in the treatment of seed with A8207M data from two Seed-TROPEX studies carried out in 1993 have been used, one study in the UK monitored operators' exposure to 'Baytan' containing triadimenol, applied at 370 g/tonne seed[1] and one study in France monitored the exposure of operators to 'Germinate Double' containing anthraquinone[2], applied at 500 g/tonne seed. In the studies, operator exposure was assessed separately for the activities of equipment calibration, slurry preparation ("mixing and loading"), bagging of treated seed and cleaning of the equipment.

Data from both these studies have been combined to form a generic database that can be used to calculate potential exposure to other seed treatment products. The overview[3] summarises the UK and French data and provides guidance on how to calculate exposure to a seed treatment product using the generic data in the form of a worked example.

For all tasks, except for bagging, it is assumed that operator exposure is a result of contact with the (neat or diluted) seed dressing liquid. Therefore, the generic exposure figures are expressed in mL/operation so that the respective concentration of active substance present in the neat formulation or in the diluted seed dressing liquid is taken into account. For bagging, a constant generic exposure figure – expressed as mg/hr – is used, meaning that the amount of product applied to the seeds is not taken into account. For this assessment bagging into 25 kg bags has been considered as the worst case scenario. The generic exposure values are given in Table 7.2.1-2.

Table 7.2.1-2: Generic Seed-TROPEX exposure values for seed treatment activities (geometric mean values)

TASK	Data normalisation	Estimated Actual Dermal Exposure	Inhalation Exposure [1)]
Calibration	[mL/operation]	0.014	0.0014
Mixing / Loading (pre-mix)	[mL/operation]	0.001	0.0001
Mixing / Loading (fast-coupling) [2)]	[mL/operation]	0.005	0.0001
Bagging (25 kg bags)	[mg/hour]	0.862	0.131
Cleaning	[mL/operation]	0.083	0.016

1) Based on an average ventilation rate of 29 L/min
2) Baytan in 10L bags-in-boxes was used in the original Seed-TROPEX studies performed in the UK. These bags were directly linked to the treater. This system did not have a high level of operator protection built in, and potential dermal exposure in mL/operator was the same for both loading systems, pre-mix and fast-couple. The 10L bags-in-boxes have now been replaced by more sophisticated packaging designs. The Seed-TROPEX data are therefore of limited relevance for the use of more modern fast-coupling systems.

Loading and sowing of treated seeds

In order to estimate the likely exposure to operators involved in the loading and sowing of treated seed data combined from two worker exposure studies carried out in 1993 the UK and France have been used as a source of generic exposure data (Seed-TROPEX Studies).

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[1] Findlay, M.L., Chester, G., Mallyon B. Worker Exposure During Treatment of Seed with 'Baytan'. Report No. RJ 1621B. 12th December 1994.
[2] Leplay, M.A., Vergnon, JC., Zell, S. Worker Exposure During Treatment of Wheat Seed With 'Germinate Double'. Report No. 93002 HI 557/037/95.
[3] Chester, G., Wiseman, M., Pontal, P-G., Worker Exposure During Seed Treatment and Sowing of Treated Seed in the UK and France: An Overview. Zeneca Agrochemicals, Fernhurst, Haslemere. Report No. TMF 4896. The data are property of the Seed-TROPEX Group of which Syngenta is a member.

The UK study monitored the sowing of wheat seed treated with 'Baytan' and measured exposure to the triadimenol component of the formulation[4]. The French study measured exposure to anthraquinone during a day of sowing of wheat seed treated with 'Germinate Double'[5].

The generic Seed-TROPEX exposure figures to estimate dermal and inhalation exposure of the operator cover exposure during both activities, loading and sowing of treated seed. These exposure figures are normalised to mg/hour and accordingly do not take into account the amount of product applied to the seed. Exposure by inhalation of operators loading and sowing of treated seed was based on an average ventilation rate of 29 L/min.

As for bagging, for loading and sowing activities dust is likely to be the main source of exposure, whereby it is reasonable to conclude that the contents of active substance in the dust is related to the loading of active substances on the seed. Thus, for all active substances applied to seed in lower doses than those used in the 1993 Seed-TROPEX studies (i.e. UK-study: 370 g a.s./t, French-study: 500 g a.s./t) a pure time-dependent exposure figure is likely to result in a significant overestimation of operator exposure.

The generic Seed-TROPEX exposure values for loading and sowing treated seeds are given in Table 7.2.1-3 below. Exposure is normalised to time and accordingly the amount of product applied to the seeds is not taken into account.

Table 7.2.1-3: Generic Seed-TROPEX exposure values for loading and sowing treated seeds (geometric mean values)

TASK	Unit of exposure	Estimated Actual Dermal Exposure	Inhalation Exposure [1)]
Loading and sowing seeds	[mg/person/hour]	0.733	0.02

1) Based on an average ventilation rate of 29 L/min.

Exposure by inhalation of operators loading and sowing of treated seed is based on an average ventilation rate of 29 L/min, which is in accordance with the value applied in the 1993 Seed-TROPEX studies for this activity. Although loading of the hopper may be physically demanding where manual handling of seed bags is involved, this activity is usually of short duration compared to the actual sowing of the seed. During the latter the operator is driving the tractor, possibly leaving it once in a while to verify the drilling depth or to check and equalise remaining amount of seeds in the hopper. An average ventilation rate of 29 L/min for the combined loading and sowing task is therefore considered conservative.

Fludioxonil

Potential operator exposure estimations were compared to the endpoints summarised in Table 7.2-1 for fludioxonil; see Table 7.2.1-4.

[4] Findlay, M., Chester G. - Worker Exposure During Sowing of Treated Seed with 'Baytan'. Report No. WER001, issued 3 February 1995.
[5] Leplay, M.A. - Worker Exposure During Drilling of Wheat Seed Treated With Germinate Double. Report No. 93003 HI 5/42, issued March 1995.

Table 7.2.1-4: Summary of estimated operator exposure to fludioxonil

Model data	Level of PPE	Total absorbed dose (mg/kg bw/day)	% of AOEL (0.59 mg/kg bw/day)
Industrial seed treatment 200 mL of product/100 kg seed			
Seed-TROPEX 60 kg operator			
Calibration	Gloves	0.00060	0.10
Mixing/loading – pre-mix	Gloves	0.00036	0.06
Mixing/loading –fast-coupling	Gloves	0.00041	0.07
Bagging (25 kg bags)	Standard Work Clothing	0.01788	3.03
Cleaning	Gloves	0.00675	1.14
Multi Activity Task[1]	Gloves while handling product and cleaning equipment.	0.02564	4.3
Seed TROPEX 70 kg operator			
Calibration	Gloves	0.00051	0.09
Mixing/loading – pre-mix	Gloves	0.00030	0.05
Mixing/loading –fast-coupling	Gloves	0.00035	0.06
Bagging (25 kg bags)	Standard Work Clothing	0.01532	2.60
Cleaning	Gloves	0.00579	0.98
Multi Activity Task[1]	Gloves while handling product and cleaning equipment	0.02197	3.7
Loading and Sowing Treated Seed			
Seed-TROPEX 60 kg operator	Gloves while loading hopper	0.0038	0.65
Seed-TROPEX 70 kg operator	Gloves while loading hopper	0.0033	0.56

Standard Work Clothing Seed-TROPEX Model: Operator wearing long sleeved jacket and long trousers but no gloves.

[1)] Sum of absorbed doses and AOELs for a single operator performing calibration, fast couple mixing/loading, bagging and cleaning.

Therefore, according to the model calculations, it can be concluded that the risk of exposure to fludioxonil for the operator using A8207M on cereals is acceptable with the use of personal protective equipment.

Upon request by the RMS (France) the risk assessment for operators based on the French Seed-TROPEX model have been added:

Risk assessment for operators based on French Seed-TROPEX model

Operator exposure was assessed against the AOEL of fludioxonil. Data on dermal absorption of A8207M was provided for fludioxonil and considered acceptable. Operator exposure was modelled using the French Seed- TROPEX[6] for seed treatment and loading and sowing of treated seeds[7].

According to the model calculations, it can be concluded that the risk for the operator treating cereals with A8207M and bagging the treated seed is acceptable without the use of personal protective equipment. It can also be concluded that the risk for the operator sowing cereal seed treated with A8207M is acceptable without the use of personal protective equipment.

Evaluation

The formulation is a flowable suspension for seed treatment containing 25 g/L fludioxonil. Estimations of operator exposure have been undertaken for A8207M using the critical uses (Table 7.2.1-1).

This critical use pattern has been defined following evaluation of the GAP for each crop.

A 20 L container has been selected as the worst-case scenario for a medium sized industrial cereal treatment facility treating 75 tonnes of cereals per day.

Estimations of potential operator exposure have been undertaken for fludioxonil using the critical uses (Table 7.2.1-1) and the French Seed TROPEX predictive model.

Fludioxonil

Potential operator exposure estimations using the French Seed TROPEX predictive model were compared to EU agreed data for fludioxonil; see Table 7.2.1-5.

[6] Worker Exposure During Seed Treatment in the UK and France, Overview; Provisional Document prepared by the „Methodology“ Working Party of the French Advisory Committee on Pesticides, Operator risk evaluation in seed protection, Version 4 of 19 May 2006

[7] Provisional Document prepared by the „Methodology“ Working Party of the French Advisory Committee on Pesticides, Worker risk assessment during loading/sowing of treated seeds, Version 3 of June 1st, 2006

Table 7.2.1-5: Summary of estimated operator exposure to fludioxonil using the French Seed TROPEX predictive model

Model data	Level of PPE	Total absorbed dose (mg/kg bw/day)	% of AOEL (0.59 mg/kg bw/day)
Industrial seed treatment **2 L of product/t seed**			
Seed-TROPEX data Industrial treatment 8 hour workday 8 x Mixing and Loading 7 hours bagging/day Bagging [mg a.s./hour] 70 kg operator 75th percentiles	No PPE One layer of work clothing	0.0335	5.7
Seed-TROPEX data Industrial treatment 8 hour workday 8 x Mixing and Loading 7 hours bagging/day Bagging [mg a.s./hour] 70 kg operator 90th percentiles	No PPE One layer of work clothing	0.0463	7.8
Loading and Sowing Treated Seed			
Seed-TROPEX data 8 hours/day 70 kg operator 75th percentiles	No PPE One layer of work clothing	0.00408	0.7

[#] 20 L container was considered as a worst case to be used in calculations if 150 L of product are handled per day resulting in 8 mixing/loading operations per day

Therefore, according to the French Seed-TROPEX model (75th percentile), it can be concluded that the risk of exposure to fludioxonil for the operator using A8207M on cereals is acceptable with the use of personal protective equipment.

CP 7.2.1.1 Estimation of operator exposure

Seed-TROPEX

Estimation of operator exposure without personal protective equipment

The total potential dermal exposure (TPDE) values given in the Seed-TROPEX data base represent the sum of residues detected on outer clothing (long-sleeved work jacket, long trousers, Tyvek coverall where worn), inner clothing (long underwear representing the operators' skin), cap (representing the unprotected face) or face/neck wipes, hands and gloves of the operators. The TPDE is therefore the total amount of active substance of the skin of an operator would potentially be exposed to if no clothing at all was worn. Given the nature of the task and the potential for exposure during seed treatment operations, operators are assumed to wear suitable protective clothing and in addition protective gloves for activities such as opening and/or connecting product containers, calibrating or cleaning machinery.

Estimation of operator exposure using personal protective equipment

Operator exposure is assessed by using the Seed-TROPEX model based on the 1993 studies. This model is based on treatment of cereal seeds.

Industrial Scale seed treatment

Table 7.2.1.1-1: Scenario variables for seed treatment

Formulation	A8207M
Active substance concentration	Fludioxonil: 25 mg/mL
Application rate	200 mL product / 100 kg seed; equivalent to : Fludioxonil: 5 g / 100 kg seed
Dilution factor	No factor applied: undiluted product has been taken as the worst case scenario
Dermal absorption	Fludioxonil: 0.4%
Operator body weight	60 and 70 kg
Amount of seed treated	75 t/day
Amount of active substance applied	3.75 kg fludioxonil/day
Amount of product used	150 L/day
Cleaning tasks performed	1 per day
Mixing/Loading tasks performed	8 per day (20 L container size)
Calibration tasks performed	1 per day
Duration of bagging	8 hours

Personal Protective Equipment (PPE)

In the 1993 Seed-TROPEX studies the operators wore a long-sleeved work jacket and long trousers as usual work wear during all tasks and in addition gloves when handling formulated product and treated seeds and cleaning machinery.

Calculation of operator exposure to fludioxonil during treatment of seed is presented in Table 7.2.1.1-2.

Table 7.2.1.1-2: Estimation of operator exposure to fludioxonil during equipment calibration, slurry preparation (mixing/loading), bagging of treated seed and equipment cleaning – Based on 1993 Seed-TROPEX data, with PPE[§]

OPERATOR EXPOSURE (Geometric Mean Values)					
Task	**Estimated Actual dermal Exposure**	**Inhalation Exposure [1)]**	**Frequency of operation per day**	**Estimated Actual Dermal Exposure**	**Inhalation Exposure [1)]**
	[mg/person per operation]			[mg/person per day]	
Calibration [2)]	0.356	0.035	1	0.356	0.035
Mixing/Loading					
pre-mix [2)]	0.029	0.003	8	0.228	0.020
fast-coupling [2)]	0.130	0.003	8	1.038	0.020
Bagging (25 kg bags)	0.862	0.131	8 hours	6.900	1.045
Cleaning [2)]	2.084	0.397	1	2.084	0.397
SYSTEMIC EXPOSURE					
Task	**Dermal [3)]**	**Inhalation [4)]**	**Total**	**Total [mg/kg bw/day]**	
	[mg/person per day]	[mg/person per day]	[mg/person per day]	60 kg body weight	70 kg body weight
Calibration	0.00142	0.035	0.03595	0.00060	0.00051
Mixing/Loading					
pre-mix	0.00091	0.020	0.02132	0.00036	0.00030
fast-coupling	0.00415	0.020	0.02456	0.00041	0.00035
Bagging (25 kg bags)	0.0276	1.045	1.0726	0.01788	0.01532
Cleaning	0.0083	0.397	0.4051	0.00675	0.00579
SYSTEMIC EXPOSURE as a percentage of the AOEL (			**0.59**	**mg/kg bw/day)**	
Task	**Total [mg/kg bw/day]**		**% AOEL**		
	60 kg body weight	70 kg body weight	60 kg body weight	70 kg body weight	
Calibration	0.00060	0.00051	**0.10**	**0.09**	
Mixing/Loading					
pre-mix	0.00036	0.00030	**0.06**	**0.05**	
fast-coupling	0.00041	0.00035	**0.07**	**0.06**	
Bagging (25 kg bags)	0.01788	0.01532	**3.03**	**2.60**	
Cleaning	0.00675	0.00579	**1.14**	**0.98**	
Multiple Activity Task [5)]	0.02564	0.02197	**4.3**	**3.7**	

[§] Long-sleeved jacket and long trousers as usual work wear and in addition gloves.
[1)] Based on an average ventilation rate of 29 L/min.
[2)] Generic exposure value in mL x 25 mg/mL fludioxonil in the undiluted product
[3)] Estimated actual dermal exposure multiplied by percentage of dermal absorption (0.4%).
[4)] Inhalation exposure multiplied by percentage of inhalation exposure.
[5)] Sum of AOELs for a single operator performing calibration, fast couple mixing/loading, bagging and cleaning.

It is concluded that the calculations according to the Seed-TROPEX model predict an acceptable exposure to fludioxonil for operators treating cereals with A8207M when a long-sleeved work jacket and long trousers are worn with the addition of chemical resistant gloves during the handling of product or treated seeds. For the hypothetical operator performing the multiple activities of calibration, fast couple mixing/loading, bagging and cleaning, exposure was estimated as 4.3% and 3.7% of the AOEL for body weights of 60 and 70 kg for fludioxonil.

Mobile treaters

The Seed-TROPEX model does not contain data for the assessment of exposure of operators treating seeds on mobile equipment.

For the following reasons exposure to operators treating seed on mobile equipment is considered to be in the same range or less than the exposure to operators working in static plants:

- Treatment on mobile equipment is usually done outside. This will most likely lead to lower levels of dust in the vicinity of the operators compared to working in a closed environment.

- Treatment capacities are estimated to be lower (0.5 to 2 tonnes/hour) on mobile equipment compared to static industrial equipment (estimated to be in the range of 2 to 9 tonnes/hour).

- Exposure time is likely to be shorter than in static plants because part of the working day is used for movement of the treatment equipment to the farms or between farms.

Calculations for industrial seed treatment according to the Seed-TROPEX model predict acceptable levels of exposure to fludioxonil for operators wearing a long sleeved work jacket and long trousers with the addition of chemical resistant gloves during the handling of product or treated seeds. The degree of protection defined is considered to be appropriate for operators working on mobile treatment equipment.

On-farm treatment

The Seed-TROPEX model does not contain data for the assessment of exposure of operators treating seeds using on-farm treatment equipment.

For the following reasons exposure to operators treating seed on-farm is considered to be in the same range or less than the exposure to operators working in static plants:

- Treatment on-farm is usually done outside. This will most likely lead to lower levels of dust in the vicinity of the operators compared to working in a closed environment.

- Treatment capacities are estimated to be lower (0.5 to 2 tonnes/hour) with on-farm equipment compared to static industrial equipment (estimated to be in the range of 2 to 9 tonnes/hour).

- Exposure time is likely to be shorter than in static plants because the operator will only treat sufficient seed for planting on the farm.

Calculations for industrial seed treatment according to the Seed-TROPEX model predict acceptable levels of exposure to fludioxonil for operators wearing a long sleeved work jacket and long trousers with the addition of chemical resistant gloves during the handling of product or treated seeds. The degree of protection defined is considered to be appropriate for operators working with on-farm treatment equipment.

Loading and sowing of treated seeds

Calculations of operator exposure to fludioxonil during loading and sowing of treated seed are presented in Table 7.2.1.1-3.

VARIABLES	ACTIVE SUBSTANCES
Active substance	Fludioxonil
AOEL (mg/kg bw/day)	0.59
Working time (h)	10
Body weight (kg)	60 and 70
Dermal absorption (%)	0.4
Inhalation absorption (%)	100

Table 7.2.1.1-3: Estimated operator exposure to fludioxonil during loading and sowing of treated seed - Based on 1993 Seed-TROPEX data (geometric mean values), with PPE[§]

Route of exposure	Calculation of exposure					Systemic Exposure	
	Specific exposure		Working time		Route specific exposure	Absorption	Absorbed Dose
	[mg/person/hr]		[hrs/day]		[mg/person/day]	[%]	[mg/person/day]
Dermal	0.733	x	10	=	7.33	0.4	0.029
Inhalation	0.02	x	10	=	0.20	100	0.20
Total systemic dose [mg/person/day]							0.229
SYSTEMIC EXPOSURE as a percentage of the AOEL (					**0.59**	**mg/kg bw/day)**	
Task	**Total [mg/kg bw/day]**				**% AOEL**		
	60 kg body weight		70 kg body weight		60 kg body weight	70 kg body weight	
Loading and sowing	0.0038		0.0033		**0.65**	**0.56**	

[§]: Long-sleeved jacket and long trousers as usual work wear and in addition gloves when handling treated seed

It is concluded that the exposure calculations based on generic Seed-TROPEX data predict the systemic exposure to fludioxonil to be within the AOEL. This indicates an acceptable toxicological risk to operators involved in loading and sowing seed treated with A8207M and no adverse effects upon the health of operators would be expected.

Adequate work clothing should be worn. When handling treated seed or contaminated equipment protective gloves should be worn in addition.

French Seed-TROPEX

Estimation of operator exposure without personal protective equipment

Estimation of exposure during seed treatment of cereals

The French Seed-TROPEX model for seed treatment activities was derived from the original Seed-TROPEX database[8], but adopted the following changes, which were in line with the comments from

[8] Chester, G., Wiseman, M., Pontal, P-G., Worker Exposure During Seed Treatment and Sowing of Treated Seed in the UK and France: An Overview. Zeneca Agrochemicals, Fernhurst, Haslemere. Report No. TMF4896.

the Methodology group of the Advisory Committee on Pesticides of the French Ministry of Agriculture.

In the French Seed-TROPEX model for seed treatment four different scenarios regarding personal protective equipment (PPE) are considered. In "Scenario 1" no protection is worn during any of the seed treatment activities. "Scenario 2" considers gloves worn during all phases except bagging. For "Scenario 3" gloves are worn during all phases except bagging and respiratory protection worn (level P2 minimum) during the cleaning phase. In "Scenario 4" gloves and non-woven clothing is considered during all phases except gloves during bagging and respiratory protection (level P2 minimum) during the cleaning and bagging phases.

According to the French Advisory Council on Pesticides for evaluation the 75th and 90th percentiles of the Seed-TROPEX database will be used. The 75th percentile of the exposure data will be compared to the AOEL, this ratio will be used as criterion for a decision of acceptability of the risk. The risk evaluation with the use of the 90th percentile of the exposure data in comparison with AOEL will be also used.

Based on the application rates of 50 g fludioxonil/t seed for cereals, as well as the seed throughput per day of 75 t, the total amount of a.s. handled per day is 3.75 kg fludioxonil/day. A body weight of 70 kg is taken into account. As representative worst-case for industrial treatment, the use of 20 L containers is assumed. As a first tier approach exposure of operators according to Scenario 1 is calculated. This scenario represents an operator who does not wear any PPE.

The assumptions and parameters applied in the estimations are summarised below.

Fludioxonil

Scenario variables applied for the estimation of operator exposure to fludioxonil during seed treatment (Scenario 1)

- Type of seed: Cereals
- Application rate: 2 L A8207M/t seed (50 g a.s./t)
- Dilution factor: 1
- Throughput: 75 t/day
- Amount product handled per day: 150 L/day
- Amount a.s. handled per day: 3.75 kg/day
- Pack size: The use of 20 L containers is modelled as a worst-case scenario
- Operator body weight: 70 kg
- Dermal absorption: 0.4%
- Absorption by inhalation: 100%.

Frequency or duration of tasks assumed to occur in a stationary seed treatment plant with a moderate level of automation that achieves a throughput of about 75 tonnes of cereal seed per day:

- Total duration of a work shift: 8 hours/day (according to French conditions)
- Mixing/loading: 8 operations /day (calculated from pack size and amount product used)
- Calibration: 1 operation/day
- Bagging: 7 hours/day
- Cleaning: 1 operation /day
- Operator clothing (Scenario 1): In the Seed-TROPEX studies the operators wore one-layer cotton clothing (long sleeved work jacket and long trousers) as usual work wear during all activities. According to the French Seed-TROPEX model as tier one approach no protection like gloves are worn during calibration, mixing/loading and cleaning.

Details of the exposure estimations taking into account the 75th and 90th percentiles are presented in the following Tables 7.2.1.1-4 and 7.2.1.1-5. The comparison of the estimated exposure values with the AOEL for fludioxonil is shown in Table 7.2.1.1-6.

Table 7.2.1.1-4: Operator exposure to fludioxonil during seed treatment activities, based on French Seed-TROPEX model (75th percentiles)

Formulation concentration	25	mg/ml
Dilution factor	1	
Dermal penetration	0.40%	
Application dose	50	g a.s./ton
Systemic AOEL	0.59	mg/kg/day
Body weight	70	kg

CALIBRATION

Number of operations	1
Protective gloves	no
Mask	no
Protective overall	no

	Gloves	Actual dermal	Inhalation
	0.209 ml/ope	0.014 ml/ope	0.062 mg/ope
	5.22 mg/ope	0.362 mg/ope	0.062 mg/ope
	5.22 mg/day	5.58 mg/day	0.062 mg/day

Mixing/Loading

Number of operations	8
Protective gloves	no
Mask	no
Protective overall	no

	Gloves	Actual dermal	Inhalation
	0.0113 ml/ope	0.0011 ml/ope	0.050 mg/ope
	0.28 mg/ope	0.028 mg/ope	0.050 mg/ope
	2.257 mg/day	2.48 mg/day	0.400 mg/day

BAGGING (normal)

Number of hours	7
Protective gloves	no
Mask	no
Protective overall	no

	Gloves	Actual dermal	Inhalation
	0.00 mg/hr	1.14 mg/hr	0.031 mg/hr
	0.00 mg/day	7.95 mg/day	0.214 mg/day

CLEANING

Number of operations	1
Protective gloves	no
Mask	no
Ventilated helmet	no
Protective overall	no

	Gloves	Actual dermal	Inhalation
	18.50 mg/ope	4.53 mg/ope	1.515 mg/ope
	18.50 mg/day	23.03 mg/day	1.515 mg/day

Table 7.2.1.1-5: Operator exposure to fludioxonil during seed treatment activities, based on French Seed-TROPEX model (90th percentiles)

Formulation concentration	25	mg/ml
Dilution factor	1	
Dermal penetration	0.40%	
Application dose	50	g a.s./ton
Systemic AOEL	0.59	mg/kg/day
Body weight	70	kg

CALIBRATION

Number of operations	1
Protective gloves	no
Mask	no
Protective overall	no

Gloves	Actual dermal	Inhalation
0.406 ml/ope	0.014 ml/ope	0.062 mg/ope
10.15 mg/ope	0.362 mg/ope	0.062 mg/ope
10.15 mg/day	10.51 mg/day	0.062 mg/day

Mixing/Loading

Number of operations	8
Protective gloves	no
Mask	no
Protective overall	no

Gloves	Actual dermal	Inhalation
0.0518 ml/ope	0.0016 ml/ope	0.051 mg/ope
1.30 mg/ope	0.040 mg/ope	0.051 mg/ope
10.360 mg/day	10.68 mg/day	0.405 mg/day

BAGGING (normal)

Number of hours	7
Protective gloves	no
Mask	no
Protective overall	no

Gloves	Actual dermal	Inhalation
0.00 mg/hr	2.25 mg/hr	0.040 mg/hr
0.00 mg/day	15.72 mg/day	0.279 mg/day

CLEANING

Number of operations	1
Protective gloves	no
Mask	no
Ventilated helmet	no
Protective overall	no

Gloves	Actual dermal	Inhalation
23.50 mg/ope	9.80 mg/ope	2.21 mg/ope
23.50 mg/day	33.30 mg/day	2.21 mg/day

Table 7.2.1.1-6: Operator exposure to fludioxonil during seed treatment activities in comparison with the AOEL

	75th percentiles		90th percentiles	
	Estimated actual dermal exposure	Inhalation exposure	Estimated actual dermal exposure	Inhalation exposure
Total route specific exposure (mg/person/day)	39.03	2.191	70.21	2.959
Dermal absorption / inhalation absorption (%)	0.4	100	0.4	100
Route specific absorbed dose (mg/person/day)	0.156	2.191	0.281	2.959
Total absorbed dose (mg/person/day)	2.347		3.240	
Total absorbed dose (mg/kg bw/day)*	0.0335		0.463	
Percentage of AOEL (%)	5.7		7.8	

* Assuming 70 kg body weight

Conclusion

The exposure calculations based on generic Seed-TROPEX data (75th percentile) predict the systemic exposure to fludioxonil during the treating of cereal seed with A8207M to be within the AOEL.

Estimation of exposure during loading and sowing of treated seeds

Estimations are done using the French Seed-TROPEX model for sowing activities[7].

In the Seed-TROPEX studies the operators wore a long-sleeved work jacket and long trousers as usual work wear during all tasks and in addition gloves when handling treated seeds. Thus, in the Seed-TROPEX model the estimated actual dermal exposure (EADE) values, therefore, reflect this level of PPE.

The French Seed-TROPEX model for sowing activities[7] was derived from the original Seed-TROPEX database[8]. According to the French Advisory Council on Pesticides exposure values normalised to mg per hour were considered the most appropriate. In addition, for estimations the 75th percentile of the exposure data base and a working day of 8 hours was taken into account. Exposure by inhalation of operators loading and sowing of treated seed is based on an average ventilation rate of 29 L/min, which is in accordance with the value applied in the 1993 Seed-TROPEX studies for this activity. Although loading of the hopper may be physically demanding where manual handling of seed bags is involved, this activity is usually of short duration compared to the actual sowing of the seed. During the latter the operator is driving the tractor, possibly leaving it once in a while to verify the drilling depth or to check and equalise remaining amount of seeds in the hopper. An average ventilation rate of 29 L/min for the combined loading and sowing task is therefore considered to be conservative.

In the Seed-TROPEX studies the operators wore a long-sleeved work jacket and long trousers as usual work wear during all tasks and in addition gloves when handling treated seeds. Thus, in the Seed-TROPEX model the estimated actual dermal exposure (EADE) values reflect this level of PPE. In contrast in the French Seed-TROPEX model the actual dermal exposure corresponds to the contamination of hands not protected by gloves, contamination of the body protected by clothes and contamination of the head. Thus, in the case of the workers who were wearing gloves, the actual dermal contamination was assessed by adding the contamination measured on gloves. The 75th

percentile exposure values were considered in the French Seed-TROPEX model for exposure evaluation during loading and sowing activities.

The generic exposure values used for evaluation are presented in Table 7.2.1.1-7 below.

Table 7.2.1.1-7: Generic Seed-TROPEX exposure values for loading and sowing treated seeds (75th percentile values according to the French Advisory Council on Pesticides) – no PPE

TASK	Unit of exposure	Estimated Actual Dermal Exposure	Inhalation Exposure [1)]
Loading and sowing seeds	[mg/person/hour]	1.73	0.0288

[1)] Based on an average ventilation rate of 29 L/min.

The assumptions and parameters applied for the estimation of operator exposure during loading and sowing of cereal seeds treated with A8207M are given in the following.

Fludioxonil

Scenario variables applied for the estimation of operator exposure during loading and sowing of treated cereal seeds:

- Type of seed: Cereals
- Seed treated with: 2 L A8207M/t seed (50 g a.s./t)
- Working time: 8 hours/day
- Operator body weight: 70 kg
- Dermal absorption: 0.4%
- Absorption by inhalation: 100%
- Operator clothing: In the 1993 Seed-TROPEX studies the operators wore a long-sleeved work jacket and long trousers as usual work wear during all tasks. The estimated actual dermal exposure (EADE) values, therefore, reflect this level of clothing.

Details of the calculation are given in Table 7.2.1.1-8 below. The results are summarised in Table 7.2.1-5.

Table 7.2.1.1-8: Estimated operator exposure to fludioxonil during loading and sowing of treated cereals - French Seed-TROPEX model (75th percentile values), no PPE[§]

Route of exposure	Calculation of exposure					Systemic exposure	
	Specific exposure		Working time		Route-specific exposure	Absorption	Absorbed dose
	[mg/person/hr]		[hrs/day]		[mg/person/day]	[%]	[mg/person/day]
Dermal	1.73	x	8	=	13.84	0.4	0.0554
Inhalation	0.0288	x	8	=	0.230	100	0.230
Total systemic dose	[mg/person/day]						0.2854
	[mg/kg bw/day]*						0.00408
% of AOEL	(%)						0.7

[§]: Long-sleeved jacket and long trousers as usual work wear
*: Assuming a body weight of 70 kg

Conclusion

The exposure calculations based on generic Seed-TROPEX data predict the systemic exposure to fludioxonil during the loading and sowing of cereal seed treated with A8207M to be within the AOEL.

CP 7.2.1.2 Measurement of operator exposure

Seed treatment

Calculations based on generic data indicate acceptable levels of exposure, therefore no measurement was necessary and no operator exposure studies have been performed with A8207M.

Loading and sowing treated seed

Calculations based on generic data indicate acceptable levels of exposure, therefore no measurement was necessary and no operator exposure studies have been performed with A8207M.

CP 7.2.2 Bystander and resident exposure

In industrial seed treatment facilities the incidental presence of bystanders can be excluded by technical management measures. If occurring, exposure of bystanders would be of short duration and normally lower than that of seed treatment operators who are occupationally exposed all day long. The same applies for seed loading and sowing activities. Therefore, it is reasonable to assume that there will be no undue risk to persons being incidentally exposed to seed treatment or seed sowing operations.

It is concluded that there is no undue risk of exposure to the bystander after incidental short-term exposure to A8207M. This has no labelling implications.

CP 7.2.2.1 Estimation of bystander and resident exposure

Not applicable for seed treatment products (see Point CP 7.2.2).

CP 7.2.2.2 Measurement of bystander and resident exposure

Not applicable for seed treatment products (see Point CP 7.2.2).

CP 7.2.3 Worker exposure

The only intended use of A8207M is treatment of seed prior to sowing. Consequently, no re-entry scenario is given and therefore there is no unacceptable risk.

CP 7.2.3.1 Estimation of worker exposure

Not applicable for seed treatment products (see Point CP 7.2.3).

CP 7.2.3.2 Measurement of worker exposure

Not applicable for seed treatment products (see Point CP 7.2.3).

CP 7.3 Dermal Absorption

RMS Comment

Please provide a table with individual data for each cell, the mean and standard deviation.

Syngenta response:

As requested, the tables are presented below:

Table 7.3-1 Distribution of Radioactivity (% applied dose) at 24 h Post Dose following Topical Application of [^{14}C]-Fludioxonil in Formulation Concentrate (25 g/L) to Human Split-Thickness membrane

	Cell Number and Donor Number								Mean	SD
	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8		
	0663	0663	0666	0666	0623	0623	0671	0671		
Skin Wash 6 h	31.88	43.31	13.90	30.08	24.87	33.63	22.49	37.62	29.72	9.20
Tissue Swab 6 h	66.24	60.23	89.50	73.15	75.36	67.72	79.92	63.12	71.91	9.64
Pipette Tip 6 h	0.01	0.01	0.01	0.01	0.01	0.03	0.01	0.07	0.02	0.02
Skin Wash 24 h	0.07	0.15	0.10	0.09	0.05	0.61	0.07	0.31	0.18	0.19
Tissue Swab 24 h	0.07	0.07	0.07	0.06	0.06	0.46	0.07	0.17	0.13	0.14
Pipette Tip 24 h	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Donor Chamber Wash	0.05	0.29	0.24	0.57	0.04	0.43	0.09	0.31	0.25	0.19
Stratum Corneum 1-2	0.01	0.05	0.01	0.05	0.01	0.08	0.01	0.02	0.03	0.03
Stratum Corneum 3-5	0.00	0.02	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.00
Stratum Corneum 6-10	0.01	0.01	0.01	0.01	0.00	0.02	0.01	0.01	0.01	0.00
Stratum Corneum 11-15	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.00
Stratum Corneum 16-20	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.01	0.00
Unexposed Skin	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Exposed Skin	0.09	0.11	0.14	0.18	0.07	0.15	0.13	0.12	0.12	0.04
Receptor Fluid	0.13	0.14	0.22	0.18	0.05	0.05	0.05	0.05	0.11	0.07
Receptor Chamber Wash	0.01	0.01	0.02	0.03	0.00	0.01	0.01	0.01	0.01	0.01
Mass Balance	98.57	104.41	104.24	104.45	100.54	103.22	102.87	101.84	102.52	2.10

Table 7.3-2 Cumulative Absorption (% applied dose) of [^{14}C]-Fludioxonil into Receptor Fluid Following Topical Application of the [^{14}C]-Fludioxonil in Formulation Concentrate (25 g/L) to Human Split-Thickness Membranes

Time (h)	Cell Number and Donor Number								Mean	SD
	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8		
	0663	0663	0666	0666	0623	0623	0671	0671		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.78	1.76	0.00	0.00	0.00	0.00	0.32	0.64
4	2.75	0.00	3.59	0.11	0.00	0.00	0.00	0.00	0.81	1.48
6	15.11	14.38	3.48	0.11	0.00	5.99	5.08	0.00	5.52	6.16
8	36.63	10.50	76.55	0.11	0.00	0.36	0.30	3.14	15.95	27.48
12	117.50	70.75	260.10	0.11	42.03	30.00	2.71	19.67	67.86	86.76
24	358.54	375.30	591.02	492.63	124.22	139.03	130.07	140.45	293.91	185.83

RMS Comment:

It should be noted that according to EFSA guidance on dermal absorption (point 5.1) the first 2 tape strips can be excluded when calculating dermal absorption. For the other tape strips (3-20 in this case) they should be included since less than 75% of total absorption occurred within half of the study duration. Anyway taking into account the amount in receptor fluidD, receptor chamber wash, exposed skinD and tape strips 3-20, the mean of the absorbable values of the 8 cells plus the standard deviation, a value of 0,4% is obtained when rounded up in line with EFSA guidance.

Syngenta response:

The wording in the original submission was incorrect. The tape strips 1-2 had been excluded, the values presented were correct.

Report:	K-CP 7.3/01 Blackstock, C. (2015) Fludioxonil FS (A8207M) – The *In Vitro* Percutaneous Absorption of Radiolabelled Fludioxonil in the Concentrate Formulation Through Human Skin. Charles River Laboratories Edinburgh Ltd., Elphinstone Research Centre, Tranent, East Lothian, EH33 2NE. Laboratory Report No. 36950, 11 DEC 2015. Unpublished. (Syngenta File No. A8207M_10597)

Guidelines: OECD 428; *In Vitro* Dermal Absorption (Human)

GLP: Signed and dated GLP and Quality Assurance statements were provided. There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

The rate and extent of absorption of fludioxonil following topical application as a flowable concentrate for seed treatment (FS) formulation (A8207M) through human split-thickness skin was measured *in vitro*. The concentration of the fludioxonil in the concentrate FS formulation was 25 g/L.

The Formulation Concentrate was applied at *ca* 10 µL/cm^2 and left unoccluded for an experimental period of 24 h, with an interim wash at 6 h post-application.

The absorption process was followed by taking samples of the receptor fluid (phosphate buffered saline containing polyoxyethylene 20 oleyl ether (*ca* 6%, w/v), sodium azide (*ca* 0.01%, w/v),

streptomycin (0.1 mg/mL) and penicillin (100 units/mL)) at recorded intervals throughout the experimental period.

The distribution of fludioxonil within the test system and a 24 h absorption profile were determined using liquid scintillation counting. Before conducting the main study, stability and solubility assessments were carried out.

The study demonstrated that the amount of fludioxonil absorbed through human split-thickness skin membranes over 24 h (following a 6 h exposure) from the Formulation Concentrate (25 g/L) was 0.12% of the applied dose, as measured in the receptor fluid and receptor chamber wash.

The dermal absorption value used for the operator exposure risk assessment calculations is greater than 0.12%. The value of 0.4% also includes fludioxonil measured in the exposed skin ~~and in~~ but excluding tape strips 1-2 (mean = 0.15%); and since the Standard Deviation was more than 25% of the mean the SD (0.10%) was then added, arriving at 0.37%. Finally, the value was rounded up to the nearest 1 decimal place in line with the EFSA guidance on dermal absorption.

MATERIALS AND METHODS

Materials:

Test Material:	Fludioxonil Technical
Description:	Brownish powder
Product Code:	CGA173506
Purity:	97.7%
Storage Conditions:	<30°C
Radiolabelled Test Material:	[Phenyl-U-^{14}C]-CGA173506 [^{14}C]-Fludioxonil
Batch Number:	MJD-I-12
Radiochemical Purity:	98.8%
Specific Activity:	57.0 µCi/mg
Stability of Test Compound:	Confirmed
Storage Conditions:	Refrigerated at 4°C/protected from light
Commercial Formulation:	Fludioxonil FS (A8207M)
Design Code:	A8207M
Batch Number:	SSP3D1566M
Physical Appearance:	Liquid
Storage Conditions:	<30°C
Blank Formulation:	Blank Formulation of A8207M
Batch Number:	BAR001-063-001-BLANK
Physical Appearance:	Liquid
Storage Conditions:	<30ºC

Study Design and Methods:

In-life dates: Start: 28 August 2015 End: 17 October 2015

Diffusion cell: Diffusion of [^{14}C]-Fludioxonil into and across the skin to receptor fluid was measured using glass diffusion cells in which the split-thickness skin formed a horizontal membrane and provided an application area of 0.64 cm^2.

Receptor fluid: The receptor fluid chosen was: phosphate buffered saline containing polyoxyethylene 20 oleyl ether (6%, w/v), sodium azide (*ca* 0.01%, w/v), streptomycin (0.1 mg/mL) and penicillin (100 units/mL). The pH was adjusted to 7.4 ± 0.1. It was degassed and stored in a refrigerator set to maintain a temperature of +4ºC prior to use on the study.

Skin preparation integrity: The integrity of the membranes was checked by measurement of the electrical resistance across the skin. Only those membranes with an acceptable resistance (>10.9 kΩ), thereby showing that they were intact, were used on the study.

Test substance: The dose for [^{14}C]-Fludioxonil was prepared to mimic the commercial formulation using the technical material, [^{14}C]-labelled test item and blank formulation.

Application to the skin: [^{14}C]-Fludioxonil in Formulation Concentrate was applied over the stratum corneum surface of the exposed skin of 8 split-thickness skin samples each using an MR25 Rainin positive displacement pipette set to deliver *ca* 6.4 µL (10 µL/cm^2). Following application of the test preparation, the test system remained unoccluded for an experimental period of 24 h, with a wash at 6 h and 24h post-application.

Temperature: Throughout the experiment the receptor fluid was stirred and maintained at a normal skin temperature of 32 ± 1^{o}C by a circulating water bath.

Duration of exposure and sampling: The skin was exposed to the test preparations for 6 hours and receptor fluid samples were collected at 2, 4 and 6 hours post dose. To allow adequate characterisation of the absorption profile, receptor fluid samples were also collected at 8 hours and 12 hours post dose.

Terminal exposure (6 h Post Dose): At 6 h post dose, the exposure was terminated. Commercial hand wash soap (50 µL) was applied to the skin and gently rubbed in with a tissue swab. The skin was then rinsed with *ca* 5 mL of a *ca* 2% (v/v) commercial soap solution. The soap solution was applied in aliquots (0.5 mL), aspirated three times, and each aliquot was added and removed with a pipette. The skin was dried with a tissue swab. This process was repeated and the skin was dried with an additional tissue swab. The soap solution (skin wash) was pooled into a single pre-weighed vial for each cell. Samples were weighed, and duplicate weighed aliquots (1 mL) were taken from all skin wash samples, mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting. Acetone (10 mL) was added to the tissue swabs, and the vials were allowed to extract overnight. The tissue swab vials were sonicated for 10 min. The samples were then split into 5 vials for each cell (2 mL of solvent and one tissue swab in each vial) and mixed with scintillation fluid (10 mL). These were mixed by vortex for *ca* 20 s prior to analysis by liquid scintillation counting. The tips were retained separately, cut in half, mixed with methanol:scintillation fluid (1:5, v/v; 12 mL) and analysed by liquid scintillation counting.

Terminal post exposure procedure (24 h Post Dose): After an 18 h monitoring period, *i.e.* at 24 h post dose, the skin was washed and all samples analysed as described above, with the exception that the 24 h tissue swabs were mixed with methanol:scintillation fluid (1:5, v/v; 12 mL) and analysed by liquid scintillation counting. The donor chambers were transferred to a pre-weighed pot containing acetone (*ca* 40 mL). Donor wash pots were left to extract for >30 min prior to sonication for 10 min and the apparatus was removed for washing. Duplicate weighed aliquots (1 mL) were taken from each donor wash pot, mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting. The skin was removed from each cell and placed on a piece of tissue to remove any remaining receptor fluid from the underside of the skin. This tissue was placed into the pre-weighed receptor chamber wash pot for that particular cell.

The stratum corneum was removed with 20 successive tape strips. The skin sample was rotated 90° after each tape strip. Each tape strip was placed into an individual vial containing methanol:scintillation fluid (1:5, v/v; 12 mL) and then analysed by liquid scintillation counting. The skin under the cell flange (unexposed skin) was cut away from the exposed skin. The exposed and unexposed skin samples were placed into separate vials containing Solvable® (2 mL). The skin samples were placed into a waterbath set to 60°C to aid solubilisation. When fully dissolved, stannous chloride solution (0.2 g/mL in ethanol; 150 µL) and scintillation fluid (10 mL) was added to the skin samples and analysed by liquid scintillation counting.

Bulk receptor fluid was removed from each receptor chamber and retained in a vial. This was split into two. Scintillation fluid (10 mL) was added to the new vial and the original bulk receptor fluid vial. All receptor fluid samples were then analysed by liquid scintillation counting.

The receptor chambers were rinsed with acetone (20 mL). The solvent was pooled as a single sample into the pre-weighed receptor wash pot. Duplicate weighed aliquots (1 mL) of the solvent were mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting.

Analysis: All components of the test system (e.g. receptor fluid, skin wash, donor chamber, tape strips) were analysed by liquid scintillation counting and the recovery determined.

Data: Results of the analysis of the samples of receptor fluid collected in the study were expressed as amounts of [^{14}C]-Fludioxonil in the receptor solution in terms of µg equiv./cm^2, 'percentage of dose absorbed' and rates of absorption (ng equiv./cm^2/h). The results of the mass balance and distribution determinations are expressed in terms of amount (µg equiv./cm^2) and 'percentage of applied dose'.

Definition of absorbed test material: The absorbed (systemically available) dose is considered to be the test material detected in the receptor fluid and receptor chamber wash. Material removed from the surface of the skin by the washing procedure is regarded as unabsorbed. The test material recovered from the skin at the end of the exposure is also considered to be unabsorbed, although it is recognised that a proportion of this material may be absorbed beyond the duration of the exposure investigated in this study.

RESULTS AND DISCUSSION

[^{14}C]-Fludioxonil Formulation Concentrate (25 g/L) in human split-thickness skin membranes

A total of 8 samples of human split-thickness skin membranes obtained from 4 different donors were dosed topically with [^{14}C]-Fludioxonil in the Formulation Concentrate (25 g/L). Overall, the absorption profiles looked similar for all samples. The mass balance for all individual samples was within 100 ± 10%. The following results are provided as mean values (n = 8).

The mean absorption rate of [^{14}C]-Fludioxonil from the Formulation Concentrate through human split-thickness skin was 12.3 ng equiv./cm^2/h during the 24 h experimental period. The amount penetrated at 24 h, as measured in the receptor fluid, was 0.29 µg equiv./cm^2 (0.11% of the applied dose).

Following the skin wash at 6 h, 101.65% of the applied dose of [^{14}C]-Fludioxonil was washed off. At 24 h post dose, a further 0.31% was removed during the wash. A proportion of the dose applied was recovered from the donor chamber (0.25%), exposed skin (0.12%) and receptor chamber wash (0.01%). The mean total recovery was 102.52% of the applied dose.

Table 7.3-~~1~~3: Summary of Fludioxonil Distribution Through Human Split Thickness Membranes

Test Preparation	Formulation Concentrate (25 g/L)
Test Item	Fludioxonil
Distribution	% Applied Dose
Dislodgeable Dose 6 h*	101.65
Total Dislodgeable Dose**	102.21
Donor Chamber Wash	0.25
Tape Strips 1-2	0.03
Tape Strips 3-20	0.03
Unexposed Skin	<0.01
Exposed Skin	0.12
Receptor Fluid	0.11
Receptor Chamber Wash	0.01
Mass Balance	102.52
Distribution	µg equiv./cm^2
Dislodgeable Dose 6 h*	273.75
Total Dislodgeable Dose**	275.27
Donor Chamber Wash	0.68
Tape Strips 1-2	0.09
Tape Strips 3-20	0.08
Unexposed Skin	0.01
Exposed Skin	0.33
Receptor Fluid	0.29
Receptor Chamber Wash	0.04
Mass Balance	276.10

* Dislodgeable Dose 6 h = Skin wash 6 h + Tissue swab 6 h + Pipette tip 6 h

** Total Dislodgeable Dose = Dislodgeable Dose 6 h + Dislodgeable Dose 24 h + Donor chamber wash

Table 7.3-~~2~~4: Summary of Fludioxonil Absorption through Human Split-Thickness Membranes

Application of Test Materials and Actual Concentration of Dose Preparation	Mean Absorption Rates	
	Time Period (h)	Absorption Rate
Formulation Concentrate		ng equiv./cm^2/h ± SEM
(27.06 g/L Fludioxonil)	0-2	0.16 ± 0.11
9.95 µL/cm^2 (269.32 µg ai/cm^2)	2-6	1.35 ± 0.55
Unoccluded	6-24	16.02 ± 3.63
Duration of experiment: 24 h, n = 8	0-24	12.25 ± 2.74

CONCLUSION: The study demonstrated that the amount of fludioxonil absorbed through human split-thickness skin membranes over 24 h (following a 6 h exposure) from the Formulation Concentrate (25 g/L) was 0.12% of the applied dose, as measured in the receptor fluid and receptor chamber wash.

The dermal absorption value used for the operator exposure risk assessment calculations is greater than 0.12%. The value of 0.4% also includes fludioxonil measured in the exposed skin ~~and in~~ but excluding tape strips 1-2 (mean = 0.15%); and since the Standard Deviation was more than 25% of the mean the SD (0.10%) was then added, arriving at 0.37%. Finally, the value was rounded up to the nearest 1 decimal place in line with the EFSA guidance on dermal absorption.

(Blackstock, C, 2015)

CP 7.4 Available Toxicological Data Relating to Co-Formulants

CONFIDENTIAL information - data provided separately (Document J)