REGISTRATION REPORT

Part B
Section 3: Mammalian Toxicity

Detailed summary of the risk assessment

TRANSFORM (GF-2372)

500 g/Kg Sulfoxaflor

Southern Zone

Zonal Rapporteur Member State: France

CORE ASSESSMENT

Applicant: DOW AgroSciences

Date: October 2017

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IIIA 7 TOXICOLOGICAL STUDIES

Sulfoxaflor is a new active substance which is listed in Annex I of Directive 91/414. Ireland (Pesticide Registration and Control Division, PRCD) is the rapporteur Member State (RMS). A dossier for the active substance was submitted by Dow AgroSciences, under Regulation (EC) 1107/2009, to the RMS in July 2011. The evaluation of the dossier and review report (i.e. full report) including an outline of the technical questions was finalised May 29th, 2015. A Commission decision following Annex I inclusion was published August 18th, 2015 (Reg. (EU) 2015/1295).
The active substance submission followed a zonal approach where the evaluation was shared by four Member States participating under a work-share umbrella as follows: Ireland - RMS, lead reviewer for the sections Toxicology, Residues and Metabolism along with coordination of the work-share project. France: Lead reviewer for the sections Identity/Physical-Chemical properties, Methods of Analysis and Efficacy/Biology. Poland: Lead reviewer for the section Environmental Fate. Czech Republic: Lead reviewer for the section Ecotoxicology.

The Draft Assessment Report (DAR) on sulfoxaflor was finalised and distributed by Ireland in November 2012, with a recommendation for approval of the active substance according to Regulation (EC) 1107/2009. The EFSA peer review process was conducted and the EFSA conclusion was published in May 2014.

Dow AgroSciences submitted an EU MRL dossier to the RMS, Ireland, in April 2011. The EU MRL evaluation was integrated with the active substance evaluation under 1107/2009. The adoption of MRL/Import tolerances was therefore aligned with the same timing as that of the active substance approval.

There were two representative formulations for the EU active approval submission. These were GF-2372 (500 g/kg WG) and GF-2626 (120 g/L SC).

This current submission is for one of these two formulations, GF-2372. This is the first submission for authorisation of plant protection products containing sulfoxaflor in EU Member States. The proposed zonal RMS for Central Zone and Southern Zone are Ireland and France respectively.

Where appropriate, this document refers to the conclusions of the EFSA review report (EFSA Journal 2014; 12(5):3692) of sulfoxaflor. This will be where: the active substance data are relied upon in the risk assessment of the formulation; or when the EU review concluded that additional data/information should be considered at national registration.

This Part B document only reviews data (active substance or plant protection product) and additional information that has not previously been considered within the EU review process, as part of the active approval decision. Studies for the active substance which have already been evaluated during the approval process are not summarised. New active substance data are only included if they are considered essential for the evaluation and a full study summary is provided.

Details of the active substance, the active approval Regulation and the Commission Review Report are provided in Table #-1.

Table #-1: Details for the active substance

Active Substance	Approval Regulation	Commission Review Report	EFSA Scientific Report
Sulfoxaflor	Regulation (EU) 2015/129 (18 August 2015)	SANTE/10665/2015 rev 2, 29 May 2015	EFSA Journal 2014; 12(5):3692

The review report for sulfoxaflor (SANTE/10665/2015 rev 2, 29 May 2015) is considered to provide the relevant review information or a reference to where such information as considered appropriate for this application can be found. The following table provides the EU endpoints to be used in the evaluation.

Active substance	Acceptable Daily Intake (ADI) mg/kg bw/d	Acute reference dose (ARfD) mg/kg bw	Acceptable operator exposure level (AOEL) mg/kg bw/d	Classification
Sulfoxaflor approved	**0.04 (2-years rat oral study, SF 100)**	**0.25 (90-day rat oral study, 90-day dog study and 1-year dog study, SF 100)**	**0.06 (Rat acute neurotoxicity study, SF 100)**	Cat4 H302 Cat1 H400 Cat1 H410
Origin	UE, 2015	UE, 2015	UE, 2015	CEE

The active approval Regulation for sulfoxaflor provides specific provisions which need to be considered by the applicant in the preparation of their product submissions and by the Member States prior to granting an authorisation:

For sulfoxaflor, Member States shall pay particular attention to [to be defined].

These concerns have been addressed within the current submission.

NOTE
Sulfoxaflor is also referred to as manufacture's code numbers X11422208, XR-208, XDE-208 and DE-208 in the section.

IIIA 7.1 Acute toxicity

Overall Summary

Table 7.1-1: GF-2372 Acute toxicity data

Parameter	Species (sex)	Result	EU Classification	Reference
Acute oral	Rat/ Fischer 344 (both)	LD_{50} >2000 mg/kg	None	[redacted] 2009a
Acute dermal	Rat/ Fischer 344 (both)	LD_{50} >5000 mg/kg	None	[redacted] 2009b
Acute inhalation	Rat/ Fischer 344 (both)	LC_{50} >5.35 mg/L (4h)	None	[redacted] 2010
Skin irritation	Rabbit/ NZW (female)	Minimal irritation	None	[redacted] 2010a
Eye irritation	Rabbit/ NZW (female)	Moderate irritation	None	[redacted] 2010b
Skin sensitisation	Mouse/ CBA/J (female)	No sensitization	None	[redacted] 2010

Overall Conclusions: IIIA 7.1 Acute toxicity

GF-2372 exhibited low acute oral, dermal and inhalation toxicity in the rat with no mortality at limit doses of 2000 mg/kg, 5000 mg/kg and 5.35 mg/L, respectively. Skin irritation was minimal and resolved by 72 hours. Eye irritation included minimal corneal effects which resolved in all animals by Day 1 or 10. No skin sensitization potential was evident in a mouse local lymph node assay.

Classification of formulation GF-2372 in accordance with Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures, as follows:

- Unclassified

IIIA 7.1.1 Acute oral toxicity

Report	KIIIA1 7.1.1/01 [redacted] (2009a)
Title	Acute Oral Up and Down Procedure in Rats. [redacted] August 18, 2009
Document No	EPSL Study Number 27566, Dow Study Number 090348
Guideline	Acute Oral Toxicity – Rat; OPPTS 870.1100; OECD 425
GLP	Yes

EXECUTIVE SUMMARY: An acute oral toxicity test (Up and Down Procedure) was conducted with Fischer 344 rats to determine the potential for GF-2372 to produce toxicity from a single dose via the oral route. Under the conditions of this study, the acute oral LD_{50} of GF-2372 was greater than 2,000 mg/kg of body weight in female and male rats.

A limit dose of 2,000 mg/kg bw was administered to 4 male and 4 female rats by oral gavage. All animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for up to 14 days after dosing. Body weights were recorded prior to administration and again on Days 7 and 14 (termination) following dosing. Necropsies were performed on all animals at sacrifice.

All animals survived test substance administration and gained body weight during the study. Following administration, all rats exhibited clinical signs including facial staining, ano-genital staining and/or reduced fecal volume, but all animals recovered from these symptoms by Day 8 and appeared active and healthy for the remainder of the observation period. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

Under the conditions of this study, the acute oral LD_{50} of GF-2372 was greater than 2,000 mg/kg of body weight in female and male rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	GF-2372
	Description:	White granular solid
	Lot #:	E2110-28
	Test Substance Number	031070-0004
	Purity:	49.9% XDE-208

2. Vehicle and/or positive control: None

3.	**Test animals:**		
	Species:	Rat	
	Strain:	Fischer 344	
	Age/weight at dosing:	9-12 weeks/ females: 130-140 grams and males: 160-172 grams at experimental start	
	Source:	[redacted]	
	Housing:	Singly housed in suspended stainless steel caging with mesh floors	
	Diet:	Purina Certified Rodent Diet, PMI #5002 *ad libitum*	
	Water:	Filtered tap water *ad libitum*	
	Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	19-22ºC 38-71%RH 15/hr 12 hrs dark/12 hrs light
	Acclimation period:	7-30 days	

B. STUDY DESIGN and METHODS:

1. In life dates - Start: May 21, 2009 End: July 14, 2009

2. Animal assignment and treatment - Animals were assigned to the test group as noted in Table 7.1.1/1-1. Following an overnight fast, rats were given a single dose of GF-2372 by gavage then observed (first several hours post-dosing and at least once daily thereafter) and weighed prior to test substance administration (initial) and again on Days 7 and 14 (termination). An initial dose was administered to one healthy female rat by oral gavage. Due to the absence of mortality in this animal, three additional females received the same dose level, sequentially. Since these animals survived, four males received the test substance at a dose level of 2,000 mg/kg, simultaneously. In the absence of mortality in either sex, no additional animals were tested. All rats were euthanized via CO_2 inhalation at the end of the 14-day observation period. Gross necropsies were performed on all euthanized animals. The external surface of the body and all orifices, tissues, and organs of the thoracic and abdominal cavities were examined.

II. RESULTS AND DISCUSSION:

A. Mortality is given below in Table 7.1.1/1-1.

Table 7.1.1/1-1. Doses, mortality/animals treated

Dose (mg/kg bw)	Females	Males
2,000	0/4	0/4

B. Clinical observations: All animals survived test substance administration. Following administration, all rats exhibited clinical signs including facial staining, ano-genital staining and/or reduced fecal volume, but all animals recovered from these symptoms by Day 8 and appeared active and healthy for the remainder of the observation period.

C. Body weight: All animals gained body weight during the study.

D. Necropsy observations: No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

E. Applicant's Conclusions: Under the conditions of this study, the acute oral LD_{50} of GF-2372 was greater than 2,000 mg/kg of body weight in female and male rats.

IIIA1 7.1.1/01 Study comments	The study is acceptable.
IIIA1 7.1.1/01 Agreed endpoint	Acute oral LD_{50} > 2000 mg/kg bw in the rat. According to the classification criteria of **CLP Regulation (EC) No. 1272/2008**, the test material **does not require classification** for acute oral toxicity.

IIIA 7.1.2 Acute percutaneous (dermal) toxicity

Report	KIIIA1 7.1.2/01 [redacted] (2009b)
Title	Acute Dermal Toxicity in Rats. [redacted] August 18, 2009
Document No	EPSL Study Number 27567, Dow Study Number 090349
Guideline	Acute Dermal Toxicity - Rat; OPPTS 870.1200; OECD 402
GLP	Yes

EXECUTIVE SUMMARY: An acute dermal toxicity test was conducted with Fischer 344 rats to determine the potential for GF-2372 to produce toxicity from a single topical application.

A limit dose of 5000 mg/kg bw was moistened with a 0.5% w/w solution of carboxymethylcellulose (CMC) in distilled water and then applied to the skin of ten healthy rats for 24 hours. The animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days. Body weights were recorded prior to application and again on Days 7 and 14 (termination). Necropsies were performed on all animals at terminal sacrifice.

All animals survived, gained body weight and appeared active and healthy during the study. There were no signs of gross toxicity, dermal irritation, adverse clinical signs, or abnormal behavior. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

Under the conditions of this study, the single dose acute dermal LD_{50} of the test substance was greater than 5,000 mg/kg of body weight in male and female rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	GF-2372
	Description:	White granular solid
	Lot #:	E2110-28
	Test Substance Number	031070-0004
	Purity:	49.9% XDE-208

2. Vehicle and/or positive control: CMC in distilled water

3.	**Test animals:**		
	Species:	Rat	
	Strain:	Fischer 344	
	Age/weight at dosing:	12 weeks/males: 218-243 and females: 151-164 grams at experimental start	
	Source:	[redacted]	
	Housing:	Singly housed in suspended stainless steel caging with mesh floors	
	Diet:	Purina Certified Rodent Diet, PMI #5002 *ad libitum*	
	Water:	Filtered tap water *ad libitum*	
	Environmental conditions:	**Temperature:** **Humidity:** **Air changes/Hour:** **Photoperiod:**	19-22ºC 38-68%RH 15//hr 12 hrs dark/12 hrs light
	Acclimation period:	30 days	

B. STUDY DESIGN and METHODS:

1. In life dates - Start: May 21, 2009 End: June 4, 2009

2. Animal assignment and treatment - Animals were assigned to the test groups noted in Table 7.1.2/1-1. On the day prior to application, a group of animals was prepared by clipping the dorsal area and the trunk. Prior to use the test substance was ground and then moistened with a 0.5% w/w solution of CMC in distilled water to achieve a dry paste by preparing a 60% w/w mixture. Five thousand mg/kg of body weight of the ground test substance was placed on a 2-inch x 3-inch, 4-ply gauze pad and applied evenly over a dose area of approximately 2 inches x 3 inches (approximately 10% of the body surface) on each animal. The gauze pad and entire trunk of each animal were then wrapped with 3-inch Durapore tape to avoid dislocation of the pad and to minimize loss of the test substance. The rats were then returned to their designated cages. The day of application was considered Day 0 of the study. All animals were observed during the first several hours after application and at least once daily thereafter for up to 14 days, and weighed prior to test substance application (initial) and again on Days 7 and 14 (termination). All rats were euthanized via CO_2 inhalation at the end of the 14-day observation period. Gross necropsies were performed on all animals. The external surface of the body and all orifices, tissues, and organs of the thoracic and abdominal cavities were examined.

II. RESULTS AND DISCUSSION:

A. Mortality is given below in Table 7.1.2/1-1.

Table 7.1.2/1-1. Doses, mortality/animals treated

Dose (mg/kg bw)	Females	Males	Combined
5,000	0/5	0/5	0/10

B. Clinical observations – All animals survived exposure to the test substance. There were no signs of gross toxicity, dermal irritation, adverse clinical signs, or abnormal behavior.

C. Body Weight – All animals gained body weight throughout the 14-day observation period.

D. Necropsy - No gross abnormalities were noted for the animals when necropsied at the conclusion of the 14-day observation period.

E. Applicant's Conclusions:

The dermal LD_{50} of GF-2372 was greater than 5,000 mg/kg of body weight in male and female rats.

IIIA1 7.1.2/01 Study comments	The study is acceptable.
IIIA1 7.1.2/01 Agreed endpoint	Acute dermal LD_{50} > 5000 mg/kg bw in the rat. According to the classification criteria of **CLP Regulation (EC) No. 1272/2008**, the test material **does not require classification** for acute dermal toxicity.

IIIA 7.1.3 Acute inhalation toxicity

Report	KIIIA1 7.1.3/01 [redacted] (2010).
Title	GF-2372: Acute Dust Aerosol Inhalation Toxicity Study in F344/DuCrl Rats. [redacted] (10 June 2010)
Document No	Study ID: 101024
Guideline	Acute Inhalation Toxicity – (rats); OPPTS 870.1300; OECD 403; JMAFF; EEC
GLP	Yes

EXECUTIVE SUMMARY: an acute inhalation toxicity test was conducted with Fischer 344 rats to determine the potential for GF-2372 to produce toxicity from an inhaled dose. Groups of five F344/DuCrl rats/sex were exposed for four hours, using a nose-only inhalation exposure system, to a time-weighted average chamber concentration of 5.35 mg GF-2372 per liter of air. The mass median aerodynamic diameter (MMAD) of particulate GF-2372 present in the exposure chamber test atmosphere averaged 3.31 microns with an average geometric standard deviation of 1.52 microns.

All animals survived the four-hour exposure to the test material as well as the two-week post-exposure period. Clinical effects noted during the exposure were limited to soiling of the haircoat in all five female rats. In-life observations noted post-exposure were limited to perineal soiling in one female rat. All animals appeared normal by test day 3. Mean body weight losses of 7.5 and 5.7% were noted for male and female rats, respectively, on test day 2. Pre-exposure mean body weight values for males and females were exceeded on test days 4 and 8 respectively. There were no visible treatment-related lesions noted in any of the rats exposed to GF-2372 at the test day 15-

scheduled necropsy. One female was noted to have a hiatal hernia of the liver that was determined to be unrelated to treatment.

Based on these data, the four-hour LC_{50} of inhaled particulate GF-2372 is greater than 5.35 mg/L for male and female F344/DuCrl rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	GF-2372
	Lot/Batch #:	Lot # E2110-28, TSN031070-0004
	Description:	White dust formulation
	Purity:	49.9% XDE-208
	CAS #:	XDE-208: 946578-00-3

2. Vehicle and/or positive control: None

3.	**Test animals:**		
	Species:	Rat	
	Strain:	Fischer 344	
	Age/weight at dosing:	8 weeks at the time of exposure	
	Source:	[redacted]	
	Housing:	Singly housed in suspended stainless steel caging with mesh floors	
	Food and Water:	LabDiet Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in pelleted form. Feed and municipal water was provided *ad libitum* except during the 2-hour acclimation period the day prior to exposure and during the 4-hour exposure period. Drinking water obtained from the municipal water source was periodically analyzed for chemical parameters and biological contaminants by the municipal water department.	
	Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	22 ± 1°C (maximum permissible excursion of ± 3°C) 40-70% 12-15 times/hour Photoperiod: 12-hour light/dark (on at 6:00 a.m. and off at 6:00 p.m.)
	Acclimation period:	The acclimation period was one week prior to the start of the study. Animals were acclimated to the nose cones for at least two hours on the day preceding exposure to the test material.	

B. STUDY DESIGN AND METHODS:

1. In life dates: - Start: 07 April 2010 End: 21 April 2010.

2. Exposure conditions: Exposure room temperature, chamber temperature, humidity and airflow were monitored continuously and recorded approximately every 30 minutes during the exposure period.

3. Animal assignment and treatment: Before administration of test material began, animals were stratified by body weight and then randomly assigned to treatment groups using a computer program designed to increase the probability of uniform group mean weights and standard deviations at the start of the study. Animals placed on study were

uniquely identified via subcutaneously implanted transponders (BioMedic Data Systems, Seaford, Delaware) that were correlated to unique alphanumeric identification numbers.

4. Generation of the test atmosphere / chamber description:

Exposure: The exposures occurred under dynamic airflow conditions.

Chambers: A 42-liter, Dow-modified ADG nose-only chamber [30 centimeters (cm) in diameter by 60 cm high] was used for the study. Compressed filtered air supplied to the chamber was at ambient temperature. Airflow through the chamber was determined with a manometer which measured the pressure drop across a calibrated orifice plate and was maintained at approximately 30 liters per minute, which was sufficient to provide the normal concentration of oxygen to the animals and approximately 43 air changes per hour. The manometer was calibrated with a gas meter (Model DTM-115, Singer Aluminum Diaphragm Meter, American Meter Division, Philadelphia, Pennsylvania) prior to the start of the study. The chamber was operated at a slightly positive pressure relative to the surrounding area and was contained within a secondary vented area. Chamber and exposure room temperature were recorded from two thermocouples attached to an electronic digital thermometer (Control Company, Friendwood, Texas), one thermocouple extended into the exposure chamber and the second was stationed next to the chamber. Chamber relative humidity was monitored by a hygrometer (Brooklyn Thermometer Co., Inc., Farmingdale, New York) stationed in the interior of the chamber. Based on the 30 liter per minute flow rate, the theoretical equilibrium time to 99% (T_{99}) of the target concentration was 6.4 minutes. The animals were placed on the chamber after the T_{99} had elapsed and were removed after 240 minutes of exposure.

Generation System: The test material was first ball-milled prior to use in order to reduce the particle size. A dust aerosol of GF-2372 was generated using a Jet Mill (Model 00 Jet-O-Mizer, Fluid Energy Aljet, Plumsteadville, Pennsylvania). An AccuRate Dry Material Feeder (Whitewater, Wisconsin) was used with vibration added to continuously feed test material into the jetmill.

Exposure Conditions
Exposure room temperature, chamber temperature, humidity and airflow were monitored continuously and recorded approximately every 30 minutes during the exposure period.

Exposure Concentration: The mass concentration of aerosol present in the chamber was determined gravimetrically 7 times during the exposure period. Samples were taken by drawing air, at 1 L/minute, through a sample probe located in the breathing zone of the animals. Aerosol particles were collected on preweighed glass fiber filters (PALL Corporation, Ann Arbor, Michigan). Background measurements of the chamber were taken prior to starting the exposure. The time-weighted average (TWA) exposure concentration was calculated from the gravimetric measurements, after subtraction of the average background measurements.

The nominal concentration was calculated based on the mass of test material fed into the generation system divided by the total chamber airflow during the exposure period.

Particle Size: The aerodynamic particle size was determined twice during the exposure period by drawing samples from within the animal breathing zone, at a set rate using a constant flow air sampling pump through a multi-stage mercer-style cascade impactor. The MMAD and geometric standard deviation (GSD) were determined for each sample as well as the average of the samples.

A sorbent tube was placed in-line following the cascade impactor to trap vapors to prevent contamination of the pump used to draw the samples.

5. **Statistics:** Means and standard deviations were calculated for descriptive purposes for chamber concentration (mean only), animal body weights, exposure room temperature and chamber temperature, humidity, and airflow.

II. RESULTS AND DISCUSSION:

A. **Chamber Summary Data:** The resulting time-weighted average concentration was 5.35 mg/L; the nominal concentration was 14.05 mg/L. The difference between the gravimetric and the nominal concentration was due to loss of test material coating the walls of the generation apparatus and exposure chamber, and the inefficiency of the generation system employed.

The average chamber temperature and relative humidity were 22°C and 35.1 ± 1.5%, respectively. The average exposure room temperature was 22 °C. The chamber O_2 level was determined to be 20.8 % and the CO_2 level was determined to be 373 ppm. Airflow was maintained at approximately 30 liters per minute.

Based on two determinations, the mean MMAD of the particles was 3.31 microns with an average geometric standard deviation of 1.52 microns. Approximately 92% of the particulate mass was present in size fractions with an aerodynamic diameter less than 6 microns. Approximately 15% of the particle mass was contained in a size fraction with an aerodynamic diameter less than 1 micron.

B. **Mortality:** All animals survived the four-hour exposure to the test material as well as the two-week post-exposure period (Table 7.1.3/1-1).

Table 7.1.3/1-1. Concentrations, exposure conditions, mortality/animals treated

Nominal Conc. (mg/L)	Gravimetric Conc. (mg/L)	MMAD µm	GSD	Mortality (# dead/total)		
				Males	Females	Combined
14.05	5.35	3.31	1.52	0/5	0/5	0/10

C. **Clinical observations:** Clinical effects noted during the four-hour exposure period were limited to soiling of the haircoat in all five female rats.

In-life observations noted post-exposure were limited to perineal soiling in one female rat.

All animals appeared normal by test day 3

D. **Body Weight:** Mean body weight losses of 7.5% and 5.7% were noted for male and female rats, respectively, on test day 2. Pre-exposure mean body weight values for males and females were exceeded on test days 4 and 8 respectively.

E. **Necropsy:** There were no treatment-related visible lesions noted in any of the rats exposed to GF-2372 at the test day 15-scheduled necropsy. One female was noted to have a hiatal hernia of the liver that was determined to be unrelated to treatment.

F. **Applicant's Conclusions:** Based on these data, the four-hour LC_{50} of inhaled GF-2372 is greater than 5.35 mg/L for male and female F344/DuCrl rats.

IIIA1 7.1.3/01 Study comments	The study is acceptable.
IIIA1 7.1.3/01 Agreed endpoint	Acute inhalation LC_{50} >2.21 mg/L in the rat, the maximum attainable concentration. According to the classification criteria of **CLP Regulation (EC) No. 1272/2008**, the test material **does not require classification** for acute inhalative toxicity.

IIIA 7.1.4 Skin irritation

Report	KIIIA1 7.1.4/01 [redacted] (2010a)
Title	Primary Skin Irritation in Rabbits. [redacted] May 27, 2010
Document No	EPSL Study Number 29208, Dow Study Number 101055
Guideline	Primary Dermal Irritation - Rabbit; OPPTS 870.2500: OECD 404
GLP	Yes

EXECUTIVE SUMMARY: A primary skin irritation test was conducted with New Zealand albino rabbits to determine the potential for GF-2372 to produce irritation after a single topical application.

A dose of 0.5 gram of the test substance was moistened with a small amount of distilled water to create a paste that was applied to a gauze patch that was applied to a small area (6-cm^2) of skin of three healthy rabbits and held in place and in contact with the skin by a semi-occlusive dressing for 4 hours. Following exposure, dermal irritation was evaluated by the method of Draize *et al.*[1].

There was no edema noted for any treated dose site during the study. One hour after patch removal, very slight erythema was observed at all treated dose sites. The overall incidence and severity of irritation decreased with time. All animals were free of dermal irritation by 72 hours.

[1] Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.* 1944; 82:377-390.

Under the conditions of this study, GF-2372 caused very slight erythema, which cleared by 72 hours.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	GF-2372
	Description:	White, granular solid
	Lot #:	E2110-28
	Test Substance Number	031070-0004
	Purity:	XDE-208 – 49.9%

2. Vehicle and/or positive control: Distilled water

3.	**Test animals:**		
	Species:	Rabbit	
	Strain:	New Zealand, albino	
	Age/weight at treatment:	Young Adult (14-15 weeks)/ 2280-2304 grams at experimental start	
	Source:	[redacted]	
	Housing:	Singly housed in suspended stainless steel caging with mesh floors	
	Diet:	Purina Certified High Fiber Rabbit Diet (PMI #5325) *ad libitum*	
	Water:	Filtered tap water *ad libitum* by an automatic water dispensing system.	
	Environmental conditions:	**Temperature:** **Humidity:** **Air changes/Hour:** **Photoperiod:**	19-21°C 30-35%, RH 14/hr 12 hrs dark/12 hrs light
	Acclimation period:	13 days	

B. STUDY DESIGN and METHODS:

1. In life dates - Start: March 2, 2010 End: March 5, 2010

2. Animal assignment and treatment - Prior to application, the test substance was moistened with distilled water to achieve a dry paste by preparing a 60% w/w mixture. Five-tenths of a gram of the test substance (0.83 g of the test mixture) was placed on a 1-inch x 1-inch, 4-ply gauze pad and applied to one 6-cm^2 intact dose site on each animal. The pad and entire trunk of each animal were then wrapped with semi-occlusive 3-inch Micropore™ tape to avoid dislocation of the pad. Elizabethan collars were placed on each rabbit and they were returned to their designated cages.

After 4 hours of exposure to the test substance, the pads and collars were removed and the test sites were gently cleansed with a 3% soap solution followed by tap water and a clean towel to remove any residual test substance.

Individual dose sites were scored according to the Draize scoring system[2] at approximately 30-60 minutes, 24, 48 and 72 hours after patch removal.

All animals were observed for signs of gross toxicity and behavioral changes at least once daily during the test period. Body weights of the animals were recorded prior to test substance administration (initial) and again at study termination.

II. RESULTS AND DISCUSSION:

A. Results:

All animals appeared active and healthy and gained body weight during the study. Apart from the skin irritation noted (Table 7.1.4/1-1), there were no other signs of gross toxicity, adverse clinical signs, or abnormal behavior.

Table 7.1.4/1-1: Mean of scores for skin irritation at 24, 48 and 72 hours

Animal	Erythema	Oedema
1	0.33	0.0
2	0.66	0.0
3	0.33	0.0
EC trigger values	≥ 2.3, ≤ 4.0*	≥ 2.3, ≤ 4.0*

*Any irreversible effect in 2/3 animals d14

B. Applicant's Conclusions:

Under the conditions of this study, GF-2372 caused very slight erythema, which cleared by 72 hours.

IIIA1 7.1.4/01 Study comments	The study is acceptable.
IIIA1 7.1.4/01 Agreed endpoint	Under the conditions of this study, GF-2372 caused very slight erythema, which cleared within 24 hours. According to the classification criteria of **CLP Regulation (EC) No. 1272/2008**, the test material **does not require classification** for dermal irritancy.

IIIA 7.1.5 Eye irritation

Report	KIIIA1 7.1.5/01 [redacted] (2010b)
Title	Primary Eye Irritation in Rabbits. [redacted] June 24, 2010
Document No	EPSL Study Number 29207, Dow Study Number 101056

[2] Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.* 1944; 82:377-390.

Guideline	Primary Eye Irritation - Rabbit; OPPTS 870.2400; OECD 405
GLP	Yes

EXECUTIVE SUMMARY: A primary eye irritation test was conducted with New Zealand albino rabbits to determine the potential for GF-2372 to produce irritation from a single instillation via the ocular route.

One-tenth of a milliliter (50 mg) of the air-milled test substance was instilled into the right eye of three healthy rabbits. The left eye remained untreated and served as a control. Ocular irritation was evaluated by the method of Draize *et al.*[3].

One hour after test substance instillation, all three treated eyes exhibited corneal opacity, iritis and conjunctivitis. The overall incidence and severity of irritation decreased gradually with time. All animals were free of ocular irritation by Day 10 (study termination).

Under the conditions of this study, GF-2372 caused corneal opacity, iritis and conjunctivitis, all of which cleared by Day 10.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	GF-2372
	Description:	White solid (air-milled)
	Lot #:	E2837-93
	Test Substance Number	031070-0011
	Purity:	Sulfoxaflor – 49.8%

2. Vehicle and/or positive control: None

3.	**Test animals:**		
	Species:	Rabbit	
	Strain:	New Zealand, albino	
	Age/weight at dosing:	Young Adult (13-14 weeks)/ 2277-2608 grams at experimental start	
	Source:	[redacted]	
	Housing:	Singly housed in suspended stainless steel caging with mesh floors	
	Diet:	Purina Certified High Fiber Rabbit Diet (PMI #5325) *ad libitum*	
	Water:	Filtered tap water *ad libitum* by an automatic water dispensing system.	
	Environmental conditions:	**Temperature:**	19-21ºC
		Humidity:	50-70%RH
		Air changes:	14/hr
		Photoperiod:	12 hrs dark/12 hrs light

[3] Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.* 1944; 82:377-390.

	Acclimation period:	19 days

B. STUDY DESIGN and METHODS:

1. In life dates - Start: March 8 and May 17, 2010 End: May 27, 2010

2. Animal assignment and treatment - One-tenth of a milliliter (50 mg) of the air-milled test substance was instilled into the conjunctival sac of the right eye of three rabbits by pulling the lower lid away from the eyeball. The upper and lower lids were then gently held together for about one second before releasing to minimize loss of the test substance. The other eye of each rabbit remained untreated with the test substance and served as a control. The rabbits were then returned to their designated cages.

Ocular irritation was evaluated using a high-intensity white light (Mag Lite®) in accordance with Draize *et al*.[4] at 1, 24, 48, and 72 hours and at 4, 7 and 10 days post-instillation. One drop of 2% ophthalmic fluorescein sodium was instilled into the treated eyes of each rabbit. The eyes were rinsed with physiological saline (0.9% NaCl) approximately 30 seconds after instillation of the fluorescein and then evaluated for corneal damage using an ultraviolet light source at 24 hours and as needed at subsequent scoring intervals to evaluate the extent of corneal damage or to verify reversal of effects. In addition to observations of the cornea, iris, and conjunctivae, any other observed lesions were noted. The average score for all rabbits at each scoring period was calculated to aid in data interpretation.

The animals were observed for signs of gross toxicity and behavioral changes at least once daily during the test period. Individual body weights of the animals were recorded prior to test substance instillation (initial) and again on study termination following scoring.

II. RESULTS AND DISCUSSION:

A. All animals appeared active and healthy and gained body weight during the study. Apart from the eye irritation noted (Table 7.1.5/1-1), there were no other signs of gross toxicity, adverse clinical signs, or abnormal behavior.

One hour after test substance instillation, all three treated eyes exhibited corneal opacity, iritis and conjunctivitis. The overall incidence and severity of irritation decreased gradually with time. All animals were free of ocular irritation by Day 10 (study termination).

[4] Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther*. 1944; 82:377-390.

Table 7.1.5/1-1: Mean values for ocular lesions 24, 48 and 72 hours after instillation

Animals	Corneal	Iridial	Conjunctival	
	Opacity	Lesions	Redness	Chemosis
1	0	0	1.66	0.66
2	1	0	2	1
3	0	0	1	0.66
EC trigger values*: (H319)	≥ 1	≥ 1.0	≥ 2	≥ 2.0
EC trigger values*: (H318)	≥ 3	≥ 1.5	na	na

**Classification triggered if any EC value is attained by two or more animals*
*** Any Irriversible effect in 1/3 animals d21*
H318 also triggered by corneal or iris effects present at the end of the test in any animal
na not applicable

B. <u>Applicant's Conclusions</u>: Under the conditions of this study, GF-2372 caused corneal opacity, iritis and conjunctivitis, all of which cleared by Day 10.

IIIA1 7.1.5/01 Study comments	The study is acceptable.
IIIA1 7.1.5/01 Agreed endpoint	Under the conditions of this study, GF-2372 caused very slight erythema, which cleared within 24 hours. According to the classification criteria of **CLP Regulation (EC) No. 1272/2008**, the test material **does not require classification** for dermal irritancy.

IIIA 7.1.6 Skin sensitisation

Report	KIIIA1 7.1.6/01 [redacted] (May 2010)
Title	GF-2372: Local Lymph Node Assay in CBA/J Mice. [redacted] 26 May 2010
Document No	Study ID: 101020
Guideline	Local lymph node assay [mice]; OPPTS 870.2600; OECD 429
GLP	Yes

EXECUTIVE SUMMARY: The Local Lymph Node Assay (LLNA) was conducted to assess the potential of GF-2372 to cause contact sensitization by measuring lymphocyte proliferative responses from auricular lymph nodes following topical application of the test material to the mouse ear. GF-2372 contains XDE-208 as the active ingredient.

Screening Study: Three daily topical applications of 1%, 5%, 10%, 20%, 40% or 80% GF-2372 were given to one animal at each dose level. Erythema was absent and body weights were unaffected in all

dose groups. Results from this study were used to determine the dosing concentrations for GF-2372 in the LLNA.

LLNA: Six female mice/group received 5%, 20% or 80% of GF-2372, or vehicle (1% L92) or 30% α-hexylcinnamaldehyde (HCA; positive control) on days 1-3. On day 6, uptake of 3H-thymidine into the auricular lymph nodes draining the site of chemical application was measured five hours post administration. Proper conduct of the LLNA was confirmed via a positive response using 30% α-hexylcinnamaldehyde (HCA), a moderate contact sensitizer, which elicited proliferation that was 5.3 in comparison to vehicle-treated mice.

Erythema was absent and body weights were unaffected in all dose groups.

GF-2372 at concentrations of 5%, 20% and 80% elicited stimulation indices that were, 0.9, 1.2, and 1.1, respectively, in comparison to the vehicle-treated mice.

Under the conditions of this study, GF-2372 did not demonstrate dermal sensitization potential in the mouse LLNA as the lymph nodes draining the area of topical application did not demonstrate a 3-fold increase in proliferation when compared to vehicle-treated mice.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Radioisotope**	
	Radioisotope :	^{3}H-Thymidine
	Purity of Radioisotope :	>97%
	Date of Isotope Activity Assay	March 3, 2010

2.	**Test Material:**	GF-2372
	Lot/Batch #:	E2110-28
	Purity:	49.9% XDE-208 by high performance liquid chromatography with identification by retention time match to known standards
	Compound Stability:	Not Applicable
	CAS #:	XDE-208: 946578-00-3

3.	**Test Animals:**		
	Species:	Mice	
	Strain:	CBA/J	
	Age at study initiation:	Approximately 9-12 weeks	
	Source:	[redacted]	
	Housing:	Animals were housed up to six per cage in filter tubs containing corncob bedding, food pellets and a water bottle. On the day the animals were euthanized and following the injection of ^{3}H-thymidine, each treatment group of mice was housed in shoebox cages containing corncob bedding, food pellets, and a crock filled with water. The mice were euthanized five hours later.	
	Feed and Water:	Animals were provided LabDiet Certified Rodent Diet #5002 [redacted] in pelleted form. Feed and municipal water was provided *ad libitum*. Drinking water obtained from the municipal water source was periodically analyzed for chemical parameters and biological contaminants by the municipal water department.	
	Environmental conditions:	Temperature: Humidity: Air changes:	22 ± 1°C 40-70% 12-15 times/hour

		Photoperiod:	12-hour light/dark (on at 6:00 a.m. and off at 6:00 p.m.)
	Acclimation period:	For at least one week prior to the start of the study.	

B. STUDY DESIGN:

1. In life dates: 17 March 2010 – 29 March 2010

2. Animal assignment: Before administration of test material began, animals were stratified by body weight and then randomly assigned to treatment groups using a computer program designed to increase the probability of uniform group mean weights and standard deviations at the start of the study. Animals placed on study were uniquely identified via subcutaneously implanted transponders (BioMedic Data Systems, Seaford, Delaware) that were correlated to unique alphanumeric identification numbers.

3. Dose selection: The dermal route is a relevant route for evaluation of skin contact allergy potential and is consistent with the animal model developed by Kimber and Weisenberger (1989) to predict dermal sensitization potential using the mouse LLNA.

Concentrations tested for the irritancy screen were selected based upon maximum miscibility or solubility in an appropriate LLNA vehicle while maintaining a solution suitable for application. Toxicity data regarding irritation potential and lethality doses were also taken into consideration.

Concentrations tested in the LLNA were based on this information and previous dermal sensitization data.

5. Statistics:

1. The Stimulation Index (SI) was calculated for each mouse using the following equation:

$$SI = \frac{\text{Disintegration per minute (dpm) of individual mouse}}{\text{Average dpm of the VH control mice}}$$

2. EC_3 Calculation:

$$EC_3 = X_L + [(3-Y_L)/(Y_h-Y_L)](X_h-X_L)$$

Where, Y_L = SI value below 3
X_L = chemical concentration that elicits Y_L
Y_h = SI value above 3
X_h = chemical concentration that elicits Y_h

Means and standard deviation (SD) were generated for body weight data (absolute and gain) and the LLNA response (dpm & SI values).

These body weight and dpm data were analyzed by a one-way analysis of variance (Steele and Torrie, 1960). When differences were indicated by the ANOVA, a comparison of treated vs. control groups was done using a Dunnett's t-test (Steele and Torrie, 1960). The alpha level at which all tests were conducted was 0.05. The final interpretation of the

biological significance of the responses was based on both statistical outcome and scientific judgment.

C. METHODS:

1. Observations: Twice each day a cage-side examination was conducted by animal care personnel, and to the extent possible the following parameters were evaluated: skin, fur, mucous membranes, respiration, nervous system function (including tremors and convulsions), animal behavior, morbidity, mortality, and the availability of feed and water. The ears were evaluated for erythema prior to application of test material solutions and ^{3}H-thymidine injections as follows:

Table 7.1.6/1-1. Erythema Evaluation

	Value
No visual effect	0
Slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Eschar	4

2. Body weight: The initial and terminal body weights were obtained and recorded.

3. Sacrifice and Pathology: Approximately five hours post administration, the mice were euthanized via CO_2 asphyxiation and both auricular lymph nodes located at the bifurcation of the jugular veins were excised and placed in PBS.

a. Gross necropsy: N/A

b. Tissue preparation/histopathology: A single cell suspension of the auricular lymph nodes from one mouse was prepared by gentle mechanical disaggregation using a tissue homogenizer (Stomacher 80 Lab System, Seward Ltd., London, United Kingdom). The cells were washed two times and were suspended in 3 ml of 5% trichloroacetic acid (TCA) for approximately 18 hours. The suspended precipitates were centrifuged (200 x g for 10 minutes) and the supernatant removed. The pellet from each mouse was reconstituted in 1 ml of 5% TCA and subsequently transferred to a scintillation vial containing 10 ml of Aquasol-2 scintillation cocktail (Packard Instrument Company, Meridan, Connecticut). Two additional 2 ml aliquots of water were used to rinse the tubes and the rinses were added to the scintillation vials containing the 1 ml of pellet in TCA and cocktail. The radioactivity in each precipitate was measured using a β-scintillation counter and reported as disintegrations per minute (dpm) per mouse.

II. RESULTS During the screening study, the mice were treated with three daily applications of 1%, 5%, 10%, 20%, 40% or 80% GF-2372. Erythema was absent and body weights were unaffected in all dose groups.

Based on the results of the screen, 80% GF-2372 was tested in the LLNA along with 20% and 5% to characterize the dose response. Erythema was absent (Table 7.1.6/1-2), and body weights were unaffected in all dose groups (Table 7.1.6/1-3).

Proper conduct of the LLNA was demonstrated via the positive response from the positive control, 30% HCA which elicited a stimulation index (SI) of 5.3 in comparison to vehicle-treated mice (Table 7.1.6/1-4).

GF-2372 at concentrations of 5%, 20% and 80% elicited stimulation indices that were, 0.9, 1.2, and 1.1, respectively, in comparison to the vehicle-treated mice (Table 7.1.6/1-4). GF-2372 did not demonstrate dermal sensitization potential in the mouse LLNA as the lymph nodes draining the area of topical application did not demonstrate a 3-fold increase in proliferation when compared to vehicle-treated mice.

A. Observations:

1. Clinical signs of toxicity: Not Applicable

Table 7.1.6/1-2. Individual Erythema Scores of Animals Treated with Vehicle (1% L92), 30% α-hexylcinnamaldehyde (HCA) or 5%, 20%, and 80% GF-2372

DOSE %	ANIMAL NUMBER	DAYS ON TEST 1	2	3	6
VH (1% L92)	2257	0	0	0	0
	2258	0	0	0	0
	2259	0	0	0	0
	2260	0	0	0	0
	2261	0	0	0	0
	2262	0	0	0	0
5% GF-2372	2269	0	0	0	0
	2270	0	0	0	0
	2271	0	0	0	0
	2272	0	0	0	0
	2273	0	0	0	0
	2274	0	0	0	0
20% GF-2372	2275	0	0	0	0
	2276	0	0	0	0
	2277	0	0	0	0
	2278	0	0	0	0
	2279	0	0	0	0
	2280	0	0	0	0
80% GF-2372	2281	0	0	0	0
	2282	0	0	0	0
	2283	0	0	0	0
	2284	0	0	0	0
	2285	0	0	0	0
	2286	0	0	0	0
30% HCA	2263	0	1	2	0
	2264	0	1	2	0
	2265	0	1	2	0

2266	0	1	2	0
2267	0	1	2	0
2268	0	1	2	0

Table 7.1.6/1-3. Individual Body Weights (g) and Body Weight Gain (g) of Animals Treated with Vehicle (1% L92), 30% α-hexylcinnamaldehyde (HCA) or 5%, 20%, and 80% GF-2372

DOSE %	ANIMAL NUMBER	DAYS ON TEST 1	6	GAIN
VH (1% L92)	2257	24.4	24.9	0.5
	2258	22.9	23.9	1.0
	2259	23.2	24.3	1.1
	2260	24.0	24.9	0.9
	2261	21.3	22.7	1.4
	2262	23.2	24.4	1.2
	MEAN	23.2	24.2	1.0&
	S.D.	1.1	0.8	0.3
	N=	6	6	6
5% GF-2372	2269	22.1	23.5	1.4
	2270	23.8	26.1	2.3
	2271	22.6	24.9	2.3
	2272	23.5	24.5	1.0
	2273	23.1	25.2	2.1
	2274	22.9	22.7	-0.2
	MEAN	23.0	24.5	1.5&
	S.D.	0.6	1.2	1.0
	N=	6	6	6
20% GF-2372	2275	24.2	24.2	0.0
	2276	24.1	25.5	1.4
	2277	22.9	24.2	1.3
	2278	23.2	23.7	0.5
	2279	24.3	26.4	2.1
	2280	21.1	23.3	2.2
	MEAN	23.3	24.6	1.2&
	S.D.	1.2	1.2	0.9
	N=	6	6	6
80% GF-2372	2281	22.4	22.3	-0.1
	2282	23.2	24.6	1.4
	2283	22.7	24.8	2.1
	2284	24.3	24.6	0.3
	2285	21.5	22.9	1.4
	2286	24.0	25.2	1.2
	MEAN	23.0	24.1	1.1&
	S.D.	1.0	1.2	0.8
	N=	6	6	6
30% HCA	2263	24.3	23.6	-0.7
	2264	22.3	23.6	1.3
	2265	22.1	23.4	1.3
	2266	21.9	22.6	0.7
	2267	25.2	24.6	-0.6
	2268	24.8	23.2	-1.6
	MEAN	23.4	23.5	0.1&
	S.D.	1.5	0.7	1.2
	N=	6	6	6

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05

Table 7.1.6/1-4. Disintegrations Per Minute (DPM) and Stimulation Indices (SI) of Animals Treated with Vehicle (1% L92), 30% α-hexylcinnamaldehyde (HCA) or 5%, 20%, and 80% GF-2372

DOSE %	ANIMAL NUMBER	DPM	SI
VH (1% L92)	2257	1480.0	1.0
	2258	240.00	0.2
	2259	1761.0	1.2
	2260	2272.0	1.5
	2261	1908.0	1.2
	2262	1502.0	1.0
	MEAN	1527.2	1.0&
	S.D.	694.86	0.4
	N=	6	6
5% GF-2372	2269	1367.0	0.9
	2270	1836.0	1.2
	2271	1663.0	1.1
	2272	362.00	0.2
	2273	2023.0	1.3
	2274	1007.0	0.7
	MEAN	1376.3	0.9&
	S.D.	612.70	0.4
	N=	6	6
20% GF-2372	2275	1650.0	1.1
	2276	2450.0	1.6
	2277	1327.0	0.9
	2278	1958.0	1.3
	2279	1479.0	1.0
	2280	1833.0	1.2
	MEAN	1782.8	1.2&
	S.D.	399.01	0.2
	N=	6	6
80% GF-2372	2281	2280.0	1.5
	2282	2045.0	1.3
	2283	1889.0	1.2
	2284	1746.0	1.1
	2285	1783.0	1.2
	2286	334.00#	0.2#
	MEAN	1679.5	1.1&
	S.D.	687.54	0.5
	N=	6	6
30% HCA	2263	5775.0	3.8
	2264	6937.0	4.5
	2265	10207	6.7
	2266	7584.0	5.0
	2267	6222.0	4.1
	2268	12089	7.9
	MEAN	8135.7*	5.3&
	S.D.	2485.8	1.6
	N=	6	6

\# STATISTICAL OUTLIERS INCLUDED
& INDICATES NO STATISTICAL COMPARISON OF MEANS.
* STATISTICALLY DIFFERENT FROM CONTROL MEAN BY DUNNETT'S TEST,

III. DISCUSSION

Investigator's conclusions: GF-2372 did not elicit a stimulation index (SI) that met the 3X threshold, thus indicating a lack of dermal sensitization potential in the mouse LLNA.

IIIA1 7.1.6/01 Study comments	The study is acceptable.
IIIA1 7.1.6/01 Agreed endpoint	Under the conditions of this study, GF-2372 indicates a lack of dermal sensitisation potential in the mouse LLNA, which cleared within 24 hours. According to the classification criteria of **CLP Regulation (EC) No. 1272/2008**, the test material **does not require classification** for skin sensitisation.

IIIA 7.1.7 Supplementary studies for combinations of plant protection products

This formulation contains a single active, therefore additional data are not required

IIIA 7.2 Short-term toxicity studies

This is not an EC data requirement according to Directive 91/414/EEC or Regulation (EC) No. 1107/2009.

IIIA 7.3 Operator exposure

GF-2372 is a water dispersible granule (WDG) insecticidal formulation containing a nominal 500 g/kg sulfoxaflor as the active ingredient. Details of intended uses and packaging of GF-2372 in the European Union are contained in IIIA 3 and IIIA 4 of this dossier.

Applications of GF-2372 to crops (see Table 7.3-1 for details) will be made using a tractor-mounted boom sprayer. Water is the intended diluent/carrier.

Sulfoxaflor has a low vapour pressure (*ca.* $\leq 2.5 \times 10^{-6}$ Pa @ 25 °C). This means that any additional exposure from inhalation exposure would be negligible.

Information pertinent to operator exposure is summarized in Table 7.3-1, Table 7.3-2 and Table 7.3-3.

Table 7.3-1: Summary of application information for GF-2372

Crop	**Conc. in product** g/kg	**Application rate**		**Spray volume** L/ha
		Product kg/ha	**Active substance** kg/ha	
Cereals*	500	0.048	0.024	100-600
Cotton				300-1000
Oilseed Rape				100-300

* Wheat, Barley, Oats, Rye, Spelt, Triticale (spring and winter)

Table 7.3-2: Summary of critical use patterns (i.e. worst case)

Crop	**Application rate**		**Spray volume** L/ha	**Mode of application**
	Product kg/ha	**Active substance** kg/ha		
Cereals	0.048	0.024	**100**-600	**Tractor-mounted/trailed boom sprayer**

Table 7.3-3: EU endpoints for non-dietary human exposure

Endpoint		**EU agreed endpoint**[1]	**Endpoint used in risk assessment**
Dermal absorption			
Active	Concentrate	0.8%	0. 4%
	Spray dilution	6% (0.024 g/L)	6% (0.024 g/L)

AOEL		
Sulfoxaflor	0.06 mg/kg bw/day (EFSA, 2014)	
Default body weights (kg):	UK POEM	60
	German/Greenhouse Model	70
Application parameters:	Area treated (UK POEM)	50 ha per day
	Area treated (German Model)	20 ha per day
	Duration of spraying (UK POEM)	6 hours
	Duration of Spraying (German Model)	N/A

[1] *EFSA Journal 2014; 12(5):3692*

IIIA 7.3.1 Estimation of operator exposure without personal protection

Estimations of potential operator exposure to sulfoxaflor associated with applications of GF-2372 were made using the UK POEM and the German model for intended uses.

Predicted systemic exposures are summarised in Table 7.3.1-1. Individual spreadsheets are presented in Appendix 3, Tables 1-2.

Table 7.3.1-1: Estimation of operator exposure for GF-2372 without PPE

Model data	Level of PPE	Total absorbed dose (mg/kg/day)	% of AOEL
Tractor boom sprayer application outdoors to low crops (ornamentals) *Application rate: 0.048 kg* sulfoxaflor */ha*			
German Model 20 ha/day 70 kg operator	no PPE	0.0010	**1.6**
UK POEM 50 ha/day, 6 h/day 100 L/ha 60 kg operator	no PPE	0.0114	**19.0**

No PPE German Model: Operator wearing long work wear (coverall) but no gloves.
UK POEM: Operator wearing long sleeved shirt, long trousers ("permeable") but no gloves

An additional evaluation has been performed with the German model with similar entry parameters in the model as presented in the dRR; however taking into account a protection factor of 90% for the working coverall. With this consideration the estimation of operator exposure, when GF-2372 is applied on:

- cereals using a tractor-mounted boom sprayer represented 0.3 % of the AOEL of sulfoxaflor with working coverall and with gloves during mixing/loading and application.

IIIA 7.3.2 Estimation of operator exposure using personal protective equipment

Estimation of operator exposure assuming PPE is used is required when the AOEL may be exceeded in the absence of PPE, or based on hazard classification requirements.

Exposure based on no PPE indicates that PPE will not be required for use of GF-2372 according to predicted exposures using the German and UK models.

In addition, GF-2372 is not classified for acute toxicity in accordance with criteria in Council Directive 1272/2008/EC (Classification, labelling and packaging of substances and mixtures) and 99/45/EEC (Dangerous Preparations Directive).

Therefore, an estimation of operator exposure assuming PPE is used is not required and has not been performed.

IIIA 7.3.3 Measurement of operator exposure

Measurement of operator exposure is required where, based on estimated exposure, the AOEL may be exceeded. Estimations of operator exposure indicate that the AOEL will not be exceeded by the proposed uses of GF-2372 during mixing, loading and application. Therefore, measurement of operator exposure is not required and has not been performed.

IIIA 7.4 Bystander and resident exposure

Sulfoxaflor is a new active substance developed by Dow AgroSciences. GF-2372, containing sulfoxaflor is the representative formulation for the EU registration of sulfoxaflor.

All relevant data and risk assessments are provided and are considered adequate.

IIIA 7.4.1 Estimation of bystander exposure

Bystanders are defined as persons who are not occupationally involved in the application or application related activities. The exposure is considered to be accidental, not occurring repeatedly to the same individual and therefore less frequent, of less duration and at a lower level compared to the operator. The AOEL is therefore considered to be a very conservative toxicological reference value.

The potential routes for bystanders are via dermal and inhalation exposure to drift of spray material. The exposure is likely to be brief and in any case will be significantly lower than the operator exposure (see data point IIIA 7.3).

As the objective of this dossier is to apply for approval of GF-2372 in Europe the bystander risk assessment presented has been based according to the EU requirements on the following model:

- EUROPOEM II; Bystander exposure to pesticides – Report of the bystander working group, Europoem II Project, Fair3 CT96-1406, December 2002, 1-43.

One worst-case scenario has been identified to assess potential bystander exposure. This is the application of GF-2372 to cereals at a rate of 0.048 kg product/ha (equivalent to 0.024 kg sulfoxaflor/ha in a water volume of 100 L/ha. Usage information pertinent to operator exposure is summarised in Table 7.4.1-1 and Table 7.4.1-2.

Table 7.4.1-1: Usage scenarios for which bystander exposure has been considered

Crop (field use)	Application rate (kg a.s./ha)	Minimum water volume (L/ha)	Application equipment
Cereals	0.048 kg/ha (0.024 kg sulfoxaflor/ha)	100	Tractor mounted/trailed boom sprayer

Table 7.4.1-2: Parameters used for calculation:

Estimation of bystander exposure was calculated according to the following formula (proposed by EUROPOEM II):

Active substance		Sulfoxaflor
Application rate (g a.s./ha)		24
Minimum spray volume (L/ha)		100
Inhalation absorption (%)		100 %
AR	Maximum Application Rate (mg/m²)	2.4
C	Maximum spray Concentration (mg a.s./mL)	2.4
DA	Dermal Absorption, diluted formulation (%)	6%
D	Drift deposition at a distance of x m (% of applied dose)	0.41 %
BS	Exposed Body Surface (m^2)	1
BW Body Weight (kg)		60
IE	Inhalation Exposure (mL spray/h)	0.063
T	Exposure duration (h)	0.083

Dermal exposure has been calculated with the following formula:

D = AR (mg/m²) x D at 7 meters x BS (m²)

Inhalation exposure [mg/person/day]

I = C (mg a.s./mL) x IE (mL spray/h) x T(h)

Total systemic exposure [mg/kg bw/day]

T = (Dermal exposure x DA % + Inhalation exposure) / bw (kg)

Table 7.4.1-3: Estimated bystander exposure to GF-2372 and % of the AOEL

Uses	% A.O.E.L. Sulfoxaflor (0.06 mg/kg bw/day)

Cereals	**0.03**

Total bystander exposure to sulfoxaflor from spray drift following application is acceptable according to EUROPOEM II.

It is concluded that there is no significant risk to incidental bystanders (adults or children) from sulfoxaflor in the formulation GF-2372 for the commercial uses/rates illustrated in the GAP when applied via tractor mounted/trailed boom sprayer.

IIIA 7.4.2 Estimation of resident exposure

Spray drift fallout may be deposited in gardens adjacent to the treated area and users of these gardens may become exposed through contact with deposits. The level of exposure in this situation can be estimated using the spray drift fallout values for field crop sprayers used for the aquatic risk assessment[5] and US EPA values for residential exposure resulting from contact with treated lawns[6].
As the objective of this dossier is to apply for approval GF-2372 in Europe the resident risk assessment presented has been based according to the EU requirements on the following model: .

- Martin S. et al. (2008) Guidance for Exposure and Risk Evaluation for Bystanders and Residents exposed to Plant Protection Products during and after Application [see 2008/1070089 Martin S. et al. 2008 a] drift distances adopted according to the recommendation published by the German BVL: B. Nolting (2012) Bekanntmachung ueber Mindestabstände, die bei der Anwendung von Pflanzenschutzmitteln zum Schutz von Umstehenden und Anwohnern einzuhalten sind [see 2012/1162091 Nolting H.-G. 2011 a] for the bystander and resident.

According to Martin et al. 2008, residents may possibly live or work near areas where the application of plant protection products (PPPs) is in progress or has recently taken place. They may be exposed to PPPs mainly via the dermal route from spray drift deposits and by inhalation of vapour drift (depending on the vapour pressure of the a.s). 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);

For the calculation of the exposure of residents the following default parameters and the specific parameters for sulfoxaflor were taken into account:

Dermal Exposure (via deposits caused by spray drift):

$$SDER = (AR \times D \times TTR \times TC \times H \times DA) / BW$$

[5] Rautmann, D., Streloke, M. and Winkler, R. (2001). New basic drift values in the authorisation procedure for plant protection products. In Forster, R. and Streloke, M. Workshop on risk assessment and risk mitigation measures in the context of the authorisation of plant protection products (WORMM). Mitt. Biol. Bundesanst. Land-Forstwirtsch. Berlin-Dahlem, Heft 381.
[6] USA EPA (1998). Occupational and residential exposure test guidelines: Group B, Post-application exposure monitoring test guidelines. Series 875 v 5.4.

Where: SDER = Systemic Exposure of Residents via the Dermal Route (mg/kg bw/day)
AR = Application Rate (mg/cm^2)
Sulfoxaflor: 0.024 kg a.s./ha = 0.00024 mg/cm^2
D = Drift (%): 2.77 (field crops)
TTR = Turf Transferable Residues (%): 5
TC = Transfer Coefficient (cm^2/hour): 7300 (adult), 2600 (child)
H = Exposure Duration (hours): 2
DA = Dermal Absorption (%):
Sulfoxaflor: 15 %
BW = Body Weight (kg/person): 60 (adult), 16.15 (child)

Inhalation Exposure (Vapour Drift):

SIER = (ACV x IR x IA) / BW

Where: SIER = Systemic Exposure of Residents via Inhalation (mg/kg bw/day)
ACV = Airborne Concentration of Vapour (mg/m^3): vapour pressure of a.s. is very low i.e. Sulfoxaflor: ≤ 2.5 x 10^{-6} Pa at 25°C. Acc. to guideline all compounds are non volatile substances (vapour pressure <1 x 10^{-5} Pa at 20°C). Thus, resident inhalation exposure can be estimated as negligible. (i.e. airborne conc. of 0 mg/m^3)
IR = Inhalation Rate (m^3/day): 16.57 (adult), 8.31 (child)
IA = Inhalation Absorption (%): 100
BW = Body Weight (kg/person): 60 (adult), 16.15 (child)

Child Oral Exposure

Children's hand-to-mouth exposure

SOEH = (AR x D x TTR x SE x SA x Freq x H x OA) / BW

Where: SOEH = Systemic Oral Exposure via the Hand to Mouth Route (mg/kg bw/day)
AR = Application Rate (mg/cm^2)
Sulfoxaflor: 0.024 kg a.s./ha = 0.00024 mg/cm^2
D = Drift (%): 2.77 (field crops)
TTR = Turf Transferable Residues (%): 5
SE = Saliva Extraction Factor (%): 50
SA = Surface Area of Hands (cm^2): 20
Freq = Frequency of Hand-to-Mouth (events/hour): 20
H = Exposure Duration (hours): 2
OA = Oral Absorption (%): 100
BW = Body Weight (kg/person): 16.15

Children's object-to-mouth exposure

SOEO = (AR x D x DFR x IgR x OA) / BW

Where: SOEO = Systemic Oral Exposure via the Object to Mouth Route (mg/kg bw/day)
AR = Application Rate (mg/cm^2)
Sulfoxaflor: 0.024 kg a.s./ha = 0.00024 mg/cm^2
D = Drift (%):2.77 (field crops)
DFR = Dislodgeable Foliar Residues (%): 20
IgR = Ingestion Rate for Mouthing of Grass/Day (cm^2): 25
OA = Oral Absorption (%): 100
BW = Body Weight (kg/person): 16.15

Total Systemic Exposure of Residents

Adults: SER = SDER + SIER (mg/kg bw/day)
Children: SER = SDER + SIER + SOEH + SOEO (mg/kg bw/day)

Where: SER = Systemic Exposure of Residents (mg/kg bw/day)
SDER = Systemic Dermal Exposure of Residents (mg/kg bw/day)
SIER = Systemic Inhalation Exposure of Residents (mg/kg bw/day)
SOEH = Systemic Oral Exposure via the Hand to Mouth Route (mg/kg bw/day)
SOEO = Systemic Oral Exposure via the Object to Mouth Route (mg/kg bw/day)

Table 7.4.2-1: Detailed calculations of resident exposure to sulfoxaflor, absorbed dose and % of systemic AOEL

Crop	Exposed population	Dermal (absorbed dose) (mg/kg bw/day)	Hand-to-mouth exposure (mg/kg bw/day)	Object-to-mouth exposure (mg/kg bw/day)	Vapour inhalation[1] (mg/kg bw/day)	Total systemic (mg/kg bw/day)	Total systemic as % of AOEL
Cereals	Children	0.0000064	0.0000082	0.0000021	NA	0.0000167	**0.03**
	Adults	0.0000049	NA	NA	NA	0.0000049	**0.01**

Based on these exposure estimates there is no unacceptable risk anticipated for residents when being (accidentally) exposed to GF-2372.

IIIA 7.5 Worker exposure

Re-entry worker exposure to GF-2372 was evaluated as part of the EU review of sulfoxaflor. Therefore, all relevant data and risk assessments are provided here and are considered adequate. Table 7.3-1 summarizes the GAPs evaluated for re-entry worker exposure assessment.

GF-2372 is applied in the field to cereals via tractor mounted field crop sprayers. No manual activities are necessary for maintaining the crop. Harvesting is performed by appropriate machines. Hence, there is in general no scenario for which worker exposure needs to be addressed.

However, in single cases a certain re-entry scenario might occur (e.g. crop inspection, irrigation). For such a post-application scenario exposure to workers is estimated. The estimation of worker exposure to cereal was calculated anyway, according to the EUROPOEM II model.

One worst-case scenario has been identified to assess potential worker exposure. This is the application of GF-2372 to cereal crop at a rate of 0.048 kg product/ha (equivalent to 0.024 kg sulfoxaflor/ha) in a water volume of 100 L/ha. Usage relevant information to operator exposure is summarised in Table 7.5-1 and Table 7.5-2.

Table 7.5 -1: Usage scenarios for which worker exposure has been considered

Crop (field use)	Application rate (kg a.s./ha)	Minimum water volume (L/ha)	Re-entry activities
Cereals	0.024	100	Harvesting activities

Table 7.5-2: Parameters applied for the assessment of worker exposure

Estimation of worker exposure was calculated according to the following formula (proposed by EUROPOEM II):

Parameters and units		Sulfoxaflor
DFR	Dislodgeable Foliar Residues (µg/cm²/kg a.i./ha)	3
AR	Application rate (kg a.i./ha)	0.024
TC	Transfer coefficient (cm²/ person/h)	5000
T	Task duration (h)	2
BW	Body weight (kg)	60
DA	Dermal absorption (worst-case between diluted and undiluted formulations)	6%
TSF	Transfer Specific Factor (%)	0
PPE	Personal Protection Equipment	1 or 0.1

The potential dermal exposure of a worker is calculated by the following approach:

D (mg/person/d) = 0.001 x DFR (µg/cm²/kg a.i./ha) x AR (kg a.i./ha) x TC (cm²/person/h) x T (h/day)

The potential inhalation exposure of a worker is calculated by the following approach:

I (mg/person/d) = AR (kg a.i./ha) x TSF (%) x T (h/day)

As the potential inhalation exposure is considered to be negligible (outdoor applications), the worker total systemic exposure is calculated as follows:

Total systemic exposure (mg/kg bw/d) = $\frac{\text{D (mg/person/d) x DA (\%) + I (mg/person/d)}}{\text{BW (kg)}}$

Table 7.5-2: Estimated worker exposure to GF-2372 and % of the AOEL

Uses	Personal Protective Equipment	% A.O.E.L. Sulfoxaflor (0.06 mg/kg bw/day)
Cereals	Without PPE	**1.2**
	With PPE	**0.1**

Total worker exposure to sulfoxaflor from spray drift following application is acceptable according to EUROPOEM II.

IIIA 7.5.1 Estimation of worker exposure without personal protective equipment

See IIIA 7.5.

IIIA 7.5.2 Estimation of worker exposure with personal protective equipment

See IIIA 7.5.

IIIA 7.5.3 Measurement of worker exposure

Measurement of worker exposure is required where, on the basis of estimated exposure, the AOEL may be exceeded. Estimations of worker exposure indicate that the AOEL of sulfoxaflor will not be exceeded by proposed uses of GF-2372 and therefore measurement of worker exposure is not required and has not been conducted.

IIIA 7.6 Dermal absorption

Dermal absorption of sulfoxaflor formulated in the Suspension Concentrate (SC) GF-2032 was evaluated as part of the EU review. Therefore, all relevant data and risk assessments are provided here and are considered adequate.

No dermal absorption studies have been conducted on the sulfoxaflor solid water dispersible granule (WG) formulation GF-2372 (500 g/kg). This test preparation can be adequately represented by the sulfoxaflor SC formulation, GF-2032, which had already been extensively tested for dermal absorption in a rat *in vivo* study and a rat/human *in vitro* study. Based on GAP, spray dilutions of GF-2032 are comparable surrogates for those of GF-2372. Details of the studies on GF-2032 are supplied in this dossier.

Table 7.6-1: Dermal absorption end-points for the risk assessment

End-Point	
Dermal absorption	Concentrate: 0.8 % Dilution: 6 %

IIIA 7.6.1 Dermal absorption, *in vivo*, in the rat

Report:	KIIIA1 7.6.1/01 [redacted] (June 2010)
Title:	XDE-208: The *In Vivo* Percutaneous Absorption of Radiolabelled XDE-208 in Formulation (GF-2032) and Two In-Use Spray Dilutions in the Rat (OECD 427). [redacted]
Document No:	191168
Guidelines:	*In Vivo* Dermal Absorption – *Rat Sprague Dawley*; OECD 427.
GLP	Yes

EXECUTIVE SUMMARY:

GF-2032 is a suspension concentrate (SC) formulation containing the active substance XDE-208, an experimental insecticide under development by Dow AgroSciences. The nominal concentration of the active substance in GF-2032 is 240 g per litre. The highest in-use spray dilution concentration is produced by mixing 0.4 L of formulation with 200 L of water to give 0.48 g XDE-208/L. The lowest in-use spray dilution concentration is produced by mixing 0.1 L of formulation with 1000 L of water to give 0.024 g XDE-208/L.

As part of the safety evaluation of XDE-208, this study was designed to assess its rate and extent of absorption through rat skin following topical application of the formulation and two typical in-use spray dilutions that cover the concentration range of these sprays.

The study was divided into 3 phases, each phase corresponded to one of the test preparations studied (i.e. undiluted formulation, highest and lowest spray dilutions concentrations),

For each test preparation five groups of four male CD rats were tested. The contact time was 10 hours, in order to be consistent with the US EPA Guideline 870.7600 and the first group was killed at 24 h and the remaining groups at 48, 96, 144 and 192 h post dose to provide additional data on the stratum corneum reservoir/application site and long term absorption, distribution and excretion of XDE-208.

Following dose administration, the application site, which was protected by an O-ring that enclosed the treated area, was then covered with a protective dressing. The animals were placed in all-glass metabowls designed specifically for the quantitative collection of urine and faeces. Urine and faeces were quantitatively collected for the periods predose, 0-10 and 10-24 h then daily up to termination. Cages were washed with water at the time of each collection and the wash retained. At 10 h post dose, the dressing was removed and retained for analysis. The exposed area was gently wiped clean using a single cotton wool swab soaked in handwash soap concentrate followed by 3 cotton wool swabs soaked in lukewarm soapy water. The skin was dried with a further 3 cotton wool swabs. The washing process was repeated once. The washes and cotton wool were retained. A new protective dressing was then applied.

A terminal blood sample (*ca* 5-10 mL) was taken from the *vena cava* or by cardiac puncture into tubes containing lithium heparin. A sample of whole blood (*ca* 0.5 mL) was retained separately for analysis. Plasma was separated by centrifugation and the blood cells discarded. The exposed area of skin was washed as described above prior to being clipped and the hair clippings retained along with the dressings. The stratum corneum was removed by 20 successive tape strips and each tape strip was retained separately. The exposed area was dissected out and retained. The remaining carcass and gastrointestinal tract were retained.

Each sample retained was analysed for total radioactivity.

The mass balance was complete for [^{14}C]-XDE-208 from all three test preparations and across all time points was (99%, 95% and 94%, of the applied dose, respectively).

The results for the test preparations can be expressed as *absorbed*, (excreted dose plus dose retained in the body, excluding the application site), or as the *dermal delivery* (excreted dose plus dose retained in the body, plus the *absorbable dose* (residue remaining at the application site).

As this study design incorporated the separation of the stratum corneum from the application site, the definition of the *dermal delivery* was refined to exclude the stratum corneum. The inclusion of the stratum corneum was covered by an additional definition of *potential absorbable dose*.

This study was designed to determine the dermal absorption and the fate of that residual material (application site), in particular the portion absorbed and the rate at which it is absorbed (EPA 870/7600).

For all three test preparations (formulation , highest and lowest spray dilutions concentrations), the amount of the applied dose removed across all time points, by washing the skin at 10 h post dose; was 96%, 71% and 67% of the applied dose, respectively.

Formulation (GF-2032)

At 24 h following topical application of [^{14}C]-XDE-208 in GF-2032, the *absorbed dose* of was 1.5% (42.61 µg equiv./cm^2) and at subsequent sampling times (48, 96,144 at 192 hours), the absorbed dose was 1.7 % (49.35 µg equiv./cm^2), 1.1% (37.20 µg equiv./cm^2), 1.4% (42.44 µg equiv./cm^2) and 1.2% (43.47 µg equiv./cm^2 indicating that absorbed dose was low and complete by 24 hours with a maximum absorbed dose of 1.7%

Highest Concentration Spray Dilution (0.48g/L)

At 24 h following topical application of [^{14}C]-XDE-208 in highest concentration in-use dilution (0.48 g/L), the absorbed dose of [^{14}C]-XDE-208 was 2.01% (98.89 ng equiv./cm^2) and at subsequent sampling times (48, 96, 144 at 192 hours), the absorbed dose was 2.88 % (132.94 ng equiv./cm^2), 8.16% (392.91 ng equiv./cm^2), 11.24% (549.82 ng equiv./cm^2) and 11.35% (555.56 ng equiv./cm^2).

This indicates that absorption was initially increasing until reaching a plateau at 144 h to give a dermal absorption value of ca. 11%.

Lowest Concentration Spray Dilution (0.024g/L)

At 24 h following topical application of [^{14}C]-XDE-208 in lowest concentration in-use dilution (0.024 g/L), the absorbed dose of [^{14}C]-XDE-208 was 2.50% (6.50 ng equiv./cm^2) and at subsequent sampling times (48, 96, 144 at 192 hours), the absorbed dose was 1.25 % (4.42 ng equiv./cm^2), 6.02% (16.05 ng equiv./cm^2), 12.51% (34.31 ng equiv./cm^2) and 10.77% (29.87 ng equiv./cm^2).

This indicates that absorption was initially increasing until reaching a plateau at 12.5 % at 144 h.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Preparations:

Three test preparations were tested.

Test Preparation 1: Radiolabelled Formulation GF-2032, (240g/L)

[^{14}C]-XDE-208 in GF-2032 (1.5 mL) was added to unlabeled GF-2032 (4.5 mL) and stirred until visually confirmed as homogenous. This adjustment was required to ensure an appropriate dose in terms of MBq per rat. The homogeneity and radioactive concentration were confirmed by removing nine 10 µL weighed aliquots (3 from the top, 3 from the middle and 3 from the bottom) from the test preparation and analyzed by liquid scintillation counting. The concentration of XDE-208 in the test preparation was calculated to be 236.3 g/L (1.63 MBq/g).

Test Preparation 2: Spray Dilution Formulation of Lowest In-Use Spray Dilution/ Highest Concentration (ca 0.48 g/L)

XDE-208 (10.49 mg) was transferred into a vial. GF-2032 formulation blank (105 µL) was added. The sample was mixed and the new concentration of XDE-208 was calculated to be 81.51 mg/g. The [^{14}C]-XDE-208 (100 µCi, 3.7 MBq) was removed from *ca* -20°C freezer storage and allowed to reach ambient temperature. An aliquot (22 µL, 21.13 mg) of the 81.51 mg/g formulation was added to the [^{14}C]-XDE-208. Water (pH 7, 4.98 mL, 4.96503 g) was added and the sample mixed by vortex and sonication. The homogeneity, radioactive concentration and concentration of XDE-208 were determined by removing nine 10 µL aliquots (3 from the top, 3 from the middle and 3 from the bottom) from the test preparation and analysed by liquid scintillation counting.

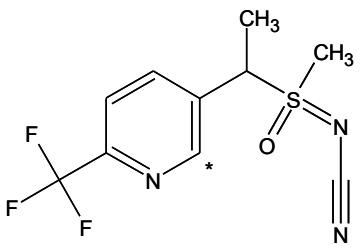
The concentration of XDE-208 in the test preparation was calculated to be 0.48 g/L (0.8 MBq/g).

Test Preparation 3: Formulation of Highest In-Use Spray Dilution/ Lowest Concentration (*ca* 0.024 g/L)

The [^{14}C]-XDE-208 (20 µCi, 0.74 MBq) was removed from *ca* -20°C freezer storage and allowed to reach ambient temperature. An aliquot (10 µL, 9.62 mg) of GF-2032 formulation blank was added to the [^{14}C]-XDE-208. Water (pH 7, 4.50 mL, 4.47170 g) was added and the sample stirred until visually confirmed as homogenous. The homogeneity, radioactive concentration and concentration of XDE-208 were determined by removing nine 10 µL aliquots from the test preparation and analysed by liquid scintillation counting. The results of this analysis showed that the sample concentration was too low. Therefore the sample was stirred and sonicated again. Two 10 µL aliquots were taken and analysed and the concentration calculated. This concentration was found to be too high. Therefore, an aliquot (3.15 mL) of the resultant sample was taken, water (1.3 mL) was added and the sample mixed. Six aliquots (10 µL) were taken from the test preparation (at random and not from top, middle and bottom) and analysed by liquid scintillation counting.

The concentration of XDE-208 in the test preparation was calculated to be 0.025 g/L (0.15 MBq/g).

Radiolabelled GF-2032:	[^{14}C]-XDE-208, batch no. XS9-37562-34
Radiochemical purity	97% [determined by HPLC,]
Specific Activity	1.63 Mbq/g
Lot/Batch #:	E3026-33
Radiolabelled XDE 208	[^{14}C]-XDE-208, batch no. XS9-37562-34
Radiochemical purity	99.3% [determined by HPLC,]
Specific Activity	45.2 McI/mmo;
Lot/Batch #:	Batch no. XS9-37562-34
Non-Radiolabelled Test Material:	XDE-208
Description:	Analytical Standard
Lot/Batch #:	TSN105878
Purity:	99.8%
Contaminants:	n/a
CAS #:	946578-00-3



* - position of ^{14}-C [2-^{14}C]

Vehicle and/or positive control: not appropriate

3. Test animals:

Species:	Rat
Strain:	Spraque Dawley (male selected as the elimination rate is slower compared to female)
Age/weight at study initiation:	6-8 Weeks 200-300g
Source:	[redacted]
Housing:	The animals were multiply housed in polypropylene and stainless steel cages with wood shavings as bedding during pre-trial periods. For collection of pre-dose urine samples and immediately following dose administration, animals were housed in all-glass metabowls designed specifically for the quantitative collection of urine and faeces. Each animal was uniquely identified by tail marking.
Feed and Water:	A complete diet of known formulation (SDS Rat and Mouse Maintenance Diet No. 1, Special Diet Services, 1 Stepfield, Witham, Essex, UK) was offered *ad libitum* to the animals. Domestic mains quality water was available *ad libitum*
Environmental conditions:	**Temperature:** 19-21ºC **Humidity:** 35-76%
Acclimation period:	

4. Preparation of dosing solutions:

Refer to section I. MATERIALS AND METHODS for preparation of dose

B. STUDY DESIGN AND METHODS:

1. Group Arrangements: 4 rats per time point Sacrifice at 24, 48, 96, 144 and 192 hours.

2. Dosing and sample collection:

A single 100 µL dose of the concentrate and in-use spray dilution was applied within the exposed area (O-ring) onto the skin on the back of the animal. The test preparation was evenly applied over the area defined within the O-ring using the pipette tip used to apply the test preparation. The pipette was weighed before and after dosing to calculate the weight of test preparation delivered to the skin surface. A pervious protective dressing (Micropore® tape) was wrapped around the animal to protect the exposed area of the skin.

Approximately 24 h prior to dose application, the fur was clipped from the back of each animal and the area wiped with lukewarm water. Care was taken not to abrade the skin. Any animal whose skin was observed to be damaged was rejected from the study and replaced.

Approximately 1 h prior to dosing, a rubber O-ring of internal area *ca* 10 cm^2 was glued, using acrylic glue, to the back of each animal.
Following dose administration, the animals were placed in all-glass metabowls designed specifically for the quantitative collection of urine and faeces. Urine and faeces were quantitatively collected for the periods predose, 0-10, 10-24 h then daily up to 192 h post dose (or up until termination). Cages were washed with water at the time of each collection and the wash retained.

At 10 h post dose, the pervious dressing was removed and retained for analysis. The expose area was gently wiped clean using a single cotton wool swab soaked in handwash soap concentrate followed by 3 cotton wool swabs soaked in lukewarm soapy water. The skin was dried with a further 3 cotton wool swabs. The washing process was repeated once. The washes and cotton wool were retained. A new pervious protective dressing was then placed on the animals.

Groups of four male rats were humanely killed (CO_2 narcosis) at 24, 48, 96, 144 and 192 h post dose administration. A terminal blood sample (*ca* 5-10 mL) was taken from the *vena cava* or by cardiac puncture into tubes containing lithium heparin.

A sample of whole blood (*ca* 0.5 mL) was retained separately for analysis. Plasma was separated by centrifugation and the blood cells discarded.

The exposed area of skin was washed as described above prior to being clipped and the hair clippings retained along with the dressings. The stratum corneum was removed by 20 successive tape strips and each tape strip was retained separately. The exposed area was dissected out and retained. The remaining carcass and gastrointestinal tract were retained.

3. Statistics:

Individual date was collected and the mean values summarised.

II. RESULTS

The report contains a summary table for each test preparation, detailed tables for each test preparation, distribution of the dose for each sacrifice time point for each individual rat. The data is

presented as % of dose applied and s absolute mass (µg equiv /cm^2). In addition the distribution through the Stratum Corneum is reported by the analysis of the individual tape strips. The data is also presented graphically.

The overall summary of the data is presented in Table 1

Table 1: Summary of the Results Following a Single Percutaneous Administration of [^{14}C]-XDE-208 for each Test Preparation Studied Results expressed as % administered dose

Test Preparation	Concentrate				
Target XDE-208 Concentration	240 g/L				
XDE-208 Concentration in Test Preparation by Radioactivity	236 g/L				
Application Site	Dermal				
Time	24 h	48 h	96 h	144 h	192 h
Dislodgeable Dose 10 h	94.44	95.16	93.09	98.06	98.86
Total Dislodgeable Dose	95.66	97.44	94.38	98.96	99.69
Unabsorbed Dose	95.78	97.63	94.65	99.30	99.99
Absorbed Dose	1.50	1.66	1.05	1.41	1.22
Dermal Delivery	1.91	2.03	1.27	1.61	1.35
Potentially Absorbable Dose	1.95	2.11	1.36	1.72	1.52
Mass Balance	97.70	99.80	96.10	101.00	101.30

Test Preparation	Highest In-Use Concentration				
Target XDE-208 Concentration	0.48 g/L				
XDE-208 Concentration in Test Preparation by Radioactivity	0.48 g/L				
Application Site	Dermal				
Time	24 h	48 h	96 h	144 h	192 h
Dislodgeable Dose 10 h	61.70	78.81	66.03	71.62	75.81
Total Dislodgeable Dose	73.75	84.50	71.21	74.22	78.02
Unabsorbed Dose	77.81	89.61	79.22	80.21	81.41
Absorbed Dose	2.01	2.88	8.16	11.24	11.35
Dermal Delivery	11.72	10.41	12.16	16.01	17.71
Potentially Absorbable Dose	13.40	13.59	15.53	17.95	18.86
Mass Balance	89.60	100.03	91.33	96.20	99.10

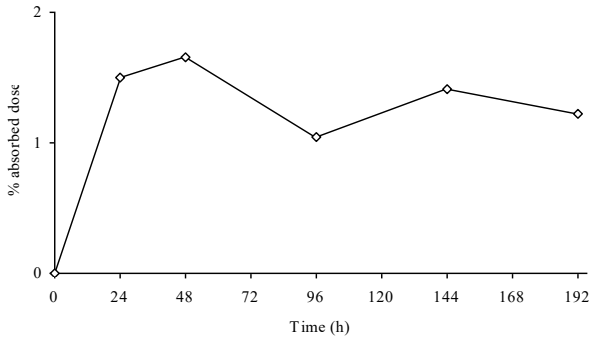
Test Preparation	Lowest In-Use Concentration				
Target XDE-208 Concentration	0.024 g/L				
XDE-208 Concentration in Test Preparation by Radioactivity	0.025 g/L				
Application Site	Dermal				
Time	24 h	48 h	96 h	144 h	192 h
Dislodgeable Dose 10 h	67.40	64.42	70.52	63.01	70.80
Total Dislodgeable Dose	76.75	73.51	76.12	67.17	71.59
Unabsorbed Dose	82.88	83.36	83.83	74.45	79.49
Absorbed Dose	2.50	1.25	6.02	12.51	10.77
Dermal Delivery	9.09	7.05	11.23	19.16	16.87
Potentially Absorbable Dose	10.05	8.66	14.50	20.51	20.11
Mass Balance	92.00	90.37	96.10	95.40	96.40

Detailed analysis for each preparation was undertaken.

Preparation 1 (GF-2032)

The absorption of XDE 208 over the duration of the study is expressed in Table 1 and Figure 1.

Figure 1: % Absorption over the Study Period Following a Single Percutaneous Administration of [14C]-XDE-208 in the Concentrate Test Preparation



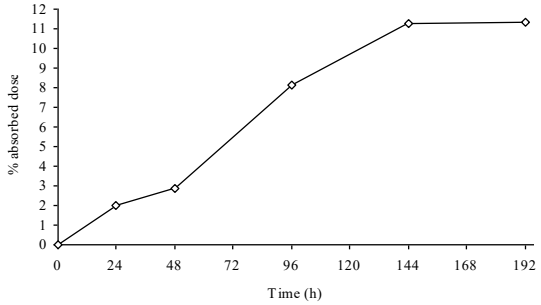
This indicates that absorption essentially ceased at 24 hours.

The concentration of total radioactivity in plasma (Table 63) gave a highest mean concentration at 24 h post application with a mean of 0.23 µg equiv./mL, decreasing to 0.21 µg equiv./mL at 48 h post application. The mean concentration of total radioactivity decreased to 0.01, 0.05 and 0.03 µg equiv./mL at 96, 144 and 192 h post application, respectively. The concentration of total radioactivity in whole blood (Table 64) was below the limit of reliable measurement at all times analysed.

The plasma data combined with the data obtained from the excretion rate profiles at 144 and 192 hrs (Figure 15 and 18), confirm that absorption had ceased by the end of the study and that the absorbed dose (1.7%) based on the sum of the urine (0.86%), faeces (0.20%), cage wash (0.24%), gastrointestinal tract (0.00%) and carcass (0.40%) at 48 hours is representative of the absorbed dose.

Preparation 2 (Spray Dilution) (*ca* 0.48 g/L)
The absorption of XDE 208 over the duration of the study is expressed in Table 1 Figure 2.

Figure 2: % Absorption over the Study Period Following a Single Percutaneous Administration of [^{14}C]-XDE-208 in the Highest In-Use Spray Dilution



This figure indicates that absorption has essentially ceased at 144 hours.

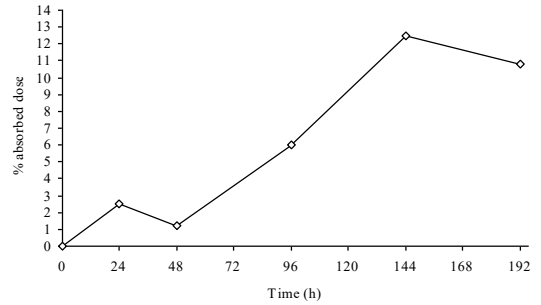
The concentration of total radioactivity in plasma and blood are presented in Table 65 and 66. The highest mean concentration was noted at 24 h post application with a mean of 2.0 ng equiv./mL, respectively, decreasing to 0.6 ng equiv./mL , respectively at 192hrs. It should be noted that these later low values were very close the background levels.

The plasma data combined with the data obtained from the excretion profiles at 144 and 192 hrs (Figure 30 and 33) confirm that the peak absorption had passed and the excreted dose particularly urine, was returning to pre dose values.

The absorbed (11.35%) was the sum of the urine (4.20%), faeces (4.73%), cage wash (2.38%), gastrointestinal tract (0.10%) and carcass (0.00%) at 192 hrs and is representative of the absorbed dose.

Preparation 3 (Spray Dilution) (*ca* 0.024 g/L)
The absorption of XDE 208 over the duration of the study is expressed in Figure 1.

Figure 3: % Absorption over the Study Period Following a Single Percutaneous Administration of [^{14}C]-XDE-208 in the Lowest In-Use Spray Dilution



This indicates that absorption essentially ceased at 144 hours.

The concentration of total radioactivity in plasma and blood following percutaneous administration of [^{14}C]-XDE-208 in the lowest in-use spray dilution is shown in Table 67 and 68. The mean concentration of total radioactivity in both was below the limit of reliable measurement at all times analysed.

The data obtained from the excretion profiles at 144 and 192 hrs (Figure 45 and 48) which confirm that the peak absorption had passed and the excreted dose, particularly urine was returning to pre dose values.

The absorbed dose (12.51%) was the sum of the urine (6.76%), faeces (2.18%), cage wash (3.58%), gastrointestinal tract (0.00%) and carcass (0.00%) at 144 hours is representative of the absorbed dose.

III. DISCUSSION

A. Applicants conclusions

The mass balance was complete for [^{14}C]-XDE-208 from all three test preparations and across all time points was (99%, 95% and 94%,of the applied dose, respectively).

For all dose groups following skin washing, some radioactivity remained in the skin at the application site and was therefore recognised as potentially absorbable. Because each skin site was tape-stripped, it was possible to discriminate between radioactivity present in the stratum corneum, which was subject either to loss from the skin surface by desquamation or to subsequent absorption, and radioactivity in the epidermis, which had translocated the stratum corneum and was therefore was potentially available for absorption, although it had not yet entered systemic circulation.

After 8 days, the excretion of the absorbed dose was minimal suggesting absorption of skin residues was no longer significant.

At each dose level, elimination of the absorbed dose in urine and faeces appeared to be fairly slow, with the greater proportion excreted *via* the urine. At the end of the collection period, excretion of radioactivity was considered to be essentially complete. However in some cases at 192 h once the animals were removed from their cages an extensive final excreta collection gave a slight rise in the excretion value at this time. This rise was considered to be related to residues of excreta products from earlier timepoints rather than a sudden increase in elimination of absorbed components. This is supported by the low levels of radioactivity in the residual carcasses and gastrointestinal tract after 8 days which are entirely consistent with limited absorption in the latter stages of each experiment.

All radioactivity concentrations in blood were low throughout each experiment. After an initial early peak all values were near to or below the limit of detection, particularly in the low dose group (0.024 g/L).

Within each dose group there was some variance in individual results however group data were consistent across the study, with good recoveries of applied radioactivity, thereby providing confidence in these absorption values determined for each dose preparation.

To conclude, following a dermal exposure period of 10 h to XDE-208 formulation concentrate (240 g/L), *ca* 93-99% of the applied radioactivity was readily removed from the skin surface by a mild detergent wash. Approximately 1-2% of the applied dose was absorbed over the exposure interval and subsequent post exposure collection periods up to 192 h after exposure. The highest absorption was observed within the 48 hour group with 1.7%.

After a 10 h dermal exposure to the 0.48g/L spray dilution, the dosed material was not as readily

removed from the skin surface with *ca* 62-79% of the applied radioactivity removed by a mild detergent wash. Approximately 2-3% of the applied radioactivity was absorbed after 24 h and 48 h, increasing to *ca* 8% at 96 h. By 144 h and 192 h the absorption levels were similar at *ca* 11%, indicating that absorption of the dose was essentially complete.

After a 10 hour dermal exposure to the 0.024g/L spray, the amount of dislodgeable dose was similar to the 0.48g/L spray dilution with *ca* 63-71% of the applied radioactivity removed from the skin surface by the detergent wash. Approximately 1-3% of the applied radioactivity was absorbed after 24 h and 48 h increasing to *ca* 6% at 96 h. By 144 h and 192 h the absorption levels were similar at *ca* 13% and *ca* 11%, respectively, indicating that absorption of the dose was essentially complete.

IIIA1 7.6.1/01 Study comments	The study was evaluated during the inclusion of the active substance. The study is not revalued.
IIIA1 7.6.1/01 Agreed endpoint	The results for the test preparations are expressed as: (1) **absorbed**, (excreted dose plus dose retained in the body, excluding the application site), (2) **dermal delivery,** (excreted dose plus dose retained in the body, plus the residue remaining at the application site excluding the *stratum corneum* (tape strips 2-20). (3) **potential absorbable dose,** (excreted dose plus dose retained in the body, plus residue remaining at the application site plus the *stratum corneum.*

Table 6.12.1-1: Summary of the Results Following a Single Percutaneous Administration of [^{14}C]-sulfoxaflor for each Test Preparation. (Expressed as mean values of % administered dose)

Summary of the Results Following a Single Percutaneous Administration of [^{14}C]-sulfoxaflor for each Test Preparation. (Expressed as mean values of % administered dose)					
Test Preparation: 240 g/L					
Time	24 h	48 h	96 h	144 h	192 h
Dislodgeable Dose 10h	94.44	95.16	93.09	98.06	98.86
Total Dislodgeable Dose	95.66	97.44	94.38	98.96	99.69
Unabsorbed Dose	95.78	97.63	94.65	99.30	99.99
Absorbed Dose	1.50	1.66	1.05	1.41	1.22
Dermal Delivery	1.91	2.03	1.27	1.61	1.35
Potentially Absorbable Dose	1.95	***2.11***	1.36	1.72	1.52
Carcass	0.00	0.40	0.00	0.00	0.00
Urine + Cage Wash	1.17	1.10	0.85	1.04	1.14
Faeces	0.25	0.20	0.50	0.38	0.08
GI Tract	0.09	0.00	0.00	0.00	0.00
Plasma[a]	0.23	0.21	0.01	0.05	0.03
Whole Blood[a]	0.03	0.06	0.05	0.00	0.07
Mass Balance	97.70	99.80	96.10	101.00	101.30
Test Preparation: 0.48 g/L					
Dislodgeable Dose 10h	61.70	78.81	66.03	71.62	75.81
Total Dislodgeable Dose	73.75	84.50	71.21	74.22	78.02
Unabsorbed Dose	77.81	89.61	79.22	80.21	81.41
Absorbed Dose	2.01	2.88	8.16	11.24	11.35
Dermal Delivery	11.72	10.41	12.16	16.01	17.71
Potentially Absorbable Dose	13.40	13.59	15.53	17.95	***18.86***
Carcass	0.00	0.00	0.00	0.00	0.00
Urine + Cage Wash	1.58	2.16	4.02	7.39	6.58
Faeces	0.27	0.69	3.10	3.85	4.73

GI Tract	0.16	0.03	0.03	0.00	0.10
Plasma[a]	2.0	1.0	1.1	0.7	0.6
Whole Blood[a]	2.1	1.1	1.4	0.5	0.2
Mass Balance	89.60	100.03	91.33	96.20	99.10
Test Preparation: 0.024 g/L					
Dislodgeable Dose 10h	67.40	64.42	70.52	63.01	70.80
Total Dislodgeable Dose	76.75	73.51	76.12	67.17	71.59
Unabsorbed Dose	82.88	83.36	83.83	74.45	79.49
Absorbed Dose	2.50	1.25	6.02	12.51	10.77
Dermal Delivery	9.09	7.05	11.23	19.16	16.87
Potentially Absorbable Dose	10.05	8.66	14.50	***20.51***	20.11
Carcass	0.00	0.00	0.00	0.00	0.00
Urine + Cage Wash	2.41	1.25	5.14	10.34	8.85
Faeces	0.09	0.00	0.90	2.18	1.93
GI Tract	0.00	0.00	0.00	0.00	0.00
Plasma[a]	0.0	0.0	0.0	0.0	0.0
Whole Blood[a]	0.0	0.0	0.0	0.0	0.0
Mass Balance	92.00	90.37	96.10	95.40	96.40

[a]expressed as ng equiv./cm^2; numbers in ***bold italic*** indicate maximum dermal absorption. The maximum time to complete absorption was 48 for the concentrate and 144 to 192h for the two dilutions.

The mass balance for [^{14}C]-sulfoxaflor from all three test preparations and across all time points was (99%, 95% and 94%, of the applied dose, respectively). The inclusion of the *stratum corneum* in the calculations for the absorbed dose is found in the *potential absorbable dose*. Following the EFSA guidance on dermal absorption (2012), tape strips 2-20 (the *stratum corneum*), should always be included unless >75% of the total absorption occurs within half of the study duration. As this is not the case (see the following absorption profile figures) for the two dilutions tested in this study, the potentially Absorbable Dose represents the most appropriate estimate of the dermal absorption for sulfoxaflor in these two cases. The undiluted formulation shows a different absorption profile in that the majority of the total absorption occurs within 48h out of the 192h study duration so technically tape strips 2-20 can be discarded. In this case the dermal delivery value is more appropriate (it just lacks the amount of sulfoxaflor attributable to the tape strips).

Conclusion:
To conclude, following a dermal exposure period of 10 h to sulfoxaflor formulation concentrate (240 g/L), 93-99% of the applied radioactivity was readily removed from the skin surface by a mild detergent wash. Approximately 1-2% of the applied dose was absorbed over the exposure interval and subsequent post exposure collection periods up to 192 h after exposure. The highest absorption was observed within the 48 hour group with 2.03%.

After a 10 h dermal exposure to the 0.48g/L spray dilution, the dosed material was not as readily removed from the skin surface with 62-79% of the applied radioactivity removed by a mild detergent wash. Approximately 13% of the applied radioactivity was absorbed after 24h and 48h, increasing to 15% at 96h. By 144h and 192h the absorption levels were similar at 18-19%, indicating that absorption of the dose was essentially complete near the end of the study.

After a 10 hour dermal exposure to the 0.024g/L spray, the amount of dislodgeable dose was similar to the 0.48g/L spray dilution with 63-71% of the applied radioactivity removed from the skin surface by the detergent wash. Approximately 10% of the applied radioactivity was absorbed after 24 h and 48 h increasing to 14% at 96h. By 144h and 192h the absorption levels had peaked and were similar at 20.5% and 20.1%, respectively, indicating that absorption of the dose was essentially complete near the end of the study.

The study finds dermal absorption values of 2.11%, 18.86% and 20.51% for the concentrate and two dilutions respectively. These values appear to correspond to appropriate time points for cessation of dermal absorption. In the case of the two dilutions, the values include the

	substance remaining on the skin in the *stratum corneum* as required by current guidance on dermal absorption (EFSA 2012). Guidance mandates the use of the *dermal delivery* value. Hence, dermal absorption values for the *in vivo* rat should be established as follows: **Formulation (GF-2032)** *Dermal delivery* (excreted dose plus dose retained in the body, plus the *absorbable dose* (residue remaining at the application site) **2.11%** **Highest Concentration Spray Dilution (0.48g/L)** *Dermal delivery* (excreted dose plus dose retained in the body, plus the *absorbable dose* (residue remaining at the application site) **18.86%** **Lowest Concentration Spray Dilution (0.024g/L)** *Dermal delivery* (excreted dose plus dose retained in the body, plus the *absorbable dose* (residue remaining at the application site) **20.51%**

IIIA 7.6.2 Comparative dermal absorption, in vitro, using rat and human skin

Report:	KIIIA1 7.6.2/01 Clive S Roper BSc PhD CBiol MSB, 2010
Title:	XDE-208: The *In Vitro* Percutaneous Absorption of Radiolabelled XDE-208 in Formulation (GF-2032) and Two In-Use Spray Dilutions Through Rat and Human Skin (OECD 428).
Document No:	Report No 786740
Guidelines:	*In Vitro* Dermal Absorption – Isolated Rat Sprague Dawley/Human Skin; OECD 428
GLP	Yes

EXECUTIVE SUMMARY:

GF-2032 is a suspension concentrate (SC) formulation containing the active substance XDE-208, an experimental insecticide. The nominal concentration of the active substance in GF-2032 is 240 g per litre. The highest in-use spray dilution concentration is produced by mixing 0.4 L of formulation with 200 L of water to give *ca* 0.48 g XDE-208/L. The lowest in-use spray dilution concentration is produced by mixing 0.1 L of formulation with 1000 L of water to give *ca* 0.024 g XDE-208/L.

As part of the safety evaluation of XDE-208, a study was required to assess the rate and extent of absorption of XDE-208 following topical application of the formulation and two typical in-use spray dilutions to human and rat skin.

The experimental procedure is summarised as follows:

Split-thickness human and rat skin membranes were mounted into flow-through diffusion cells. Receptor fluid, a tissue culture medium containing polyoxyethylene 20-oleyl ether (PEG, *ca* 6%, w/v), sodium azide (*ca* 0.01%, w/v), glucose (*ca* 1%, w/v), streptomycin (*ca* 0.1 mg/mL) and penicillin G (*ca* 100 units/mL), was pumped underneath the skin at a flow rate of *ca* 1.5 mL/h. 5% CO_2 in air was bubbled over the surface of the receptor fluid reservoir. The skin surface temperature was maintained at *ca* 32°C throughout the experiment. A tritiated water barrier integrity test was performed and any

human and rat skin sample exhibiting absorption greater than 0.6% of the applied tritiated water was excluded from subsequent absorption measurements.

Three test preparations containing [^{14}C]-XDE-208 were prepared and applied, at an application volume of 10 µL/cm^2, to human and rat split-thickness skin membranes mounted into flow-through diffusion cells *in vitro*.

Percutaneous absorption was assessed by collecting receptor fluid in hourly fractions from 0 to 6 h post application and then in 2-hourly fractions from 6 to 24 h post application. At 10 h post application, exposure was terminated by washing the skin surface with a concentrated commercial soap followed by rinsing with a dilute soap solution and drying the skin surface with tissue paper (tissue swabs). At 24 h post application (*ie* after a 14 h post exposure monitoring period), the underside of the skin was rinsed with receptor fluid (receptor rinse). The receptor rinse represented the absorbed test item, which was in the receptor chamber but had not been collected in the 22 to 24 h receptor fluid fraction. The skin surface receiving the dose was washed and dried again. The skin was then removed from the flow-through diffusion cells, dried and the stratum corneum was removed with 20 successive tape strips. The remaining skin was divided into exposed and unexposed skin and solubilised with Solvable® tissue solubiliser. All samples were analysed by liquid scintillation counting.

The results for the test preparations can be expressed as absorbed dose (receptor fluid excluding the treated skin) or as the dermal delivery (receptor fluid plus the treated skin). This study incorporated the separation of the stratum corneum from the treated skin. The definition of the dermal delivery was refined to exclude the stratum corneum. The inclusion of the stratum corneum was covered by an additional definition of potentially absorbable dose.

A summary of the mean results are provided in the tables below:

Test Preparation	1			
Target XDE-208 Concentration	240 g/L			
XDE-208 Concentration in Test Preparation by Radioactivity	244.51 g/L			
Species	Human		Rat	
Distribution	% Applied Dose	µg equiv./cm^2	% Applied Dose	µg equiv./cm^2
Dislodgeable Dose 10 h	94.73	2287.15	95.00	2293.66
Total Dislodgeable Dose	95.06	2295.13	95.46	2304.65
Unabsorbed Dose	95.16	2297.60	95.66	2309.67
Absorbed Dose	0.26	6.26	1.30	31.33
Dermal Delivery	0.31	7.51	1.63	39.24
Potentially Absorbable Dose	0.35	8.39	1.67	40.34
Mass Balance	95.48	2305.12	97.29	2348.92

Test Preparation	2			
Target XDE-208 Concentration	0.48 g/L			
XDE-208 Concentration in Test Preparation by Radioactivity	0.47 g/L			
Species	Human		Rat	
Distribution	% Applied Dose	ng equiv./cm^2	% Applied Dose	ng equiv./cm^2
Dislodgeable Dose 10 h	92.28	4336.15	84.53	3971.95
Total Dislodgeable Dose	93.99	4416.65	87.52	4112.44
Unabsorbed Dose	94.62	4445.98	89.34	4198.01

Absorbed Dose	1.54	72.57	3.94	185.03
Dermal Delivery	1.93	90.59	7.63	358.68
Potentially Absorbable Dose	2.44	114.63	8.72	409.97
Mass Balance	96.54	4536.57	96.97	4556.68

Test Preparation	3			
Target XDE-208 Concentration	0.024 g/L			
XDE-208 Concentration in Test Preparation by Radioactivity	0.0255 g/L			
Species	Human		Rat	
Distribution	% Applied Dose	ng equiv./cm^2	% Applied Dose	ng equiv./cm^2
Dislodgeable Dose 10 h	92.69	236.44	85.35	217.73
Total Dislodgeable Dose	94.45	240.94	88.00	224.49
Unabsorbed Dose	95.05	242.45	89.07	227.21
Absorbed Dose	1.15	2.92	4.34	11.08
Dermal Delivery	1.94	4.94	7.21	18.38
Potentially Absorbable Dose	2.38	6.07	8.02	20.45
Mass Balance	96.98	247.40	96.27	245.59

In conclusion, the dermal delivery for [^{14}C]- XDE-208 from GF-2032 (240 g/L) essentially ceased after 8 to 12 hours. Comparing the mean results generated as µg equiv./cm^2 or percentage of dose, the dislodgeable and unabsorbed dose and the stratum corneum profiles for the rat and human were similar. The comparative absorbed dose was 0.26% (6.26 µg equiv./cm^2) and 1.30% (31.33 µg equiv./cm^2) for human and rat, respectively. The absorbed dose for the rat skin was greater than the human skin by a factor of 5-fold. A similar difference in this ratio was observed in the dermal delivery and potentially absorbable dose.

The dermal delivery of [^{14}C]-XDE-208 from the highest concentration in-use spray dilution (0.48 g/L) for rat and human skin based on the absorption profiles had a degree of similarity. However, there was a wash-in effect observed in the rat after the 10 h wash and not in the human. The absorption rate also reduced more evidently after washing in the rat than in the human skin. Absorption at 24 h was continuing although at a decreased rate. The dermal delivery was 1.93% (90.59 ng equiv./cm^2) and 7.63% (358.68 ng equiv./cm^2) for human and rat, respectively. The dermal delivery for the rat skin was greater than the human skin by a factor of 4-fold. A similar difference in this ratio was observed when the potentially absorbable doses were compared, but a lower ratio (2.5-fold) was observed for the absorbed dose.

The dermal delivery of [^{14}C]-XDE-208 from the lowest concentration in-use spray dilution (0.024 g/L) for rat and human skin based on the absorption profiles had a degree of similarity. However, the rate of absorption fell more evidently in the human than in the rat at *ca* 4 h post dose and the reduction in the rat was increased after washing at 10 h post dose. Absorption at 24 h was continuing although at a significantly decreased rate. The dermal delivery was 1.94% (4.94 ng equiv./cm^2) and 7.21% 18.38 ng equiv./cm^2) for human and rat, respectively; which indicated that the rat skin had absorbed more XDE-208 than human skin by a factor of 3.7-fold. A similar difference in this ratio was observed for the potentially absorbable dose and absorbed dose.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Preparations:

Three test preparations were tested.

Test Preparation 1: Radiolabelled Formulation GF-2032, (240g/L)

The supplied [^{14}C]-XDE-208 in GF-2032 was used as supplied. The homogeneity and radioactive concentration were confirmed by removing nine 6.4 µL weighed aliquots (3 from the top, 3 from the middle and 3 from the bottom) from the test preparation and analysed by liquid scintillation counting. The results of the homogeneity confirmation are provided in the Table below.

	Radioactive Concentration (µCi/g)		
Region	Mean	SD	CV (%)
Top	179.40	10.33	5.76
Middle	173.86	5.07	2.92
Bottom	173.95	2.04	1.17

This was considered to be acceptable for dosing.

Test Preparation 2: Spray Dilution Formulation of Lowest In-Use Spray Dilution/ Highest Concentration (*ca* 0.48 g/L)

The following work was performed, as per protocol, under Charles River Study No. 191168. XDE-208 (10.49 mg) was transferred into a vial. GF-2032 formulation blank (105 µL) was added. The sample was mixed and the new concentration of XDE-208 was calculated to be 81.51 mg/g. The [^{14}C]-XDE-208 (100 µCi, 3.7 MBq) was removed from *ca* -20C freezer storage and allowed to reach ambient temperature. An aliquot (22 µL, 21.13 mg) of the 81.51 mg/g formulation was added to the [^{14}C]-XDE-208. Water (pH 7, 4.98 mL, 4.96503 g) was added and the sample mixed by vortex and sonication. The homogeneity, radioactive concentration and concentration of XDE-208 were determined by removing nine 10 µL aliquots (3 from the top, 3 from the middle and 3 from the bottom) from the test preparation and analysed by liquid scintillation counting. The results of the homogeneity confirmation are provided in the Table below.

	Radioactive Concentration (µCi/g)		CV
Region	Mean	SD	(%)
Top	17.97	0.09	0.48
Middle	18.33	0.40	2.16
Bottom	18.50	0.42	2.25

Note: one of the replicates from the bottom region was rejected, therefore, the results for the bottom region are reported from two aliquots only.

The mean concentration of XDE-208 in the test preparation was calculated to be 0.46 g/L. This was 95.07% of target concentration of 0.48 g/L. This was considered to be acceptable for dosing.

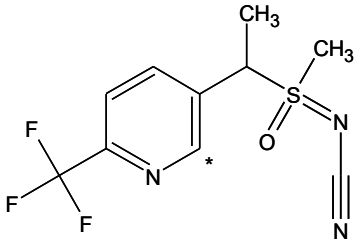
Test Preparation 3: Formulation of Highest In-Use Spray Dilution/ Lowest Concentration

(*ca* 0.024 g/L)

The following work was performed, as per protocol, under Charles River Study No. 191168. The [^{14}C]-XDE-208 (20 µCi, 0.74 MBq) was removed from *ca* -20C freezer storage and allowed to reach ambient temperature. An aliquot (10 µL, 9.62 mg) of GF-2032 formulation blank was added to the [^{14}C]-XDE-208. Water (pH 7, 4.50 mL, 4.47170 g) was added and the sample mixed by vortex and sonication. The homogeneity, radioactive concentration and concentration of XDE-208 were determined by removing nine 10 µL aliquots from the test preparation and analysed by liquid scintillation counting. The results of this analysis showed that the sample concentration was too low. Therefore the sample was mixed by vortex and sonication again. Two 10 µL aliquots were taken and analysed and the concentration calculated. This concentration was found to be too high. Therefore, an aliquot (3.15 mL) of the resultant sample was taken, water (1.3 mL) was added and the sample mixed. Six aliquots (10 µL) were taken from the test preparation (at random and not from top, middle and bottom) and analysed by liquid scintillation counting.

The mean concentration of XDE-208 in the test preparation was calculated to be 0.0248 g/L with a CV of 0.71%. This was 103.28% of target concentration of 0.024 g/L. The mean (SD) radioactive concentration was 4.04 µCi/g (0.03 µCi/g). This was considered to be acceptable for dosing.

Radiolabelled GF-2032:	[^{14}C]-XDE-208, batch no. XS9-37562-34
Radiochemical purity	97% [determined by HPLC,]
Specific Activity	1.63 Mbq/g
Lot/Batch #:	E3026-33
Radiolabelled XDE 208	[^{14}C]-XDE-208, batch no. XS9-37562-34
Radiochemical purity	99.3% [determined by HPLC,]
Specific Activity	45.2 McI/mmo;
Lot/Batch #:	Batch no. XS9-37562-34
Non-Radiolabelled Test Material:	XDE-208
Description:	Analytical Standard
Lot/Batch #:	TSN105878
Purity:	99.8%
Contaminants:	n/a
CAS #:	946578-00-3



* - position of ^{14}-C [2-^{14}C]

. **Vehicle and/or positive control:** not appropriate

Test Species

3.

Species:	Rat/ Human skin
Preparation of Skin (Rat)	Eight male CD rats (Crl:CD(SD)), aged 35-42 days, weighing 190-200 g were obtained from Charles River UK Limited, Manston Road, Margate, Kent, CT9 4LT, UK. The animals were held in the animal rooms for 8 days. The dorsal hair on the animal was then clipped. The animals were then killed *ca* 25 h later by rising carbon dioxide narcosis with death confirmed by cervical dislocation. The weights of the carcass were determined and are provided in Appendix 8. All animals were within the desired age (6 to 8 weeks) and weight (200 g to 300 g) range when killed. The clipped area of the pelt was then removed using a scalpel. The pelts were divided into 2 along the mid lateral line, placed onto aluminium foil, placed onto self sealing bags and then stored at *ca* -20C until use.
Preparation of Skin (Human) :	A total of 10 samples of human skin, obtained from 5 different donors. The skin samples were removed from storage and allowed to thaw at ambient temperature. The thickness of the uncut skin membranes was measured using a micrometer. Split-thickness membranes were prepared by pinning the full-thickness skin, stratum corneum uppermost, onto a raised cork board and cutting at a setting equivalent to 200-400 µm depth using a Zimmer® electric dermatome. The membranes were then laid out onto aluminium foil and the thickness of the membranes measured using a micrometer. The split-thickness membranes were stored at *ca* -20°C. The thickness of the human and rat full-thickness and split-thickness membranes is provided in Appendix 9.
Integrity Testing	The method as described by Meidan and Roper (2008) and modified by Runciman *et al.* (2009) was used. Tritiated water (250 µL, *ca* 100,000 disintegrations per minute [d.p.m.]) was applied to the surface of each skin sample and the donor chamber occluded. Penetration of tritiated water was assessed by collecting receptor fluid for 1 h and analysing the sample by liquid scintillation counting. The mean d.p.m. applied for the tritiated water was calculated from the seven mock tritiated water samples taken at the time of dosing. The percentage absorption was then calculated for each skin sample from the 1 h receptor fluid sample collected. Any human skin sample exhibiting a percentage absorption value greater than 0.6% was excluded from subsequent absorption measurements. A cross reference for skin cell number, donor number and % absorption is presented in Appendix 13. At the end of the 1 h period, residual tritiated water was removed from the skin surface by rinsing with water (*ca* 2 mL). The residual water was then removed from the skin surface using a plastic pastette, rinsed with water (*ca* 1 to 2 mL) and dried with tissue paper. An equilibration period of *ca* 90 min was allowed prior to collection of the pre-dose sample which was collected for *ca* 45 min.
Solubility in Receptor Fluid	Receptor fluid, tissue culture medium containing polyoxyethylene 20-oleyl (PEG, *ca* 6%, w/v), sodium azide (*ca* 0.01%, w/v), glucose (*ca* 1%, w/v), streptomycin (0.1 mg/mL) and penicillin G (100 units/mL) was used as the

receptor fluid. 5% CO_2 in air was bubbled over the surface of the receptor fluid reservoir.

XDE-208 has a water solubility of *ca* 570 mg/L. For an application of 10 µL/cm^2 of the highest concentration formulation (GF-2032, 240 g/L) over a 0.64 cm^2 application area (*ca* 1536 µg/ 0.64 cm^2), if 100% was absorbed in 24 h (36 mL), then this was equivalent to 43 µg/mL (43 mg/L). Therefore, this receptor fluid was not considered to be rate limiting for solubility.

4. Preparation of dosing solutions:

Refer to section I. MATERIALS AND METHODS for preparation of dose

B. STUDY DESIGN AND METHODS:

1. Design:

An automated flow-through diffusion cell apparatus (Scott/Dick, University of Newcastle-upon-Tyne, UK) was used (see photograph overleaf). The flow-through cells were placed in a steel manifold heated *via* a circulating water bath to maintain the skin surface temperature at *ca* 32°C (Appendix 10). The cells were connected to multi-channel peristaltic pumps from their afferent ports, with the receptor fluid effluent dropping *via* fine bore tubing into scintillation vials on a fraction collector.

The surface area of exposed skin within the cells was 0.64 cm^2. The receptor chamber volume was 0.25 mL. The peristaltic pumps were adjusted to maintain a flow-rate of *ca* 1.5 mL/h

2. Dosing and sample collection:

Dosing

Test Preparation 1 (GF-2032) was applied over the surface of the stratum corneum of the exposed skin using a Gilson Microman positive displacement pipette set to deliver *ca* 6.4 µL (*ca* 10 µL/cm^2). The donor chambers were left open to the atmosphere. To accurately quantify the radioactivity applied to the skin samples, eight *ca* 6.4 µL aliquots of test preparation were taken and weighed. These samples were mixed with methanol (1 mL) and scintillant (10 mL) for analysis by liquid scintillation counting. Test Preparation 2 and Test Preparation 3 (in use dilutions of GF-2032) were applied to skin samples and analysed in a similar manner except that the representative dose confirmation samples were not weighed.

A summary of the cells to which each of the test preparations were applied are shown in the Table below.

Test Preparation No.	Cells Nos
1	28-37, 39-46 and 54
2	1-5, 7, 8, 10, 11, 13, 16-19 and 21-25
3	58-71, 73,76, 77, 79, 81 and 83

Sample Collection

Receptor fluid was collected in hourly fractions from 0 to 6 h post dose and then in 2 hourly fractions from 6 to 24 h post dose. All receptor fluid samples were mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting.

The exposure period was terminated at 10 h by applying concentrated commercial hand wash soap (*ca* 50 µL) to each cell which was rubbed in with a tissue swab. Each cell was then washed with commercial soap diluted in water (2%, v/v, 5 mL). Each aliquot was aspirated three times. The skin surface was dried with a tissue swab. This process was then repeated except that the skin surface was dried with two tissue swabs. The tissue swabs were retained in scintillation vials, mixed with methanol (1 mL) and scintillation fluid (10 mL) and analysed by liquid scintillation counting. The skin wash was pooled in one pre weighed skin wash vial per skin sample. Duplicate weighed aliquots (1 mL) were removed from each skin wash vial, mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting. The pipette tips were mixed with methanol (1 mL) and scintillation fluid (10 mL) and analysed directly by liquid scintillation counting.

At 24 h post dose, the diffusion cell was disconnected from the receptor fluid pump lines. The underside of the skin was washed (receptor rinse) with receptor fluid (1 to 2 mL), which was collected into vials, mixed with scintillation fluid (10 mL) and then analysed by liquid scintillation counting. The receptor rinse represented the absorbed test item, which was in the receptor chamber but had not been collected in the 22 to 24 h receptor fluid fraction. The skin was washed and dried as previously performed at 10 h post dose.

The cell was dismantled and the skin removed from the cell. The donor and receptor chambers were placed into pot containing ethanol (*ca* 20 mL and 40 mL, respectively). The solvent was then allowed to extract the test item for *ca* 30 min during this time, the sample were sonicated for *ca* 10 min. The equipment was then removed from the pots. Duplicate weighed aliquots (2 mL) were removed from each of the posts, mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting.

The stratum corneum was removed with 20 successive D-Squame® discs. Where a small piece of epidermis was removed, the tape strip number was recorded and the tape stripping process was stopped. These samples are provided in Appendix 15. The discs were placed into vial containing methanol (1 mL) and scintillant (10 mL) and analysed by liquid scintillation counting.

The skin under the cell flange (unexposed skin) was cut away from the exposed skin with scissors. The samples were placed into individual vials containing Solvable® (1 mL) to dissolve the skin. The samples were placed into a water bath at *ca* 60-65°C for *ca* 2-5 h to aid tissue solubilisation. All samples were mixed with stannous chloride solution (0.2 g/mL, 50 µL) and scintillation fluid (10 mL) and analysed by liquid scintillation counting. Stannous chloride was added to reduce quenching of the liquid scintillant by Solvable®.

3. Statistics:

$$\text{Sample amount (µg equiv./cm}^2\text{)} = \frac{\text{sample radioactivity (d.p.m.)}}{\text{SA (d.p.m./µg equiv.) x exposure area (cm}^2\text{)}}$$

$$\text{Sample absorbed dose (\%)} = \frac{\text{sample radioactivity (d.p.m.)}}{\text{applied dose (d.p.m.)}} \times 100\%$$

II. RESULTS

The results for the test preparations can be expressed as absorbed dose (receptor fluid excluding the treated skin) or as the dermal delivery (receptor fluid plus the treated skin). This study incorporated the separation of the stratum corneum from the treated skin. The definition of the dermal delivery was refined to exclude the stratum corneum. The inclusion of the stratum corneum was covered by an additional definition of potentially absorbable dose.

The overall summary of the data is presented in the following Table

Test Preparation	1			
Target XDE-208 Concentration	240 g/L			
XDE-208 Concentration in Test Preparation by Radioactivity	244.51 g/L			
Species	Human		Rat	
Distribution	% Applied Dose	µg equiv./cm^2	% Applied Dose	µg equiv./cm^2
Dislodgeable Dose 10 h	94.73	2287.15	95.00	2293.66
Total Dislodgeable Dose	95.06	2295.13	95.46	2304.65
Unabsorbed Dose	95.16	2297.60	95.66	2309.67
Absorbed Dose	0.26	6.26	1.30	31.33
Dermal Delivery	0.31	7.51	1.63	39.24
Potentially Absorbable Dose	0.35	8.39	1.67	40.34
Mass Balance	95.48	2305.12	97.29	2348.92

Test Preparation	2			
Target XDE-208 Concentration	0.48 g/L			
XDE-208 Concentration in Test Preparation by Radioactivity	0.47 g/L			
Species	Human		Rat	
Distribution	% Applied Dose	ng equiv./cm^2	% Applied Dose	ng equiv./cm^2
Dislodgeable Dose 10 h	92.28	4336.15	84.53	3971.95
Total Dislodgeable Dose	93.99	4416.65	87.52	4112.44
Unabsorbed Dose	94.62	4445.98	89.34	4198.01
Absorbed Dose	1.54	72.57	3.94	185.03
Dermal Delivery	1.93	90.59	7.63	358.68
Potentially Absorbable Dose	2.44	114.63	8.72	409.97
Mass Balance	96.54	4536.57	96.97	4556.68

Test Preparation	3			
Target XDE-208 Concentration	0.024 g/L			
XDE-208 Concentration in Test Preparation by Radioactivity	0.0255 g/L			
Species	Human		Rat	
Distribution	% Applied Dose	ng equiv./cm^2	% Applied Dose	ng equiv./cm^2
Dislodgeable Dose 10 h	92.69	236.44	85.35	217.73
Total Dislodgeable Dose	94.45	240.94	88.00	224.49
Unabsorbed Dose	95.05	242.45	89.07	227.21
Absorbed Dose	1.15	2.92	4.34	11.08
Dermal Delivery	1.94	4.94	7.21	18.38
Potentially Absorbable Dose	2.38	6.07	8.02	20.45
Mass Balance	96.98	247.40	96.27	245.59

The report contains detailed tables for each test preparation, distribution of the dose , dose /collection period and distribution through the stratum corneum . The data is presented as % of dose applied and s absolute mass (µg equiv /cm^2). In addition the distribution through the Stratum Corneum is reported by the analysis of the individual tape strips. The data is also presented graphically.

III. DISCUSSION

A. Applicants conclusions

In conclusion, the dermal delivery for [^{14}C]- XDE-208 from GF-2032 (240 g/L) essentially ceased after 8 to 12 hours. Comparing the mean results generated as µg equiv./cm^2 or percentage of dose, the dislodgeable and unabsorbed dose and the stratum corneum profiles for the rat and human were similar. The comparative absorbed dose was 0.26% (6.26 µg equiv./cm^2) and 1.30% (31.33 µg equiv./cm^2) for human and rat, respectively. The absorbed dose for the rat skin was greater than the human skin by a factor of 5-fold. A similar difference in this ratio was observed in the dermal delivery and potentially absorbable dose.

The dermal delivery of [^{14}C]-XDE-208 from the highest concentration in-use spray dilution (0.48 g/L) for rat and human skin based on the absorption profiles had a degree of similarity. However, there was a wash-in effect observed in the rat after the 10 h wash and not in the human. The absorption rate also reduced more evidently after washing in the rat than in the human skin. Absorption at 24 h was continuing although at a decreased rate. The dermal delivery was 1.93% (90.59 ng equiv./cm^2) and 7.63% (358.68 ng equiv./cm^2) for human and rat, respectively. The dermal delivery for the rat skin was

greater than the human skin by a factor of 4-fold. A similar difference in this ratio was observed when the potentially absorbable doses were compared, but a lower ratio (2.5-fold) was observed for the absorbed dose.

The dermal delivery of [^{14}C]-XDE-208 from the lowest concentration in-use spray dilution (0.024 g/L) for rat and human skin based on the absorption profiles had a degree of similarity. However, the rate of absorption fell more evidently in the human than in the rat at *ca* 4 h post dose and the reduction in the rat was increased after washing at 10 h post dose. Absorption at 24 h was continuing although at a significantly decreased rate. The dermal delivery was 1.94% (4.94 ng equiv./cm^2) and 7.21% 18.38 ng equiv./cm^2) for human and rat, respectively; which indicated that the rat skin had absorbed more XDE-208 than human skin by a factor of 3.7-fold. A similar difference in this ratio was observed for the potentially absorbable dose and absorbed dose.

IIIA1 7.6.2/01 Study comments	The study was evaluated during the inclusion of the active substance. The study is not revalued.
IIIA1 7.6.2/01 Agreed endpoint	In conclusion, the dermal delivery for [14C]- XDE-208 from GF-2032 (240 g/L) essentially ceased after 8 to 12 hours. Comparing the mean results generated as µg equiv./cm^2 or percentage of dose, the dislodgeable and unabsorbed dose and the stratum corneum profiles for the rat and human were similar. The dermal delivery was 0.35% (8.39 µg equiv./cm^2) and 1.67% (40.34 µg equiv./cm^2) for human and rat, respectively. The dermal delivery of [14C]-XDE-208 from the highest concentration in-use spray dilution (0.48 g/L) for rat and human skin based on the absorption profiles had a degree of similarity. However, there was a wash-in effect observed in the rat after the 10 h wash and not in the human. The absorption rate also reduced more evidently after washing in the rat than in the human skin. Absorption at 24 h was continuing although at a decreased rate. The dermal delivery was 2.44% (114.63 ng equiv./cm^2) and 8.72% (409.97 ng equiv./cm^2) for human and rat, respectively. The dermal delivery for the rat skin was greater than the human skin by a factor of 4-fold. A similar difference in this ratio was observed when the potentially absorbable doses were compared, but a lower ratio (2.5-fold) was observed for the absorbed dose. The dermal delivery of [14C]-XDE-208 from the lowest concentration in-use spray dilution (0.024 g/L) for rat and human skin based on the absorption profiles had a degree of similarity. However, the rate of absorption fell more evidently in the human than in the rat at *ca* 4 h post dose and the reduction in the rat was increased after washing at 10 h post dose. Absorption at 24 h was continuing although at a significantly decreased rate. The dermal delivery was 2.38% (6.07 ng equiv./cm^2) and 8.02% (20.45 ng equiv./cm^2) for human and rat, respectively; which indicated that the rat skin had absorbed more XDE-208 than human skin by a factor of 3.7-fold. A similar difference in this ratio was observed for the potentially absorbable dose and absorbed dose. **Overall Conclusions:** The *in vivo* rat dermal absorption for undiluted (240 g/L) was 1-2% of the applied dose measured for up to 192 h after exposure. The highest absorption was 2.11%, observed within the 48-hour group with skin included as per current guidance. For the most concentrated spray dilution (0.48 g/L spray dilution) absorption

increased to *ca* 18% at 144 - 192 h indicating that absorption was complete.
For the most dilute spray dilution (0.024 g/L spray), absorption increased to *ca* 20.5% at 144 h and was *ca* 20% at 192 h, indicating that absorption was complete.
Data from the study of absorption through rat and human skin ***in vitro*** gave a value for undiluted GF-2032 (240 g/L) of 1.67% for rat skin, which was very similar to the value of 1-2% for rat skin in the in vivo study. The corresponding value for human skin was 0.35%.
Dermal absorption of sulfoxaflor from the most concentrated spray dilution (0.48 g/L) was continuing for rat and human skin at 24 h although at a decreased rate. The potentially absorbable dose was 8.72% and 2.44% for rat and human skin, respectively.
Dermal absorption of sulfoxaflor from the most dilute spray dilution (0.024 g/L) was continuing for rat and human skin at 24 h although at a significantly decreased rate. The dermal delivery was 8.02% and 2.38% for rat and human skin, respectively.
The *in vivo* human equivalent values can be calculated from the *in vivo* rat data, by dividing the *in vivo* rat data by the *in vitro* rat/human ratio:

Suspension Concentration	(240 g/L)	(*in vivo* rat)/ (*in vitro rat/ in vitro* human) = 0.4 % (2.11)/(1.67/0.35)
Spray dilution	(0.48 g/L)	(*in vivo* rat)/ (*in vitro rat/ in vitro* human) = 5 % (18.86)/(8.72/2.44)
Spray dilution	(0.024 g/L)	(*in vivo* rat)/ (*in vitro rat/ in vitro* human) = 6 % (20.51)/(8.02/2.38)

Endpoints used in risk assessment
The dermal absorption values for sulfoxaflor were generated using a reference formulation (GF-2032) that contained a nominal 240 g/L sulfoxaflor as described above. Since GF-2372 is a granular formulation but contains 500 g/L sulfoxaflor, which represents a 2-fold concentration with respect to the reference product, a *pro rata* correction is not considered necessary because the dermal absorption value for GF-2032 will overestimate that for GF-2372 to provide a conservative estimate of operator exposure. Therefore the RMS employs an unchanged and conservative dermal absorption value of 0.4% for the undiluted GF-2372 in this human health risk assessment for exposure to sulfoxaflor. The proposed in-use dilution for the smallest application volume per hectare corresponds to 0.024 g/L which is similar to the tested dilution of the reference formulation at 0.024g/L. A *pro rata* correction is not considered necessary in this case results in a dermal absorption value identical to that of the reference formulation, i.e. 6%.

GF-2372 (500 g/kg)
Neat product = 0.4% dermal abs
Spray dilution = 6% dermal abs

IIIA 7.7 Dislodgeable residues

IIIA 7.7.1 Dislodgeable residues – foliar

Citation: Rotondaro, A, McKellar, R. 2010. Dissipation of Dislodgeable Foliar Sulfoxaflor Residues from Treated Wheat. Dow AgroSciences Report No. 101090. 08 July 2010.

Executive Summary:

Two applications at 50 g/Ha of two different formulations were made to evaluate the foliar dissipation of sulfoxaflor on wheat in two growing regions of the United States. The trials were conducted in California and Georgia with the suspension concentrate (240SC) and water dispersible granule (500WDG) formulations in the spring of 2010. The samples were shipped to Dow AgroSciences LLC for analysis. Field fortifications were conducted a total of five times for both trials; two in California and three in Georgia. The recoveries ranged from 75.5% to 109% with an overall average of 97.4%. Dislodgeable residues at the California site ranged from 0.0516 µg/cm2 at time zero after the second application of the 240 SC formulation to 0.000236 ug/cm2 at the 22 day sampling interval. Dislodgeable residues at the California site ranged from 0.131 µg/cm2 at time zero after the second application of the 500 WDG formulation to 0.000588 ug/cm2 at the same 22 day sampling interval. Dislodgeable residues at the Georgia site ranged from 0.0331 µg/cm2 at time zero after the second application of the 240 SC formulation to 0.000259 ug/cm2 at the 20 day sampling interval. Dislodgeable residues at the Georgia site ranged from 0.0287 µg/cm2 at time zero after the second application of the 500 WDG formulation to 0.000222 ug/cm2 at the same 20 day sampling interval. The foliar half life of sulfoxaflor for both formulations across both locations ranged from 2.9 to 3.9 days. The overall average half life for the two formulations at both sites was 3.3 days. Both formulations showed similar results.

IIIA1 7.7.1/1 Study comments	This study is not assessed.
IIIA1 7.7.1/1 Agreed endpoint	The results of the present study are not taken into account. Furthermore, this study is judged not necessary.

IIIA 7.7.2 Dislodgeable residues – soil

This is not an EC data requirement in Regulation 1107/2009/EC.

IIIA 7.7.3 Dislodgeable residues – indoor surface and volatilisation

This is not an EC data requirement in Regulation 1107/2009/EC.

IIIA 7.8 Epidemiology

This is not an EC data requirement in Regulation 1107/2009/EC.

IIIA 7.9 Data on Formulants

IIIA 7.9.1 Material Safety Data Sheet for each formulant

Data on formulants are considered CONFIDENTIAL BUSINESS INFORMATION as public disclosure would compromise trade secrets regarding the composition of the formulation.

IIIA 7.9.2 Available toxicological data for each formulant

Data on formulants are considered CONFIDENTIAL BUSINESS INFORMATION as public disclosure would compromise trade secrets regarding the composition of the formulation.

IIIA 7.10 Domestic animal/livestock assessment

This is not an EC data requirement in Regulation 1107/2009/EC.

IIIA 7.11 Other/special studies

No other/special studies.

Appendix 1 List of data submitted in support of the application

Data protection statement
Dow AgroSciences is the first applicant for approval of this active substance. Data protection for the studies and tests indicated in the following reference list is claimed for a period of 10 years from the first authorisation of the formulation in each Member State in accordance with Article 59 of Regulation (EC) No 1107/2009.

These tests and studies are submitted to a Member State for first time and as such, all are considered necessary for the authorisation. Relevant studies (as listed in SanCo Guidance Document 7109/VI/1995) have been conducted in compliance with the principles of GLP or GEP.

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner	Essential data
KIIIA 7.1.1	[redacted]	2009a	Acute Oral Up and Down Procedure in Rats [redacted] Report No.: 090348 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS	Y
KIIIA 7.1.2	[redacted]	2009b	Acute Dermal Toxicity Study in Rats [redacted] Report No.: 090349 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS	Y
KIIIA 7.1.3	[redacted]	2010	GF-2372: Acute Dust Aerosol Inhalation Toxicity Study in F344/Ducrl Rats [redacted] Report No.: 101024 GLP/GEP (Y/N): Y	Y	DAS	Y

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner	Essential data
			Published (Y/N): N			
KIIIA 7.1.4	[redacted]	2010a	Primary Skin Irritaton Study in Rabbits [redacted] Report No.: 101055 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS	Y
KIIIA 7.1.5	[redacted]	2010b	Primary Eye Irritation Study in Rabbits [redacted] Report No.: 101056 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS	Y
KIIIA 7.1.6	[redacted]	2008	GF-2372: Local Lymph Node Assay in CBA/J Mice [redacted] Report No.: 101020 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS	Y
KIIIA 7.6.1	[redacted]	2010	XDE-208: The In Vivo Percutaneous Absorption of Radiolabelled XDE-208 in Formulation (GF-2032) and Two In-Use Spray Dilutions in the Rat (OECD 427) [redacted] Report No.: 191168	Y	DAS	Y

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner	Essential data
			GLP/GEP (Y/N): Y Published (Y/N): N			
KIIIA 7.6.2	[redacted]	2010	XDE-208: The *In Vitro* Percutaneous Absorption of Radiolabelled XDE-208 in Formulation (GF-2032) and Two In-Use Spray Dilutions through Rat and Human Skin (OECD 428) [redacted] Report No.: 786740 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS	Y
KIIIA 7.7.1/1	[redacted]	2010a	Dissipation of Dislodgeable Foliar Sulfoxaflor Residues from Treated Wheat [redacted] Report No.: 101090 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS	Y

Appendix 2 Table of intended uses, GAP and justification for the risk envelope

Crop and/or situation (a)	Member State or Country	Product Name	F or G (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI days (k)	Remarks (l)
					Type (d-f)	Conc. of a.s. (i) g/L	Method Kind (f-h)	Growth stage (j)	Number min max	Interval between applications (min)	kg as/hl min max	Water (l/ha) min max	kg as./ha min max		
Cotton	South (EL)	GF-2372	F	Aphids	WG	500 g/kg	Ground applied foliar spray, broadcast	BBCH 20-87 May-Sep	1-2	7	0.004-0.0016	300 - 1000	0.024	14	Two applications would be minimum 7 days interval.
Oilseed Rape	South (FR)	GF-2372	F	Aphids	WG	500 g/kg	Ground applied foliar spray, broadcast	BBCH 10 - 29 Sep-Dec BBCH 30 – 87 Apr-Jun	1-2	21	0.004-0.016	100-600	0.024	28	Two applications would be minimum 21 days interval. Only 1 application is allowed in the Sep-Dec interval followed by 1 application in the April-June period. If no autumn application, 2 spring applications are possible.
Cereal (Wheat, Barley, Oats, Rye, Spelt, Triticale) [W, S]	South (FR, IT)	GF-2372	F	Aphids	WG	500 g/kg	Ground applied foliar spray, broadcast	BBCH 12-29 Sep-Dec BBCH 30 – 87 Mar-Jul	1-2	21	0.004-0.016	100-600	0.024	21	Two applications would be minimum 21 days interval. Only 1 application is allowed in the Sep-Dec interval followed by 1 application in the March-July period. If no autumn application, 2 spring applications are possible.

Remarks:
(a) For crops the EU and Codex classifications (both) should be used.
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) e.g. biting and sucking insects, soil borne insects, foliar fungi, weeds
(d) e.g. wettable powder (WP),emulsifiable concentrate (EC), granule (GR)
(e) GIFAP Codes - GIFAP Technical Monograph No. 2, 1989
(f) All abbreviations must be explained
(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants
(i) g/kg or g/l
(j) Growth stage at last treatment, including where relevant information on season at time of application
(k) The minimum and maximum number of applications possible under practical conditions must be given
(l) PHI - Pre-harvest interval
(m) Remarks may include: Extent of use/ economic importance/restrictions (e.g. feeding/grazing)/minimal intervals between applications. Indicate uses not yet authorised.

Appendix 3 Operator and bystander calculations

Appendix 3 Table 1a

Estimation of operator exposure to the active ingredient sulfoxaflor upon application of GF-2372 (cereal, BBA model, field crop, tractor mounted boom sprayer, no PPE)

THE GERMAN MODEL (GEOMETRIC MEAN VALUES)

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles				
Product	**TRANSFORM**		Active substance		**Sulfoxaflor**
Formulation type	WG		a.s. concentration		**500** g/kg
Dermal absorption from product	**0,4** %		Dermal absorption from spray		**6** %
RPE during mix/loading	None		RPE during application		None
PPE during mix/loading	None				
PPE during application: Head	None	Hands	None	Body	None
Dose	**0,048** kg product/ha		Work rate/day		**20** ha

DERMAL EXPOSURE DURING MIXING AND LOADING

Hand contamination/kg a.s.	2	mg/kg a.s.
Hand contamination/day	0,96	mg/day
Protective clothing	none	
Transmission to skin	100	%
Dermal exposure to a.s.	0,96	mg/day

INHALATION EXPOSURE DURING MIXING AND LOADING

Inhalation exposure/kg a.s.	0,008	mg/kg a.s.
Inhalation exposure/day	0,00384	mg/day
RPE	none	
Transmission through RPE	100	%
Inhalation exposure to a.s.	0,00384	mg/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique: Tractor-mounted/trailed boom sprayer: hydraulic nozzles

	Head	Hands	Rest of body	
Dermal contamination/kg a.s.	0,06	0,38	1,6	
Dermal contamination/day	0,0288	0,1824	0,768	
Protective clothing	none	none	none	
Transmission to skin	100	100	100	%
Total dermal exposure to a.s.	0,9792	mg/day		

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure/kg a.s.	0,001	mg/kg a.s.
Inhalation exposure/day	0,00048	mg/day
RPE	none	
Transmission through RPE	100	%
Inhalation exposure to a.s.	0,00048	mg/day

ABSORBED DOSE

	Mix/load		Application	
Dermal exposure to a.s.	0,96	mg/day	0,9792	mg/day
Percent absorbed	0,4	%	6	%
Absorbed dose (dermal route)	0,00384	mg/day	0,058752	mg/day
Inhalation exposure to a.s.	0,00384	mg/day	0,00048	mg/day
Total systemic exposure	0,00768	mg/day	0,059232	mg/day

PREDICTED EXPOSURE

Total systemic exposure	0,066912	mg/day
Operator body weight	70	kg
Operator exposure	**0,000955886**	**mg/kg bw/day**

AOEL (mg/kg/day) =	0,0600
% of AOEL =	**1,6%**

Appendix 3 Table 1a

Estimation of operator exposure to the active ingredient sulfoxaflor upon application of GF-2372 (cereal, UKPOEM model, field crop, tractor mounted boom sprayer, no PPE)

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM) WITH GERMAN MODEL MIX/LOAD DATA (75th PERCENTILE)

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Product	**GF-2372**	Active substance	**Sulfoxaflor**	
Formulation type	WG or SG	a.s. concentration	**500**	mg/g
Dermal absorption from product	**0,4** %	Dermal absorption from spray	**6**	%
PPE during mix/loading	None	PPE during application	None	
Dose	**0,048** kg product/ha	Work rate/day	**50**	ha
Application volume	**100** l/ha	Duration of spraying	**6**	h

DERMAL EXPOSURE DURING MIXING AND LOADING

Hand contamination/kg a.s.	5,72	mg/kg a.s.
Hand contamination/day	6,864	mg/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to a.s.	6,864	mg/day

INHALATION EXPOSURE DURING MIXING AND LOADING

Inhalation exposure/kg a.s.	0,0358	mg/kg a.s.
Inhalation exposure/day	0,04296	mg/day
RPE	None	
Transmission through RPE	100	%
Inhalation exposure to a.s.	0,04296	mg/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	
Penetration	100%	5%	15%	
Dermal exposure	6,5	0,05	0,375	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	41,55	ml/day		
Concentration of a.s. in spray soluti	0,24	mg/ml		
Dermal exposure to a.s.	9,972	mg/day		

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure to spray	0,01	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0,24	mg/ml
Inhalation exposure to a.s.	0,0144	mg/day
Percent absorbed	100	%
Absorbed dose	0,0144	mg/day

ABSORBED DOSE

	Mix/load		Application	
Dermal exposure to a.s.	6,864	mg/day	9,972	mg/day
Percent absorbed	0,4	%	6	%
Absorbed dose (dermal route)	0,027456	mg/day	0,59832	mg/day
Inhalation exposure to a.s.	0,04296	mg/day	0,0144	mg/day
Absorbed dose	0,070416	mg/day	0,61272	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0,683136	mg/day
Operator body weight	60	kg
Operator exposure	0,0113856	mg/kg bw/day

AOEL (mg/kg/day) =	0,0600
% of AOEL =	**19,0%**

Appendix 3 Table 3
Bystander Exposure Estimation BfR Calculator – cereals

Estimation of bystander and resident exposure (adults and children)

Active substance (a.s.)			**Sulfoxaflor**
Product			**GF-2372**
Intended uses	**Cereal**		**Field Crops, Tractor Mounted (FCTM)**
Treated area per day (A)		**20**	**ha/d**
Application rate (AR)		**0,024**	kg a.s./ha
Number of applications (NA)		**1**	[1]

[1] Consideration of more than two applications are not necessary if degradation of the active substance on foliage of at least 50 % can be assumed between two applications (otherwise use multiple application factor).

Dermal absorption (DA)	**6**	% (worst case, e.g. during application)
Inhalation absorption (IA)	**100**	%
Oral absorption (OA)	**100**	%
Systemic AOEL	**0,06**	mg/kg bw/d
Body weight (BW)	**60**	kg/person (adults)
	16,15	kg/person (children)

Distance between application and bystander or resident:

FCTM:	**1**	m
High crops not selected		
		m
Home & garden not selected		
		m

Drift deposit (D) for 1 appl. based on appl. technique and distance: **2,77 % (FCTM, 1 m)**

Airborne vapour concentration (ACv) mg/m³ [2]

[2] 1 µg/m³ for semivolatile substances, i.e. vapour pressure (20 °C): ≥ $1x10^{-5}$ - < $5x10^{-3}$ Pa; 15 µg/m³ for volatile substances, i.e. vapour pressure (20 °C): ≥ $5x10^{-3}$ Pa

Estimation of resident exposure after application in Field Crops, Tractor Mounted (FCTM)

Input parameters considered for the estimation of resident exposure:

Intended use(s):	Cereal		**Drift (D):**	2,77	% (FCTM, 1 m)
Application rate (AR):	0,024	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):	6,00	% ('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	0,06	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^2/d

Resident exposure towards Sulfoxaflor					
Adults			**Children**		
Residents: Dermal exposure after application in Cereal (via deposits caused by spray drift)					
$SDE_R = (AR \times NA \times D \times TTR \times TC \times H \times DA) / BW$			$SDE_R = (AR \times NA \times D \times TTR \times TC \times H \times DA) / BW$		
(0,00024 x 1 x 2,77% x 5% x 7300 x 2 x 6%) / 60			(0,00024 x 1 x 2,77% x 5% x 2600 x 2 x 6%) / 16,15		
External exposure	0,00485304	mg/person	External exposure	0,00172848	mg/person
External exposure	8,0884E-05	mg/kg bw/d	External exposure	0,00010703	mg/kg bw/d
Absorbed dose:	**0,0000049**	**mg/kg bw/d**	**Absorbed dose:**	**0,0000064**	**mg/kg bw/d**
Residents: Inhalation exposure to vapour					
$SIE_R = (AC_V \times IR \times IA) / BW$			$SIE_R = (AC_V \times IR \times IA) / BW$		
(0 x 16,57 x 100%) / 60			(0 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)		
			$SOE_H = (AR \times NA \times D \times TTR \times SE \times SA \times Freq \times H \times OA) /$		
			(0,00024 x 1 x 2,77% x 5% x 50% x 20 x 20 x 2 x 100%) / 16,15		
			External exposure	0,00013296	mg/person
			External exposure	8,2328E-06	mg/kg bw/d
			Absorbed dose	**0,0000082**	**mg/kg bw/d**
			Residents: Oral exposure (object-to-mouth transfer)		
			$SOE_O = (AR \times NA \times D \times DFR \times IgR \times OA) / BW$		
			(0,00024 x 1 x 2,77% x 20% x 25 x 100%) / 16,15		
			External exposure	0,00003324	mg/person
			External exposure	2,0582E-06	mg/kg bw/d
			Absorbed dose	**0,0000021**	**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)	0,00029118	mg/person	Total systemic exposure (absorbed dose)	0,00026991	mg/person
Total systemic exposure (absorbed dose)	**0,0000049**	**mg/kg bw/d**	**Total systemic exposure** (absorbed dose)	**0,0000167**	**mg/kg bw/d**
% of AOEL	**0,01**	**%**	**% of AOEL:**	**0,03**	**%**